

Nanotechnology: Delivering on the Promise Volume 2

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**Nanotechnology: Delivering
on the Promise
Volume 2**

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Foreword

The ACS Symposium Series was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of the series is to publish timely, comprehensive books developed from the ACS sponsored symposia based on current scientific research. Occasionally, books are developed from symposia sponsored by other organizations when the topic is of keen interest to the chemistry audience.

Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previous published papers are not accepted.

ACS Books Department

Foreword

Nanotechnology represents a huge R&D investment worldwide. The global funding of nanotechnologies was estimated to be about \$7 billion in 2011 and has increased about 20% per year since then, according to various studies. The U.S. is certainly investing heavily in nanotechnology. It started the National Nanotechnology Initiative (NNI) about 16 years ago, pulling together the efforts of 20 federal departments and independent agencies. Since 2001, the NNI has spent a total of \$22 billion; in fiscal 2015 the NNI had a budget of \$1.5 billion. Many exciting programs have been initiated by the NNI, and several commercial products have appeared in the market.

As a scientist myself, I am very interested in nanotechnology and the promise that it holds. Thus, when I was elected to the American Chemical Society (ACS) Presidential Succession, I made nanotechnology part of my scientific platform during my Presidential year in 2015. A major action item was to organize a high-profile Presidential symposium on nanotechnology to be held at the ACS national meeting in Denver in 2015. It was gratifying that the symposium was well attended with leading researchers and representatives of U.S. funding and regulatory agencies sharing valuable information and updates. My thanks to all the speakers and especially Larry Doemeny and Chuck Geraci who played leading roles in helping me organize this successful symposium.

The second part of my nanotechnology initiative was to initiate an ACS book on nanotechnology. The purpose of the two volumes of this book is to compile the latest R&D findings and to assess the current progress towards commercialization of nanotech products. I am pleased that H. N. Cheng, Larry Doemeny and Chuck Geraci were willing to collaborate with me to co-edit these books. We have invited the speakers from the Denver symposium and also many other well-known nanotechnology experts to contribute articles to these books. I am delighted that this book project has come to fruition. Thanks are due to all of the authors and my co-editors for their wonderful efforts.

The two volumes of this book contain a wealth of information on research, product development, commercialization, and regulatory issues related to nanotechnology. My hope is that these books will be a valuable resource and reference for students and active practitioners alike.

Diane Grob Schmidt
2015 ACS President

Preface

The two volumes of this book are based on the successful Presidential symposium on “Nanotechnology: Delivering on the Promise”, which took place at the ACS Spring national meeting in Denver in March 2015. The symposium featured leading researchers in U.S. academia, industry and government labs as well as representatives of major U.S. funding and regulatory agencies. The topics covered included cutting-edge research on nanotechnology and useful information on product development, manufacturing and commercialization of selected nanotech products. Their reports noted impressive progress in all aspects of nanotechnology with a huge (and growing) number of publications and numerous products already in the marketplace. However, there were also some cautionary voices on the health, safety and environmental issues related to nanotechnology. Concurrently, all of the symposium speakers were invited to provide chapters to this ACS book. We were pleased that many of them did. Additional nanotechnology experts were also invited to submit manuscripts in order to strengthen the coverage of specific technical areas.

A total of 25 chapters are included; for the reader’s convenience, this book is divided into two volumes. Volume 1 describes many U.S. government nanotechnology programs and issues relating to health, safety, environment, and regulation. Volume 2 contains four sections: energy and electronics, materials, bio/medical areas, and agriculture; these sections were authored by leading scientists in their fields. They cover promising developments, not only discoveries made in the laboratory, but also product development, collaborative research, manufacturing, and (in some cases) commercialization of nanotech products. Several chapters provide case histories that may be useful to people interested in the commercial aspects of nanotechnology.

We appreciate the efforts of all the authors who took time to prepare their manuscripts and the many reviewers for their time and talent during the peer review process. Thanks are also due to the personnel at ACS Books, particularly Arlene Furman, Elizabeth Hernandez, Bob Hauserman, and Mary Calvert for their efficient handling of the manuscripts. We hope these volumes will stand out as a source of up-to-date information for researchers, a useful guide for practitioners interested in product development, and a handy reference for people who want to learn more about nanotechnology.

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Chapter 1

Progress Towards a Synergistically Integrated, Scalable Solar Fuels Generator

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The development of an artificial photosynthetic system involves obtaining desired functionalities on the nanoscale. A viable blueprint for an artificial photosynthetic system involves two complementary, current-matched and voltage-adding photosystems, in conjunction with two different catalysts: one to oxidize water, and the other to reduce either water and/or carbon dioxide to solar fuels. Recent progress towards a robust, efficient, inexpensive and safe solar-fuels generator provides an example of nanoscale materials-by-design. The light-absorbing semiconductors have been designed and grown as high-aspect-ratio microwires which simultaneously allow minimization of ionic transport pathways, sufficient depth for light absorption in the semiconductor, efficient collection of charge carriers, and high surface areas for catalyst loading. Non-noble-metal catalysts for the redox reactions have been discovered, and methods for protecting the semiconductors against corrosion have been developed.

Introduction

Artificial photosynthesis, i.e., the direct production of fuels from sunlight, is arguably an inevitable technology. It was inevitable that vacuum tubes would be replaced with transistors. It is inevitable that vehicles will have drivetrains that use electric motors, due to their much higher efficiency than internal combustion engines. And similarly, it is inevitable that the energy from the biggest source known to mankind, the sun, will eventually be readily stored in chemical bonds, the densest form of energy known other than the nucleus of an atom.

The direct production of fuels from sunlight would also fill two formidable technological gaps in constructing a full energy system based on renewable energy sources. One such gap is massive grid-scale energy storage, to allow for compensation of the intermittency of wind and solar energy. The second gap is to provide a carbon-neutral, high energy-density transportation fuel for the 40% of global transportation that cannot viably be battery-powered: specifically aircraft, ships, and long-distance trucks.

Construction of an artificial photosynthetic system requires, at minimum, a material to absorb sunlight effectively, as well as catalysts to perform the desired fuel-forming reactions. To achieve a sustainable fuel cycle, water must also be oxidized to form O_2 . The O_2 could then be recovered from the air and used in conjunction with the combustion of solar fuel or in a fuel cell, to complete a carbon-neutral cycle of energy production, storage, and consumption. The fuel production process could entail the reduction of water to form H_2 , and/or the reduction of water and CO_2 to form a hydrocarbon or alcohol. Additionally, N_2 could be reduced to form ammonia, for use in agriculture as well as in transportation.

The construction of a viable artificial photosynthetic system is arguably a frontier for nanoscience and nanotechnology, because achieving such a system involves obtaining the desired functionality on the nanoscale, utilizing components derived from hard and soft materials and their interfaces. Although birds provided inspiration for the development of machines that could fly, aircraft are not built out of feathers. By analogy, natural photosynthesis serves as an inspiration to construct artificial photosynthetic systems that produce fuels directly from sunlight, but the goal is to have artificial photosynthetic systems provide higher efficiency and an improved value proposition relative to the natural photosynthetic system.

Background and Perspective

Photoelectrochemistry has been known to enable the direct conversion of sunlight into chemical fuel for over forty years. For instance, directing sunlight onto minerals such as $SrTiO_3$ readily facilitates the sustained, high quantum-yield splitting of water into H_2 and O_2 (1, 2). Figure 1 shows a schematic depiction of an experiment that demonstrated the “wireless”, spontaneous production of fuel from sunlight by such materials. Four attributes are required of such a technology: the system must be robust, efficient, cheap, and safe. At present, materials and system implementations offer at most two out of these four desired attributes. A major problem is that a single material must be simultaneously both oxidatively

and reductively stable in sunlight. Additionally, the gases must be produced separately to meet the safety criterion; otherwise the system would produce a potentially explosive mixture of hydrogen and oxygen.

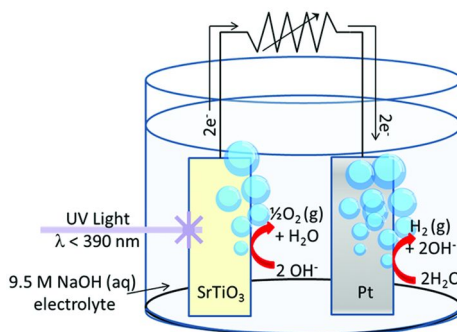


Figure 1. Schematic of a spontaneous water-splitting device using a single, wide-band-gap semiconducting light absorber (SrTiO₃) demonstrated experimentally in 1976 (2).

The blueprint provided by natural photosynthesis (Figure 2) provides a more viable design than a water-splitting system conceived around a single photoelectrode. Photosynthesis does not use one photosystem, and does not use one light absorber that must absorb light in the near-ultraviolet to obtain enough energy from each incident photon to perform the chemical bond-making and bond-breaking that is needed to generate an energy-rich fuel. Instead, nature uses two photosystems, involving two chlorophyll-based chromophores that are the basis for Photosystem I and Photosystem II, respectively. The photosystems are arranged in series, so that two 1.7 eV photons can provide a voltage equivalent to that produced by absorption of a single, higher energy, ultraviolet photon. Photosynthesis is, however, non-optimal in other aspects of its system design for the purposes of solar energy conversion. For example, both chlorophylls absorb at ~ 670 nanometers, and thus compete with each other for photons. Optimally, one material should absorb the higher-energy photons, leaving lower-energy photons for the other material.

Additionally, for production of fuels, the first law of thermodynamics provides an important constraint. Specifically, the voltages produced by each photosystem must combine to produce the voltage needed to produce fuel, including the thermodynamically required voltage as well as any kinetic overpotentials and resistance losses that will be present in a real system. In a solar cell, a system engineer can trade voltage for current with no penalty on the overall system efficiency: for example, a solar cell that provides 0.5 V of voltage and 20 mA of current produces exactly the same power as a solar cell that provides 1.0 V of voltage and 10 mA of current. In contrast, for fuel production, specifically for water splitting under standard conditions, providing 1.20 V produces no fuel, regardless of how much current is produced, whereas the production of 1.23 V or larger can yield a functioning solar-driven water-splitting system having

a rate of H_2 production that is specified by the current that flows through the system. Hence a viable blueprint for an artificial photosynthetic system involves two complementary, current-matched and voltage-adding photosystems, in conjunction with two different catalysts: one to oxidize water, and the other to reduce either water and/or carbon dioxide to generate solar fuels.

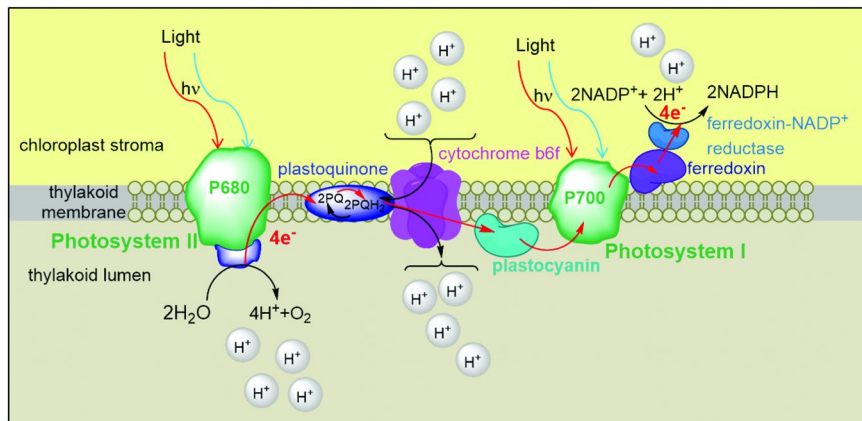


Figure 2. Schematic diagram of natural photosynthesis. The light-dependent reactions occur in the thylakoid membranes of chloroplasts, where water is oxidized to O_2 (g) and the liberated electrons are passed to an electron-transport chain that ultimately leads to the reduction of NADP to NADPH.

Another design principle implemented by natural photosynthesis is to separate the sites of reduction and oxidation. The manganese-based oxygen-evolving complex in Photosystem II is the source of all O_2 in the atmosphere. Although this metal complex is not reductively stable, the functioning Mn complex never sees a reducing environment. Similarly, many of the key reducing enzymes in a cell, such as NADH, hydrogenases and nitrogenases, are the source of photosynthetically formed fuels such as fossil fuels and biofuels. These enzymes are not oxidatively stable, but in an operating photosynthetic system they do not experience an oxidizing environment. Hence, compartmentalizing these catalysts avoids the constraint involved with ensuring that all of the catalysts and materials are chemically stable under the same conditions at the same time. This compartmentalization strategy also produces flexibility in the choice of materials as well as in the choice of designs to achieve a viable, operational artificial photosynthetic system. A catalyst-separated design also requires a method to ensure robust separation of the products; otherwise the energy-rich fuels and O_2 will tend to recombine, lowering the efficiency of the system. In addition, the mixture could potentially explode if, for example, stoichiometric mixtures of $\text{H}_2(\text{g})$ and $\text{O}_2(\text{g})$ were produced over active catalysts for their recombination. The membrane that separates the products must also be permeable to ions, to maintain charge neutrality in the system. Chemically, oxidation of water liberates protons, whereas the reduction of water and/or CO_2 and H_2O consumes protons. Hence,

protons and/or hydroxide ions must cross the membrane to maintain the pH of the system in each compartment, or a continually increasing pH gradient will result and will eventually force the system to cease operation.

System Architecture

Implementation of these design principles requires more than just the development of individual functional components, in the same way that design of a viable airplane requires more than just an engine or a wing or a fuselage. In addition to functional components, a viable architecture is required to obtain a full, functional, artificial photosynthetic system. In the mid-2000s, one such full system architecture was developed by my research group at Caltech. We reasoned that the morphology of the light absorber should optimally be permeable to protons, to produce the shortest possible path for movement of the ionic species needed to neutralize the electrical charge flow in the system. The second key feature of the architecture is to use non-planar materials, such as arrays of highly asymmetric structures, including for example nanowires or microwires. This aspect of the design would allow for use of a different class of materials than are used in solar panels, because a highly asymmetric morphology decouples the direction of light absorption from the direction of charge-carrier motion in the solid. To illustrate this point, consider for example a Si-based solar cell. Approximately 100 μm of Si is needed to fully absorb the incident sunlight; use of thinner Si results in transmission rather than absorption, and use of thicker Si samples simply wastes material. However, the $\sim 100\text{ }\mu\text{m}$ absorption depth in turn dictates the purity required of the Si. In a planar structure, charge carriers that are created deep within the structure must have an excited-state lifetime that is sufficient to allow the photoexcited carriers to diffuse to the front contact region, where they can be separated and produce electricity, before the carriers recombine and produce heat. This purity constraint limits the types of materials that can be used in efficient solar cells, and also imposes an expense associated with obtaining the requisite purity and charge-carrier collection length in such designs.

Our favored solar-fuels system architecture instead exploited favorably the morphological attributes of our system design. Using the tools of nanoscience, we imagined constructing a high-aspect-ratio set of structures that would be long enough to fully absorb incident sunlight, but instead of moving carriers back the way they came, the structure would allow for charge carriers to move sideways, in a direction orthogonal to the direction of light absorption (Figure 3). In this way, impure Si, with a 2 μm charge-carrier collection length, for example, could still in principle yield full absorption and simultaneously allow for efficient charge-carrier collection. We referred to this concept as the “orthogonalization” principle, whose implementation would require the synthesis of a “photon forest” of light absorbers, analogous to a forest of aspen trees. An additional favorable feature of the envisioned morphology and system architecture was that the nanostructures could be embedded in a membrane, to separate the products. Hence, the protons would follow the electrons along the shortest possible path, from the top to the bottom of the structure.

The preferred implementation of this architectural vision would utilize two such nanowire or microwire arrays, one that appears red to the human eye, because that material would absorb in the green, and one that looks blue to the eye, because that material would absorb in the red. The absorbers should be current-matched under sunlight, and should have band gaps of 1.7 eV and 1.1 eV, respectively, to generate the voltage needed to spontaneously split water while producing the maximum current capable of solar fuels production (3). The current-matched assembly would be connected to a water-oxidation catalyst on the top and to a water-reduction and/or CO₂-reduction catalyst on the bottom, with the absorbers and products separated by a proton-permeable membrane that would robustly separate the gas products, thereby facilitating safe operation of the system. Figure 3 provides a conceptual rendering of this design and system architecture.

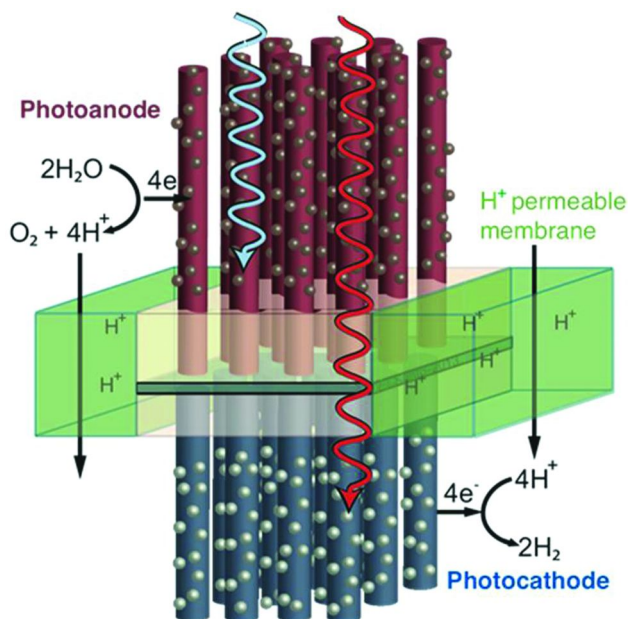


Figure 3. Schematic of a water-splitting device based on high-aspect-ratio light absorbers embedded in an ion-exchange membrane. The long axis of the light absorbers allows optimal absorption of light, particularly for semiconductors with indirect band gaps, while the short radial axis improves charge separation and collection for semiconducting materials with short minority-carrier diffusion lengths. The high surface areas of the photoelectrodes allow enhanced loading of catalysts for the fuel-forming and oxygen-evolving reactions. (Courtesy of E. A. Santori; used with permission.)

Our efforts to construct such an artificial photosynthetic system based on this vision and architecture provides an excellent example of materials-by-design using nanoscience. A major constraint is that the system must operate under conditions

where every piece works in conjunction with every other piece. Below we describe the progress that has been made in bringing this vision to reality and enabling the construction of a full, integrated, artificial photosynthetic system.

Synthesis and Characterization of Si Microwire Arrays

We first constructed a device-physics model of architectures that adhered to the orthogonalization principle, to see if they indeed offered the expected performance advantages based on the qualitative conceptualization of the approach. The device-physics model quantitatively verified the validity of the architecture, and furthermore indicated that the wires need not be oriented perfectly normal to the surface (4). For materials like Si, the modeling further indicated that microwires would provide superior performance to nanowires, and thus microwires were the target of our subsequent materials-synthesis efforts. This prediction was based on the observation of $> 1\ \mu\text{m}$ minority-carrier diffusion lengths in Si, because decreases in the diameter of the microwires to $< 1\ \mu\text{m}$ would merely produce more junction area, which would enhance junction-based recombination but not provide substantial advantages for minority-carrier collection.

For several reasons, Si was our first target material to implement the microwire/nanowire array structure. Silicon cannot readily be reduced, and hence is stable as a cathode. Furthermore, Si is stable in acid for hydrogen evolution for extended time periods. Additionally, Si has a nearly ideal band gap, 1.1 eV, for use in the bottom cell of the membrane-bound architecture. Hence Si provided an excellent material to realize the bottom component of the desired photon forests in a tandem microwire-array architecture.

We subsequently developed a process that produced Si microwires of the desired orientation, diameter, and purity for use in a solar-fuels generator. The vapor-liquid-solid growth of Si was exploited, in conjunction with a patterned catalyst such as Au, Ni or Cu, to obtain high-fidelity Si microwire arrays. Importantly, the metal catalysts were isolated and confined spatially by patterning holes into a Si oxide buffer layer, which prevented Ostwald ripening and migration of the metal along the surface when the substrate was exposed to the high temperatures needed for epitaxial growth of Si by the VLS method (5). Subsequent process improvements involved using sufficient metal such that the surface tension of the molten drop in the VLS growth step fully confined the metal on the top of the desired diameter of a growing Si microrod. Excess metal led to catalyst dripping down the side of the microwire, like wax flowing down a candle, and consequently produced highly branched structures. In contrast, use of too little metal led to pooling of the catalyst on top of the growing microwires, which also produced branched structures. When the size of the catalyst droplet matched the diameter of the growing Si crystal, high-fidelity, uniform-diameter, microwires were formed reproducibly over large areas of a patterned Si wafer. Figure 4 shows an array of such Si microwires, each grown along the (111) growth direction, as shown by scanning-electron microscopy, on a (111)-oriented Si substrate (5).

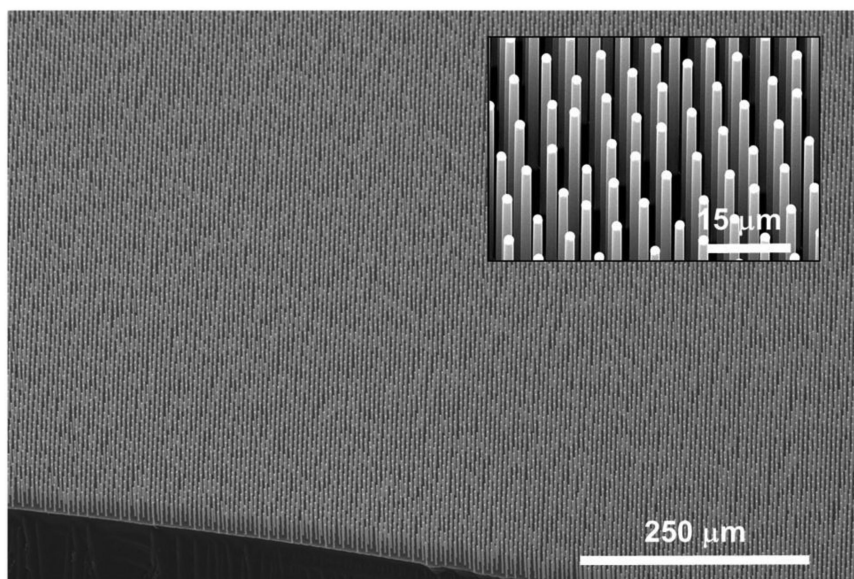


Figure 4. Scanning-electron microscope view of a tilted silicon microwire array grown using a copper catalyst. The array has nearly 100% fidelity over a large ($> 1 \text{ cm}^2$) area.

Even though Au is a mid-gap trap in Si, and the minority-carrier diffusion length was $\sim 2 \text{ }\mu\text{m}$ in the microwires, as expected from the known solubility of Au in Si at the growth temperature as well as the carrier-capture cross section of Au in Si, efficient radial carrier collection was predicted in such microwires. Photoelectrochemical experiments confirmed that the microwire arrays exhibited high quantum yields for charge-carrier collection (6), whereas planar Si samples with the same minority-carrier diffusion length would show low quantum yields in the visible region of the solar spectrum, under otherwise the same conditions. The photolithography step has recently been replaced by a nano-imprint lithography process to generate the patterned oxide, allowing convenient re-use of the stamp as well as facile regeneration of the stamp from the master mold (7).

Use of Au as the growth catalyst produced Si microwires that were undoped or intrinsically doped n-type (5, 6), neither of which was suitable for use as a photocathode. We thus developed a process for the production of Si microwire arrays that instead utilized an alternative VLS-growth catalyst, Cu (5, 8). Even though Cu is well-tolerated as an impurity in Si, the diffusion coefficient of Cu at 300 K in Si is $10^{-7} \text{ cm}^2 \text{ s}^{-1}$, preventing the persistent formation of abrupt doped homojunctions in Si. However, Cu can be deposited by electroplating or by electroless plating, and furthermore, the large diffusion coefficient of Cu in Si facilitates gettering of impurities such as Cu into the oxide at a Si oxide/Si interface. Exploiting these characteristics led to the development of a process for formation of controllable, p-type doped Si microwire arrays that are well-suited for use as photocathodes for solar fuels production (8).

We subsequently explored the optical properties of such Si microwire arrays. The optical behavior is interesting because with microwires having a 2 μm diameter and positioned on a 7 μm pitch, the optical characteristics of a microwire array are neither adequately described by the ray-tracing limit nor by Bruggeman effective-medium theory. Fourier transform finite-difference wave optics modeling was used to understand the behavior of such arrays, and to understand the reasons why an array that is 4% by projected area in Si light absorbing material nevertheless absorbed $\sim 25\%$ of the incoming light at normal incidence (9).

Membrane-Embedded Microwire Arrays

We then developed a process that allowed removal of the microwires from the Si substrate, and facilitated embedding the wires into a free-standing polymer membrane. Poly(dimethylsiloxane), PDMS, has a very interesting property in that its viscosity depends on the rate at which the polymer is peeled. Specifically if peeled rapidly, PDMS is stiff, whereas if peeled slowly, PDMS is quite flexible. This process works remarkably well, and produces free-standing, flexible, processable polymer-embedded Si microwire arrays (10). Nanomechanical measurements on such materials have elucidated the adhesive and bonding forces between Si microwires that have been functionalized with various alkyl monolayers having a variety of chemically different terminating groups, and have also correlated the adhesive forces with the strength of magnetic fields needed to torque Si microwires off of the Si substrate in the presence of various solvents and other ambients (11–13). The thickness of the polymer can also be controlled by addition of a high-vapor-pressure monomer to the polymer casting solution, resulting in microwires that are either fully embedded in the polymer or instead are only partially supported by the polymer. The peel-off process has additionally been extended to a variety of polymers, such as Nafion, that also are used in electrolyzers and fuel cells, and which also allow for gas-blocking as well as permselective ionic conductivity of protons or hydroxide ions (14).

The combination of VLS growth on patterned substrates and peel-off processing allows for facile re-use of the Si substrate. After peeling off the polymer-embedded microwires, stubs of Si are present in the holes in the Si oxide. The stubs are readily etched in 1.0 M KOH(aq), to reveal the holes in the oxide. Cu is then electrodeposited in the holes, and selectively plates onto the conducting Si substrate as opposed to the insulating Si oxide. The VLS-growth is then performed, and the resulting array is then transferred into the desired polymer by the peel-off step, to complete the cycle (Figure 5) (15).

The polymer-embedded arrays exhibit beautiful optical diffraction patterns that reflect the periodicity and spacing of the microwires (Figure 6) (9). Although the arrays exhibited very large optical absorption for light incident at most angles other than normal regardless of the pattern that was used, the arrays exhibited substantial optical transmission for light at normal incidence. To overcome this drawback, a metallic back reflector can be introduced at the bases of the microwires (9). This reflector is effective but may not be useful for integrated solar fuels

generators. In contrast, infiltration of the void spaces in the array with optical scatterers made from silica, alumina, or titania was found to effectively minimize the optical transmission at normal incidence, and produces high absorption with minimal reflection and transmission over a wide range of wavelengths and angles of incidence (16).

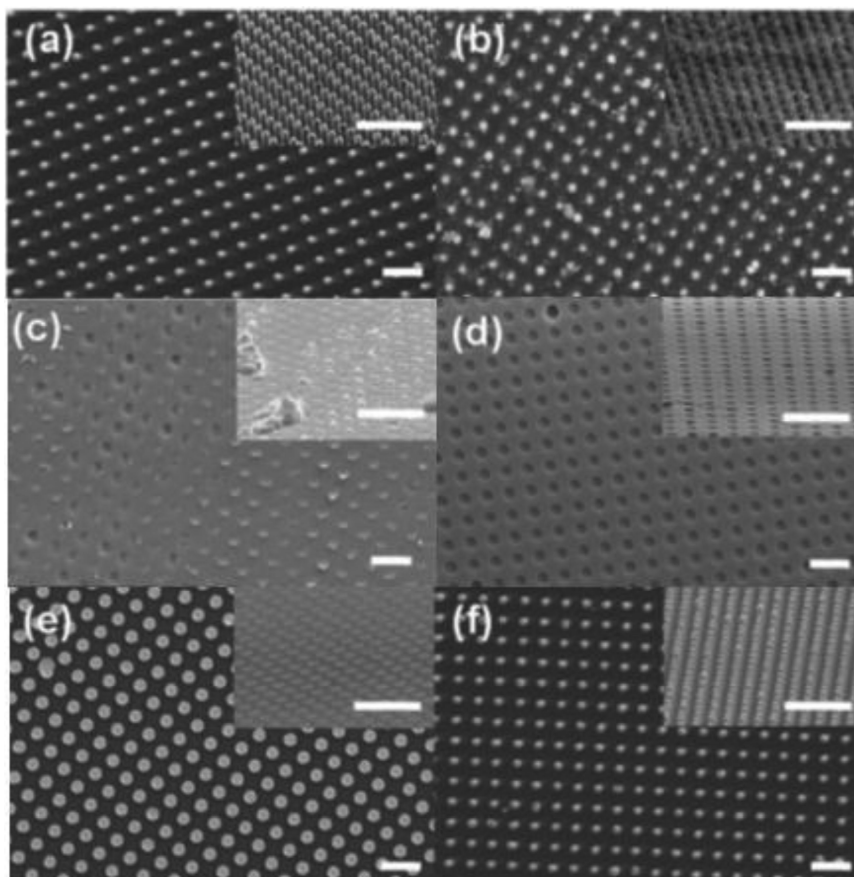


Figure 5. Top-down and tilted view (insets) scanning-electron micrographs of the silicon microwire regrowth process. The scale bars are 10 μm for the top-down images and 20 μm for the insets. a) The first-generation wire array. b) The first-generation array peeled in poly(dimethylsiloxane), PDMS. c) The wafer left behind after peeling, with wire stubs and polymer residue on its surface. d) The oxide pattern was recovered following an aqueous potassium hydroxide etch. e) The catalyst (Au in this case) was electrodeposited into the oxide-patterned holes. f) The second-generation wire array grown from the reused wafer. (Reprinted with permission from ref. (15). Copyright 2008, AIP Publishing LLC.)

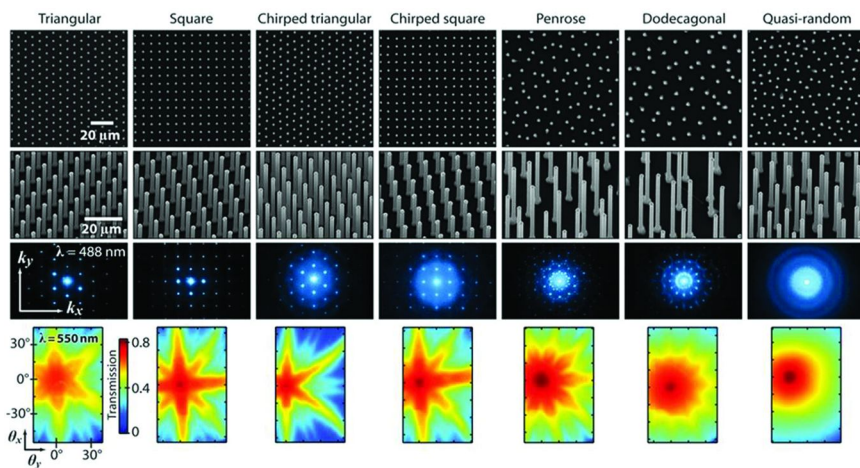


Figure 6. Top-down (first row) and tilted-view (second row) scanning-electron micrographs of as-grown silicon microwire arrays. Transmitted optical diffraction patterns ($\lambda = 488$ nm, third row) for polymer-embedded silicon microwire arrays on a quartz slide. Integrated transmission of each wire array observed at $\lambda = 550$ nm (bottom row) as a function of the incidence angle of the beam (θ_x, θ_y). (Reproduced from ref. (9))

Photoelectrochemical H₂ Evolution with Si Microwire Arrays

To determine the photovoltage that can be produced by the microwires, we then formed radial n⁺-p junctions by performing a multi-step masking, doping, and diffusion process. An oxide “boot”, which would not be needed in a membrane-embedded dual microwire architecture, was also formed to prevent shunt pathways to the conducting Si substrate. When the photovoltaic behavior of a representative radial-junction microwire was probed in an optically thin geometry that only allowed for absorption of ~50% of the incident light, a 9% efficient photovoltaic was obtained, based on light incident onto the microwire (17). Accordingly, the Si microwires are therefore capable of providing an 18% efficient microwire-based Si photovoltaic device, based on the estimated light absorbed by the Si microwire. Consistently, when a transparent conducting oxide was used to form a top contact to an array of n⁺p radial-junction microwires, 7% photovoltaic efficiency was observed, for a system that only absorbed ~50% of the incident light (16).

We then used these radial-junction microwire arrays as photocathodes for H₂ evolution from water (18). The flat-band potential of p-Si is not sufficiently positive to provide high photovoltages at the Si/H₂O interface; hence methods of functionalizing the Si surface to shift the band edges positively are being explored at present. In the meantime, the buried radial p-n junction was used as

a demonstration system to show the performance that could be obtained when the microwire arrays are integrated with a low-overpotential electrocatalyst for hydrogen evolution. The resulting Si microwire-array photoelectrode exhibited ~6% ideal regenerative cell efficiency for $\text{H}_2(\text{g})$ production from sunlight, even though the photoelectrode only absorbed ~50% of the incident light in the experiment (Figure 7) (8, 18).

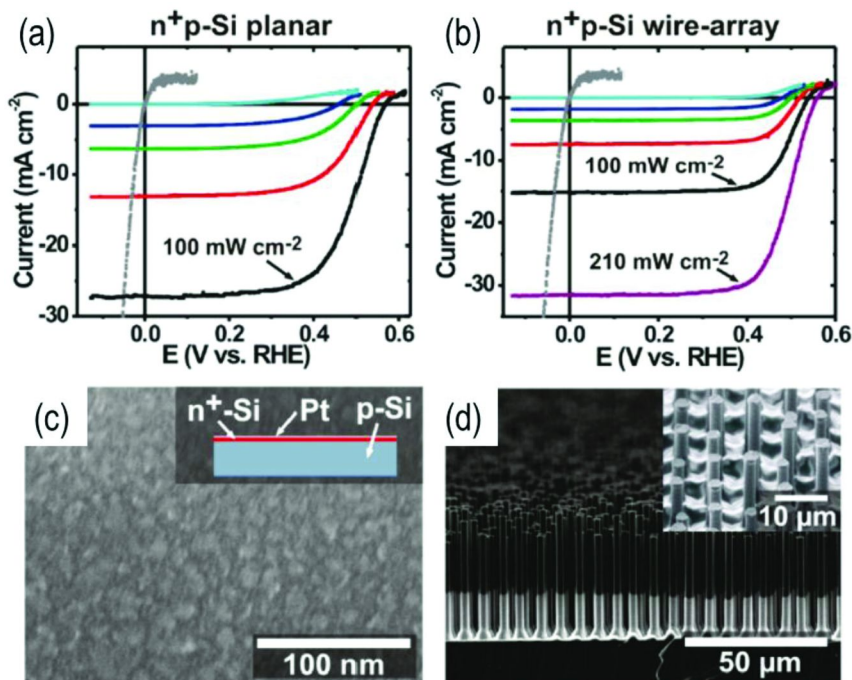


Figure 7. Current-density versus voltage behavior for a) planar n⁺p-Si and for b) radial-junction n⁺p-Si microwire-array photocathodes, both loaded with platinum for the electrocatalysis of the hydrogen-evolution reaction, in contact with 0.5 M H_2SO_4 (aq), and under simulated solar illumination from an ELH-type bulb. Scanning-electron micrographs of the Pt layer deposited on c) the planar electrode, and d) the n⁺p-Si microwire array. The inset to (d) shows a tilted SEM image of an electrode after partial infilling with wax. (Reproduced from ref. (18). Copyright 2011 American Chemical Society.)

We subsequently attempted to replace the Pt with a non-precious-metal, acid-stable electrocatalyst for the HER. Because electrochemical H_2 evolution and thermochemical hydrodesulfurization share a common surface-bound metal hydride putative intermediate, we hypothesized that earth-abundant hydrodesulfurization catalysts might make excellent electrocatalysts for the HER. Covalent metal phosphides are generally stable in acid, so we explored a series

of transition metal phosphides as HER electrocatalysts in 1.0 M H₂SO₄ (aq). Indeed, theoretical predictions in 2003 arrived at the same conclusion specifically regarding the potential of Ni₂P as a HER catalyst (19). In collaboration with the Schaak group at Penn State, we rapidly found that nanoparticles of Ni₂P exhibited a high HER activity, requiring an overpotential of < 150 mV to produce 10 mA/cm² of cathodic current density for H₂ production in 1.0 M H₂SO₄(aq) (20). Further experiments indicated that CoP exhibited still lower overpotentials for the HER, and FeP exhibited the lowest overpotentials of all of the metal phosphides that were explored in the series (21, 22). All of the binary metal phosphides that we explored were generally stable in acidic media, and thus are viable candidates for use in solar fuels generators in place of Pt, as might be needed to achieve scalability to TW levels.

We also showed that dual microwire arrays could be fabricated by preparing two individual polymer-embedded assemblies, and laminating the materials together into one assembly (14). Because the microwires are not aligned between both films, an intermediate electrically conductive layer is required to provide ohmic contact between the microwires on the top and bottom of the laminate. Such an ohmic contact was obtained in the case of Si microwires by use of the conducting polymer poly(3,4-ethylenedioxythiophene) polystyrene sulphonate, PEDOT-PSS. Microprobe measurements were used to evaluate the contact resistance and electrical properties of a series of polymer/Si microwire junctions, as a function of the doping level, oxide thickness, and surface functionalization chemistry of the Si microwire arrays (23–27). The dual microwire arrays have been embedded in Nafion and in the alkaline equivalent of Nafion that instead has fixed cationic sites instead of fixed anionic sites, allowing for conductance of hydroxide ions instead of protons for operation in locally alkaline conditions in a solar fuels generator system (Figure 8) (14).

Stabilization of Small-Band-Gap Photoanodes for Water Oxidation

Such arrays could provide all of the functionality that we initially sought, except that they cannot split water, for two important reasons. First, the 1.12 eV band gap of Si only allows for production of 0.6 V of photovoltage under 1 Sun of illumination. Hence, even two high-performance Si photovoltaic junctions cannot provide sufficient photovoltage to effect unassisted water splitting with high efficiency under standard conditions. Instead, operating voltages of 1.6–1.7 V are required at maximum power under typical optimal operating conditions. Secondly, Si, like most small-band-gap semiconductors, is not oxidatively stable under anodic current flow for water oxidation in aqueous solutions. Hence, either a new photoanode material needs to be identified that can provide the stable, efficient oxidation of water to O₂(g), or a method needs to be developed to stabilize otherwise unstable semiconductors for use as photoanodes for water oxidation, under conditions that are compatible with the production of a full solar fuels-generator system.

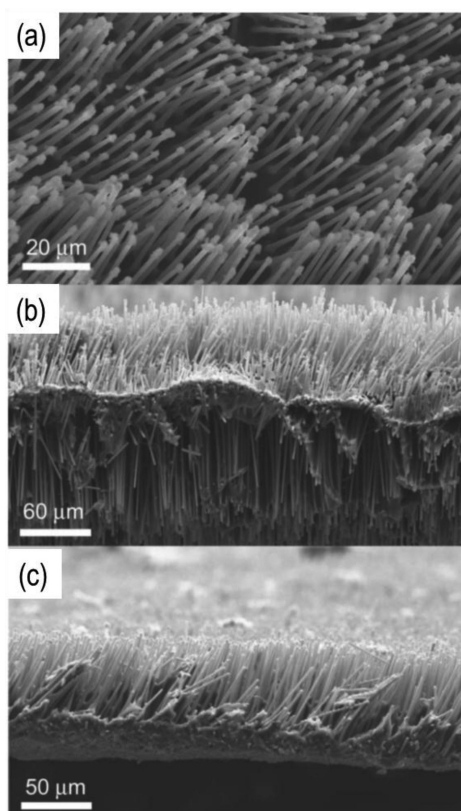


Figure 8. Scanning-electron microscope images of the (a) top-down view and (b) cross-sectional view of a Si microwire array embedded in Nafion and laminated with an intervening layer of a conducting polymer, PEDOT-PSS. c) Cross-sectional SEM of a Si microwire array embedded in an anion-exchange membrane, QAPSF. (Reproduced from ref (14) with permission from The Royal Society of Chemistry.)

Although we are vigorously searching, using high-throughput experimentation methods, for a new, stable, efficient photoanode material, in the meantime we have developed two strategies for stabilization of small band-gap semiconductors for use as photoanodes for water oxidation. One approach uses relatively thick films of amorphous TiO_2 formed by atomic-layer deposition, in conjunction with thin films or islands of Ni or other active electrocatalysts for the OER in alkaline electrolytes (28). Although TiO_2 has a large band gap, 3.0–3.2 eV depending on the crystal structure, and should produce a barrier for the conduction of holes into the electrolyte, ALD-formed TiO_2 films instead exhibit

significant hole conductivity, especially when contacted with low-work-function metals such as Ni. The surface of the Ni oxidizes in 1.0 M KOH(aq) to form Ni oxide, which is an active electrocatalyst for the OER especially after intercalation of trace Fe to form FeNi oxide, hence the system allows for the formation of a stable, catalytic, protective layer that enables a variety of small-band-gap semiconductors to be used as efficient photoanodes for water oxidation in alkaline electrolytes. This protection scheme has been exploited to stabilize Si, GaAs, GaP, GaAs_xP_{1-x}, CdTe, and BiVO₄ as water-oxidation photoanodes for extended periods of time in alkaline electrolytes (28–30). The interfacial energetics at the Si/TiO₂, and at the TiO₂/Ni/electrolyte interfaces have been investigated using operando ambient pressure X-ray photoelectron spectroscopy, AP-XPS, in conjunction with XPS and UPS measurements in UHV as well as electrical transport measurements (31, 32). These measurements collectively indicate that the Si/TiO₂ interface forms a rectifying contact, whereas the TiO₂/Ni/electrolyte contact is electrically ohmic and thus facilitates water oxidation by the photogenerated holes that are conducted through the TiO₂. The highest photovoltages produced by Si/TiO₂ interfaces are obtained when buried emitters are formed, to produce n-p⁺ junctions that are then coated with the amorphous TiO₂ deposited by ALD. Because ALD yields conformal films, the TiO₂ protection scheme has also been demonstrated to allow extended continuous operation (> 1000 h) of p⁺n-Si radial junction microwire arrays as photoanodes for water oxidation, to yield O₂(g) quantitatively and efficiently (33).

The other protection strategy that we have developed involves the use of a single-component film that is stable, conductive, optically transparent, and inherently catalytic for water oxidation. Reactively sputtered NiO_x films meet all of these criteria, and enable Si, InP, and CdTe to be used as photoanodes for water oxidation in aqueous alkaline electrolytes. Because the sputtered films have defects and pinholes, this approach is useful for semiconductors that passivate under operating conditions, but does not impart extended stability to semiconductors that undergo corrosion by dissolution, such as GaAs or other related III-V materials (34–36). The deposition of CoO_x films by ALD allows for control over the interfacial energetics of Si/oxide contacts, and allows for high photovoltage systems to be formed without the need to fabricate a buried emitter p⁺-n homojunction under the interfacial oxide layer (37). Achieving such high photovoltages without the need for a diffusion and drive-in step is important because during the drive-in step, dopants preferentially migrate down grain boundaries in polycrystalline material, introducing majority-carrier shunts and minority-carrier recombination sites that preclude efficient operation of such low-cost, readily prepared materials as photoanodes.

Synergistic Integration of Components

We have also performed an engineering design analysis of solar fuels generators, to determine the dimensions, orientation, geometry, and other variables that will optimize the efficiency while preserving the intrinsically safe operation of the system (38). Several designs are viable in either acidic or alkaline

electrolytes, adapted from designs used for commercial electrolyzers. In contrast, for electrolytes that are buffered at near-neutral pH, efficient systems co-evolve stoichiometric mixtures of $\text{H}_2(\text{g})$ and $\text{O}_2(\text{g})$, whereas membrane-containing systems that are intrinsically safe exhibit electro dialysis that eventually shuts down the system due to the resulting large ion-concentration gradients in the electrolyte (39). These design guidelines have been confirmed experimentally by the behavior of amorphous hydrogenated Si triple junctions at near-neutral pH with a variety of electrocatalysts for the OER and HER. Consistent with the modeling and simulation analysis, such systems were found to be inefficient and/or not intrinsically safe (40). In contrast, use of alkaline electrolytes allows for construction of efficient, intrinsically safe systems, as has been demonstrated by incorporation of a TiO_2 -protected III-V tandem junction photoanode in conjunction with a Ni OER catalyst, a Ni-Mo HER electrocatalyst, and a hydroxide-conducting membrane in 1.0 M KOH(aq), which has yielded >10% solar-to-hydrogen efficiencies for extended periods of time under 1 Sun of simulated solar illumination (Figure 9) (41). A sensitivity analysis indicated that the remaining increases in efficiency will predominantly be achieved by obtaining better performance from the light absorbers, and a reduction in the electrocatalyst overpotentials in alkaline media will produce relatively little gain in efficiency for systems that contain optimally configured tandem light absorbers (38).

The remaining challenges moving forward therefore reside primarily in assembling the available components together into a functional system under mutually compatible operational conditions. Tandem junctions from lattice-matched microwire- or nanowire-array light absorbers will need to be combined with appropriate tunnel junctions, with the whole system grown by inexpensive, scalable methods such as those demonstrated for Si microwires by the VLS method. The electrocatalysts will then need to be strategically placed on these light absorbers to allow for efficient transport of reactants into the structure and egress of products from the structure, while providing minimal optical obscuration and achieving excellent light management of incident photons into the structured light absorbers. Protection schemes will have to be implemented in a conformal fashion, preferably without the formation of buried junctions, on inexpensive polycrystalline semiconductors, to minimize the manufacturing cost of the light absorbers.

Hence, pursuant to implementation of the vision of a viable architecture for artificial photosynthesis, the principles of construction of efficient, safe, robust, scalable solar fuels generators have been elucidated and the needed components have been developed and demonstrated to exhibit the desired functionality, leveraging concepts of materials-by-design and taking advantage of advances in nanoscience and nanotechnology. The culmination of this effort in a fully assembled, functional artificial photosynthetic system that is simultaneously efficient, scalable, robust, and safe is thus within reach, and will arguably represent a compelling demonstration of the power and promise of nanotechnology to allow for the development of better options to provide a clean, sustainable energy future by the direct production of fuels from sunlight.

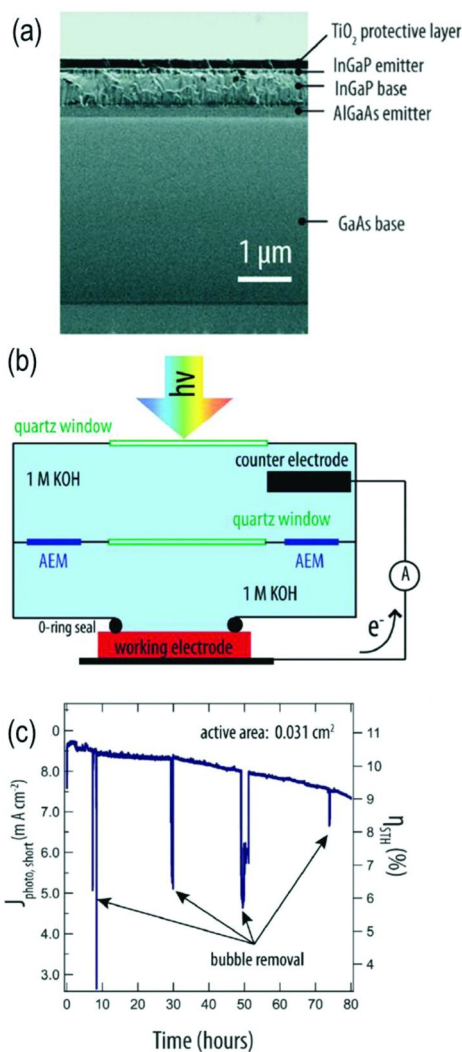


Figure 9. a) Cross-sectional scanning-electron micrograph of a GaAs/InGaP photoelectrode coated with a TiO₂/Ni protective layer. b) Schematic of a two-electrode cell configuration employing a Ni–Mo cathode (counter electrode) and a GaAs/InGaP/TiO₂/Ni working electrode. The photoanode and the cathode are separated by an anion-exchange membrane (AEM). c) The short-circuit current density, $J_{photo,short}$, and corresponding solar-to-hydrogen conversion efficiency, η_{STH} , as a function of time for the assembled two-electrode cell utilizing 1.0 M KOH (aq) as an electrolyte and under simulated solar illumination. (Reproduced from ref. (41) with permission from The Royal Society of Chemistry.)

Acknowledgments

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References

1. Nozik, A. J. *Annu. Rev. Phys. Chem.* **1978**, *29*, 189–222.
2. Wrighton, M. S.; Ellis, A. B.; Wolczanski, P. T.; Morse, D. L.; Abrahamson, H. B.; Ginley, D. S. *J. Am. Chem. Soc.* **1976**, *98*, 2774–2779.
3. Hu, S.; Xiang, C. X.; Haussener, S.; Berger, A. D.; Lewis, N. S. *Energy Environ. Sci.* **2013**, *6*, 2984–2993.
4. Kayes, B. M.; Atwater, H. A.; Lewis, N. S. *J. Appl. Phys.* **2005**, *97*, 114302.
5. Kayes, B. M.; Filler, M. A.; Putnam, M. C.; Kelzenberg, M. D.; Lewis, N. S.; Atwater, H. A. *Appl. Phys. Lett.* **2007**, *91*, 103110.
6. Maiolo, J. R.; Kayes, B. M.; Filler, M. A.; Putnam, M. C.; Kelzenberg, M. D.; Atwater, H. A.; Lewis, N. S. *J. Am. Chem. Soc.* **2007**, *129*, 12346–12347.
7. Audesirk, H. A.; Warren, E. L.; Ku, J.; Lewis, N. S. *ACS Appl. Mater. Interfaces* **2015**, *7*, 1396–1400.
8. Boettcher, S. W.; Spurgeon, J. M.; Putnam, M. C.; Warren, E. L.; Turner-Evans, D. B.; Kelzenberg, M. D.; Maiolo, J. R.; Atwater, H. A.; Lewis, N. S. *Science* **2010**, *327* (5962), 185–187.
9. Kelzenberg, M. D.; Boettcher, S. W.; Petykiewicz, J. A.; Turner-Evans, D. B.; Putnam, M. C.; Warren, E. L.; Spurgeon, J. M.; Briggs, R. M.; Lewis, N. S.; Atwater, H. A. *Nat. Mater.* **2010**, *9*, 239–244.
10. Plass, K. E.; Filler, M. A.; Spurgeon, J. M.; Kayes, B. M.; Maldonado, S.; Brunschwig, B. S.; Atwater, H. A.; Lewis, N. S. *Adv. Mater.* **2009**, *21*, 325–328.
11. Cho, C. J.; O’Leary, L.; Lewis, N. S.; Greer, J. R. *Nano Lett.* **2012**, *12*, 3296–3301.
12. Gallant, B. M.; Gu, X. W.; Chen, D. Z.; Greer, J. R.; Lewis, N. S. *ACS Nano* **2015**, *9*, 5143–5153.
13. Beardslee, J. A.; Sadtler, B.; Lewis, N. S. *ACS Nano* **2012**, *6*, 10303–10310.
14. Spurgeon, J. M.; Walter, M. G.; Zhou, J. F.; Kohl, P. A.; Lewis, N. S. *Energy Environ. Sci.* **2011**, *4*, 1772–1780.
15. Spurgeon, J. M.; Plass, K. E.; Kayes, B. M.; Brunschwig, B. S.; Atwater, H. A.; Lewis, N. S. *Appl. Phys. Lett.* **2008**, *93*, 032112.

16. Putnam, M. C.; Boettcher, S. W.; Kelzenberg, M. D.; Turner-Evans, D. B.; Spurgeon, J. M.; Warren, E. L.; Briggs, R. M.; Lewis, N. S.; Atwater, H. A. *Energy Environ. Sci.* **2010**, *3*, 1037–1041.
17. Kelzenberg, M. D.; Turner-Evans, D. B.; Putnam, M. C.; Boettcher, S. W.; Briggs, R. M.; Baek, J. Y.; Lewis, N. S.; Atwater, H. A. *Energy Environ. Sci.* **2011**, *4*, 866–871.
18. Boettcher, S. W.; Warren, E. L.; Putnam, M. C.; Santori, E. A.; Turner-Evans, D.; Kelzenberg, M. D.; Walter, M. G.; McKone, J. R.; Brunschwig, B. S.; Atwater, H. A.; Lewis, N. S. *J. Am. Chem. Soc.* **2011**, *133*, 1216–1219.
19. Rodriguez, J. A.; Kim, J. Y.; Hanson, J. C.; Sawhill, S. J.; Bussell, M. E. *J. Phys. Chem. B* **2003**, *107*, 6276–6285.
20. Popczun, E. J.; McKone, J. R.; Read, C. G.; Biacchi, A. J.; Wilttrout, A. M.; Lewis, N. S.; Schaak, R. E. *J. Am. Chem. Soc.* **2013**, *135*, 9267–9270.
21. Callejas, J. C.; McEnaney, J. M.; Read, C. G.; Crompton, J. C.; Biacchi, A. J.; Popczun, E. J.; Gordon, T. R.; Lewis, N. S.; Schaak, R. E. *ACS Nano* **2014**, *8*, 11101–11107.
22. Popczun, E. J.; Read, C. G.; Roske, C. W.; Lewis, N. S.; Schaak, R. E. *Angew. Chem, Int. Ed.* **2014**, *53*, 5427–5430.
23. Yahyaie, I.; McEleney, K.; Walter, M.; Oliver, D. R.; Thomson, D. J.; Freund, M. S.; Lewis, N. S. *J. Phys. Chem. Lett.* **2011**, *2*, 675–680.
24. Yahyaie, I.; McEleney, K.; Walter, M. G.; Oliver, D. R.; Thomson, D. J.; Freund, M. S.; Lewis, N. S. *J. Phys. Chem. C* **2011**, *115*, 24945–24950.
25. Yahyaie, I.; Ardo, S.; Oliver, D. R.; Thomson, D. J.; Freund, M. S.; Lewis, N. S. *Energy Environ. Sci.* **2012**, *5*, 9789–9794.
26. Bruce, J. P.; Asgari, S.; Ardo, S.; Lewis, N. S.; Oliver, D. R.; Freund, M. S. *J. Phys. Chem. C* **2014**, *118*, 27741–27748.
27. Bruce, J. P.; Oliver, D. R.; Lewis, N. S.; Freund, M. S. *ACS Appl. Mater. Interfaces* **2015**, *7*, 27160–27166.
28. Hu, S.; Shaner, M. R.; Beardslee, J. A.; Lichterman, M.; Brunschwig, B. S.; Lewis, N. S. *Science* **2014**, *344*, 1005–1009.
29. Lichterman, M. F.; Carim, A. I.; McDowell, M. T.; Hu, S.; Gray, H. B.; Brunschwig, B. S.; Lewis, N. S. *Energy Environ. Sci.* **2014**, *7*, 3334–3337.
30. McDowell, M. T.; Lichterman, M. F.; Spurgeon, J. M.; Hu, S.; Sharp, I. D.; Lewis, N. S. *J. Phys. Chem. C* **2014**, *118*, 19618–19624.
31. Lichterman, M. F.; Hu, S.; Richter, M. H.; Crumlin, E. J.; Axnanda, S.; Favaro, M.; Drisdell, W.; Hussain, Z.; Mayer, T.; Brunschwig, B. S.; Lewis, N. S.; Liu, Z.; Lewerenz, H.-J. *Energy Environ. Sci.* **2015**, *8*, 2409–2416.
32. Hu, S.; Richter, M. H.; Lichterman, M. F.; Beardslee, J.; Mayer, T.; Brunschwig, B. S.; Lewis, N. S. *J. Phys. Chem. C* **2016**, *120*, 3117–3129.
33. Shaner, M. R.; Hu, S.; Sun, K.; Lewis, N. S. *Energy Environ. Sci.* **2015**, *8*, 203–207.
34. Sun, K.; McDowell, M. T.; Nielander, A. C.; Hu, S.; Shaner, M. R.; Yang, F.; Brunschwig, B. S.; Lewis, N. S. *J. Phys. Chem. Lett.* **2015**, *6*, 592–598.
35. Sun, K.; Kuang, Y.; Verlage, E. A.; Brunschwig, B. S.; Tu, C. W.; Lewis, N. S. *Adv. Energy Mater.* **2015**, *5*, 1402276.

36. Sun, K.; Saadi, F. H.; Lichterman, M.; Hale, W. G.; Wang, H.-P.; Zhou, X.; Plymale, N. T.; Omelchenko, S.; He, J.-H.; Papadantonakis, K. M.; Brunschwig, B. S.; Lewis, N. S. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 3612–3617.
37. Zhou, X.; Liu, R.; Sun, K.; Friedrich, D.; McDowell, M. T.; Yang, F.; Omelchenko, S.; Saadi, F. H.; Nielander, A. C.; Yalamanchili, S.; Papadantonakis, K. M.; Brunschwig, B. S.; Lewis, N. S. *Energy Environ. Sci.* **2015**, *8*, 2644–2649.
38. Chen, Y.; Hu, S.; Xiang, C. X.; Lewis, N. S. *Energy Environ. Sci.* **2015**, *8*, 876–886.
39. Singh, M. R.; Papadantonakis, K. M.; Xiang, C. X.; Lewis, N. S. *Energy Environ. Sci.* **2015**, *8*, 2760–2767.
40. Jin, J.; Walczak, K.; Singh, M. R.; Karp, C.; Lewis, N. S.; Xiang, C. X. *Energy Environ. Sci.* **2014**, *7*, 3371–3380.
41. Verlage, E.; Hu, S.; Liu, R.; Jones, R. J. R.; Sun, K.; Xiang, C.; Lewis, N.; Atwater, J. H. A. *Energy Environ. Sci.* **2015**, *8*, 3166–3172.

Chapter 2

Molecular Plasmonics: From Molecular-Scale Measurements and Control to Applications

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Nanophotonics is a prominent field within nanotechnology aimed at researching the light-matter interactions occurring at the nanoscale. In case of metal nanostructures, collective oscillation of electrons leads to nanoscale confinement of optical fields with high thermal energy and elevated near-field optical forces. The interactions of these effects with molecules known as molecular plasmonics have generated novel devices with implications in energy, biology, and healthcare. In this chapter, we will review our research on molecular plasmonics in three correlated sections: molecular-scale measurements and control, directed and self-assembled nanofabrication, and real-life applications. The first section presents the measurements and control of molecular-scale events using active molecules as a framework. We further summarize two unconventional nanofabrication techniques based on directed and self-assembly, i.e., moiré nanosphere lithography and multi-photon plasmonic lithography. The success in these two arenas positions molecular plasmonics to be propelled for real-life applications. We finally present the potential of plasmonics in achieving its promise of creating futuristic technologies.

1. Introduction

Tasks which were once inconceivable are now commonplace due to rapid technological advancements. Specifically, the curiosity to understand and develop novel phenomena and devices has led to multidisciplinary convergence of scientists to explore and develop nanotechnology. Nanotechnology focuses on the study and manipulation of unique fundamental property differences that originate when the size of material is scaled down to a collection of several atoms. Advancements in nanotechnology have been steered by the ability to fabricate, measure and optimize nanoscale structures using sophisticated tools. Among its various research areas, nanophotonics has been an active and prominent field due to the interesting optical properties that arise at sub-wavelength scales. Traditionally, it has been challenging to study the optical interactions at the nanoscale due to the diffraction limit, which restricts the focusing of light to dimensions roughly above one half of the wavelength. This barrier has been overcome in the past decades, leading to intense research in the area of nanophotonics.

Nanophotonics aims to design and manipulate the behavior of optical field at the scale of a fraction of the wavelength. Research on nanophotonics has resulted in immense applications in various realms such as optical fiber technology, solar energy conversion, molecular diagnosis and therapy, and biosensors. According to Forbes, market for nanophotonic components and devices has been projected to reach \$100 billion (1). In the past two decades, nanophotonics has been primarily approached by the following three strategies: (i) confinement of light waves in various structures, (ii) confinement of atoms to create materials in the sub-100 nm regime, and (iii) characterization and control of photophysical and photochemical processes at the nanoscale. Based on the above strategies, two prominent areas in nanophotonics being investigated are photonic crystals and plasmonics.

1.1. Photonic Crystals and Plasmonics

Photonic crystals (PCs) are periodic dielectric nanostructures with lattice parameters comparable to the wavelength of light. The periodic modulation of the dielectric medium creates photonic band gaps or stop gaps, which limit the propagation of certain wavelengths of light. The refractive-index contrast between the materials, structural symmetry and lattice parameters of the particles can be altered to tune the bandwidth and frequency of PCs. The transmission and reflection properties of PCs can be rationally designed by using modeling methods involving either plane-wave expansion (2), or transfer matrices (3) of the Maxwell equation. PCs have found applications in a variety of industries such as telecommunications (PC fibers, and integrated optical components), medicine (micro-lasers, and biosensors), and displays (image sensors). A recent market report estimated PC component market as \$6 billion in 2014, with a projection to reach \$17.4 billion in 2020 (4).

Plasmonics involves the study of the interaction between electromagnetic radiations and free electrons of a metal. In general, materials which possess a negative real and a small imaginary dielectric constant can support a surface

plasmon resonance (SPR). SPR is the coherent oscillation of the free electrons in response to an incident electromagnetic radiation, and has achieved prominence due to its ability to circumvent the diffraction limit. SPR has important applications in surface-enhanced spectroscopies (5) and biological sensing (6). With molecular spectroscopy market projected to reach \$5.9 billion by 2018, plasmonics is expected to make significant contributions. For instance, commercial instruments such as BIAcore® employ SPR spectroscopy to monitor thermodynamics and kinetics of biological binding processes (7).

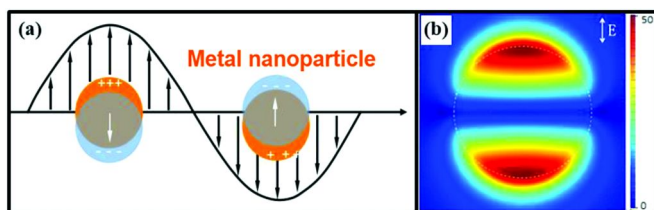


Figure 1. (a) Schematic illustration of localized surface plasmon at metal nanoparticles. (b) Optical simulation shows the hot spots of an Ag nano-disk on a substrate upon incidence of electromagnetic radiation at plasmon resonance. Reproduced with permission (8). Copyright 2015 Springer Science+Business Media New York.

SPR can exist either as propagating or localized surface plasmon. Surface plasmon polaritons (SPPs) are electromagnetic excitations which occur at a metal/dielectric interface. They propagate parallel to the surface, but decay exponentially in both media along the perpendicular direction. This property can be exploited to localize and guide light in subwavelength metal structures to fabricate nanoscale optoelectronic circuits and components (9). For sensing applications, the interaction between the SPP and the molecular surface layer results in plasmon resonance shift, which can be characterized via reflectivity measurements as a function of either wavelength or incident angle (10). In the case of localized surface plasmon resonances (LSPRs), light interacts with nanoscale particles which are smaller than the incident wavelength (Figure 1), leading to the light-coupled coherent oscillation of electrons confined within the nanoparticle (NP). The LSPR is extremely sensitive to local dielectric environment, which can be sensed via a wavelength shift. The excitation of LSPR creates localized enhancement of electromagnetic fields in the vicinity of the NP. Figure 1(b) shows the field enhancement around a 10nm Au NPs.

1.2. Molecular Plasmonics

Molecular plasmonics fuses light and molecules at the nanoscale. In this emerging field, one exploits the plasmon-enhanced nanoscale light for molecular analysis or seeks the synergy of the response of molecules and the nanoscale light manipulation by surface plasmons for active nanophotonic devices. For example, the light confined at the nanoscale can interact with cells

and biomolecules of variable sizes. An efficient control and understanding of the interactions (optical, thermal and mechanical) between the molecules and SP can lead to superior sensing, trapping and manipulation at molecular and cellular levels (11–15). For instance, the high scattering and absorption cross sections help improve the sensitivity of molecular spectroscopy (16). The promising developments in nanolaser, non-linear quantum optics, terahertz nanoelectronics and ultra-high fluorescence enhancements have also been enabled by harnessing the plasmon-molecule interactions (17–19). The success of molecular plasmonics has been and will continue to be contingent upon simultaneous development of plasmonic and molecular nanotechnology.

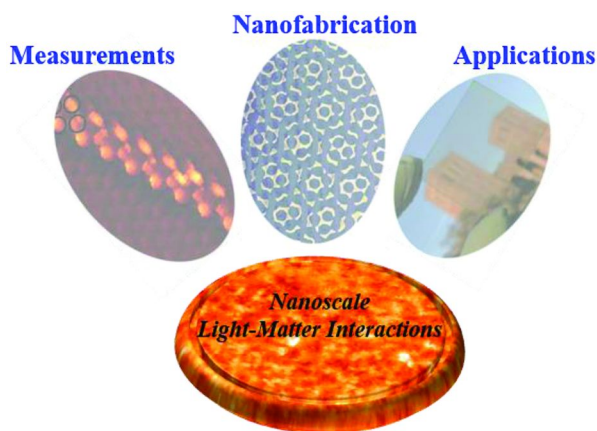


Figure 2. An overview of book chapter focused on our fundamental study and applications of molecular plasmonics and nanoscale light-matter interactions: (i) molecular-scale measurements and control, (ii) directed and self-assembled nanofabrication, and (iii) applications in energy, biology and healthcare.

In this chapter, we review our research on molecular plasmonics and the applications of plasmonics in the domains of energy, biology, and healthcare. As shown in Figure 2, the chapter is divided into three synergistic sections. The first section concentrates on the methods and tools available for the measurements and control of molecules. These techniques are essential for the understanding of molecular-scale processes and for the scaling-up of molecular units for device applications. We exploit the sophisticated measurement tools to study active molecules such as rotaxane and azobenzene, which can change their properties in response to external stimuli. The second section discusses a couple of unconventional nanofabrication techniques based on directed and self-assembly, including moiré nanosphere lithography (M-NSL) and multi-photon plasmonic lithography (MPPL), which provide versatility and cost-effectiveness in nanofabrication. The final section provides a snapshot of our recent work on the applications of plasmonics in the energy, biology, and healthcare.

2. Molecular-Scale Measurements and Control

Due to the extremely small size of molecules, it is imperative to have tools that are capable of measuring events at the scale of few nanometers. The ability to resolve and separate the plasmonic and molecular changes is beneficial. To this end, imaging techniques such as scanning electron microscopy (SEM), atomic force microscopy, scanning tunneling microscopy (STM), and near-field optical scanning microscopy have been developed. Further, optical characterization techniques such as single-particle dark-field scattering spectroscopy, surface-enhanced Raman spectroscopy (SERS) and tip-enhanced Raman spectroscopy have emerged. In this section, we summarize our approaches towards the nanoscale characterization of active molecules using STM and SERS. Our progress in developing controlled molecular environments based on directed and self-assembly techniques are also summarized.

2.1. Self-Assembly

Self-assembly is an attractive strategy to obtain surface functionalization in a controlled, organized manner via the tuning of the molecule-molecule and molecule-substrate interactions. It is defined as the spontaneous assembly of molecules into structured, stable, non-covalently joined aggregates under equilibrium (20). Typically self-assembly has been employed for creating monolayers of molecules (21) or polymer micro/nanoparticles (22). Due to its ease of preparation, high versatility, high throughput, and low cost, self-assembly of molecules has been used for numerous applications beyond the surface functionalization (23), including molecular electronics (24) and biomolecule immobilization (25). Self-assembled monolayers (SAMs) of thiols on Au are one of the well-researched platforms due to their high stability in the ambient atmosphere and their ability for precise modification (21, 26–28). At relatively low concentrations of ~1mM, ethanolic solution of thiols swiftly organize on Au surfaces via covalent bonds formed between the thiol head group and Au. The tail groups are exposed in ordered lattices and a few types of disorders or defects, including step edges, vacancy islands, and monolayer defects (tilt domain boundaries and improper ordering of molecules) exist. Figure 3 depicts the scanning tunneling microscopy image of decanethiol SAMs on Au{111} along with the marked defect regions (29). These defects in SAMs are exploited to position desired active molecules with precise placement and orientation control (30, 31).

Molecules are inserted onto the SAMs in the solution and vapor phases, as well as through microcontact insertion printing. In most scenarios, SAMs with thickness of few nanometers are ideal for molecular plasmonics. Further, these molecules have a tendency to preferentially attach at the defect sites in the SAMs since these are the most reactive and sterically accessible sites on the Au surface (32–34). SAMs can also be applied to functional molecules. For example, to extend the applicability of rotaxanes, we formed the SAMs on Au surfaces through the modification of rotaxanes with disulfide-based anchoring groups (35). The SAMs enable the applicability of molecular machines by facilitating their

coordinated functioning at the nanoscale. In another example, Huang, Weiss, Stoddart, and co-workers utilized SAMs of rotaxane molecules on Au surfaces to drive a mechanical cantilever (36).

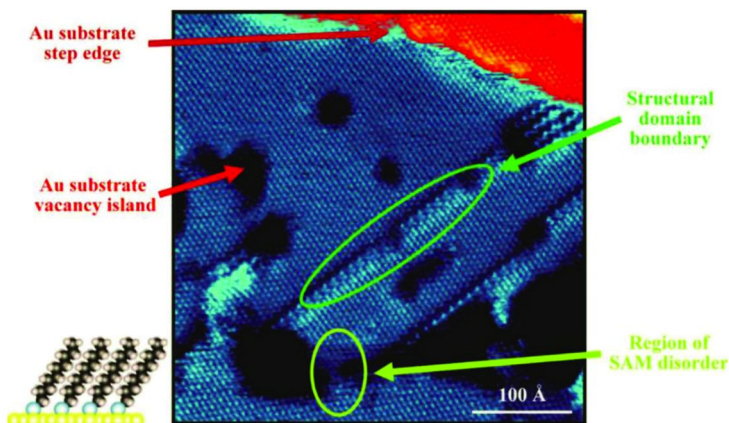


Figure 3. Scanning tunneling micrograph of decanethiol SAM on Au{111} surface depicting the various types of defects such as step edges, vacancy islands, improperly ordered molecules, and tilt domain boundaries. Reproduced with permission (29). Copyright 2008 American Chemical Society.

2.2. Scanning Tunneling Microscopy

The ability of characterizing the properties of molecules on surfaces is of great interest. For instance, it is crucial to understand whether the molecules retain their functionality when positioned on surfaces (37). While techniques such as cyclic voltammetry (38) and X-ray photoemissions spectroscopy (39) are useful for ensemble measurements, to achieve single-molecule resolution to gain significant insights is plausible with STM. STM can be used for imaging surfaces at an atomic resolution (40). STM measurements can be used to estimate the insertion efficiency, arrangement and orientation of molecules on surfaces, providing information about the quality of the SAMs. STM can also monitor conformational changes in active molecules (e.g., molecular switches and motors) upon exposure to external stimuli. In order to obtain statistically significant data, a large number of measurements are needed from the sites of interest (41). Because the activity of individual active molecules can be altered by the environment and the neighboring active molecules (29, 42, 43), it is imperative to isolate and characterize individual molecules in controlled environments.

Weiss and co-workers have achieved isolation of single molecular motors and switches in ordered alkanethiol SAM matrices (30, 31, 44, 45). STM images are recorded as the convolution of topography and electronic properties of the molecules. The images provide the apparent height profile of active molecules relative to the SAM matrix (46), and can be used to study molecular switching at an ultra-high resolution. As an example, Figure 4 shows the schematic of azobenzene

undergoing reversible photoisomerization within the alkanethiol domain. The use of 1-decanethiol as the matrix ensures that the azobenzene molecules protruding out of the matrix have ample degrees of freedom for structural changes to occur (30). The inserted azobenzene-functionalized molecules within alkanethiol monolayer also limits the stochastic switching events which are otherwise observed due to the movement of molecules bound at the defect sites (32, 45, 47). The co-deposition is performed by immersing annealed Au substrates in a solution of azobenzene and alkanethiol at a temperature of 80 °C. This leads to the insertion of azobenzene molecules within the ordered alkanethiol domains.

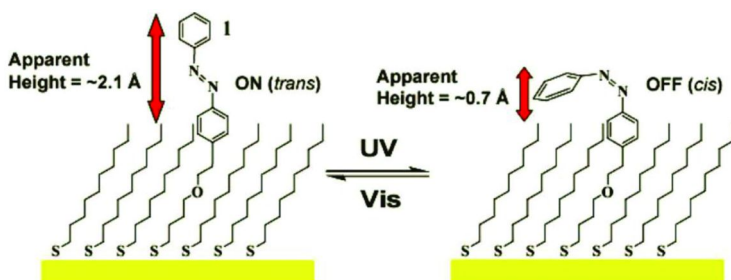


Figure 4. Isolated single azobenzene molecules embedded in a matrix of 1-decanethiol over Au {111} surface. The photoisomerization results in a change from 2.1 ± 0.3 Å to 0.7 ± 0.2 Å with respect to the SAM matrix upon switching from trans to cis form. Reproduced with permission (30). Copyright 2011, American Chemical Society.

The isolated active molecule within the matrix is characterized by a STM which is equipped with a light source to irradiate the tunneling junction while the scanning tip is still present. This ensures the on-demand illumination of azobenzene molecules for specific periods of time along with continued imaging. Figure 5 summarizes the conformational changes of azobenzene under different irradiation conditions. Initially, the individual trans-azobenzene molecules possess an apparent height of ~ 2 Å with respect to the SAM matrix. Upon UV (365 nm) irradiation, the molecules gradually change the apparent height with an increase in the exposure time. The reduction in apparent height to ~ 0.7 Å upon photoisomerization is a result of (i) lower conductance of cis-azobenzene with respect to trans since it is non-planar, and (ii) change in the height of the molecule after the isomerization. The cis form can return back to the thermodynamically stable trans orientation through visible light illumination. This phenomena is observed in Figure 5 (F) where some of the molecules revert back to the trans conformation after 30 mins of visible light exposure. STM imaging has been performed on other alternative substrates such as GaAs (48).

Although the measurements on single molecules provide valuable insights into the physics and chemistry at the nanoscale, real-life devices mandate a large number of molecules in one-dimensional (1D), two-dimensional (2D) and even three-dimensional (3D) assemblies, which involve intermolecular interactions and charge transport (29, 49, 50). The 1D assemblies are obtained

via the assembly of compact azobenzene molecules assembled around the domain boundaries of 1-decanethiol monolayers (51). In this scenario, we observe a reduced switching speed although all molecules undergo switching coherently. This suggests the presence of electronic coupling along the chain. By exploiting the cooperative functions, it is plausible to create novel 2D assemblies with superior photoswitching capabilities (52–54).

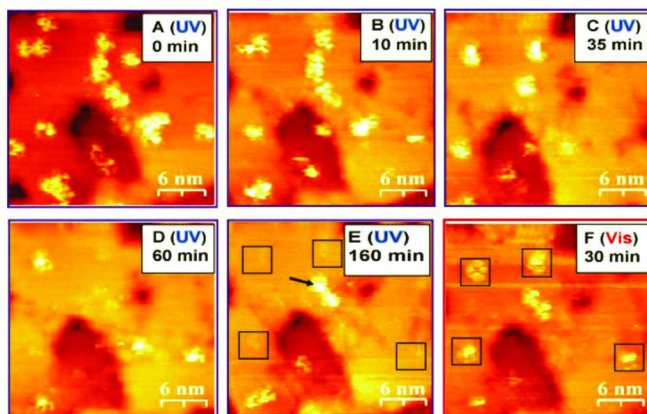


Figure 5. STM images of azobenzene molecules in SAM matrix upon irradiation with UV ($\sim 365\text{nm}$) light for (A) 0 min, (B) 10 min, (C) 35 min, (D) 60 min, and (E) 160 min, as well as subsequently under visible light ($\sim 450\text{nm}$) exposure for (F) 30 min. The images show the switching of azobenzene between high-conductive trans and low-conductive cis forms. STM imaging was performed with $V_{\text{sample}} = 1\text{V}$ and $I_t = 2\text{pA}$. Reproduced with permission (30). Copyright 2011, American Chemical Society.

2.3. Surface-Enhanced Raman Spectroscopy

Since the first report on single-molecule SERS in 1977 (55), intense research in various disciplines, including physics, chemistry and life sciences, has led to the rapid development of SERS over the past two decades. SERS is a nanoscale phenomenon where the enhancement in Raman signal is observed due to the coupling between vibrational modes of the molecule and the LSPR of the metal nanostructure upon optical excitation (56). It has immense applications in a plethora of avenues such as biosensing (57) of various diseases such as cancer (58, 59) and Alzheimer's disease (60, 61), spectroelectrochemistry (62), chemical warfare agent detection (63), and single-molecule SERS (64). SERS provides a comprehensive information on the vibrational modes of the molecule along with high sensitivity towards conformational changes (65). By exploiting the surface selection rules that vibrational modes perpendicular to the substrate are enhanced while parallel modes remain constant, it is possible to obtain information regarding the orientation of the molecule on a specific substrate (66, 67).

Specific to active molecules, SERS provides a unique opportunity to probe ensemble averages of the molecular events. As an example, we have employed SERS to study photoisomerization of alkanethiolate-tethered azobenzene inserted in dodecanethiolate SAM matrix. The host-guest structures and tethers help reduce the surface quenching and steric hindrance for photoswitching of molecules on metal surfaces (68). The nanohole arrays on Au{111}/mica were carefully designed to provide the necessary field enhancement to obtain large SERS intensity (Figure 6) (45).

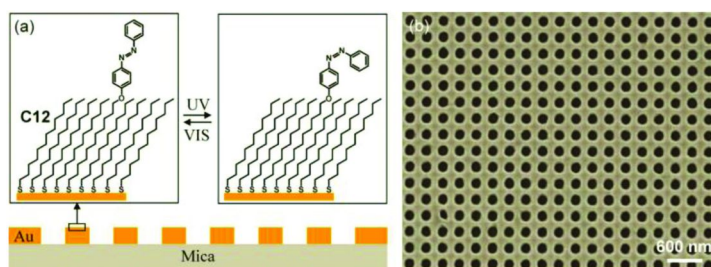


Figure 6. (a) Schematic illustration of the cross-sectional view of azobenzene molecule inserted in the dodecanethiolate SAM matrix on Au {111} with nanohole arrays. (b) SEM image of the nanohole array on Au {111}. Reproduced with permission (45). Copyright 2011, American Chemical Society.

The SERS spectra obtained at specific time intervals of alternating UV and blue light irradiation on the molecules are shown in Figure 7. The vibrational modes that contribute to different Raman peaks denoted as C1-C5 are illustrated in the diagrams in the lower panel. The switching characteristics are analyzed by processing the ratio of the peak areas of each spectrum. From the data for various C_i/C_j (i, j in the range of 1-5), we observe a monotonic and reversible trend in the peak-area ratio ($C3:C4$) upon alternative UV and blue light exposure (Figure 7d). The peak ratio is decreased upon UV irradiation (*trans* to *cis* transformation) and blue light switches it back to *trans*, which is manifested as an increase in $C3/C4$ ratio. Unlike STM, SERS tool enables ensemble measurements of isolated molecules and assemblies in controlled nano-environments. SERS can be exploited to understand many other types of active molecules and their functional devices (69). In a separate work, we have utilized SERS to probe the reaction pathways for self-assembled anthracene molecules on Au substrate, and study the influence of nanoscale morphology on the regioselective reactions (70). It is possible to further gain dynamic information by integrating SERS with ultra-fast optics (71). With recent developments in the creation and manipulation of hot spots, SERS can be efficiently employed for studying molecule-plasmon interactions at high spatial and temporal resolution (72).

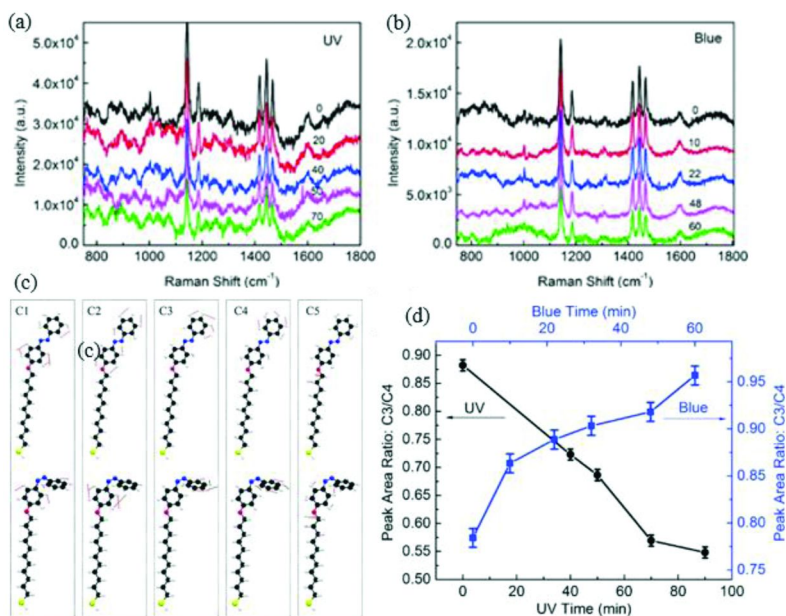


Figure 7. A series of time-dependent SERS spectra of azobenzene obtained after alternate exposure of (a) UV (~350nm) and (b) blue (~450nm) light over the sample. The spectra are shifted along the y-axis for clarity. (c) Schematic showing the various modes (C1-C5) for trans and cis configuration. (d) Experimental C3/C4 Raman peak ratio as a function of UV and blue light exposure times. Reproduced with permission (45). Copyright 2011, American Chemical Society.

3. Directed and Self-Assembled Nanofabrication

Progress in nanofabrication techniques has been an integral component for propelling the research in nanophotonics. It involves making functional structures with well-defined features having the least dimension to be <100 nm. Majority of the patterning has been accomplished via photolithography, and has been primarily aimed at the semiconductor industry. Over the years, various nanofabrication techniques have been developed in parallel to combat the high cost and diffraction limit of light in photolithography and to fabricate complex 2D and 3D structures for novel optical properties. Scanning beam techniques such as electron-beam lithography and focused ion-beam lithography can create arbitrary precise features. However, they feature low throughput since patterns are carved out line-by-line with high-energy (>10 kV) electron and ion beams. These limitations have motivated researchers to further explore the unconventional nanofabrication techniques based on directed and self-assembly. In this section, we highlight our research on M-NSL and MPPL.

3.1. Moiré Nanosphere Lithography

Over the past two decades, nanosphere lithography (NSL) has emerged as a low-cost, large-scale nanofabrication technique for creating periodic nanostructures. NSL, which is also known as colloidal lithography (73) or natural lithography, is a method of organizing nanospheres into tightly packed patterns to aid the creation of metasurfaces and nanostructures (74). The patterns obtained by metal deposition using the monolayer of nanospheres as the mask feature hexagonal arrays of metal nanotriangles (75). The use of bilayer sphere as masks leads to quasi-spherical NPs (76). With a variable combination of materials deposition and etching steps, it is possible to create myriads of structures such as nanodisks (77, 78), nanorings (79), nanopillars (80, 81), and nanoholes (82, 83). A few variants of NSL such as angle-resolved NSL and shadow NSL have emerged (84, 85). They involve the dynamic and non-dynamic deposition of materials at non-zero incident angle with respect to the substrate normal. This can result in exotic shapes such as nanooverlaps, nanogaps, nanochains, and nanocups. The major motivation for the creation of these periodic metal nanostructures is to exploit their plasmonic properties. These nanostructures have proved as strong candidates for applications in surface-enhanced spectroscopy, photocatalysis (86), and biochemical sensing (87). In the context of active molecular plasmonics, we have employed nanodisk arrays to study the tunable plasmon-exciton resonant coupling, which has potential applications in active nanophotonic devices (88, 89).

Recently, we have developed M-NSL to further extend the capability of NSL in fabrication of novel nanostructures (90). Conventional NSL involves convective self-assembly of nanospheres, in which colloidal spheres assemble into crystalline patterns as a result of capillary and hydrodynamic forces. M-NSL is a modified version of NSL, which can be used to create complex nanostructures with moiré patterns by depositing layers of close-packed spheres one at a time and applying an in-plane rotation between the layers. Figure 8 illustrates the spontaneous self-assembly of bilayers in NSL and the layer-by-layer deposition of bilayers in M-NSL.

Depending on the relative in-plane rotation between the top and bottom layers, the resultant moiré patterns vary. With a small angle of rotation ($<10^\circ$), the moiré patterns have a large unit cell with simple structures. As the angle increases to 10° - 30° , the moiré patterns have a smaller unit cell with increased complexity. Once the angle of rotation is larger than 30° , the patterns start to repeat due to the symmetry of the two layers. Figure 9 shows the low- and high-magnification SEM images of the plasma-etched M-NSL bilayer with moiré pattern. Like NSL, the M-NSL bilayers can be used as lithographic masks to create various metal nanostructures on general substrates. The resultant metasurfaces exhibit tunable multiband optical responses ranging from visible (~ 600 nm) to mid IR (~ 4200 nm), enabling their applications in ultrabroadband absorbers and multiband SERS substrates (91).

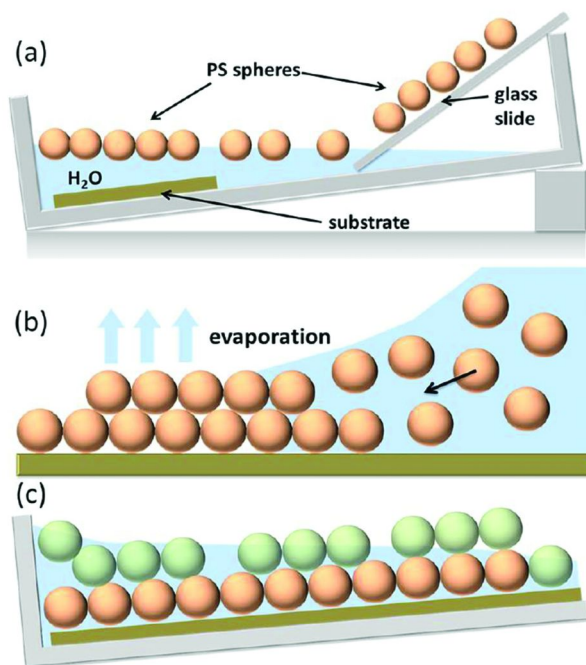


Figure 8. (a) Schematic of the formation of monolayer of polystyrene spheres at water-air interface and its transfer to glass slide. (b) Schematic of spontaneous self-assembly of bilayer of spheres in NSL. (c) Schematic of layer-by-layer stacking of spheres with the in-plane rotation in M-NSL. The different colors indicate the angle rotation between the top and bottom layers. Reproduced with permission (90). Copyright 2015, American Chemical Society.

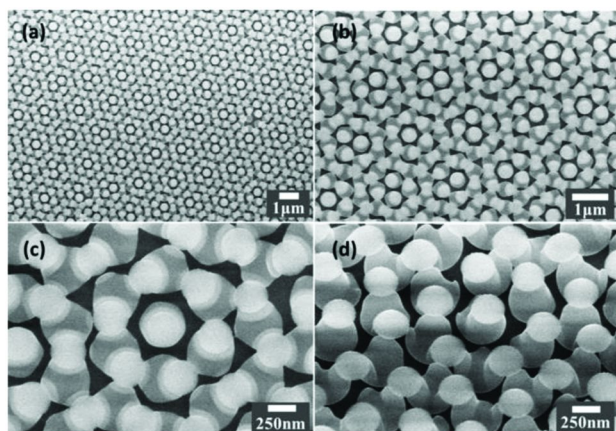


Figure 9. (a-c) SEM images of the etched bilayer in M-NSL with different magnifications. (d) 30° tilted view of the bilayer. Reproduced with permission (90). Copyright 2015, American Chemical Society.

3.2. Multiphoton Plasmonic Lithography

In recent years, multiphoton fabrication (MPL) has emerged as a powerful technique for direct laser writing of complex 3D structures with resolutions beyond the diffraction limit (92). This is typically achieved by scanning a tightly focused beam of a femtosecond laser inside polymer materials. The unique aspect of MPL is that the photo-crosslinking occurs at the vicinity of the focal point, where the laser intensity is high enough for photoreactants to absorb two or more photons simultaneously, a process known as non-linear absorption. This high localization of reaction and freeform fabrication capability has been utilized to create novel photonic devices (93). The dependence of MPL on the intensity threshold has motivated researchers to couple MPL process with plasmonics, resulting in the development of MPPL. MPPL relies on the fact that LSPR can be used to trigger and control photophysical and photochemical reactions, which occur via the coupling between the LSPR energy and the chemical system (94).

The metal NPs have intense field enhancements at the plasmonic hot spots, which lead to an enhanced energy density exceeding the non-linear absorption threshold of polymers within these regions. The nanoscale confinement of the hot spots has been utilized to fabricate highly resolved nanostructures using photoresists such as SU-8 and TSMR-V90 (95). It has also been used to visualize the plasmon modes in nanostructures (96) and to enhance SERS performance via selective adhesion of molecules within the hot spots (97). MPPL has also been used in conjunction with atomic force microscopy to visualize the non-resonant electromagnetic effects in Au nanorods (98).

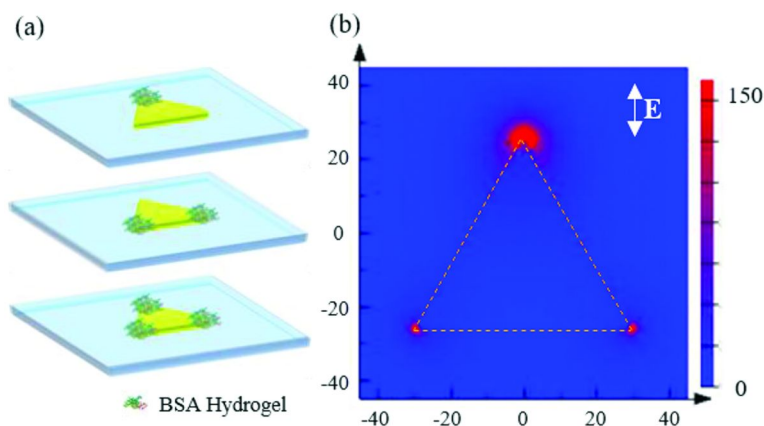


Figure 10. (a) Schematic showing the immobilization of BSA hydrogel on the one, two and three tips of a single AuNT (b) The FDTD simulated field-intensity distribution over a single AuNT upon incidence on linearly polarized light. The intensity enhancement ($|E|/|E_0|$) is shown in the color scale.

We have recently extended the capability of MPPL to regioselectively localize protein molecules at the hot spots of a single Au nanotriangle (AuNT) (99). We direct bovine serum albumin (BSA) hydrogels at the tips of a single AuNT

along with control over their position and quantity. In MPPL, the intensity and polarization of incident light was tuned to achieve BSA hydrogel localization at one, two and three tips of a single AuNT (Figures 10a). Specifically, Rose Bengal, the photosensitizer used in the fabrication medium undergoes facile intersystem crossing within the plasmonic “hot spots”, which promote the intermolecular BSA crosslinking through singlet-oxygen and/or direct hydrogen-transfer mechanism (100). In Figure 10 (b), simulated data using finite-difference time domain (FDTD) method depict the hot spot present at the tips of the AuNT when irradiated with linearly polarized light. The regioselective process was further characterized using single-particle dark-field scattering spectroscopy. With the extension of MPPL to biological domains, we envisage the use of the hybrid metal-biomolecule nanostructures for the better understanding of various biological processes at the nanoscale such as cell-matrix interactions. With the better understanding and finer control, MPPL will significantly impact nanophotonics by enabling various novel nanostructures such as tunable nanoantennas, nonlinear nanostructures, and protein-based micro-lens (101).

4. Applications in Energy, Biology, and Healthcare

Novel and exciting applications arise from the intriguing photonic phenomena at the nanoscale. The engineering of size, shape and composition of metal NPs can lead to different kinds of optical responses. As a result of the extensive research over the past two decades, plasmonics is on the verge of delivering its promise of transforming various basic research and industries such as healthcare, energy and telecommunication. In the first sub-section, we discuss our research aimed at the exploitation of plasmonics in solar-energy harvesting technologies such as photoelectrochemical (PEC) cells and transparent solar cells. The second sub-section focuses on the applications in biology and healthcare sectors, with an emphasis on the development of point-of-care devices. One of the most promising biological applications of plasmonics is in biosensors, whose industry is projected to grow from \$13 billion in 2014 to \$22.5 billion in 2020 (102).

4.1. Solar-Energy Harvesting

With an ever-increasing energy demand, the need for harnessing alternative sources of energy has become inevitable. Over the past few decades, technologies utilizing the incident solar radiation have resulted in significant environmental benefits and sustainable development. The solar energy is utilized primarily by its conversion to either electrical energy (photovoltaic), chemical energy (photocatalysis) or heat energy (solar heaters). For creation of viable technologies, it is critical for the devices to achieve a high conversion efficiency. Among the various approaches for efficiency enhancement, the ability of metal NPs to concentrate electromagnetic fields, along with the option of engineering the scattering and absorption of radiation, makes them an efficient medium for enhancing the device performance.

PEC water splitting is a promising technology, which mimics natural photosynthesis to generate chemical fuels such as hydrogen and oxygen using solar energy. Traditional PEC cell employs a semiconductor photoelectrode and a metal counter electrode. Although the concept was introduced in 1972 (103), real-life applications of robust and efficient PEC cells have been limited. The criteria for achieving a high performance include: (i) a high absorption of solar radiation obtained via appropriate band energies and structures, (ii) low electron-hole pair recombination, (iii) a high charge-carrier conductivity, and (iv) a fast surface reaction kinetics. Since most of the semiconductors fail to meet all the requirements, inclusion of plasmonic NPs can offset the limitations (104). Semiconductors such as TiO_2 and ZnO with wide band gaps (UV absorption) can be sensitized to utilize visible light via absorption and subsequent electron transfer from Au NPs to the semiconductors. For instance, Li and co-workers have demonstrated photoactivity over an entire UV-visible spectrum on TiO_2 by incorporating Au NPs (105). A similar effect with increased absorption has been observed on ZnO (106). Recently, absorption of 27% of solar radiation in 300-1100 nm has been achieved using Ag-Si nanocones (107). Further, metal NP can act as an antenna to localize optical energy at the semiconductor-NP interface. This can reduce the travel distance of minority charge carriers, thereby limiting recombination (108). Integration of large NPs with efficient scattering can increase the path length of the incident light. The cumulative effects improve device efficiency and reduce active semiconductor volume.

The mechanisms for plasmonic enhancement include (i) plasmon resonance energy transfer (PRET) from metal NPs to the adjacent semiconductors via dipole-dipole interactions. This was exploited by Cronin and co-workers to achieve 66 times enhancement in photocurrent with Au- TiO_2 anode under 633 nm irradiation (109); (ii) hot-electron injection, where electrons are generated in the Au NPs and injected into the conduction band of semiconductors upon non-irradiative decay of surface plasmons. This mechanism is possible only when the metal NP and semiconductor have direct contact, resulting in a Schottky barrier with the work function of the metal NPs higher than that of an n-type semiconductor or lower than that of a p-type semiconductor. This mechanism has proved to enhance performance in titanium oxide (110), zinc oxide (111), cerium oxide (112), and tungsten oxide (113); (iii) plasmonic heating, which in most situations has a minor role; and (iv) local electromagnetic field concentration for the enhanced light absorption by semiconductors (114, 115).

For a further reduction in cost and improvement in performance, the earth-abundant, stable and visible-light active photoanodes of metal oxides such as haematite (Fe_2O_3) and bismuth vanadate (BiVO_4) are being explored. Among these photoanode candidates, BiVO_4 is a promising material due to a direct band gap of 2.4 eV and suitable band position for oxygen evolution, which lead to a low onset potential and utilization of blue portion of the visible spectrum (116). Compared to bulk BiVO_4 , which suffers from high charge recombination, nanostructured BiVO_4 along with co-catalyst has been reported to achieve high photocurrent density of 2.73 mAcm^{-2} at a potential as low as 0.6 V versus reversible hydrogen electrode (RHE) (117). In addition, we have demonstrated the role of hydrogen treatment in improving BiVO_4 performance, which is

attributed to the enhanced carrier density and conductivity (118). Further, Zhang and co-workers demonstrated the plasmonic enhancements in BiVO_4 photonic crystals (119). Recently, we have demonstrated Au-NP-decorated nanoporous BiVO_4 photoanodes in which we synergize multiple enhancement mechanisms, including enhanced charge and carrier collection, plasmon-induced electron and energy transfer, and plasmon-enhanced electromagnetic field (120). Figure 11 shows scanning electron micrograph of the Au- BiVO_4 photoanode. The high-resolution transmission electron micrograph (inset of Figure 11) reveals the Au nanoparticles.

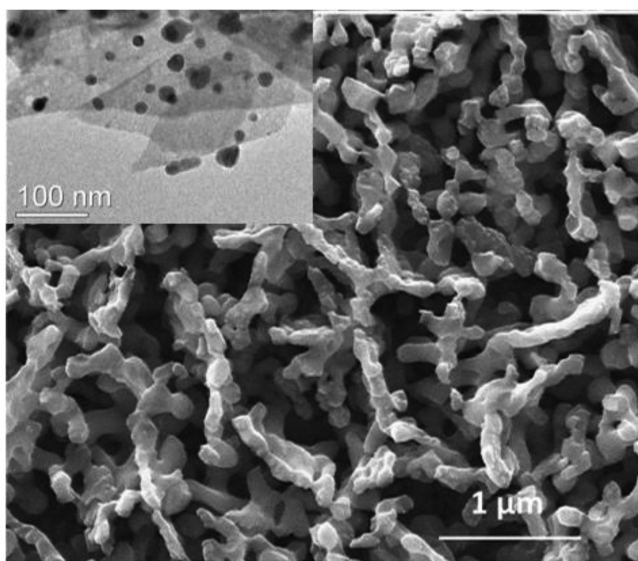


Figure 11. Scanning electron micrograph of Au- BiVO_4 photoanode. Inset is the high-resolution transmission electron micrograph revealing the Au nanoparticles.

Photovoltaics, the most prominent application of solar-energy harvesting also benefits from the unique properties of metal NPs. Similar to their advantages in PEC cells, plasmonic nanostructures can enhance light absorption and scattering as well as reduce light reflection (121). In addition, they can serve as back contact of solar cells and aid in the creation of transparent solar cells (122). We have demonstrated transparent polymer solar cells (with an efficiency of 4%) with Ag nanowire networks as electrodes (123). A further exploitation of plasmonic effects in the electrodes could improve the efficiency. By creating or improving

next-generation energy materials, plasmonics is expected to play a pivotal role in enhancing our energy-harvesting capabilities.

4.2. Biology and Healthcare

Intense research on plasmonic materials has greatly benefited biomedical -- and healthcare sectors. Numerous applications such as multifunctional theranostics and ultra-sensitive portable multi-assay biosensors have been realized (124). The enhanced photo-thermal effects have gained prominence for minimally invasive cancer treatment. Efficient penetration of light into tissues to perform *in vivo* treatments requires the use NIR light. With their LSPRs in the NIR window, Au nanoshells, nanorods and nanocages are actively investigated (125).

We are exploiting plasmonic NPs to detect biomolecules and cellular signals (126, 127). The LSPR peak wavelength, intensity and bandwidth can be modulated by events such as adsorption or conformational changes of molecules on NPs (128). NPs can provide a high spatial resolution for *in vivo* applications. Recently, transfer of chirality between chiral analyte and achiral dye molecule was demonstrated in the vicinity of a single AgNP (129). This can be utilized to study biomolecular processes with ultra-high resolution. Attaining single-molecule detection has been the crown jewel of SPR sensing (130). This is achieved by engineering highly sensitive single or an array of nanoparticles with hot spots, designing high-sensitive spectrometers, and improving a figure-of-merit (FOM, ratio of wavelength sensitivity to the line-width of LSPR). For instance, Altug and co-workers have achieved naked-eye detection of single protein monolayers by designing asymmetric Fano resonance in plasmonic nanohole arrays with a large FOM of 162 (131). Alternatively, Zhang and co-workers designed a zero-mode waveguide within Al nanoholes with light fields confined at the bottom to detect single-molecules (132). Further, rational design can be exploited to modify the emission of molecules via Purcell effect to achieve single-molecule measurements and sensing (133, 134).

Metal nanoparticle arrays with plasmonic-photonic coupling have attracted interests for sensor applications due to high FOM (135). We have recently designed Au bowtie nanoantenna arrays (BNAs) with metal-insulator-metal (MIM) configuration to achieve high field enhancement and wide spectral response specific to sensing applications (136). Figure 12(a) shows the schematic of the design of the Au BNAs in MIM configuration. In this design, we can tune the spacer thickness, lattice parameters along *x-y* direction, and the bowtie gap distance for performance optimization. In particular, we have developed coordinated multiple couplings to achieve sensors with an ultra-high FOM of 254, wide refractive-index (RI) working range, and high signal-to-noise ratio. Figures 12 (b) demonstrates the sensitivity of Au BNAs with RI changing from 1 to 1.4.

The high sensitivity and compactness have motivated researchers to extend plasmonic technologies for point-of-care diagnostics. A viable strategy to achieve this is to integrate plasmonics with microfluidics (137, 138). In general, label-free sensing is achieved by immobilizing a recognition element on detection substrate. For instance, a SPR sensor was used to differentiate interleukin-8 (expressing agent for prostate cancer) concentration in saliva between healthy

and cancerous individuals (139). In a recent study, Tamayo and co-workers have detected cancer biomarkers (carcinoembryonic antigen and the prostate specific antigen) in serum using a hybrid mechanical and optoplasmonic sensor (139). A detection limit of 10^{-16} gml⁻¹ with very low false positives/negatives (10^{-4}) was achieved. A portable microfluidic SPR device has been developed for fast (~30 mins) detection of antibiotics (phenolic antibiotic families) in milk without any processing requirements (140). A similar approach has been employed to detect biotoxin (141). Researchers have also employed commercial SPR equipment in Kretschmann configuration to detect B-type natriuretic peptide up to 10 pg/ml (A typical range in blood is from ~20 pg/ml to 2 ng/ml) in a microfluidic channel (142).

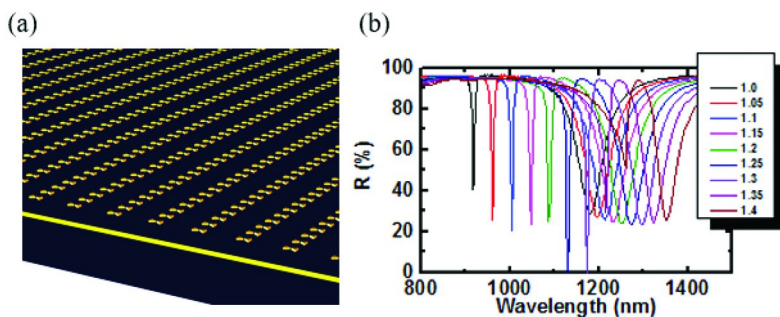


Figure 12. (a) Side view of Au BNAs with MIM configuration. (b) Normalized reflection dips of the BNAs as a function of the superstrate refractive index depicting large spectral range.

LSPR has been traditionally used in conjunction with DNA as a plasmonic ruler (143), and in label-free DNA biosensing (144). It has been extended to clinical application in biomarker detection for Alzheimer's disease (145) and cancer diagnostics (146). We have realized sensitive detection of a herbicide (paraquat) by engineering the supramolecule-modified Au NPs (147). We have also demonstrated the integration of an LSPR chip with a microfluidic channel to realize bulk RI sensing (148). Other researchers have applied LSPR-microfluidic spectroscopy to study DNA hybridization (149). A unique advantage of LSPR integrated with microfluidics is in the multiplexed analysis of various biomarkers. Endo et al. used specific antibodies immobilized at pre-determined locations on a nanochip containing 100 nm silica nanoparticles sandwiched between two Au films (150). The nanochip was utilized to detect six antigens, C-reaction protein and fibrinogen. Acimovic et al. have achieved 32 parallel sensing sites for cancer markers in serum by utilizing microfluidic systems with multiple channels (8) and microvalves (151). A similar approach was employed in real-life testing to monitor inflammatory response of infants by tracking time-course variation of their serum cytokines (152). The advancements in fast multiplexed sensing, along with high sensitivity, are crucial differentiators, positioning plasmonics in a unique position to create a significant impact in the biosensing industry.

5. Conclusions and Perspectives

Molecular plasmonics, which explores and exploits the interactions of plasmons with molecules, is an emerging field. It presents us with immense opportunities for basic research in multiple fields such as quantum optics, surface chemistry and molecular photonics, and for various engineering applications. Tremendous progress has been made in molecular plasmonics because of the developments in tools for molecular-scale measurements and control, nanofabrication techniques, as well as quantum and classic optical simulations. With meticulous engineering and fabrication of plasmonic nanostructures, single-molecule detection can be achieved in both SERS and LSPR sensors. Plasmonics has been exploited to improve the efficiency and reduce cost in solar-energy harvesting devices such as PEC cells and solar cells. In the healthcare sector, plasmonics has a plethora of applications such as photothermal therapy, molecular diagnosis, and *in vivo* imaging.

There has been a gradual shift from standalone devices to highly integrated lab-on-chip systems comprising of plasmonic and microfluidic components. Such plasmofluidic systems support multifunctional capabilities such as sensing, drug delivery, and molecular manipulation. A major challenge in plasmonics is the translation from a laboratory concept to real-life application. Some companies have combated this challenge and managed to create marketable products. Companies such as Renishaw Diagnostics, Carbot Corporation, and Causeway sensors are already selling SERS materials and detection methods. Major companies such as General Electric have also invested in plasmonics to create high-quality SPR sensors. Intel and IBM are forging plasmonics into information technology where plasmon-enhanced detectors and nanolasers are pursued for integrated photonic circuits. With these initial leaps, the future of plasmonics and molecular plasmonics is bright and prosperous.

References

1. Forbes. *Why IBM and Intel Are Chasing the \$100B Opportunity in Nanophotonics*; 2012; available from <http://www.forbes.com/sites/joshwolfe/2012/12/13/why-ibm-and-intel-are-chasing-the-100b-opportunity-in-nanophotonics/> (accessed Jan. 15, 2016).
2. Shi, S. Y.; Chen, C. H.; Prather, D. W. Plane-wave expansion method for calculating band structure of photonic crystal slabs with perfectly matched layers. *J. Opt. Soc. Am. A* **2004**, *21*, 1769–1775.
3. Li, Z. Y.; Lin, L. L. Photonic band structures solved by a plane-wave-based transfer-matrix method. *Phys. Rev. E* **2003**, *67*, 046607.
4. ReportLinker. *Global Markets and Technologies for Photonic Crystals*; 2015; <http://www.reportlinker.com/p01105923/Global-Markets-and-Technologies-for-Photonic-Crystals.html> (accessed Jan. 15, 2016).
5. Sonntag, M. D.; Klingsporn, J. M.; Zrimsek, A. B.; Sharma, B.; Ruvuna, L. K.; Van Duyne, R. P. Molecular plasmonics for nanoscale spectroscopy. *Chem. Soc. Rev.* **2014**, *43*, 1230–1247.

6. Brolo, A. G. Plasmonics for future biosensors. *Nat. Photonics* **2012**, *6*, 70–713.
7. Willets, K. A.; Van Duyne, R. P. Localized surface plasmon resonance spectroscopy and sensing. *Annu. Rev. Phys. Chem.* **2007**, *58*, 267–297.
8. Wu, Z.; Zheng, Y. Radiative Enhancement of Plasmonic Nanopatch Antennas. *Plasmonics* **2015**, 1–10.
9. Barnes, W. L.; Dereux, A.; Ebbesen, T. W. Surface plasmon subwavelength optics. *Nature* **2003**, *424*, 824–830.
10. Brockman, J. M.; Nelson, B. P.; Corn, R. M. Surface plasmon resonance imaging measurements of ultrathin organic films. *Annu. Rev. Phys. Chem.* **2000**, *51*, 41–63.
11. Stewart, M. E.; Anderton, C. R.; Thompson, L. B.; Maria, J.; Gray, S. K.; Rogers, J. A.; Nuzzo, R. G. Nanostructured plasmonic sensors. *Chem. Rev.* **2008**, *108*, 494–521.
12. Im, H.; Shao, H. L.; Park, Y. I.; Peterson, V. M.; Castro, C. M.; Weissleder, R.; Lee, H. Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. *Nat. Biotechnol.* **2014**, *32*, 490–495.
13. Shevchenko, Y.; Camci-Unal, G.; Cuttica, D. E.; Dokmeci, M. R.; Albert, J.; Khademhosseini, A. Surface plasmon resonance fiber sensor for real-time and label-free monitoring of cellular behavior. *Biosens. Bioelectron.* **2014**, *56*, 359–367.
14. Min, C. J.; Shen, Z.; Shen, J. F.; Zhang, Y. Q.; Fang, H.; Yuan, G. H.; Du, L. P.; Zhu, S. W.; Lei, T.; Yuan, X. C. Focused plasmonic trapping of metallic particles. *Nat. Commun.* **2013**, *4*, 2891.
15. Grigorenko, A. N.; Roberts, N. W.; Dickinson, M. R.; Zhang, Y. Nanometric optical tweezers based on nanostructured substrates. *Nat. Photonics* **2008**, *2*, 365–370.
16. Li, Y.; Jing, C.; Zhang, L.; Long, Y. T. Resonance scattering particles as biological nanosensors in vitro and in vivo. *Chem. Soc. Rev.* **2012**, *41*, 632–642.
17. Noginov, M. A.; Zhu, G.; Belgrave, A. M.; Bakker, R.; Shalae, V. M.; Narimanov, E. E.; Stout, S.; Herz, E.; Suteewong, T.; Wiesner, U. Demonstration of a spaser-based nanolaser. *Nature* **2009**, *460*, 1110–1112.
18. Tan, S. F.; Wu, L.; Yang, J. K. W.; Bai, P.; Bosman, M.; Nijhuis, C. A. Quantum Plasmon Resonances Controlled by Molecular Tunnel Junctions. *Science* **2014**, *343*, 1496–1499.
19. Russell, K. J.; Liu, T. L.; Cui, S. Y.; Hu, E. L. Large spontaneous emission enhancement in plasmonic nanocavities. *Nat. Photonics* **2012**, *6*, 459–462.
20. Whitesides, G. M.; Mathias, J. P.; Seto, C. T. Molecular Self-Assembly and Nanochemistry - a Chemical Strategy for the Synthesis of Nanostructures. *Science* **1991**, *254*, 1312–1319.
21. Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. Self-assembled monolayers of thiolates on metals as a form of nanotechnology. *Chem. Rev.* **2005**, *105*, 1103–1169.
22. Yang, S. M.; Jang, S. G.; Choi, D. G.; Kim, S.; Yu, H. K. Nanomachining by colloidal lithography. *Small* **2006**, *2*, 458–475.

23. Spinke, J.; Liley, M.; Schmitt, F. J.; Guder, H. J.; Angermaier, L.; Knoll, W. Molecular recognition at self-assembled monolayers: Optimization of surface functionalization. *J. Chem. Phys.* **1993**, *99*, 7012–7019.
24. Fan, F. R. F.; Yang, J. P.; Cai, L. T.; Price, D. W.; Dirk, S. M.; Kosynkin, D. V.; Yao, Y. X.; Rawlett, A. M.; Tour, J. M.; Bard, A. J. Charge transport through self-assembled monolayers of compounds of interest in molecular electronics. *J. Am. Chem. Soc.* **2002**, *124*, 5550–5560.
25. Chaki, N. K.; Vijayamohan, K. Self-assembled monolayers as a tunable platform for biosensor applications. *Biosens. Bioelectron.* **2002**, *17*, 1–12.
26. Maksymovych, P.; Voznyy, O.; Dougherty, D. B.; Sorescu, D. C.; Yates, J. T. Gold adatom as a key structural component in self-assembled monolayers of organosulfur molecules on Au(111). *Prog. Surf. Sci.* **2010**, *85*, 206–240.
27. Costelle, L.; Jarvi, T. T.; Raisanen, M. T.; Tuboltsev, V.; Raisanen, J. Binding of deposited gold clusters to thiol self-assembled monolayers on Au(111) surfaces. *Appl. Phys. Lett.* **2011**, *98*, 043107.
28. Newton, L.; Slater, T.; Clark, N.; Vijayaraghavan, A. Self assembled monolayers (SAMs) on metallic surfaces (gold and graphene) for electronic applications. *J. Mater. Chem. C* **2013**, *1*, 376–393.
29. Weiss, P. S. Functional Molecules and Assemblies in Controlled Environments: Formation and Measurements. *Acc. Chem. Res.* **2008**, *41*, 1772–1781.
30. Kumar, A. S.; Ye, T.; Takami, T.; Yu, B. C.; Flatt, A. K.; Tour, J. M.; Weiss, P. S. Reversible photo-switching of single azobenzene molecules in controlled nanoscale environments. *Nano Lett.* **2008**, *8*, 1644–1648.
31. Kim, M.; Hohman, J. N.; Cao, Y.; Houk, K. N.; Ma, H.; Jen, A. K. Y.; Weiss, P. S. Creating Favorable Geometries for Directing Organic Photoreactions in Alkanethiolate Monolayers. *Science* **2011**, *331*, 1312–1315.
32. Donhauser, Z. J.; Mantoosh, B. A.; Kelly, K. F.; Bumm, L. A.; Monnell, J. D.; Stapleton, J. J.; Price, D. W.; Rawlett, A. M.; Allara, D. L.; Tour, J. M.; Weiss, P. S. Conductance switching in single molecules through conformational changes. *Science* **2001**, *292*, 2303–2307.
33. Mullen, T. J.; Srinivasan, C.; Hohman, J. N.; Gillmor, S. D.; Shuster, M. J.; Horn, M. W.; Andrews, A. M.; Weiss, P. S. Microcontact insertion printing. *Appl. Phys. Lett.* **2007**, *90*, 063114.
34. Smith, R. K.; Lewis, P. A.; Weiss, P. S. Patterning self-assembled monolayers. *Prog. Surf. Sci.* **2004**, *75*, 1–68.
35. Zheng, Y. B.; Yang, Y. W.; Jensen, L.; Fang, L.; Juluri, B. K.; Flood, A. H.; Weiss, P. S.; Stoddart, J. F.; Huang, T. J. Active Molecular Plasmonics: Controlling Plasmon Resonances with Molecular Switches. *Nano Lett.* **2009**, *9*, 819–825.
36. Juluri, B. K.; Kumar, A. S.; Liu, Y.; Ye, T.; Yang, Y. W.; Flood, A. H.; Fang, L.; Stoddart, J. F.; Weiss, P. S.; Huang, T. J. A Mechanical Actuator Driven Electrochemically by Artificial Molecular Muscles. *ACS Nano* **2009**, *3*, 291–300.
37. Browne, W. R.; Feringa, B. L. Light Switching of Molecules on Surfaces. *Annu. Rev. Phys. Chem.* **2009**, *60*, 407–428.

38. Klajn, R.; Fang, L.; Coskun, A.; Olson, M. A.; Wesson, P. J.; Stoddart, J. F.; Grzybowski, B. A. Metal Nanoparticles Functionalized with Molecular and Supramolecular Switches. *J. Am. Chem. Soc.* **2009**, *131*, 4233–4235.
39. Vance, A. L.; Willey, T. M.; van Buuren, T.; Nelson, A. J.; Bostedt, C.; Fox, G. A.; Terminello, L. J. XAS and XPS characterization of a surface-attached rotaxane. *Nano Lett.* **2003**, *3*, 81–84.
40. Li, Z.; Schouteden, K.; Iancu, V.; Janssens, E.; Lievens, P.; Van Haesendonck, C.; Cerda, J. I. Chemically modified STM tips for atomic-resolution imaging of ultrathin NaCl films. *Nano Res.* **2015**, *8*, 2223–2230.
41. Ye, T.; Kumar, A. S.; Saha, S.; Takami, T.; Huang, T. J.; Stoddart, J. F.; Weiss, P. S. Changing Stations in Single Bistable Rotaxane Molecules under Electrochemical Control. *ACS Nano* **2010**, *4*, 3697–3701.
42. Kushmerick, J. G.; Holt, D. B.; Yang, J. C.; Naciri, J.; Moore, M. H.; Shashidhar, R. Metal-molecule contacts and charge transport across monomolecular layers: Measurement and theory. *Phys. Rev. Lett.* **2002**, *89*, 086802.
43. Lewis, P. A.; Inman, C. E.; Maya, F.; Tour, J. M.; Hutchison, J. E.; Weiss, P. S. Molecular engineering of the polarity and interactions of molecular electronic switches. *J. Am. Chem. Soc.* **2005**, *127*, 17421–17426.
44. Cygan, M. T.; Dunbar, T. D.; Arnold, J. J.; Bumm, L. A.; Shedlock, N. F.; Burgin, T. P.; Jones, L.; Allara, D. L.; Tour, J. M.; Weiss, P. S. Insertion, conductivity, and structures of conjugated organic oligomers in self-assembled alkanethiol monolayers on Au{111}. *J. Am. Chem. Soc.* **1998**, *120*, 2721–2732.
45. Zheng, Y. B.; Payton, J. L.; Chung, C. H.; Liu, R.; Cheunkar, S.; Pathem, B. K.; Yang, Y.; Jensen, L.; Weiss, P. S. Surface-Enhanced Raman Spectroscopy to Probe Reversibly Photoswitchable Azobenzene in Controlled Nanoscale Environments. *Nano Lett.* **2011**, *11*, 3447–3452.
46. Bumm, L. A.; Arnold, J. J.; Dunbar, T. D.; Allara, D. L.; Weiss, P. S. Electron transfer through organic molecules. *J. Phys. Chem. B* **1999**, *103*, 8122–8127.
47. Moore, A. M.; Mantooth, B. A.; Donhauser, Z. J.; Maya, F.; Price, D. W.; Yao, Y. X.; Tour, J. M.; Weiss, P. S. Cross-step place-exchange of oligo(phenylene-ethynylene) molecules. *Nano Lett.* **2005**, *5*, 2292–2297.
48. Pechenezhskiy, I. V.; Cho, J.; Nguyen, G. D.; Berbil-Bautista, L.; Giles, B. L.; Poulsen, D. A.; Frechet, J. M. J.; Crommie, M. F. Self-Assembly and Photomechanical Switching of an Azobenzene Derivative on GaAs(110): Scanning Tunneling Microscopy Study. *J. Phys. Chem. C* **2012**, *116*, 1052–1055.
49. Carroll, G. T.; London, G.; Landaluce, T. F.; Rudolf, P.; Feringa, B. L. Adhesion of Photon-Driven Molecular Motors to Surfaces via 1,3-Dipolar Cycloadditions: Effect of Interfacial Interactions on Molecular Motion. *ACS Nano* **2011**, *5*, 622–630.
50. Dri, C.; Peters, M. V.; Schwarz, J.; Hecht, S.; Grill, L. Spatial periodicity in molecular switching. *Nat. Nanotechnol.* **2008**, *3*, 649–653.

51. Zheng, Y. B.; Pathem, B. K.; Hohman, J. N.; Thomas, J. C.; Kim, M.; Weiss, P. S. Photoresponsive Molecules in Well-Defined Nanoscale Environments. *Adv. Mater.* **2013**, *25*, 302–312.
52. Wan, P. B.; Jiang, Y. G.; Wang, Y. P.; Wang, Z. Q.; Zhang, X. Tuning surface wettability through photocontrolled reversible molecular shuttle. *Chem. Commun.* **2008**, *44*, 5710–5712.
53. Baisch, B.; Raffa, D.; Jung, U.; Magnussen, O. M.; Nicolas, C.; Lacour, J.; Kubitschke, J.; Herges, R. Mounting Freestanding Molecular Functions onto Surfaces: The Platform Approach. *J. Am. Chem. Soc.* **2009**, *131*, 442–443.
54. Jacob, H.; Ulrich, S.; Jung, U.; Lemke, S.; Rusch, T.; Schutt, C.; Petersen, F.; Strunskus, T.; Magnussen, O.; Herges, R.; Tuczek, F. Monitoring the reversible photoisomerization of an azobenzene-functionalized molecular triazatriangulene platform on Au(111) by IRRAS. *Phys. Chem. Chem. Phys.* **2014**, *16*, 22643–22650.
55. Kneipp, K.; Wang, Y.; Kneipp, H.; Perelman, L. T.; Itzkan, I.; Dasari, R.; Feld, M. S. Single molecule detection using surface-enhanced Raman scattering (SERS). *Phys. Rev. Lett.* **1997**, *78*, 1667–1670.
56. Schlucker, S. Surface-Enhanced Raman Spectroscopy: Concepts and Chemical Applications. *Angew. Chem. Int. Ed.* **2014**, *53*, 4756–4795.
57. Tripp, R. A.; Dluhy, R. A.; Zhao, Y. P. Novel nanostructures for SERS biosensing. *Nano Today* **2008**, *3*, 31–37.
58. Stevenson, R.; Ingram, A.; Leung, H.; McMillan, D. C.; Graham, D. Quantitative SERRS immunoassay for the detection of human PSA. *Analyst* **2009**, *134*, 842–844.
59. Song, J. B.; Zhou, J. J.; Duan, H. W. Self-Assembled Plasmonic Vesicles of SERS-Encoded Amphiphilic Gold Nanoparticles for Cancer Cell Targeting and Traceable Intracellular Drug Delivery. *J. Am. Chem. Soc.* **2012**, *134*, 13458–13469.
60. Zengin, A.; Tamer, U.; Caykara, T. A SERS-Based Sandwich Assay for Ultrasensitive and Selective Detection of Alzheimer's Tau Protein. *Biomacromolecules* **2013**, *14*, 3001–3009.
61. Choi, I.; Huh, Y. S.; Erickson, D. Ultra-sensitive, label-free probing of the conformational characteristics of amyloid beta aggregates with a SERS active nanofluidic device. *Microfluid. Nanofluid.* **2012**, *12*, 663–669.
62. Paxton, W. F.; Kleinman, S. L.; Basuray, A. N.; Stoddart, J. F.; Van Duyne, R. P. Surface-enhanced Raman spectroelectrochemistry of TTF-modified self-assembled monolayers. *J. Phys. Chem. Lett.* **2011**, *2*, 1145–1149.
63. Stuart, D. A.; Biggs, K. B.; Van Duyne, R. P. Surface-enhanced Raman spectroscopy of half-mustard agent. *Analyst* **2006**, *131*, 568–572.
64. Radziuk, D.; Moehwald, H. Prospects for plasmonic hot spots in single molecule SERS towards the chemical imaging of live cells. *Phys. Chem. Chem. Phys.* **2015**, *17*, 21072–21093.
65. Tuma, R. Raman spectroscopy of proteins: from peptides to large assemblies. *J. Raman Spectrosc.* **2005**, *36*, 307–319.
66. Moskovits, M.; Suh, J. S. Surface Selection-Rules for Surface-Enhanced Raman-Spectroscopy - Calculations and Application to the Surface-

- Enhanced Raman-Spectrum of Phthalazine on Silver. *J. Phys. Chem.* **1984**, 88, 5526–5530.
67. Moskovits, M.; Dilella, D. P.; Maynard, K. J. Surface Raman-Spectroscopy of a Number of Cyclic Aromatic-Molecules Adsorbed on Silver - Selection-Rules and Molecular-Reorientation. *Langmuir* **1988**, 4, 67–76.
 68. Comstock, M. J.; Levy, N.; Kirakosian, A.; Cho, J.; Lauterwasser, F.; Harvey, J. H.; Strubbe, D. A.; Frechet, J. M. J.; Trauner, D.; Louie, S. G.; Crommie, M. F. Reversible photomechanical switching of individual engineered molecules at a metallic surface. *Phys. Rev. Lett.* **2007**, 99, 178301.
 69. Witlicki, E. H.; Johnsen, C.; Hansen, S. W.; Silverstein, D. W.; Bottomley, V. J.; Jeppesen, J. O.; Wong, E. W.; Jensen, L.; Flood, A. H. Molecular Logic Gates Using Surface-Enhanced Raman-Scattered Light. *J. Am. Chem. Soc.* **2011**, 133, 7288–7291.
 70. Zheng, Y. B.; Payton, J. L.; Song, T. B.; Pathem, B. K.; Zhao, Y. X.; Ma, H.; Yang, Y.; Jensen, L.; Jen, A. K. Y.; Weiss, P. S. Surface-Enhanced Raman Spectroscopy To Probe Photoreaction Pathways and Kinetics of Isolated Reactants on Surfaces: Flat versus Curved Substrates. *Nano Lett.* **2012**, 12, 5362–5368.
 71. Keller, E. L.; Brandt, N. C.; Cassabaum, A. A.; Frontiera, R. R. Ultrafast surface-enhanced Raman spectroscopy. *Analyst* **2015**, 140, 4922–4931.
 72. Shiohara, A.; Wang, Y.; Liz-Marzán, L. M. Recent approaches toward creation of hot spots for SERS detection. *J. Photochem. Photobiol., C* **2014**, 21, 2–25.
 73. Zhang, G.; Wang, D. Colloidal lithography—the art of nanochemical patterning. *Chem. Asian. J.* **2009**, 4, 236–245.
 74. Hulteen, J. C.; Van Duyne, R. P. Nanosphere lithography: a materials general fabrication process for periodic particle array surfaces. *J. Vac. Sci. Technol., A* **1995**, 13, 1553–1558.
 75. Haynes, C. L.; Van Duyne, R. P. Nanosphere lithography: A versatile nanofabrication tool for studies of size-dependent nanoparticle optics. *J. Phys. Chem. B* **2001**, 105, 5599–5611.
 76. Choi, J.-Y.; Alford, T.; Honsberg, C. B. Solvent-controlled spin-coating method for large-scale area deposition of two-dimensional silica nanosphere assembled layers. *Langmuir* **2014**, 30, 5732–5738.
 77. Zheng, Y. B.; Jensen, L.; Yan, W.; Walker, T. R.; Juluri, B. K.; Jensen, L.; Huang, T. J. Chemically tuning the localized surface plasmon resonances of gold nanostructure arrays. *J. Phys. Chem. C* **2009**, 113, 7019–7024.
 78. Zheng, Y. B.; Juluri, B. K.; Mao, X.; Walker, T. R.; Huang, T. J. Systematic investigation of localized surface plasmon resonance of long-range ordered Au nanodisk arrays. *J. Appl. Phys.* **2008**, 103, 014308.
 79. Kosiorek, A.; Kandulski, W.; Glaczynska, H.; Giersig, M. Fabrication of nanoscale rings, dots, and rods by combining shadow nanosphere lithography and annealed polystyrene nanosphere masks. *Small* **2005**, 1, 439–444.
 80. Huang, Z.; Fang, H.; Zhu, J. Fabrication of silicon nanowire arrays with controlled diameter, length, and density. *Adv. Mater.* **2007**, 19, 744–748.

81. Li, Y.; Zhang, J.; Zhu, S.; Dong, H.; Wang, Z.; Sun, Z.; Guo, J.; Yang, B. Bioinspired silicon hollow-tip arrays for high performance broadband anti-reflective and water-repellent coatings. *J. Mater. Chem.* **2009**, *19*, 1806–1810.
82. Lee, S. H.; Bantz, K. C.; Lindquist, N. C.; Oh, S.-H.; Haynes, C. L. Self-assembled plasmonic nanohole arrays. *Langmuir* **2009**, *25*, 13685–13693.
83. Zheng, Y. B.; Juluri, B. K.; Kiraly, B.; Huang, T. J. Ordered Au Nanodisk and Nanohole Arrays: Fabrication and Applications. *J. Nanotechnol. Eng. Med.* **2010**, *1*, 031011.
84. Haynes, C. L.; McFarland, A. D.; Smith, M. T.; Hulteen, J. C.; Van Duyne, R. P. Angle-resolved nanosphere lithography: Manipulation of nanoparticle size, shape, and interparticle spacing. *J. Phys. Chem. B* **2002**, *106*, 1898–1902.
85. Kosiorok, A.; Kandulski, W.; Chudzinski, P.; Kempa, K.; Giersig, M. Shadow nanosphere lithography: simulation and experiment. *Nano Lett.* **2004**, *4*, 1359–1363.
86. Honda, M.; Kumamoto, Y.; Taguchi, A.; Saito, Y.; Kawata, S. Plasmon-enhanced UV photocatalysis. *Appl. Phys. Lett.* **2014**, *104*, 061108.
87. Haes, A. J.; Van Duyne, R. P. A nanoscale optical biosensor: sensitivity and selectivity of an approach based on the localized surface plasmon resonance spectroscopy of triangular silver nanoparticles. *J. Am. Chem. Soc.* **2002**, *124*, 10596–10604.
88. Zheng, Y. B.; Juluri, B. K.; Jensen, L. L.; Ahmed, D.; Lu, M. Q.; Jensen, L.; Huang, T. J. Dynamic Tuning of Plasmon-Exciton Coupling in Arrays of Nanodisk-J-aggregate Complexes. *Adv. Mater.* **2010**, *22*, 3603–3607.
89. Liu, Y. J.; Zheng, Y. B.; Liou, J.; Chiang, I. K.; Khoo, I. C.; Huang, T. J. All-Optical Modulation of Localized Surface Plasmon Coupling in a Hybrid System Composed of Photoswitchable Gratings and Au Nanodisk Arrays. *J. Phys. Chem. C* **2011**, *115*, 7717–7722.
90. Chen, K.; Rajeeva, B. B.; Wu, Z.; Rukavina, M.; Dao, T. D.; Ishii, S.; Aono, M.; Nagao, T.; Zheng, Y. Moiré Nanosphere Lithography. *ACS Nano* **2015**, *9*, 6031–6040.
91. Wu, Z.; Chen, K.; Menz, R.; Nagao, T.; Zheng, Y. Tunable multiband metasurfaces by moiré nanosphere lithography. *Nanoscale* **2015**, *7*, 20391–20396.
92. Paz, V. F.; Emons, M.; Obata, K.; Ovsianikov, A.; Peterhansel, S.; Frenner, K.; Reinhardt, C.; Chichkov, B.; Morgner, U.; Osten, W. Development of functional sub-100 nm structures with 3D two-photon polymerization technique and optical methods for characterization. *J. Laser Appl.* **2012**, *24*, 042004.
93. Ergin, T.; Stenger, N.; Brenner, P.; Pendry, J. B.; Wegener, M. Three-Dimensional Invisibility Cloak at Optical Wavelengths. *Science* **2010**, *328*, 337–339.
94. Chen, H. J.; Ming, T. A.; Zhao, L.; Wang, F.; Sun, L. D.; Wang, J. F.; Yan, C. H. Plasmon-molecule interactions. *Nano Today* **2010**, *5*, 494–505.

95. Ueno, K.; Takabatake, S.; Nishijima, Y.; Mizeikis, V.; Yokota, Y.; Misawa, H. Nanogap-Assisted Surface Plasmon Nanolithography. *J. Phys. Chem. Lett.* **2010**, *1*, 657–662.
96. Gruber, C.; Hirzer, A.; Schmidt, V.; Trugler, A.; Hohenester, U.; Ditzbacher, H.; Hohenau, A.; Krenn, J. R. Imaging nanowire plasmon modes with two-photon polymerization. *Appl. Phys. Lett.* **2015**, *106*, 081101.
97. Diebold, E. D.; Peng, P.; Mazur, E. Isolating Surface-Enhanced Raman Scattering Hot Spots Using Multiphoton Lithography. *J. Am. Chem. Soc.* **2009**, *131*, 16356–16357.
98. Deeb, C.; Zhou, X.; Gérard, D.; Bouhelier, A.; Jain, P. K.; Plain, J.; Soppera, O.; Royer, P.; Bachelot, R. Off-resonant optical excitation of gold nanorods: nanoscale imprint of polarization surface charge distribution. *J. Phys. Chem. Lett.* **2010**, *2*, 7–11.
99. Rajeeva, B. B.; Hernandez, D. S.; Wang, M.; Perillo, E.; Lin, L.; Scarabelli, L.; Pingali, B.; Liz-Marzán, L. M.; Dunn, A. K.; Shear, J. B. Regioselective Localization and Tracking of Biomolecules on Single Gold Nanoparticles. *Adv. Sci.* **2015**, *2*.
100. Pitts, J. D.; Campagnola, P. J.; Epling, G. A.; Goodman, S. L. Submicron multiphoton free-form fabrication of proteins and polymers: Studies of reaction efficiencies and applications in sustained release. *Macromolecules* **2000**, *33*, 1514–1523.
101. Sun, Y.-L.; Dong, W.-F.; Niu, L.-G.; Jiang, T.; Liu, D.-X.; Zhang, L.; Wang, Y.-S.; Chen, Q.-D.; Kim, D.-P.; Sun, H.-B. Protein-based soft micro-optics fabricated by femtosecond laser direct writing. *Light Sci. Appl.* **2014**, *3*, e129.
102. PersistenceMarketResearch. *Global Market Study on Biosensor: Asia-Pacific to Witness Highest Growth by 2020*; 2015; <http://www.persistencemarketresearch.com/market-research/biosensor-market.asp> (accessed Jan. 15, 2016).
103. Fujishima, A.; Honda, K. Photolysis-decomposition of water at the surface of an irradiated semiconductor. *Nature* **1972**, *238*, 37–38.
104. Linic, S.; Christopher, P.; Ingram, D. B. Plasmonic-metal nanostructures for efficient conversion of solar to chemical energy. *Nat. Mater.* **2011**, *10*, 911–921.
105. Pu, Y. C.; Wang, G. M.; Chang, K. D.; Ling, Y. C.; Lin, Y. K.; Fitzmorris, B. C.; Liu, C. M.; Lu, X. H.; Tong, Y. X.; Zhang, J. Z.; Hsu, Y. J.; Li, Y. Au Nanostructure-Decorated TiO₂ Nanowires Exhibiting Photoactivity Across Entire UV-visible Region for Photoelectrochemical Water Splitting. *Nano Lett.* **2013**, *13*, 3817–3823.
106. Chen, H. M.; Chen, C. K.; Tseng, M. L.; Wu, P. C.; Chang, C. M.; Cheng, L. C.; Huang, H. W.; Chan, T. S.; Huang, D. W.; Liu, R. S.; Tsai, D. P. Plasmonic ZnO/Ag Embedded Structures as Collecting Layers for Photogenerating Electrons in Solar Hydrogen Generation Photoelectrodes. *Small* **2013**, *9*, 2926–2936.
107. Zhou, L.; Yu, X. Q.; Zhu, J. Metal-Core/Semiconductor-Shell Nanocones for Broadband Solar Absorption Enhancement. *Nano Lett.* **2014**, *14*, 1093–1098.

108. Warren, S. C.; Thimsen, E. Plasmonic solar water splitting. *Energ. Environ. Sci.* **2012**, *5*, 5133–5146.
109. Liu, Z. W.; Hou, W. B.; Pavaskar, P.; Aykol, M.; Cronin, S. B. Plasmon Resonant Enhancement of Photocatalytic Water Splitting Under Visible Illumination. *Nano Lett.* **2011**, *11*, 1111–1116.
110. Zhang, X.; Liu, Y.; Lee, S. T.; Yang, S. H.; Kang, Z. H. Coupling surface plasmon resonance of gold nanoparticles with slow-photon-effect of TiO₂ photonic crystals for synergistically enhanced photoelectrochemical water splitting. *Energy Environ. Sci.* **2014**, *7*, 1409–1419.
111. Chen, H. M.; Chen, C. K.; Chen, C. J.; Cheng, L. C.; Wu, P. C.; Cheng, B. H.; Ho, Y. Z.; Tseng, M. L.; Hsu, Y. Y.; Chan, T. S.; Lee, J. F.; Liu, R. S.; Tsai, D. P. Plasmon Inducing Effects for Enhanced Photoelectrochemical Water Splitting: X-ray Absorption Approach to Electronic Structures. *ACS Nano* **2012**, *6*, 7362–7372.
112. Primo, A.; Marino, T.; Corma, A.; Molinari, R.; Garcia, H. Efficient Visible-Light Photocatalytic Water Splitting by Minute Amounts of Gold Supported on Nanoparticulate CeO₂ Obtained by a Biopolymer Templating Method. *J. Am. Chem. Soc.* **2011**, *133*, 6930–6933.
113. Chen, X. Q.; Li, P.; Tong, H.; Kako, T.; Ye, J. H. Nanoarchitectonics of a Au nanoprism array on WO₃ film for synergistic optoelectronic response. *Sci. Technol. Adv. Mater.* **2011**, *12*, 044604.
114. Chen, H. M.; Chen, C. K.; Liu, R. S.; Zhang, L.; Zhang, J. J.; Wilkinson, D. P. Nano-architecture and material designs for water splitting photoelectrodes. *Chem. Soc. Rev.* **2012**, *41*, 5654–5671.
115. Zhang, P.; Wang, T.; Gong, J. Mechanistic Understanding of the Plasmonic Enhancement for Solar Water Splitting. *Adv. Mat.* **2015**, *27*, 5328–5342.
116. Park, Y.; McDonald, K. J.; Choi, K. S. Progress in bismuth vanadate photoanodes for use in solar water oxidation. *Chem. Soc. Rev.* **2013**, *42*, 2321–2337.
117. Kim, T. W.; Choi, K. S. Nanoporous BiVO₄ Photoanodes with Dual-Layer Oxygen Evolution Catalysts for Solar Water Splitting. *Science* **2014**, *343*, 990–994.
118. Gan, J.; Lu, X.; Rajeeva, B. B.; Menz, R.; Tong, Y.; Zheng, Y. Efficient Photoelectrochemical Water Oxidation over Hydrogen-Reduced Nanoporous BiVO₄ with Ni–Bi Electrocatalyst. *ChemElectroChem* **2015**, *2*, 1385–1395.
119. Zhang, L. W.; Lin, C. Y.; Valev, V. K.; Reisner, E.; Steiner, U.; Baumberg, J. J. Plasmonic Enhancement in BiVO₄ Photonic Crystals for Efficient Water Splitting. *Small* **2014**, *10*, 3970–3978.
120. Gan, J.; Rajeeva, B. B.; Wu, Z. L.; Tong, Y. X.; Zheng, Y. B. Plasmon-Induced Hot-Electron and Energy Transfer Enhancement in Nanoporous BiVO₄ for Efficient Photoelectrochemical Water Oxidation. Submitted for publication, 2015.
121. Atwater, H. A.; Polman, A. Plasmonics for improved photovoltaic devices. *Nat. Mater.* **2010**, *9*, 205–213.
122. Ellmer, K. Past achievements and future challenges in the development of optically transparent electrodes. *Nat. Photonics* **2012**, *6*, 809–817.

123. Chen, C.-C.; Dou, L.; Zhu, R.; Chung, C.-H.; Song, T.-B.; Zheng, Y. B.; Hawks, S.; Li, G.; Weiss, P. S.; Yang, Y. Visibly transparent polymer solar cells produced by solution processing. *ACS Nano* **2012**, *6*, 7185–7190.
124. Rajeeva, B. B.; Menz, R.; Zheng, Y. Towards rational design of multifunctional theranostic nanoparticles: what barriers do we need to overcome? *Nanomedicine (London)* **2014**, *9*, 1767–1770.
125. Huang, X.; Jain, P. K.; El-Sayed, I. H.; El-Sayed, M. A. Plasmonic photothermal therapy (PPTT) using gold nanoparticles. *Laser Med. Sci.* **2008**, *23*, 217–228.
126. Guo, L.; Kim, D.-H. LSPR biomolecular assay with high sensitivity induced by aptamer–antigen–antibody sandwich complex. *Biosens. Bioelectron.* **2012**, *31*, 567–570.
127. Oh, B.-R.; Huang, N.-T.; Chen, W.; Seo, J. H.; Chen, P.; Cornell, T. T.; Shanley, T. P.; Fu, J.; Kurabayashi, K. Integrated nanoplasmonic sensing for cellular functional immunoanalysis using human blood. *ACS Nano* **2014**, *8*, 2667–2676.
128. Hsiao, V. K.; Zheng, Y. B.; Juluri, B. K.; Huang, T. J. Light-driven plasmonic switches based on au nanodisk arrays and photoresponsive liquid crystals. *Adv. Mater.* **2008**, *20*, 3528–3532.
129. Pour, S. O.; Rocks, L.; Faulds, K.; Graham, D.; Parchansky, V.; Bour, P.; Blanch, E. W. Through-space transfer of chiral information mediated by a plasmonic nanomaterial. *Nat. Chem.* **2015**, *7*, 591–596.
130. Mayer, K. M.; Hao, F.; Lee, S.; Nordlander, P.; Hafner, J. H. A single molecule immunoassay by localized surface plasmon resonance. *Nanotechnology* **2010**, *21*, 255503.
131. Yanik, A. A.; Cetin, A. E.; Huang, M.; Artar, A.; Mousavi, S. H.; Khanikaev, A.; Connor, J. H.; Shvets, G.; Altug, H. Seeing protein monolayers with naked eye through plasmonic Fano resonances. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 11784–11789.
132. Zhao, C. L.; Liu, Y. M.; Yang, J.; Zhang, J. S. Single-molecule detection and radiation control in solutions at high concentrations via a heterogeneous optical slot antenna. *Nanoscale* **2014**, *6*, 9103–9109.
133. Cang, H.; Liu, Y. M.; Wang, Y.; Yin, X. B.; Zhang, X. Giant Suppression of Photobleaching for Single Molecule Detection via the Purcell Effect. *Nano Lett.* **2013**, *13*, 5949–5953.
134. Kinkhabwala, A.; Yu, Z. F.; Fan, S. H.; Avlasevich, Y.; Mullen, K.; Moerner, W. E. Large single-molecule fluorescence enhancements produced by a bowtie nanoantenna. *Nat. Photonics* **2009**, *3*, 654–657.
135. Shen, Y.; Zhou, J. H.; Liu, T. R.; Tao, Y. T.; Jiang, R. B.; Liu, M. X.; Xiao, G. H.; Zhu, J. H.; Zhou, Z. K.; Wang, X. H.; Jin, C. J.; Wang, J. F. Plasmonic gold mushroom arrays with refractive index sensing figures of merit approaching the theoretical limit. *Nat. Commun.* **2013**, *4*, 2381.
136. Lin, L.; Zheng, Y. Optimizing plasmonic nanoantennas via coordinated multiple coupling. *Sci. Rep.* **2015**, *5*, 14788.
137. Wang, M. S.; Zhao, C. L.; Miao, X. Y.; Zhao, Y. H.; Rufo, J.; Liu, Y. J.; Huang, T. J.; Zheng, Y. B. Plasmofluidics: Merging Light and Fluids at the Micro-/Nanoscale. *Small* **2015**, *11*, 4423–4444.

138. Niu, L.; Zhang, N.; Liu, H.; Zhou, X.; Knoll, W. Integrating plasmonic diagnostics and microfluidics. *Biomicrofluidics* **2015**, *9*, 052611.
139. Kosaka, P. M.; Pini, V.; Ruz, J. J.; da Silva, R. A.; Gonzalez, M. U.; Ramos, D.; Calleja, M.; Tamayo, J. Detection of cancer biomarkers in serum using a hybrid mechanical and optoplasmonic nanosensor. *Nat. Nanotechnol.* **2014**, *9*, 1047–1053.
140. Fernandez, F.; Hegnerova, K.; Piliarik, M.; Sanchez-Baeza, F.; Homola, J.; Marco, M. P. A label-free and portable multichannel surface plasmon resonance immunosensor for on site analysis of antibiotics in milk samples. *Biosens. Bioelectron.* **2010**, *26*, 1231–1238.
141. Feltis, B. N.; Sexton, B. A.; Glenn, F. L.; Best, M. J.; Wilkins, M.; Davis, T. J. A hand-held surface plasmon resonance biosensor for the detection of ricin and other biological agents. *Biosens. Bioelectron.* **2008**, *23*, 1131–1136.
142. Kurita, R.; Yokota, Y.; Sato, Y.; Mizutani, F.; Niwa, O. On-chip enzyme immunoassay of a cardiac marker using a microfluidic device combined with a portable surface plasmon resonance system. *Anal. Chem.* **2006**, *78*, 5525–5531.
143. Sonnichsen, C.; Reinhard, B. M.; Liphardt, J.; Alivisatos, A. P. A molecular ruler based on plasmon coupling of single gold and silver nanoparticles. *Nat. Biotechnol.* **2005**, *23*, 741–745.
144. Endo, T.; Kerman, K.; Nagatani, N.; Takamura, Y.; Tamiya, E. Label-free detection of peptide nucleic acid-DNA hybridization using localized surface plasmon resonance based optical biosensor. *Anal. Chem.* **2005**, *77*, 6976–6984.
145. Haes, A. J.; Chang, L.; Klein, W. L.; Van Duyne, R. P. Detection of a biomarker for Alzheimer's disease from synthetic and clinical samples using a nanoscale optical biosensor. *J. Am. Chem. Soc.* **2005**, *127*, 2264–2271.
146. El-Sayed, I. H.; Huang, X. H.; El-Sayed, M. A. Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: Applications in oral cancer. *Nano Lett.* **2005**, *5*, 829–834.
147. Li, H.; Chen, D. X.; Sun, Y. L.; Zheng, Y. B.; Tan, L. L.; Weiss, P. S.; Yang, Y. W. Viologen-mediated assembly of and sensing with carboxylatopillar[5]arene-modified gold nanoparticles. *J. Am. Chem. Soc.* **2013**, *135*, 1570–1576.
148. Zheng, Y. B.; Juluri, B. K.; Mao, X. L.; Walker, T. R.; Huang, T. J. Systematic investigation of localized surface plasmon resonance of long-range ordered Au nanodisk arrays. *J. Appl. Phys.* **2008**, *103*, 014308.
149. Huang, C.; Ye, J.; Wang, S.; Stakenborg, T.; Lagae, L. Gold nanoring as a sensitive plasmonic biosensor for on-chip DNA detection. *Appl. Phys. Lett.* **2012**, *100*, 173114.
150. Endo, T.; Kerman, K.; Nagatani, N.; Hiepa, H. M.; Kim, D.-K.; Yonezawa, Y.; Nakano, K.; Tamiya, E. Multiple label-free detection of antigen-antibody reaction using localized surface plasmon resonance-based core-shell structured nanoparticle layer nanochip. *Anal. Chem.* **2006**, *78*, 6465–6475.

151. Acimovic, S. S.; Ortega, M. A.; Sanz, V.; Berthelot, J.; Garcia-Cordero, J. L.; Renger, J.; Maerkl, S. J.; Kreuzer, M. P.; Quidant, R. LSPR Chip for Parallel, Rapid, and Sensitive Detection of Cancer Markers in Serum. *Nano Lett.* **2014**, *14*, 2636–2641.
152. Chen, P. Y.; Chung, M. T.; McHugh, W.; Nidetz, R.; Li, Y. W.; Fu, J. P.; Cornell, T. T.; Shanley, T. P.; Kurabayashi, K. Multiplex Serum Cytokine Immunoassay Using Nanoplasmonic Biosensor Microarrays. *ACS Nano* **2015**, *9*, 4173–4181.

Chapter 3

Crafting Multidimensional Nanocomposites: Functional Materials for Application in Energy Conversion, Energy Storage, and Optoelectronics

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Depending on the scale, not all nanocomposites are created equal. Whereas physical mixing is the gold standard for effectively realizing many useful bulk nanocomposites, it has been shown to be much less so when applied to the nanoscale where controlling the interaction between light, electrons and atoms are of prime concern. To that end, our group works to rationally design nanomaterials that incorporate essential connectivity and surface modification to facilitate the movement and interaction between these fundamental species. Spanning the range from fundamental research to device fabrication and performance measurement, this review details some of the work in crafting nanocomposites for applications in photovoltaics, energy storage, photocatalysis, ferroelectricity and optics through the use of novel templating strategies, polymers and chemistries.

Introduction

Nanocomposites, broadly described as materials possessing two or more different materials which together work to demonstrate useful properties of the constituents, are a popular and active area of research across all scientific and engineering disciplines. A common nanocomposite formulation involves hard

(inorganic/metallic) and a soft (polymeric /ligand) components brought together through many different physical and/or chemical approaches. Depending on the particular area of application, the soft component will typically confer solubility or film formability of the integrated hard components. The hard material often incorporates the primary functionality desired but which is difficult, or impossible, to realize in the bulk or process without some sort of compatibilizer (such as a soft component).

Unlike bulk composites, nanocomposites capitalize on the property changes, oftentimes significant, which occur when the dimensions of systems approach the wavelength of light or below (100-1000nm). This property, termed the quantum size effect, enables the formation of distinct energy bands (quantized energy) in contrast to the continuous energy structure of many bulk materials. What this means is that the way nanoscale materials behave (i.e. absorbance, emission, conductivity etc.) is highly dependent on the size, shape, inter-particle separation and surface chemistry within this nanometer range (1). Another important property of nanoscale materials (including nanocomposites) is the large increase in surface area-to-volume ratio which occurs at such small sizes. What this essentially means is that more and more of the atoms are being displayed on the surface with less and less fully shielded in the inside volume (Figure 1).

This property has a pronounced effect on transport properties (heat and mass) for example (2). As a result, nanocomposites have become an extremely popular area of research with the number of publications on nanocomposites quickly rising from less than 250 in 2000 to nearly 3000 in 2015 (Figure 2) most of which have occurred in the 12 years since the passing of the National Nanotechnology Initiative (NNT) in 2003 in which more than 20 United States departments or agencies are actively participating in funding nanotechnology-related research (3).

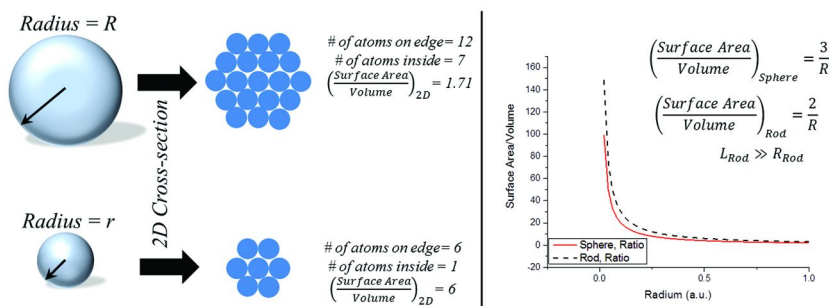


Figure 1. (Left) Simplified diagram representing two differently-sized particles and their respective cross sections. The atoms (blue circles) are divided into “edge” (representing surface area in 3D space) and “inside” (representing volume in 3D space). The ratio of these values is demonstrated to increase substantially as particle size reduces. (Right) Ratio equations and respective plots for a sphere and rod (length much larger than radius) showing the rapid growth of the surface area-to-volume ratio.

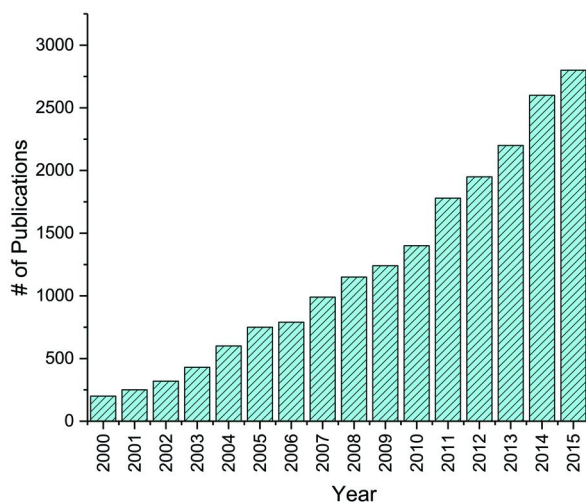


Figure 2. Plot showing the number of papers published containing the work 'nanocomposite' in the title. Data Source: ISI Web of Knowledge.

Areas of particular focus for nanocomposites include electronics, energy capture/storage, medicine and optics among others.

This chapter will review our group's work in nanocomposites within these categories in particular. The overall goals to be addressed cover the spectrum from fundamental research, including novel materials development, to processing improvement, device performance enhancement and cost reduction. Throughout many of the works to be addressed, one overarching concept is employed in many of the efforts: the concept of star-like block copolymer "nanoreactors." This essential aspect of the group's research is first reviewed in brief.

In photovoltaics, areas to be addressed include various types of solar cells such as perovskite, dye-sensitized solar cells (DSSCs) and quantum dot-sensitized solar cells (QD-SSCs) which incorporate various inorganic photo-responsive materials of varying size, shape and dimension as well as organic-inorganic nanocomposites possessing conducting polymers as the soft component. The emphasis is on improving the power conversion efficiency (PCE), ease of device formation, and developing an understanding of the structure–property relationships inherent in both the hard and soft components. In energy storage, areas to be addressed include the design and characterization of novel electrode materials for improved capacitance, cycle stability and longevity. For batteries, two major areas identified as limiting battery performance are poor surface-electrolyte interface (SEI) formation and pulverization of battery materials due to volume changes associated with charge cycling. In photocatalysis, areas to be addressed include the design of complex assemblies (wrapped, sandwiched) or morphologies of both conventional (e.g., metal oxides and graphene) and

novel (e.g., perovskites) electrocatalytic materials with the goal of improving the rate of pollutant degradation or product formation, catalytic stability and reduction of cost compared to traditional catalytic materials. In addition to the above major areas of interest in the group, several smaller research areas of growing importance include nanocomposites for ferroelectric, thermoelectric and optoelectronic applications. It is the intention to review the major findings within the group in a manner accessible to researchers as well as people outside of the scientific and engineering fields and address possible future research directions.

“Nanoreactors” for Well-Defined Organic Nanostructures

Among the many different techniques available for producing inorganic and metallic nanostructures, few are able to achieve well-defined shapes of deliberate size, minimal size variation and general applicability. Owing to the, oftentimes significant, size-dependent properties inherent in nanomaterials; this can present a problem when trying to produce nanostructured devices with consistent and well-defined properties. It is for these reasons, that the group developed a nanoparticle templating technique which employs star-like block copolymers to preferentially nucleate nanoparticles (Figure 3) (4).

This approach relies on star-like unimolecular block copolymer templates based on β -cyclodextrin. The motivation behind this method is to enable the synthesis of well-defined inorganic structures otherwise inaccessible via micellar-based templating techniques due to high temperatures, extreme pH or high ionicity requirements.

The choice of polymers used in templating is important as well. Polystyrene (PS) serves as both a spacer layer preventing particle nucleation and a coating layer facilitating well-defined particle growth (e.g., a cage). Poly(*tert*-butyl acrylate) (pBA) can be hydrolyzed to poly(acrylic acid) (PAA) which is known to preferentially coordinate inorganic and metallic precursors and enable preferential nucleation and growth within the template. The poly(4-vinylpyridine) (P4VP) works in a similar fashion for coordinating noble metal ions. This strategy has been shown to be capable of templating many different metals and metal oxides each with unique nanoscale properties which are capitalized on in the research subsequently detailed (Figure 4).

This approach can be readily adjusted to produce core@shell and hollow structures of the same composition by changing the order and type of polymers grown from the star-like initiator. For example, first growing gold in an inner P4VP core followed by Fe_3O_4 shell growth in the intermediate PAA block and lastly solubility and shape control in the outer PS block enables an organic solvent-soluble core@shell structure with dual plasmonic and superparamagnetic properties. It should follow then that the size of the core and shell components can be controlled by varying the degree of polymerization of the respective polymer blocks. In several of the subsequent research areas to be reviewed, this template strategy (or a variation thereof) is employed to create some of the essential nanocomposite materials investigated.

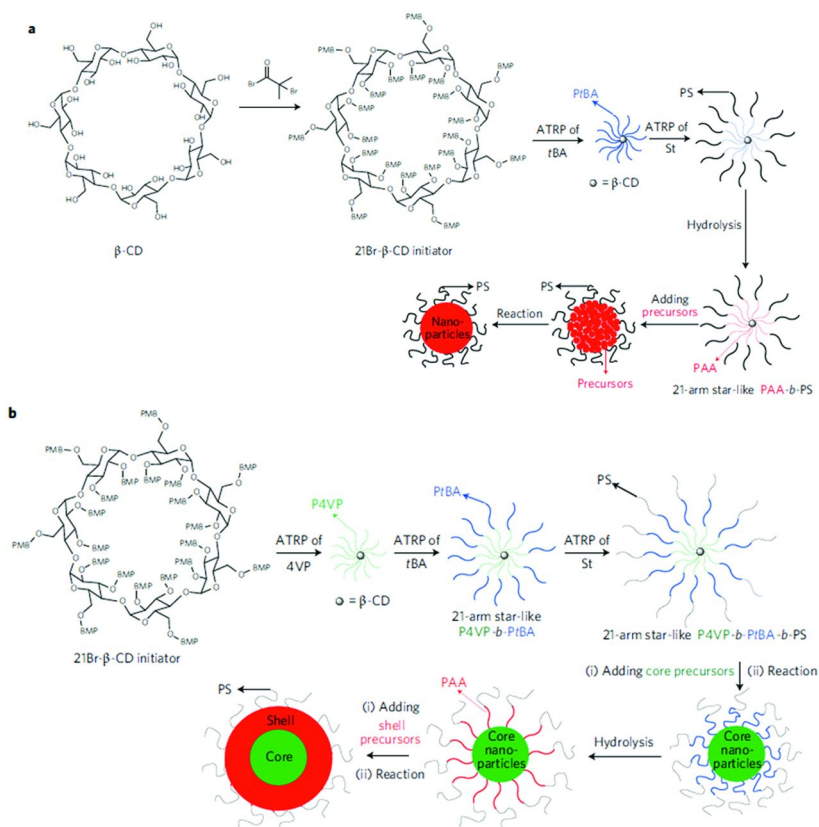


Figure 3. (a) Synthetic pathway for producing 21-arm star-like PAA-b-PS (diblock) starting from a low cost cyclic polysaccharide β -CD (4). Sequential ATRP of the respective arms followed by TFA hydrolysis of the inner core yields a well-defined polymeric template for single-core nanoparticles capped in PS. (b) Synthetic pathway in which a 21-arm starlike P4VP-b-PAA-b-PS (triblock), produce in similar fashion, is used to template core@shell nanoparticles capped with PS. Reproduced from (4). Copyright 2013 Nature Publishing Group.

Nanocomposites for Photovoltaics

When trying to understand the trends and emerging technologies in photovoltaics, it is easiest to divide the immense body of work by the different major solar cell device types. Consequently the work by our group in this area is divided into three groups: dye-sensitized solar cells (DSSCs), hybrid solar cells and perovskite solar cells. In each case, the materials employed as well as their respective roles are different. In all cases, however, the underlying operation of the various devices is similar and with similar goals.

Dye-Sensitized Solar Cells

In DSSCs, a photosensitized (dye adsorbed) semiconductor absorbs incident photons and injects electrons into the semiconducting network. These electrons are transported to the fluorine-doped tin oxide/indium-doped tin oxide (FTO/ITO) contact and the dye is refreshed by a redox couple. The spent redox couple is then refreshed at the counter electrode and the process repeats.

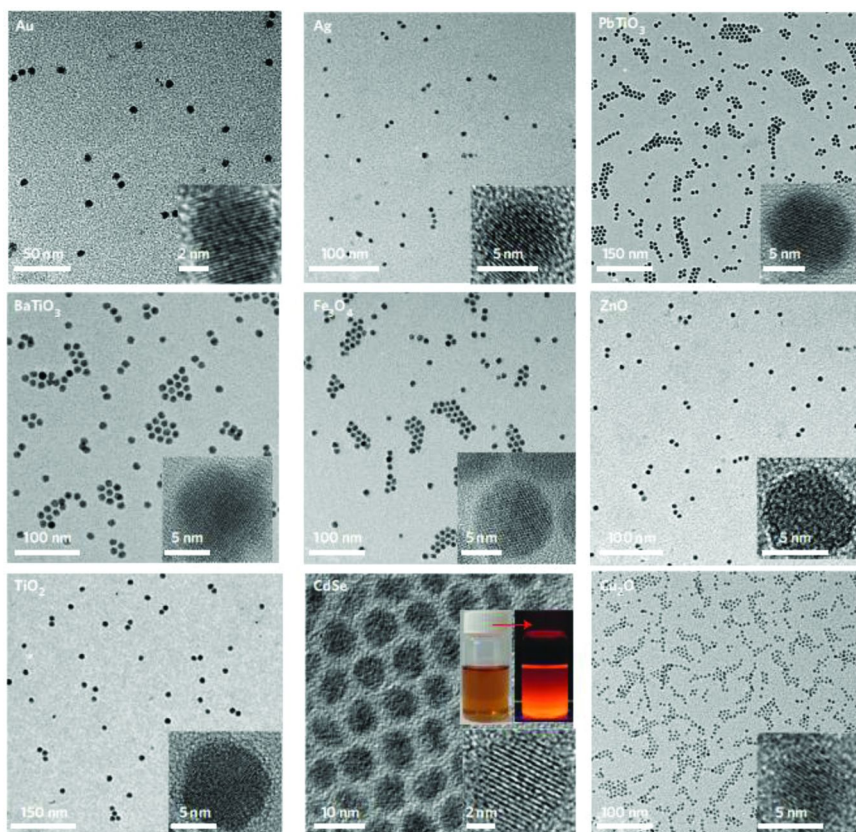


Figure 4. Summary of the various PS-capped solid-core particles that can be produced using the “nanoreactor” approach design by the group (4). Plasmonic noble metal particles (Au, and Ag), ferroelectric particles (PbTiO₃, and BaTiO₃), superparamagnetic particles (Fe₃O₄), semiconducting (ZnO, TiO₂ and Cu₂O) and photoluminescent particles (CdSe) are just some of the types of particles that can be produced using this method. Core@shell and hollow forms of these particles and their combinations are also possible. Reproduced from (4). Copyright 2013 Nature Publishing Group.

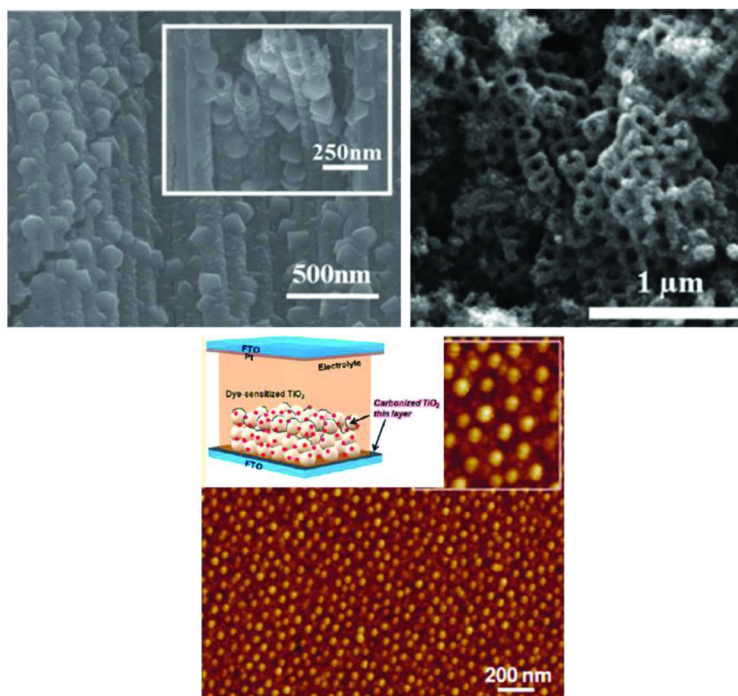


Figure 5. (Left) SEM of hierarchically-structured TiO_2 nanotubes with TiO_2 protuberances decorating the surface after subsequent hydrothermal treatment.

Reproduced from (5). Copyright 2013 Wiley-VCH. (Right) SEM of TiO_2 nanotubes after post-treatment with TiCl_4 yielding a pristine coating layer of TiO_2 . Reproduced from (6). Copyright 2012 Royal Society of Chemistry. (Bottom) AFM of TiO_2 particles in a graphitic (carbonaceous) matrix; (inset) shows the structure of titania particles and graphitic layer incorporation on the bottom and within the nanoparticle structure. Reproduced from (7). Copyright 2012 American Chemical Society.

Among the many components in this device, the semiconducting material is the most heavily researched as it controls the degree and dye adsorption and the effectiveness of the electron migration and redox couple diffusion. By far the most heavily investigated material for this purpose is TiO_2 owing to its chemical and photo-stability, low cost as well as appropriate energy level structure for transporting injected electrons. The work by the group, then, has focused heavily on controlling the phase, shape and morphology of this component. Several efforts focused on improving the power conversion efficiency through designing hierarchical TiO_2 nano-tubular structures (Figure 5, upper left) (5) and hybrid “neat” nanotube/nanoparticle (P-25) arrangements (Figure 5, upper right) (6)

or graphitic TiO₂ structures (Figure 5, bottom) (7) and nanoreactor-templated carbon-coated plasmonic Au@TiO₂ structures (8). In all cases the PCEs and dye loading were improved compared to particle-only and non-hierarchical structures via simple hydrothermal treatments and post treatments. This is attributed to the fractal-like (i.e. fractal fern) structure of these materials which increases the surface area for dye loading as well as producing a neat titania layer on the surfaces to reduce charge recombination. In the case of nanotube structures the one-dimensional pathways have been shown to play a large role in providing pathways for electron diffusion that minimize hopping. The presence of plasmonic gold cores and graphitic coatings have both also shown to improve the PCE noticeably.

Owing to the wealth of different, and truly beautiful, structures that TiO₂ can form in either pure or mixed phases (typically rutile and anatase); other complex structures have been investigated for use in DSSCs including peach-like microspheres composed of TiO₂ nano-needles (9), hierarchical flower-like structures (10), and hollow dual-shell TiO₂-upconverting nanostructures (11). In the first work, titania microspheres composed of needles were produced via a low temperature, acidic bath approach with a poly(ethylene glycol) steric dispersant (Figure 6, top left). This method is particularly ideal because of the mild conditions which, coupled with a post treatment by TiCl₄, enabled a PCE increase from 2.55% to 5.25%. In the second work, a highly-dense network of flower-like nanoclusters were reported and shown to be controllable by the addition of different valence cations (in addition to other hydrothermal variables). The high surface area and spiky tips of the structures facilitate high connectivity to neighboring clusters as well as facilitate high loading of dye molecules (Figure 6 top right). In the third study, a novel hybrid material was developed to capitalize on the large IR portion of the spectrum that cannot be absorbed by the standard N719 dye used in DSSCs. This is accomplished by synthesizing hollow upconversion nanospheres composed of either Na_xGdF_yO_z:Yb/Er (green emitting) or Gd₂O₃:Yb(20%)/Er(2%) (red emitting) subsequently coated with titania (Figure 6, bottom left). Upconverting materials essentially absorb photons in the near IR or IR and emit the energy back at a wavelength that the dye can then absorb and convert to electrons. The effect is that more energy is captured from solar radiation which leads to an improved PCE value (7.58%) compared to pristine devices (6.81%) (Figure 6, bottom right).

DSSCs are indeed a popular area of research, despite the relatively slow growth of their PCE, with ongoing aggressive work in this area. Efforts, however, should continue as there are still likely many unrealized morphologies and hierarchical structures of TiO₂ (and other related materials such as ZnO and SnO₂) to be investigated as well as a wealth of nanocomposites to be made from these besides the few addresses herein (flowers, hierarchical rods, microcapsules composed of needles, and rod sphere bilayers). The work by our group highlights a few of these additions (graphitic coatings, upconverting layers). Simulation work has also supported the use of quantum dots as sensitizers in an equivalent fashion to expensive ruthenium-based dyes typically used (12). This work suggests that the interfacial electronic states between CdSe or PbSe quantum dots and a titanium semiconductor are favorable toward electron injection.

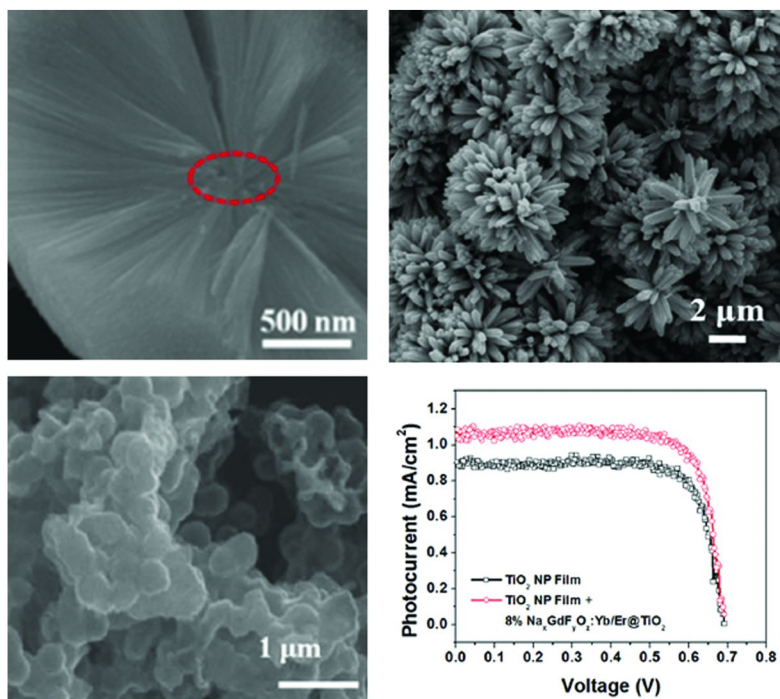


Figure 6. (top left) SEM showing a cracked titania microparticle and its hierarchical composition of needle-like titania structures. Reproduced from (9). Copyright 2014 American Chemical Society. (top right) SEM showing a collection of titania flower clusters grown on top of a compact titania film via a cation-mediated hydrothermal growth procedure. Reproduced from (10). Copyright 2013 Wiley-VCH. (Bottom left) hollow dual-shell $\text{TiO}_2@\text{Na}_x\text{GdF}_x\text{O}_x:\text{Yb/Er}$ semiconducting@upconverting nanoparticles and (bottom right) J-V curves showing the impact of the additional upconverting layer (top curve) leading to PCE improvement. Reproduced from (11). Copyright 2015 Royal Society of Chemistry.

Hybrid Solar Cells

Unlike DSSCs, which are essentially inorganic apart from the small organometallic dye, hybrid solar cells are composed of both an inorganic nanocrystal (such as CdSe, PbS or CuInSe_2) and a conducting polymer (such as P3HT or MEH-PPV). The two major challenges that need to be overcome to improve the performance and processibility of hybrid solar cells are the proper design of the electronic band structures, alignment of donors and acceptors as well as the improvement of the interfacial contact between conducting polymer and nanocrystal (13). This is because the generated excitons must diffuse to the interface of the two materials before they can be separated into holes and electrons (or otherwise be recombined and contribute nothing towards performance). It is

for these reasons that the PCEs of hybrid solar cells are generally lower than the highest-performing DSSCs and typically less than 10% (14). Figure 7 outlines the general working principal of hybrid solar cells and helps to underscore the importance of maximizing the interfacial contact between the organic and inorganic components to minimize the diffusion length required for excitons and thus improve the PCE of the overall device.

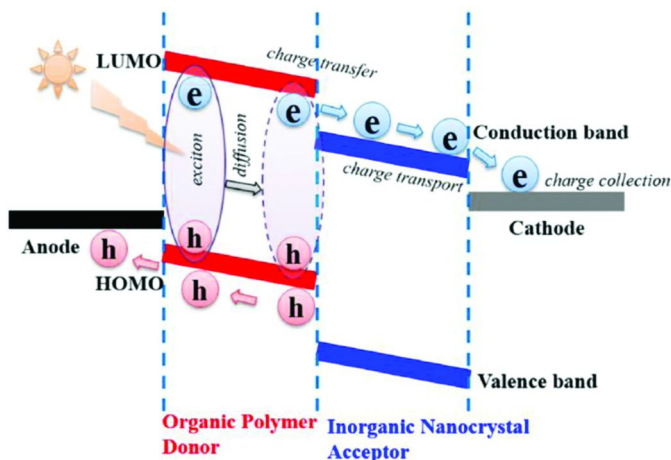


Figure 7. Summary of photovoltaic process in hybrid solar cell devices. The main point of difference, and consequently the main challenge to overcome, is the exciton diffusion to the interface. The highly bound excitons cannot separate until this point and this leads to lower PCEs. Reproduced from (13). Copyright 2013 American Chemical Society.

Of the two major PCE limitations inherent in hybrid solar cell devices, the group's focus has been primarily on improving the interfacial connectivity between conducting polymers and nanocrystals. The work in this area has focused on covalently-tethering conducting polymers (such as P3HT) to the surface of metal chalcogenide nanocrystals (CdSe) (15). The representative work for this involves using click-chemistry to covalently attach P3HT to the surface of CdSe tetrapod structures (16). This work capitalizes on the use of colloidal synthesis when producing nanocrystals. Specifically, the nonfunctional ligands typically used to produce such nanocrystals can be substituted for bifunctional ligands that can both coordinate and stabilize the nanocrystal. The bifunctional bromobenzyl phosphonic acid possesses a bromine group that can be converted to an azide group. In parallel, the P3HT can be modified to have an ethynyl bond on one end. Through their copper catalyzed azide-alkyne coupling (CuAAC) the two can be intimately and permanently connected (Figure 8).

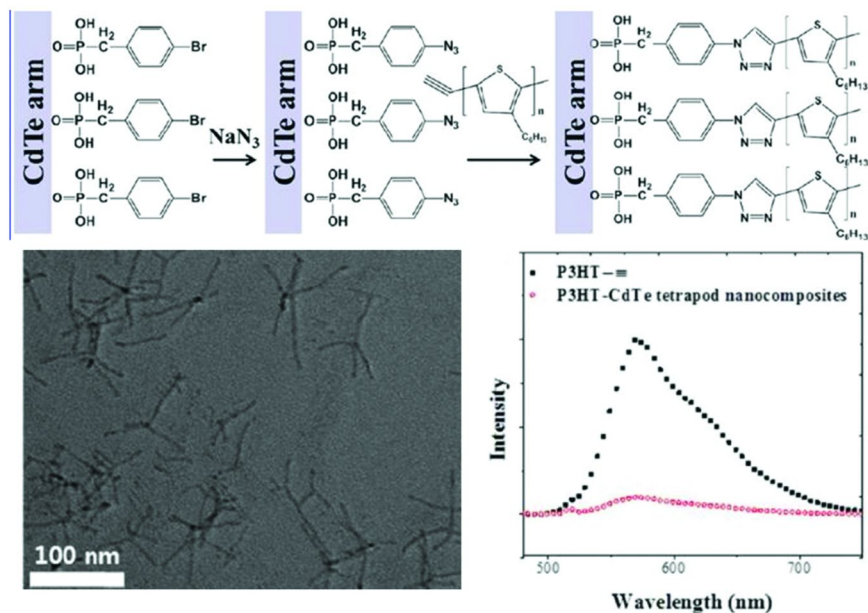


Figure 8. (Left) reaction schematic demonstrating the coordination of the bifunctional ligand to the surface, the conversion of the bromine to an azide group ($-N_3$) and subsequent click reaction with ethynyl-functionalized P3HT. (Bottom, right) TEM showing CdSe—P3HT tetrapod nanocomposite. (Bottom, left) UV-Vis of pure P3HT (top curve) and tetrapod CdSe-P3HT nanocomposite (bottom curve) demonstrating the quenched emission of P3HT in support of redirected exciton transfer to tetrapod. Reproduced from (16). Copyright 2013 American Chemical Society.

It was found that the covalent attachment nearly completely quenched the emission of the nanocomposite. This provides strong evidence for the efficient charge transfer at the organic-inorganic interface. This example can be readily generalized to other ethynyl-functionalized conducting polymers as well as other colloidal nanocrystals with different absorption ranges from infrared to visible. Furthermore, the shape effects (in this case a tetrapod) may also serve to improve the film formability as well as interconnectedness of the two components when deposited on the conducting substrate. Further work on the relationship between the nanocrystal type and shape and the conducting polymer orientation within the film is important as the latter has been demonstrated to play a major role in ultimate device performance (17).

Perovskite Solar Cells

Since 2009, perovskite solar cells have quickly emerged as a promising way to realized high efficiency solar cells. Our group has also recently taken on the challenge of studying these materials. Perovskite takes on an ABX_3 -type structure

(A=Pb²⁺, B=methyl ammonium, X=halide). Specifically methylammonium lead halide-based perovskites [CH₃NH₃Pb(I_{1-n}Br_n)₃] (0 ≤ n ≤ 1) are the focus of most research of late. Among the many advantages of perovskite materials, the essential aspects are the absorption/band gap tunability by varying the iodide/bromide ratio, direct band gap, high carrier mobility and high absorption coefficient (18). The group's work in this area combines the upconversion properties of NaYF₄:Yb/Er with the nanoreactor template approach to produce well-defined upconversion nanocrystals for use in the electrode for methylammonium lead iodide perovskite solar cells (19). This work is particularly interesting because not only did the device achieve a respectable PCE of 18.1%, it did so while incorporating near-infrared irradiation (Figure 9). It was observed that by adding near-IR irradiation on top of the standard AM1.5G solar spectrum an increase in the current density occurs. Cycling the additional irradiation on and off produced a reversible increase and decrease in the current density and PCE. Granted, the efficiency of perovskite devices can go even higher without the incorporation of near-IR, this study is an essential validation of broad spectrum perovskite solar cells.

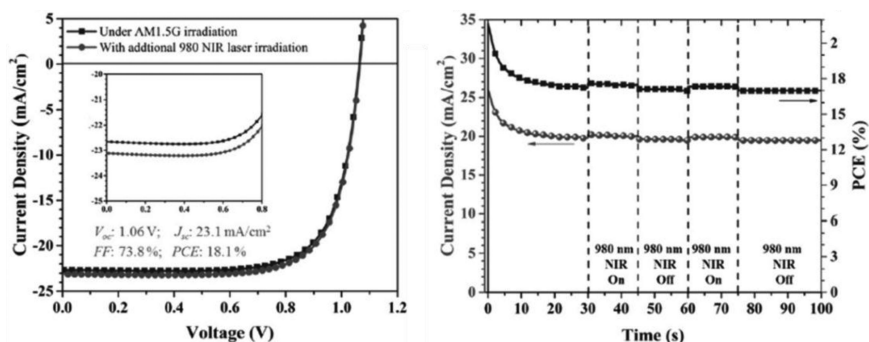


Figure 9. (left) J-V curve showing the increase in fill factor and PCE with the introduction of an additional near-IR irradiation source. (Right) Time-resolved current density study showing the increase in current density under extra near-IR irradiation as well as cycling up and down when turned from on to off. Reproduced from (19). Copyright 2016 Wiley-VCH.

The research of photovoltaics by our group covers many different types including dye-sensitized solar cells (DSSCs), hybrid solar cells, perovskite solar cells and other niche variants. In each class, different challenges remain to be addressed. In the case of DSSCs, investigations into new dye materials and other sensitizers is a way to improve the range of the spectrum that can be captured and converted to injected electrons as well as investigations into creating titania morphologies with even higher surface areas and loading potentials. These and other limitations may help push the PCE of DSSCs above 10% while also retaining the low cost and roll-to-roll processibility which make them attractive to industry. For hybrid solar cells, boosting the PCE requires improving the matching of the energy levels of the conducting polymer and inorganic nanocrystal. Furthermore, developing strategies for achieving improved interfacial contact (via direct

tethering of polymers to the nanocrystal surface) and minimizing the diffusion length of excitons and their resulting electrons will maximize the number of photo-generated excitons that lead to injected electrons. Finally, perovskite solar cell performance is likely to be improved by optimizing the thickness and grain size of the perovskite active layer in planar heterojunctions as well as by minimizing the number of pinhole defects. In addition, the elimination of heavy metals and improved environmental stability are additional practical considerations for real-world implementation.

Nanocomposites for Energy Storage

The importance of developing high energy density storage devices is more than apparent. A part of this challenge is designing novel, high performance components for use in lithium ion batteries. To date our group's efforts have focused on designing new anode materials to replace conventional graphite-based materials which cannot handle high charge/discharge rates at low temperatures and possess limited power capabilities. The projects that follow each address a different type of anode material with their own respective advantages and disadvantages. Different lithium cycling modes are also addressed by the different works. In alloying, the lithium forms an intermetallic compound with the metallic anode. In intercalation the lithium ions diffuse into the spaces between stacked materials. Lastly, in conversion the lithium ions react with transition metal oxides in a reversible fashion.

In the first study, a spinel-type $\text{Li}_4\text{Ti}_5\text{O}_{12}$ lithium titanate (LTO) is investigated due to its cycling stability, thermal stability and minimal volume change during lithiation and de-lithiation. This study employed a new titanium precursor (TiN) in conjunction with $\text{LiOH}\cdot\text{H}_2\text{O}$ and a polyvinyl pyrrolidone (PVP) stabilizer during hydrolysis (20). This enabled the formation of small (up to 150 nm) well defined LTO nanospheres when the pH is maintained throughout the process (Figure 10, bottom). These materials exhibited excellent cycle stability and high specific capacities even at high current densities (from 159.5 mAh g^{-1} at 1C to 108 mAh g^{-1} at 80°C) (Figure 10, top).

In a related study, hollow porous TiO_2 nanoparticles were used as the anode material (21). Similar to LTO, titania undergoes relatively small volume changes upon lithiation/delithiation cycling in addition to a large surface area for uptake and small diffusion length of the migrating lithium ions (intercalation/deintercalation cycle mode). It was found that the material's reversible capacitance performance is greatly improved when the material is completely dried (remove all water and surface hydroxyl groups).

Whereas the first group involved materials that underwent minimal volume change, in many other battery materials there is a substantial volumetric change of the anode material during lithiation and de-lithiation. This volume change leads to pulverization of the anode materials which reduces the amount of available lithium ions and thus reduces the capacitance (Figure 11). Another study attempted to address this issue by forming well-defined Sn nanoparticles possessing a high quality solid electrolyte interface (SEI) which preserves the structural integrity

of the particles during multiple cycles (22). Since tin is abundant, inexpensive and possesses a high theoretical capacity ($7260 \text{ mA h cm}^{-3}$), efforts to minimize pulverization and extend performance to high cycle numbers are justifiable. Figure 11 provides a schematic showing the effect of having a high quality SEI (with FEC) or a low quality SEI (without FEC) for both nano- and microparticles.

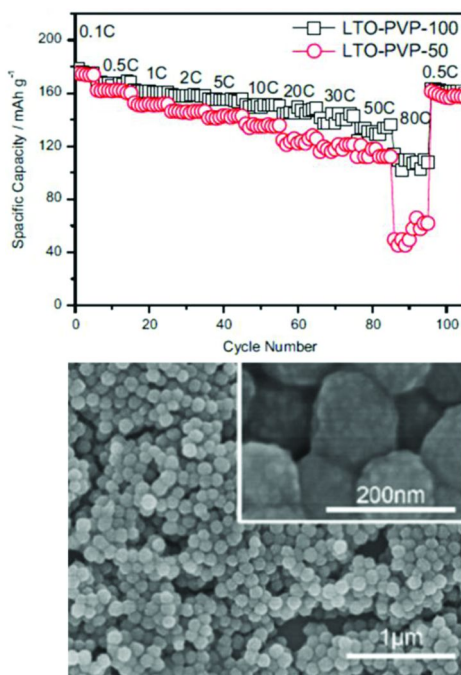


Figure 10. (Top) Cycling performance of two devices with differently-sized LTO nanoparticles over different charging rates. The devices showed excellent stability across all charge/discharge rates. (Bottom) SEM showing well-defined LTO nanoparticles. Reproduced from (20). Copyright 2016 Elsevier.

Whereas the previous two investigations employed different lithium cycling mechanisms, both involved materials with minimal volume change. The next study involves the newer anode material zinc ferrite, ZnFe_2O_4 , which possesses a higher theoretical capacity (1000 mAh g^{-1}) but a larger volume change during the lithium alloying and conversion cycling modes. This study produced zinc ferrite nanoparticles via precursor reaction in a core@shell PS@PAA polymer template (23). Subsequently, the precursor-loaded PS@PAA nanospheres are annealed to produce an interconnected carbonaceous network between the zinc ferrite nanoparticles. This carbonization procedure has been shown to reduce the degree of pulverization of the anode material and leads to increased cycle lifetime and high capacity retention. This study is advantageous because it can be generalized to other ternary metal oxides.

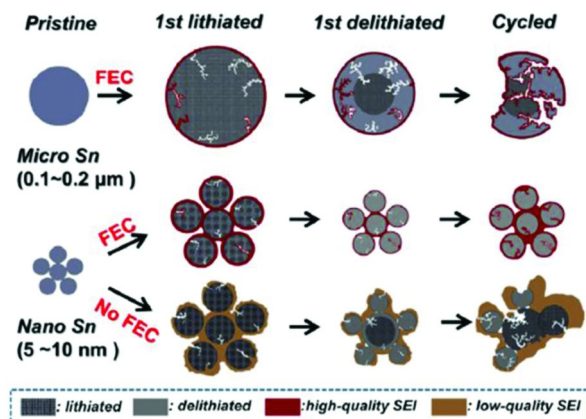


Figure 11. Schematic showing how a thin, high quality SEI layer can preserve the particles through multiple cycles and minimize the degree of pulverization of the anode material. High quality SEI layers on micro Sn particles are not able to prevent pulverization due to the larger volume that needs to be contained. Reproduced from (22). Copyright 2015 Elsevier.

The final anode material investigated is a nanocomposite composed of CuGeO_3 nanowires sandwiched between graphene layers. This study involves a combined conversion/alloying lithium cycling mode wherein the lithium ions transport along the nanowires during cycling (24). Furthermore, the layered structure keeps the different components separated during many cycles (i.e. keep graphene layers from stacking together and nanowires from aggregating) and facilitates the migration of the lithium ions into and out of the layered structure (Figure 12) (25). This contributes to minimized volume changes in the overall structure and excellent performance over many cycles (853 mAh g^{-1} after 50 cycles).

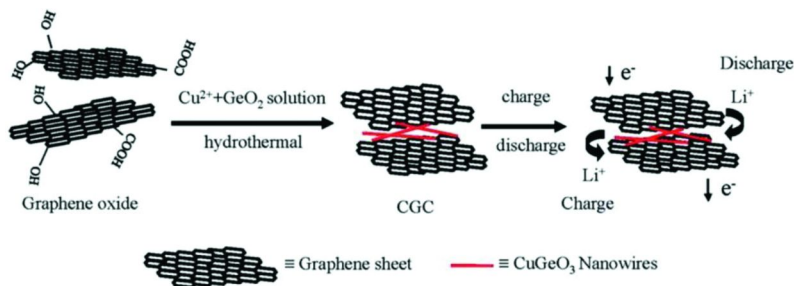


Figure 12. Schematic showing the formation of the CuGeO_3 nanowire and graphene sandwich structure formed in a one-pot hydrothermal process. The graphene serves to shuttle the lithium ions into and out of the structure under charging and discharging. Reproduced from (25). Copyright 2015 Wiley-VCH.

The group's efforts to date largely focus on designing novel anode materials to replace current graphite-based materials. The different materials exhibit different lithium cycling mechanisms, different theoretical capacities and different degrees of volumetric expansion under cycling. Investigation into reducing the damaging effects of anode pulverization involved the introduction of carbonaceous coatings throughout the anode matrix and this has proven successful at extending the cycle performance. Further investigation into improving the SEI quality in other systems is a promising future development in particular for metal-based anode materials.

Nanocomposites for Photocatalysis

Similar to photovoltaics, photocatalysts take advantage of the inexhaustible solar radiation hitting the planet. The difference in this case is that the formed electrons are used to enable other reactions to occur. The two most important reactions are organic pollutant degradation and electrolytic water splitting. The major group of photocatalytic materials investigated in the group involve deposition or modification of a titania layer by various functional materials. Titania is popular for photocatalysis for the same reasons it is heavily employed in photovoltaics; possessing chemical and photo-stability, corrosion resistance, low toxicity and high oxidizing potential. However, titania is not sufficient for photocatalysis on its own owing to the ease with which charge recombination can occur and its large band gap ($E_g = 3.2$ eV) (26). Thus efforts in the group have focused on combining titania with various functional materials to improve on the properties.

In the first group, titania nanotubes were functionalized with a metal oxide (Cu_2O) semiconductor. Since Cu_2O has a narrower bandgap ($E_g = 2.17$ eV) compared to titania and its electronic band structure is aligned above that of titania, photogenerated electrons from Cu_2O can be injected into titania (26). This work is ideal for its simplicity. The titania nanotube arrays are produced by established electrochemical anodization procedures and subsequently immersed in a bath of copper precursors and in-situ oxidized under sonication. The Cu_2O forms on the inside and outside of the nanotube arrays. The resulting nanocomposite structures proved successful at degrading organic pollutant analogues by capitalizing on both UV and extended visible wavelength photoexcitation (Figure 13, left panel). In addition to photocatalytic degradation, certain types of decorated titania nanotube arrays have proven to be excellent for water splitting (into molecular hydrogen and oxygen). Our group investigated palladium-decorated titania nanotube arrays for the purpose of water splitting. This small work demonstrated a simple modified hydrothermal approach to coating the nanotubes with small (3 nm) well-defined palladium nanoparticles (27).

Two related studies also employ TiO_2 in both nanotube arrays (28) and nanoflowers (29), which are partially etched and the surface is replaced with strontium ions to produce STO perovskite-decorated (SrTiO_3) TiO_2 arrays of two different kinds. The rationale for using perovskite additives is because it provides good thermal and photo-stability as well as enhanced exciton separation (and

therefore minimal charge recombination). The STO-decorated titania nanotube arrays afforded higher photocurrent (6 mA cm^{-2} compared to 1.8 mA cm^{-2} for Cu_2O loaded titania) and more than 3 times the degradation rate compared to titania nanotubes (Figure 13, center panel). In the case of STO-decorated titania nanoflowers, the photocatalytic degradation rate was more than 5 times that of neat titania nanoflowers (Figure 13, right panel).

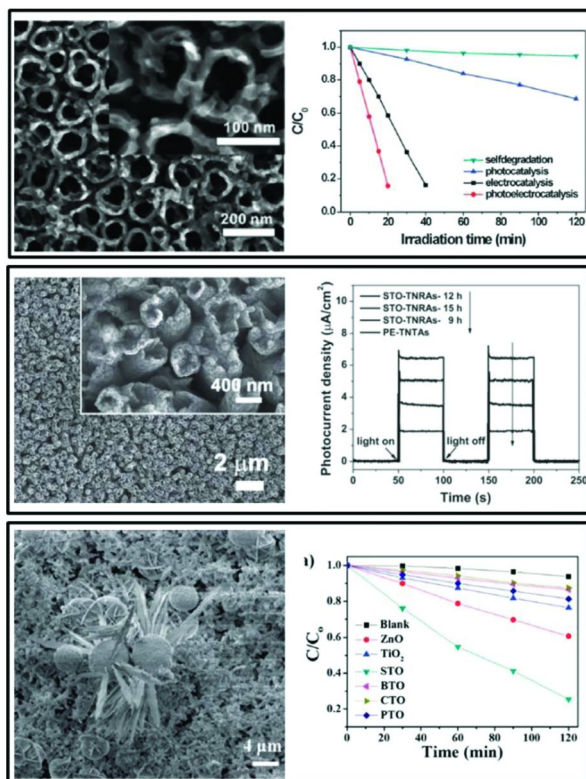


Figure 13. (Top panel) TEM of Cu_2O -decorated TiO_2 nanotube arrays and the resulting degradation behavior under different modes. The photoelectrocatalytic mode is the most effective and achieves an 80% pollutant degradation in 20 minutes. Reproduced from (26). Copyright 2013 Royal Society of Chemistry. (Middle panel) TEM of STO-decorated TiO_2 nanotube arrays and the large photocurrent density achievable for the resulting nanocomposites. Reproduced from (28). Copyright 2014 Wiley-VCH. (Bottom panel) TEM of STO-decorated TiO_2 nanoflowers. The plot compares the relative photocatalytic degradation rates to other metal oxides and perovskites and shows that the STO-decorated flowers achieve the fastest degradation rate. Reproduced from (29). Copyright 2014 Royal Society of Chemistry.

Besides strategies for decorating titania nanotube arrays, efforts have also been directed towards producing hollow TiO₂ mesospheres (30). The advantages of hollow TiO₂ mesospheres include improved permeability, larger surface area and larger light-harvest capacity. Furthermore, the use of a low cost single component makes these structures ideal from an industrial scalability standpoint. It was found that the hollow titania particles achieved photocatalytic degradation performance values larger than many of the STO-decorated materials and Cu₂O-decorated materials already addressed for certain organic pollutants. A one-pot, single-component system with a more complex morphology can thus perform comparably to multicomponent materials.

Besides titania-based materials, graphene-based photocatalyst have also been a focus in the group owing to their low cost and high carrier mobility. Thus Cu₂O has also been incorporated into wrapped reduced graphene oxide nanocomposites via ultra-sonication (31). Compared to other materials previously reviewed, these wrapped architectures still require some improvements however; the degradation of pollutants is nonetheless respectable with a photocatalytic degradation of organic pollutants that is 20 times the rate of P25 titania. These studies on photocatalysts underscore the importance of hierarchically-structured and multidimensional materials in aspects of photocatalysis and photodegradation.

Nanocomposites for Other Applications

This last section focuses on relatively new nanocomposite projects within the group. Areas including ferroelectric, thermoelectric and optical nanocomposite materials are herein addressed. Ferroelectric materials are an interesting class of inorganic compounds that possess a nonzero polarization under no applied field as well as tunable, large magnitude permittivity. Consequently, this makes ferroelectric materials useful in capacitors. Furthermore, the hysteresis of the material response enables the capability for information storage. These properties are extremely important for the development of high performance nanostructured materials for emerging portable electronic devices and computing.

The first work involves the use of the nanoreactor template method to synthesize polystyrene-capped BaTiO₃ (barium titanate, BTO) (32). The use of the β -CD templating method afforded PS-capped nanoparticles. This fact is extremely important as it allows the particles to be selectively incorporated into the PS domain of PS-*b*-PMMA copolymers and thereby be self-assembled into the resulting domain structures of polymeric films. The dielectric properties are greatly enhanced by the presence of the well-dispersed BTO particles enabling an increase in ϵ' from 2 to 20 (Figure 14, left panel). A related study employed a modified template method based on PVDF-*b*-PAA to produce a wholly-ferroelectric organic-inorganic PVDF-BTO nanocomposite (33). In many ways, this work is an extension of the first in that the piezoresponse of an individual BTO nanocomposite was characterized and used to support ferroelectricity even on an individual BTO nanoparticle (Figure 14, right panel). This study offers an interesting first step to understanding the structure-property

relationship of size-dependent ferroelectric nanostructures of BTO as well as other materials of interest for batteries and capacitors.

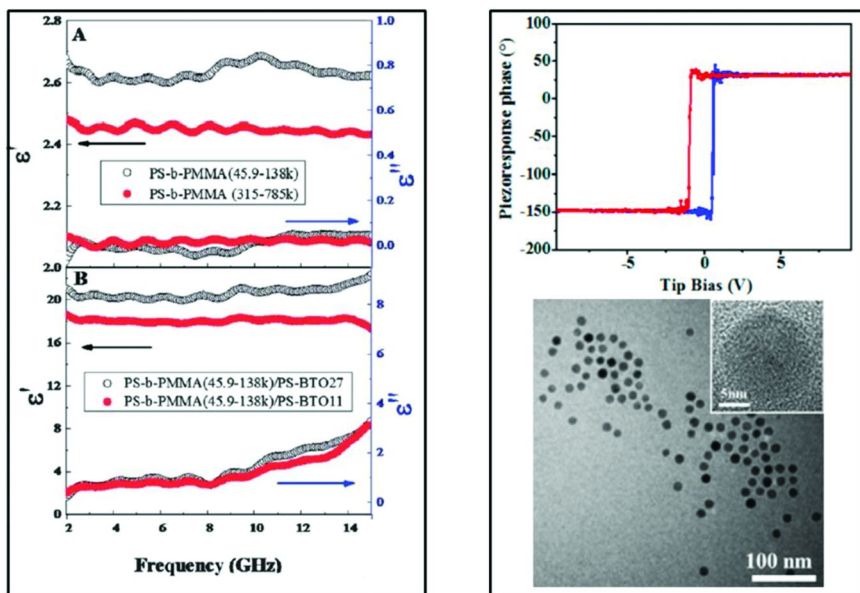


Figure 14. (Left panel) plots of the dielectric constant for the PS-PMMA copolymer (open circles) and the PS-PMMA film containing BTO-PS nanocomposites sequestered in the PS domains (closed circles). There is a significant increase upon addition of BTO. Reproduced from (32). Copyright 2013 Royal Society of Chemistry. (Right panel) Piezoforce microscopy (PFM) of an individual BTO-PVDF particle showing the 180° switch in the polarization associated with ferroelectric materials. TEM shows the well-defined BTO-PVDF nanocomposites demonstrating highly regular size and shape. Reproduced from (33). Copyright 2015 American Chemical Society.

Among the many attractive properties of some materials, thermoelectricity is one of the most interesting and potentially useful. This property describes some material's ability to convert errant heat directly into electricity. Considering that, in many systems, there is leftover heat, and in many systems a majority of energy is actually lost as heat; the use of such materials is immediately evident. Two projects in the area of thermoelectric materials have been undertaken by the group. The first involves the template-free synthesis of thermoelectric bismuth telluride-poly(3-hexylthiophene) films (Bi_2Te_3 -P3HT) (34). The goal of this work is to increase the Seebeck coefficient and the power factor simultaneously by capitalizing on a theorized interfacial filtering effect whereby only low energy carriers are scattered. This study showed a seven-fold increase in the Seebeck coefficient and a power factor increase three times that of neat P3HT (Figure

15, top). This likely attributed to an energy filtering effect resulting from the intimate and strong contact between the nanorods and conducting polymer. This study underscored the importance of intimate contact at the interface in such nanocomposite materials and informed heavily on the next study which attempted to address this issue more directly. The next study employed the nanoreactor template method previously described by employing a PAA-b-PEDOT star-like block copolymer to template well-define PbTe nanoparticles in the inner PAA domain (35). In this instance the PEDOT (another conducting polymer) is covalently connected to the inner PAA and thus the connection between the formed PbTe and PEDOT is also very intimate. Furthermore the size of the inorganic core is highly regular and possesses long-term stability (Figure 15, bottom). Subsequent combination with poly(styrene sulfonate) (PSS) would enable this thermoelectric nanocomposite to be processed via aqueous spin coating and film forming techniques which makes it particularly attractive for low cost industrial applications.

It is clear that the presence of materials such as lead and tellurium are not ideal for applications. Thus developing greener inorganic functional materials is essential where the strategies developed here can be directly applied. Thermoelectricity is a natural property to include in solar cell devices not only to improve their efficiency outright (IR-induced heating) but also capture additional energy that would otherwise not be used in any capacity. A final brief work to follow addresses the importance of interfacial and surface properties as it relates to optical materials.

The last study by the group looked to investigate the optical properties of a complex dual plasmonic-superparamagnetic nanocomposite synthesized by the nanoreactor approach. More specifically, it was concerned with the potential coupling effect of the plasmonic and superparamagnetic properties of a core@shell Au@Fe₃O₄-PS nanocomposite under systematic variation of the core and shell (36). Though only an introductory foray into coupled nano-scale material properties, it is unique due to the fact that not all nanomaterials can be placed in intimate contact with one another due to things such as lattice mismatch or incompatible wet chemical synthesis. It is for this reason that the nanoreactor templating approach is ideal as it enables such functionality (independent nucleated growth via template coordination). The results of this work demonstrated that there is indeed a coupled effect of the two (Figure 16).

In the case of a fixed Fe₃O₄ core and varied Au shell thickness, it was found that the plasmonic peak blue-shifted from 537 nm to 528 nm with an increase in the shell thickness from 5 nm to 18 nm. In the opposite case (fixed Au shell thickness and varied Fe₃O₄ core) an opposite trend was overserved whereby the plasmonic peak red-shifted with an increase in the Fe₃O₄ diameter from approximately 540 nm to 558 nm with a core diameter increase from 6 nm to 20 nm. The use of core@shell materials to confer two useful concomitant properties has been discussed. However, the additional layer of functionality imposed by introducing a coupling effect between the two is an exciting premise for novel, tunable functional materials for optical applications. The nanoreactor template method would allow a wealth of crystallographically-incompatible materials to be combined in new, potentially useful arrangements.

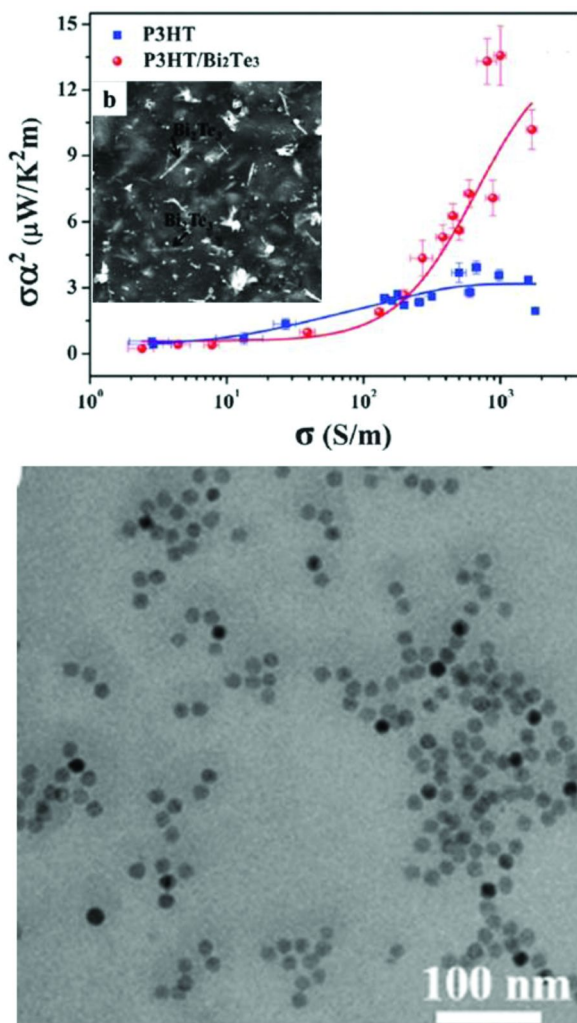


Figure 15. (Top) Plot of the power factor (y-axis) versus the electrical conductivity (x-axis) for pure P3HT (blue) and the Bi₂Te₃-P3HT nanocomposite (red). Inset shows SEM of the thermoelectric nanorods integrated into a film of P3HT. Note the more pronounced growth of the power factor for the nanocomposite compared to the polymer-only system which is attributable to the increased Seebeck coefficient for the former. Reproduced from (34). Copyright 2012 Royal Society of Chemistry. (Bottom) TEM of nanoreactor-templated PbTe nanoparticles capped with PEDOT conducting polymer. The particles demonstrated well-defined sizes and shapes. Preproduced from (35). Copyright 2015 Wiley-VCH.

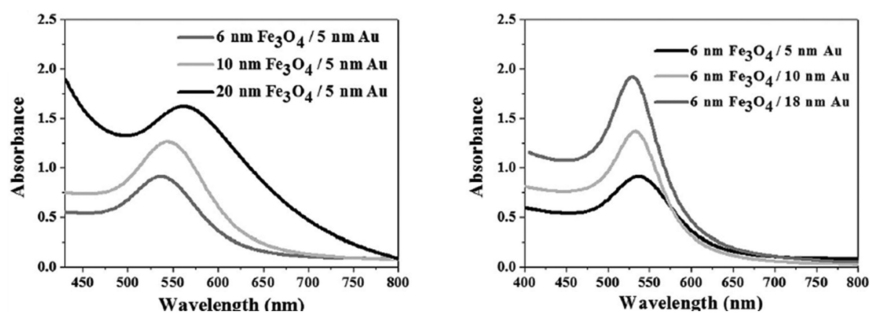


Figure 16. (Left) UV-Vis plot demonstrating the red-shift in the plasmonic peak as a function of increasing Fe₃O₄ diameter. (Right) UV-Vis plot demonstrating the blue-shift in the plasmonic peak as a function of increasing shell thickness. reproduced from (36). Copyright 2015 Wiley-VCH.

Conclusions

In this brief introductory review of our group's recent efforts, the focus has centered on the rational design of nanocomposites. Key factors essential on the nanoscale, including size, shape, interface and surface area, play important roles in controlling, understanding and ultimately informing on the relationship between the structure and property of nanoscale devices. In many of the areas reviewed, the use of a novel nanoreactor method has enabled the precise tunability of a few of these critical properties. As one can see, depending on the particular end use, the relative influence and therefore importance of these properties can vary. When addressing photovoltaic materials, the importance of high dye loading for DSSCs as well as minimum exciton diffusion lengths of all PV devices emphasized the use of hierarchically-structured materials for the electron acceptor material. Efforts in hybrid solar cell design focused on improving the degree of intimate contact at the interface between the quantum dot/tetrapod and the conducting polymer. In the case of energy storage materials, improving the cycle stability and performance was an essential property. However, large volume changes leading to pulverization of the anode material and the formation of low quality SEI layers often hinder battery performance. Successful attempts at improving or reducing the impact of these factors focused on engineering the interface of the anode materials with respect to the surrounding electrolyte solution and neighboring anode nanostructures. This was accomplished by introducing carbonaceous layers and improving the SEI quality to minimize the impact of particle volume change. In the case of photocatalysts, hierarchical TiO₂ nanostructures were modified to either improve electron injection onto titania to form surface species for degradation or improve the exciton diffusion and separation upon formation. These were accomplished by investigating the effects of surface modification of various titania nanostructures including tubes, flowers and hollow spheres through either decoration (Cu₂O) or surface replacements (STO and other perovskites). For ferroelectric materials, again the importance of

intimate interfacial contact between the inorganic component and the conducting polymer proved to be essential to realizing property improvements (in Seebeck coefficient and power factor). Similarly in our fundamental optical properties work, achieving intimate contact between two crystallographically-incompatible materials enabled interesting coupling between plasmonic and superparamagnetic materials. It is clear that the rational design of nanocomposites, fraught with all manner of nanoscale interfacial modification, placement and tuning, is far more complicated than that for bulk nanocomposites: the sum is truly greater than the parts when it comes to properties on the nanoscale. Further improving our understanding of such materials will depend heavily on our understanding of what goes on in the in-between.

References

1. Daniel, M.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293–346.
2. Paul, D. R.; Robeson, L. M. *Polymer* **2008**, *49*, 3187–3204.
3. Hayes, A. W.; Sahu, S. C.; Hulla, J. E. *Hum. Exp. Toxicol.* **2015**, *34*, 1318–1321.
4. Pang, X.; Zhao, L.; Han, W.; Xin, X.; Lin, Z. *Nat. Nanotechnol.* **2013**, *8*, 426–431.
5. Ye, M.; Zheng, D.; Lv, M.; Chen, C.; Lin, C.; Lin, Z. *Adv. Mater.* **2013**, *25*, 1–6.
6. Xin, X.; Wang, J.; Han, W.; Ye, M.; Lin, Z. *Nanoscale* **2012**, *4*, 964–969.
7. Jang, Y.; Xin, X.; Byun, M.; Jang, Y.; Lin, Z.; Kim, D. *Nano Lett.* **2012**, *12*, 479–485.
8. Zheng, D.; Pang, X.; Wang, M.; He, Y.; Lin, C.; Lin, Z. *Chem. Mater.* **2015**, *27*, 5271–5278.
9. Ye, M.; Zheng, D.; Wang, M.; Chen, C.; Liao, W.; Lin, C.; Lin, Z. *ACS Appl. Mater. Interfaces* **2014**, *6*, 2893–2901.
10. Ye, M.; Liu, H.; Lin, C.; Lin, Z. *Small* **2013**, *9*, 312–321.
11. Liao, W.; Zheng, D.; Tian, J.; Lin, Z. *J. Mater. Chem. A* **2015**, *3*, 23360–23367.
12. Xin, X.; Jung, J.; Yoon, Y.; Biswas, R.; Lin, Z. *Part. Part. Syst. Charact.* **2015**, *32*, 80–90.
13. He, M.; Qiu, F.; Lin, Z. *J. Phys. Chem. Lett.* **2013**, *4*, 1788–1796.
14. Jung, J.; Yoon, Y.; He, M.; Lin, Z. *J. Polym. Sci., Part B: Polym. Phys.* **2014**, *52*, 1641–1660.
15. Zhao, L.; Lin, Z. *Adv. Mater.* **2012**, *24*, 4353–4368.
16. Jung, J.; Pang, X.; Feng, C.; Lin, Z. *Langmuir* **2013**, *29*, 8086–8092.
17. He, M.; Wang, M.; Lin, C.; Lin, Z. *Nanoscale* **2014**, *6*, 3984–3994.
18. He, M.; Zheng, D.; Wang, M.; Lin, C.; Lin, Z. *J. Mater. Chem. A* **2014**, *2*, 5994–6003.
19. He, M.; Pang, X.; Liu, X.; Jiang, B.; He, Y.; Snaith, H.; Lin, Z. *Angew. Chem., Int. Ed.* **2016**, *128*, 4352–4356.
20. Wang, C.; Wang, S.; Tang, L.; He, Y.; Gan, L.; Li, J.; Du, H.; Li, B.; Lin, Z.; Kang, F. *Nano Energy* **2016**, *21*, 133–144.

21. Han, C.; Yang, D.; Yang, Y.; Jiang, B.; He, Y.; Wang, M.; Song, A.; He, Y.; Li, B.; Lin, Z. *J. Mater. Chem. A* **2015**, *3*, 13340–13349.
22. Eom, K.; Jung, J.; Lee, J.; Lair, V.; Joshi, T.; Lee, S.; Lin, Z.; Fuller, T. *Nano Energy* **2015**, *12*, 314–321.
23. Jiang, B.; Han, C.; Li, B.; He, Y.; Lin, Z. *ACS Nano* **2015**, *10*, 2728–2735.
24. Wu, S.; Wang, R.; Wang, Z.; Lin, Z. *Nanoscale* **2014**, *6*, 8350–8358.
25. Wu, S.; Xu, R.; Lu, M.; Iocozzia, J.; Han, C.; Jiang, B.; Lin, Z. *Adv. Energy Mater.* **2015**, *5*, 1–40.
26. Wang, M.; Sun, L.; Lin, Z.; Cai, J.; Xie, K.; Lin, C. *Energy Environ. Sci.* **2013**, *6*, 1211–1220.
27. Ye, M.; Gong, J.; Lai, Y.; Lin, C.; Lin, Z. *J. Am. Chem. Soc.* **2012**, *134*, 15720–15723.
28. Wang, M.; Zheng, D.; Ye, M.; Zhang, C.; Xu, B.; Lin, C.; Sun, L.; Lin, Z. *Small* **2015**, *11*, 1436–1442.
29. Ye, M.; Wang, M.; Zheng, D.; Zhang, N.; Lin, C.; Lin, Z. *Nanoscale* **2014**, *6*, 3576–3584.
30. Yang, D.; Wang, M.; Zou, B.; Zhang, G.; Lin, Z. *Nanoscale* **2015**, *7*, 12990–12997.
31. Liu, X.; Li, Z.; Zhao, W.; Zhao, C.; Wang, Y.; Lin, Z. *J. Mater. Chem. A* **2015**, *3*, 19148–19154.
32. Pang, X.; He, Y.; Jiang, B.; Iocozzia, J.; Zhao, L.; Guo, H.; Liu, J.; Akinc, M.; Bowler, N.; Tan, X.; Lin, Z. *Nanoscale* **2013**, *5*, 8695–8702.
33. Jiang, B.; Pang, X.; Li, B.; Lin, Z. *J. Am. Chem. Soc.* **2015**, *137*, 11760–11767.
34. He, M.; Ge, J.; Lin, Z.; Feng, X.; Wang, X.; Lu, H.; Yang, Y.; Qiu, F. *Energy Environ. Sci.* **2012**, *5*, 8351–8358.
35. Xu, H.; Pang, X.; He, Y.; He, M.; Jung, J.; Xia, H.; Lin, Z. *Angew. Chem., Int. Ed.* **2015**, *54*, 4636–4640.
36. Yang, D.; Pang, X.; He, Y.; Wang, Y.; Chen, G.; Wang, W.; Lin, Z. *Angew. Chem., Int. Ed.* **2015**, *127*, 12259–12264.

Chapter 4

Nanoparticle-Decorated Polymer Single Crystals for Nanoscale Materials

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Polymer single crystals have been extensively studied since 1957. They are typically used as model systems to determine crystalline structures, or as markers to investigate the crystallization process. In this chapter, we review the recent progresses on using nanoparticle-decorated polymer single crystals as nanoscale materials. We show that the unique quasi-two dimensional features of the polymer single crystals, combining with the long chain nature of polymers, can lead to an abundance of functional materials for numerous applications.

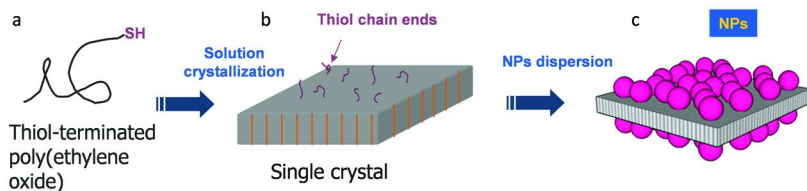
Introduction

Polymer crystallization was first reported in 1938, when Storks described gutta-percha (*trans*-polyisoprene) single crystals obtained by casting thin films from dilute chloroform solution, and suggested a possible chain folding mechanism in polymer crystallization (1). His work was however overlooked till 1957, when Till, Keller, and Fisher independently reported the growth of single crystals of linear polyethylene (2–5). Since then, a library of polymer single crystals (PSCs) has been prepared and reported. It is generally agreed that PSCs can be considered as markers of the corresponding crystallization process, allowing one to unambiguously determine crystal structures and chain conformation using techniques such as electron diffraction (6).

A typical polymer lamella is approximately 10 nm thick and a few nanometers to a few micrometers wide, and they can be considered as quasi-two dimensional nano plates. On the other hand, nanoparticles (NPs) by definition have at least one dimension less than 100 nm; the similar size of these two seemingly different objects also ensures an interesting marriage between the fields of PSCs and NPs. The ability to form regular chain packing suggests that crystalline polymers may be able to offer much more than simply passivating NPs for dispersion purposes. In this chapter, we will discuss how quasi-two dimensional polymer lamellae can be used as functional nanomaterials.

Nanoparticle-Decorated Polymer Single Crystals

Metal NPs formed *in situ* on the surface of inorganic and polymer crystals by vapor deposition have been used to reveal crystal defect structures since 1958 (7, 8). Recently, we have demonstrated that pre-formed NPs can also be immobilized onto the surface of PSCs *via* coupling with the polymer chain ends (9, 10). Gold nanoparticles (AuNPs) and thiol-terminated poly (ethylene oxide) (HS-PEO) were used as a model system. As the chain ends are different from the rest of the polymer backbone, given the right crystallization conditions, they are excluded onto the surface of the lamellar crystals. Judiciously selected nanoparticles can then be bound onto the single crystal surface *via* chemisorption (Scheme 1). If we consider a polymer lamella as a thin sheet of paper, lamella with functional groups on the surface can then be regarded as a nanoscale sticky tape (double- or single-sided, depending on the polymer chemistry) that can immobilize different nanoparticles, which directly leads to intriguing NP/PSC/NP “nanosandwiches”.



Scheme 1. Schematic Representation of Polymer Single Crystal-Templated Nanoparticle Assembly

Figure 1a shows the TEM images of AuNPs on the HS-PEO (48.5k) single crystal (inset shows the entire single crystal). AuNPs can be clearly seen on the surface as the dark dots. The density of AuNP population is relatively low and the NPs are randomly located. Much denser arrangement of AuNPs can be seen in the case of the 2k HS-PEO, as shown in Figure 1b, where most of the single crystal

surface was covered with AuNPs. The inset shows a 6x6 μm , square-shaped AuNP assemble and the size can be easily controlled by crystallization time. The difference of the AuNP area density on 2k and 48.5k PEO crystals can be attributed to the different thiol group density on the PEO single crystal surface as shown in Figures 1c-d. For 48.5k PEO, the polymer chain end density is much lower than that of 2k PEO (~24 times less assuming similar the lamellar thickness). Furthermore, low molecular weight PEO undergoes integral folding which renders most of the chain ends on the crystal surface while for relatively long chain PEO, non-integral folding occurs. In this case, despite the fact that thiol groups are different from the rest of the polymer chain, they could be embedded in the lamellar crystals as shown in Figure 1c. This further reduces the thiol population on the 48.5k crystal surface. Therefore, PEO molecular weight can be used as a controlling factor to tune the thiol (and thus AuNP) density on the PEO crystal surface. Following similar methods, magnetic, platinum, and semiconducting nanoparticles have been successfully immobilized on the PEO, polycaprolactone (PCL), and PE-*b*-PCL single crystal surfaces (11, 12). The previously discussed adsorption process occurred after polymer single crystal formation. We have also demonstrated that much more complex nanoparticle patterns on these single crystals can be obtained by *in situ* assembling NPs during polymer crystallization (13).

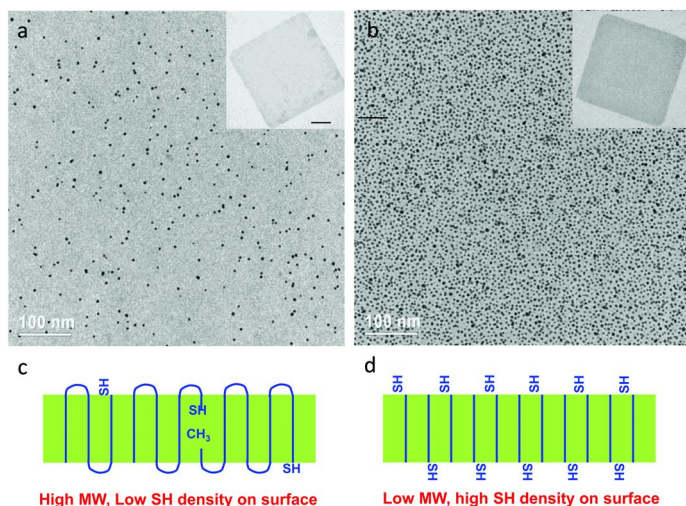


Figure 1. AuNPs on the surface of HS-PEO (a) 48.5k and (b) 2k single crystals (inset shows the entire single crystal, scale bar 2 μm). Schematic representation shows low thiol density on 48.5k PEO single crystals (c) and relatively high thiol density on 2k PEO single crystals (d). Note that extended chain single crystals are shown in d; folding could occur depending on MW and crystallization conditions. Reproduced from reference (9). Copyright 2007 American Chemical Society.

Applications of the Nanoparticle-Decorated Polymer Single Crystals

PSC-NP conjugates provide a unique pre-assembled system which can broaden nanoparticle applications. In this section, we shall focus on three examples, e.g. using this system for catalysis, surface enhanced Raman spectroscopy (SERS), and nanomotors. We recently demonstrated the utilization of PSCs as magnetically recyclable support for NP catalysts (14). Small NPs would be ideal candidates for surface catalysis. However, because of their high specific surface energy, NPs are subject to aggregation which undermines their efficacy. On the other hand, large NPs have reduced specific surface energy and lower surface area/volume ratio, likely making them relatively inefficient for this application. In recent work, we used PSCs to isolate catalyst NPs as a means to take advantage of their high specific surface energy without having to contend with NP aggregation. As a proof of concept, we selected platinum nanoparticles (PtNPs) as the catalytic component and Fe_3O_4 NPs as the magnetically responsive materials (14). In order to attach both nanoparticles, the support is composed of PSCs with two different types of surface groups, *i.e.* SH- and hydroxyl- (-OH). In this way, -SH can attach to a variety of metal surfaces, such as platinum, gold, silver, copper, etc., while -OH can bond to iron oxide. α -Hydroxy- ω -thiol terminated polycaprolactone (HO-PCL-SH) with a molecular weight of 13.2 kg/mol was synthesized. Uniformly sized HO-PCL-SH single crystal was obtained using the self-seeding method. The as-fabricated HO-PCL-SH single crystal has a hexagon shape. It is 13 μm long and 5 μm wide. The thickness was determined by using atomic force microscopy (AFM) to be around 8 nm (Figure 2). Since PSCs have a relatively constant thickness which is determined by the crystallization temperature, their surface area to volume ratio can be estimated to be $\sim 2/t$, where t is the thickness of the PSC, which is independent of the single crystal lateral size. The surface area to volume ratio in the present case therefore can be calculated to be $\sim 2.5 \times 10^8 \text{ m}^{-1}$ (equivalent to that of a nanosphere with a 12 nm radius!). In contrast, a sphere with similar volume ($\sim 0.45 \mu\text{m}^3$) has only a surface area to volume ratio of about $6.3 \times 10^6 \text{ m}^{-1}$, ~ 40 times less than what PSCs can offer. This implies thin polymer lamellar crystal could be an excellent candidate for catalyst support. To this end, after crystallization, PtNP was immobilized on single crystal surfaces through thiol-platinum bond. Fe_3O_4 NPs were then introduced to bond to OH groups on the PSC surface. Figure 2c shows the TEM image of the HO-PCL-SH single crystal surface after incubating in the PtNP solution, which indicates that PtNPs have been immobilized on HO-PCL-SH single crystal surface through thiol-platinum bond. The areal number density of PtNPs is about $440/\mu\text{m}^2$ and the catalyst loading can be estimated to be $\sim 20\%$ by weight. Figure 2d shows the TEM image of a HO-PCL-SH single crystal after adsorbing two types of nanoparticles. The number density of PtNPs after Fe_3O_4 NP attachment remains unchanged, indicating there is no PtNP detachment during the second nanoparticle adsorption process.

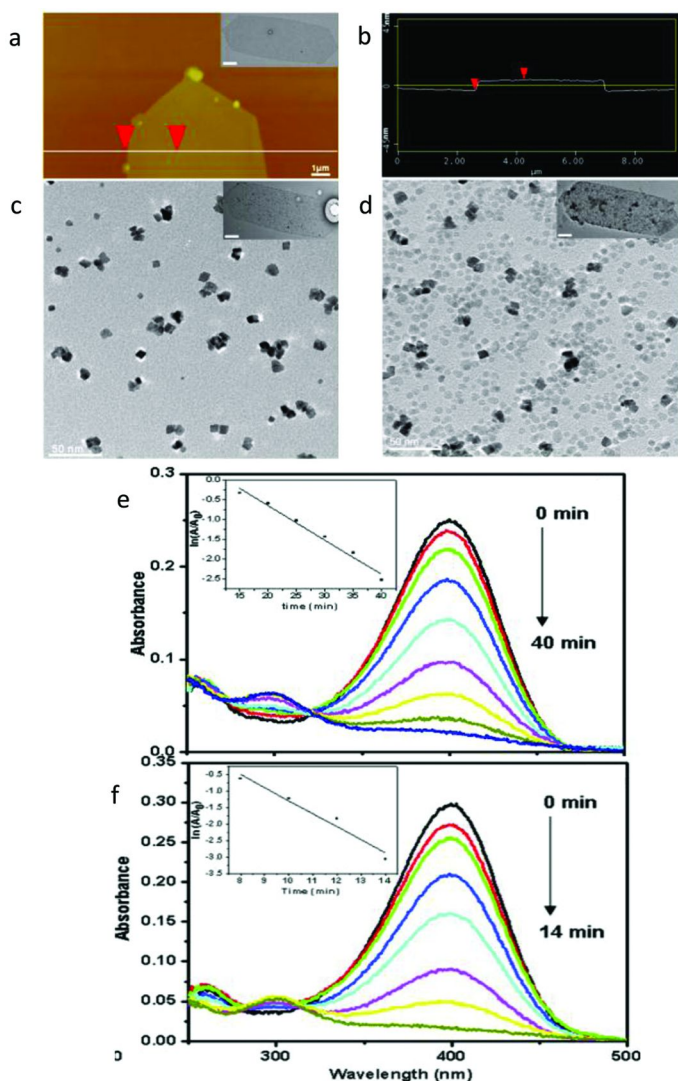


Figure 2. (a) Height mode AFM image of HO-PCL-SH single crystal. (b) Cross section analysis showing the thickness of HO-PCL-SH single crystal is around 8 nm. (c) and (d) show TEM images of 7 nm PtNP-coated, and 7 nm PtNP/10 nm Fe₃O₄NP-decorated HO-PCL-SH single crystals, respectively. Insets of (a), (c) and (d) show the zoom-out view of the corresponding single crystals. Scale bar for all insets: 1 μ m. (e-f) UV spectra of nitrophenol reduction reaction for (e) PSC/PtNP during 40 mins reaction with 5 mins interval. (f) PSC/PtNP/Fe₃O₄NP during 14 mins reaction with 2 mins interval. Inset: linear relationship of $\ln(A/A_0)$ as a function of time. Reproduced from reference (14). Copyright 2012 American Chemical Society.

Reduction of 4-nitrophenol to 4-aminophenol by sodium borohydride has been frequently used as a model reaction to check the catalytic activity of metallic nanoparticles (15). Figure 2e shows successive UV-vis spectra of 4-nitrophenol reduction solution at the presence of HO-PCL-SH single crystal supported 7 nm PtNP (PSC/PtNP in abbreviation). Those spectra were taken at 5 mins interval. The 4-nitrophenolate anion, which forms after adding sodium borohydride, shows a strong absorption peak at 400 nm. As time lapses, the peak intensity decreases. At the same time, the peak at 300 nm, which corresponds to 4-aminophenol, increases. The appearance of the isosbestic point at 314 nm indicates that there is no side reaction. The apparent rate constant was calculated in this case to be around 0.08 min^{-1} , as shown in Figure 2e inset. Figure 2f shows the UV-vis spectra of 4-nitrophenol reaction when using HO-PCL-SH single-crystal-supported PtNP and $\text{Fe}_3\text{O}_4\text{NP}$ (PSC/PtNP/ $\text{Fe}_3\text{O}_4\text{NP}$ in short) as catalyst. The amount of PtNP added into the reaction solution was kept the same as that of PSC/PtNP without $\text{Fe}_3\text{O}_4\text{NPs}$. The reduction was completed in 40 mins for Figure 2e and in 14 mins for Figure 2f. The apparent rate constant increased from 0.08 min^{-1} in Figure 2e to about 0.4 min^{-1} in Figure 2f. Since the HO-PCL-SH supported $\text{Fe}_3\text{O}_4\text{NP}$ has negligible catalytic activity, the 5 times increase in the apparent rate constant must be derived from the interaction between PtNP and $\text{Fe}_3\text{O}_4\text{NP}$ (16). Moreover, we have compared the present rate constant of PSC/PtNP with that of similar systems (17), which was fabricated by first synthesizing nanoparticles and then immobilizing them onto polymer support. The catalytic activity is characterized under the same sodium borohydride (30 mM) and 4-nitrophenol concentration (0.015 mM). The rate constant in our current system is estimated to be $0.01 \text{ s}^{-1}\text{m}^{-2}\text{L}$ when normalized by nanoparticle surface area and $0.08 \text{ s}^{-1}\text{g}^{-1}\text{L}$ when normalized by the total weight of catalyst and support, which are 2.5 and 26 times higher than the reported literature value (17), respectively. Furthermore, as evidenced in Figure 2d, the surface of HO-PCL-SH single crystal can be heavily loaded with a dense layer of $\text{Fe}_3\text{O}_4\text{NPs}$. Such high loading of $\text{Fe}_3\text{O}_4\text{NP}$ ensures efficient nanoparticle recycling. All PSC/PtNP/ $\text{Fe}_3\text{O}_4\text{NP}$ can be quickly separated out to the side wall of the test tube under magnetic field, demonstrating that it is recyclable. The nanocatalyst can be magnetically recycled and reused for four times with only slight changes in apparent rate constant.

PSC-nanoparticle conjugates can also be used for SERS study (18). For example, we recently reported the fabrication of a novel PSC@Au nanoparticle (PSC@AuNP) nanosandwich structure for SERS applications. The detailed fabrication procedure starts with solution growth of a 2D PSC with -SH end groups exposed on the PSC surface. The PSC can then be used to immobilize 6 nm AuNPs through thiol-gold bonds by solution mixing. Then, 6nm-AuNP-decorated PSCs are immersed in 10mg/ml AuCl_3 solution for electroless deposition. 1 hour deposition time is utilized to ensure the formation of a dense layer of AuNPs approximately 50nm in diameter on the PSC surface. The spontaneous AuNP formation in the absence of a reducing agent may be attributed to the nanoparticle-induced autocatalytic growth mechanism. The as-fabricated substrate can then be used for SERS study. Figure 3 shows the optical image of large AuNP-decorated PCL-SH single crystal after electroless deposition (AuCl_3 treatment for 1hr), *i.e.* PSC@AuNP nanosandwich (which exhibits a band in the

near-IR) and Figure 3b,c shows a low magnification TEM image of this structure, the surface of which is decorated with densely packed AuNPs. The average size of AuNPs is approximately 50nm based on the image analysis of Figure 3c. Figure 3e is a high resolution TEM image showing the lattice structure (Au(111)) of the edge of a large AuNP. Furthermore, AFM was utilized to monitor the changes in thickness. Figure 3f,h show pure PCL-SH single crystal has a thickness of about 8 nm. After the deposition of 50nm AuNP, the thickness is increased to around 110 nm, which is about 2 times the average diameter of AuNP plus the thickness of PCL-SH single crystal. This, combined with TEM analysis, demonstrates that the AuNPs have decorated both sides of PCL-SH single crystal forming a PSC@AuNP nanosandwich structure.

The as-fabricated PSC@AuNP nanosandwich can be used as SERS substrates in two ways. On the one hand, it can be utilized by mixing the hybrid material with an analyte solution. On the other hand, detection is also possible using the tip-enhanced Raman Spectroscopy (TERS) configuration, where PSC@AuNP is placed onto the analyte surface, as will be described below. Raman signals from the analyte lies under PSC@AuNP can be probed. As a proof of concept, we have used 4-aminothiophenol (4-ATP) as the probe molecule to evaluate the SERS effect in both cases. In the first case, before 4-ATP adsorption, the pristine PSC@AuNP had only negligible signals (Figure 4a red curve). When 4-ATP molecules were attached using solution mixing method, it exhibited strong SERS signals, as shown in Figure 4a black curve. We performed a control experiment to study SERS of 4-ATP chemically adsorbed on 6nm-AuNP-decorated PSC (the AuNP-decorated PSC prior to electroless deposition of additional gold from AuCl solution); much weaker Raman signals were observed (Figure 4b black curve). According to the vibration of C-S at 1077 cm^{-1} , the enhancement factor (EF) for PSC@AuNP nanosandwich was calculated to be approximately 1×10^7 , while the 6nm-AuNP-decorated PCL-SH single crystal had a relatively modest EF of about 2×10^5 .

In the second case, PSC@AuNP was cast on self assembled monolayer (SAM) of 4-ATP molecules chemically adsorbed on Pt/Pd (80/20) surface. Figure 4c black curve shows the strong Raman spectrum obtained at this configuration. Without applying PSC@AuNP, 4-ATP SAMs on metal surface had very weak Raman signals (Figure 4c red curve). The EF (based on C-S vibration) is calculated to be around 4×10^6 . Because of their different sizes, not all AuNPs contacted the SAM surface. The contact area should also be smaller than the footprint area of a nanoparticle; this EF is therefore an underestimation. It is worth noting that although the EF is the same order of magnitude as that of TERS (up to 10^6), the overall Raman intensity is much stronger because each PSC@AuNP mimics thousands of TERS probes. Furthermore, due to the thinness and flexibility of PSC, this hybrid structure can coat onto non-flat surfaces, such as yeast cells. SERS of the latter can then be obtained adopting the similar TERS configuration. As can be seen from Figure 4d inset, a PSC@AuNP nanosandwich can cover a single yeast cell. Strong SERS signals from amide, protein, etc. of yeast cell can be obtained (Figure 4d black curve).

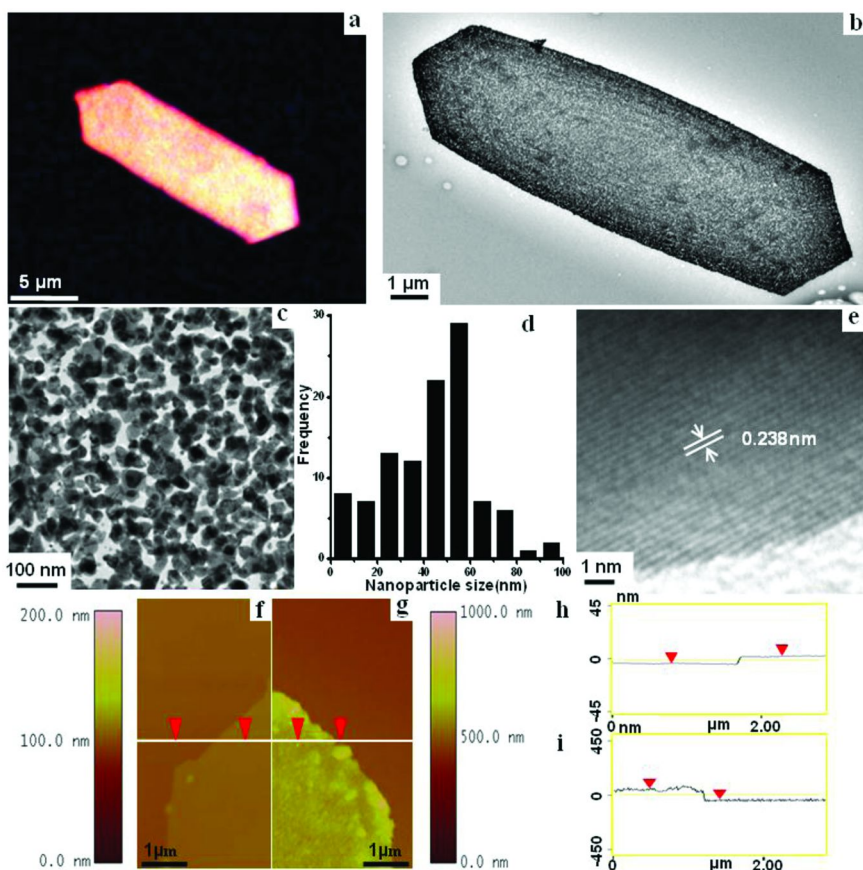


Figure 3. (a) Optical image of a PSC@AuNP nanosandwich cast on a glass slide. (b,c) TEM images of PSC@AuNP nanosandwich at different magnifications. (d) Size distribution histogram of AuNPs measured from (c). (e) High resolution TEM image showing the lattice structure of AuNP (111). AFM images of pure PSC (f) and PSC@AuNP (g). AFM section analysis of (f) and (g) is shown in (h) and (i), respectively. Reproduced from reference (18). Copyright 2012 The Royal Society of Chemistry.

Another interesting application of the PSC/NP nanosandwiches is for nanomotors. Inspired by biological motors (19), man-made catalytic nanomotors, which are nanoscale devices that are capable of converting energy into forces and movement, have recently attracted increasing attention (20–25). Since the discovery of the first man-made catalytic motor by Whitesides *et al.* (20), the research field has experienced rapid progresses (2, 21–34). We recently showed that PSCs can be used as a platform to fabricate nanomotors (35). As a

proof-of-concept, AuNP, Fe₃O₄NPs, and PtNPs were directly self-assembled onto the surface of a quasi-two dimensional PSC to form a nanomotor. The functions of nanoparticles are as follows: AuNPs with tunable SPR absorption serve as markers to make the nanomotor clearly visible under optical microscope; Fe₃O₄NPs allow for remote control with a magnetic field; PtNPs are able to catalyze the decomposition of H₂O₂ to generate oxygen bubbles, providing the propulsion force.

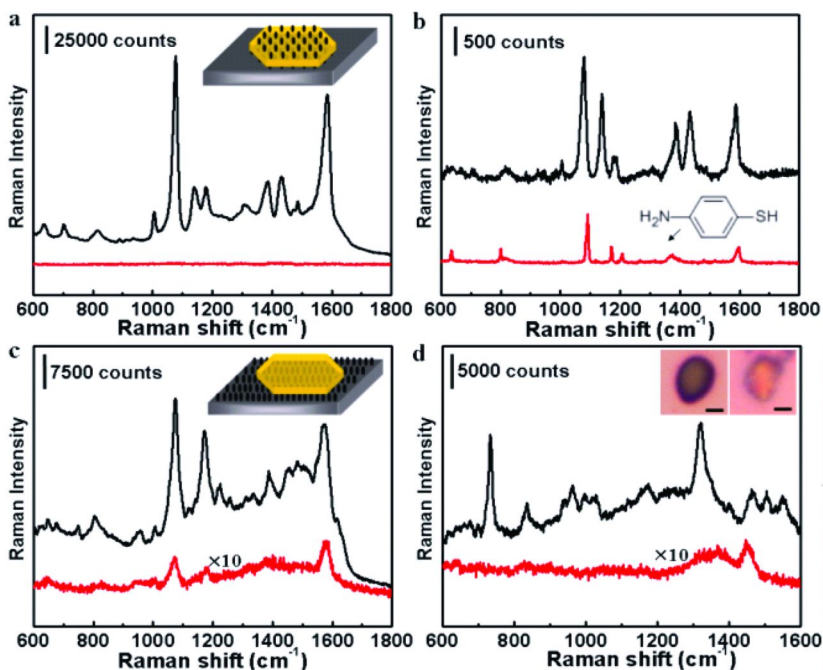


Figure 4. (a) SERS of PSC@AuNP before (red curve) and after (black curve) 4-ATP molecule adsorption. Inset: schematic of detection adopting conventional configuration with yellow hexagon and dark dots representing gold coloured PSC@AuNP and 4-ATP molecules, respectively. (b) Raman spectrum from pure 4-ATP molecules (red curve) and SERS from 4-ATP molecules chemically adsorbed on 6nm-AuNP-coated PCL-SH single crystal (black curve). Inset: molecular structure of 4-ATP. (c) Raman spectrum of 4-ATP SAMs on Pt/Pd (80/20) surface (red curve) and SERS spectrum obtained after casting PSC@AuNP on top (black curve). Inset: schematic of detection adopting TERS configuration. (d) Raman spectrum of yeast cell (red curve) and SERS spectrum obtained after casting PSC@AuNP on top (black curve). Inset: optical image of yeast cell before (left) and after (right) coating with PSC@AuNP, scale bar 2 μ m. Reproduced from reference (18). Copyright 2012 The Royal Society of Chemistry.

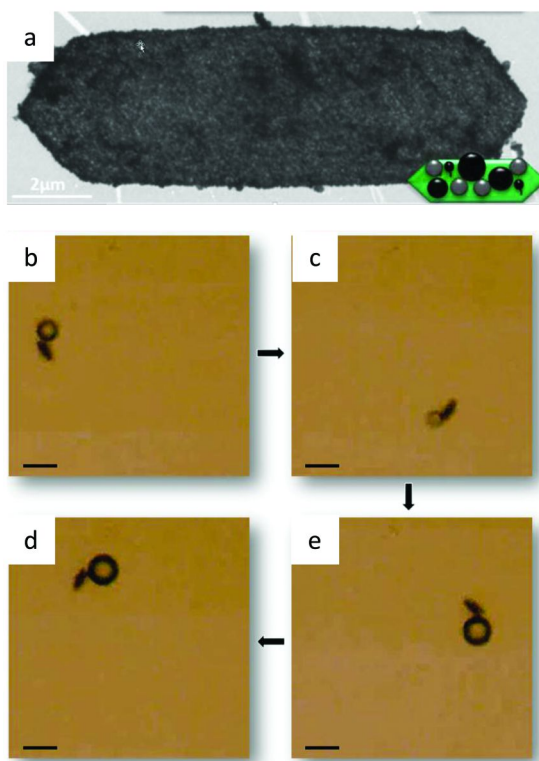


Figure 5. (a) Low magnification TEM image of PSC-AuNP-PtNP-Fe₃O₄NP. (b-e) Series of optical images taken at 1 second interval showing the autonomous movement of the nanoparticle based nanomotor. Scale bar: 20 μm. Reproduced from reference (35). Copyright 2013 American Chemical Society.

Figure 5a shows the TEM image of the resulting PSC-AuNP-PtNP-Fe₃O₄NP. The dimension of the PSC-based hybrid structure is 12 μm x 4 μm x 90 nm (length/width/thickness). Note that the length and width of the PSC can be easily decrease to sub-micrometer range. The entire PSC-nanoparticle hybrid structure can also be viewed as a smart nanoshuttle, with PtNPs as the engine, Fe₃O₄NPs as the steering wheel, and AuNPs as the marker. This nanoparticle-decorated PSC nanomotor (PSC-AuNP-PtNP-Fe₃O₄NP) moves autonomously when placed in a 15% H₂O₂ solution. The average speed is estimated to be around 30 μm per second. Figure 5b shows an individual motor moving autonomously in circles. Interestingly, the weight ratio between the catalytic part of current motor (PtNP) and the non-catalytic part of PSC-based nanomotor to be approximately 1:100 through TEM image analysis. This indicates that, due to the high catalytic activity, PtNP is capable of carrying the whole motor which is about 100 times of its own body weight at a high speed (30 μm/s), demonstrating the superiority

of nanoparticle-based nanomotors. Recent efforts have been focused on tuning the speed, functionality as well as biodegradability of the PSC-based nanomotors (36, 37).

Conclusions

Combining PSCs and NPs leads to new types of hybrid materials. Not only do they offer novel properties, but also they provide opportunities for studying the fundamentals of polymer physics. From a technological standpoint, the functionalized single crystals are of great interests because they are nano “tapes” which can immobilize desired nanoparticles, viruses, and proteins. Nano tapes can therefore be used in drug delivery, NP recycling, controlled nanoparticle synthesis, etc. The NP-coated PSCs have a sandwich structure; they might find applications in microelectromechanical systems and nanoelectromechanical systems as well as microfluidic applications.

Acknowledgments

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References

1. Storks, K. H. *J. Am. Chem. Soc.* **1938**, *60*, 1753–1761.
2. Dreyfus, R.; Baudry, J.; Roper, M. L.; Fermigier, M.; Stone, H. A.; Bibette, J. *Nature* **2005**, *437*, 862–865.
3. Keller, A. *Philos. Mag* **1957**, *2*, 1171–1175.
4. Till, P. H. *J. Polym. Sci.* **1957**, *24*, 301–306.
5. Fisher, E. W. *Naturforsch* **1957**, *12a*, 753–754.
6. Lotz, B.; Cheng, S. Z. D. *Polymer* **2005**, *46*, 577–610.
7. Bassett, G. A. *Philos. Mag.* **1958**, *3*, 1042–1045.
8. Li, C. Y. *J. Polym. Sci.; Part B: Polym. Phys.* **2009**, *47*, 2436–2440.
9. Li, B.; Li, C. Y. *J. Am. Chem. Soc.* **2007**, *129*, 12–13.
10. Wang, B. B.; Li, B.; Zhao, B.; Li, C. Y. *J. Am. Chem. Soc.* **2008**, *130*, 11594–11595.
11. Wang, B.; Li, B.; Ferrier, R. C. M.; Li, C. Y. *Macromol. Rapid Commun.* **2010**, *31*, 169–175.
12. Li, B.; Ni, C.; Li, C. Y. *Macromolecules* **2008**, *41*, 149–155.
13. Li, B.; Wang, B. B.; Ferrier, R. C. M.; Li, C. Y. *Macromolecules* **2009**, *42*, 9394–9399.
14. Dong, B.; Miller, D. L.; Li, C. Y. *J. Phys. Chem. Lett.* **2012**, *3*, 1346–1350.
15. Wunder, S.; Polzer, F.; Lu, Y.; Mei, Y.; Ballauff, M. *J. Phys. Chem. C* **2010**, *114*, 8814–8820.
16. Wang, C.; Daimon, H.; Sun, S. H. *Nano Lett.* **2009**, *9*, 1493–1496.
17. Mahmoud, M. A.; Snyder, B.; El-Sayed, M. A. *J. Phys. Chem. Lett.* **2010**, *1*, 28–31.

18. Dong, B.; Wang, W.; Miller, D. L.; Li, C. Y. *J. Mater. Chem.* **2012**, *22*, 15526–15529.
19. Schnitzer, M. J.; Block, S. M. *Nature* **1997**, *388*, 386–390.
20. Ismagilov, R. F.; Schwartz, A.; Bowden, N.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **2002**, *41*, 652–654.
21. Ozin, G. A.; Manners, I.; Fournier-Bidoz, S.; Arsenault, A. *Adv. Mater.* **2005**, *17*, 3011–3018.
22. Paxton, W. F.; Sen, A.; Mallouk, T. E. *Chem.–Eur. J.* **2005**, *11*, 6462–6470.
23. Wang, J. *ACS Nano* **2009**, *3*, 4–9.
24. Ebbens, S. J.; Howse, J. R. *Soft Matter* **2010**, *6*, 726–738.
25. Wang, J.; Manesh, K. M. *Small* **2010**, *6*, 338–345.
26. Mirkovic, T.; Zacharia, N.; Scholes, G. D.; Ozin, G. A. *Small* **2010**, *6*, 159–167.
27. Pantarotto, D.; Browne, W. R.; Feringa, B. L. *Chem. Commun.* **2008**, 1533–1535.
28. Vicario, J.; Eelkema, R.; Browne, W. R.; Meetsma, A.; La Crois, R. M.; Feringa, B. L. *Chem. Commun.* **2005**, 3936–3938.
29. Mano, N.; Heller, A. *J. Am. Chem. Soc.* **2005**, *127*, 11574–11575.
30. Mirkovic, T.; Zacharia, N. S.; Scholes, G. D.; Ozin, G. A. *ACS Nano* **2010**, *4*, 1782–1789.
31. Ghosh, A.; Fischer, P. *Nano Lett.* **2009**, *9*, 2243–2245.
32. Hong, Y.; Blackman, N. M. K.; Kopp, N. D.; Sen, A.; Velegol, D. *Phys. Rev. Lett.* **2007**, *99*, 178103.
33. Gibbs, J. G.; Kothari, S.; Saintillan, D.; Zhao, Y. P. *Nano Lett.* **2011**, *11*, 2543–2550.
34. Gibbs, J. G.; Zhao, Y. *Small* **2010**, *6*, 1656–1662.
35. Dong, B.; Zhou, T.; Zhang, H.; Li, C. Y. *ACS Nano* **2013**, *7*, 5192–5198.
36. Liu, M.; Liu, L. M.; L., G. W.; Su, M. D.; Ge, Y.; Shi, L. L.; Zhang, H.; Dong, B.; Li, C. Y. *Nanoscale* **2014**, *6*, 8601–8605.
37. Liu, M.; Liu, L.; Gao, W.; Su, M.; Ge, Y.; Shi, L.; Zhang, H.; Dong, B.; Li, C. Y. *Nanoscale* **2015**, *7*, 4949–4955.

Chapter 5

Applications of Nanoparticles through Surface Functionalization

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Modification of the titania surface with organic and inorganic groups can significantly improve the practical potential of this material as a component of sunscreen and toothpastes. Functionalization of titania and silica nanoparticles improves their adhesion to human dentin, which opens their future use for treating tooth hypersensitivity by occlusion of dentin tubules and carriers of remineralizing and other active components. The affinity of organic and inorganic molecules to titania can suppress photodegradation of caffeic acid and work toward new formulations of sunscreens.

Introduction

The booming field of applied nanotechnology spurs the growing interest to the surface functionalization as a versatile tool for fine-tuning of nanoscale devices and compositions, and for tailoring their properties to a specific practical application (1–4). Among those properties is conjugation with different materials by electrostatic (5–7) or covalent (8, 9) interaction.

One of the most common nanomaterials is titania (TiO_2), also known as titanium dioxide, an inorganic compound occurring in three different polymorphs: anatase, brookite and rutile (10–12) which have found applications in catalysis (13, 14), water purification (15, 16) medicine (17–19) optoelectronics (20) and in charge-transfer process (21). The least expensive commercial rutile-anatase nanopowder is widely used as a constituent of sunscreens (22) due to its ability to absorb (23) and scatter UV-light (24), and as a component of toothpastes due to its physiochemical properties (25).

Our recent results demonstrate that modification of the titania surface with organic and inorganic groups can significantly improve the practical potential of this material as a component of sunscreen and toothpastes. This study is based on the known affinity of the caffeic acid's catechol group to titania (26) (Figure 1), which can be fine-tuned by the competitive binding of other ligands to the titania surface.

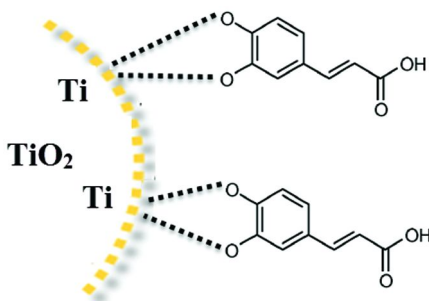


Figure 1. Binding of caffeic acid to titania particles.

Results and Discussion

Application of the Affinity of Caffeic Acid toward Titania

Occlusion of the dentin tubules is a known approach to the treatment of tooth hypersensitivity (27, 28). Hydroxyapatite crystallizing in the crystal unit $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is the major component of human dentin (29), which can bind with the carboxy-groups of aminoacids (30, 31).

Functionalization of nanoparticles with carboxy-groups as a method of increasing their affinity to human dentin and, therefore, ability to occlude the dentin tubules, have been explored. First, adsorption of caffeic acid on titania increases its affinity to the hydroxyapatite component of dentin and capability to occlude the tubules, due to the binding of the imparted carboxy-groups with calcium ions. For comparison, affinity of non-functionalized and covalently functionalized by carboxy-groups silica nanoparticles to dentin has been also tested.

The SEM images of cross-sections of human dentin before and after treatment with titania nanoparticles are presented in Figure 2. Sonication was selected as a reproducible method to access how well the particles are retained by the dentin surface and tubules.

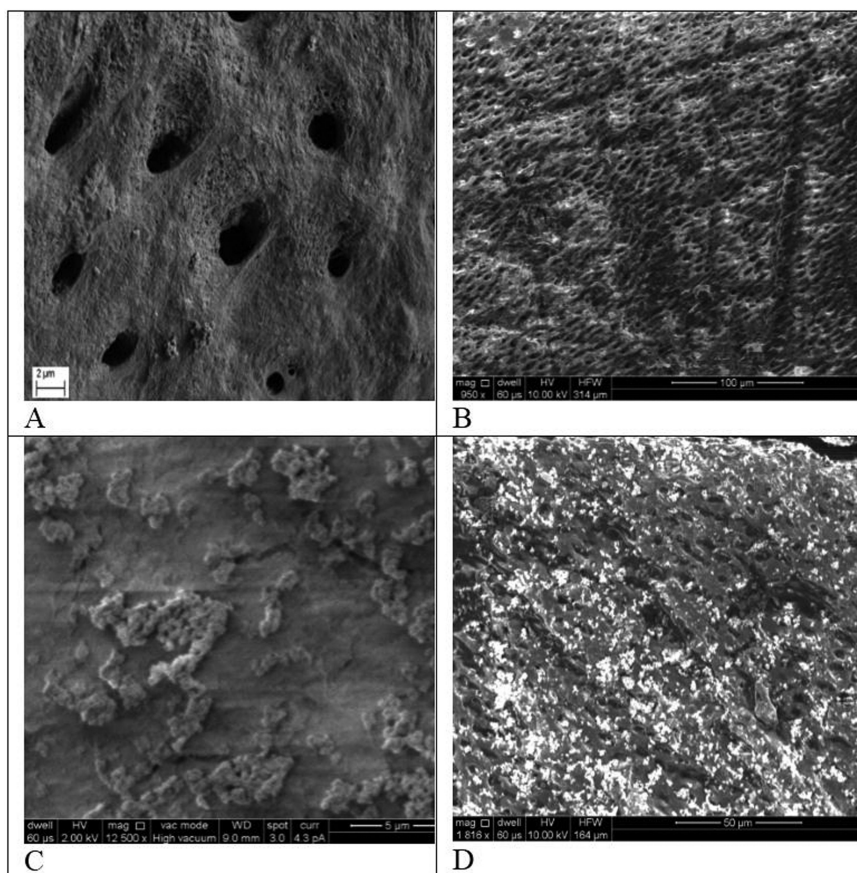


Figure 2. (A) Untreated dentin; (B) Dentin exposed to untreated titania after rinsing with water; (C) Dentin exposed to caffeic acid-functionalized titania after rinsing with water; (D) Dentin exposed to caffeic acid-functionalized titania after rinsing with water and sonication for 10 s.

As expected, surface functionalization of titania with caffeic acid increased adhesion of titania to hydroxyapatite. As opposed to untreated titania, after sonication, a significant fraction of the applied caffeic acid – functionalized titania particles still remained on the dentin surface (Figure 2).

Next, the possibility of harnessing another common component of toothpastes – silica nanoparticles – for the treatment of tooth hypersensitivity was explored. However, unfunctionalized silica nanoparticles did not exhibit a sustainable affinity to dentin, and have been mostly removed from the surface after 10 seconds sonication (Figure 3A). This explains why ordinary silica-containing toothpastes do not address the hypersensitivity problem. The modification of silica nanoparticles with carboxy-groups (by APTES amination followed by carboxylation using succinic anhydride) noticeably improve their adhesion to dentin and occlusion of the tubules (Figure 3B).

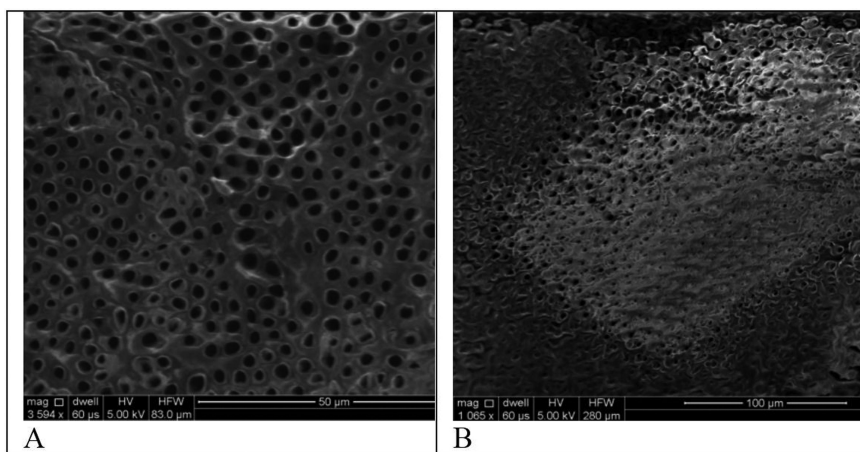


Figure 3. (A) Dentin exposed to unfunctionalized silica nanoparticles after rinsing with water and sonication for 10 s; (B) Dentin exposed to carboxylated silica nanoparticles after rinsing with water and sonication for 10 s.

While carboxylated silica nanoparticles are not currently used in dentifrices, it was found that modification of titania with caffeic acid can be immediately employed for improving existing toothpastes. In order to avoid using any chemicals outside of the realm of approved dentifrice components and food supplements, a composition containing titania nanoparticles from an existing toothpaste Crest® - Cavity Protection), and caffeic acid from a green coffee bean extract (a food supplement) was tested.

The SEM images in Figure 4 illustrate that the Crest® toothpaste forms a layer, covering most of the dentin tubules after rinsing with water (Figure 4A), but was completely cleared out after sonication (Figure 4B). In contrast, the toothpaste – green coffee bean extract composition was retained by the dentin surface even after sonication (Figure 4C and 4D).

Suppressing the Affinity of Caffeic Acid toward Titania

While adsorption of caffeic acid on titania may lead to novel therapeutical toothpastes, it also makes caffeic acid unusable in titania-containing sunscreen compositions. Caffeic acid has been patented as a component of sunscreen composition due to its ability to adsorb the most damaging to skin UV-bands, and provide additional health benefits due to its anti-oxidant properties (32). However, to the best of our knowledge, caffeic acid has not been used in any commercial sunscreen compositions, perhaps, because of its low photostability, especially in the presence of a titania photocatalyst. In addition, the reactive oxygen species generated upon photoexcitation of titania, are damaging to skin (33). We have demonstrated that competitive adsorption of phosphate-ions and caffeic acid on titania significantly slows down photodecomposition of caffeic acid, perhaps, due

to dissociation of photoactive titania-caffeic acid surface complexes that separates caffeic acid from the photocatalyst.

Sunlight-induced photodegradation of caffeic acid has been mimicked for 5h in a solar simulator. Concentration of caffeic acid in a solution of centrifuged samples was monitored by measuring its optical density at 328 nm.

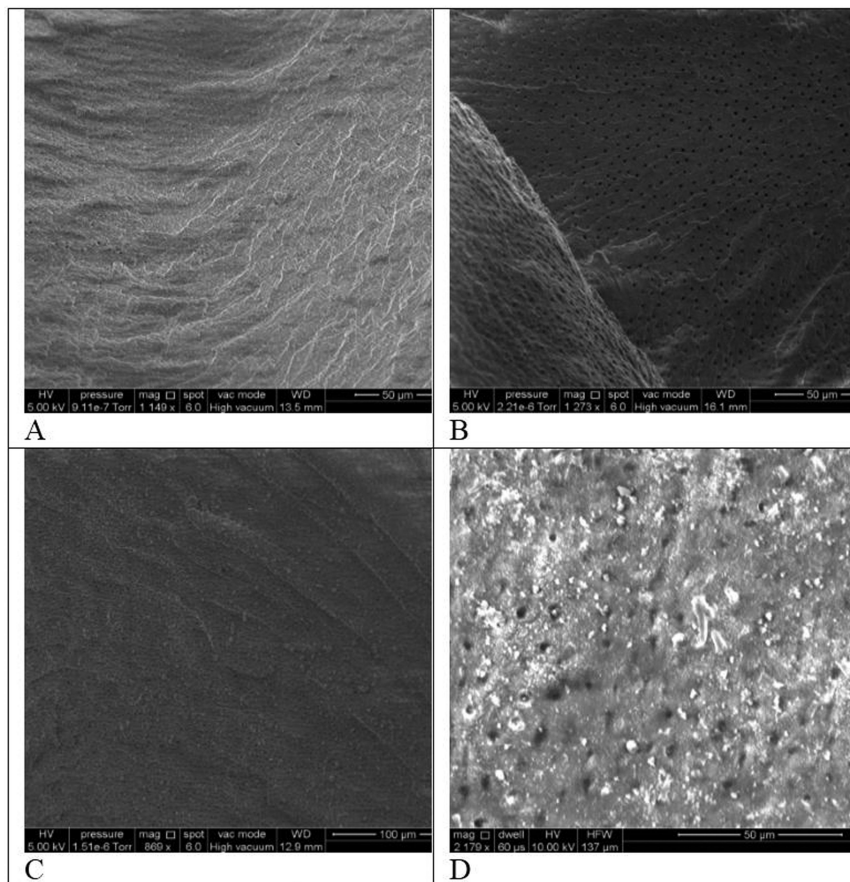


Figure 4. (A) Dentin exposed to Crest® - Cavity Protection toothpaste after rinsing with water; (B) Dentin exposed to Crest® - Cavity Protection toothpaste after rinsing with water and sonication for 10 s; (C) Dentin exposed to the Crest® - Cavity Protection toothpaste and green coffee bean extract after rinsing with water; (D) Dentin exposed to the Crest® - Cavity Protection toothpaste and green coffee bean extract after rinsing with water and sonication for 10 s.

In order to mimic stability of sunscreen compositions containing caffeic acid and titania, photodegradation of caffeic acid in the presence of titania for about 3h was followed. As shown in Figure 5, the process follows the zero-order kinetics, which is consistent with literature (34).

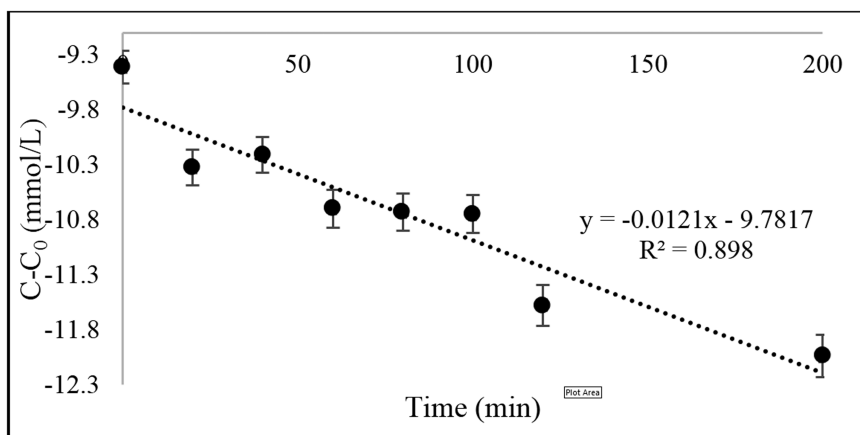


Figure 5. Photodegradation of caffeic acid in the presence of titania (zero order plot.)

Interestingly, the kinetic of photodegradation also fits the first-order kinetic law (Figure 6). Further discussion regarding these reaction kinetics is provided in a subsection entitled “Further discussion on the kinetics of decomposition of caffeic acid over titania”

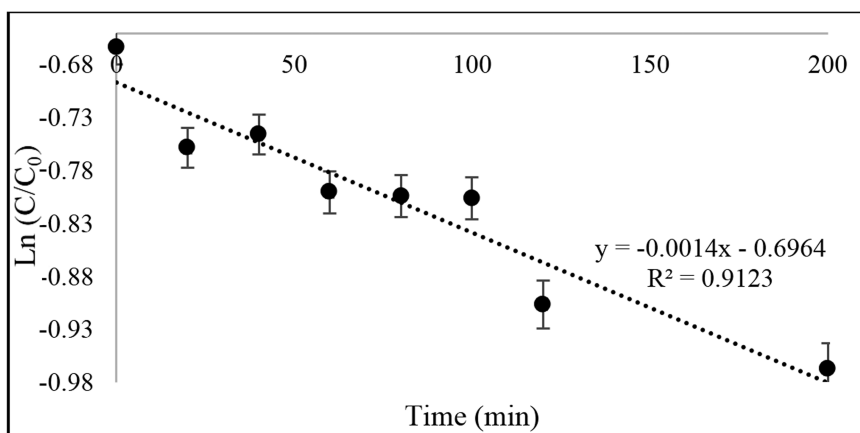


Figure 6. Photodegradation of caffeic acid in the presence of titania (first order plot.)

Regardless of the reaction order, the effect of addition of phosphate-ions, chondroitin sulfate, and β -cyclodextrin on the photodegradation rate of caffeic acid in the presence of titania was explored (Figures 7,8.)

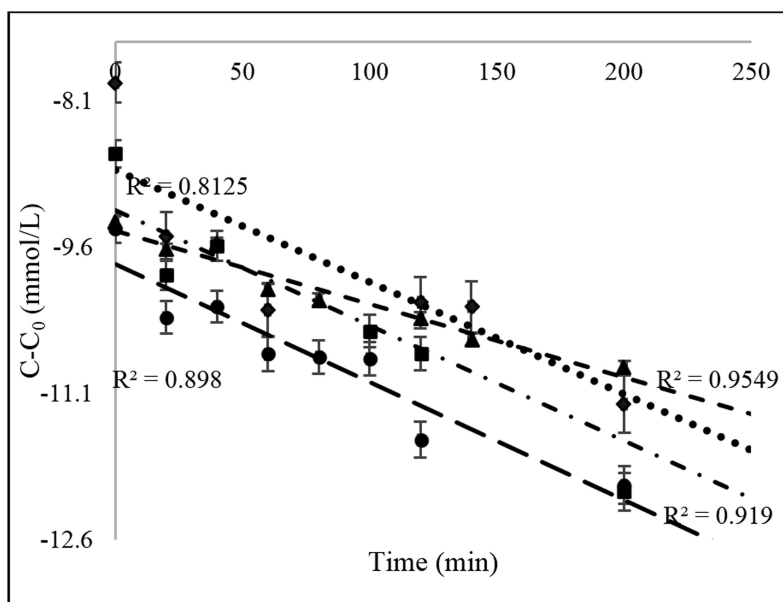


Figure 7. Titania-catalyzed photodegradation of caffeic acid in the absence (●) and presence of phosphate-ions (▲), chondroitin sulfate (◆), and β -cyclodextrin (◼) additives (zero order plot.)

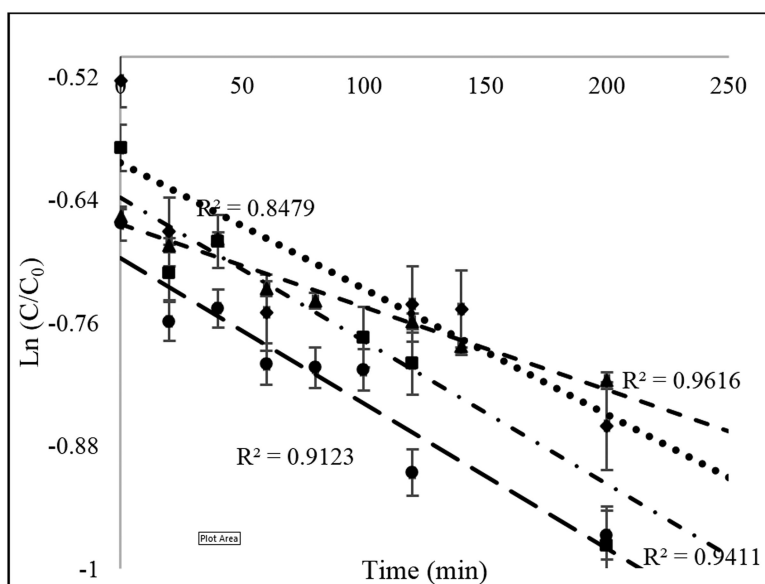


Figure 8. Titania-catalyzed photodegradation of caffeic acid in the absence (●) and presence of phosphate-ions (▲), chondroitin sulfate (◆), and β -cyclodextrin (◼) additives (first order plot.)

Not surprisingly, neither photodegradation process exhibits a distinctive zero or first order kinetics. However, addition of 4.9×10^{-5} M of phosphate significantly suppresses photodegradation of caffeic acid, increasing its concentration in solution roughly by the factor of 2. A similar effect of phosphate is known for photooxidation of ferulic acid by KBrO_3 under anaerobic conditions (35). Perhaps, this suppressing effect is due to adsorption of phosphate on the surface of titania, which decreases the surface concentration of caffeic acid on the titania and, therefore, reduces effectiveness of photocatalysis. This is consistent with our observation that adsorption of caffeic acid on titania is significantly suppressed by the presence of phosphate ions. When 11 g of commercial titania was mixed with 2 mL of 6.9 mmol/L solution of caffeic acid in an ~20 % aqueous methanol for ~20 minutes, concentration of caffeic acid dropped to 6.4 mmol/L (by 6%) because of adsorption on titania. When 2 mL of 0.1 M KH_2PO_4 was added into the mixture, the concentration of caffeic acid increased nearly to the original value. Although we used about 2.8 times higher concentration of caffeic acid for the photodegradation experiments for the practical purposes, the effect of phosphate ions on the photostability of caffeic acid was quite evident.

The observed competitive adsorption of caffeic acid and another solution component demonstrate that any reliable study of adsorption or photodegradation in the presence of titania must specify type of the employed buffer.

Interestingly, neither chondroitin sulfate nor β -cyclodextrin significantly affected the photodegradation rate, despite their known affinity to titania (36, 37). It is consistent with our observation that either of those additives released a noticeable amount of the adsorbed caffeic acid on titania into the solution (Figure 7). The effect of desorption of caffeic acid from titania was likely counteracted by the formation of supramolecular complexes interfacing titania and caffeic acid and stimulating photodegradation. Such an equivocal effect on photodegradation of bisphenol has been reported for several cyclodextrins (38). In addition, the exact orientation of the molecule of caffeic acid at the surface of titania may be affected by the surface supramolecular complexes. According to our recent computational study (39), the mode of adsorption of a molecule of caffeic acid at the surface of titania significantly affect the energy of adsorption essential for the photoexcited state dynamics.

Further Discussion on the Kinetics of Decomposition of Caffeic Acid over Titania

Apparently, at the studied range of concentrations for caffeic acid and titania mixture, the difference between the zeroth and first kinetics is within the experimental error, which is illustrated in Figure 7, which shows $\ln C$ plotted against C , and one calculated point ($\ln C = 0$ at $C = 1$).

Figure 9 shows that the zeroth and first kinetic orders of photodecomposition cannot be confidently distinguished in the studied concentration range. According to another study (40), even in lower concentrations, the first reaction order becomes evident only after extended reaction times (several hours). The zero- and first order photodegradation rate constants calculated (36, 37) from the linear regression parameters, are summarized in Table 1. For zero-order; $[C]$ versus t

derived from the equation $[C] = [C]_0 - kt$; where $[C]$ is the concentration of caffeic acid remaining in the solution (mol/L), $[C]_0$ is the initial concentration of caffeic acid (mol/L), k is the rate constant (mol/L.min) and t is time (min.) For first-order kinetics; $\ln [C]$ versus t derived from the equation $[C] = [C_0] e^{-k t}$; where k is the rate constant (min^{-1}).

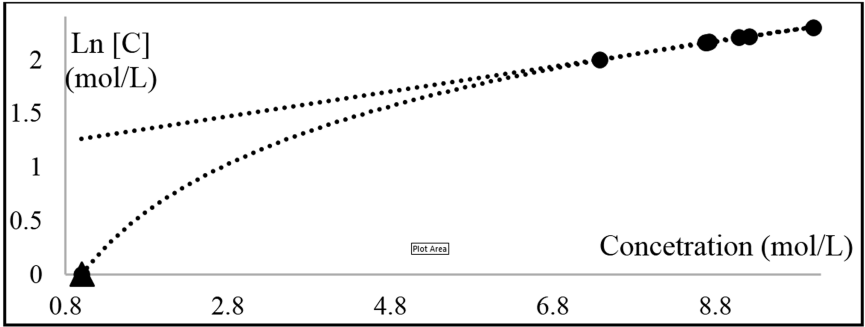


Figure 9. Plot of the logarithm of caffeic acid concentration against the concentration. One calculated point has been added (marked by a triangle).

Table 1. Titania-Catalyzed Photodegradation of Caffeic Acid Rate Constants (K), Their Margin Errors ($\pm$) and Line Regression Fitting (R^2) in the Absence ($\bullet$) and Presence of Phosphate-Ions ($\blacktriangle$), Chondroitin Sulfate ($\blacklozenge$), and β -Cyclodextrin ($\blacksquare$) Additives (First Order Plot)

	Zero Order			First Order		
	k (mol/L.min) $\ast 10^{-3}$	$\pm$	R^2	k (min ⁻¹) $\ast 10^{-4}$	$\pm \ast 10^{-5}$	R^2
$\bullet$	12.1	1.7	0.90	14.2	17.9	0.91
$\blacktriangle$	7.5	0.7	0.95	8.1	6.6	0.96
$\blacklozenge$	10.3	2.5	0.81	12.3	23.3	0.85
$\blacksquare$	11.8	1.6	0.92	14.0	15.6	0.94

Conclusion

Controlled adsorption of caffeic acid on titania can markedly increase its practical applicability for biomedical applications. The bifunctional molecules of caffeic acid can serve as a linker between titania and human dentin, enabling application of this material for the treatment of tooth hypersensitivity by dental

tubule occlusion. On the other hand, modification of titania surface significantly affects the photodegradation rate of caffeic acid, which will aid in developing new sunscreen formulations that combine advantages of both organic and inorganic UV-protectors.

Experimental Section

Caffeic acid, (3-aminopropyl) triethoxysilane, 98% (APTES) and succinic anhydride were purchased from *Alfa Aesar*, tetraethoxysilane (TEOS) from *Sigma-Aldrich*, chondroitin sulfate from *Bioalternatives*, β -cyclodextrin from *Lancaster*, potassium phosphate monobasic from *J. T. Baker Chemical Co.*, titania from *Fisher Scientific (Cat. No. 79586)*. The experiments were conducted with the solar stimulator (*Arc Lamp Housing, Single Element FS, F/1.5, Collimated, 50-500W, 1.5 In, Newport co.*), Cary® 50 UV-Vis Spectrophotometer, and *FEI Quanta 450 FEG Environmental Scanning Electron Microscope (SEM)*.

Preparation of Tooth Samples

A human tooth was rinsed with tap water. The outer enamel layer was removed using a dental bur. Next, the tooth was wrapped in a paper towel and split with a hammer into pieces about 2X2 mm, which were shaken 5 mL water and sonicated for 20 s. The turbid liquid was drained out, and sonication was repeated with a fresh 5 mL of water. The sonication step was repeated until no more turbid liquid was produced (usually 2-3 steps). The purified samples were dried on air overnight at 35°C.

Application of Materials to a Tooth Sample (The General Procedure)

Approximately A 2 mL portion of a ~10 mg/mL of an aqueous nanoparticle suspension or 50 mg of the paste of a solid material mixed well with 1 mL of water was applied to the tooth sample followed by brushing with a toothbrush for 1 min. The treated tooth sample was washed by a medium stream of tap water for 20 s. Persistence of adhesion and occlusion was tested by sonicating the sample in 2 mL of water for 20 s.

Functionalization of Titania with Caffeic Acid

A 30 mg portion of titania was added to a solution of 40 mg of caffeic acid in 20 mL of 50% v/v aqueous ethanol. The mixture was left for 48 h. Then 4 mL of the mixture was centrifuged at 8K RPM for 10 minutes, and the precipitate was washed with DI water (4 mL X 3), using centrifugation on each step. The titania-caffeic acid conjugate was stored in 2.5 mL of water.

Synthesis of Silica Nanoparticles

Silica nanoparticles (100 nm average size) were prepared according to a known procedure (41, 42). Briefly, in a plastic flask, 2 mL of TEOS were added to 20 mL of absolute ethanol at stirring. After another 15 min, 2 mL of 30% ammonium hydroxide were added, and the mixture was stirred for 2 h. The white turbid solution of silica nanoparticles was centrifuged at 8K RPM for 10 minutes, and the precipitate was washed with absolute ethanol (10 mL X 3), using centrifugation on each step. The silica nanoparticles were suspended in 15 mL of absolute ethanol and stored in a plastic vial.

Carboxylation of Silica Nanoparticles

Carboxylated silica nanoparticles were prepared by amination of silica nanoparticles followed by the reaction with succinic anhydride, according to the known method (43). Briefly, 15 mL of the ethanol suspension of silica nanoparticles, 150 μ L of APTES, and 50 μ L of 30% aqueous ammonium hydroxide was stirred overnight. The white turbid solution of silica nanoparticles was centrifuged at 8K RPM for 10 minutes, and the precipitate was washed with absolute ethanol (10 mL X 3), then DMF (dimethylformamide) (10 mL X 2) using centrifugation on each step. This material (aminated silica nanoparticles) was carboxylated by stirring overnight with a solution of 0.266 mg of succinic anhydride in 10 mL DMF. The mixture was centrifuged at 8K RPM for 10 minutes, and the precipitate was washed with DMF (10 mL X 3) using centrifugation on each step.

Modification of a Toothpaste

A solution of 0.225 g potassium citrate (a desensitizing agent (44–46) that can inhibit tooth pain via its capability of passing through the exposed dentin tubules to reach the tooth nerve) and 0.155 g citric acid in 0.5 mL of DI water was added to 4.5 g of a titania-containing toothpaste (Crest®) at stirring. Next, the mixture was thoroughly mixed with 0.022 g of caffeic acid.

Photodegradation of Caffeic Acid

A solution of ~0.035 g of caffeic acid and an additive (~0.57 g of KH_2PO_4 , or 1.1 g of β -cyclodextrin or 0.2 g of chondroitin, or no additive) in 10 mL of 55% v/v aqueous ethanol was stirred overnight with 30 mg of titania in a light protected quartz tube. The quartz tube was subjected to UV-light at 15 cm from a 300 watt solar simulator under magnetic stirring. After the specified time, a 0.5 mL sample was withdrawn from the reaction tube and centrifuged at 8000 RPM for 10 min. A 0.03 mL portion of the supernatant solution was diluted with DI water to 4 mL, and concentration of caffeic acid was determined photometrically.

Acknowledgments

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References

1. Liu, J.; Lu, Y. Preparation of aptamer-linked gold nanoparticle purple aggregates for colorimetric sensing of analytes. *Nat. Protoc.* **2006**, *1*, 246–252.
2. Lu, A.-H.; Salabas, E. L.; Schüth, F. Magnetic Nanoparticles: Synthesis, Protection, Functionalization, and Application. *Angew. Chem., Int. Ed.* **2007**, *46*, 1222–1244.
3. Wang, H.; Lin, W.; Fritz, K. P.; Scholes, G. D.; Winnik, M. A.; Manners, I. Cylindrical Block Co-Micelles with Spatially Selective Functionalization by Nanoparticles. *J. Am. Chem. Soc.* **2007**, *129*, 12924–12925.
4. Westcott, S. L.; Oldenburg, S. J.; Lee, T. R.; Halas, N. J. Formation and Adsorption of Clusters of Gold Nanoparticles onto Functionalized Silica Nanoparticle Surfaces. *Langmuir* **1998**, *14*, 5396–5401.
5. Li, H.; Rothberg, L. Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14036–14039.
6. Warner, M. G.; Hutchison, J. E. Linear assemblies of nanoparticles electrostatically organized on DNA scaffolds. *Nat. Mater.* **2003**, *2*, 272–277.
7. Kim, B.; Sigmund, W. M. Functionalized Multiwall Carbon Nanotube/Gold Nanoparticle Composites. *Langmuir* **2004**, *20*, 8239–8242.
8. He, F.; Fan, J.; Ma, D.; Zhang, L.; Leung, C.; Chan, H. L. The attachment of Fe₃O₄ nanoparticles to graphene oxide by covalent bonding. *Carbon* **2010**, *48*, 3139–3144.
9. Zhang, S.; Wang, N.; Yu, H.; Niu, Y.; Sun, C. Covalent attachment of glucose oxidase to an Au electrode modified with gold nanoparticles for use as glucose biosensor. *Bioelectrochemistry* **2005**, *67*, 15–22.
10. Reyes-Coronado, D.; Rodríguez-Gattorno, G.; Espinosa-Pesqueira, M. E.; Cab, C.; Coss, R. d.; Oskam, G. Phase-pure TiO₂ nanoparticles: anatase, brookite and rutile. *Nanotechnology* **2008**, *19*, 145605.

11. Yan, W.; Chen, B.; Mahurin, S. M.; Schwartz, V.; Mullins, D. R.; Lupini, A. R.; Pennycook, S. J.; Dai, S.; Overbury, S. H. Preparation and Comparison of Supported Gold Nanocatalysts on Anatase, Brookite, Rutile, and P25 Polymorphs of TiO₂ for Catalytic Oxidation of CO. *J. Phys. Chem. B* **2005**, *109*, 10676–10685.
12. Serpone, N.; Dondi, D.; Albini, A. Inorganic and organic UV filters: Their role and efficacy in sunscreens and suncare products. *Inorg. Chim. Acta* **2007**, *360*, 794–802.
13. Topsoe, N. Y.; Topsoe, H.; Dumesic, J. A. Vanadia/Titania Catalysts for Selective Catalytic Reduction (SCR) of Nitric-Oxide by Ammonia: I. Combined Temperature-Programmed in-Situ FTIR and On-line Mass-Spectroscopy Studies. *J. Catal.* **1995**, *151*, 226–240.
14. Stangland, E. E.; Stavens, K. B.; Andres, R. P.; Delgass, W. N. Characterization of Gold–Titania Catalysts via Oxidation of Propylene to Propylene Oxide. *J. Catal.* **2000**, *191*, 332–347.
15. Shannon, M. A.; Bohn, P. W.; Elimelech, M.; Georgiadis, J. G.; Marinas, B. J.; Mayes, A. M. Science and technology for water purification in the coming decades. *Nature* **2008**, *452*, 301–310.
16. Lachheb, H.; Puzenat, E.; Houas, A.; Ksibi, M.; Elaloui, E.; Guillard, C.; Herrmann, J.-M. Photocatalytic degradation of various types of dyes (Alizarin S, Crocein Orange G, Methyl Red, Congo Red, Methylene Blue) in water by UV-irradiated titania. *Appl. Catal., B* **2002**, *39*, 75–90.
17. Vallet-Regí, M.; Balas, F.; Arcos, D. Mesoporous Materials for Drug Delivery. *Angew. Chem., Int. Ed.* **2007**, *46*, 7548–7558.
18. Wu, K. C. W.; Yamauchi, Y.; Hong, C.-Y.; Yang, Y.-H.; Liang, Y.-H.; Funatsu, T.; Tsunoda, M. Biocompatible, surface functionalized mesoporous titania nanoparticles for intracellular imaging and anticancer drug delivery. *Chem. Commun.* **2011**, *47*, 5232–5234.
19. Sereda, G., Application of Mesoporous Materials for Drug Delivery. In *Comprehensive Guide for Mesoporous Materials*; Aliofkhazraei, M., Ed.; Nova Science Publishers: New York, 2015; Vol. 4, pp 151–166.
20. Vogel, R.; Meredith, P.; Kartini, I.; Harvey, M.; Riches, J. D.; Bishop, A.; Heckenberg, N.; Trau, M.; Rubinsztein-Dunlop, H. Mesostructured Dye-Doped Titanium Dioxide for Micro-Optoelectronic Applications. *ChemPhysChem* **2003**, *4*, 595–603.
21. Jakob, M.; Levanon, H.; Kamat, P. V. Charge Distribution between UV-Irradiated TiO₂ and Gold Nanoparticles: Determination of Shift in the Fermi Level. *Nano Lett.* **2003**, *3*, 353–358.
22. Botta, C.; Labille, J.; Auffan, M.; Borschneck, D.; Miche, H.; Cabié, M.; Masion, A.; Rose, J.; Bottero, J.-Y. TiO₂-based nanoparticles released in water from commercialized sunscreens in a life-cycle perspective: Structures and quantities. *Environ. Pollut.* **2011**, *159*, 1543–1550.
23. Li, W.; Wang, Y.; Lin, H.; Ismat Shah, S.; Huang, C. P.; Doren, D. J.; Rykov, S. A.; Chen, J. G.; Barteau, M. A. Band gap tailoring of Nd³⁺-doped TiO₂ nanoparticles. *Appl. Phys. Lett.* **2003**, *83*, 4143–4145.

24. Choi, Y.-S.; Kim, B.-W. Photocatalytic disinfection of E coli in a UV/TiO₂-immobilised optical-fibre reactor. *J. Chem. Technol. Biotechnol.* **2000**, *75*, 1145–1150.
25. Li, Q.; Luo, G.; Feng, J. Direct Electron Transfer for Heme Proteins Assembled on Nanocrystalline TiO₂ Film. *Electroanalysis* **2001**, *13*, 359–363.
26. Barreto, W. J.; Ando, R. A.; Estevão, B. M.; Zanoni, K. P. d. S. Adsorption of caffeic acid on titanium dioxide: A spectroscopic study. *Spectrochim. Acta, Part A* **2012**, *92*, 16–20.
27. Kerns, D. G.; Scheidt, M. J.; Pashley, D. H.; Horner, J. A.; Strong, S. L.; Van Dyke, T. E. Dentinal Tubule Occlusion and Root Hypersensitivity. *J. Periodontol.* **1991**, *62*, 421–428.
28. Pashley, D. H. Dentin permeability, dentin sensitivity, and treatment through tubule occlusion. *J. Endod.* **1986**, *12*, 465–474.
29. Sarkar, N. K.; Caicedo, R.; Ritwik, P.; Moiseyeva, R.; Kawashima, I. Physicochemical Basis of the Biologic Properties of Mineral Trioxide Aggregate. *J. Endod.* **2005**, *31*, 97–100.
30. Fujisawa, R.; Wada, Y.; Nodasaka, Y.; Kuboki, Y. Acidic amino acid-rich sequences as binding sites of osteonectin to hydroxyapatite crystals. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1996**, *1292*, 53–60.
31. Koutsopoulos, S.; Dalas, E. The effect of acidic amino acids on hydroxyapatite crystallization. *J. Cryst. Growth* **2000**, *217*, 410–415.
32. Andary, C.; Andre, P. Derivative of caffeic acid, orapoxide, and cosmetic or pharmaceutical compositions, in particular dermatological compositions, containing it. U.S. Patent 5,719,129, February 17, 1998.
33. Xiao, J.; Chen, W.; Wang, F.; Du, J. Polymer/TiO₂ Hybrid Nanoparticles with Highly Effective UV-Screening but Eliminated Photocatalytic Activity. *Macromolecules* **2013**, *46*, 375–383.
34. Carlotti, M. E.; Ugazio, E.; Sapino, S.; Peira, E.; Battaglia, L.; Cavalli, R. Photodegradation of Caffeic Acid in W/O/W Emulsions in the Absence and in the Presence of TiO₂. *J. Dispersion Sci. Technol.* **2008**, *29*, 1435–1444.
35. Parrino, F.; Augugliaro, V.; Camera-Roda, G.; Loddo, V.; López-Muñoz, M. J.; Márquez-Álvarez, C.; Palmisano, G.; Palmisano, L.; Puma, M. A. Visible-light-induced oxidation of trans-ferulic acid by TiO₂ photocatalysis. *J. Catal.* **2012**, *295*, 254–260.
36. Kandiah, K.; Venkatachalam, R.; Wang, C.; Valiyaveetil, S.; Ganesan, K. In vitro and preliminary in vivo toxicity screening of high-surface-area TiO₂-chondroitin-4-sulfate nanocomposites for bone regeneration application. *Colloids Surf., B* **2015**, *128*, 347–356.
37. Kim, J.-W.; Nichols, W. T. Hierarchically assembled titania-cyclodextrin nano-networks. *Mater. Lett.* **2012**, *67*, 11–13.
38. Wang, G.; Wu, F.; Zhang, X.; Luo, G.; Deng, N. Enhanced Photocatalytic Degradation of Bisphenol F by β -cyclodextrin in aqueous TiO₂ dispersion. *Fresenius Environ. Bull.* **2006**, *15*, 61–67.
39. Rashwan, K.; Sereda, G.; Kilin, D. Adsorption patterns of caffeic acid on titania: affinity, charge transfer and sunscreen applications. *Mol. Phys.* **2015**, 1–11.

40. Baransi, K.; Dubowski, Y.; Sabbah, I. Synergetic effect between photocatalytic degradation and adsorption processes on the removal of phenolic compounds from olive mill wastewater. *Water Res.* **2012**, *46*, 789–798.
41. Rossi, L. M.; Shi, L.; Quina, F. H.; Rosenzweig, Z. Stöber Synthesis of Monodispersed Luminescent Silica Nanoparticles for Bioanalytical Assays. *Langmuir* **2005**, *21*, 4277–4280.
42. Banerjee, S.; Das, J.; Santra, S. Native silica nanoparticle catalyzed anti-Markovnikov addition of thiols to inactivated alkenes and alkynes: a new route to linear and vinyl thioethers. *Tetrahedron Lett.* **2009**, *50*, 124–127.
43. Rashwan, K.; Brakke, E.; Sereda, G. Fluorescent labeling of materials using silica nanoparticles. *Nanotechnol. Rev.* **2014**, *3*, 591.
44. Norfleet, J.; Carter, W. J.; Frankel, M. J.; Gaffar, A. Desensitizing anti-tartar dentifrice. U.S. Patent 5,352,439, October 4, 1994.
45. Shen, S.-Y.; Tsai, C.-H.; Yang, L.-C.; Chang, Y.-C. Clinical efficacy of toothpaste containing potassium citrate in treating dentin hypersensitivity. *J. Dent. Sci.* **2009**, *4*, 173–177.
46. Yates, R.; Ferro, R.; Newcombe, R. G.; Addy, M. A comparison of a reformulated potassium citrate desensitising toothpaste with the original proprietary product. *J. Dent.* **2005**, *33*, 19–25.

Chapter 6

Nanostructured Organosilica Hybrids as Highly Efficient and Regenerable Sorbents for Rare Earth Extraction

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Due to the rapidly growing energy demand and increasing production of high technology devices, the development of new sequestration materials for rare earth elements (REEs) has become critical. Nowadays, lanthanides (Ln) play a major role as supplies for the transition to cleaner energy and production of economically important modern devices, like wind turbines (Pr, Nd, Sm, Dy), batteries (La), fluorescent and luminescent phosphor lamps (La, Gd, Tb, Eu, Yb), car catalysts (Ce) or hybrid vehicles (Dy, La, Nd). However, for all these applications, only a high purity fraction of a single rare earth element can be used. Frequently, the presence of other REEs as contaminants in a pure single-element REE fraction alters the efficiency of electronic/optic devices. Although, several methods have been used for REEs extraction, such as liquid-liquid extraction (LLE) or liquid-solid extraction (supported liquid-liquid extraction, SLE, solid-phase extraction, SPE), the selective separation and purification of REEs still remain challenging. Mostly, the difficulties arise from the fact that these metals have very similar chemical properties. Among various extracting agents used for lanthanide separation, the diglycolamide (DGA)-based materials have attracted great attention as some of the most effective agents. In this contribution, we review our advances concerning the development of mesoporous hybrid sorbents for REEs. In particular, series of DGA-modified mesoporous silica (KIT-6)

sorbents were synthesized and tested for the REEs separation. Moreover, it has been proposed that by tuning the ligand *bite angle* and/or its environment it becomes possible to tune the extraction selectivity.

Introduction

Nowadays, the natural REEs resources are very limited and most of the common extraction/separation processes industrially used are not adequate for sustainable development, and current green chemistry trends (1). Industrially, the extraction and purification of REEs are commonly based on multiple liquid-liquid extractions (LLE) or chromatographic-based resins separation techniques, such as *supported* liquid-liquid extraction (SLE) or solid-phase extraction (SPE). However, the extraction chromatographic-based (EXC) resins, a branch of the SLE family, are plagued with several issues, such as lack of reusability, degradation of stationary phase, elution of organic phase or high risk of sample cross-contamination. On the other hand, the main drawback associated to ion-exchange type resins (IEC) is related to the lack of selectivity (2). Consequently, new materials with well-developed structure, high extraction capacity, selectivity and stability, are rapidly needed. In this context, we believe that well-designed selective chelating ligands, properly attached to mesoporous silica supports, should provide a better and greener alternative to the commercial resins. In our recent studies, we showed that phosphorous-modified mesoporous materials (e.g., KIT 6 P, SBA 15 P sorbents) can be considered as potential sorbents for the efficient extraction and separation of actinides (3, 4). As previously reported, these sorbents show very fast adsorption (with equilibrium reached within 1 min) and high uranium uptake, being around 10000 mL g⁻¹ (3). Moreover, these materials exhibit good stability in the extraction condition tested (pH=4, HNO₃, 30 min extraction time), which allowed us to recycle these sorbents several times with no loss of extraction performance. Among different supports tested for actinide extraction, the functionalized KIT-6 material showed the highest extraction capacity, and therefore this material was used in our supplementary studies. KIT 6 mesoporous silica can be regarded as a three-dimensional (cubic) analog of SBA-15 silica material with well-ordered large cylindrical pores, high surface area and large pore volume (5). Similar to its SBA-15 counterpart, the pores of KIT-6 material can easily be tuned by varying the synthesis conditions, such as aging time and/or temperature (6). Based on these studies, we developed a new family of sorbents for selective separation of REE elements. In that case, we used large-pore KIT-6 material modified with DGA-functionalities (i.e., diglycolamide-based ligands) and obtained sorbents with enhanced selectivity towards heavier REEs, in comparison to the commercially available products (7, 8). Moreover, the investigation of the sequestration capability of these mesoporous sorbents revealed that adsorption equilibrium was achieved much faster than with commercial EXC resins (<1 min vs. 10 min) (9). Additionally, the synthesized materials displayed better selectivity towards targeted lanthanides than other competitive elements, such as

Al, Fe U or Th ions, which commonly co-exist with lanthanides. Our subsequent studies also demonstrated that by tuning the ligand composition and structure (i.e., the ligand *bite angle*) it is possible to tune the REE coordination environment and, consequently, shifts the REE selectivity of the sorbents (8).

Research Background

The rare earth elements are crucial for the transition to a green and low-carbon economy, owing to their essential role in permanent magnets, phosphors lamps, rechargeable NiMH batteries or catalysts, to name a few only (10). Recent growing needs of REEs (especially needs of heavier elements, HREEs) increased the demand and price of REEs (11, 12). Additionally, the difficult situation of the REEs market has been recently acknowledged by several government agencies across the globe. The EU commission, for instance, considers the REEs as the most critical raw material group with the highest supply risk (13). Furthermore, in the statement from 2011 from the US Department of Energy, it can be found that among 13 critical elements, five are heavy lanthanides (14). They are considered as critical because of the high risk of supply interruptions. The main manufacturer of REEs is China that produces more than 90% of all REEs. However, China recently tightened its REEs export quota from ~50,000 tons in 2009 to only 31,000 tons in 2012 (15). Moreover, despite all this information, the commercial recycling of REEs is unfortunately still in its infancy. For instance, fewer than 3% of the REEs were being recycled in 2011 (16). The traditional route for the recovery of REEs is through the hydrometallurgy process. In general, in the first step the minerals or ores are subjected to the acidic or basic treatments. Most often, the leaching process is not selective and beside the targeted elements huge amounts of the competing metals are also extracted. Therefore, often additional purification steps are needed prior to the lanthanide separation. Moreover, the acidic or basic digestion is usually time-consuming and creates large volumes of non-recyclable and environmentally harmful reagents, such as H₂SO₄, NaOH or HF. Then, the real separation procedure of REEs starts after this leaching process. As mentioned previously, the liquid-liquid extraction technique is viewed as one of the most predominant methods for the separation of rare earth metals. Nevertheless, this approach is often hampered by the lack of selectivity and consumption of the large amount of organic solvents. Therefore, researchers proposed to use the ionic liquids (ILs) as a better alternative to the conventional solvents (17). However, this separation method is also limited by several factors, such as low solubility of different extractants in ILs, especially phosphorous-based ligands. Sometimes, it is also difficult to recover the metal species due to the strong interactions between ILs and targeted metals (18, 19). Differently, in the solid-liquid extraction systems, one of the liquid phases is reduced/eliminated and therefore this approach should be considered as a greener alternative to the LLE process. Nowadays, ion-exchange or extraction resins are commonly used for REEs separation/purification. For instance, it has been demonstrated that ammonium citrate / citric acid loaded onto Amberlite support can effectively extract REEs. Furthermore, REEs separation was achieved owing

to different stability of REEs complexes with slightly different REEs ionic radius (20). Recently, SLE resins containing impregnated ligands have been used for REE purification (21, 22). Among them, the diglycolamide (DGA) resin which consists of a DGA derivative (tetraoctylDGA) impregnated on a solid support is commonly used for separation of REEs from other elements (23). Unfortunately, as the ligand in this system is only supported (i.e., adsorbed) on the stationary phase, leaching into the aqueous phase is frequently observed. As a consequence, these materials have limited reusability. To overcome this problem, Zhang and coworkers adsorbed a DGA-polymer composite on macroporous silica support (24). In addition, it is well-documented that small particles lead to significant improvement of efficiency and shorten the analysis time. In relation to the particle size, efficiency is inversely proportional to the particle size (diameter); therefore when the particle size decreases, an enhancement in terms of peak efficiencies and resolution is observed (25). In contrast, retention capacity is directly proportional to the column length. Nevertheless, it is very difficult to build well-packed chromatographic columns with small particle size which lead to often occurring back-pressure and permeability problems. Ordered mesoporous materials with well-developed porous structure could provide better solvent transport in the packed-bed column systems, and therefore, they could solve transportation/pressure problems. Further, the high surface area of these materials combined with a high density of OH groups on the pore surfaces provides an easy way for functionalization, which in turns increases the overall sorption capacity of these materials. The density of the functional groups, introduced by a post-synthesis grafting step, is easily tunable by varying the conditions of the surface modification process. In our studies, we modified the silica support with diglycolamide derivative (in analogy to the commercially available products) by post-synthesis modification using a DGA-functionalized silane (26), and tested these materials for the separation of lanthanides in batch extraction conditions.

Sorbent Synthesis and Characterization

In the first step, high quality mesoporous KIT-6 material was synthesized as described by Kleitz et al. (5). After synthesis and template removal, the support was subjected to DGA-modification, whereby the organic functionalities were introduced either in one step or in a step-by-step approach (7). In case of the one-step modification procedure, 3-aminopropyltriethoxysilane (APTS) was first reacted with diglycolyl chloride (DGACl) and this modified silane was further attached to the silica support (material label as KIT-6-N-DGA-1). Differently, in the 2-step modification sequence, the KIT-6 material was first modified with APTS followed by a reaction with DGACl (material noted as KIT-6-N-DGA-2). In case of the former material, beside the targeted functional groups, some residual unmodified amino groups can also be present on the silica surface (Figure 1).

The structure and chemical composition of the mesoporous sorbents were validated using several techniques, such as solid state NMR, thermogravimetric and elemental analyses, N₂ sorption analysis at 77 K, and electron microscopy

studies (SEM/TEM). According to the thermogravimetric analysis (TGA), both materials possess almost the same amount of organic functional groups, with a mass loss being around 24 wt%. The carbon solid state (^{13}C) NMR analysis confirmed that all carbon atoms of the DGA ligand are present in the modified materials, suggesting that no ligand damage or cleavage of the ligand occurred upon the functionalization procedure.

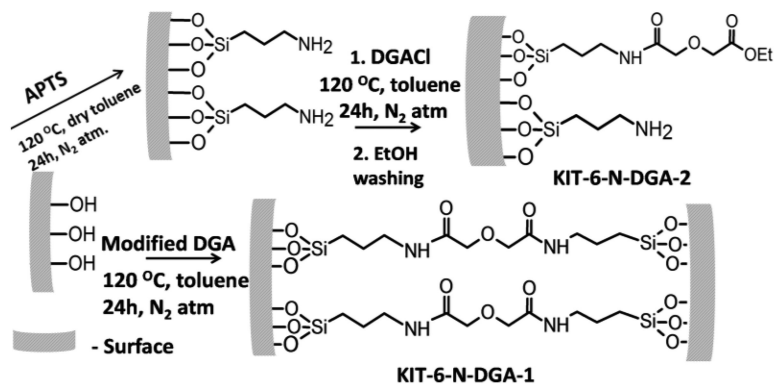


Figure 1. Schematic representation of the one- and two-step KIT-6 surface modification. Reproduced with permission from ref. (7). Copyright 2014 Wiley.

A first difference between one- and two-step modified materials was observed by the ^{13}C CP and ^{29}Si MAS NMR analysis. For instance, KIT-6-N-DGA-2 showed more a pronounced peak associated with the ethoxy species from the grafted silane, in line with the lower amount of T³ ((SiO)₃SiR) species on the silicon NMR spectrum, in comparison to the material modified in one-step procedure. The thermogravimetric analysis, similarly to solid state NMR analysis, exposed again some differences between these two samples. The one-step modified sample exhibits, for instance, slightly higher decomposition temperature (356 °C) than the material modified step-by-step (345 °C). This difference most probably can be associated with some residual (unmodified) amino groups that decomposed at lower temperature (310 °C) in case of the latter sorbent. As expected, the introduction of the functional group decreased the values of specific surface area, pore size and pore volume of the mesoporous sorbents (Figure 2a). The highest drop of these parameters was observed for the step-by-step modified material (see Table 1 and Figure 2a). Furthermore, high resolution (HR) TEM (Figure 2b) and (HR) SEM analyses validated the well-ordered cubic pore structure of the sorbents and showed maintained open mesopores, even after several grafting/modification steps.

Table 1. Physicochemical Parameters Derived from Low Temperature N₂ Physisorption Measurements for the Different Samples

Sample	S_{BET} (m ² g ⁻¹)	V_{pore} (cm ³ g ⁻¹)	Pore size (nm)
KIT-6	831	1.17	8.4
KIT-6-N	392	0.68	7.8
KIT-6-N-DGA-1	621	0.81	7.6
KIT-6-N-DGA-2	351	0.57	7.0

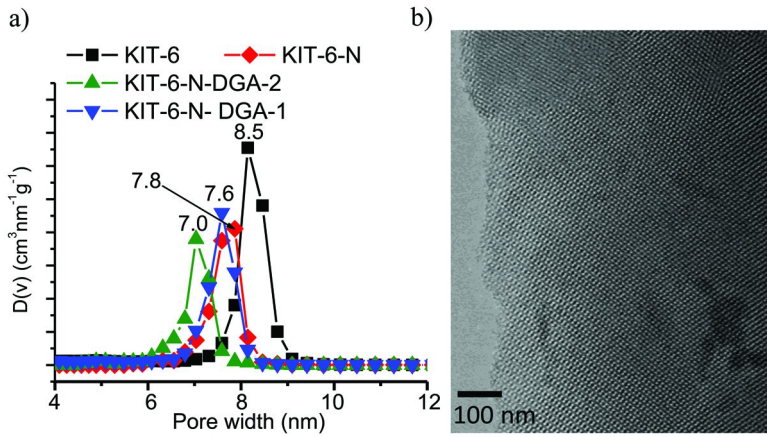


Figure 2. (a) Pore size distribution plots for pristine and modified materials and (b) TEM image of KIT-6-N-DGA-1 silica sorbent (Prof. R. Ryoo, Dr. Y. Seo (KAIST, South Korea) are acknowledged for high resolution-TEM analysis).

Performance Studies

The performances of our materials were checked in batch extraction tests from the mixture of all REE elements and the results can be seen in Figure 3a. The results are presented as the function of atomic number (Z) vs. the distribution coefficient (K_d). The K_d were calculated based on the following equation (Eq. 1.):

$$K_d = \frac{(C_i - C_f)}{C_f} \times \frac{V}{m}$$
 (Eq. 1.)

Where, C_i and C_f are initial and final concentration, respectively; and V and m are volume of the solution and mass of sorbent taken for the batch extraction tests, respectively.

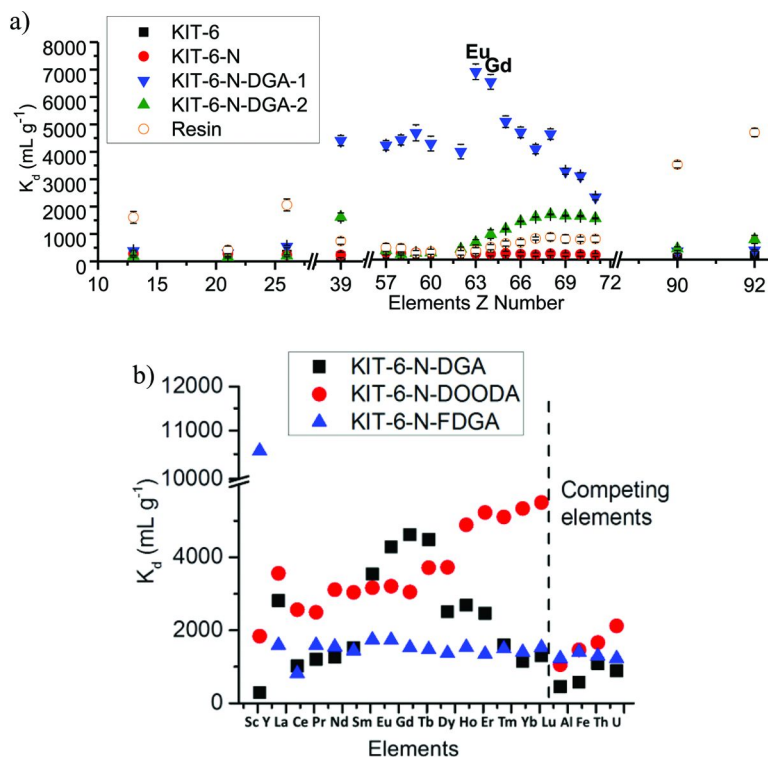


Figure 3. (a) Distribution coefficient (K_d) values and standard deviations for a mixture of all the lanthanides and competitive elements for the mesoporous sorbents and commercial resin, as a function of the atomic number Z . Reproduced with permission from ref. (7). Copyright 2014 Wiley. (b) Comparison of distribution coefficients (K_d) for all lanthanides and competing elements, for different silica sorbents.

According to the literature, the K_d value directly describes the quality of the sorbent (27). In general, the higher the K_d value, the better is the extraction capacity. For instance, materials with good extraction performances are characterized by the K_d values above 5000 mL g^{-1} , while outstanding sorbents display distribution coefficients at about 50000 mL g^{-1} . As it can be seen from Figure 3, the modification with the DGA-type ligand significantly increased the overall uptake of lanthanides, in comparison with non- or amino-modified reference materials. Interestingly, significant differences in the extraction selectivity were observed between the one-step and two-step functionalized materials. For example, much higher extraction capacity and sharper selectivity towards mid-size elements were observed for the KIT-6-N-DGA-1 material than for the two-step functionalized counterpart. Yet, both materials retain much higher K_d values than commercially available DGA resin. Furthermore, it has been shown that the synthesized mesoporous sorbents also possess better selectivity towards targeted species, such as lower uptake of other competing elements

(e.g., Al, Fe, U or Th ions), than amino-modified material or the commercial product. In case of the one-step functionalized samples, the separation factor between Eu/Th and Eu/U was 6.3 and 3.4, respectively. The separation factors between Europium and radioactive elements were calculated from the ratio of the distribution coefficients obtained for each radioactive element in the batch extraction test from the mixture of all elements. Such high separation factors were not observed for other materials. Indeed, in case of the commercial product, the uptake of competing elements were even higher than lanthanides entrapment. For example, the commercial resin displayed higher uptake of Uranium ions than targeted REEs, in the extraction conditions tested (batch extraction in pH=4, HNO₃, 30 min extraction). In addition to magnified extraction capacity and enhanced selectivity, the mesoporous sorbents exhibit also significant stability after extraction. The mesoporous hybrid materials could be reused several times with no significant loss in the extraction performance or decrease in the amount of ligand attached. Several methods, such as N₂ sorption analysis at 77 K, thermogravimetric analysis or solid state NMR, did not reveal any changes in the structure of the sorbent and/or ligand, even after 5 loading-release cycles. Such an increased extraction stability of the DGA-functionalized silica sorbents can be attributed to the chemically bonded ligand through the formation of the siloxane bonds (Si-O-Si-DGA) which appear stable and resistant in the extraction conditions tested.

Recently, we also varied the synthesis of the DGA-chelating ligand and obtained a family of analogs of the DGA compound, such as FDGA (furan-2,4-diamido-propyltriethoxysilane) and DOODA (3,6-dioxaoctanediamido-propyltriethoxysilane) ligands that display different *bite angle* compared to the previously synthesized DGA-ligand. These recent results show that REEs selectivity was changed depending on the ligand attached to the silica support (8). For instance, in comparison to the KIT-6-N-DGA-1 material, the sorbent modified with DOODA ligand (KIT-6-N-DOODA) showed higher selectivity towards heavier lanthanides, in agreement with a smaller bite angle of this ligand, i.e., smaller bite angle between ligand and La was observed for La-DOODA (57.90-58.27°) compared to La-DGA complexes (60.35-60.93°). On the other hand, KIT6-N-FDGA sorbent did not show particular selectivity towards lanthanides but revealed very high selectivity for Scandium (Figure 3b) (8).

Conclusions

Because of the broad range of applications of REEs and increasing energy demands, the ability to selectively separate lanthanides, rapidly and with high efficiency, from ores or commercial/industrial wastes is critically required. Clearly, methods used nowadays suffer from several limitations or fail under real process conditions. Mesoporous sorbents with chemically bonded selective ligands have higher ability to selectively capture REEs, in the presence of other competing elements, compared to the commercial resins. In addition, because of the grafting procedure used in the sorbent synthesis, these materials demonstrate a higher degree of reusability. Finally, it has been shown that tailoring the ligand

geometry and its environment also allows tuning the extraction selectivity of the sorbents. All these attributes make these materials ideal candidates in the perspective of developing new environmental remediation technologies and critical resource recovery processes without necessarily the need for multiple additional steps.

References

1. Massari, S.; Ruberti, M. Rare earth elements as critical raw materials: Focus on international markets and future strategies. *Resour. Policy* **2013**, *38*, 36–43.
2. Larivière, D.; Cumming, T. A.; Kiser, S.; Li, C.; Cornett, R. J. Automated flow injection system using extraction chromatography for the determination of plutonium in urine by inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **2008**, *23*, 352–360.
3. Lebed, P. J.; de Souza, K.; Bilodeau, F.; Larivière, D.; Kleitz, F. Phosphonate-functionalized large pore 3-D cubic mesoporous (KIT-6) hybrid as highly efficient actinide extracting agent. *Chem. Commun.* **2011**, *47*, 11525–11527.
4. Lebed, P. J.; Savoie, J. D.; Florek, J.; Bilodeau, F.; Larivière, D.; Kleitz, F. Large Pore Mesoporous Organosilica-Phosphonate Hybrids as Highly Efficient and Regenerable Sorbents for Uranium Sequestration. *Chem. Mater.* **2012**, *24*, 4166–4176.
5. Kleitz, F.; Choi, S. H.; Ryoo, R. Cubic Ia3d large mesoporous silica: synthesis and replication to platinum nanowires, carbon nanorods and carbon nanotubes. *Chem. Commun.* **2003**, 2136–2137.
6. Kleitz, F.; Bérubé, F.; Guillet-Nicolas, R.; Yang, C.-M.; Thommes, M. Probing Adsorption, Pore Condensation, and Hysteresis Behavior of Pure Fluids in Three-Dimensional Cubic Mesoporous KIT-6 Silica. *J. Phys. Chem. C* **2010**, *114*, 9344–9355.
7. Florek, J.; Chalifour, F.; Bilodeau, F.; Larivière, D.; Kleitz, F. Nanostructured Hybrid Materials for the Selective Recovery and Enrichment of Rare Earth Elements. *Adv. Funct. Mater.* **2014**, *24*, 2668–2676.
8. Florek, J.; Mushtaq, A.; Larivière, D.; Cantin, G.; Fontaine, F.-G.; Kleitz, F. Selective Recovery of Rare Earth Elements Using Chelating Ligands Grafted on Mesoporous Surfaces. *RSC Adv.* **2015**, *5*, 103782–103789.
9. Horwitz, E. P.; Dietz, M. L.; Diamond, H.; Maxwell, S. L.; Nelson, M. R. Separation and Preconcentration of Actinides by Extraction Chromatography Using a Supported Liquid Anion Exchanger: Application to the Characterization of High-Level Nuclear Waste Solutions. *Anal. Chim. Acta* **1995**, *310*, 63–78.
10. Tzanetakis, N.; Scott, K. Recycling of nickel–metal hydride batteries. I: Dissolution and solvent extraction of metals. *J. Chem. Technol. Biotechnol.* **2004**, *79*, 919–926.
11. Jordens, A.; Cheng, Y. P.; Waters, K. E. A review of the beneficiation of rare earth element bearing minerals. *Miner. Eng.* **2013**, *41*, 97–114.

12. Kanazawa, Y.; Kamitani, M. Rare earth minerals and resources in the world. *J. Alloys Compd.* **2006**, *408-412*, 1339–1343.
13. Binnemans, K.; Jones, P. T.; Blanpain, B.; Van Gerven, T.; Yang, Y.; Walton, A.; Buchert, M. Recycling of rare earths: a critical review. *J. Cleaner Prod.* **2013**, *51*, 1–22.
14. U.S. Department of Energy *Critical materials strategy. Congressional Research Service, Rare Earth Metals*; (December 2011) Parliamentary, DOE/PI-0009; January 10 2012; available at http://energy.gov/sites/prod/files/DOE_CMS2011_FINAL_Full.pdf (accessed November 2015).
15. Chen, Z. Global rare earth resources and scenarios of future rare earth industry. *J. Rare Earths* **2011**, *29*, 1–5.
16. Alonso, E.; Sherman, A. M.; Wallington, T. J.; Everson, M. P.; Field, F. R.; Roth, R.; Kirchain, R. E. Evaluating Rare Earth Element Availability: A Case with Revolutionary Demand from Clean Technologies. *Environ. Sci. Technol.* **2012**, *46*, 3406–3414.
17. Renner, R. Ionic liquids: An industrial cleanup solution. *Environ. Sci. Technol.* **2001**, *35*, 410A–413A.
18. Sun, X.; Luo, H.; Dai, S. Mechanistic investigation of solvent extraction based on anion-functionalized ionic liquids for selective separation of rare-earth ions. *Dalton Trans.* **2013**, *42*, 8270–8275.
19. Sun, X.; Waters, K. E. Development of Industrial Extractants into Functional Ionic Liquids for Environmentally Friendly Rare Earth Separation. *ACS Sustainable Chem. Eng.* **2014**, *2*, 910–1917.
20. Spedding, F. H.; Fulmer, E. I.; Butler, T. A.; Gladrow, E. M.; Gobush, M.; Porter, P. E.; Powell, J. E.; Wright, J. M. The Separation of Rare Earths by Ion Exchange. III. Pilot Plant Scale Separations. *J. Am. Chem. Soc.* **1974**, *69*, 2812–2818.
21. Kumar, B. N.; Radhika, S.; Reddy, B. R. Solid–liquid extraction of heavy rare-earths from phosphoric acid solutions using Tulsion CH-96 and T-PAR resins. *Chem. Eng. J.* **2010**, *160*, 138–144.
22. Dave, S. R.; Kaur, H.; Menon, S. K. Selective solid-phase extraction of rare earth elements by the chemically modified Amberlite XAD-4 resin with azacrown ether. *React. Funct. Polym.* **2010**, *70*, 692–698.
23. Zhang, A.; Mei, C.; Wei, Y.; Kumagai, M. Preparation of a Novel Macroporous Silica-based Diglycolamide Derivative-impregnated Polymeric Composite and its Adsorption Mechanism for Rare Earth Metal Ions. *Adsorpt. Sci. Technol.* **2007**, *25*, 257–272.
24. Zhang, A.; Mei, C.; Wei, Y.; Kumagai, M. Preparation of a Novel Macroporous Silica-Based Diglycolamide Derivative Impregnated Polymeric Composite and Its Adsorption Mechanism for Rare Earth. *Adsorpt. Sci. Technol.* **2007**, *25*, 257–272.
25. Nguyen, D. T.-T.; Guillarme, D.; Rudaz, S.; Veuthey, J.-L. Fast analysis in liquid chromatography using small particle size and high pressure. *J. Sep. Sci.* **2006**, *29*, 1836–1848.
26. Hoffmann, F.; Cornelius, M.; Morell, J.; Fröba, M. Silica-based mesoporous organic-inorganic hybrid materials. *Angew. Chem., Int. Ed.* **2006**, *45*, 3216–3251.

27. Lin, Y.; Fiskum, S. K.; Yantasee, W.; Wu, H.; Mattigod, S. V.; Vorpapel, E.; Fryxell, G. E.; Raymond, K. N.; Xu, J. Incorporation of Hydroxypyridinone Ligands into Self-Assembled Monolayers on Mesoporous Supports for Selective Actinide Sequestration. *Environ. Sci. Technol.* **2005**, *39*, 1332–1337.

Chapter 7

Advanced Materials and Membranes for Gas Separations: The UOP Approach

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Development of advanced membrane technologies for gas separations has gained significant attention in recent years due to the energy and environmental efficiency of membrane processes. This chapter reviews recent advances in gas separation membranes by considering the materials, membrane fabrication, membrane modules, and the industrial applications. Design of novel membrane materials and establishment of advanced membrane fabrication capability enabled breakthrough development of new UOP Separex™ Flux+ and Select membrane elements for natural gas upgrading and other gas separations. So far, over 170 UOP Separex™ membrane systems have been installed around the world for natural gas upgrading and H₂ purification. Development of new robust and high performance membranes with a nanometer scale selective layer is critical to the future success in membrane gas separation technologies.

Introduction

Membrane-based technologies have the advantages of both low capital cost and high energy efficiency compared to the other techniques such as cryogenic distillation, absorption and adsorption. Membranes, alone or in combinations with other technologies, provide a comprehensive approach for solving energy, environmental resource recovery, medical and many other technical problems. Membrane-based separation processes are widely adopted today in semiconductor, water, food, pharmaceutical, biotechnology industries and a wide range of

environmental applications. For seawater desalination, membrane technology or its combination in integrated systems has been a successful approach for solving the situation of freshwater demand in many regions of the world at lower costs and minimum environmental impact. Membranes are a factor of 10 times more energetically efficient than thermal options for water desalination (*1*). World-wide sales of membrane products and systems have more than doubled in a decade. The drive towards greater economic and environmental efficient separations will result in more aggressive future growth in membrane-based separations.

Gas Separation Membranes

Membrane-based technologies for non-aqueous feed separations, such as gas, vapor, and organic/organic liquid separations, are less mature than those for aqueous feed separations. Membranes have been barely commercialized for CO₂ removal from flue gas, olefin/paraffin separations, and aromatics separations. However, membrane gas separation for some large volume applications has rapidly become a competitive separation technology with the successful production of commercial asymmetric polymeric membranes. Different from conventional separation unit operations such as cryogenic distillation, membrane gas separation does not require a phase change. Moreover, the modular design and small footprint make membrane systems particularly attractive for remote natural gas upgrading applications such as off-shore gas-processing platforms, floating liquefied natural gas (FLNG), and floating, processing, storage and offloading (FPSO) vessel applications.

Typical polymeric membranes for gas separation applications are asymmetric membranes with either an integrally skinned or a thin-film composite structure. Such membranes are characterized by a thin, nanometer scale, dense, selectively semipermeable, selective layer and a less dense, void-containing (or porous), non-selective support layer, with pore sizes ranging from large in the support layer to very small in nanometer scale proximate to the selective layer.

Membrane gas separation is a pressure-driven process. The membrane gas separation performance is characterized by the flux of a gas component across the membrane. This flux can be expressed as permeance (P/L) or permeability (P). P/L is a pressure-normalized flux and P is a pressure- and thickness-normalized flux of a given gas component. The separation of a gas mixture is achieved by a membrane material that permits a faster permeation rate for one gas (i.e., higher permeability) over that of another gas. The efficiency of the membrane in enriching one gas component over another in the permeate stream can be expressed as a quantity called selectivity or separation factor. Selectivity (α) is defined as the ratio of the permeabilities of the gas components across the membrane (i.e., $\alpha_{A/B} = P_A/P_B$, where A and B are the two gas components). The permeability and selectivity of a gas separation membrane are the intrinsic properties of the membrane material, and thus these properties are ideally constant with feed pressure, flow rate and other process conditions. However, permeability and selectivity are both temperature-dependent.

The transport and separation in a polymeric gas separation membrane is based on a solution-diffusion mechanism which involves molecule-scale interactions of the permeating gas component with the polymer. The mechanism assumes that each feed gas dissolves into the membrane at one interface, transports by diffusion across the membrane through the voids between the polymer chains (or so-called free volumes) and desorbs at the other interface. According to this solution-diffusion mechanism, the permeation of gas components through a polymeric membrane is controlled by two parameters: the solubility coefficient (S) and the diffusivity coefficient (D). Both permeance and selectivity influence the economics of a gas separation membrane process. Selectivity is a key parameter to achieve high product purity at high recoveries. Membrane gas separation has the potential to grow enormously if higher selectivity membranes are available. To obtain high selectivity, either a membrane material with high intrinsic selectivity is needed or a membrane post-treatment process such as chemical or physical cross-linking is required to enhance the selectivity of the membrane. Furthermore, a defect-free selective layer that performs the separation is critical to achieve high selectivity. To obtain high membrane permeance, a membrane material with high intrinsic permeability is preferred. More importantly, the selective layer of gas separation membranes must be extremely thin. Therefore, a defect-free, thin, nanometer scale, dense, selective layer formed from a membrane material with high intrinsic selectivity and permeability will provide the gas separation membranes with both high selectivity and high permeance.

Most commercially available gas separation membranes are made from polymeric membrane materials developed in the 1970s and 1980s, which have reached their performance limits for applications where membranes are promising. New robust, high permeance and selectivity membranes are required for refinery, petrochemical, and natural gas processes that are simply not possible with traditional polymeric materials.

The separation properties of gas separation membranes not only depend upon membrane material, membrane morphology, membrane module and system design, but also rely on the pre-treatment of the feed gas. Failure of any of these aspects will result in the overall failure of membrane technology for gas separations. The choice of a membrane material for gas separation application is based on its specific physical and chemical properties. The membrane materials need to be tailored in an advanced way specific to a particular gas mixture. Moreover, robust materials are required for most of membrane gas separation processes. As an example, Honeywell UOP Separex™ membrane technology for natural gas upgrading removes acid gases such as CO₂ and H₂S from natural gas. The technology includes not only the membrane, but also the regenerable MemGuard™ feed pre-treatment system to remove water and heavy hydrocarbons from the feed gas before it is introduced to the membrane.

Figure 1 summarizes the key steps in the development of a robust UOP Separex™ spiral wound gas separation membrane process for acid gas removal from natural gas. Normally, the process starts with the identification of the market needs and targets setting for membrane performance. The current major challenge for UOP Separex™ commercial cellulose acetate (CA) membrane for CO₂/CH₄ separation is the relatively low CO₂/CH₄ selectivity that results in low natural

gas recovery. The market for natural gas upgrading needs new membranes with enhanced selectivity. Steps 2 and 3 involve the identification of a membrane material that can meet the target performance and proof-of-concept (POC) of the membrane that can perform the separation. To screen and identify the membrane material and measure the intrinsic separation properties, dense film membranes with a symmetric nonporous structure and a known thickness are used. Lab-scale, commercially viable, asymmetric flat sheet membranes are fabricated and tested after the selection of the target membrane material. Asymmetric flat sheet membrane has a very thin, nanometer scale, selective skin layer on a highly porous non-selective support layer (Figure 2). Normally, the porous non-selective support layer is supported on a fabric support backing. The porous non-selective support layer and the fabric support backing in the asymmetric flat sheet membrane provide the mechanical strength for the thin selective layer. Sometimes, a highly permeable, thin, nanometer scale, coating layer is applied to the surface of the thin, nanometer scale, selective skin layer to plug the minor defects and protect the nanometer scale selective layer. Extensive studies on formulations of membrane casting solutions, casting process conditions, and post-treatments are required to optimize the membrane performance to meet the performance targets. With the POC of the asymmetric membrane, a detailed business case will be established before moving from research experiments to next step development work for membrane scale-up and selection of a robust formula and fabrication process for membrane manufacturing. Preparation of spiral wound membrane modules from the optimized large-scale flat sheet membranes is needed to conduct field tests and study the effects of feed composition (CO_2 concentration), operation temperature, pressure, and time, and contaminants on the performance of the membrane in a spiral wound module. Moving from membrane research and development steps to the final step of membrane commercialization requires the successful demonstration of target membrane performance from the field tests on commercial-scale spiral wound membrane elements.

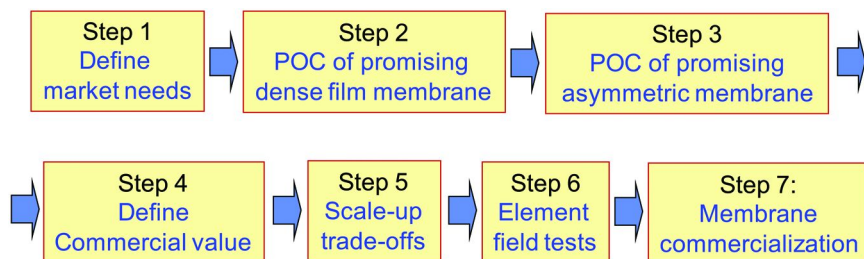


Figure 1. UOP Separex™ process for the development of gas separation membranes.

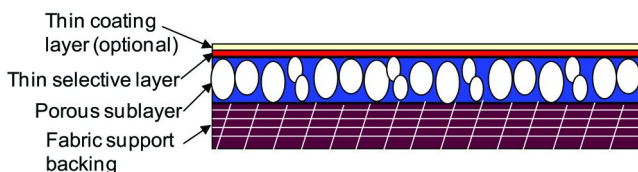


Figure 2. Schematic illustration of an asymmetric flat sheet membrane.

Commercial Gas Separation Membrane Materials

Polymeric materials have been used to make almost all the commercial gas separation membranes. Polymeric membranes have a number of advantages for gas separations including low cost, ease of processability, good mechanical stability and reasonably good selectivity and permeability. In the past, both rubbery polymers and glassy polymers had been used for making commercial polymeric membranes. Polymeric membrane-based gas separation processes have undergone a major evolution since the introduction of the first polymer membrane-based industrial hydrogen separation process by Permea (now a division of Air Products) in 1980. Permea used polysulfone polymer to produce PRISM® hollow fiber membrane for hydrogen separation and air separation (2). A list of the principal gas separation applications, producers, and types of membrane materials and modules is shown in Table 1.

The UOP Separex™ spiral wound membrane systems made from CA and new UOP proprietary polymers have been extensively used for acid gases and He removal from natural gas and are the leading membrane systems for this application. UOP also supplies Polysep™ hollow fiber membrane systems for H₂ separation processes. Over 170 gas separation membrane units have been successfully installed around the world by UOP since late 1990s. Cameron has been using cellulose triacetate hollow fiber membrane for CO₂ removal from natural gas (3). Ube Industries in Japan has commercial polyimide hollow fiber membranes for nitrogen separation from air (3). Air Products and Air Liquide also supply commercial hollow fiber membranes for air separation (3). GKSS and MTR have commercial silicone rubber polymer membranes for vapor/gas separation and other applications (3).

Advanced Gas Separation Membrane Materials

Despite the large number of polymeric materials investigated and developed by industry and academia for gas separation applications, the number of polymers used in commercial membrane system is still limited (4). High cost, lack of commercial availability, poor contaminant resistance, and poor processability are their key limitations. In particular, there is a well-known trade-off between permeability and selectivity (or so-called polymer upper bound limit) of polymeric membranes. By comparing the experimental data of hundreds of different polymers, Robeson demonstrated in 1991 that the selectivity and permeability of polymeric gas separation membranes were inseparably linked to one another, in

a relation where selectivity increases as permeability decreases and vice versa (5). In 2008, Robeson revisited the polymer upper bound for gas separations (6). Figure 3 shows Robeson’s 1991 and 2008 polymer upper bound relationships between CO₂ permeability and CO₂/CH₄ selectivity for over 300 glassy and rubbery polymers (5, 6).

Table 1. Membrane Gas Separation Applications, Producers, and Types of Membrane Materials and Modules

<i>Application</i>	<i>Company</i>	<i>Membrane Material</i>	<i>Membrane Module</i>
CO ₂ /CH ₄ , H ₂ /CH ₄ , He/CH ₄	UOP, Honeywell (Separex™, PolySep™)	Cellulose acetate, others	Spiral wound, Hollow fiber
CO ₂ /CH ₄	Cameron (Cynara®)	Cellulose triacetate	Hollow fiber
CO ₂ /CH ₄ , H ₂ /CH ₄ , O ₂ /N ₂	Ube	Polyimide	Hollow fiber
H ₂ separation, O ₂ /N ₂	Air Products (PRISM®)	Polysulfone	Hollow fiber
H ₂ separation, O ₂ /N ₂	Air Liquide (MEDAL™)	Polyimide/polyaramide	Hollow fiber
O ₂ /N ₂	Generon IGS	Tetrabromo polycarbonate	Hollow fiber
Vapor/gas, other	MTR	Silicone rubber, others	Spiral wound
Vapor/gas, air dehydration	GKSS	Silicone rubber	Plate-and frame, spiral wound

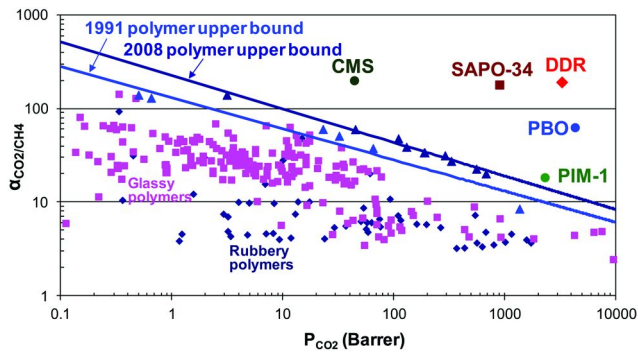


Figure 3. Polymer upper bound correlation and some advanced materials for CO₂/CH₄ separation. (Data are from references (5, 6, 8–12).)

Substantial research effort has been directed to overcoming the limits imposed by the polymer upper bound limit since 1991. Figure 3 shows some of the recent advanced polymeric membrane materials including high permeability polymers with intrinsic microporosity (PIMs) (7, 8) and polybenzoxazoles (PBOs) (9) for CO₂/CH₄ separation. These new polymeric membrane materials showed intrinsic permeabilities and selectivities above 1991 polymer upper bound limit for CO₂/CH₄ separation. They also showed improved intrinsic separation properties for other gas separation applications compared to conventional polymeric materials. However, no current commercial gas separation membranes made from PIM and PBO materials have been reported in the literature. This is mainly due to the fact that in addition to the significantly improved permeability and selectivity, the new polymeric materials must be cost effective and have good processability to form a stable, thin, nanometer scale, selective layer supported on a finely microporous substrate made from the same or different material that provides mechanical strength. The selectivity and permeability of inorganic membrane materials are not limited by the polymer upper bound limit. For example, microporous inorganic molecular sieves (e.g., DDR (10) and SAPO-34 (11)) and carbon molecular sieves (CMSs) (12) with well-defined micropores exhibited performance far above 2008 polymer upper bound limit for CO₂/CH₄ separation (Figure 3).

Polymers

Recently, high performance polymers such as PIMs, tetrazole-functionalized PIMs, and PBOs have been developed to make gas separation membranes. McKeown et al. reported the synthesis of PIMs with a randomly contorted molecular structure, bridging the void between inorganic microporous and polymeric membrane materials (7, 8). The rotational freedom of these PIM materials has been removed from the polymer backbone. These polymers exhibit properties analogous to those of conventional inorganic microporous materials including large and accessible surface areas, interconnected micropores of less than 2 nm in size, as well as high chemical and thermal stability. Moreover, these PIMs possess some favorable properties of conventional polymers including good solubility in certain organic solvents and relatively easy processability for the preparation of membranes. PIM dense film membranes have shown extremely high intrinsic gas permeabilities for the separation of commercially important gas pairs of O₂/N₂ and CO₂/CH₄ (Figure 3). The exceptionally high permeabilities of gases arise from the rigid but contorted molecular structures of PIMs. Membranes from PIMs, however, have much lower selectivities for gas separations such as for O₂/N₂ and CO₂/CH₄ separations, although their gas permeabilities are significantly higher than those of commercial polymeric membranes made from conventional polymers such as CA, polyimides, and polyetherimides.

Recently, Guiver et al. reported CO₂-philic tetrazole-functionalized PIM membrane materials (TZPIMs) for CO₂-capture applications (13). The TZPIM materials were prepared by [2+3] cyclo-addition modification of the aromatic nitrile groups on PIM-1 polymer to form tetrazole groups. The TZPIM dense film

membranes showed enhanced intrinsic CO₂-philic separation selectivities due to interactions between CO₂ and the tetrazole compared to PIM-1 membrane.

Aromatic PBOs, polybenzothiazoles (PBTs), and polybenzimidazoles (PBIs) are highly thermally stable ladderlike glassy polymers with flat, stiff, rigid-rod phenylene-heterocyclic ring units. The stiff, rigid ring units in such polymers pack efficiently, leaving very small penetrant-accessible free volume elements that are desirable for gas separation membranes with both high permeability and high selectivity. These aromatic PBO, PBT, and PBI polymers with high thermal and chemical stability, however, have poor solubility in common organic solvents, preventing them from being used as common materials for making membranes by the most practical solution casting or dip coating method. Thermal conversion of soluble aromatic polyimides containing pendent functional groups ortho to the heterocyclic imide nitrogen in the polymer backbone to aromatic PBOs or PBTs (14) could provide an alternative method for creating PBO or PBT polymer membranes that are difficult or impossible to obtain directly from PBO or PBT polymers by solvent casting method. Park et al. reported the preparation of PBO dense film membranes from polyimide polymer containing pendent hydroxyl groups ortho to the heterocyclic imide nitrogen via thermal rearrangement between 350 and 450 °C (9). These PBO dense films exhibited CO₂/CH₄ selectivity comparable to commercial CA polymer, but extremely high intrinsic CO₂ permeability (>1000 Barrers), which is over 100 times higher than that of CA and similar to that of some inorganic molecular sieve membranes. Therefore, if a PBO membrane is used instead of the conventional CA membrane with the same selective layer thickness for natural gas upgrading, it would require at least 100 times less membrane area.

These advanced high performance membrane materials such as PIM-1, TZPIM, and PBOs, however, have not been successfully used for the production of commercially viable asymmetric gas separation membranes with a very thin selective layer supported on a microporous substrate made from the same or a different material.

Inorganic Molecular Sieves and Zeolitic Imidazolate Frameworks (ZIFs)

Some zeolitic imidazolate frameworks (ZIFs) with zeolite topologies and inorganic molecular sieve membranes such as molecular sieve and carbon molecular sieve membranes have shown superior separation properties for gas separations. Under conditions where polymeric membranes cannot be used, inorganic molecular sieve and ZIF membranes have the potential for separations with high efficiency and high productivity by taking advantage of their superior thermal and chemical stability, good erosion resistance, and high plasticization resistance to condensable gases.

Membranes made from molecular sieve or ZIF materials with well-defined micropores provide separation properties mainly based on molecular sieving and/or surface diffusion mechanism. Separation with large pore molecular sieve or ZIF membranes is mainly based on surface diffusion when their pore sizes are much larger than the molecules to be separated. The selectivity of this type of

large pore molecular sieve or ZIF membranes is normally very low. Separation with small pore molecular sieve or ZIF membranes is mainly based on molecular sieving when the pore sizes are smaller or similar to one molecule but are larger than other molecules in a gas mixture to be separated. Some small-pore molecular sieve membranes, such as DDR (0.36-0.44 nm) (10) and SAPO-34 (0.38 nm) (11), have shown high selectivities for CO₂/CH₄ separation. These membranes possess pores that are similar in size to CH₄ but larger than CO₂, resulting in high CO₂/CH₄ selectivities. For example, a DDR-type membrane showed much higher CO₂ permeability and CO₂/CH₄ selectivity compared to the polymeric membranes (Figure 3) (10). SAPO-34 molecular sieve membranes also exhibited high selectivity for separation of certain gas mixtures, including mixtures of CO₂ and CH₄ (Figure 3) (11). Small pore ZIF-8 membranes showed very promising separation property for propylene/propane separation (15). Sub-micron thick ZIF-67 membranes displayed further improved propylene/propane separation performance compared to ZIF-8 membranes (16). Controlling the microstructures of the polycrystalline ZIF-67 membranes resulted in high propylene/propane selectivity.

The unique properties of microporous molecular sieve and ZIF materials combined with the continuous separation properties of membranes make molecular sieve and ZIF membranes very attractive for a wide range of gas separation applications. Molecular sieve membranes, however, have poor processability, low mechanical stability, micrometer scale thick molecular sieve separation layer, and are much more expensive than the commercial polymeric membranes with current state-of-the-art membrane manufacturing process. Further advancement in making molecular sieve and ZIF membranes with thinner selective separation layer (preferably in nanometer scale) and continuous improvement in membrane production techniques and reproducibility will make inorganic molecular sieve and ZIF membranes more successful in commercial gas separation applications.

Graphenes and Graphene Oxides

Recently, new graphene and graphene oxide membranes for gas separations have attracted significant attention. Graphene and graphene oxide are attractive membrane materials for the development of gas separation membranes due to their atomic thickness, excellent mechanical strength, and high chemical stability. Graphene is impermeable to all gases due to the electron density of its aromatic rings. However, researchers have explored both chemical and physical approaches to form nanometer-sized pores in the graphene material for membrane application. As an example, an UV-induced oxidative etching method has been used to create pores in graphene membranes and the transport of a range of gases such as H₂, CO₂, Ar, N₂, CH₄, and SF₆ through the pores has been measured (17). Most recently, Hang et al. reported the preparation of a thin, molecular-sieving graphene oxide membrane by a facile filtration process (18). The membrane showed significantly higher selectivity than inorganic molecular sieve membranes for H₂/CO₂ separation due to the formation of selective structure

defects within the graphene oxide flakes. However, significant challenges such as low permeance and large-scale membrane fabrication processability are still facing this new type of graphene and graphene oxide membranes for commercial gas separation applications.

Inorganic Molecular Sieve/Polymer; Metal-Organic Framework/Polymer; and Graphene/Polymer Composites

To overcome the drawbacks of polymeric membranes and inorganic membranes for gas separations, inorganic molecular sieve/polymer, metal-organic framework (MOF)/polymer, and graphene/polymer composite membranes, or called mixed matrix membranes, have been investigated. Inorganic molecular sieve/polymer, MOF/polymer, and graphene/polymer composite membranes combine the excellent transport properties of microporous molecular sieve, MOF, or graphene materials with the low cost and ease of processability of polymeric membranes. Inorganic molecular sieve/polymer, MOF/polymer, and graphene/polymer composite membranes comprise dispersed microporous inorganic molecular sieve particles, MOF particles, or graphene thin plates in a continuous organic polymeric matrix. The membrane may be fabricated with only minor adjustment to the existing polymeric manufacturing process. The concept of inorganic molecular sieve/polymer, MOF/polymer, and graphene/polymer composite membranes for gas separations with improved selectivity or/and greater permeability is to combine the solution-diffusion mechanism of polymeric membranes with the molecular sieving or surface diffusion mechanism of microporous molecular sieve, MOF, or graphene membranes.

The superior properties of mixed matrix membranes depend on the proper selection of the inorganic material such as the microporous molecular sieve material used as the dispersed phase and the polymeric material used as the continuous phase in the membranes. Both rubbery polymers and glassy polymers have been studied as the continuous polymer matrices in the mixed matrix membranes. Small pore microporous molecular sieves such as SAPO-34 and AIPO-18, as well as small pore MOF materials such as MAMS-1 have been used to prepare inorganic molecular sieve/polymer and MOF/polymer composite membranes for CO₂/CH₄ separation. Some of these composite membranes have shown remarkably improved intrinsic selectivities and permeabilities for gas separations compared to the polymeric membranes. However, most of the asymmetric mixed matrix membranes with a thin selective layer could not show any selectivity improvement due to the issues of poor compatibility and adhesion between the molecular sieve or MOF and the polymer, as well as the availability of large production scale nano-sized molecular sieve and MOF particles (19).

Successful synthesis of nano-sized inorganic molecular sieve or MOF particles without agglomeration and the development of novel approaches to uniformly disperse the nano-sized inorganic molecular sieve or MOF particles in the continuous polymer matrix will significantly advance the field of mixed matrix membranes for gas separations.

Membrane processes for gas separations are of great interest to UOP. Natural gas upgrading and purification of hydrogen are key membrane business areas for UOP.

In order to maintain UOP existing membrane leadership and enable new gas separation membrane applications, UOP has developed a series of advanced membrane materials with significantly improved separation performance for gas separations such as CO₂ removal from natural gas, He recovery from natural gas, H₂ purification, air separation, and olefin/paraffin separations. Figure 4 shows the intrinsic separation properties of new UOP membrane materials for CO₂ removal from natural gas. New UOP group A membrane materials shown in Figure 4 have higher P_{CO2} than the commercial polymers such as CA polymer. New UOP groups B and C membrane materials have further enhanced P_{CO2} compared to the commercial polymers and group A membrane materials. Groups B and C membrane materials have shown separation performance above Robeson's 1991 polymer upper bound limit for CO₂/CH₄ separation. In addition to these groups A, B, and C membrane materials, UOP has also invented new high selectivity membrane materials (groups D and E in Figure 4). Groups D and E materials have significantly higher $\alpha_{\text{CO}_2/\text{CH}_4}$ and higher P_{CO2} than the commercial polymers and the performance is far beyond Robeson's 2008 polymer upper bound limit for CO₂/CH₄ separation. Several of these UOP proprietary membrane materials have been used for the development of UOP new commercial Separex™ membrane elements such as Flux+ and Select for CO₂/CH₄ (Figure 5) and other gas separations. As an example, one of the group A membrane materials as shown in Figure 4 has been produced in large scale and used successfully for the production of UOP commercial Separex™ Select spiral wound membrane elements. As shown in Figure 5, the Select membrane element enables much higher natural gas recovery due to the much higher selectivity than the Flux membrane element for CO₂/CH₄ separation. UOP is continuing on the development of next generation high performance membranes for CO₂/CH₄ and other gas separations through the introduction of new membrane materials and advanced membrane fabrication technologies.

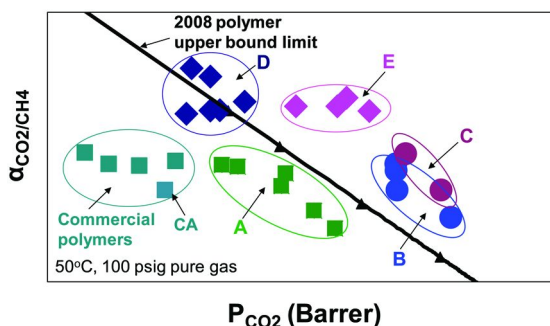


Figure 4. UOP advanced membrane materials for CO₂/CH₄ separation.

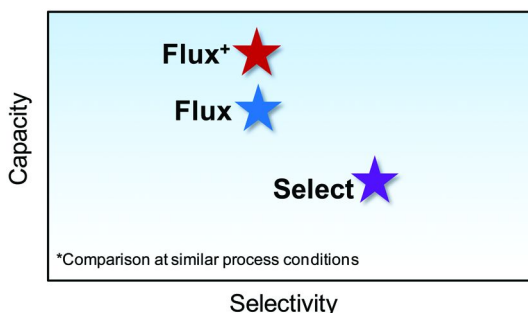


Figure 5. UOP commercial SeparexTM membrane elements for CO₂/CH₄ separation.

Membrane Configurations

Membranes for gas separations are available in both hollow fiber and flat sheet configurations. Flat sheet membranes, for example, may be formed into either spiral wound or plate and frame modules. Hollow fiber membranes are typically formed into hollow fiber modules.

The efficiency of a gas separation process can be described by essentially two parameters: the purity of the product gas and the fraction of that gas in the feed recovered as product (the recovery). Although these two parameters are influenced by membrane properties such as permeance and selectivity, membrane module, process design and operating conditions strongly impact system performance.

Flat Sheet Membranes

Most commercial flat sheet membranes for gas separations are asymmetric membranes with either an integrally skinned or a thin-film composite (TFC) structure. The asymmetric integrally skinned flat sheet membranes are manufactured from a casting dope by a dry-wet phase inversion process. The membrane has a thin, nanometer scale, dense, nonporous selective skin layer that performs the separation, supported on a highly porous substrate layer made from the same material. The phase inversion method used to prepare asymmetric integrally skinned membranes limits the number of materials that can be formed into high performance gas separation membranes.

TFC flat sheet membranes made by lamination or dip coating technique are also commercially available for gas separations. TFC membranes comprise a thin, nanometer scale, dense, nonporous selective layer that performs the separation and a highly porous substrate layer made separately from a different or same material.

TFC flat sheet membranes offer two major advantages relative to asymmetric integrally skinned flat sheet membranes:

- a) The two or more layers of TFC flat sheet membranes can be made from different materials, so that it allows the use of new high performance, high cost material as the selective layer and cheap commercial material as the porous substrate layer to provide the mechanical strength. The porous substrate layer can be made by the phase inversion process using a casting dope. Therefore, a low cost TFC flat sheet membrane can be economically fabricated even if expensive, custom-made new materials are used for the selective layer.
- b) The dip coating or lamination method for making TFC flat sheet membranes allows the use of high performance new materials which cannot be processed via the phase inversion technique.

UOP Separex™ commercial membranes are flat sheet membranes. The membranes are formed into spiral wound membrane elements for natural gas upgrading (Figure 6). The spiral wound membrane element has the key features of cross-flow, high pressure tolerance, high fouling resistance, high reliability, and ease of installation into space-efficient, skid-mounted units.

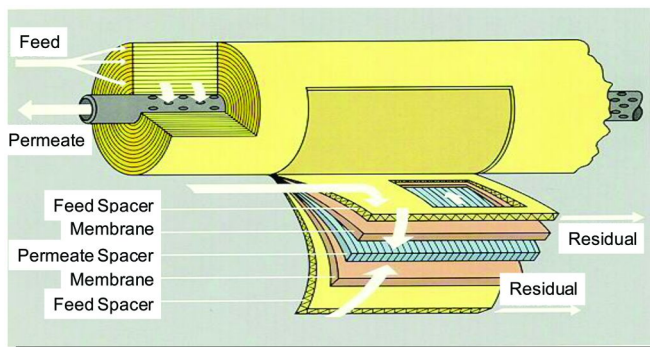


Figure 6. Diagram of spiral wound membrane element.

Hollow Fiber Membranes

Gas separation membranes are also available in hollow fiber configuration and are formed into hollow fiber modules. Same as flat sheet membranes, hollow fiber membranes can be either integrally skinned or TFC membranes.

The hollow fiber membrane spinning processes allows multiple parallel spinning lines for high throughput fabrication. Most hollow fiber membrane modules have higher membrane area packing density, but lower pressure tolerance, high bore-side pressure drop, and more difficulty to repair defects compared to spiral wound membrane modules. Hollow fiber membranes are normally used for the low pressure separation of clean gas streams such as separation of N₂ or water from air, free of components that might foul or plasticize the membrane. Some hollow fiber modules have also been used for high pressure

gas separations such as CO₂/CH₄ separation and H₂ purification. On the other hand, spiral wound membrane units have been extensively used for high pressure gas separations such as natural gas upgrading and H₂ purification.

Gas Separation Membrane Applications

Gas separation processes using membranes have undergone a major evolution since the introduction of the first large membrane-based industrial hydrogen separation process by Permea in 1980. Gas separation membranes are now used in a wide variety of application areas and their major applications are in the production, separation, recovery, drying, and/or purification of O₂, N₂, CO₂, H₂S, H₂, He, hydrocarbons, and water vapor.

Natural Gas Upgrading

Raw natural gas varies substantially in composition from source to source. CH₄ is the major component, typically 75-90% of the total. Natural gas also contains significant amounts of ethane, some propane and butane, and 1-3% of other higher hydrocarbons. In addition, the gas contains undesirable impurities such as water, CO₂, H₂S, and N₂. In most recent years, more and more raw natural gas has been found to contain high concentration of CO₂ (> 40%). CO₂ removal from natural gas is required to meet natural gas pipeline specifications, since CO₂ reduces the heating value of natural gas, is corrosive, and freezes at a relatively high temperature, forming blocks of dry ice that can clog equipment lines and damage pumps. Typical U.S. natural gas pipeline specifications are summarized in Table 2.

Table 2. Composition Specifications for Natural Gas for Delivery to the U.S. National Pipeline Grid

<i>Component</i>	<i>Specification</i>
CO ₂	<2%
Water	<120 ppm
H ₂ S	<4 ppm
C ₃₊	950-1050 Btu/SCF Dew point -20 °C
Total inert gases (N ₂ , He, etc.)	<4%

The technology most widely used for this separation is amine absorption. Amine plants, however, suffer from a number of problems such as high capital cost, complicated operation, and expensive maintenance. Membrane technology

is attractive for CO₂ and H₂S removal from natural gas because many membrane materials are selective for CO₂ and H₂S over CH₄. Polymeric membranes are more developed and commercially available for acid gas removal from natural gas. A high natural gas recovery (>95%) can be achieved using a multistage membrane system. The UOP Separex™ spiral wound membrane systems have been extensively used for CO₂ removal from natural gas for both onshore and offshore applications.

Cameron produces commercial cellulose triacetate polymeric hollow fiber membrane modules for CO₂ removal from natural gas (3). MTR has commercial NitroSep™ membrane system, which is more permeable to methane, ethane, and other hydrocarbons than to N₂, for separating N₂ from natural gas (3).

The high solubility of CO₂ and H₂S in polymeric membranes induces plasticization of the membrane, causing the polymer to swell with the disruption of the polymer matrix and an increase in the mobility of the polymer chains. Therefore, the membrane selectivity can drop significantly when the raw natural gas has high CO₂ and H₂S concentrations. To overcome plasticization issues, new membrane materials have been developed at UOP (Figure 4). The new membrane materials have high chemical and mechanical stability, high CO₂ and hydrocarbon plasticization resistance, as well as significantly improved CO₂/CH₄ selectivity and CO₂ permeability compared to the traditional polymer materials.

H₂ Separation

The first large-scale commercial application of gas separation membrane technology was for H₂ recovery from ammonia purge gases using Permea polysulfone hollow fiber Prism membrane system. Since then, membrane technology has been extended to other H₂ recovery applications such as adjustment of H₂/CH₄ or H₂/CO ratios for syngas production. UOP supplies Polysep™ hollow fiber membrane systems for H₂ separation from a variety of feed sources. H₂ recovery from refinery streams to meet the increased demand of H₂ is an emerging field for gas separation membranes for hydrotreating, hydroprocessing, or hydrodesulfurization processes. Commercial polymer membranes are currently used for H₂ recovery in refineries.

Vapor/Vapor Separation

Large opportunities for membranes in the separation of light hydrocarbon mixtures exist. However, extending the membrane technology from current gas separation applications to large volume organic vapor/vapor separations such as olefin/paraffin separations and the separation of hydrocarbon isomers needs new robust membranes with both high selectivity and high permeance. Otherwise, a high purity and high recovery product cannot be produced using membrane systems. In addition, high hydrocarbon plasticization resistance, contaminant resistance, and long-term performance stability are critical for commercial membrane success for large volume organic vapor/vapor separations. Both spiral

wound and hollow fiber membrane modules that are resistant to plasticization and hydrocarbon contamination are likely to be used in vapor/vapor separation applications. Current gas separation membranes are not suitable for most of these vapor/vapor separations. The development of new membranes for vapor/vapor separations could open up large new membrane markets.

Conclusions

Membrane technologies compete with cryogenic distillation, absorption, and pressure swing adsorption for gas and vapor separations. Gas separation membrane systems have been successfully used for several industrial applications such as the production of N₂ from air, CO₂ removal from natural gas, and H₂ recovery from refinery streams. Membrane-based removal of natural gas contaminants is the most rapidly growing segment of membrane gas separation industry, particularly in applications for the separation of CO₂, H₂S, He, and N₂ from natural gas. Research on improved membrane materials for gas separations has been significantly advanced over the past 20 years. Breakthrough development of new robust and high performance membranes is critical to the future success in membrane technologies for gas and vapor separations in challenging and harsh environments to compete with conventional separation technologies.

References

1. Koros, W. J. Three Hundred Volumes. *J. Membr. Sci.* **2007**, *300*, 1.
2. Henis, J. M. S. In *Polymeric Gas Separation Membranes*; Paul, D. R., Yampol'skii, Y. P., Eds.; CRC Press: Boca Raton, FL, 1994; pp 441–530.
3. Bernardo, P.; Drioli, E.; Golemme, G. Membrane Gas Separation: A Review/State of the Art. *Ind. Eng. Chem. Res.* **2009**, *48*, 4638–4663.
4. Nunes, S. P.; Peinemann, K. -V. In *Membrane Technology: in the Chemical Industry*; Nunes, S. P., Peinemann, K. -V., Eds. Wiley-VCH: Weinheim, 2001; p 1.
5. Robeson, L. M. Correction of Separation Factor Versus Permeability for Polymeric Membranes. *J. Membr. Sci.* **1991**, *62*, 165–185.
6. Robeson, L. M. The Upper Bound Revisited. *J. Membr. Sci.* **2008**, *320*, 390–400.
7. McKeown, N. B.; Budd, P. M.; Msayib, K. J.; Ghanem, B. S.; Kingston, H. J.; Tattershall, C. E.; Makhseed, S.; Reynolds, K. J.; Fritsch, D. Polymers of Intrinsic Microporosity (PIMs): Bridging the Void between Microporous and Polymeric Materials. *Chem.-Eur. J.* **2005**, *11*, 2610–2620.
8. Budd, P. M.; Msayib, K. J.; Tattershall, C. E.; Ghanem, B. S.; Msayib, K. J.; McKeown, N. B.; Fritsch, D. Gas Separation Membranes from Polymers of Intrinsic Mesoporosity. *J. Membr. Sci.* **2005**, *251*, 263–269.
9. Park, H. B.; Jung, C. H.; Lee, Y. M.; Hill, A. J.; Pas, S. J.; Mudie, S. T.; Wagner, E. V.; Freeman, B. D.; Cookson, D. J. Polymers with Cavities Tuned for Fast Selective Transport of Small Molecules and Ions. *Science* **2007**, *318*, 254–258.

10. van den Bergh, J.; Zhu, W.; Gascon, J.; Moulign, J. A.; Kapteijn, F. Separation and Permeation Characteristics of a DD3R Zeolite Membrane. *J. Membr. Sci.* **2008**, *316*, 35–45.
11. Li, S.; Falconer, J. L.; Noble, R. D. SAPO-34 Membranes for CO₂/CH₄ Separations: Effect of Si/Al Ratio. *Microporous Mesoporous Mater.* **2008**, *110*, 310–317.
12. Steel, K. M.; Koros, W. J. Investigation of Porosity of Carbon Materials and Related Effects on Gas Separation Properties. *Carbon* **2003**, *41*, 253–266.
13. Du, N.; Park, H. B.; Robertson, G. P.; Dal-Cin, M. M.; Visser, T.; Scoles, L.; Guiver, M. D. Polymer Nanosieve Membranes for CO₂-capture Applications. *Nat. Mater.* **2011**, *10*, 372–375.
14. Tullios, G. L.; Powers, J. M.; Jeskey, S. J.; Mathias, L. J. Thermal Conversion of Hydroxy-containing Imides to Benzoxazoles: Polymer and Model Compound Study. *Macromolecules* **1999**, *32*, 3598–3612.
15. Kwon, H. T.; Jeong, H.-K. *In Situ* Synthesis of Thin Zeolitic–Imidazolate Framework ZIF-8 Membranes Exhibiting Exceptionally High Propylene/Propane Separation. *J. Am. Chem. Soc.* **2013**, *135*, 10763–10768.
16. Kwon, H. T.; Jeong, H.-K.; Lee, A. S.; An, H. S.; Lee, J. S. Heteroepitaxially-grown Zeolitic Imidazolate Framework Membranes with Unprecedented Propylene/Propane Separation Performances. *J. Am. Chem. Soc.* **2015**, *137*, 12304–12311.
17. Koenig, S. P.; Wang, L.; Pellegrino, J.; Bunch, J. S. Selective Molecular Sieving through Porous Graphene. *Nat. Nanotechnol.* **2012**, *7*, 728–732.
18. Hang, L.; Song, Z.; Zhang, X.; Huang, Y.; Li, S.; Mao, Y.; Ploehn, H. J.; Bao, Y.; Yu, M. Ultrathin, Molecular-Sieving Graphene Oxide Membranes for Selective Hydrogen Separation. *Science* **2013**, *342*, 95–98.
19. Liu, C.; Kulprathipanja, S. In *Zeolites in Industrial Separation and Catalysis*; Kulprathipanja, S., Ed.; Wiley-VCH: Weinheim, 2010; pp 329–353.

Chapter 8

Multifunctional Magnetic Nanomaterials for Diverse Applications

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Multifunctional nanostructures have been at the center of attention in nanoscience and technology based on their enhanced functionality and diverse applicability in a plethora of devices. Fusing two distinctly different properties in a single nanostructure provides plenty of opportunities to tune these nanomaterials for diverse applications. Of these usage of multifunctional nanomaterials in biomedical fields have been on the rise since their multiple functionalities can be utilized for diagnosis, therapeutic, targeting uses as well as increasing cellular uptake with proper functionalization. In this chapter we will discuss about some multifunctional magnetic nanostructure, especially focusing on the Au-Fe₃O₄ nanostructures and their potential application biomedical fields. The catalytic uses of these nanostructures for water oxidation reaction has been also discussed with the multifunctional iron oxides as well as the metal selenide nanostructures.

Introduction

Multifunctional nanomaterials have recently attracted the attention of the materials science community owing to their vast applicability in a diverse range of applications (1–6). One of the most promising applications for these multifunctional nanomaterials is related to the biomedical field where they have been used as photothermal killing of cancerous cells (7), magnetic resonance and fluorescence imaging (8–11), cell targeting and sorting (12), and drug

delivery (13–15). In these multifunctional nanostructures which are made by fusion of two entirely different materials with diverse properties into one single nanostructure, the capability to tune the size, shape and morphology in addition to the composition of these multifunctional nanomaterials provides them with even more versatility. Multifunctional nanomaterials can be categorized according to their morphologies as core-shell (where heterocomposition is expressed radially), dumbbell shaped (where heterocompositions are segregated along the long axis of the nanostructure) and barcode structures (2). The functionalities of the individual regions can be varied to obtain various types of multifunctional nanomaterials. The dumbbell shaped nanoparticles containing two compositions of widely different functionalities sharing a common interface is especially lucrative since it provides opportunities to fully utilize both the functionalities in the nanoparticle ensemble. These types of multifunctional particles are also referred to as *Janus* particles when they are more spherical than elongated. Amongst these, nanostructures including a metallic and a magnetic composition have been of considerable interest due to their promising applications in magneto-optic and optoelectronic devices and Au has been the preferred choice as one of the components. The optical properties of the Au imparts major advantages, when couple with the magnetic properties of the nanostructure. In this chapter we will discuss about two diverse applications of the multifunctional magnetic nanostructures in: (i) biomedical and (ii) energy-related fields.

Traditionally, Au nanoparticles have demonstrated their importance in biological and medicinal applications, including immuno-sensing (16), phagokinetic studies (17), as carrier vehicles for delivery of nucleic acids via covalent and non-covalent conjugation (18), labelling and cell visualization by photothermal or photo acoustic methods (19), separation and purification of biomolecules, hyperthermia agents (local heat generation for tumor destruction), contrast enhancer, tissue engineering and highly selective bio-sensors (16–18). The reason for their extensive applications in biology and medicine is not only because of their robust interaction with biomolecules containing thiol and disulfide functional groups but also due to their unique optical properties. However, despite the numerous advantages, the ability to manipulate Au non-invasively is rather limited. Recently, many researchers have tried to find a solution to this problem by using multifunctional nanomaterials where Au is combined with other suitable magnetic materials thereby producing an optically active magnetic nanostructure. Among these, magnetite (Fe_3O_4) is the most appropriate candidate to be coupled with Au, due to its low toxicity, high saturation magnetization and high susceptibility. Magnetite nanoparticles have also been used as drug delivery vehicle (therapeutics) (20), to generate local heat in alternating magnetic field leading to necrosis of cancer cells (hyperthermia) (21), MRI contrast enhancer and magnetic separator when labelled with appropriate biomolecules (22–26). The functionality of the magnetic nanoparticles depends predominantly on their unique magnetic properties in the nanoscale including superparamagnetism. As the size of a ferromagnetic particle is reduced from the bulk state to below several tens of nanometers (i.e. critical size), the particle behaves as a monodomain magnetic particle. This behavior is termed as superparamagnetism and the material is called superparamagnetic (27). The minimum temperature above

which spontaneous flipping of the particle moment under an applied field occurs is called as the blocking temperature, T_B . Above T_B the particles behave as superparamagnets with randomly oriented particle moments, while below T_B they may exist in blocked (i.e. ordered) state.

As a bifunctional nanoparticle, gold-iron oxide nanoparticle can inherit excellent surface chemistry characteristics, unique optical properties (attributed to Au) and superparamagnetic characteristics attributed to Fe-oxides. Drugs attached to these bifunctional nanoparticles can have more advantages over ordinary drugs. First, they offer size controllability, ranging from few to hundreds of nanometers with different and unique size-dependent properties. Second, they can be easily controlled and manipulated from outside with the help of external magnetic field being operated from a distance. Third, they can provide enhanced contrast in medical imaging that can be used to diagnose the situation efficiently. Fourth, with their highly selective binding properties, drug molecules can also be attached to the surface of these nanoparticles. Fifth, they also exhibit high rate of absorption in the human body due to their high surface area to volume ratio. These characteristics would further enhance and broaden the application of these nanoparticles for *theranostic* applications.

The transition metal chalcogenides (ME_x) [$M = \text{Fe, Co, Mn}$; $E = \text{Se, S, Te}$] on the other hand, have attracted the solid state chemists for a long time owing to their interesting electronic and magnetic properties (28). Most of these chalcogenides are semiconducting with variable bandgaps which decreases down the chalcogen series. Transition metal chalcogenides have found applications in various semiconductor devices including photovoltaic, thermoelectric as well as water splitting catalysts (29). Recently the later property, viz. the water oxidation/reduction (OER and HER, respectively) catalytic activity has come into prominence for the transition metal chalcogenides especially the Ni and Co-based selenides (30–34). As the oxygen atoms are replaced by the chalcogen atoms in the lattice, it is expected to reduce the bandgap and alter the position of the valence band and conduction band edges thereby enhancing catalytic activities. A multifunctional magnetic nanostructure combining these transition metal chalcogenides with a metallic component like Au can further increase the electrocatalytic activity by facilitating charge transfer at the semiconductor electrode interface, and possibly altering the surface electronic states, while the magnetic interactions can lead to better absorption of paramagnetic oxygen at the catalytic site for efficient oxygen reduction reaction.

Among the various transition metal chalcogenides, Ni- and cobalt based selenides have shown promising catalytic activity for water oxidation and oxygen reduction reactions. Several theoretical as well as experimental studies have been conducted to understand why Ni-based electrocatalysts show superior activity and it was learnt that this can be attributed to various factors including the band alignment and occupancy of the d -levels of the transition metal (35–37). Studies led by Shao-Horn, Goodenough and other research groups have predicted that an e_g occupancy of 1e helps the catalytic activity of the transition metal center (38). These groups have used several descriptors to understand their influence on the catalytic properties and had observed that the molecular orbital descriptor was the most influential to predict and optimize catalyst efficiency (38, 39). However,

according to those studies, apparently Co(II) based systems with a low spin octahedral coordination for Co should produce the best water oxidation catalyst. In fact, organometallic complexes of Co does show high catalytic performance which has been researched extensively by Nocera, and other groups (40–44). CoSe is a semiconducting material with the band gap of 1.52 eV which can potentially show high catalytic activity for water oxidation. Cobalt selenides typically show compositions ranging from the stoichiometric CoSe, CoSe₂ phases to the non-stoichiometric Co_{0.85}Se phase (45–48). Cobalt selenide is known to be a metallic conductor and exchange-enhanced Pauli paramagnet in its ground state with a T_c of approximately 125 K (45–48). Recently cobalt selenides have shown lot of promise as catalysts for oxygen reduction, decomposition of hydrazine hydrate, magnetic refrigeration and as electrodes for Li-ion batteries (49). CoSe in bulk form has been synthesized through electrochemical deposition technique (50), high pressure solid—state synthesis (51) and through mechanical alloying (52). However, reports of cobalt selenide nanostructures are very limited (53–55). CoSe nanoparticles have been synthesized through microwave assisted methods (53) while CoSe nanocrystals were also formed using metal acetate-paraffin approach, in the presence of oleylamine (54), and by hydrothermal method in presence of hydrazine, cobalt chloride and selenium (55).

Au-Fe₃O₄ Nanostructures for Biomedical Applications

Given the importance of gold-iron oxide nanoparticles in *theranostic* applications, there have been considerable efforts directed towards synthesis of these bifunctional nanoparticles. Reported synthesis protocols for Au-Fe₃O₄ include decomposition of iron precursors (e.g. iron acetylacetonate) on gold nanoparticle seeds, reduction of Au³⁺ on iron oxide nanoparticles with porous silica shell, by chemical bond linkage using intermediary molecules to form core-shell, core-hollow shell, and dumbbell-like nanostructures (56–62). However, most of these methods are multi-step processes, which might be detrimental for large scale synthesis of these potentially transformative nanoparticles. Hence, there is a need for producing these gold-iron oxide nanoparticles through simpler reactions involving less number of steps and more biocompatible precursors.

The authors have recently synthesized Au-Fe₃O₄ crystalline nanostructures through one-step *hot injection* technique involving reaction between Fe(CO)₅ and HAuCl₄ at 300 °C in a solvent medium in presence of oleic acid and oleyl amine (Figure 1) (63). The synthesis was carried out in air by simultaneous addition of Fe(CO)₅ and HAuCl₄ with excess oleylamine (OLAM) and oleic acid (OLAC). 5 mL Triton® X-100 was added to a three neck round bottom flask equipped with a magnetic stir bar and air condenser. The solution was heated to 85 °C. 2.5 mM of Fe(CO)₅, 0.25 mM of HAuCl₄, 2.5 mM OLAC, and 2.5 mM of OLAM were injected in this hot solution. The temperature was then ramped to 300 °C. Upon injection of the Au and Fe-precursors, the solution turned black with rapid evolution of gases. After 10 min the gases subsided and the black solution was

allowed to reflux for 1 h. After 1 h, heating was stopped and the reaction was cooled to room temperature. The product was isolated from the reaction mixture by magnetic filtration since it adhered strongly to a lab magnet, followed by washing and centrifugation at least 3-4 times with ethanol using ultrasonication to remove excess Triton® X-100 and any unreacted precursors. The powder collected at the bottom of the centrifuge tube was dried in air.

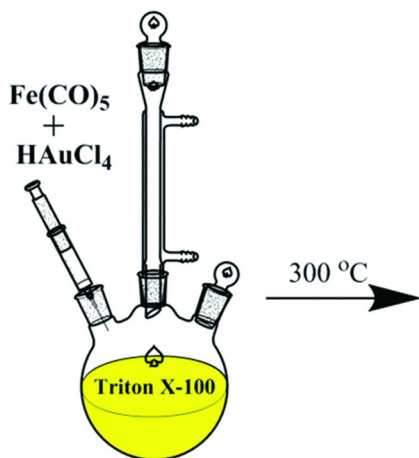


Figure 1. Reaction set-up for synthesis of Au-Fe₃O₄ nanostructures through hot-injection technique.

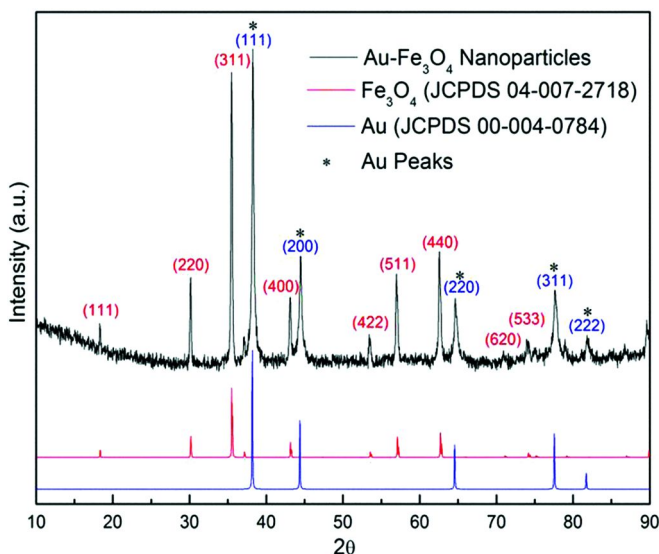


Figure 2. PXRD pattern of Au-Fe₃O₄ bifunctional nanoparticles synthesized at 300 °C.

The *as-synthesized* Au-Fe₃O₄ nanoparticles was seen to be highly crystalline as confirmed by PXRD pattern shown in Figure 2, which corroborated very well with the standard patterns of Fe₃O₄ with inverse spinel structures (JCPDS 04-007-2718) and Au (JCPDS 00-004-0784). The PXRD pattern was very clean and did not show any impurity peaks corresponding to other iron oxide phases like Fe₂O₃ and FeOOH. From Scherrer equation the size of these particles was calculated to be 75 nm with the Fe₃O₄ region being around 60 nm while the Au part was around 15 nm in average (64). The size of nanoparticles could be tuned by controlling the time at which HAuCl₄ was injected, presence of OLAM in the reaction mixture (excess OLAM led to bigger gold particles), reflux time and the HAuCl₄/Fe₃O₄ ratio. Specifically, injecting Fe(CO)₅ in large excess compared to HAuCl₄ (~20:1 Fe:Au molar ratio) led to overgrowth of Fe₃O₄ part while refluxing the reaction mixture for longer time resulted in agglomeration of Au-Fe₃O₄ particles. Presence of OLAM helps in reduction of Au⁺³ to Au⁰ onto the Fe₃O₄ surface. The role of Triton® X-100 was more as a highly viscous solvent which inhibited overgrowth of the Fe₃O₄ part and changing the concentration of Triton® X-100 had very minimal effect on the morphology of the Au-Fe₃O₄ nanoparticles.

The morphology and composition of the bifunctional nanoparticles were characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Investigations from TEM and SEM showed a high yield of bifunctional nanoparticles in the product with uniform particle size distribution. Figure 3(A) shows a typical distribution of the Au-Fe₃O₄ nanoparticles where each Fe₃O₄ region is decorated with several Au dots. Typically the Fe₃O₄ regions (lighter contrast in the images) were 80 nm while the Au part (darker contrast) was approximately 20 nm. Several wide area SEM images were obtained to calculate the average particles size. The size of the Au and Fe₃O₄ particles ranged from 5-20 nm and 50-80 nm, respectively, with average bifunctional particle size around 80 nm. This kind of morphology is slightly different than the dumbbell shaped particles and is closer to multifaceted Janus particles (65, 66). This kind of morphology (Au-decorated Fe₃O₄) gives the added advantage that both Au and Fe₃O₄ functionalities are available for full utilization of their potential, while, several Au dots on the Fe₃O₄ host ensures that there are maximum number of spots for molecular recognition since Au-terminal acts as anchor for the aptamers used as targeting agents. Au-Fe₃O₄ nanoparticles were highly crystalline as revealed by the high resolution TEM (HRTEM) image shown in Figure 3(B). The HRTEM image exhibited lattice fringes corresponding to (111) planes of Au and (311) lattice planes of Fe₃O₄. Through these HRTEM images we also looked in detail at the interfaces between the Au and Fe₃O₄ regions. The interface in these nanoparticles was very clean and well-defined with minimal mixing of the Au and Fe₃O₄ phases. There was no fuzziness or presence of any other crystalline phase at the interface. Interfacial surface can be very important in these superparamagnetic nanoparticles as depending on the composition on both sides of the interface they might lead to exchange bias magnetic interactions leading to interparticle interaction and ordering.

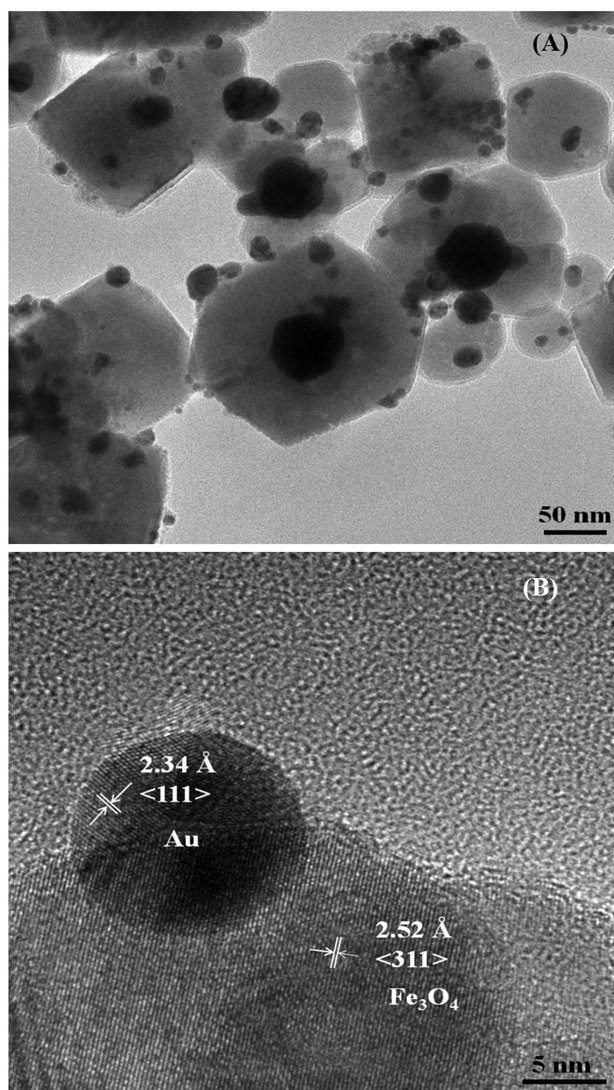


Figure 3. (A) TEM image of the bifunctional nanoparticles showing decoration of Fe_3O_4 with Au. (B) HRTEM showing the attachment of Au to Fe_3O_4 and the crystallinity of the individual regions. Lattice fringes from the Au and Fe_3O_4 regions corresponds to $\langle 111 \rangle$ and $\langle 311 \rangle$ planes, respectively.

Gold nanoparticles ranging in size from 5-20 nm are reported to show a plasmon resonance band at 520 nm. This phenomenon can be attributed to the resonance condition satisfied between the incoming light frequency and natural frequency of the surface electrons oscillating against the positive force of nuclei. The bifunctional Au- Fe_3O_4 nanoparticles reported here show red shift of plasmon

resonance band due to the attachment of Au with Fe_3O_4 nanoparticles although the exact position of the absorption maxima varied with the gold particle size attached to Fe_3O_4 part. A typical spectrum exhibited that the Au- Fe_3O_4 nanoparticles in ethanol shows a plasmon resonance band ~ 540 nm (Figure 4), which is shifted by ~ 20 nm from pure Au nanoparticles. This red shift can be accounted for the deficiency of electrons on Au due to the interaction with Fe_3O_4 (67, 68). The absorption spectra collected from pure Fe_3O_4 nanoparticles did not show any peak in the 300 – 800 nm regions.

These bifunctional Au- Fe_3O_4 nanoparticles deserve a very careful magnetic characterization since *Magnetite* (Fe_3O_4) is one of the oldest and interesting magnetic materials known to the scientific community. Bulk magnetite is ferrimagnetic with Curie temperature around 858 K. In 1939, Verwey (69) observed first order transition on cooling below 120 K which resulted in increased resistivity and distortion of the cubic symmetry of magnetite. Charge ordering of different states of iron (Fe^{2+} and Fe^{3+}) on the octahedral sites in alternating layers was ascribed to be the reason for this transition (70). The temperature below which this transition occurs is termed Verwey temperature (T_V). To better understand the magnetic properties of these gold-magnetite nanoparticles, we performed detailed magnetization measurements as a function of temperature and applied magnetic field. The magnetization behavior of the sample as a function of temperature was measured under ZFC and FC conditions under an applied magnetic field of 100 Oe are shown in Figure 5(A). These nanoparticles show a clear Verwey transition at 120 K visibly prominent in the ZFC curve. For the superparamagnetic nanoparticles there are two basic criterions. First is that the magnetization as a function of H/T for non-interacting single domain particles must be fitted by *Langevin* equation (1) (27, 71) as shown below.

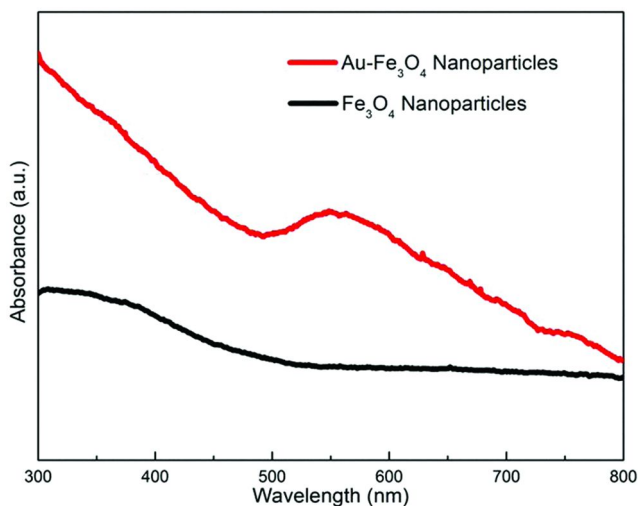


Figure 4. Absorption spectra of the bifunctional Au- Fe_3O_4 nanoparticles and Fe_3O_4 nanoparticles.

$$M = M_0 \left[\coth \left(\frac{\mu H}{k_B T} \right) - \frac{1}{\left(\frac{\mu H}{k_B T} \right)} \right] \quad \dots(1)$$

where M = magnetization, M_0 = saturation magnetization, H = applied magnetic moment, μ = magnetic moment, T = temperature and k_B = Boltzmann constant. The plot of M versus H/T at 5 K, 100 K and 300 K converge into a single universal curve and could be fitted using *Langevin* equation with $R^2=0.9981$ as shown in Figure 5(B). Second condition for superparamagnetic particles is the anhysteretic nature of isothermal magnetization against applied magnetic field with zero coercivity, which is also temperature independent above blocking temperature (T_B).

Figure 5(C) shows the isothermal magnetization curves as a function of applied magnetic field at 5 K, 100 K and 300 K for the bifunctional nanoparticles. The presence of coercivity was very apparent at 5K and 100K. At 300 K on the other hand, the M vs H was anhysteretic in nature typical for a superparamagnetic nanoparticle, as shown in Figure 5D. This indicates that the Au-Fe₃O₄ nanoparticles were superparamagnetic at 300 K with T_B lying in between 100 – 300 K. Generally for magnetite nanoparticles, it is assumed that T_v is greater than blocking temperature with the exemption of any chain formation of the particles which is absent in our particles (72). However, it is also known that for superparamagnetic nanoparticles the T_B increases with increasing particle size within the single domain range (27, 71). For Fe₃O₄ nanoparticles it has been estimated that the *maximum allowable* size for single domain particle is ~120 nm (71). The average size of Fe₃O₄ region in the Au-Fe₃O₄ ensemble was ~60 nm which indicates that these nanoparticle ensembles may be on the realm of single domain superparamagnetic behavior and multi-domain characteristics. Skumryev have recently shown that it is possible to beat the superparamagnetic limit by formation of exchange bias interactions (73) caused by several factors including magnetic coupling at the interface, uncompensated spins on the surface, as well as the presence of multiple interfaces in these composite magnetic nanoparticles (74). Previously, Sun *et al.* have shown that higher T_B and higher coercivity could be obtained due to exchange bias in nanoflower-like Au-Fe₃O₄ nanoparticles (74). According to the literature Fe₃O₄ nanoparticles with an average size in the range of 50 nm shows a T_B close to room temperature (75). In the current case the anhysteretic nature of M vs H curve for the Au-Fe₃O₄ nanoparticle ensemble at 300 K along with the nature of the ZFC-FC curves indicate that the T_B is very close to room temperature. At 300 K the Au-Fe₃O₄ nanoparticle ensemble is right at the interface of superparamagnetic and ordered states thereby possessing high magnetic moment as well as the thermally activated spontaneous particle

moment fluctuations. The coercivity observed at 5 K and 100 K might indicate that near the Verwey phase transition, changes in resistivity and distortion of symmetry may occur due to charge ordering. The saturation magnetization for these Au-Fe₃O₄ nanoparticles was 73.68 emu/g at 300 K which is almost 80% of the saturation magnetization of bulk magnetite (~92 emu/g) (76). Such enhanced magnetic properties can be also attributed to the typical morphology of these Au-Fe₃O₄ nanoparticles where each Fe₃O₄ is decorated with several non-magnetic Au dots which can function as surface passivation agents thereby pushing the superparamagnetic limit. Additionally, if the nanoparticles are not absolutely immobilized and well separated, inter-particle interactions will influence the relaxation and if the magnetic interaction energy exceeds the thermal energy, it will lead to a degree of ordering of the particle moments, resulting in behavior typically referred to as superferromagnetism (77). These phenomena can give rise to higher saturation magnetization compared to bare Fe₃O₄ nanoparticles with smaller sizes.

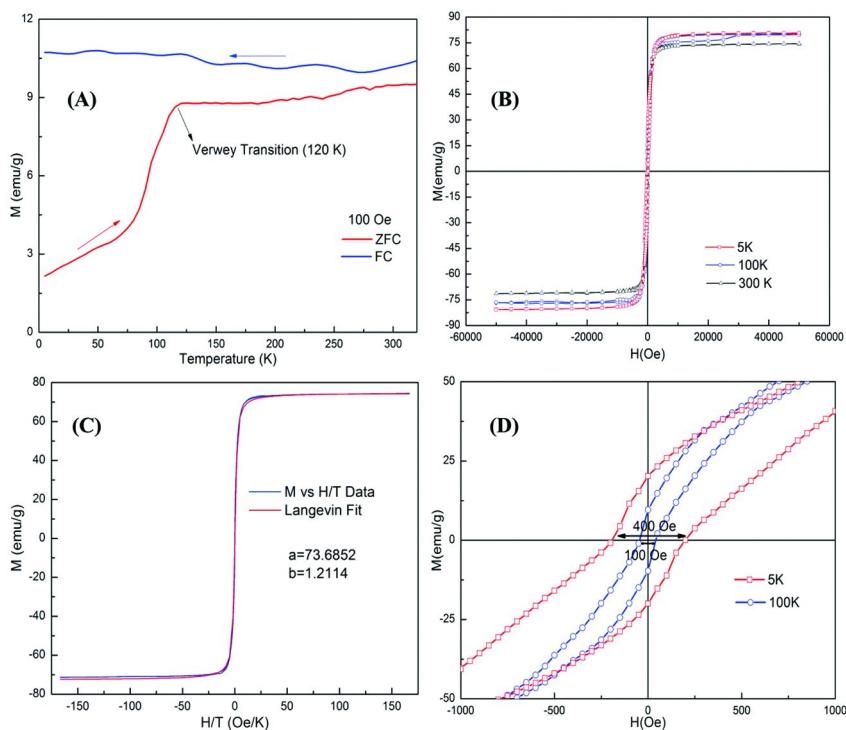


Figure 5. (A) ZFC and FC curves of the Au-Fe₃O₄ nanoparticles under an applied field of 100 Oe. (B) M vs H plots at 5 K, 100 K and 300 K. (C) The Langevin fit at 300 K. (D) M vs H plots at 5 and 100 K magnified to show the coercive fields.

The average particle volume of superparamagnetic nanoparticles could be also estimated using the following equations along with the *Langevin* equation (1).

$$y = a \left[\coth(bx) - \frac{1}{(bx)} \right] \quad \dots(2)$$

where $x = H/T$; $y = M$; $a = M_0$ and $b = \mu/k_B$.

$$\text{Particle moment, } \mu = M_S \langle V \rangle \quad \dots(3)$$

where M_S = saturation moment of Fe_3O_4 nanoparticles; and $\langle V \rangle$ = average particles volume.

The observed saturation moment of Fe_3O_4 is ~ 74 emu/g from the *Langevin* fit. Parameters a and b could be obtained from the *Langevin* fit of M vs H/T at 300 K and through proper substitution of these parameters in equation 3, the average particle volume can be calculated (the density of Fe_3O_4 was taken to be 5.197 g/cc corresponding to the literature). Assuming the particles were spherical, the average particle diameter obtained using this approach was approximately 58 nm. Since this approach only accounts for the superparamagnetic part in these bifunctional nanoparticles (i.e. Fe_3O_4 region), the overall size of the nanoparticles including the average diameter of the Au region (~ 20 nm) would be approximately 78 nm, which was close to the average particle size observed through STEM and TEM investigations (~ 80 nm). This is an interesting aspect of these Au- Fe_3O_4 nanoparticles, which retain their superparamagnetic nature even with larger size. A larger superparamagnetic volume also ensures better heat generation and hyperthermia effect since the energy barrier scales proportionately with the superparamagnetic volume.

As mentioned previously, one of the most potentially transformative applications of these magnetic nanoparticles is in biomedical fields. Hence, to demonstrate the feasibility of using these bifunctional nanoparticles for biological applications, we have functionalized the nanoparticles with biologically relevant molecules such as L-cysteine and (-)epigallocatechin gallate [EGCG]. We have successfully attached L-cysteine to Au terminal of the bifunctional nanoparticles taking advantage of Au-thiol facile interactions. The EGCG attachment was facilitated by interactions between Au and the hydroxylic groups of the polyphenol EGCG. The process for functionalization with L-cysteine and EGCG along with the expected morphology has been schematically shown in Figure 6.

Biofunctionalization of these Au-IO nanoparticles with EGCG was achieved by treating the pre-synthesized nanoparticles with large excess of EGCG in aqueous medium. Typically 20 mg of Au-IO nanoparticles were incubated with 150 – 200 mg of EGCG in 10 ml of Millipore water for 12 h on an orbital shaker. The attachment of EGCG occurs mostly by *via* ligand exchange, whereby the surface ligands of the Au-IO nanoparticles are replaced with EGCG. Cysteine attachment on the other hand, was carried out by incubating a fixed amount of Au- Fe_3O_4 nanoparticles with 10 mg of cysteine in 10 ml of Millipore water for

48 h on an orbital shaker. The solution remained colorless for the entire time indicating no sample degradation. After incubation the magnetic nanoparticles were separated by magnetic filtration.

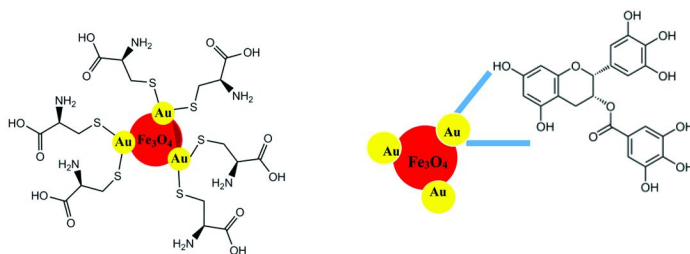


Figure 6. Scheme showing grafting of cysteine (left) and EGCG (right) on $\text{Au-Fe}_3\text{O}_4$ nanoparticles through attachment to Au -end.

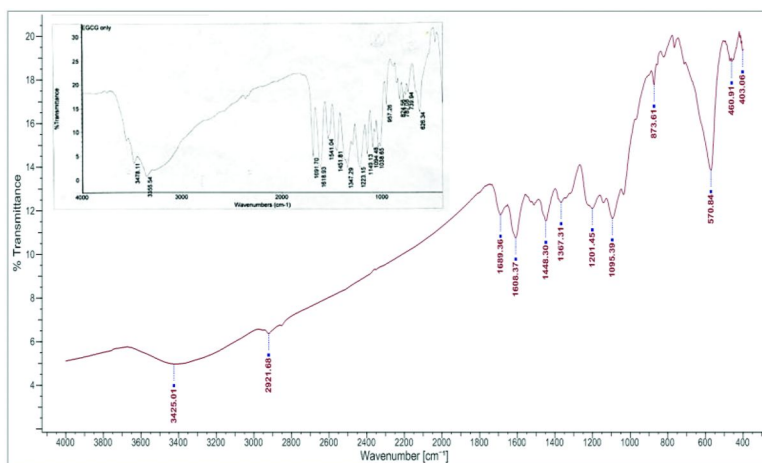


Figure 7. FTIR spectra of EGCG coated $\text{Au-Fe}_3\text{O}_4$ nanoparticles. Inset shows the FTIR spectra of pure EGCG.

The functionalization with EGCG is of special significance. EGCG is flavonoid found in green tea and through extensive *in vitro* and *in vivo* studies it has been observed that EGCG can be beneficial in treating brain, prostate, cervical and bladder cancers (78). A major advantage of using EGCG is that it targets the Laminin receptors (Lam 67R) which are over expressed on human prostate cancer cells (79) and hence the EGCG functionalized Au-IO nanostructures expectedly will have specificity with regards to prostate cancer cells and hence can populate near the affected tissue. Such target recognition and specificity is crucial for any *theranostic* tool, which makes the EGCG functionalization very significant. Additionally, the EGCG functionalization also renders these

bifunctional nanoparticles water soluble yielding a reddish-brown solution, thereby making it possible to transfer the *theranostic* agents in physiological conditions. On drying the EGCG-coated nanoparticles could be recollected which retained their crystalline nature.

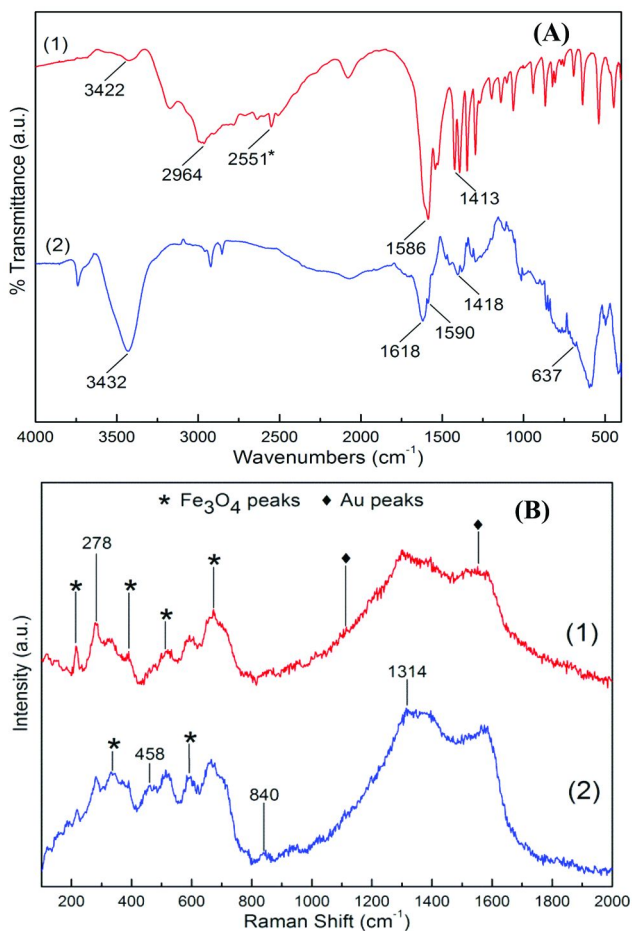


Figure 8. (A) FTIR spectra of (1) pure L-cysteine and (2) L-cysteine modified-bifunctional Au-Fe₃O₄ nanoparticles (B) Raman spectra of (1) bifunctional Au-Fe₃O₄ nanoparticles and (2) L-cysteine modified Au-Fe₃O₄ nanoparticles.

The biofunctionalized Au-Fe₃O₄ nanoparticles were characterized through both qualitative (FTIR and Raman) and quantitative (acid ninhydrin assay for cysteine functionalized particles) analysis. Figure 7 shows the FTIR spectra of the EGCG functionalized Au-Fe₃O₄ nanoparticles as compared with pure EGCG powder. Specifically, after incubation in the EGCG containing solvent, the nanoparticles were separated with magnetic filtration washed with copious

quantities of water, and then analyzed in the FTIR spectrometer. As can be seen from the figure the coated nanoparticles show distinct FTIR peaks characteristic of EGCG confirming the presence of the coating. Figure 8(A) shows the FTIR spectra of L-cysteine-modified Au-Fe₃O₄ bifunctional nanoparticles (curve 2) as compared with that of free L-cysteine (curve 1). While most of the bands assigned to cysteine were visible for both the samples, the absence of band at $\sim 2551\text{ cm}^{-1}$ corresponding to the stretching vibration of S-H bond was prominent in the FTIR spectra of the cysteine functionalized nanoparticles as shown in curve b. This indicates breakage of the S-H bond on attachment of cysteine to the Au-terminal through S, and has been accepted as a signatory evidence for successful molecule attachment (80–82). Trans C-S stretching vibrational modes can be assigned to the presence of band at $\sim 637\text{ cm}^{-1}$. The band at $\sim 3422\text{ cm}^{-1}$ can be assigned to N-H stretching vibrations (82), while bands in the range $1510\text{ to }1680\text{ cm}^{-1}$ can be assigned to carbonyl and N-H stretching vibrations (83). Detailed analysis of the FTIR spectra confirmed the attachment of L-cysteine to the Au-Fe₃O₄ bifunctional nanoparticles.

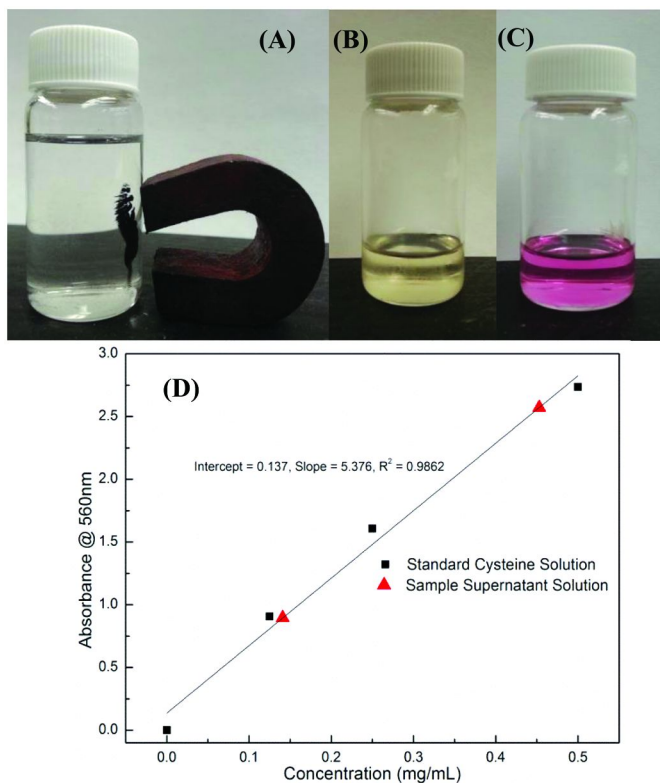


Figure 9. (A) Magnetic separation of the nanoparticles from the L-cysteine solution. (B) Color of supernatant after adding acid ninhydrin and (C) after heating in water bath for 15 minutes. (D) Standard curve of absorbance versus L-cysteine concentration.

While qualitative analysis showed that the Au-Fe₃O₄ nanoparticles could be actually functionalized with L-cysteine attachment, the quantitative analysis was performed by estimating the amount of leftover cysteine in the supernatant through acid ninhydrin assay, an instant spectrophotometric method (81). In this assay, the supernatant was analyzed for L-cysteine after magnetically separating the nanoparticles from the L-cysteine solution. This indirect approach was employed due to presence of Au-Fe₃O₄ nanoparticles in the product, where Au exhibits unique optical properties, which could interfere with the absorbance readings. After 24 h vigorous shaking of nanoparticles in L-cysteine solution, the functionalized nanoparticles were separated using a magnet since the nanoparticles were quickly attracted to the magnet as shown in Figure 9(A). Then acid ninhydrin reagent was added to a portion of the supernatant and was kept in boiling water bath for 10 min. Figures 9(B) and 9(C) show the color change in the reaction solution after boiling. Figure 9(D) shows the standard curve of absorbance versus L-cysteine concentration (in mg/mL). From an initial concentration of 10 mg L-cysteine, 4.53 mg of L-cysteine was found to be in the supernatant (i.e. unattached to the nanoparticles). Assuming 100% of L-cysteine activity, the loading capacity of the bifunctional nanoparticles used in the study is approximately 1 mg of L-cysteine per 4 mg of nanoparticles. We are presently trying to attach various other biomolecules to gain a better insight into the loading capacity of these bifunctional nanoparticles.

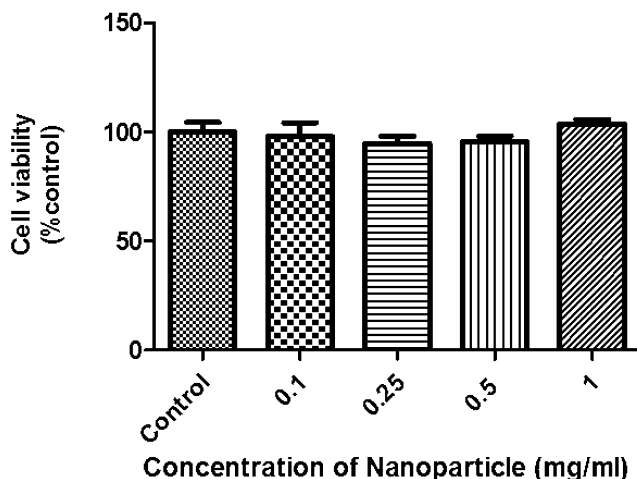


Figure 10. Cytotoxicity of Au-Fe₃O₄ nanoparticles at various concentrations for CHO cells after 48 h incubation.

Au-Fe₃O₄ nanoparticles are intended to be applied for magnetic fluid hyperthermia and delivery of biomolecules. Hence the cytotoxicity of these nanoparticles was also evaluated. For this study, the cell viability of CHO cells was evaluated after 48 h incubation at various increasing concentration of nanoparticles ranging from 0.1 – 1 mg/ml by MTS assay. Since the toxicity

cell viability estimation from MTS assay is determined through spectroscopic techniques, slight interference from the nanoparticles might be expected. To eliminate any such interference from the nanoparticles, we repeated these studies by simultaneously running some control experiments as detailed below and accumulated the data from a statistical analysis. Typically, the MTS assay was carried out in a 96 well plate where three batches were compared. The first batch contained the CHO cells in the culture medium without any nanoparticles (referred to as control cells). The second batch contained the cells exposed to varying concentrations of Au-Fe₃O₄ nanoparticles present in the culture medium. The third batch contained just the nanoparticles in the culture medium with the same concentration gradient as the second batch but containing no cells (referred to as nanoparticle blank). This third batch was expected to show the un-perturbed behavior of the nanoparticles under similar conditions. MTS is bio-reduced by cells into a formazan product that is soluble in tissue culture medium. Typically after exposing the cells to the nanoparticles in the growth medium for a specific time, the nanoparticles were removed by centrifugation which reduced interference from them. The absorbance (i.e. optical density, OD) of only the supernatant was measured from each well plate which was directly proportional to the number of living cells in culture. To get an accurate value, the OD obtained from the nanoparticle blank (i.e. 3rd batch) was subtracted from that of the nanoparticles + cell supernatant (i.e. the 2nd batch). The resulting OD was then compared with that obtained from the control cells to determine the relative percentage of viable cells. The nanoparticles didn't show any sign of cytotoxicity even at 1 mg/mL (Figure 10). The results obtained corroborates well with polymer coated magnetite nanoparticles and other morphologies (84–88).

Multifunctional Magnetic Nanostructures as Oxygen Evolution Catalysts

Among the many applications of photoactive semiconductors, the ones related to energy conversion process has been at the center of attention in the materials research community mainly due to their potential of solar energy harvesting and enriching alternative energy resources. In this regard, the water splitting catalysts enhancing oxygen evolution reaction (OER) and hydrogen evolution reaction (HER) deserves special mention. Based on their favorable band gap lying in the visible range and favorable positioning of the valence and conduction band edges, iron oxide shows immense potential as photoactivated OER catalyst. It is expected that in these multifunctional Au-Fe₃O₄ nanostructures the coupling of Au plasmonic mode will increase the photoresponse and subsequently the catalytic properties of the nanostructures. The metallic nature of Au is also expected to facilitate charge transport in the matrix and hence increase the exchange current density.

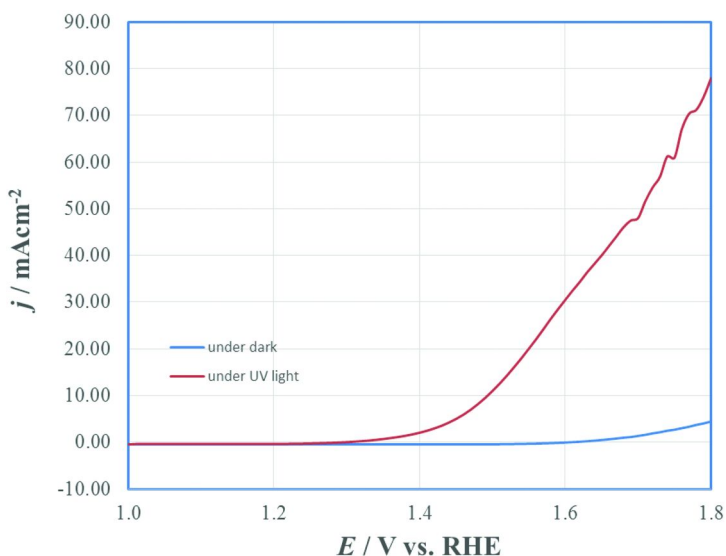


Figure 11. Linear scan voltammetry showing O_2 evolution from anode coated with $Au-Fe_3O_4$ nanoparticles acting as OER electrocatalysts in 1M KOH.

We have measured the OER catalytic efficiency of an ensemble of the $Au-Fe_3O_4$ nanoparticles through detailed electrochemical measurements (89). Typically the nanoparticles were dispersed in iso-propanol and mixed with Nafion with a 20% loading of the nanoparticles (by weight). The slurry containing Nafion, iso-propanol and the nanoparticles was then dried at 180 °C on a GC electrode, which led to formation of a uniform film of known geometric area. The GC electrode was then connected as the anode in a electrochemical cell with $Ag/AgCl$ as the reference electrode, Pt as counter electrode, and 1M KOH as the electrolyte.

The $Au-Fe_3O_4$ nanoparticle ensemble shows moderate oxygen evolution under non-focused laboratory light with the onset potential being 1.53 V (vs RHE) and a very gradual rise in exchange current density corresponding to the electron transfer process in O_2 evolution as shown in Figure 11. A UV light source was used to illuminate the $Au-Fe_3O_4$ -GC electrode and on illumination it was observed that the catalyst's activity improved dramatically. The onset potential for O_2 evolution was shifted to a much lower value (1.40 V vs RHE) while the exchange current density showed a sharp rise reaching 10 mA/cm² at 1.53 V, which corresponds to an overpotential of 300 mV. However, it should be noted here that, a distinct photovoltage was not observed with the $Au-Fe_3O_4$ loaded electrode, rather what is observed can be described a photoelectrochemical effect. Recently magnetite has been reported as semiconducting with the bandgap being dependent on the particle size (90). It may be possible that the $Au-Fe_3O_4$ can be

made a better photoabsorber by controlling size of the Fe_3O_4 segment which, in turn may make these ensembles as efficient photocatalysts for water oxidation. Typically the OER catalysts are benchmarked according to the overpotential at 10 mA/cm^2 current density, and it was observed that the $\text{Au-Fe}_3\text{O}_4$ nanoparticles showed much better performance (lower onset potential) than the conventionally used precious metal catalysts like IrO_2 and RuO_x . Fe being more in abundance and less cost-prohibitive, these kind of OER catalysts will lead to significant advancements of the field. This also presents the true essence of multifunctional nanostructures where these nanostructures show distinctly different properties, such as superparamagnetism and catalytic activity thereby increasing their applicability range.

Recently selenides have been identified as very promising OER catalysts which includes the Ni-based and Co-based selenides mainly attributed to the increased covalency and proper positioning of the valence and conduction bands in the selenides. Additionally the interesting magnetic properties observed in the selenides might have an influence on the catalytic activities. Hence, bifunctional Au-CoSe nanostructures have been synthesized and tested as OER catalysts.

$\text{Au}_3\text{Pd-CoSe}$ nanoparticles were synthesized by catalyst aided CVD reaction carried out in a horizontal tube furnace at 800 °C under a flow of N_2 as carrier gas (120 sccm) (91). A Au-Pd (3:2) coated Si wafer used as the substrate for growth was kept at the central region of the horizontal furnace at 800°C. With the help of a mass flow controller the reaction assembly was maintained at a continuous N_2 flow of 120 sccm. Both the Co(acac)_3 and Se sublime at elevated temperatures and hence they were strategically placed within the reaction tube such that the temperature at the precursors just exceeded their sublimation temperature when the central zone of the furnace was at 800 °C. Selenium shots were positioned at 400°C, while the Co(acac)_3 was kept in the 250 °C region. Initially, The Co(acac)_3 and Se were kept outside the heating zone by pushing the ceramic liner to the extreme left. Once the central zone of the furnace reached the reaction temperature (800°C), the ceramic liner was pushed to the right such that the Se and Co(acac)_3 were at 400 °C and 250 °C respectively. These steps were crucial for reproducibility of the reaction, as it avoids the sublimation and escape of the reactants (Se and Co(acac)_3 vapors) before the Au/Pd catalyst reaches the melting temperature. The reaction was conducted out for 30 minutes, and the furnace was cooled down at the rate of 8 °C/min. After completion of the reaction a golden brown deposition was observed on the Si substrate. This deposition was further characterized for elucidation of the morphology and composition of the product.

The structural and compositional analysis of these nanoparticles confirmed formation of crystalline CoSe phase fused with Au_3Pd with distinct segregation of the Au_3Pd and CoSe regions as shown in Figure 12. This compositional segregation was also confirmed through detailed EDS analysis including elemental mapping and line scan analysis. The shape and nature of these nanoparticles are very representative of the bifunctional *Janus* particles (92). HRTEM images collected near the interface revealed that the junction between CoSe and Au(Pd) phases were very clean and sharply defined (Figure 12a and b). There was minimal mixing at the interface and there was no loss of crystalline order across the interface. The nature of the interface is very crucial since in these magnetic

nanostructures pinning of magnetic flux at the interface by another magnetic material may lead to exchange bias interactions. However, in this case, the interface was very clean indicating that there was no major magnetic phase other than CoSe present in the interface.

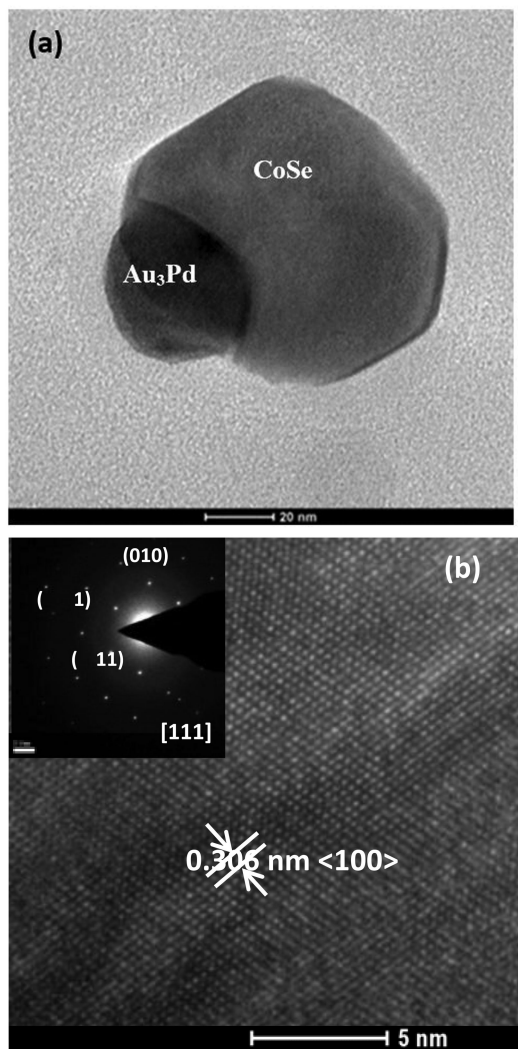


Figure 12. (a). Magnified view of single nanoparticle, clearly showing the union of two nanoparticles, through a common interface. (b) HRTEM image of the CoSe region showing lattice fringes which could be matched with $\langle 100 \rangle$ lattice planes of CoSe phase. Inset showing the SAED pattern depicting high degree of crystallinity.

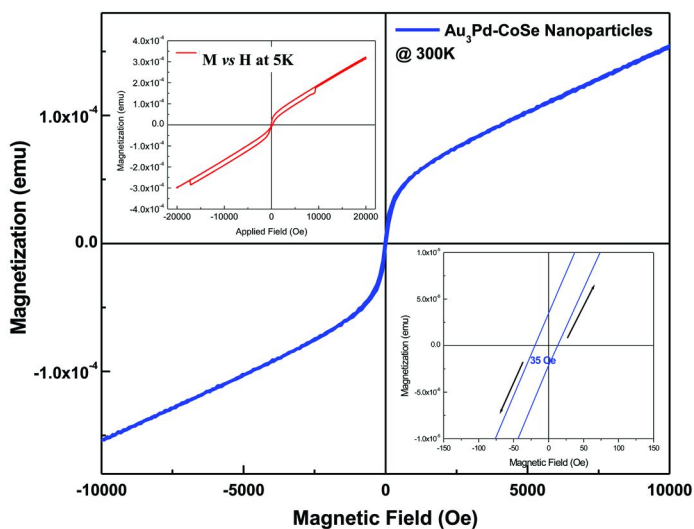


Figure 13. Magnetic behavior of the $\text{Au}_3\text{Pd-CoSe}$ nanostructures showing superparamagnetic behavior at room temperature along with soft ferromagnetism at low temperature.

Magnetic measurement of the bifunctional $\text{Au}_3\text{Pd-CoSe}$ nanoparticles revealed that they have soft ferromagnetic interactions. At 300K the hysteresis loop showed a very small coercive field of ~ 40 Oe (Figure 13, right insert). At low temperatures (5K) a clear hysteresis was also observed (Figure 13, left insert). However, there was a marked absence of coercivity and the magnetization had a cross-over near 0 Oe. This kind of behavior has been observed in single molecule magnets and mesoscopic granular ferromagnetic particles (93, 94). It is explained mainly by the occurrence of magnetic relaxation by quantum tunneling at low temperatures near zero fields. These kinds of magnetic relaxation are very dependent on the particle size and anisotropy. Also the presence of surface spin states causes anomalous behavior in the low temperature hysteresis loops of the nanosized magnetic particles. Hence, it was suspected that the polydispersity of the CoSe regions along with varying degree of anisotropy between particles and the presence of interface with Au_3Pd give rise to competing magnetic interactions, especially at low temperatures, contributing to the complex nature of the hysteresis curve. However, the nature of the hysteresis loops also indicated that the nanoparticles had considerable ferromagnetic interactions within the ensemble. The very low value of the coercive field also suggested superparamagnetic behavior, which is expected for ferromagnetic nanoparticles with sizes below the critical limit for forming monodomain magnetic particles. It was observed that the $\text{Au}_3\text{Pd-CoSe}$ nanoparticle ensemble was weakly attracted to a common laboratory magnet and could be magnetically separated over several days. Their diminishing hysteresis loop and nature of the ZFC-FC curves indicate that at 300 K they might be very near to the superparamagnetic blocking temperature separating the ordered state with the superparamagnetic state.

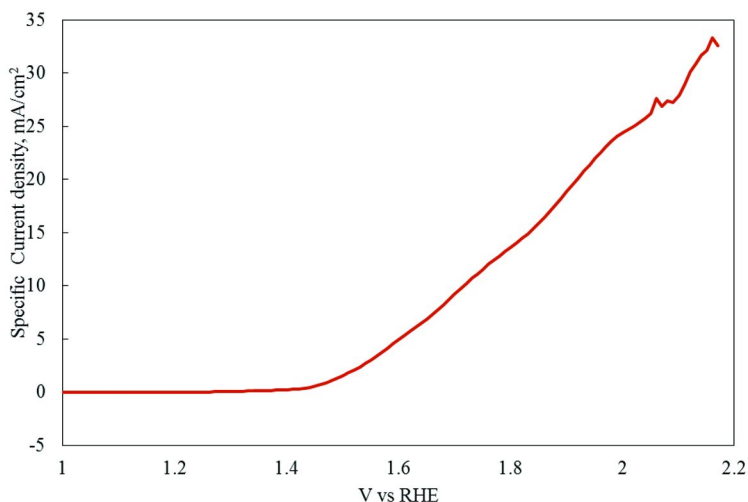


Figure 14. OER catalytic activity of $\text{Au}_3\text{Pd-CoSe}$ bifunctional nanostructures assembled on Au-coated Si electrode measured in 1M KOH.

The OER activities on these $\text{Au}_3\text{Pd-CoSe}$ nanostructures was investigated by growing them directly on a conducting surface such as Au-coated Si (*n*-type doped) and then connecting the as-synthesized nanostructures coated substrate as anode in the water oxidation cell with Ag|AgCl and Pt mesh as reference and counter electrodes, respectively. The onset potential for O_2 evolution was observed to be 1.5 V (*vs* Ag|AgCl) corresponding to a overpotential of 270 mV as shown in Figure 14 (95). This overpotential is comparable to that obtained for the precious metal oxides such as IrO_x , RuO_x , and some Ni-oxides. Having the metallic region attached to these semiconducting catalyst is expected to influence charge transfer within the electrocatalyst thereby enhancing the catalytic efficiency and exchange current density.

It should be noted here that the OER catalytic activities for some of these multifunctional nanostructures are comparable or even better than the conventional precious metal oxides such as IrO_x and RuO_x in acidic and alkaline medium, respectively (96). Recently several OER catalysts based on chalcogenides has been identified, which outperform the state-of-art oxide electrocatalysts. In keeping with this recent observation, the $\text{Au}_3\text{Pd-CoSe}$ electrocatalysts shows low onset potential for water oxidation as well low overpotential at $10 \text{ mA}\cdot\text{cm}^{-2}$, both of which are more than 50 mV lower than the state-of-the-art oxide based electrocatalysts.

Conclusions

While multifunctional nanomaterials are slowly keeping up with their promise of delivering high performance nanostructures applicable in a diverse areas, the current challenges are also focusing more on designing such multifunctional nanostructures through chemical intuition and targeting specific composition.

A more targeted approach to create well-defined, uniform multifunctional nanostructures in bulk quantities will make a significant contribution in advancing this field.

References

1. Sotiriou, G. A. Biomedical applications of multifunctional plasmonic nanoparticles. *Wiley Interdisp Rev.: Nanomed. Nanobiotechnol.* **2013**, *5*, 19–30.
2. Zeng, H.; Sun, S. Syntheses, Properties, and Potential Applications of Multicomponent Magnetic Nanoparticles. *Adv. Funct. Mater.* **2008**, *18*, 391–400.
3. Kim, J.; Piao, Y.; Hyeon, T. Multifunctional nanostructured materials for multimodal imaging, and simultaneous imaging and therapy. *Chem. Soc. Rev.* **2009**, *38*, 372–390.
4. Yue, X-L.; Ma, F.; Dai, Z-F. Multifunctional magnetic nanoparticles for magnetic resonance image-guided photothermal therapy for cancer. *Chin. Phys. B* **2014**, *23*, 044301.
5. Yan, K.; Li, P.; Zhu, H.; Zhou, Y.; Ding, J.; Shen, J.; Li, Z.; Xu, Z.; Chu, P. K. Recent advances in multifunctional magnetic nanoparticles and applications to biomedical diagnosis and treatment. *RSC Adv.* **2013**, *3*, 10598–10618.
6. Hao, R.; Xing, R.; Xu, Z.; Hou, Y.; Gao, S.; Sun, S. Synthesis, Functionalization, and Biomedical Applications of Multifunctional Magnetic Nanoparticles. *Adv. Mater.* **2010**, *22*, 2729–2742.
7. Sotiriou, G. A.; Starsich, F.; Dasargyri, A.; Wurnig, M. C.; Krumeich, F.; Boss, A.; Leroux, J-C.; Pratsinis, S. E. Photothermal Killing of Cancer Cells by the Controlled Plasmonic Coupling of Silica-Coated Au/Fe₂O₃ Nanoaggregates. *Adv. Funct. Mater.* **2014**, *24*, 2818–2827.
8. Kim, J.; Kim, H. S.; Lee, N.; Kim, T.; Kim, H.; Yu, T.; Song, I. C.; Moon, W. K.; Hyeon, T. Multifunctional Uniform Nanoparticles Composed of a Magnetite Nanocrystal Core and a Mesoporous Silica Shell for Magnetic Resonance and Fluorescence Imaging and for Drug Delivery. *Angew. Chem., Int. Ed.* **2008**, *47*, 8438–8441.
9. McCarthy, J. R.; Weissleder, R. Multifunctional magnetic nanoparticles for targeted imaging and therapy. *Adv. Drug Delivery Rev.* **2008**, *60*, 1241–1251.
10. Huh, Y-M.; Jun, Y-W.; Song, H-T.; Kim, S.; Choi, J-S.; Lee, J-H.; Yoon, S.; Kim, K-S.; Shin, J-S.; Suh, J-S.; Cheon, J. In Vivo Magnetic Resonance Detection of Cancer by Using Multifunctional Magnetic Nanocrystals. *J. Am. Chem. Soc.* **2005**, *127*, 12387–12391.
11. Fang, C.; Zhang, M. Multifunctional magnetic nanoparticles for medical imaging applications. *J. Mater. Chem.* **2009**, *19*, 6258–6266.
12. Di Corato, R.; Bigall, N. C.; Ragusa, A.; Dorfs, D.; Genovese, A.; Marotta, R.; Manna, L.; Pellegrino, T. Multifunctional Nanobeads Based on Quantum Dots and Magnetic Nanoparticles: Synthesis and Cancer Cell Targeting and Sorting. *ACS Nano* **2011**, *5*, 1109–1121.

13. Bao, G.; Mitragotri, S.; Tong, S. Multifunctional Nanoparticles for Drug Delivery and Molecular Imaging. *Annu. Rev. Biomed. Eng.* **2013**, *15*, 253–282.
14. Bhaskar, S.; Tian, F.; Stoeger, T.; Kreyling, W.; de la Fuente, J. M.; Grazu, V.; Borm, P.; Estrada, G.; Ntziachristos, V.; Razansky, D. Multifunctional Nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. *Part. Fibre Toxicol.* **2010**, *7*, 3.
15. Gao, J.; Gu, H.; Xu, B. Multifunctional magnetic nanoparticles: design, synthesis, and biomedical applications. *Acc. Chem. Res.* **2009**, *42*, 1097–1107.
16. Daniel, M.-C.; Astruc, D. Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology. *Chem. Rev.* **2004**, *104*, 293–346.
17. Sperling, R. A.; Gil, P. R.; Zhang, F.; Zanella, M.; Parak, W. J. Biological applications of gold nanoparticles. *Chem. Soc. Rev.* **2008**, *37*, 1896–1908.
18. Ghosh, P.; Han, G.; De, M.; Kim, C. K.; Rotello, V. M. Gold nanoparticles in delivery applications. *Adv. Drug Delivery Rev.* **2008**, *60*, 1307–1315.
19. El-Sayed, I. H.; Huang, X.; El-Sayed, M. A. Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. *Cancer Lett.* **2006**, *239*, 129–135.
20. Gupta, A. K.; Gupta, M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials* **2005**, *26*, 3995–4021.
21. Laurent, S.; Dutz, S.; Häfeli, U. O.; Mahmoudi, M. Magnetic fluid hyperthermia: Focus on superparamagnetic iron oxide nanoparticles. *Adv. Colloid Interface Sci.* **2011**, *166*, 8–23.
22. Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Vander Elst, L.; Muller, R. N. Magnetic Iron Oxide Nanoparticles: Synthesis, Stabilization, Vectorization, Physicochemical Characterizations, and Biological Applications. *Chem. Rev.* **2008**, *108*, 2064–2110.
23. Qiao, R.; Yang, C.; Gao, M. Superparamagnetic iron oxide nanoparticles: from preparations to in vivo MRI applications. *J. Mater. Chem.* **2009**, *19*, 6274–6293.
24. Lewin, M.; Carlesso, N.; Tung, C.-H.; Tang, X.-W.; Cory, D.; Scadden, D. T.; Weissleder, R. Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. *Nat. Biotechnol.* **2000**, *18*, 410–414.
25. Weissleder, R.; Moore, A.; Mahmood, U.; Bhorade, R.; Benveniste, H.; Chiocca, E. A.; Basilion, J. P. In vivo magnetic resonance imaging of transgene expression. *Nat. Med.* **2000**, *6*, 351–354.
26. Zhao, M.; Beauregard, D. A.; Loizou, L.; Daveletov, B.; Brindle, K. M. Non-invasive detection of apoptosis using magnetic resonance imaging and a targeted contrast agent. *Nat. Med.* **2001**, *7*, 1241–1244.
27. Coey, J. M. D. *Magnetism and Magnetic Materials*; Cambridge University Press: New York, 2010; p 264.

28. Ido, H.; Itoh, T. *Electronic structure and Magnetism of 3d-Transition Metal Pnictides*; Springer: Berlin, 2009.
29. Yuan, H.; Wang, H.; Cui, Y. Two-dimensional layered chalcogenides: from rational synthesis to property control via orbital occupation and electron filling. *Acc. Chem. Res.* **2015**, *48*, 81–90.
30. Swesi, A. T.; Masud, J.; Nath, M. Nickel Selenide As High-efficiency Catalyst for Oxygen Evolution Reaction. *Energy Environ Sci.* **2016** DOI:10.1039/c5ee02463c.
31. Masud, J.; Swesi, A. T.; Nath, M. Cobalt Selenide Nanostructures: An Efficient Trifunctional Catalyst for Full Water Splitting with High Current Density at Low Coverage. *ACS Catalysis* submitted for publication, 2016.
32. Feng, Y. G.; He, T.; Alonso-Vante, N. In situ Free-Surfactant Synthesis and ORR- Electrochemistry of Carbon-Supported Co₃S₄ and CoSe₂ Nanoparticles. *Chem. Mater.* **2008**, *20*, 26–28.
33. Liu, Y.; Cheng, H.; Lyu, M.; Fan, S.; Liu, Q.; Zhang, W.; Zhi, Y.; Wang, C.; Xiao, W.; Wei, S.; Ye, B.; Xie, Y. Low Overpotential in Vacancy-Rich Ultrathin CoSe₂ Nanosheets for Water Oxidation. *J. Am. Chem. Soc.* **2014**, *136*, 15670–15675.
34. Vayner, E.; Sidik, R. A.; Anderson, A. B.; Popov, B. N. Experimental and Theoretical Study of Cobalt Selenide as a Catalyst for O₂ Electroreduction. *J. Phys. Chem.* **2007**, *111*, 10508–10513.
35. Rosalbino, F.; Delsante, S.; Borzone, G.; Scavino, G. Electroanalytic activity of crystalline Ni-Co-M (M = Cr, Mn, Cu) alloys on the oxygen evolution reaction in an alkaline environment. *Int. J. Hydrogen Energy* **2013**, *38*, 10170–10177.
36. Hammer, B.; Norskov, J. K. Theoretical surface science and catalysis – calculations and concepts. *Adv. Catal.* **2000**, *45*, 71–129.
37. Norskov, J. K.; Bligaard, T.; Rossmeisl, J.; Christensen, C. H. Towards the computational design of solid catalyst. *Nat. Chem.* **2009**, *1*, 37–46.
38. Suntivich, J.; May, K. J.; Gasteiger, H. A.; Goodenough, J. B.; Horn, Y. S. A Perovskite Oxide Optimized for Oxygen Evolution Catalysis from Molecular Orbital Principles. *Science* **2011**, *334*, 1383–1385.
39. Trotochaud, L.; Boettcher, S. W. Precise oxygen evolution catalysts: Status and opportunities. *Scr. Mater.* **2014**, *74*, 25–32.
40. Surendranath, Y.; Kanan, M. W.; Nocera, D. G. Mechanistic Studies of the Oxygen Evolution Reaction by a Cobalt-Phosphate Catalyst at Neutral pH. *J. Am. Chem. Soc.* **2010**, *132*, 16501–16509.
41. Sun, Y.; Bigi, J. P.; Piro, N. A.; Tang, M. L.; Long, J. R.; Chang, C. J. Molecular Cobalt Pentapyridine Catalysts for Generating Hydrogen from Water. *J. Am. Chem. Soc.* **2011**, *133*, 9212–9215.
42. Kärkäs, M. D.; Verho, O.; Johnston, E. V.; Åkermark, B. Artificial Photosynthesis: Molecular Systems for Catalytic Water Oxidation. *Chem. Rev.* **2014**, *114*, 11863–12001.
43. Faunce, T.; Styring, S.; Wasielewski, M. R.; Gary, W.; Brudvig, G. W.; Rutherford, A. W.; Messinger, J.; Lee, A. F.; Hill, C. L.; deGroot, H.; Fontecave, M.; MacFarlane, D. R.; Hankamer, B.; Nocera, D. G.; Tiede, D. M.; Dau, H.; Hillier, W.; Wang, L.; Amal, R. Artificial photosynthesis as a

- frontier technology for energy sustainability. *Energy Environ. Sci.* **2013**, *6*, 1074–1076.
44. Nocera, D. G. The Artificial Leaf. *Acc. Chem. Res.* **2012**, *45*, 767–776.
 45. Hansen, M. *Constitution of Binary Alloys*; Geminuim Publ. Co.: New York, 1985; p 502.
 46. Liu, X. H.; Zhang, N.; Yi, R.; Qiu, G. Z.; Yan, A. G.; Wu, H. Y.; Meng, D. P.; Tang, M. T. Hydrothermal synthesis and characterization of sea urchin-like nickel and cobalt selenides nanocrystals. *Mater. Sci. Eng., B* **2007**, *140*, 38–43.
 47. Zhang, J. H.; Yang, X. G.; Li, S. D.; Xie, Y.; Yu, W. C.; Qian, Y. Synthesis of nanocrystalline cobalt selenide in nonaqueous solvent. *J. Solid State Chem.* **2000**, *152*, 537–539.
 48. Zhao, J. F.; Song, J. M.; Liu, C. C.; Liu, B. H.; Niu, H. L.; Mao, C. J.; Zhang, S. Y.; Shen, Y. H.; Zhang, Z. P. Graphene-like cobalt selenide nanostructures: template-free solvothermal synthesis, characterization and wastewater treatment. *Cryst. Eng. Comm.* **2011**, *13*, 5681–5684.
 49. Zhang, L.; Zhang, C. Multifunctional Co_{0.85}Se/graphene hybrid nanosheets: controlled synthesis and enhanced performances for the oxygen reduction reaction and decomposition of hydrazine hydrate. *Nanoscale* **2014**, *6*, 1782–1789.
 50. Liu, F.; Wang, B.; Lai, Y.; Li, J.; Zhang, Z.; Liu, Y. Electrodeposition of Cobalt Selenide Thin Films. *J. Electrochem. Soc.* **2010**, *157*, D523–D527.
 51. Umeyama, N.; Tokumoto, M.; Yagi, S.; Tomura, M.; Tokiwa, K.; Fujii, T.; Toda, R.; Miyakawa, N.; Ikeda, S. Synthesis and Magnetic Properties of NiSe, NiTe, CoSe, and CoTe. *Jpn. J. Appl. Phys.* **2012**, *51*, 053001.
 52. Camposa, C. E. M.; de Lima, J. C.; Grandia, T. A.; Machado, K. D.; Pizanib, P. S. Structural studies of cobalt selenides prepared by mechanical alloying. *Physica* **2002**, *B 324*, 409–418.
 53. Wang, C.; Xu, C.; Zeng, H.; Sun, S. Structural studies of cobalt selenides prepared by mechanical alloying. *Adv. Mater.* **2009**, *21*, 3045–3052.
 54. Jin, Y.; Jia, C.; Huang, S.; O'Donnell, M.; Gao, X. Multifunctional nanoparticles as coupled contrast agents. *Nat. Commun.* **2010**, *1*, 1–8.
 55. Liu, X.; Zhang, N.; Yi, R.; Qiu, G.; Yan, A.; Wu, H.; Meng, D.; Tang, M. Hydrothermal synthesis and characterization of sea urchin-like nickel and cobalt selenides nanocrystals. *Mater. Sci. Eng. B* **2007**, *140*, 38–43.
 56. Bao, J.; Chen, W.; Liu, T.; Zhu, Y.; Jin, P.; Wang, L.; Liu, J.; Wei, Y.; Li, Y. Bifunctional Au-Fe₃O₄ Nanoparticles for Protein Separation. *ACS Nano* **2007**, *1*, 293–298.
 57. Yu, H.; Chen, M.; Rice, P. M.; Wang, S. X.; White, R. L.; Sun, S. Dumbbell-like Bifunctional Au-Fe₃O₄ particles. *Nano Lett.* **2005**, *5*, 379–382.
 58. Liang, C-H.; Wang, C-C.; Lin, Y-C.; Chen, C-H.; Wong, C-H.; Wu, C-Y. Iron Oxide/Gold Core/Shell Nanoparticles for Ultrasensitive Detection of Carbohydrate-Protein Interactions. *Anal. Chem.* **2009**, *81*, 7750–7756.
 59. Xu, Z.; Hou, Y.; Sun, S. Magnetic Core/Shell Fe₃O₄/Au and Fe₃O₄/Au/Ag Nanoparticles with Tunable Plasmonic Properties. *J. Am. Chem. Soc.* **2007**, *129*, 8698–8699.

60. Shevchenko, E. V.; Bodnarchuk, M. I.; Kovalenko, M. V.; Talapin, D. V.; Smith, R. K.; Aloni, S.; Heiss, W.; Alivisatos, A. P. Gold/Iron Oxide Core/Hollow-Shell Nanoparticles. *Adv. Mater.* **2008**, *20*, 4323–4329.
61. Ji, X.; Shao, R.; Elliott, A. M.; Stafford, R. J.; Esparza-Coss, E.; Bankson, J. A.; Liang, G.; Luo, Z-P.; Park, K.; Markert, J. T.; Li, C. Bifunctional Gold Nanoshells with a Superparamagnetic Iron Oxide-Silica Core Suitable for Both MR Imaging and Photothermal Therapy. *J. Phys. Chem. C* **2007**, *111*, 6245–6251.
62. Wang, L.; Luo, J.; Maye, M. M.; Fan, Q.; Rendeng, Q.; Engelhard, M. H.; Wang, C.; Lin, Y.; Zhong, C-J. Iron oxide–gold core–shell nanoparticles and thin film assembly. *J. Mater. Chem.* **2005**, *15*, 1821–1832.
63. Pariti, A.; Desai, P.; Nath, M. Superparamagnetic Au-Fe₃O₄ Nanoparticles: One-pot Synthesis, Biofunctionalization and Toxicity Evaluation. *Mater. Res. Exp.* **2014**, *1*, 035023–035032.
64. Patterson, A. The Scherrer Formula for X-Ray Particle Size Determination. *Phys. Rev.* **1939**, *56*, 978–982.
65. Lattuada, M.; Hatton, T. A. Synthesis, properties and applications of Janus nanoparticles. *Nano Today* **2011**, *6*, 286–308.
66. Perro, A.; Reculosa, S.; Ravaine, S.; Bourgeat-Lami, E.; Duguet, E. Design and synthesis of Janus micro- and nanoparticles. *J. Mater. Chem.* **2005**, *15*, 3745–3760.
67. Haiss, W.; Thanh, N. T. K.; Aveyard, J.; Fernig, D. G. Determination of Size and Concentration of Gold Nanoparticles from UV-Vis Spectra. *Anal. Chem.* **2007**, *79*, 4215–4221.
68. Link, D.; El-Sayed, M. A. Size and Temperature Dependence of the Plasmon Absorption of Colloidal Gold Nanoparticles. *J. Phys. Chem. B* **1999**, *103*, 4212–4217.
69. Verwey, E. J. W. Electronic conduction of magnetite and its transition point at low temperatures. *Nature* **1939**, *144*, 327–328.
70. Wang, J.; Chen, Q.; Li, X.; Shi, L.; Peng, Z.; Zeng, C. Disappearing of the Verwey transition in magnetite nanoparticles synthesized under a magnetic field: implications for the origin of charge ordering. *Chem. Phys. Lett.* **2004**, *390*, 55–58.
71. Sorensen, C. M. *Nanoscale Materials in Chemistry*; Klabunde, K. J., Ed.; Wiley: New York, 2001; p 169.
72. Prozorov, R.; Prozorov, T.; Mallapragada, S.; Narasimhan, B.; Williams, T.; Bazylinski, D. Magnetic irreversibility and the Verwey transition in nanocrystalline bacterial magnetite. *Phys. Rev. B* **2007**, *76*, 054406.
73. Skumryev, V.; Stoyanov, S.; Zhang, Y.; Hadjipanayis, G.; Givord, D.; Nogues, J. Beating the superparamagnetic limit with exchange bias. *Nature* **2003**, *423*, 850–853.
74. Chandra, S.; Huls, N. A.; Phan, M. H.; Srinath, S.; Garcia, M. A.; Lee, Y.; Wang, C.; Sun, S.; Iglesias, O.; Srikanth, H. Exchange bias effect in Au-Fe₃O₄ nanocomposites. *Nanotechnology* **2014**, *25*, 055702.
75. Goya, G. F.; Berquó, T. S.; Fonseca, F. C.; Morales, M. P. Static and dynamic magnetic properties of spherical magnetite nanoparticles. *J. Appl. Phys.* **2003**, *94*, 3520–3528.

76. Roca, A. G.; Morales, M. P.; O'Grady, K.; Serna, C. J. Structural and magnetic properties of uniform magnetite nanoparticles prepared by high temperature decomposition of organic precursors. *Nanotechnology* **2006**, *17*, 2783–2788.
77. Morup, S. Superferromagnetic nanostructures. *Hyperfine Interact.* **1994**, *90*, 171–185.
78. Khan, N.; Adhami, V. M.; Mukhtar, H. Review: Green tea polyphenols in chemoprevention of prostate cancer: Preclinical and clinical studies. *Nutr. Cancer* **2009**, *61*, 836–841.
79. Qiao, Y.; Cao, J.; Xie, L.; Shi, X. Cell growth inhibition and gene expression regulation by (-)epigallocatechin-3-gallate in human cervical cancer cells. *Arch. Pharm. Res.* **2009**, *32*, 1309–1315.
80. Gao, W.; Ji, L.; Li, L.; Cui, G.; Xu, K.; Li, P.; Tang, B. Bifunctional combined Au-Fe₂O₃ nanoparticles for induction of cancer cell-specific apoptosis and real-time imaging. *Biomaterials* **2012**, *33*, 3710–3718.
81. Lo, C. K.; Xiao, D.; Choi, M. M. F. Homocysteine-protected gold-coated magnetic nanoparticles: synthesis and characterization. *J. Mater. Chem.* **2007**, *17*, 2418–2427.
82. Kumar, A.; Mandal, S.; Selvakannan, P. R.; Pasricha, R.; Mandale, A. B.; Sastry, M. Investigation into the interaction between surface-bound alkylamines and gold nanoparticles. *Langmuir* **2003**, *19*, 6277–6282.
83. Shankar, S. S.; Ahmad, A.; Pasricha, R.; Khan, M. I.; Kumar, R.; Sastry, M. Immobilization of biogenic gold nanoparticles in thermally evaporated fatty acid and amine thin films. *J. Colloid Interface Sci.* **2004**, *274*, 69–75.
84. Salado, J.; Insausti, M.; Lezama, L.; Gil de Muro, I.; Moros, M.; Pelaz, B.; Grazu, V.; de la Fuente, J. M.; Rojo, T. Functionalized Fe₃O₄@Au superparamagnetic nanoparticles: in vitro bioactivity. *Nanotechnology* **2012**, *23*, 315102.
85. Liong, M.; Lu, J.; Kovichich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I. Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *ACS Nano* **2008**, *2*, 889–896.
86. Berry, C. C.; Wells, S.; Charles, S.; Aitchison, G.; Curtis, A. S. Cell response to dextran-derivatised iron oxide nanoparticles post internalization. *Biomaterials* **2004**, *25*, 5405–5413.
87. Yu, W. W.; Chang, E.; Sayes, C. M.; Drezek, R.; Colvin, V. L. Aqueous dispersion of monodisperse magnetic iron oxide nanocrystals through phase transfer. *Nanotechnology* **2006**, *17*, 4483–4487.
88. Fuente, J. M.; Berry, C. C.; Riehle, M. O.; Curtis, A. S. G. Nanoparticle targeting at cells. *Langmuir* **2006**, *22*, 3286–3293.
89. Ashokaan, N.; Masud, J.; Nath, M. Light induced Photocatalytic Water Oxidation with Bifunctional Au-Fe₃O₄ Nanoparticles. *J. Mater. Chem. A*, submitted for publication, 2016.
90. Ghandoor, H. E.; Zidan, H. M.; Khalil, M. M. H.; Ismail, M. I. M. Synthesis and Some Physical Properties of Magnetite (Fe₃O₄) Nanoparticles. *Int. J. Electrochem. Sci.* **2012**, *7*, 5734–5745.
91. Liyanage, W. P. R.; Mishra, S.; Nath, M. Fabrication of multifunctional ferromagnetic Au₃Pd–CoSe nanoparticles. *RSC Adv.* **2014**, *4*, 28140–28147.

92. Langlois, C.; Li, Z. L.; Yuan, J.; Alloyeau, D.; Nelayah, J.; Bochicchio, D.; Ferrando, R.; Ricolleau, C. Transition from core-shell to Janus chemical configuration for bimetallic nanoparticles. *Nanoscale* **2012**, *4*, 3381–3388.
93. Mills, D. P.; Moro, F.; McMaster, J.; Slageren, J.; Lewis, W.; Blake, A. J.; Liddle, S. T. A delocalized arene-bridged diuranium single-molecule magnet. *Nat. Chem.* **2011**, *3*, 454–460.
94. Kodama, R. H.; Seaman, C. L.; Berkowitz, A. E.; Mapple, B. Low temperature magnetic relaxation in NiFe₂O₄ nanoparticles. *J. Appl. Phys.* **1994**, *75*, 5639.
95. DeSilva, S.; Masud, J.; Nath, M. Efficient Water Electrolysis with Au₃Pd-CoSe Bifunctional Nanoparticles. *RSC Adv.* submitted for publication, 2016.
96. McCrory, C. C. L.; Jung, S.; Peters, J. C.; Jaramillo, T. F. Benchmarking Heterogeneous Electrocatalysts for the Oxygen Evolution Reaction. *J. Am. Chem. Soc.* **2013**, *135*, 16977–16987.

Chapter 9

Tailoring Soft Nanoparticles for Potential Application as Drug Carriers in Bladder Cancer Chemotherapy

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The current standard therapy for non-muscle invasive bladder cancer is surgical transurethral resection followed by intravesical immunotherapy or chemotherapy. However, the success of intravesical therapy is limited due to the bladder permeability barrier and periodic voiding of urine. With a high rate of recurrence and need for lifetime surveillance, bladder cancer is the costliest cancer to treat. This mini review highlights the development of different types of soft nanoparticles as drug carriers to address the challenges encountered in intravesical chemotherapy. These nanocarriers are prepared from non-cytotoxic mucoadhesive materials to promote interaction with the urothelium in order to provide sustained release of the drug locally and increase drug residence time in the bladder. With advances in nanotechnology, mucoadhesive nanocarriers can be designed to carry hydrophobic or hydrophilic drugs as well as a combination of synergistic drugs with different physicochemical properties and other agents besides drugs for intravesical therapy.

Introduction

Bladder cancer is the fifth most common cancer in the United States with 74,000 new cases and 16,000 deaths estimated in 2015 (1). Around 70% of bladder cancers have been identified as superficial or non-muscle invasive bladder cancers (NMIBCs) (2). The standard treatment for patients with NMIBCs is surgical transurethral resection (TUR) of tumors, which can achieve an 80% early success rate. Nonetheless, tumor recurrence occurs in almost 70% of these patients, with 25% exhibiting progression to muscle invasive disease within 5 years of TUR (3). With high rates of recurrence and risk of disease progression, and low mortality rate in bladder cancer, lifelong surveillance is often required. As a result, bladder cancer treatment incurs the highest per patient costs among cancers in the United States (2, 4), with a total direct medical cost of about \$4 billion in 2010 (5). Surveillance and treatment of recurrences were estimated to account for 60% of the lifetime cost per patient (6). Thus, effective treatment strategies that can decrease the rate of recurrence and progression would substantially reduce the economic burden and improve the quality of life of the patients.

Intravesical chemotherapy and Bacillus Calmette-Guerin (BCG) immunotherapy are widely used as adjuvant therapy after surgical TUR to reduce the risk of disease recurrence and progression. BCG is an attenuated strain of *Mycobacterium bovis* that produces an inflammatory reaction in the bladder, and BCG immunotherapy is the standard of care therapy for high risk NMIBC. It has been shown to reduce recurrence rates but it has potential local and systemic side effects that may lead to 30% of patients stopping treatment (7, 8). Furthermore, there is currently an ongoing significant issue of worldwide shortage of BCG due to production difficulties since 2012, which strongly reinforces the need to search for alternative strategies (9). Intravesical chemotherapy has been reported to be generally better tolerated than BCG immunotherapy but it is of limited efficacy on the long term (8). Intravesical chemotherapy minimizes systemic side effects since ideally, localized treatment of the diseased tissues in the bladder can be achieved by direct contact with the instilled drug. However, there are two major challenges in intravesical chemotherapy: short residence time of the drug in the bladder and low permeability of the urothelium (10, 11). Conventional formulations instilled into the bladder cavity are diluted with urine and usually lost upon the first voiding of urine after instillation. The umbrella cells and tight junctions of the urothelium, and the mucin layer on the luminal side of the urothelium contribute to its low permeability to instilled drugs (10, 11). Repeated instillation of drugs has to be carried out, but frequent catheterizations to instill the drugs can lead to bladder irritation, bladder fibrosis and infections (11). In view of these challenges, there is great interest to develop drug delivery systems that can rapidly adhere to the urothelium after instillation into the bladder, remain on the urothelium upon urine voiding and release the loaded drug in a sustained manner.

The mucin layer on the urothelium is composed of glycosaminoglycans (GAGs), which include hyaluronic acid, chondroitin sulfate, keratin sulfate and link proteins (11). GAGs are highly hydrophilic and negatively charged due to the carboxylate moiety on uronate residues and the sulfate moieties (12). One

of the ways to prolong the residence time of therapeutic agents in the bladder and increase urothelial exposure and penetration of these agents is via the use of mucoadhesive drug carriers. Polymers with charged groups or non-ionic functional groups capable of forming strong hydrogen bonds have been reported to have good ability to adhere to mucosal membranes (13). These include anionic polymers such as alginate and carboxymethyl cellulose, cationic polymers such as chitosan and polylysine, and non-ionic polymers such as polyvinylpyrrolidone and hydroxypropyl cellulose (14). Polymers containing side chains with thiol-bearing functional groups (thiomers) are also mucoadhesive since they are capable of forming disulfide bonds with cysteine-rich sub-domains of the mucus layer (13, 14). Thus, with mucoadhesive polymeric drug depots, exposure of the urothelium to the drug can be extended beyond the voiding of urine.

A range of chemotherapeutic agents, such as thiotepa, mitomycin C (MMC), doxorubicin (DOX), epirubicin, gemcitabine, docetaxel (DTX) and cisplatin (CDDP), have been tested either singly or in combination against NMIBCs (15–18). These agents have different physicochemical properties and may require different types of loading and delivery systems. Soft (organic and polymeric) materials have been used for fabricating nanoparticles such as micelles, liposomes, nanospheres, nanocapsules dendrimers and protein conjugates as drug carriers (19–22). This mini-review focusses on four different classes of promising drug nanocarriers for intravesical chemotherapy for NMIBCs. Some examples from the literature and our research are used to illustrate the design rationale of these nanocarriers, their physicochemical characteristics and efficacy against bladder cancer in *in vitro* and animal model studies.

Nanogels

Nanogels are essentially hydrogels prepared as nanoparticles. As such, they possess the combined features and characteristics of hydrogels and nanoparticles. A wide variety of hydrophilic naturally occurring and synthetic polymers can be used to prepare the three-dimensional, crosslinked networks of hydrogels. Some examples of polymers which have been used for nanogel synthesis are chitosan, hyaluronic acid, alginate, cholesterol-bearing pullulan, poly(ethylene glycol) (PEG) and poly(*N*-isopropylacrylamide) (20–27). Ideally, nanogels for drug delivery should be biocompatible, amenable to loading and sustained or controlled delivery of drugs, and if the nanogels are intended for intravenous administration, they should not initiate immune responses in the physiological environment in order to prolong their blood circulation time. Drug loading and its subsequent release from the nanogel is dependent to a large extent on the interaction of the drug with the polymer chains, and the porosity of the matrix of the nanogel. Loaded drugs can be released by diffusion through the matrix or upon erosion of the matrix, or the release may be triggered by an external stimulus such as pH, temperature or enzyme (20).

The porosity of the nanogel is controlled by the density of crosslinking between the polymer chains of the matrix (20). Crosslinking may be achieved by

physical or chemical means. The driving forces in physically crosslinked nanogels include hydrophobic interactions, hydrogen bonds, electrostatic interactions and host-guest interactions (in the case of cyclodextrin-bearing polymers) (27). While physically crosslinked nanogels can be easily prepared, the physical interactions are not as strong as chemical bonds and hence the stability of these nanogels is affected to a greater extent by the environment than chemically crosslinked nanogels. Chemically crosslinked nanogels have several crosslinking points formed by covalent bonds throughout the matrix of polymeric chains. Chemical crosslinking methods used in nanogels include amide bond formation (23), click chemistry (24), disulfide linkages (20) and photo-induced crosslinking (26). Detailed information on physical and covalent crosslinking methodologies for preparing nanogels can be found in recent reviews by Sasaki and Akiyoshi (27) and Zhang et al. (28).

Nanogels can be readily tuned to serve the requirements of a specific drug delivery system since a plethora of polymers and methods can be used in their preparation. This flexibility is illustrated by the following examples. Nanogels of ~150 nm have been prepared using hyperbranched polyglycerol crosslinked with disulfide bonds, and DOX was conjugated to the biodegradable matrix via an acid-labile hydrazone linker (29). Such nanogels were designed to capitalize on the enhanced permeation and retention (EPR) effect of tumor tissue for drug delivery by carriers in the range of 20–200 nm (30, 31). These nanogels were readily internalized into HeLa cells (human cervical cancer cell line) by endocytosis, where intracellular conditions triggered the release of the drug resulting in cell death. Instead of relying on passive targeting of tumors via EPR, nanogels can achieve active targeting by the use of targeting ligands. Folic acid (FA) is a commonly used targeting ligand for cancer cells which overexpress the folate receptor. FA has been conjugated to nanogels prepared from a diblock copolymer, poly(ethylene oxide)-*b*-poly(methacrylic acid), and loaded with DOX and CDDP (32). The delivery and anti-tumor effect of the drug-loaded nanogels in an *in vivo* model was demonstrated. Methotrexate, an analog of FA, has been used both as a targeting ligand and a chemotherapeutic agent in an amine-functionalized polyacrylamide (PAm) nanogel (33). MTX was conjugated to the amine groups of the polymers via carbodiimide chemistry and also adsorbed on gold nanoparticles loaded in the nanogel. These PAm hybrid nanogels demonstrate high killing efficacy towards KB cancer cells without significant cytotoxicity to macrophages. Another type of hybrid nanogel was prepared with quantum dots co-loaded with the anticancer drug, temozolomide, in covalently crosslinked chitosan-poly(methacrylic acid) network to simultaneously combine bioimaging and therapeutic functions (34). Core/shell poly(*N*-isopropylmethacrylamide)-based nanogels loaded with small interfering RNAs (siRNAs) and functionalized with a 12 amino acid peptide have been used to target the EphA2 receptor of HEY ovarian cancer cells (35).

Despite the interest in nanogels as a drug delivery system for cancer therapy, there is a paucity of research into the use of nanogels in intravesical therapy for bladder cancer. Herein, we highlight a mucoadhesive PAm nanogel as a hydrophobic drug carrier for potential intravesical bladder cancer therapy. One of the factors which limit the efficacy of bladder cancer intravesical therapy is the

poor penetration of drugs into the urothelium. Paclitaxel (PTX), a hydrophobic drug, shows higher penetration into the urothelium than other commonly used drugs such as MMC and DOX (36), and hence may be advantageous for intravesical therapy. Due to the poor solubility of paclitaxel, Cremophor is commonly used to dissolve the drug for clinical use. However, Cremophor may cause adverse side reactions (37) and it forms micelles in the bladder cavity, entrapping the drug inside the micelles, and consequently the free drug fraction decreases which diminishes drug penetration into the bladder tissue (38). For our study, DTX as the model hydrophobic drug for loading into the nanogel since a Phase I trial and a subsequent follow-on study have shown DTX to be a promising drug for decreasing the overall risk of recurrence in high-risk NMIBC, and it is safe for intravesical therapy with minimal systemic adsorption (39, 40).

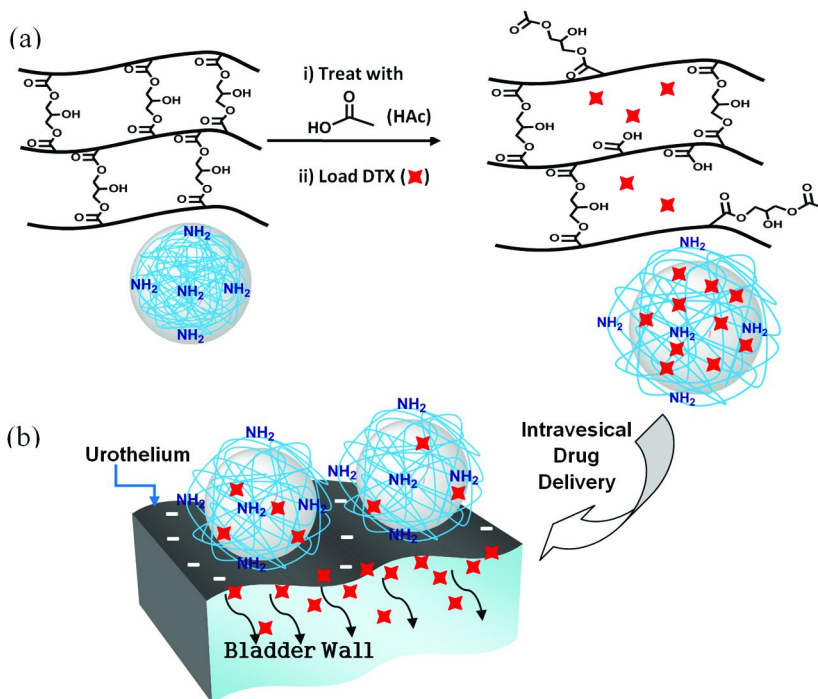


Figure 1. Schematic diagram illustrating (a) preparation of amine-functionalized DTX-loaded PAm nanogels, and (b) concept of using the nanogels as a potential intravesical drug delivery system for bladder cancer therapy.

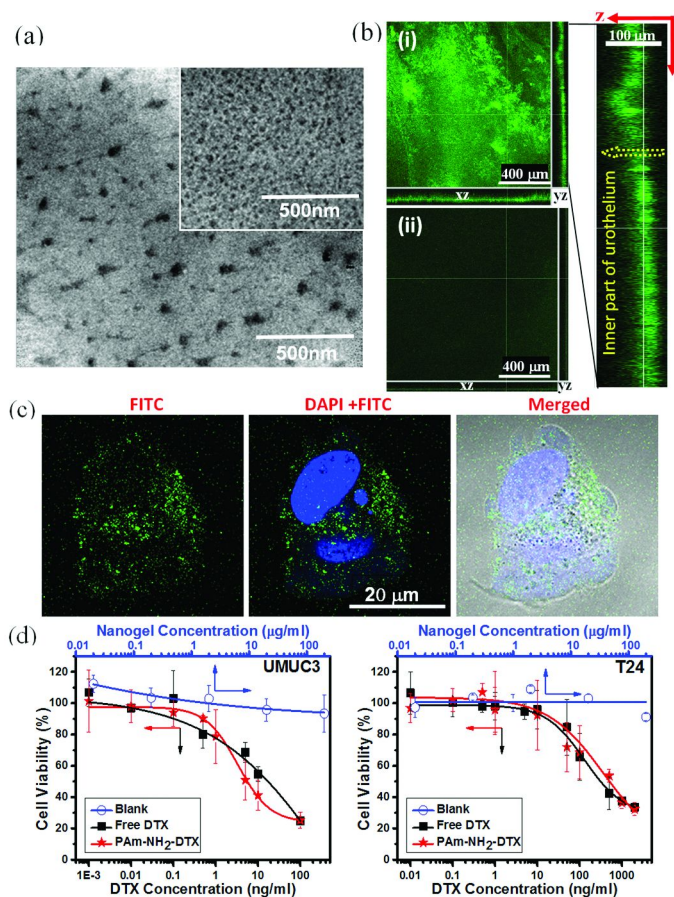


Figure 2. (a) Transmission electron microscopy (TEM) image of PAm-NH₂ nanogel (inset) and DTX-loaded PAm-NH₂ nanogel stained with 2% (w/v) phosphotungstic acid aqueous solution, (b) CLSM images of fresh porcine bladder urothelium after incubation for 4 h with (i) 5 mg/ml FITC-PAm-NH₂ nanogels or (ii) PBS (control), (c) CLSM images of UMUC3 cells after culturing in medium containing 1 mg/ml FITC-labeled PAm-NH₂ nanogels at 37 °C, and (d) in vitro viability of UMUC3 and T24 cells after exposure to “blank” and DTX-loaded PAm-NH₂ nanogels and free DTX for 4 h. (Reproduced with permission from reference (43). Copyright 2015 Elsevier.)

The concept of our mucoadhesive DTX-loaded PAm nanogel and its potential application in intravesical bladder cancer therapy is shown schematically in Figure 1. The amine-functionalized polyacrylamide nanogel (PAm-NH₂) was prepared from acrylamide and 3-(aminopropyl) methacrylamide with glycerol dimethacrylate as a crosslinker via a water/oil (W/O) microemulsion polymerization method (41–43). To load hydrophobic DTX into the hydrophilic

nanogels, PAm-NH₂ nanogels were dispersed in acetic acid for a week to swell the nanogels, and DTX in acetic acid was then added. With this simple method, the loading efficiency of DTX into PAm-NH₂ nanogels was > 90%, and the PAm-NH₂-DTX nanogel contained ~5 wt% DTX. The average size of the blank PAm-NH₂ nanogel was ~41 nm (Figure 2(a) inset), and after swelling in acetic acid and DTX loading, the PAm-NH₂-DTX nanogel became more irregular in shape and less dense, and its size increased to ~160 nm (Figure 2(a)). The change in size and shape of the PAm-NH₂ nanogel after treatment with acetic acid is likely due to the reaction of acetic acid with some of the ester bonds of the glycerol dimethacrylate crosslinker. These replacement reactions (shown schematically in Figure 1(a)) open up the nanogel network for DTX loading.

The PAm-NH₂-DTX nanogel is positively charged (zeta potential of 7.5 mV in deionized water, pH ~ 5.8) due to the amine groups from 3-(aminopropyl) methacrylamide, and as a result of its cationic nature, the nanogel is expected to adhere well to the mucin layer on the urothelium. The mucoadhesivity of the PAm-NH₂ nanogel was confirmed from confocal laser scanning microscopy (CLSM) images of ex vivo porcine bladder after treatment with fluorescein isothiocyanate (FITC)-labeled nanogel (Figure 2b). From the fluorescence signals, it is estimated that these nanogels were able to attach onto the bladder luminal surface to a depth of ~80 μ m, which approximately corresponds to the GAG layer on the urothelium surface, estimated to be 30–50 μ m in the thickness (44, 45). Cationic mucoadhesive polymers like chitosan are known to increase the permeability of the bladder wall due to the exfoliation of the superficial umbrella cells (46). Porcine bladder after treatment with PAm-NH₂ nanogels also showed signs of exfoliation of the urothelium, but not to the same extent of desquamation of the urothelium caused by chitosan (43, 47, 48). The PAm-NH₂ nanogels are also readily internalized by bladder cancer cells via endocytosis (Figure 2c), and in vitro assays confirmed that the blank PAm-NH₂ nanogels are non-cytotoxic while the cytotoxicity of PAm-NH₂-DTX against UMUC3 and T24 is similar to free DTX against the respective cell line (Figure 2d). It can be seen from this figure that DTX, both in the free form and loaded in the nanogel, has a higher efficacy against the non-muscle invasive cell line, UMUC3, than T24, a muscle invasive cell line.

Polymeric Core-Shell Nanoparticles

While nanogels are made from hydrophilic polymers, amphiphilic block copolymers can be used to prepare nanoparticles with a hydrophobic core surrounded by a hydrophilic shell. An overview of the different types of nanoparticulate structures formed from amphiphilic block copolymers and the factors that influence the formation of particular nanoparticulate formulations is given in a review paper by Letchford and Burt (49). Micelles are characterized by a fluid-like core comprising the hydrophobic regions of the amphiphilic copolymers and a corona formed by the hydrophilic block of the co-polymers. With increasing length of the hydrophobic block of the copolymer, the nanoparticles generally become larger with a more solid-like core and are termed nanospheres (49).

However, a clear distinction between these two types of nanoparticles is not always possible and the terminology is sometimes used interchangeably in the literature (49). Hence, in the present review, the terminology used by the authors of the cited papers will be retained.

Many types of biocompatible amphiphilic block copolymers have been used for preparing core-shell nanostructures as drug delivery systems. The hydrophilic block is frequently based on PEG or its derivatives while common choices for the hydrophobic block are polyesters such as poly(D,L lactic acid), poly(lactic-*co*-glycolic acid) (PLGA) or poly(caprolactone) (PCL) (50). The Pluronic[®] family of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO) tri-block copolymers is another commonly used copolymer for preparing chemotherapeutic polymeric micelles (51), and a Pluronic[®]-based micellar formulation of DOX (SP1049C, developed by Supratek Pharma, Inc.) for treating advanced esophageal adenocarcinoma has reportedly entered Phase III clinical trials (51). Loading of drugs into the hydrophobic core of the core-shell nanoparticles can be via chemical conjugation or physical entrapment, with the latter deemed more favorable for drug incorporation (52, 53). Common mechanisms for the release of the loaded drug are diffusion, polymer degradation or micellar dissolution.

In recent years, there is increasing interest in exploring the use of polymeric core-shell nanoparticles for bladder cancer therapy. Core-shell polymeric micelles with loaded DOX have been prepared from a triblock copolymer of poly(ϵ -caprolactone)-*block*-poly(glycidylmethacrylate)-*block*-poly(poly(ethylene glycol)methyl ether methacrylate) (PCL-*b*-PGMA-*b*-P(PEGMA)) with conjugated fluorescent units (54). A combination of ring-opening polymerization (ROP), reversible addition-fragmentation chain transfer (RAFT) polymerization and click chemistry was used in the synthesis of the fluorescent triblock copolymer. DOX can be loaded into the micelles at a loading efficiency of ~70%, and the drug-loaded micelles exhibited high efficacy against UMUC3 cells. Another polymeric micelle formulation based on diblock copolymer (methoxy poly(ethylene glycol)-*block*-poly (D,L-lactic acid) (MePEG-PDLLA) and loaded with PTX or DTX has been investigated with ex vivo porcine bladder (55). These micelles resulted in significantly higher urothelial tissue levels of the drugs and greater bladder wall exposures compared to their commercial formulations, PTX in Cremophor EL/ethanol or DTX in Tween 80. Since MePEG has been shown to be mucoadhesive (56), the MePEG chains at the outer surface of the micelles may penetrate into the mucin layer of the urothelium to promote the retention of the formulation at the urothelium surface. MePEG-PDLLA micellar formulation of PTX at 5 mg/ml was shown to have no adverse toxicity following intravenous (57) or intravesical administration in mice (58).

PLGA-PEG-anisamide nanoparticles of ~90–120 nm have been synthesized for ratiometric co-loading of two hydrophilic drugs, gemcitabine monophosphate and CDDP (59). In order to load these drugs into the nanoparticles, the drugs were first formulated into dioleoyl phosphatidic acid-coated “cores” of 8–12 nm and then self-assembled with PLGA and PLGA-PEG derivatives via single step solvent displacement. Anisamide, an agonist of the sigma receptor, was introduced into the PLGA nanoparticle as a ligand to enhance internalization in epithelium-

derived cancer cells which overexpress the sigma-receptor. Tests carried out with a stroma-rich xenograft bladder tumor mouse model showed that the two drugs co-loaded in the nanoparticles acted in a synergistic fashion and a more significant antitumor efficacy was observed compared to the drugs in free form or dual drugs loaded in a mixture of separate nanoparticles (59).

Drug-loaded polymeric micelles with targeting functions for potential intravesical bladder cancer therapy have also been reported (60, 61). Diblock PCL-*b*-PEO copolymers with amino and carboxylic functional groups at the end of the hydrophilic PEO block were synthesized for conjugation with FITC and cyclic Arginine–Glycine–Aspartic acid–D-Phenylalanine–Lysine (c(RGDfK)), respectively (60). DOX-loaded micelles were then prepared from these block copolymers in a 1:1 mass ratio via a dialysis method. In vitro assays with T24 bladder cancer cells confirmed the targeting action of the c(RGDfK) peptide conjugated to the micelles and the inhibitory effects of the loaded DOX on these cells (60). Another type of tumor-targeting drug-loaded micelles was reported by Lin et al. (61). These micelles (~ 23 nm in diameter) were assembled from telodendrimers, which were prepared by the conjugation of dendritic octamers of cholic acid onto linear PEG via solution-phase condensation reactions (62). The targeting ligand used was named PLZ4 (amino acid sequence: cQDGRMGFc – upper case letters represent L-amino acids and lower case letters represent unnatural D-cysteines used to cyclize and stabilize the peptide), which is the first bladder cancer-specific ligand to be identified, and has been shown to target human and canine bladder cancers (63, 64). The alkyne group on PLZ4 was conjugated to the azide group at the end of PEG on telodendrimers at a molar ratio of 1:2 (PLZ4:PEG) via aqueous phase click chemistry catalyzed by cuprous ions. PTX can be loaded at 99.4% efficiency in the hydrophobic core of the micelles assembled from the telodendrimers. In vitro assays with bladder cancer cells and an in vivo study with mice carrying subcutaneous bladder cancer xenograft showed that the PLZ4-coated micelles were more efficient than non-targeting micelles in delivering the drug load into bladder cancer cells both in vitro and in vivo.

The ability of chitosan to promote mucoadhesion and permeability of the urothelium has been exploited for the preparation of drug-loaded core-shell nanoparticles. Cationic nanoparticles based on PCL core and chitosan shell with encapsulated MMC have been tested for intravesical chemotherapy in a tumor-induced rat model (65). The nanoparticles were prepared using a water/oil/water (W/O/W) double emulsion technique. MMC in Pluronic® F-68 was first emulsified with PCL in methylene chloride. This primary emulsion was then injected into an aqueous solution of poly(vinyl alcohol) (PVA) and chitosan in Pluronic® F-68. Particles with a hydrodynamic diameter of ~300 nm and drug encapsulation efficiency of 35% were obtained. The rat model study showed that the animals treated with MMC-loaded core-shell nanoparticles have a longer survival period than those treated with commercial MMC in solution. Histopathological images of bladder biopsy samples showed that the cationic nanoparticles localized and accumulated in the bladder tissue. Interestingly, this study showed that blank chitosan nanoparticles also have a very positive effect on the survivability of the bladder tumor bearing rats. This finding is consistent with

the study by Hasegawa et al. which indicated that chitosan induces apoptosis in bladder tumor cells via caspase-3 activation (66). Another type of nanoparticulate formulation with a chitosan shell was prepared by conjugating biotinylated low molecular weight chitosan to avidin-modified PLGA nanoparticles with encapsulated survivin siRNA (67). These nanoparticles were shown to prolong survivin knockdown and reduced tumor growth in a mouse xenograft model. While higher molecular weight chitosan increased urothelial cell internalization of the nanoparticles, release of the negatively charged siRNA was inhibited due to a higher extent of binding with the chitosan chains.

The polymer chains constituting the shell of nanoparticles can also be functionalized with amine groups to increase penetration into the urothelium and uptake by cells. Very small amine terminated hyperbranched polyglycerol (HPG-C_{8/10}-MePEG-NH₂) nanoparticles (< 10 nm) have been prepared as a carrier for DTX (68). The hyperbranched polyglycerol core was derivatized with C₈/C₁₀ alkyl chains to create a hydrophobic core for DTX loading while the MePEG forms a hydrophilic shell for increasing the aqueous solubility of these carriers. As a result of the terminal amine groups, the nanoparticles are positively charged. The nanoparticles significantly increased uptake of DTX in isolated porcine bladder as well as in live mouse bladder tissues. This increase was postulated to be due to the opening of tight junctions and exfoliation of the urothelium triggered by an apoptosis mechanism. Within 24 h after instillation of the nanoparticles, recovery of the urothelium was evident. The same group has also conjugated carboxylate groups to the hydrophobically derivatized, hyperbranched polyglycerols to serve as nanoparticulate drug carriers for CDDP (69). CDDP was bound to the nanoparticles through coordination with the terminal carboxylate groups, and the release of CDDP from the carboxylate ligands is dependent on the pH and concentration of chloride and possibly other ions in the release media.

We have prepared nanocarriers based on amphiphilic PCL-*b*-poly(propargyl methacrylate-*click*-mercaptosuccinic acid-*co*-poly(ethylene glycol) methyl ether methacrylate) (PCL-*b*-P(PMA-*click*-MSA-*co*-PEGMA)) for loading multiple agents (70). PCL was first modified with 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (71) to serve as the macro-charge transfer agent for mediating RAFT copolymerization of PMA and PEGMA using azobisisobutyronitrile as initiator to form PCL-*b*-P(PMA-*co*-PEGMA). MSA was then coupled to the alkyne-functionalized PCL-*b*-P(PMA-*co*-PEGMA) via UV-initiated thiol-yne click reaction (Figure 3a). This copolymer has been designed to form nanocarriers with a number of desirable features for bladder cancer therapy (Figure 3b). Firstly, superparamagnetic iron oxide nanoparticles (SPIONs) can be loaded into the hydrophobic PCL core of the nanocarriers formed from PCL-*b*-P(PMA-*click*-MSA-*co*-PEGMA)). SPIONs are of interest since they can be used with an external magnetic field to localize drug-loaded carriers at tumor sites providing continuous exposure to high drug concentrations. Furthermore, intravesically instilled SPIONs also offer the possibility of localized hyperthermia therapy with an externally applied alternating magnetic field (72). Earlier studies indicated that heat increases the permeability of cell membranes and consequently the penetration of instilled drug into the urothelium in addition to

promoting apoptosis of tumor cells induced by the drug (73, 74). Secondly, the PEGMA hydrophilic shell will confer the nanocarriers with water-dispersible and mucoadhesive properties. Thirdly, the pendant dicarboxylic groups generated via thiol-yne click reaction between MSA and the alkyne groups of PMA in the hydrophilic shell can be used for conjugating CDDP. The free carboxylic groups can further enhance the mucoadhesivity of the nanocarriers.

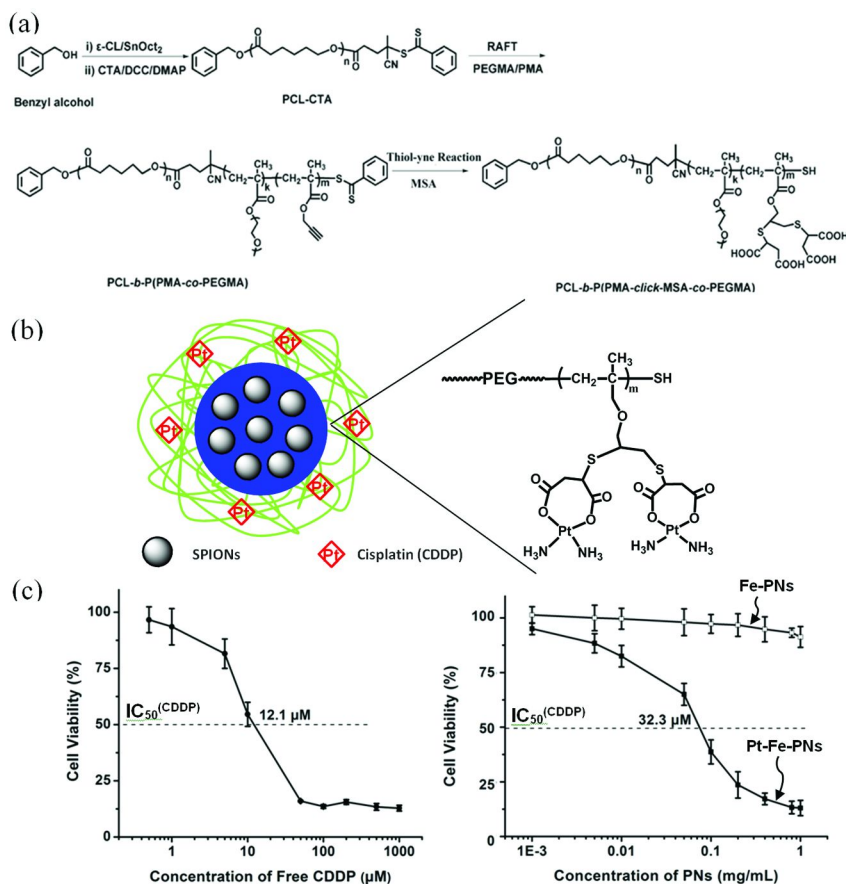


Figure 3. (a) Synthetic route for PCL-b-P(PMA-click-MSA-co-PEGMA), (b) schematic diagram illustrating concept of SPIONs-loaded, cisplatin-conjugated polymeric nanocarriers, and (c) in vitro cytotoxicity profile of free cisplatin, SPIONs-loaded polymeric nanocarriers without CDDP (Fe-PNs) and conjugated with CDDP (Pt-Fe-PNs) against UMUC3 bladder cancer cells. Cells were exposed to the drug or nanoparticles for 2 h and further cultured with fresh medium for 72 h. (Reproduced from reference (70). Copyright 2012 American Chemical Society)

CDDP was conjugated to the bidentate dicarboxylic groups of MSA of the SPIONs-loaded nanocarriers using the method of Huynh et al. (75). CDDP will be released in the presence of chloride ions due to ligand exchange with the carboxylate ligand (75, 76) or when the carboxyl groups are protonated and dechelated from the Pt center of the drug under acidic conditions (77). Thus, release is favored in artificial urine (137 mM Cl⁻, pH = 6.1) where 41% of the loaded drug was released in 4 days compared to < 5% in water. The SPIONs- and CDDP-loaded nanocarriers are also readily internalized by UMUC3 bladder cancer cells. The amount of intracellular Pt after incubation of UMUC3 cells with the CDDP-loaded nanocarriers for 2 h was almost 20 times higher than when the cells were incubated with an equivalent amount of free CDDP. However, the half maximal inhibitory concentration (IC₅₀) of CDDP in the nanocarriers is 2.7 times higher than free CDDP (Figure 3c). This is likely because the release of the drug from the nanocarriers will take time both in the medium and after uptake by the cells. This decrease in potency compared to free CDDP is consistent with earlier studies on CDDP-conjugated to polymeric nanocarriers (77, 78).

Nanocapsules

The differentiating feature between nanocapsules and the nanocarriers described in the preceding two sections is the presence of a cavity in the former for loading active agents. The cavity may be filled with an oily or aqueous phase. A summary of classical methods for preparing nanocapsules is presented in the article by Mora-Huertas et al. (79). Nanocapsules have been considered to encompass liposomes which have an aqueous inner core enclosed by unilamellar or multilamellar phospholipid bilayers (80) and polymersomes with an aqueous core and a polymeric shell (49). A recent study investigated the role of endocytosis in the uptake of liposomes prepared from *N*-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl] by cultured human urothelium UROtsa cells and rat bladder (81). After incubation of UROtsa cells with these liposomes containing colloidal gold for 2 h at 37 °C, gold was detected inside the cells, whereas after incubation at 4 °C, only extracellular binding of the gold-containing liposomes was observed. Transmission electron microscopy images of rat bladder 8 h after instillation of the liposomes containing colloidal gold also showed the presence of gold grains inside the urothelium. In contrast, intracellular gold was absent when the rat was instilled with plain colloidal gold. Based on these results, clathrin-mediated endocytosis was proposed as the primary mechanism for the liposomes entry into the bladder urothelium.

Phosphatidylcholine dipalmitoyl liposomes containing a photosensitizer, tetramethyl hematoporphyrin (TMHP), were shown to effectively deliver TMHP into two human bladder cancer cell lines within 1 h of incubation (82). Irradiation of the TMHP-treated cells with red light resulted in cytotoxicity to the cells, thereby demonstrating the potential application of photodynamic therapy in bladder cancer. On the other hand, PEG-based liposomes loaded with the photosensitizer aluminum phthalocyanine tetrasulfonate (AlPcS4) resulted in very little intracellular accumulation of AlPcS4 in rat bladder cancer cells (83). Since

there is an overexpression of transferrin receptor on the surface of certain cancer cells (83), the intracellular accumulation of AIPcS4 can be increased by two orders of magnitude if the liposomes were surface conjugated with transferrin. This increased uptake of AIPcS4 is correlated with increased photocytotoxicity in the treated cells. In an orthotopic rat model, bladders pretreated with chondroitinase ABC resulted in selective accumulation of AIPcS4 in tumor cells over the surrounding tissue after treatment with transferrin-conjugated liposomes, whereas the untargeted liposomes were virtually absent from the urothelial tumor tissue. However, there was no significant uptake of the transferrin-conjugated liposomes without pretreatment of the bladder with chondroitinase ABC, highlighting the difficulty for the liposomes to penetrate the bladder mucosa (83).

Liposomes have also been used for delivery of biological agents. Intravesical instillation of live BCG is a major treatment of choice for high risk NMIBC but the significant side effects associated with this treatment mode have been a concern (7, 8). Hence, there is interest to explore the possibility of substituting BCG cell wall skeleton (BCG-CWS), the main immune active center of BCG, for live BCG in immunotherapy. Nakamura et al. formulated a method for encapsulating BCG-CWS in 166 nm liposomes via an emulsified lipid method (84). Octaarginine (R8) peptide, a highly positively charged cell-penetrating peptide, was used to modify the surface of the liposomes to enhance delivery into mouse bladder tumor (MBT-2) cells *in vitro*. The liposomal formulation of BCG-CWS also demonstrated efficacy in inhibiting tumor growth in mice bearing MBT-2 tumors. Intravesical liposome-mediated interleukin-2 (IL-2) gene therapy was investigated in an orthotopic murine bladder cancer model using cationic liposomes prepared from positively charged lipid, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), and neutral lipid, dioleoylphosphatidylethanolamine (DOPE), in a 1:1 molar ratio (85). Forty percent of animals treated with IL-2 gene survived 60 days following the initial tumor implantation, while none in the control groups survived. The animals initially cured of pre-established tumors were found to be resistant to a subsequent tumor re-challenge. Nanocomplexes of RB94 plasmid encapsulated by a cationic liposome surface-decorated with a tumor-targeting moiety, either transferrin or an anti-transferrin receptor single-chain antibody fragment, were tested in murine bladder cancer models (86). Liposomes prepared from 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) and DOPE or DOTAP and cholesterol were found to be the most efficient, and after intravenous administration of the nanocomplexes, tumor specific transfection was observed in tumors located subcutaneously or within the bladder. Furthermore, the combination of systemically administered nanocomplexes and gemcitabine resulted in significant tumor growth inhibition/regression and induction of apoptosis in mice bearing the subcutaneous tumors. In one pre-clinical study using cationic liposomes prepared from DOTAP, intravesical liposome-mediated cytokine gene therapy with IFN- α 1 and granulocyte macrophage colony-stimulating factor demonstrated successful inhibition of tumor cell growth in an orthotopic murine bladder cancer model (87). The use of cationic liposomes for intravesical delivery of siRNA to inhibit cancer growth in murine orthotopic bladder cancer models has also been demonstrated (88).

In our study on application of nanocapsules for potential bladder cancer chemotherapy, chitosan-poly(methacrylic acid) nanocapsules were prepared according to an earlier reported method with some modifications (89). Chitosan was first dissolved in methacrylic acid and polymerization of the latter was initiated by ammonium persulfate (APS). Electrostatic interactions between the amine groups of chitosan and the carboxyl groups of poly(methacrylic acid) leads to the formation of nanocapsules (Figure 4a). The zeta potential of these nanocapsules is about +15 mV in deionized water (pH ~5.8) suggesting that the outermost layer of the nanocapsules is predominantly chitosan with protonated amine groups. These cationic nanocapsules interact strongly with mucin, possibly due to electrostatic interactions between the amine groups of the chitosan chains on the surface of the nanocapsules and the negatively charged mucin. Scanning electron microscopy (SEM) images of porcine bladder after incubation for 4 h with the nanocapsules revealed a whitish layer on the urothelium which was not removed upon rinsing with phosphate buffered saline (PBS) (Figure 4b).

Earlier studies have shown that a combination of CDDP and sunitinib malate (90, 91) or a combination of CDDP and PTX have synergistic effects which result in higher potency against NMIBC (58). Since the chitosan-poly(methacrylic acid) nanocapsules have a vesicular structure as shown in Figure 4c(i), they are expected to have a high loading capacity for drugs, and hence, may be suitable as a carrier of multiple drugs. DOX, a hydrophilic drug, was loaded into the nanocapsules by first mixing it with the nanocapsules and then converting it to a hydrophobic form by treatment with a base. CDDP was modified by conjugating N α -acetyllysine to [Pt(NH $_3$) $_2$] $^{2+}$ of CDDP (92) and then treated with a base. In this manner, DOX and peptide-modified CDDP can be loaded into these nanocapsules with loading efficiency of >90% and ~50%, respectively. The loading of these drugs is facilitated by interactions between amine groups of the drugs and carboxyl groups of the poly(methacrylic acid) chains of the nanocapsules. On the other hand, unmodified CDDP, being a neutral and water-soluble molecule with minimal interaction with the nanocapsules, has a loading efficiency of only ~1%. Nanocapsules with DOX and peptide-modified CDDP co-loaded at roughly 1:1 weight ratio and constituting ~50 wt% of the weight of the initial nanocapsules were prepared. As can be seen from Figure 4c(ii), the dual drug-loaded nanocapsules no longer exhibit a hollow structure, unlike the blank nanocapsules (Figure 4c(i)).

The efficacy of the dual drug-loaded nanocapsules in killing UMUC3 cells after 4 or 72 h-treatment is shown in Figure 5a. The IC $_{50}$ for the dual drug-loaded nanocapsules was 5 to 16 times lower than nanocapsules loaded with each drug singly. Using the Chou and Talalay method (93, 94), the combination index calculated for the dual drug-loaded nanocapsules is much less than 1, unlike the case of the combined drugs in free form where the combination index is close to 1 (Figure 5b). These results indicate that these two drugs work in synergy when co-delivered in the nanocapsules to UMUC3 cells. On the other hand, the anti-cancer effects arising from a combination of free DOX and peptide-modified CDDP are mainly attributed to the action of former as the latter has a much lower killing efficacy on UMUC3 cells due to its low uptake by the cells. The synergistic anticancer effect from the dual drug-loaded nanocapsules is also

evident from a comparison of the apoptosis assays of UMUC3 cells treated with peptide-modified CDDP in free form and loaded in nanocapsules, and the dual drug-loaded nanocapsules (Figure 5c). At a dosage of 0.1 $\mu\text{g/ml}$ [Pt] in all formulations, peptide-modified CDDP in free form induced minimal necrosis and apoptosis with 4 h- and 72 h-treatment. Treatment with the peptide-modified CDDP loaded in nanocapsules resulted in an increase in the number of necrotic and apoptotic cells (6% necrotic, 4% apoptotic for 4 h-treatment and 7% necrotic, 7% apoptotic for 72-h treatment), indicating that delivery of the peptide-modified CDDP into the cells via nanocapsules was more efficient than in the free form. With DOX and peptide-modified CDDP co-loaded in the nanocapsules, a much higher degree of cell necrosis and apoptosis was observed than without DOX for both 4 h and 72 h treatment. Thus, these mucoadhesive nanocapsules capable of simultaneous delivery of a "two-in-one" synergistic drug combination to bladder cancer cells provide an avenue for increasing therapeutic efficacy in intravesical chemotherapy.

Protein Nanocarriers

Proteins are viewed as attractive materials for formulation as nanoscale drug carriers because their amphiphilic nature facilitates interactions with both drug and solvent. Proteins also have functional groups for attachment of drugs and targeting ligands, and nanoparticles made from natural proteins are biocompatible, biodegradable and metabolizable (95). A range of protein-based nanoparticles have been investigated for cancer therapy applications. Gelatin particles ranging from ~ 600 to 1000 nm have been used to load PTX, and under optimal conditions, 0.7% drug loading was obtained. Drug release in PBS and urine was rapid with $\sim 90\%$ released at 37°C after 2 hours. After instillation of the formulation in dogs, the drug concentration in the bladder tissue was 2.6 times the concentration derived from commercial Cremophor/ethanol formulation (38). Gliadin nanoparticles carrying cyclophosphamide induced apoptosis in breast cancer cells (96) and zein nanoparticles containing 2,4-dihydroxy-5-fluorpyrimidin and quantum dot fluorophores were tested as a drug delivery and imaging system in breast cancer cells (97). Milk proteins have also been tested as a chemotherapeutic drug carrier in *in vivo* experiments (98–100). The E2 subunit of pyruvate dehydrogenase multi-enzyme complex has been used to fabricate nanoparticles and after functionalization with a targeting ligand, either folic acid (101) or antibody ABX-EGF single chain variable fragment (102), these nanoparticles demonstrated recognition of the targeted cancer cells.

Albumin is the most abundant protein in blood plasma, and nanoparticle albumin-bound (nab) technology has been shown to be a promising method for formulating chemotherapeutic drug carriers (103). Nab-PTX has been approved by the Food and Drug Administration (FDA) for treatment of metastatic breast cancer (103). Nab-PTX is prepared via an emulsion-evaporation method whereby PTX in chloroform-ethanol is added to human serum albumin in water pre-saturated with chloroform. After homogenizing the emulsion at high pressure, the albumin bound nanoparticle formulation of PTX is approximately 130 nm

(103). Encouraging response was obtained with nab-PTX in treating metastatic urethral adenocarcinoma (104) and platinum-refractory urothelial cancer (105). Recently, McKiernan et al. reported a single center, single arm Phase II trial of intravesical nab-PTX for the treatment of NMIBC after BCG treatment failure (106). The drug was administered intravesically on a weekly basis for 6 weeks as induction and on a monthly basis for 6 months as maintenance in case of response. A response rate of 35.7% was achieved, and all patients who responded remained disease-free a year later.

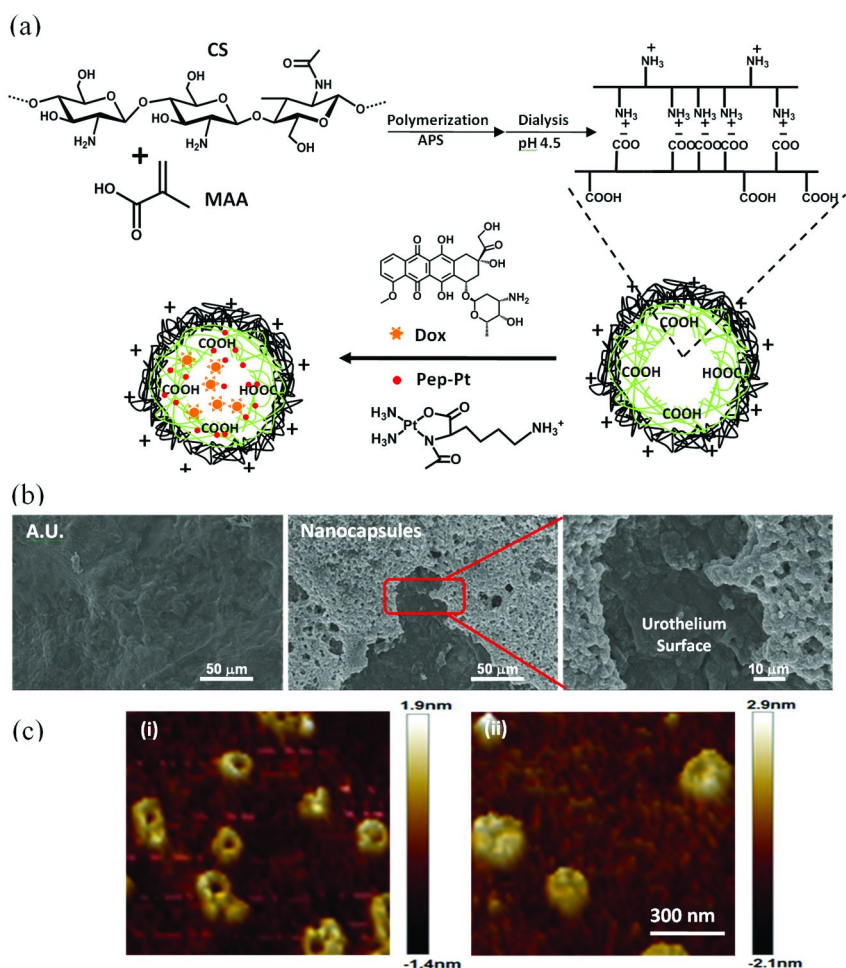


Figure 4. (a) Schematic diagram illustrating the preparation of chitosan-poly(methacrylic acid) nanocapsules, (b) SEM images of fresh porcine bladder urothelium after exposure to artificial urine (A.U.) or 5 mg/ml nanocapsules in A.U., and (c) atomic force microscopy images of (i) blank and (ii) dual drug-loaded nanocapsules.

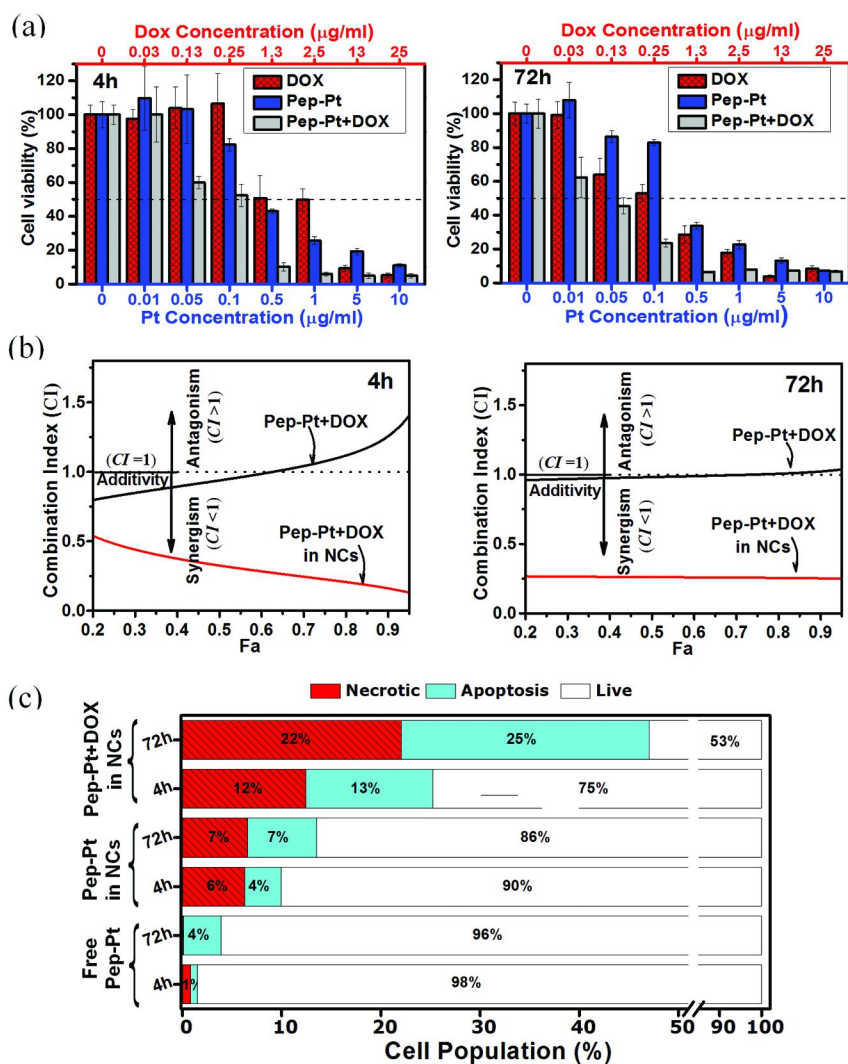


Figure 5. (a) *In vitro* viability of UMUC3 after incubation with nanocapsules loaded with either peptide-modified CDDP (Pep-Pt) or DOX or both drugs (Pep-Pt+DOX) for 4 h or 72 h, (b) Combination index (CI) versus fraction of affected cells (Fa) plots of dual drug-loaded nanocapsules (Pep-Pt+DOX in NCs) and free drug combination (Pep-Pt+DOX) against UMUC3 cells after treatment for 4 h or 72 h, (c) necrotic and apoptotic UMUC3 cells after treatment with peptide-modified CDDP in free form (Free Pep-Pt) and loaded in nanocapsules (Pep-Pt in NCs), and the dual drug-loaded nanocapsules (with equivalent [Pt] of 0.1 $\mu\text{g/ml}$) for 4 h or 72 h at 37 $^{\circ}\text{C}$.

We have investigated the use of cationic albumin for loading DTX and CDDP individually and in combination. Cationic albumin was prepared by reacting ethylenediamine with the carboxyl groups of the protein using carbodiimide chemistry. The amine groups of cationic albumin promoted greater mucoadhesion compared to albumin as shown by the results from ex vivo experiments with porcine bladder and in vivo mouse experiments (Figures 6a and 6b). The mouse bladder showed strong fluorescence 5 days after the instillation of FITC-labeled cationized albumin, whereas with FITC-labeled albumin, the fluorescence signal after 5 days was close to that of auto-fluorescence from the bladder. DTX and CDDP, which have vastly different solubility in aqueous medium, can be loaded in albumin. An earlier study has shown that the combination of intravesically instilled DTX and CDDP in a genetically engineered mouse model that progresses from carcinoma in situ to invasive, metastatic bladder cancer is more effective for reducing both tumor and metastatic burden than either component administered individually (107). In our study, CDDP was first converted to $[\text{Pt}(\text{NH}_3)_2]^{2+}$ by reaction with AgNO_3 (75) and then incubated with albumin or cationized albumin overnight. The amount of Pt in the albumin-CDDP complexes was 1.5 wt% and the corresponding amount for the cationized albumin-CDDP complexes was 0.92 wt%. A recent study has reported that the His105 and Met329 residues in human serum albumin are the main sites for coordination with CDDP (Figure 6c) (108). It is also likely that other histidine and methionine residues can serve as secondary binding sites (108, 109). Furthermore, since $[\text{Pt}(\text{NH}_3)_2]^{2+}$ has been shown to chelate with COOH-containing polymers (69, 75), the chelation between CDDP and COOH groups in the albumin would also likely occur.

Since serum albumin is known to bind to a variety of hydrophobic endogenous (e.g. cholesterol, fatty acids) or exogenous compounds (e.g. drugs) (110, 111), DTX can be easily loaded into albumin by mixing a solution of DTX in acetonitrile with albumin aqueous solution. Our results showed that DTX-albumin complexes containing up to 3 wt% of DTX were successfully obtained with a loading efficiency of ~100%. The binding sites for DTX in albumin are likely to be Sudlow's site I (in subdomain IIA) and Sudlow's site II (in subdomain IIIA) (Figure 6c), which have been shown to be the preferred binding sites for a number of drugs (110, 112). CDDP and DTX can be co-loaded into albumin (or cationized albumin) to form the albumin-dual drug complex by adding DTX to the CDDP-albumin complex in the manner described above. The cationic albumin-dual drug complex in PBS (pH = 7.0, 2 mM phosphate) has a hydrodynamic diameter of 7 nm and a zeta potential of 16 mV. In contrast, the zeta potential of the albumin-dual drug complex is -13.5 mV.

The $\text{IC}_{50}^{(\text{Pt})}$ of CDDP-cationized albumin complex against UMUC3 cells after 4 h or 72 h treatment is about one order of magnitude higher than the corresponding value for free CDDP. This increase in $\text{IC}_{50}^{(\text{Pt})}$ is consistent with an earlier report that CDDP bound to albumin is far less active than free CDDP (113). The observed slow release rate of the bound CDDP from albumin is likely a key factor for the low activity. Nevertheless, CDDP in the dual drug-loaded albumin enhances the action of DTX, as can be seen from a comparison of the IC_{50} value of the dual drug complex with those of the single drug-loaded complexes (Table 1). With CDDP present in the dual drug complex at an amount that is much lower than the IC_{50}

value of the CDDP-loaded complex, the efficacy of DTX is increased by a factor of three. Thus, a mucoadhesive carrier based on cationic albumin for intravesical co-delivery of DTX and CDDP offers the possibility of enhanced delivery of the drugs to the urothelium as well as improved drug efficacy.

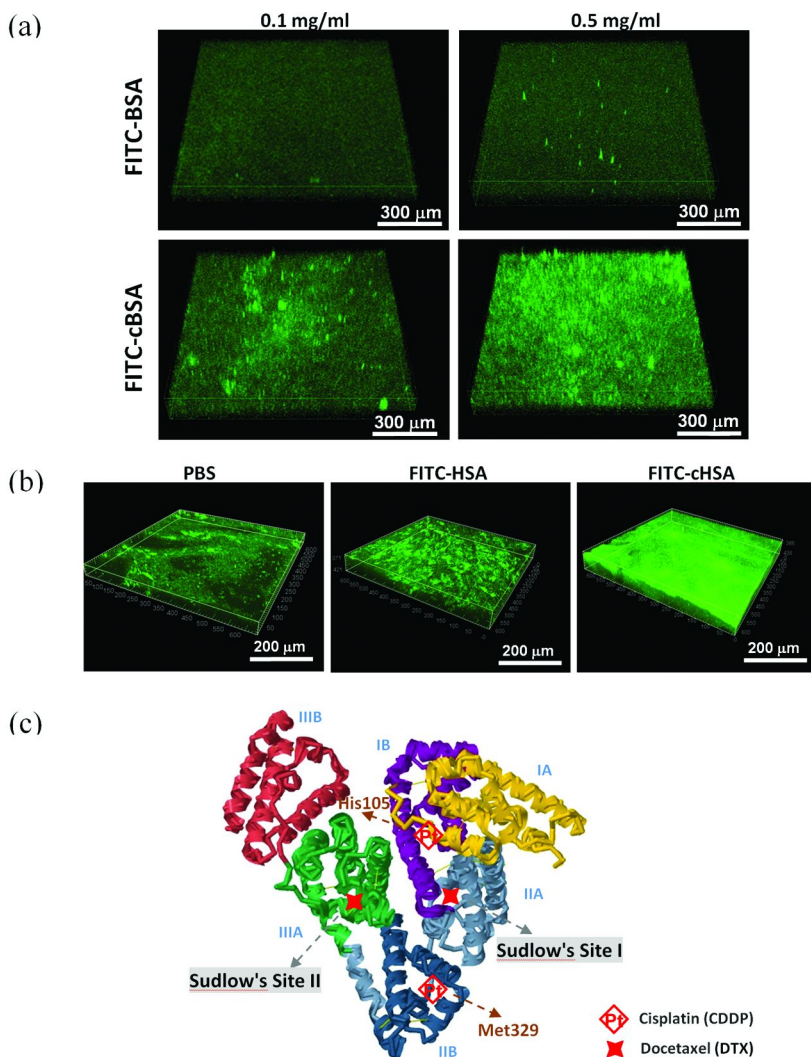


Figure 6. (a) CLSM images of fresh porcine bladder after incubation for 4 h with 0.1 and 0.5 mg/ml of FITC-labeled bovine serum albumin (FITC-BSA) or cationized bovine serum albumin (FITC-cBSA), (b) CLSM images of luminal surface of mouse bladder 5 days after intravesical instillation of PBS or 10 mg/ml FITC-labeled human serum albumin (FITC-HSA) or cationized human serum albumin (FITC-cHSA), (c) structure of albumin with possible binding sites for CDDP and DTX.

Table 1. IC₅₀ of DTX and CDDP Loaded Singly and in Combination in BSA and cBSA against UMUC3 Cells

<i>Treatment time</i>	<i>IC₅₀ (DTX-loaded cBSA)</i>	<i>IC₅₀ (CDDP-loaded cBSA)</i>	<i>IC₅₀ (dual drug-loaded cBSA)</i>	
			<i>DTX in complex</i>	<i>CDDP in complex</i>
4 h	3.6 ng/ml	9.3 µg/ml (as [Pt])	1.0 ng/ml	0.01 µg/ml (as [Pt])
72 h	1.0 ng/ml	2.7 µg/ml (as [Pt])	0.3 ng/ml	0.003 µg/ml (as [Pt])

Concluding Remarks

The high human and economic costs associated with bladder cancer have spurred research on the development of more effective means of intravesical chemotherapy. The current limited efficacy of intravesical chemotherapy in preventing tumor recurrence and progression of NMIBC is likely associated with the bladder permeability barrier and the short residence time of instilled drug in the bladder. Significant efforts have been invested in the development of soft nanoparticles as carriers of chemotherapeutic agents in a bid to overcome these limitations. As highlighted in this mini-review, with advances in the design and synthesis of nanocarriers, a multitude of options are available for delivery of different types of chemotherapeutic agents including combinations of drugs with synergistic effects but vastly different physicochemical properties, magnetic nanoparticles for localized hyperthermia therapy, siRNA and genes. Suitably engineered nanocarriers also prolong drug retention in the bladder and enhance its uptake by urothelial tissues. Decoration of these nanoparticles with ligands that target surface proteins overexpressed in bladder cancer cells can further increase the delivery of drugs. It is expected that the promising results obtained to date from in vitro experiments and limited animal studies will accelerate the development of nanoparticulate drug formulation for next generation intravesical therapy for NMIBC. Further research on the mechanisms by which nanocarriers interact with the urothelium and tumor cells will provide useful parameters for optimizing their design and for combining diagnostic and therapeutic agents in the nanocarriers to improve bladder cancer diagnosis, surveillance and treatment.

References

1. National Cancer Institute. *SEER Cancer Statistics Factsheets: Bladder Cancer*; <http://seer.cancer.gov/statfacts/html/urinb.html> (Aug 1, 2015).
2. Sievert, K. D.; Amend, B.; Nagele, U.; Schilling, D.; Bedke, J.; Horstmann, M.; Hennenlotter, J.; Kruck, S.; Stenzl, A. Economic aspects of bladder cancer: what are the benefits and costs? *World J. Urol.* **2009**, *27*, 295–300.
3. Shen, Z.; Shen, T. G.; Wientjes, M. G.; O'Donnell, M. A.; Au, J. L. S. Intravesical treatments of bladder cancer: Review. *Pharm. Res.* **2008**, *25*, 1500–1510.
4. Messing, E. M. Why should we increase public awareness of bladder cancer, and how can we do it? *Nat. Clin. Pract. Urol.* **2008**, *5*, 117.
5. Mariotto, A. B.; Yabroff, K. R.; Shao, Y.; Feuer, E. J.; Brown, M. L. Projections of the cost of cancer care in the United States: 2010–2020. *J. Natl. Cancer Inst.* **2011**, *103*, 117–128.
6. Avritscher, E. B.; Cooksley, C. D.; Grossman, H. B.; Sabichi, A. L.; Hamblin, L.; Dinney, C. P.; Elting, L. S. Clinical model of lifetime cost of treating bladder cancer and associated complications. *Urology* **2006**, *68*, 549–553.

7. Gontero, P.; Bohle, A.; Malmstrom, P. U.; O'Donnell, M. A.; Oderda, M.; Sylvester, R.; Witjes, F. The role of Bacillus Calmette-Guérin in the treatment of non-muscle-invasive bladder cancer. *Eur. Urol.* **2010**, *57*, 410–429.
8. Barocas, D. A.; Globe, D. R.; Colayco, D. C.; Onyenwenyi, A.; Bruno, A. S.; Bramley, T. J.; Spear, R. J. Surveillance and treatment of non-muscle-invasive bladder cancer in the USA. *Adv. Urol.* **2012** Article ID 421709.
9. Mostafid, A. H.; Palou Redorta, J.; Sylvester, R.; Witjes, J. A. Therapeutic options in high-risk non-muscle-invasive bladder cancer during the current worldwide shortage of Bacille Calmette-Guérin. *Eur. Urol.* **2015**, *67*, 359–560.
10. Tyagi, P.; Wu, P. C.; Chancellor, M.; Yoshimura, N.; Huang, L. Recent advances in intravesical drug/gene delivery. *Mol. Pharm.* **2006**, *3*, 369–379.
11. GuhaSarkar, S.; Banerjee, R. Intravesical drug delivery: Challenges, current status, opportunities and novel strategies. *J. Controlled Release* **2010**, *148*, 147–159.
12. Hurst, R. E. Structure, function, and pathology of proteoglycans and glycosaminoglycans in the urinary tract. *World J. Urol.* **1994**, *12*, 3–10.
13. Khutoryanskiy, V. V. Advances in mucoadhesion and mucoadhesive polymers. *Macromol. Biosci.* **2011**, *14*, 748–64.
14. Hombach, J.; Bernkop-Schnürch, A. *Handbk. Exp. Pharmacol.* **2010**, *197*, 251–266.
15. Chen, C. H.; Yang, H. J.; Shun, C. T.; Huang, C. Y.; Huang, K. H.; Yu, H. J.; Pu, Y. S. A cocktail regimen of intravesical mitomycin-C, doxorubicin, and cisplatin (MDP) for non-muscle-invasive bladder cancer. *Urol. Oncol.* **2012**, *30*, 421–427.
16. Steinberg, R. L.; Thomas, L. J.; O'Donnell, M. A.; Nepple, K. G. Sequential intravesical gemcitabine and docetaxel for the salvage treatment of non-muscle invasive bladder cancer. *Bladder Cancer* **2015**, *1*, 65–72.
17. Liu, S.; Hou, J.; Zhang, H.; Wu, Y.; Hu, M.; Zhang, L.; Xu, J.; Na, R.; Jiang, H.; Ding, Q. The evaluation of the risk factors for non muscle invasive bladder cancer (NMIBC) recurrence after transurethral resection (TURBt) in Chinese population. *PLoS One* **2015**, *10*, e0123617.
18. Barlow, L. J.; McKiernan, J. M.; Benson, M. C. The novel use of intravesical docetaxel for the treatment of non-muscle invasive bladder cancer refractory to BCG therapy: A single institution experience. *World J. Urol.* **2009**, *27*, 331–335.
19. Kumari, P.; Ghosh, B.; Biswas, S. Nanocarriers for cancer-targeted drug delivery. *J. Drug Targeting* **2015** DOI:10.3109/1061186X.2015.1051049.
20. Sun, T.; Zhang, Y. S.; Pang, B.; Hyun, D. C.; Yang, M.; Xia, Y. Engineered nanoparticles for drug delivery in cancer therapy. *Angew. Chem., Int. Ed.* **2014**, *53*, 12320–12364.
21. Larson, N.; Ghandehari, H. Polymeric conjugates for drug delivery. *Chem. Mater.* **2012**, *24*, 840–853.
22. Yang, F.; Zhang, Y.; Liang, H. Interactive association of drugs binding to human serum albumin. *Int. J. Mol. Sci.* **2014**, *15*, 3580–3595.

23. Zhuang, J.; Jiwanpanich, S.; Deepak, V. D.; Thayumanavan, S. Facile preparation of nanogels using activated ester containing polymers. *ACS Macro Lett.* **2012**, *1*, 175–179.
24. Jiang, Y.; Chen, J.; Deng, C.; Suuronen, E.J.; Zhong, Z. Click hydrogels, microgels and nanogels: emerging platforms for drug delivery and tissue engineering. *Biomaterials* **2014**, *35*, 4969–4985.
25. Yallapu, M. M.; Jaggi, M.; Chauhan, S. C. Design and engineering of nanogels for cancer treatment. *Drug Discovery Today* **2011**, *16*, 457–463.
26. Wang, Y. C.; Wu, J.; Li, Y.; Du, J. Z.; Yuan, Y. Y.; Wang, J. Engineering nanoscopic hydrogels via photo-crosslinking salt-induced polymer assembly for targeted drug delivery. *Chem. Commun.* **2010**, *46*, 3520–3522.
27. Sasaki, Y.; Akiyoshi, K. Nanogel engineering for new nanobiomaterials: From chaperoning engineering to biomedical applications. *Chem. Rec.* **2010**, *10*, 366–376.
28. Zhang, X.; Malhotra, S.; Molina, M.; Haag, R. Micro- and nanogels with labile crosslinks – from synthesis to biomedical applications. *Chem. Soc. Rev.* **2015**, *44*, 1948–1973.
29. Zhang, X.; Achazi, K.; Steinhilber, D.; Kratz, F.; Dervede, J.; Haag, R. A facile approach for dual-responsive prodrug nanogels based on dendritic polyglycerols with minimal leaching. *J. Controlled Release* **2014**, *174*, 209–216.
30. Haag, R.; Kratz, F. Polymer therapeutics: concepts and applications. *Angew. Chem., Int. Ed.* **2006**, *45*, 1198–1215.
31. Chacko, R. T.; Ventura, J.; Zhuang, J.; Thayumanavan, S. Polymer nanogels: A versatile nanoscopic drug delivery platform. *Adv. Drug Delivery Rev.* **2012**, *64*, 836–851.
32. Nukolova, N. V.; Oberoi, H. S.; Cohen, S. M.; Kabanov, A. V.; Bronich, T. K. Folate-decorated nanogels for targeted therapy of ovarian cancer. *Biomaterials* **2011**, *32*, 5417–5426.
33. Lu, S.; Neoh, K. G.; Huang, C.; Shi, Z.; Kang, E. T. Polyacrylamide hybrid nanogels for targeted cancer chemotherapy via co-delivery of gold nanoparticles and MTX. *J. Colloid Interface Sci.* **2013**, *412*, 46–55.
34. Wu, W.; Shen, J.; Banerjee, P.; Zhou, S. Chitosan-based responsive hybrid nanogels for integration of optical pH-sensing, tumor cell imaging and controlled drug delivery. *Biomaterials* **2010**, *31*, 8371–8381.
35. Dickerson, E. B.; Blackburn, W. H.; Smith, M. H.; Kapa, L. B.; Lyon, L. A.; McDonald, J. F. Chemosensitization of cancer cells by siRNA using targeted nanogel delivery. *BMC Cancer* **2010**, *10*, 10.
36. Chen, D.; Song, D.; Wientjes, M. G.; Au, J. L. S. Effect of dimethyl sulfoxide on bladder tissue penetration of intravesical paclitaxel. *Clin. Cancer Res.* **2003**, *9*, 363–369.
37. Gelderblom, H.; Verweij, J.; Nooter, K.; Sparreboom, A. Cremophor EL: The drawbacks and advantages of vehicle selection for drug formulation. *Eur. J. Cancer.* **2001**, *37*, 1590–1598.
38. Lu, Z.; Yeh, T. K.; Wang, J.; Chen, L.; Lyness, G.; Xin, Y.; Wientjes, M. G.; Bergdall, V.; Couto, G.; Alvarez-Berger, F.; Kosarek, C. E.; Au, J. L.

- S. Paclitaxel gelatin nanoparticles for intravesical bladder cancer therapy. *J. Urol.* **2011**, *185*, 1478–1483.
39. McKiernan, J. M.; Masson, P.; Murphy, A. M.; Goetzel, M.; Olsson, C. A.; Petrylak, D. P.; Desai, M.; Benson, M. C. Phase I trial of intravesical docetaxel in the management of superficial bladder cancer refractory to standard intravesical therapy. *J. Clin. Oncol.* **2006**, *24*, 3075–3080.
 40. Barlow, L.; McKiernan, J.; Sawczuk, I.; Benson, M. A single-institution experience with induction and maintenance intravesical docetaxel in the management of non-muscle-invasive bladder cancer refractory to Bacilli Calmette-Guérin therapy. *BJU Int.* **2009**, *104*, 1098–1102.
 41. Gao, D.; Xu, H.; Philbert, M. A.; Kopelman, R. Bioeliminable nanohydrogels for drug delivery. *Nano Lett.* **2008**, *8*, 3320–3324.
 42. Moreno, M. J.; Monson, E.; Reddy, R. G.; Rehemtulla, A.; Ross, B. D.; Philbert, M.; Schneider, R. J.; Kopelman, R. Production of singlet oxygen by Ru(dpp(SO₃)₂)₃ incorporated in polyacrylamide PEBBLES. *Sens. Actuators, B* **2003**, *90*, 82–89.
 43. Lu, S.; Neoh, K. G.; Kang, E. T.; Mahendran, R.; Chiong, E. Mucoadhesive polyacrylamide nanogel as a potential hydrophobic drug carrier for intravesical bladder cancer therapy. *Eur. J. Pharm. Sci.* **2015**, *72*, 57–68.
 44. Anand, M.; Wang, C.; French, J.; Isaacson-Schmid, M.; Wall, L. L.; Mysorekar, I. U. Estrogen affects the glycosaminoglycan layer of the murine bladder. *Female Pelvic Med. Reconstr. Surg.* **2012**, *18*, 148–152.
 45. Birder, L.; Andersson, K. E. Urothelial signaling. *Physiol. Rev.* **2013**, *93*, 653–680.
 46. Kerec Kos, M.; Bogataj, M.; Mrhar, A. Enhanced permeability of the urinary bladder wall: the role of polymer charge. *Pharmazie* **2009**, *64*, 232–237.
 47. Veranic, P.; Erman, A.; Kerec-Kos, M.; Bogataj, M.; Mrhar, A.; Jezernik, K. Rapid differentiation of superficial urothelial cells after chitosan-induced desquamation. *Histochem. Cell Biol.* **2009**, *131*, 129–139.
 48. Kerec-Kos, M.; Bogataj, M.; Veranic, P.; Mrhar, A. Permeability of pig urinary bladder wall: time and concentration dependent effect of chitosan. *Biol. Pharm. Bull.* **2006**, *29*, 1685–1691.
 49. Letchford, K.; Burt, H. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *Eur. J. Pharm. Biopharm.* **2007**, *65*, 259–269.
 50. Letchford, K.; Liggins, R.; Wasan, K. M.; Burt, H. In vitro human plasma distribution of nanoparticulate paclitaxel is dependent on the physicochemical properties of poly(ethylene glycol)-*block*-poly(caprolactone) nanoparticles. *Eur. J. Pharm. Biopharm.* **2009**, *71*, 196–206.
 51. Pitto-Barry, A.; Barry, N. P. E. Pluronic® block-copolymers in medicine: From chemical and biological versatility to rationalization and clinical advances. *Polym. Chem.* **2014**, *5*, 3291–3297.
 52. Ahmad, Z.; Shah, A.; Siddiq, M.; Kraatz, H. B. Polymeric micelles as drug delivery vehicles. *RSC Adv.* **2014**, *4*, 17028–17038.

53. Weissig, V.; Whiteman, K. R.; Torchilin, V. P. Accumulation of protein-loaded long-circulating micelles and liposomes in subcutaneous Lewis lung carcinoma in mice. *Pharm. Res.* **1998**, *15*, 1552–1556.
54. Chen, J.; Liu, M. Amphiphilic block copolymer micelles with fluorescence as nano-carriers for doxorubicin delivery. *RSC Adv.* **2014**, *4*, 9684–9692.
55. Tsallas, A.; Jackson, J.; Burt, H. The uptake of paclitaxel and docetaxel into ex vivo porcine bladder tissue from polymeric micelle formulations. *Cancer Chemother. Pharmacol.* **2011**, *68*, 431–444.
56. De Ascentiis, A.; De Grazia, J.; Bowman, C.; Colombo, P.; Peppas, N.A. Mucoadhesion of poly(2-hydroxyethyl methacrylate) is improved when linear poly(ethylene oxide) chains are added to the polymer network. *J. Controlled Release* **1995**, *33*, 197–201.
57. Burt, H. M.; Zhang, X.; Toleikis, P.; Embree, L.; Hunter, W. Development of copolymers of poly(D,L-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel. *Colloids Surf., B* **1999**, *16*, 161–171.
58. Hadaschik, B. A.; ter Borg, M. G.; Jackson, J.; Sowery, R. D.; So, A. I.; Burt, H. M.; Gleave, M. E. Paclitaxel and cisplatin as intravesical agents against non-muscle-invasive bladder cancer. *BJU Int.* **2008**, *101*, 1347–1355.
59. Miao, L.; Guo, S.; Zhang, J.; Kim, W. Y.; Huang, L. Nanoparticles with precise ratiometric co-loading and co-delivery of gemcitabine monophosphate and cisplatin for treatment of bladder cancer. *Adv. Funct. Mater.* **2014**, *24*, 6601–6611.
60. Zhou, D.; Zhang, G.; Gan, Z. c(RGDfK) decorated micellar drug delivery system for intravesical instilled chemotherapy of superficial bladder cancer. *J. Controlled Release* **2013**, *169*, 204–210.
61. Lin, T. Y.; Li, Y. P.; Zhang, H.; Luo, J.; Goodwin, N.; Gao, T.; de Vere White, R.; Lam, K. S.; Pan, C. X. Tumor-targeting multifunctional micelles for imaging and chemotherapy of advanced bladder cancer. *Nanomedicine (London, U.K.)* **2013**, *8*, 1239–1251.
62. Lin, T. Y.; Zhang, H.; Luo, J.; Li, Y.; Gao, T.; Lara, P. N., Jr; de Vere White, R.; Lam, K. S.; Pan, C. X. Multifunctional targeting micelle nanocarriers with both imaging and therapeutic potential for bladder cancer. *Int. J. Nanomed.* **2012**, *7*, 2793–2804.
63. Zhang, H.; Aina, O. H.; Lam, K. S.; de Vere White, R.; Evans, C.; Henderson, P.; Lara, P. N.; Wang, X.; Bassuk, J. A.; Pan, C. X. Identification of a bladder cancer-specific ligand using a combinatorial chemistry approach. *Urol. Oncol.* **2012**, *30*, 635–645.
64. Lin, T. Y.; Zhang, H.; Wang, S.; Xie, L.; Li, B.; Rodriguez, C. O.; de Vere White, R.; Pan, C. X. Targeting canine bladder transitional cell carcinoma with a human bladder cancer-specific ligand. *Mol. Cancer* **2011**, *10* (1), 9.
65. Erdoglar, N.; Iskit, A. B.; Eroglu, H.; Sargon, M. F.; Mungan, N. A.; Bilensoy, E. Cationic core-shell nanoparticles for intravesical chemotherapy in tumor-induced rat model: Safety and efficacy. *Int. J. Pharm.* **2014**, *471*, 1–9.

66. Hasegawa, M.; Yagi, K.; Iwakawa, S.; Hirai, M. Chitosan induces apoptosis via caspase-3 activation in bladder tumor cells. *Jpn. J. Cancer Res.* **2001**, *92*, 459–466.
67. Martin, D. T.; Steinbach, J. M.; Liu, J.; Shimizu, S.; Kaimakliotis, H. Z.; Wheeler, M. A.; Hittelman, A. B.; Mark Saltzman, W.; Weiss, R. M. Surface-modified nanoparticles enhance transurothelial penetration and delivery of survivin siRNA in treating bladder cancer. *Mol. Cancer Ther.* **2013**, *13*, 71–81.
68. Mugabe, C.; Raven, P. A.; Fazli, L.; Baker, J. H. E.; Jackson, J. K.; Liggins, R. T.; So, A. I.; Gleave, M. E.; Minchinton, A. I.; Brooks, D. E.; Burt, H. M. Tissue uptake of docetaxel loaded hydrophobically derivatized hyperbranched polyglycerols and their effects on the morphology of the bladder urothelium. *Biomaterials* **2012**, *33*, 692–703.
69. Ye, L.; Letchford, K.; Heller, M.; Liggins, R.; Guan, D.; Kizhakkedathu, J. N.; Brooks, D. E.; Jackson, J. K.; Burt, H. M. Synthesis and characterization of carboxylic acid conjugated, hydrophobically derivatized, hyperbranched polyglycerols as nanoparticulate drug carriers for cisplatin. *Biomacromolecules* **2011**, *12*, 145–155.
70. Huang, C.; Neoh, K. G.; Xu, L.; Kang, E. T.; Chiong, E. Polymeric nanoparticles with encapsulated superparamagnetic iron oxide and conjugated cisplatin for potential bladder cancer therapy. *Biomacromolecules* **2012**, *13*, 2513–2520.
71. Krimmer, S. G.; Pan, H. Z.; Liu, J. H.; Yang, J. Y.; Kopecek, J. Synthesis and characterization of poly(ϵ -caprolactone)-*block*-poly[*N*-(2-hydroxypropyl)methacrylamide] micelles for drug delivery. *Macromol. Biosci.* **2011**, *11*, 1041–1051.
72. Oliveira, T. R.; Stauffer, P. R.; Lee, C. T.; Landon, C. D.; Etienne, W.; Ashcraft, K. A.; McNerny, K. L.; Mashal, A.; Nouns, J.; Maccarini, P. F.; Beyer, W. F., Jr.; Inman, B.; Dewhirst, M. W. Magnetic fluid hyperthermia for bladder cancer: A preclinical dosimetry study. *Int. J. Hyperthermia* **2013**, *29*, 835–844.
73. van der Heijden, A. G.; Verhaegh, G.; Jansen, C. F.; Schalken, J. A.; Witjes, J. A. Effect of hyperthermia on the cytotoxicity of 4 chemotherapeutic agents currently used for the treatment of transitional cell carcinoma of the bladder: an in vitro study. *J. Urol.* **2005**, *173*, 1375–1380.
74. Paroni, R.; Salonia, A.; Lev, A.; Da Pozzo, L. F.; Cighetti, G.; Montorsi, F.; Rigatti, P.; Colombo, R. Effect of local hyperthermia of the bladder on mitomycin C pharmacokinetics during intravesical chemotherapy for the treatment of superficial transitional cell carcinoma. *Br. J. Clin. Pharmacol.* **2001**, *52*, 273–278.
75. Huynh, V. T.; Chen, G. J.; de Souza, P.; Stenzel, M. H. Thiol-yne and thiol-ene “click” chemistry as a tool for a variety of platinum drug delivery carriers, from statistical copolymers to crosslinked micelles. *Biomacromolecules* **2011**, *12*, 1738–1751.
76. Huynh, V. T.; de Souza, P.; Stenzel, M. H. Polymeric micelles with pendant dicarboxylate chelating ligands prepared via a Michael addition for cis-platinum drug delivery. *Macromolecules* **2011**, *44*, 7888–7900.

77. Lee, S. M.; O'Halloran, T. V.; Nguyen, S. T. Polymer-caged nanobins for synergistic cisplatin-doxorubicin combination chemotherapy. *J. Am. Chem. Soc.* **2010**, *132*, 17130–17138.
78. Uchino, H.; Matsumura, Y.; Negishi, T.; Koizumi, F.; Hayashi, T.; Honda, T.; Nishiyama, N.; Kataoka, K.; Naito, S.; Kakizoe, T. Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats. *Br. J. Cancer* **2005**, *93*, 678–687.
79. Mora-Huertas, C. E.; Fessi, H.; Elaissari, A. Polymer-based nanocapsules for drug delivery. *Int. J. Pharmaceut.* **2010**, *385*, 113–142.
80. Orive, G.; Anitua, E.; Pedraz, J. L.; Emerich, D. F. Biomaterials for promoting brain protection, repair and regeneration. *Nat. Rev. Neurosci.* **2009**, *10*, 682–692.
81. Rajaganapathy, B. R.; Chancellor, M. B.; Nirmal, J.; Dang, L.; Tyagi, P. Bladder uptake of liposomes after intravesical administration occurs by endocytosis. *PLoS One* **2015**, *10*, e0122766.
82. Bachor, R.; Reich, E.; Miller, K.; Rück, A.; Hautmann, R. Photodynamic efficiency of liposome-administered tetramethyl hematoporphyrin in two human bladder cancer cell lines. *Urol. Res.* **1995**, *23*, 151–156.
83. Derycke, A. S.; Kamuhabwa, A.; Gijssens, A.; Roskams, T.; De Vos, D.; Kasran, A.; Huwyler, J.; Missiaen, L.; de Witte, P. A. Transferrin conjugated liposome targeting of photosensitizer ALPcS4 to rat bladder carcinoma cells. *J. Natl. Cancer Inst.* **2004**, *96*, 1620–1630.
84. Nakamura, T.; Fukiage, M.; Higuchi, M.; Nakaya, A.; Yano, I.; Miyazaki, J.; Nishiyama, H.; Akaza, H.; Ito, T.; Hosokawa, H.; Nakayama, T.; Harashima, H. Nanoparticulation of BCG-CWS for application to bladder cancer therapy. *J. Controlled Release* **2013**, *176c*, 44–53.
85. Horiguchi, Y.; Larchian, W. A.; Kaplinsky, R.; Fair, W. R.; Heston, W. D. W. Intravesical liposome-mediated interleukin-2 gene therapy in orthotopic murine bladder cancer model. *Gene Ther.* **2000**, *7*, 844–851.
86. Pirolo, K. F.; Rait, A.; Zhou, Q.; Zhang, X. Q.; Zhou, J.; Kim, C. S.; Benedict, W. F.; Chang, E. H. Tumor-targeting nanocomplex delivery of novel tumor suppressor RB94 chemosensitizes bladder carcinoma cells in vitro and in vivo. *Clin. Cancer Res.* **2008**, *14*, 2190–2198.
87. Wu, Q.; Mahendran, R.; Esuvaranathan, K. Nonviral cytokine gene therapy on an orthotopic bladder cancer model. *Clin. Cancer Res.* **2003**, *9*, 4522–4528.
88. Nogawa, M.; Yuasa, T.; Kimura, S.; Tanaka, M.; Kuroda, J.; Sato, K.; Yokota, A.; Segawa, H.; Toda, Y.; Kageyama, S.; Yoshiki, T.; Okada, Y.; Maekawa, T. Intravesical administration of small interfering RNA targeting PLK-1 successfully prevents the growth of bladder cancer. *J. Clin. Invest.* **2005**, *115*, 978–985.
89. Hu, Y.; Jiang, X. Q.; Ding, Y.; Chen, Q.; Yang, C. Z. Core-template-free strategy for preparing hollow nanospheres. *Adv. Mater.* **2004**, *16*, 933–937.
90. Arantes-Rodrigues, R.; Pinto-Leite, R.; Fidalgo-Gonçalves, L.; Palmeira, C.; Santos, L.; Colaço, A.; Oliveira, P. Synergistic effect between cisplatin and sunitinib malate on human urinary bladder-cancer cell lines. *BioMed Res. Int.* **2013** Article ID 791406.

91. Sonpavde, G.; Jian, W.; Liu, H.; Wu, M. F.; Shen, S. S.; Lerner, S. P. Sunitinib malate is active against human urothelial carcinoma and enhances the activity of cisplatin in a preclinical model. *Urol. Oncol.: Semin. Orig. Invest.* **2009**, *27*, 391–399.
92. Appleton, T. G.; Hall, J. R.; Prenzler, P. D. Reaction of the *cis*-diamminediaquaplatinum(II) cation with *N*-acetylglycine. *Inorg. Chem.* **1989**, *28*, 815–819.
93. Chou, T. C. Drug combination studies and their synergy quantification using the Chou-Talalay method. *Cancer Res.* **2010**, *70*, 440–446.
94. Chou, T. C. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol. Rev.* **2006**, *58*, 621–681.
95. Lohcharoenkal, W.; Wang, L.; Chen, Y. C.; Rojanasakul, Y. Protein nanoparticles as drug delivery carriers for cancer therapy. *BioMed Res. Int.* **2014** Article ID 180549.
96. Gulfam, M.; Kim, J.; Lee, J. M.; Ku, B.; Chung, B. H.; Chung, B. G. Anticancer drug-loaded gliadin nanoparticles induced apoptosis in breast cancer cells. *Langmuir* **2012**, *28*, 8216–8223.
97. Aswathy, R. G.; Sivakumar, B.; Brahatheeswaran, D.; Fukuda, T.; Yoshida, Y.; Maekawa, T.; Kuma, D. S. Biocompatible fluorescent zein nanoparticles for simultaneous bioimaging and drug delivery application. *Adv. Nat. Sci.: Nanosci. Nanotechnol.* **2012**, *3* Article ID 025006.
98. Zhen, X.; Wang, X.; Xie, C.; Wu, W.; Jiang, X. Cellular uptake, antitumor response and tumor penetration of cisplatin-loaded milk protein nanoparticles. *Biomaterials* **2013**, *34*, 1372–1382.
99. Elzoghby, A. O.; Saad, N. I.; Helmy, M. W.; Samy, W. M.; Elgindy, N. A. Ionically-crosslinked milk protein nanoparticles as flutamide carriers for effective anticancer activity in prostate cancer-bearing rats. *Eur. J. Pharm. Biopharm.* **2013**, *85*, 444–451.
100. Golla, K.; Bhaskar, C.; Ahmed, F.; Kondapi, A. K. A target specific oral formulation of doxorubicin-protein nanoparticles: Efficacy and safety in hepatocellular cancer. *J. Cancer* **2013**, *4*, 644–652.
101. Ren, D.; Kratz, F.; Wang, S. W. Engineered drug-protein nanoparticle complexes for folate receptor targeting. *Biochem. Eng. J.* **2014**, *89*, 33–41.
102. Buecheler, J. W.; Howard, C. B.; de Bakker, C. J.; Goodall, S.; Jones, M. L.; Win, T.; Peng, T.; Tan, C. H.; Chopra, A.; Mahlera, S. M.; Lim, S. Development of a protein nanoparticle platform for targeting EGFR expressing cancer cells. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 1230–1236.
103. Fu, Q.; Sun, J.; Zhang, W.; Sui, X.; Yan, Z.; He, Z. Nanoparticle albumin-bound (NAB) technology is a promising method for anti-cancer drug delivery. *Recent Pat. Anti-Cancer Drug Discovery* **2009**, *4*, 262–272.
104. Abaza, Y. M.; Alemany, C. Nanoparticle albumin-bound-paclitaxel in the treatment of metastatic urethral adenocarcinoma: The significance of molecular profiling and targeted therapy. *Case Reports in Urology* **2014** Article ID 489686.

105. Ko, Y. J.; Canil, C. M.; Mukherjee, S. D.; Winqvist, E.; Elser, C.; Eisen, A.; Reaume, M. N.; Zhang, L.; Sridhar, S. S. Nanoparticle albumin-bound paclitaxel for second-line treatment of metastatic urothelial carcinoma: a single group, multicentre, phase 2 study. *Lancet Oncol.* **2013**, *14*, 769–776.
106. McKiernan, J. M.; Holder, D. D.; Ghandour, R. A.; Barlow, L. J.; Ahn, J. J.; Kates, M.; Badalato, G. M.; Roychoudhury, A.; Decastro, G. J.; Benson, M. C. Phase II trial of intravesical nanoparticle albumin bound paclitaxel for the treatment of nonmuscle invasive urothelial carcinoma of the bladder after Bacillus Calmette-Guérin treatment failure. *J. Urol.* **2014**, *192*, 1633–1638.
107. Delto, J. C.; Kobayashi, T.; Benson, M.; McKiernan, J.; Abate-Shen, C. Preclinical analyses of intravesical chemotherapy for prevention of bladder cancer progression. *Oncotarget* **2013**, *4*, 269–276.
108. Ferraro, G.; Massai, L.; Messori, L.; Merlino, A. Cisplatin binding to human serum albumin: A structural study. *Chem. Commun.* **2015**, *51*, 9436–9439.
109. Timerbaev, A. R.; Hartinger, C. G.; Aleksenko, S. S.; Keppler, B. K. Interactions of antitumor metallodrugs with serum proteins: Advances in characterization using modern analytical methodology. *Chem. Rev.* **2006**, *106*, 2224–2248.
110. Fasano, M.; Curry, S.; Terreno, E.; Galliano, M.; Fanali, G.; Narciso, P.; Notari, S.; Ascenzi, P. The extraordinary ligand binding properties of human serum albumin. *IUBMB Life* **2005**, *57*, 787–796.
111. Yang, F.; Zhang, Y.; Liang, H. Interactive association of drugs binding to human serum albumin. *Int. J. Mol. Sci.* **2014**, *15*, 3580–3595.
112. Krenzle, E. S.; Chen, Z. J.; Hamilton, J. A. Correspondence of fatty acid and drug binding sites on human serum albumin: A two-dimensional nuclear magnetic resonance study. *Biochemistry* **2013**, *52*, 1559–1567.
113. Møller, C.; Tastesen, H. S.; Gammelgaard, B.; Lambert, I. H.; Stürup, S. Stability, accumulation and cytotoxicity of an albumin-cisplatin adduct. *Metallomics* **2010**, *12*, 811–818.

Chapter 10

Cell Membrane-Mediated Anticancer Drug Delivery

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Integrating the conventional synthetic nanoparticle with the biological cell membrane holds tremendous promise in biomedical applications. As an emerging platform for drug delivery, cell membrane-coated nanoparticulate drug delivery system has been extensively studied for prolonged in vivo circulation time, reticuloendothelial system (RES) evasion, active tumor targeting and cancer vaccination. In this review, current advances in utilizing cell membrane-coated nanoparticles for drug delivery and cancer treatment were summarized and future challenges were discussed.

Introduction

As an important modality in disease treatment, nanotechnology-based drug delivery system has been rapidly developed and successfully used in various biomedical researches, including cancer treatment (1–3), immunotherapy (4, 5), and antibacterial therapy (6, 7). Nanoparticles offer unprecedented distinct advantages over traditional strategies for drug delivery, which can be engineered and tailored to have various physicochemical properties, including distinct size, surface charge and hydrophobic/hydrophilic drug loading, for specific functions, such as improving drug encapsulation, controlling drug release and decreasing severe side effects (8–12). More importantly, nanoparticles can be decorated with polyethylene glycol (PEG) and the specific targeting ligand to enhance the drug circulation in the blood and accumulation at the tumor site (13, 14). By taking advantages of these features, nanoparticles can protect a drug from quick clearance by evading the reticuloendothelial system (RES); active targeting property enables delivery system to transport through biological barriers and increases the availability of the drug at the targeted disease site (15–17). All of these benefits have made therapeutic nanoparticle a promising candidate to serve as the anticancer drug delivery system. However, despite the strong efforts devoted in translating therapeutic nanoparticles into clinic application, only a few nanoparticulate drug delivery formulations were applied to clinical trial and approved by Food and Drug Administration (FDA), such as liposome (18) and polymeric nanoparticles (19, 20). Thus, continuous efforts should be taken to develop the reliable drug delivery system to meet clinical criteria.

Building on the emergence of the new engineering strategies and the deep understanding of the functions of natural cells and the specific interaction between natural cells and tumor tissue, a novel bio-inspired drug delivery system has attracted great attentions and been extensively studied over recent years. Motivated by the structures and the functions found in the nature, bio-inspired drug delivery systems can be fabricated by different engineering strategies such as “top-down” method and “bottom-up” method (21). The “top-down” method is to mimic the specific components or functions of a complex biological organism (22). For example, Wang *et al.* developed the nanohairy decorated microspheres to mimic the surface morphology of the activated platelets (23). They fabricated the platelet mimicking microspheres through decorating the polyaniline (PANi) nanohairs in the shells of hollow polystyrene (PS) microspheres. These microspheres could specifically capture the circulating tumor cells through structure-based topographical interactions between the tumor cells and the biomimetic microspheres. The “bottom-up” strategy is to create the bio-inspired delivery system by combination of mimicking several features of natural biological system (24). For instance, Mitragotri *et al.* developed a synthetic microparticle to mimic the size, discoidal shape and vital function of platelet (25). They stretched the polymeric microparticles with appropriate size into a discoidal shape and crosslinked the decorated proteins to increase the stability of microparticles after removal of the inner polymeric core. The platelet-mimicking microparticles could successfully perform the adhesive

properties for selective recognition of thrombi, which is the important function of natural platelet.

Given the complexity of living cells and organism, it is evidenced that the synthetic biomaterials cannot completely mimic the whole function of the source cell (26). To address this dilemma, one emerging design is to coat the derived and purified cellular membranes on the surface of synthetic nanoparticles for development of biomimetic drug delivery system (27). Biological membranes play a vital role in natural cells by stabilizing the architecture, transporting substances in and out of the cells, and protecting the cells from their surroundings (21). Due to the preservation of integrity and functionalities of cell membrane and easy fabrication of delivery system, cell membrane-coated nanoparticles hold vast promise in anticancer drug delivery. In this review, recent advances using different cell membrane-coated nanoparticles for anticancer drug delivery were summarized and the challenges for future developments were discussed. Particularly, we highlighted the most attractive biomedical applications of biomimetic nanoparticulate drug delivery system, including 1) prolonged circulation time and RES evasion; 2) active tumor targeting; 3) cancer vaccination (Figure 1).

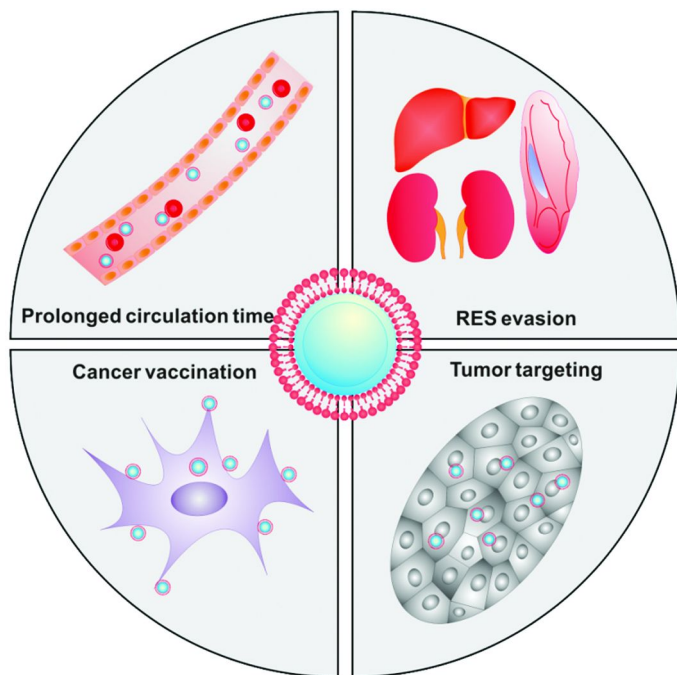


Figure 1. Various biomedical applications of cell membrane-coated nanoparticles.

Cell Membrane-Mediated Prolonged Circulation Time and RES Evasion

For effective drug delivery, a long blood circulation profile is required to maintain the sufficient drug concentration. However, synthetic nanoparticles often suffer from the short circulation time and are shuttled out of circulation to the RES, including liver and spleen quickly after administration, resulting in the nonspecific biodistribution and the potential toxicity (28, 29). To address this, poly(ethylene glycol) (PEG) has often been modified on the surface of nanoparticles to minimize the protein binding (also termed opsonization) and improve the pharmacokinetics profiles of nanoparticles (30, 31). This gold standard of PEG coating has long been applied to the development of stealth nanoparticles. However, the nondegradability of PEG chain has significantly limited the clinical application of this strategy (32). Additionally, PEGylation can significantly hinder the uptake of the nanoparticles by the tumor cells upon the accumulation of nanoparticles at the tumor site (33, 34). Furthermore, researchers recently found that the immune system could produce antibodies, which specifically bind to PEG, to increase the clearance of PEGylated therapeutics, leading to the decreased circulation time *in vivo* (35). These drawbacks of PEGylation have driven the researchers to find more reliable fabrication method to increase the prolonged circulation time of nanoparticles *in vivo*.

Inspired by the long lifespan of the natural red blood cells (RBC), which are naturally long circulating biological particulates with up to 120 days lifespan *in vivo* (36, 37), Zhang *et al.* developed a biomimetic delivery platform using RBC membrane-coated nanoparticles (RBC-NP) (Figure. 2) (38). The resulting RBC-NP was composed of two parts: RBC membrane wrapped on the surface and inner core of poly(lactic-co-glycolic acid) (PLGA) nanoparticle. The purified RBC membrane was obtained through removing the intracellular components *via* membrane disruption in a hypotonic solution. Thereafter, the RBC membrane-coated nanoparticles were prepared by extruding the PLGA nanoparticles and RBC membrane vehicles together. This fabrication strategy maximally preserved the biofunctionality of the integrative RBC membrane. The RBC-NP showed the core-shell morphology under the transmission electron microscope (TEM) observation and maintained the structure after internalization by the cancer cells. Furthermore, their results confirmed the well preservation of the membrane protein after the fabrication process. More importantly, the immunosuppressive RBC membrane protein-CD47, which plays a vital role in long circulation, was successfully transferred to RBC-NP from the source cell. The *in vitro* experiments showed the uptake of RBC-NP upon macrophage cells was significantly inhibited after RBC membrane coating. After administration, RBC-NP displayed an enhanced circulation time with the half-life time of 39.6 h, significantly longer than PEG-NP with the half-life time of 15.8 h. Their findings demonstrated the superiority of the cell membrane-coated synthetic nanoparticles over PEGylated nanoparticle in prolonged circulation *in vivo*.

Further, Tasciotti *et al* exploited another approach to develop the biomimetic nanoparticle delivery system (39). They coated cell membranes isolated from leukocytes on the nanoporous silicon particles to mimic cell-like functions, such as

immune-resistant property, long circulation and biological barrier transportation. The collected leukocyte membrane was purified by ultracentrifugation through a discontinuous sucrose density gradient and reconstituted as proteo-lipid patches. By taking advantage of the electrostatic interaction of positively charged nanoporous silicon particles and negatively charged leukocyte membranes, the leukocyte membranes could fully cover the surface of nanoporous silicon particles and self-assemble to leukolike vector (LLV) (Figure 3A). The functional components, such as CD45, CD3z, LFA-1 and an adhesion molecule, were well preserved on the particle surface, which was helpful to prevent the nonspecific uptake by the macrophages and the phagocytic cells and beneficial for the binding and transportation across the endothelial layer. The inhibition of the particle internalization was observed in murine J774 macrophages with a $\sim 75\%$ decrease in uptake and human THP-1 phagocytic cells with a $\sim 50\%$ decrease in uptake (Figure 3B). Furthermore, LLV could significantly delay the liver clearance, as evidenced by $\sim 25\%$ of adherent LLV associated with the liver endothelium rather than the surface of Kupffer cells, which was mainly attributed to the decoration of leukocytes membrane. More importantly, this long circulation time and decreased clearance resulted in two folds increase of the accumulation of LLV at the tumor site compared with the non-coated particles. Their finding had paved a new way to prolong the circulation time of nanoparticle and overcome sequential vascular barriers.

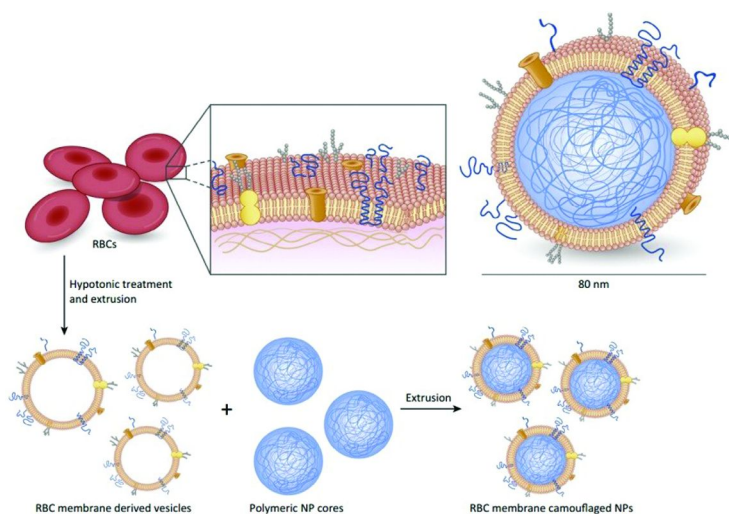


Figure 2. Schematics of the purification of RBC-membrane vesicle and coating RBC membrane on the surface of polymeric nanoparticles. Reproduced with permission from reference (38). Copyright 2011 National Academy of Sciences and reference (43). Copyright 2015 Elsevier.

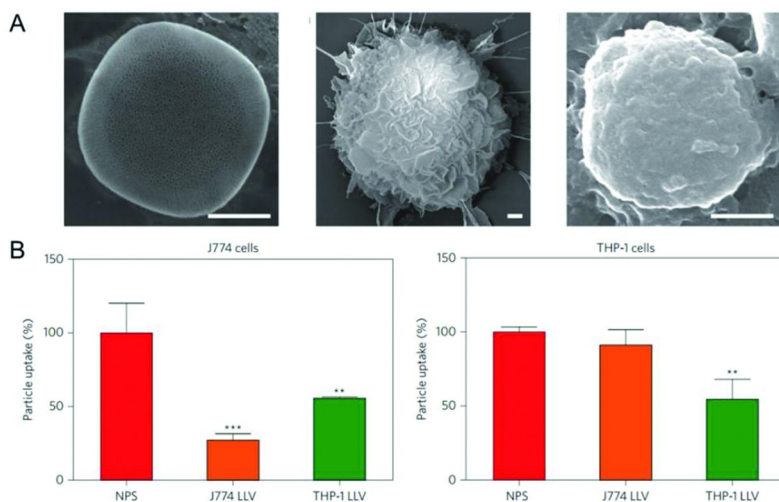


Figure 3. A) SEM images of the nanoporous silicon particle, the leukocyte and leukocyte membrane-coated nanoporous silicon particle (from left to right). Scale bar: 1 μ m. B) The internalization of nanoporous silicon particles and LLV by J774 cells and THP-1 cells. Reproduced with permission from reference (39). Copyright 2013 Macmillan Publishers Ltd.

Cell Membrane-Mediated Tumor Targeting

The development of the drug carrier that can specifically deliver the drug to the disease site is always the main focus of drug delivery research. The enhanced permeability and retention (EPR) effects in tumor tissue (40, 41), which is resulted from the leaky vasculature, could facilitate the accumulation of the drug delivery system at the tumor site. However, the EPR effects require the longevity of delivery vehicle and were size-dependent and relatively inefficient (42). To date, a lot of efforts have been devoted to develop the active targeting drug delivery system to enhance the drug concentration at the tumor site. One state-of-the-art approach is to conjugate the targeting ligand on the surface of drug delivery system to recognize the specific proteins overexpressed on the cancer cells (11, 43, 44). The typical examples of the classic targeting ligands include folic acid that can bind to folate receptor (45), lactoferrin that can bind to lactoferrin receptors (46) and RGD peptide that can bind to integrin (47). However, the active targeting strategies require the density of the targeting ligands and the corresponding receptors on the cancer cells, which is hard to control.

Additionally, the “off-target” effects could also induce the severe side effects since the receptors are often expressed on the normal cells. For example, the expression of the transferrin receptor on the tumor cells, a classic tumor-associated receptor, is only 3–5-fold higher than that on the normal cells (48, 49). Cell membrane coating strategy offers a promising approach to address these drawbacks and develops a new library of the active targeting drug delivery systems. By taking advantages of the successful translocation of the abundance and complexity of the proteins expressed on the source cell, the specific interaction of the source cell and tumor cell could be well preserved and thus the “off-target” effect could be maximally compromised.

Platelet, which is indispensable component for maintaining blood integrity and stopping bleeding by clumping and clotting blood vessel injuries, was recently found to play a crucial role in tumor metastasis through the specific recognition and interaction between platelets and circulating tumor cells in the blood (50, 51). The aggregation of platelets surrounding circulating tumor cells (CTCs) helps CTCs survive in blood stream and spread to new tissues (52). The mechanisms underlying this specific aggregation include biomolecular binding such as P-Selectin and CD44 receptors (53) and structure-based capture (54). Inspired by the interaction between platelets and tumor cells, our group has developed a platelet-mimicking drug delivery system for sequential delivery of anticancer drugs (55). In this biomimetic nanovehicle, anticancer protein drug TRAIL was decorated on the surface of isolated and purified platelet membrane and TRAIL-membrane complex was wrapped on the synthetic nanogel loaded with Dox to form core-shell structural platelet membrane-coated nanovehicle (PM-NV) (Figure 4).

The resulting PM-NV was capable of sequentially and site-specifically delivering TRAIL to the cancer cell membrane and Dox into the nuclei of the cancer cells. The aggregation of the PM-NV on the surface of the cancer cells, which is stemmed from the highly selective affinity between platelets and cancer cells, could promote the interaction of TRAIL and death receptors, triggering programmed cell death. Moreover, equipped with pH-responsive modality, the PM-NV could release Dox in the lysosome upon the cleavage of the degradable matrix after endocytosis, accompanied by the accumulation of Dox in the nuclei. The *in vitro* cellular experiments showed the location of the PM-NV on the cell membrane and distribution in the cell nuclei after internalization by the cancer cells. Additionally, the uptake efficiency of PM-NV on the macrophage cells was significantly lower than that of NV and *in vivo* half-life time of PM-NV (32.6 h) was longer than the non-coated NV (5.6 h), which were attributed to the equipment of large numbers of “self-recognized” proteins on the platelets surface. Furthermore, after *in vivo* administration, the PM-NV could efficiently aggregate on the surface of the CTCs and subsequently eliminate the CTCs, as evidenced by the decreased metastatic nodules compared with the non-coated nanovehicles. The synergistic anticancer effect of the site-specific delivery of drugs in the PM-NV was established in mice models by the significant inhibition of primary tumor growth. Our findings confirmed the active targeting capability of platelet membrane-coated nanoparticulate drug delivery system with minimized *in vivo* immunogenicity and prolonged circulation time.

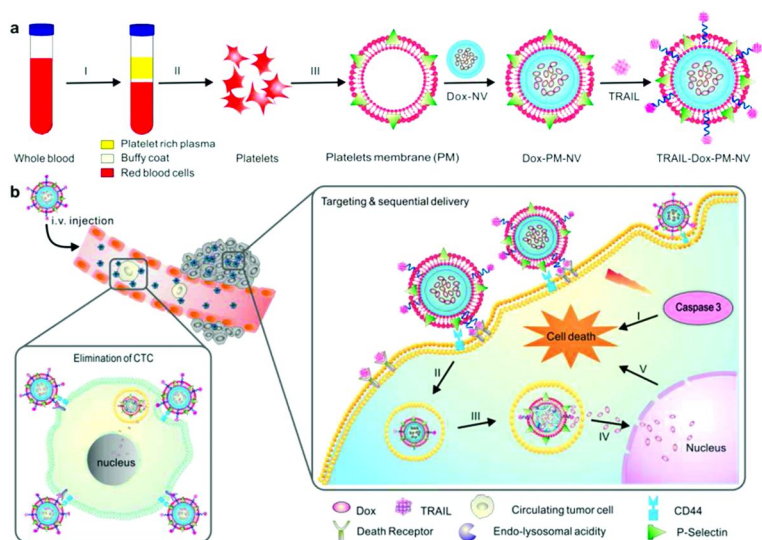


Figure 4. Schematic of design of drug-loaded PM-NV for targeting and sequential delivery of TRAIL and Dox. Reproduced with permission from reference (55). Copyright 2015 Wiley.

In addition to targeting to the cancer cells, Zhang *et al.* found that the platelet membrane-coated nanoparticles could also selectively adhere to the damaged vasculatures as well as bind to the platelet-adhering pathogens (56). By taking advantage of the different charge distribution between the outer and inner surface of the platelet membrane, the nanoparticles with negative charge could preferentially bind to the inner membrane (designated PNP) (Figure 5). They verified the preservation of the platelet membrane-expressed proteins after translocation, including immunomodulatory proteins, integrin components and subendothelial binding components, as evidenced by western blotting analysis. The PNP displayed the superiority in the collagen adhesion *via* membrane-type specific manner and enhanced the accumulation at the damaged artery site compared with the undamaged artery. After loading with docetaxel (Dtxl), PNP-Dtxl showed the significantly lower intima-to-media ratio (I/M) and luminal obliteration with 0.18 ± 0.06 and 8.0% compared with free Dtxl with 0.76 ± 0.18 and 33.6%. Furthermore, the platelet membrane-coated nanoparticles could also target to bacteria. The mechanism underlying the adherence is that bacteria often recruit the platelets to help them localize at the specific tissue and evade immune system. *In vitro* assay displayed that the bacteria showed preferential binding by PNP, exhibiting a 12-fold increase in PNP retention compared to bare NP.

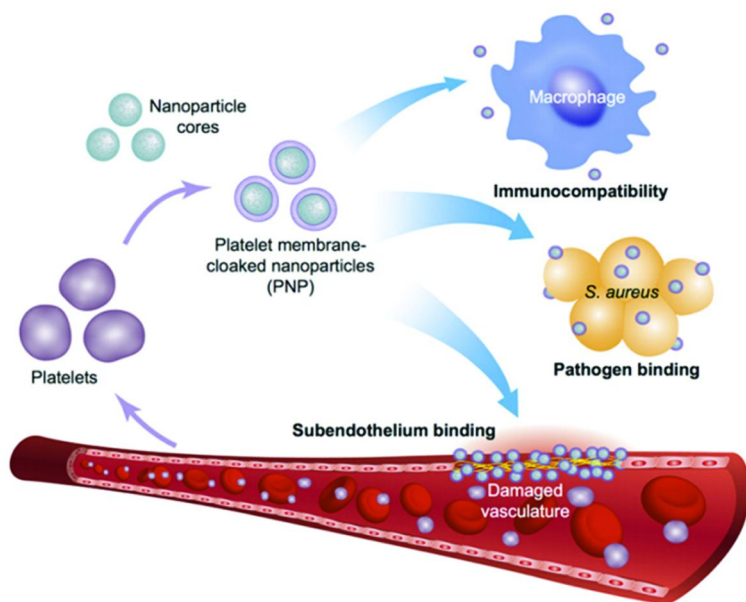


Figure 5. Schematic design of the platelet membrane-cloaked nanoparticles for immunocompatibility, subendothelium binding, and pathogen adhesion. Reproduced with permission from reference (56). Copyright 2015 Macmillan Publishers Ltd.

Cell Membrane-Mediated Cancer Vaccination

The nanotechnology has been extensively studied to develop the nanoparticle-based vaccine for carrying antigens and immunologic adjuvants to induce the strong immune responses (57). Furthermore, nanoparticles can be engineering to target to the specific tissue and cells, leading to enhanced accumulation and subsequent increased uptake by the antigen-presenting cells to elicit more effective immune responses (58). Among myriads of the successful applications of the nanoparticle-based vaccine in triggering immune reaction, cell membrane-coated nanoparticles have emerged as especially promising approach in comparison to conventional nanoscaled vaccine due to their biomimetic physicochemical nature. This membrane-coated technology could well preserve the integrity as well as biofunctionality of the cellular surface antigens, resulting in minimized loss of presented antigens during delivery process. Furthermore, the overexpression of the antigens on the cell membrane will promise the potent immune response against cancer or other infection.

Zhang *et al.* developed a cancer cell membrane-coated nanoparticle (CCNP) for anticancer vaccination (Figure 6A) (59). By taking advantages of the heterogeneity of cancer and complete replication of the surface antigenic diversity of the cancer cells, cancer cell membrane-coated nanoparticle could extensively

enhance immunogenicity of cancer vaccines and trigger immune response, leading to the recognition and elimination of these heterologous substances, which is beneficial for the cancer immunotherapy (60). B16–F10 mouse melanoma cells membranes were collected and purified through the combination of gradient centrifugation and hypotonic and mechanical co-induced membrane disruption. CCNP was prepared through the co-extrusion of the polymeric nanoparticles and the cancer cell membranes. The resulting CCNP displayed a core-shell structure with the size of 110 nm. Western blotting analysis verified the preservation of the cancer cell membrane antigens, including glycoprotein 100, a well-known tumor-associated antigen for melanoma. Additionally, the low presence of the intracellular proteins, such as histone H3—a nuclear marker and cytochrome c oxidase—a mitochondrial marker, demonstrated the preferential retention of membrane specific antigens during the fabrication process. After incubation with the dendritic cells, CCNP could elicit potent immune response, as evidenced by significant crowding of T-lymphocytes around the dendritic cells (Figure 6B) and upregulation of the cytokine interferon-gamma (IFN γ) (Figure 6C). These findings confirmed the cytotoxic T-lymphocyte stimulation by the delivery of specific cancer cell-associated antigens through CCNP, which allowed for the potent cancer immunotherapy.

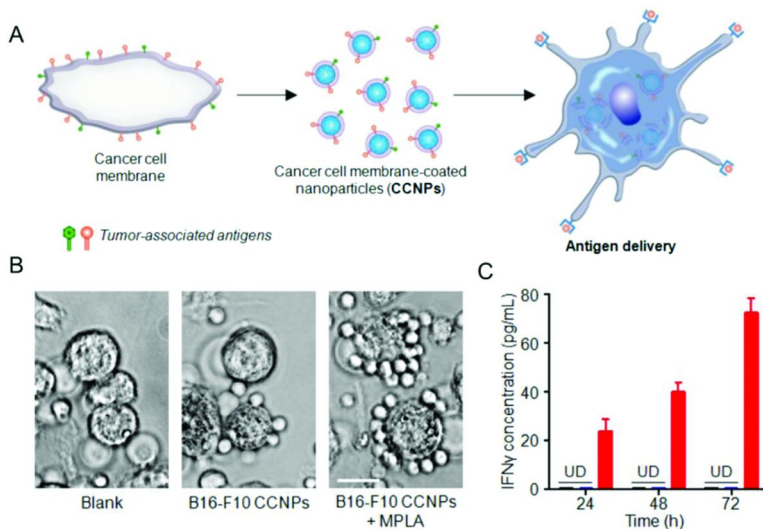


Figure 6. A) Schematic illustration of preparation of cancer cell membrane-coated nanoparticles for cancer vaccination. B) Phase contrast microscopy images of T-lymphocytes clustering around dendritic cells after different treatments. C) The upregulation of IFN γ after different treatments. Reproduced with permission from reference (59). Copyright 2014 American Chemical Society and reference (60). Copyright 2015 Wiley.

Conclusions

Integrating the natural biological cell membranes with synthetic nanoparticles, a new library of biomimetic nanoparticulate drug delivery systems has been developed for various biomedical applications (61). Cell membrane-coated nanoparticles with tremendous therapeutic potency for cancer hold incredible promise in overcoming the limitations and drawbacks of conventional drug delivery through the combination of the advantages of natural cells and synthetic nanoparticles. Additionally, this approach could further broaden the nanocarrier design flexibility and scope of applications by endowing the nanoparticles with cell-like behaviors and functionalities. For instance, Zhang *et al.* recently created the RBC membrane-coated nanoparticle to mimic the functions of the red blood cell for a wide spectrum of pore-forming toxin detainment and anchorage of toxin for effective vaccination (62, 63). Chen *et al.* developed a RBC membrane-based molecular probe to further study the interaction between cell membrane and nanoparticle (64).

Despite the promising progresses made in cell membrane-mediated anticancer drug delivery, there are still many challenges in the translation of cell membrane-coated nanoparticulate drug delivery system (12). Firstly, the resulting cell membrane-coated nanoparticles need to match the patients on the basis of their cell types to minimize the immunogenicity. For example, RBC membrane-coated nanoparticles should be prepared with the RBC derived from the patients with the same blood types. Secondly, given the complexity of the biological interaction between cells and cancer tissue, the specific affinity between the source cell and the cancer cells should be thoroughly studied to maximally reduce the “off-target” effects and decrease the unwanted side effects. Finally, to fulfill the clinical application, the scale-up production of cell membrane-coated nanoparticles should be further addressed.

As a new arrival to the drug delivery field, cell membrane-coated drug delivery system is continuously contributing to bridge the gap between synthetic materials and natural particulates by integrating the tailorability and flexibility of synthetic materials and the functionality and complexity of natural cells. Looking to the future, as a new paradigm of being inspired by the nature, cell membrane-coated approach will keep encouraging the researchers to develop more effective and versatile drug delivery system for improving disease treatment efficacy. Furthermore, using the cell membrane derived from patients themselves to functionalize drug delivery system will enable the precisely personalized treatment that can make a significant impact on the future disease treatment.

Acknowledgments

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References

1. Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* **2007**, *2*, 751–760.
2. Farokhzad, O. C.; Langer, R. Impact of nanotechnology on drug delivery. *ACS Nano* **2009**, *3*, 16–20.
3. Hu, Q.; Sun, W.; Wang, C.; Gu, Z. Recent advances of cocktail chemotherapy by combination drug delivery systems. *Adv. Drug Delivery Rev.* **2015** DOI:10.1016/j.addr.2015.10.022.
4. Cho, N.-H.; Cheong, T.-C.; Min, J. H.; Wu, J. H.; Lee, S. J.; Kim, D.; Yang, J.-S.; Kim, S.; Kim, Y. K.; Seong, S.-Y. A multifunctional core-shell nanoparticle for dendritic cell-based cancer immunotherapy. *Nat. Nanotechnol.* **2011**, *6*, 675–682.
5. Park, J.; Wrzesinski, S. H.; Stern, E.; Look, M.; Criscione, J.; Ragheb, R.; Jay, S. M.; Demento, S. L.; Agawu, A.; Limon, P. L. Combination delivery of TGF- β inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy. *Nat. Mater.* **2012**, *11*, 895–905.
6. Hajipour, M. J.; Fromm, K. M.; Ashkarran, A. A.; de Aberasturi, D. J.; de Larramendi, I. R.; Rojo, T.; Serpooshan, V.; Parak, W. J.; Mahmoudi, M. Antibacterial properties of nanoparticles. *Trends Biotechnol.* **2012**, *30*, 499–511.
7. Zhao, Y.; Tian, Y.; Cui, Y.; Liu, W.; Ma, W.; Jiang, X. Small molecule-capped gold nanoparticles as potent antibacterial agents that target gram-negative bacteria. *J. Am. Chem. Soc.* **2010**, *132*, 12349–12356.
8. Sun, W.; Lu, Y.; Gu, Z. Advances in Anticancer Protein Delivery using Micro-/Nanoparticles. *Part. Part. Syst. Charact.* **2014**, *31*, 1204–1222.
9. Lu, Y.; Sun, W.; Gu, Z. Stimuli-responsive nanomaterials for therapeutic protein delivery. *J. Control. Release* **2014**, *194*, 1–19.
10. Farokhzad, O. C.; Langer, R. Nanomedicine: developing smarter therapeutic and diagnostic modalities. *Adv. Drug Delivery Rev.* **2006**, *58*, 1456–1459.
11. Langer, R. Drug delivery and targeting. *Nature* **1998**, *392*, 5–10.
12. Mitragotri, S.; Anderson, D. G.; Chen, X.; Chow, E. K.; Ho, D.; Kabanov, A. V.; Karp, J. M.; Kataoka, K.; Mirkin, C. A.; Petrosko, S. H. Accelerating the translation of nanomaterials in biomedicine. *ACS Nano* **2015**, *9*, 6644–6654.
13. Petros, R. A.; DeSimone, J. M. Strategies in the design of nanoparticles for therapeutic applications. *Nat. Rev. Drug Discovery* **2010**, *9*, 615–627.
14. Langer, R.; Tirrell, D. A. Designing materials for biology and medicine. *Nature* **2004**, *428*, 487–492.
15. Davis, M. E.; Shin, D. M. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discovery* **2008**, *7*, 771–782.
16. Ferrari, M. Cancer nanotechnology: opportunities and challenges. *Nat. Rev. Cancer* **2005**, *5*, 161–171.
17. Lammers, T.; Hennink, W.; Storm, G. Tumour-targeted nanomedicines: principles and practice. *Br. J. Cancer* **2008**, *99*, 392–397.

18. Svenson, S.; Wolfgang, M.; Hwang, J.; Ryan, J.; Eliasof, S. Preclinical to clinical development of the novel camptothecin nanopharmaceutical CRLX101. *J. Controlled Release* **2011**, *153*, 49–55.
19. Zhang, Y.; Chan, H. F.; Leong, K. W. Advanced materials and processing for drug delivery: the past and the future. *Adv. Drug Delivery Rev.* **2013**, *65*, 104–120.
20. Eliasof, S.; Lazarus, D.; Peters, C. G.; Case, R. I.; Cole, R. O.; Hwang, J.; Schluep, T.; Chao, J.; Lin, J.; Yen, Y. Correlating preclinical animal studies and human clinical trials of a multifunctional, polymeric nanoparticle. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 15127–15132.
21. Palivan, C. G.; Goers, R.; Najer, A.; Zhang, X.; Car, A.; Meier, W. Bioinspired polymer vesicles and membranes for biological and medical applications. *Chem. Soc. Rev.* **2016** DOI:10.1039/C5CS00569H.
22. Zhang, Y.; Ruder, W. C.; LeDuc, P. R. Artificial cells: building bioinspired systems using small-scale biology. *Trends Biotechnol.* **2008**, *26*, 14–20.
23. Liu, X.; Zhang, F.; Wang, Q.; Gao, J.; Meng, J.; Wang, S.; Yang, Z.; Jiang, L. Platelet-Inspired Multiscaled Cytophilic Interfaces with High Specificity and Efficiency toward Point-of-Care Cancer Diagnosis. *Small* **2014**, *10*, 4677–4683.
24. Tu, R. S.; Tirrell, M. Bottom-up design of biomimetic assemblies. *Adv. Drug Delivery Rev.* **2004**, *56*, 1537–1563.
25. Doshi, N.; Orje, J. N.; Molins, B.; Smith, J. W.; Mitragotri, S.; Ruggeri, Z. M. Platelet mimetic particles for targeting thrombi in flowing blood. *Adv. Mater.* **2012**, *24*, 3864–3869.
26. Gao, W.; Zhang, L. Coating nanoparticles with cell membranes for targeted drug delivery. *J. Drug Target* **2015**, *23*, 619–626.
27. Luk, B. T.; Zhang, L. Cell membrane-camouflaged nanoparticles for drug delivery. *J. Control. Release* **2015**, *220*, 600–607.
28. Owens, D. E.; Peppas, N. A. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *Int. J. Pharm.* **2006**, *307*, 93–102.
29. Alexis, F.; Pridgen, E.; Molnar, L. K.; Farokhzad, O. C. Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol. Pharm.* **2008**, *5*, 505–515.
30. Jokerst, J. V.; Lobovkina, T.; Zare, R. N.; Gambhir, S. S. Nanoparticle PEGylation for imaging and therapy. *Nanomedicine* **2011**, *6*, 715–728.
31. Parveen, S.; Sahoo, S. K. Long circulating chitosan/PEG blended PLGA nanoparticle for tumor drug delivery. *Eur. J. Pharmacol.* **2011**, *670*, 372–383.
32. Veronese, F. M.; Pasut, G. PEGylation, successful approach to drug delivery. *Drug Discov. Today* **2005**, *10*, 1451–1458.
33. Sun, C. Y.; Liu, Y.; Du, J. Z.; Cao, Z. T.; Xu, C. F.; Wang, J. Facile Generation of Tumor-pH-Labile Linkage-Bridged Block Copolymers for Chemotherapeutic Delivery. *Angew. Chem.* **2015** DOI:10.1002/anie.201509507.
34. Li, S. D.; Huang, L. Stealth nanoparticles: high density but sheddable PEG is a key for tumor targeting. *J. Controlled Release* **2010**, *145*, 178–81.

35. Ishida, T.; Wang, X.; Shimizu, T.; Nawata, K.; Kiwada, H. PEGylated liposomes elicit an anti-PEG IgM response in a T cell-independent manner. *J. Controlled Release* **2007**, *122*, 349–355.
36. Muzykantov, V. R. Drug delivery by red blood cells: vascular carriers designed by mother nature. *Expert Opin. Drug Delivery* **2010**, *7*, 403–427.
37. Doshi, N.; Zahr, A. S.; Bhaskar, S.; Lahann, J.; Mitragotri, S. Red blood cell-mimicking synthetic biomaterial particles. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 21495–21499.
38. Hu, C.-M. J.; Zhang, L.; Aryal, S.; Cheung, C.; Fang, R. H.; Zhang, L. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 10980–10985.
39. Parodi, A.; Quattrocchi, N.; van de Ven, A. L.; Chiappini, C.; Evangelopoulos, M.; Martinez, J. O.; Brown, B. S.; Khaled, S. Z.; Yazdi, I. K.; Enzo, M. V. Biomimetic functionalization with leukocyte membranes imparts cell like functions to synthetic particles. *Nat. Nanotechnol.* **2013**, *8*, 61–68.
40. Iyer, A. K.; Khaled, G.; Fang, J.; Maeda, H. Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discov. Today* **2006**, *11*, 812–818.
41. Prabhakar, U.; Maeda, H.; Jain, R. K.; Sevick-Muraca, E. M.; Zamboni, W.; Farokhzad, O. C.; Barry, S. T.; Gabizon, A.; Grodzinski, P.; Blakey, D. C. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. *Cancer Res.* **2013**, *73*, 2412–2417.
42. Jain, R. K.; Stylianopoulos, T. Delivering nanomedicine to solid tumors. *Nat. Rev. Clin. Oncol.* **2010**, *7*, 653–664.
43. Xu, X.; Ho, W.; Zhang, X.; Bertrand, N.; Farokhzad, O. Cancer nanomedicine: from targeted delivery to combination therapy. *Trends Mol. Med.* **2015**, *21*, 223–232.
44. Brannon-Peppas, L.; Blanchette, J. O. Nanoparticle and targeted systems for cancer therapy. *Adv. Drug Delivery Rev.* **2012**, *64*, 206–212.
45. Stella, B.; Arpicco, S.; Peracchia, M. T.; Desmaële, D.; Hoebeke, J.; Renoir, M.; D'Angelo, J.; Cattel, L.; Couvreur, P. Design of folic acid-conjugated nanoparticles for drug targeting. *J. Pharm. Sci.* **2000**, *89*, 1452–1464.
46. Hu, K.; Li, J.; Shen, Y.; Lu, W.; Gao, X.; Zhang, Q.; Jiang, X. Lactoferrin-conjugated PEG–PLA nanoparticles with improved brain delivery: in vitro and in vivo evaluations. *J. Controlled Release* **2009**, *134*, 55–61.
47. Danhier, F.; Vroman, B.; Lecouturier, N.; Crockart, N.; Pourcelle, V.; Freichels, H.; Jérôme, C.; Marchand-Brynaert, J.; Feron, O.; Préat, V. Targeting of tumor endothelium by RGD-grafted PLGA-nanoparticles loaded with paclitaxel. *J. Controlled Release* **2009**, *140*, 166–173.
48. Chirasani, S.; Markovic, D.; Synowitz, M.; Eichler, S.; Wisniewski, P.; Kaminska, B.; Otto, A.; Wanker, E.; Schäfer, M.; Chiarugi, P. Transferrin-receptor-mediated iron accumulation controls proliferation and glutamate release in glioma cells. *J. Mol. Med.* **2009**, *87*, 153–167.

49. Zhang, B.; Shen, S.; Liao, Z.; Shi, W.; Wang, Y.; Zhao, J.; Hu, Y.; Yang, J.; Chen, J.; Mei, H. Targeting fibronectins of glioma extracellular matrix by CLT1 peptide-conjugated nanoparticles. *Biomaterials* **2014**, *35*, 4088–4098.
50. Nash, G.; Turner, L.; Scully, M.; Kakkar, A. Platelets and cancer. *Lancet Oncol.* **2002**, *3*, 425–430.
51. Labelle, M.; Begum, S.; Hynes, R. O. Platelets guide the formation of early metastatic niches. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, E3053–E3061.
52. Chaffer, C. L.; Weinberg, R. A. A perspective on cancer cell metastasis. *Science* **2011**, *331*, 1559–1564.
53. Borsig, L. The role of platelet activation in tumor metastasis. *Expert Rev. Anticancer Ther.* **2008**, *8*, 1247–55.
54. Stone, J.; Wagner, D. P-selectin mediates adhesion of platelets to neuroblastoma and small cell lung cancer. *J. Clin. Invest.* **1993**, *92*, 804–813.
55. Hu, Q.; Sun, W.; Qian, C.; Wang, C.; Bomba, H. N.; Gu, Z. Anticancer Platelet-Mimicking Nanovehicles. *Adv. Mater.* **2015**, *27*, 7043–50.
56. Hu, C.-M. J.; Fang, R. H.; Wang, K.-C.; Luk, B. T.; Thamphiwatana, S.; Dehaini, D.; Nguyen, P.; Angsantikul, P.; Wen, C. H.; Kroll, A. V. Nanoparticle biointerfacing by platelet membrane cloaking. *Nature* **2015**, *526*, 118–121.
57. Ghosh, K.; Kanapathipillai, M.; Korin, N.; McCarthy, J. R.; Ingber, D. E. Polymeric nanomaterials for islet targeting and immunotherapeutic delivery. *Nano Lett.* **2011**, *12*, 203–208.
58. Irvine, D. J.; Hanson, M. C.; Rakhra, K.; Tokatlian, T. Synthetic nanoparticles for vaccines and immunotherapy. *Chem. Rev.* **2015**, *115*, 11109–11146.
59. Fang, R. H.; Hu, C.-M. J.; Luk, B. T.; Gao, W.; Copp, J. A.; Tai, Y.; O'Connor, D. E.; Zhang, L. Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery. *Nano Lett.* **2014**, *14*, 2181–2188.
60. Fang, R. H.; Kroll, A. V.; Zhang, L. Nanoparticle-Based Manipulation of Antigen-Presenting Cells for Cancer Immunotherapy. *Small* **2015**, *11*, 5483–5496.
61. Hu, Q.; Katti, P. S.; Gu, Z. Enzyme-responsive nanomaterials for controlled drug delivery. *Nanoscale* **2014**, *6*, 12273–12286.
62. Hu, C.-M. J.; Fang, R. H.; Copp, J.; Luk, B. T.; Zhang, L. A biomimetic nanosponge that absorbs pore-forming toxins. *Nat. Nanotechnol.* **2013**, *8*, 336–340.
63. Hu, C.-M. J.; Fang, R. H.; Luk, B. T.; Zhang, L. Nanoparticle-detained toxins for safe and effective vaccination. *Nat. Nanotechnol.* **2013**, *8*, 933–938.
64. Fan, Z.; Zhou, H.; Li, P. Y.; Speer, J. E.; Cheng, H. Structural elucidation of cell membrane-derived nanoparticles using molecular probes. *J. Mater. Chem. B* **2014**, *2*, 8231–8238.

Chapter 11

Nanoelectrode Array Based Devices for Electrical Capture of Microbes Using Dielectrophoresis

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Manipulation of particles using dielectrophoresis (DEP) is a well-known technique that utilizes electric field to interact with polarizable particles. Here we summarize our studies on developing a nanostructured DEP device for the capture of bacterial cells and virus particles. A high magnitude non-uniform electric field was produced in a microfluidic channel utilizing a nanoelectrode array made of vertically aligned carbon nanofibers versus a macroscopic indium tin oxide counter electrode in a “points-and-lid” configuration. The DEP capture was found fully reversible for two types of microbes including *E. coli* bacterial cells (~1-2 micron in size) and virus particles (~80-200 NM in size), when an AC voltage (100 Hz to 1 MHz) was turned on and off. The high electric field strength focused at the nanoelectrode showed stronger interaction with virus particles, producing striking lightning patterns. For specific detection of *E. coli* strain Dhα5, SERS reporter QSY21 that was co-functionalized with polyclonal antibodies on anisotropic oval-shaped iron oxide-gold (IO-Au) core-shell nanoparticles was utilized. The signal from the QSY21 was used to specifically detect *E. coli* cells in a microfluidic channel. By integrating the SERS nanoparticles with the DEP, we have demonstrated the ability to use electric

fields to effectively concentrate microbial particles in the active areas for further SERS identification and electrical impedance sensing. This technique can be potentially utilized as a fast sample preparation module in a microfluidic chip to capture, separate, and concentrate microbes in analyzing small volume of dilute samples as a part of a portable detection system for field applications.

Introduction

The rapid detection and identification of microbial strains has emerged as a pressing issue in fields ranging from agriculture and food-borne pathogens to monitoring pathogen activity on areas of airfields, ports, power-plants and critical infrastructure (1, 2). There is a strong need for portable, miniature, low-cost sensors capable of specific detection microbes at low concentrations (3, 4). Microfluidic lab-chip technology has been recognized for their great potential for on-chip capture, sorting, and concentration of biomaterials. Second, the ease of integration of the microfluidic device with signal transduction techniques can be achieved via optical acoustic, impedance, or electrochemical measurements (5). Dielectrophoresis (DEP) is very attractive for this purpose (6, 7) and its application using micro-DEP device is been widely used for manipulation of mammalian cells (tens of microns) to bacterial cells (1 micron) (8–10). Since DEP force is proportional to the volume of the target particles, it decreases rapidly when the particle size is reduced to only ~100 nanometers (11–13), more sophisticated control requires nanostructured DEP electrodes (14, 15). We have developed a simple nanoscale DEP device based on a nanoelectrode array (NEA) made of vertically aligned carbon nanofibers (VACNFs) versus a macroscopic indium tin oxide (ITO) counter electrode, which can capture either single or large ensembles of bacterial cells and viral particles from high-velocity fluidic flows (14, 16–18). The specific detection of bacteria is based on SERS reporter QSY21 that is co-functionalized with polyclonal antibodies on anisotropic oval-shaped iron oxide-gold (IO-Au) core-shell nanoparticles on the bacterial cells. These cells are brought into the field of view of a fixed Raman probe in both confocal and portable Raman system (19). Last, we have demonstrated that the high electric field focused at the nanoelectrode (NE) tip aided on-chip impedance sensing of the *Vaccinia* viruses due to direct deposition of the viruses on an electrode array. These advantages of the nanoelectrode facilitate the simultaneous detection and identification of microbes in a microfluidic chamber (20).

Principles, Device Design, and Fabrication

DEP was first described by Pohl in 1966 (21) and has been widely used in biological science to separate live and dead bacteria, viruses, cells, and DNA. DEP is based on a spatially non-uniform electric field generated by an AC voltage

bias between a pair of electrodes, resulting in a time average net force on neutral polarizable particles that depends on the permittivity and conductivity of the particle and medium. The time average DEP force (F_{DEP}) acting on the spherical particles by the non-uniform electric field is given by (21):

$$\langle \mathbf{F}_{DEP} \rangle = 2\pi r^3 \epsilon_m \text{Re}[K(\omega)] \nabla E^2, \quad (1)$$

where r is the radius of the particle, ϵ_m is the permittivity of the suspending medium, ∇E^2 is the gradient of the square of the applied electric field strength, and $\text{Re}[K(\omega)]$ is the real component of the complex Clausius-Mossotti (CM) factor given by:

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}, \text{ where } \epsilon^* = \epsilon - j \frac{\sigma}{\omega} \quad (2)$$

with ϵ^* representing the complex permittivity and the indices p and m referring to the particle and medium, respectively. Parameter σ is the complex conductivity, ω is the angular frequency ($\omega = 2\pi f$) of the applied electric field, and $j = \sqrt{-1}$. In this study, the proper medium is chosen to give $\text{Re}[K(\omega)] > 0$ so the particles experience a DEP force directing toward higher electric field strength, i.e. positive DEP (pDEP) (21). The force is then directed toward regions of high field strength, which is positive DEP (pDEP). A system with negative value of $\text{Re}[K(\omega)]$ will push the particles toward lower electric field strength. The value of $\text{Re}[K(\omega)]$ in an aqueous medium can vary from -0.5 to 1.0, depending on the effective polarizabilities of particle and medium. In this study large pDEP is desired to capture bacteria at the exposed VACNF tip by selecting a proper frequency and the right medium composition.

According to eq 1, the DEP force (F_{DEP}) is proportional to the volume or cube of the radius (r^3) of the particle. The hydrodynamic force to carry the particles with flow (i.e. Stokes drag force F_{Drag}) is directly proportional to the radius of the particle by

$$F_{Drag} = 6\eta\pi r k v \quad (3)$$

where η is the dynamic viscosity, k is a small factor accounts for the wall effects, and v is the linear flow rate (flow velocity) (10). Sedimentation force and Brownian force are negligible. The advantage of nanostructured DEP devices is that the magnitude of ∇E^2 can be enhanced by orders of magnitude so even small viral particles can be captured (22).

Figure 1 schematically illustrates the design with a NEA as the ‘points’ electrode and a macroscopic ITO slide as the ‘lid’ electrode in a “points-and-lid” configuration (9) for DEP experiments. The NEA comprises VACNFs embedded in SiO₂ matrix with only the tip exposed. The average diameter of the VACNFs

is $\sim 100\text{--}120\text{ nm}$ (14, 17). Either a randomly distributed array with an average spacing of $\sim 1\text{--}2$ microns or a regular patterned array can be used (14, 17). The NEA is covered with a $2\text{-}\mu\text{m}$ SU-8 layer, a negative photoresist widely used in microelectronics industry, with only $200 \times 200\text{ }\mu\text{m}^2$ active area exposed. This area is aligned at the center of a 1.0 mm diameter circular chamber connected with $500\text{ }\mu\text{m}$ wide microfluidic channels etched in an $18\text{-}\mu\text{m}$ SU-8 layer on the top ITO slide. These two pieces are then permanently bonded together through the SU-8 layers.

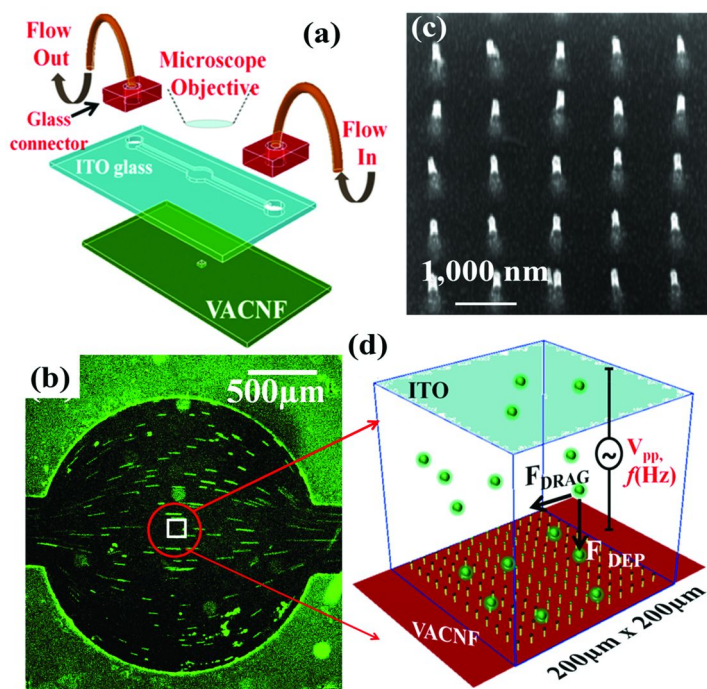


Figure 1. Schematic of the DEP device. (a) The components of the device, including a lid electrode (indium tin oxide coated glass) with a $18\text{-}\mu\text{m}$ SU-8 layer containing a microfluidic channel, a nano-electrode array chip covered with $2\text{-}\mu\text{m}$ SU-8 except exposing a $200 \times 200\text{ }\mu\text{m}^2$ area, glass fluidic connectors, and microbore tubes. (b) A low-magnification optical microscope image showing the flow profile of fluorescent labeled bacteriophage solution passing through the bonded device. (c) SEM image of a nano-electrode array made of e-beam patterned regular vertically aligned carbon nanofibers. (d) Schematic diagram of microbial particles in the active nano-DEP area, which are subjected to the hydrodynamic drag force (F_{DRAG}) along the flow direction and the dielectrophoretic force (F_{DEP}) mostly perpendicular to the NEA surface. Reproduced with permission from reference (2). Copyright 2007 American Chemical Society.

DEP Capture of Bacteria and Identification by Surface Enhanced Raman Spectroscopy

DEP capture of bacterial cells was demonstrated with nontoxic *E. coli* strain DH α 5 (18265-017, Fisher Scientific). Normally, 20.0 μ L of grown culture was incubated in 2.0 mL of fresh media to reach a cell concentration of $\sim 1 \times 10^9$ cells/mL. The cells were centrifuged at 5000 rpm. The collected cells were resuspended and washed three times with 1X phosphate buffer saline (PBS) to remove leftover media. Labeling *E. coli* cells was done in two steps. First, $\sim 3 \times 10^9$ cells/mL were incubated with FITC conjugated rabbit anti-*E. coli* Ab (AbD Serotech, NC) at 330.0 μ g/mL for 1 hr at room temperature (RT). The cells were then washed twice with the PBS buffer. Second, *E. coli* cells were incubated with Alexa 555 conjugated goat anti-rabbit second Ab (Invitrogen, CA) at 130.0 μ g/mL for 1 hr at RT. The labeled *E. coli* cells were then washed three times with PBS buffer and then with DI water. The cells were finally resuspended in DI water to a concentration of $\sim 1 \times 10^9$ cells/mL for DEP experiments.

The DEP device was placed under an upright fluorescence optical microscope (Axioskop II, Carl Zeiss) using 50X objective lens. The NEA employed in this study had an exposed CNF density of $\sim 2 \times 10^7$ CNFs/cm ^{2} , with an average spacing of ~ 2.0 μ m. A filter set with excitation wavelength of 540-552 nm and emission wavelength of 567-647 nm (filter set 20HE, Carl Zeiss) was used with an Axio Cam MRm digital camera to record fluorescence videos at an exposure time of 0.40 s using multi-dimensional acquisition mode in the Axio-vision 4.7.1 release software (Carl Zeiss MicroImaging, Inc). One of the major concerns while performing bacterial capture experiments is non-specific adsorption of bacteria. To overcome this issue, the microfluidic channel was injected with 1.0 mL BSA solution (2.0% w/v) at a flow rate of 0.2 μ L/min before performing DEP experiments. This step helped to passivate the surface of SU-8 and SiO $_2$ in the fluidic channel and substantially reduced the non-specific adsorption of *E. coli* cells. The channel was then rinsed with 2.0 mL DI water at a flow rate of 5.0 μ L/min.

DEP experiments were carried out by injecting labeled *E. coli* suspension into the passivated channel at a specified flow velocity. When no DEP force was applied on the bacteria, they flow with the media due to the hydrodynamic drag force. Figure 2a shows study to capture of *E. coli* cells flowing through the channel at a linear flow velocity of 1.6 mm/sec and varying frequencies of AC voltage. Quantitatively, the number of captured *E. coli* cells was measured by counting the fixed bright spots using the auto measure module of the Carl Zeiss software. Varying the parameters of AC voltage has concluded that the optimum DEP capture conditions for *E. coli* cells are 100.0 kHz, though any frequency from 50.0 kHz to 1.0 MHz can generate DEP effects. Higher V_{pp} gives stronger DEP force and normally reliable results can be obtained with ≥ 9.0 V $_{pp}$. Figure 2b shows the number of bright spots monotonically decreasing as the flow rate is increased. This indicates that the pDEP force would attract many cells (mostly those close to the NEA surface) toward the NEA. Once they were at the CNF tip, the lateral DEP force became larger than the hydrodynamic drag force along the flow direction and the *E. coli* cell was captured at the fixed tips of the NEA. Figure 2c is snapshot of

the *E. coli* cell flowing in the microfluidic chamber, and appears as a stretched line corresponding to the travel distance over the finite exposure time. This was used to calculate the linear flow velocity at the NEA surface. When an AC voltage of 10.0 V_{pp} at 100.0 kHz frequency is applied between the NEA and the macro-ITO electrode, pDEP force is generated, which pulled *E. coli* cells toward the CNF NEA at the bottom of the microfluidic channel and trapped them at isolated CNF tips. Once trapped, the stretched *E. coli* moving lines changed to bright fixed spots in Figure 2d. The flow velocity at 1.6 mm/sec matched the highest flow velocity (0.04-2 mm/sec) used in interdigitated micro-DEP device (10, 23).

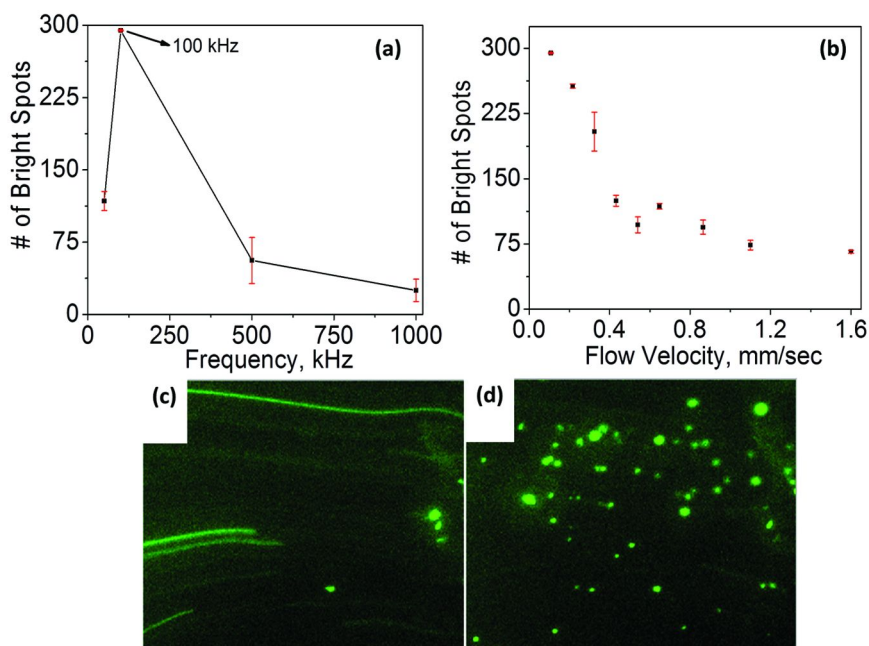


Figure 2. Capture of *E. coli* Dha5 in NEA microfluidic channel. (a) The correlation of the frequency and the number of bright spots (Number of captured *E. coli* cells) (b) The correlation of the number of bright spots (representing captured *E. coli* cells) and the flow velocity. (c) Snapshot images of the CNF NEA as *E. coli* cells flowing through at 1.6 mm/sec, with the 100 kHz 10 V_{pp} AC bias turned (c) off and (d) on, respectively. The size of the frame is about 100 x 100 μm^2 . Reproduced with permission from reference (18). Copyright 2011 Wiley-VCH.

The identification of *E. coli* DH α 5 using SERS nanotag based on nanoovals (NOVs) is summarized in Figure 3a. In brief, the NOVs were synthesized from spherical IO nanoparticle cores (~23 nm diameter) onto which an irregular-shaped Au shell was deposited, forming NOVs with the outer dimension of 35 to 50 nm to provide a large SERS enhancement factor (24, 25). The NOV surfaces were then coated with a mixture of carboxyl-polyethylene glycol-thiol (HOOC-PEG-SH, MW 5000) and methoxy-polyethylene glycol-thiol (mPEG-SH, MW 5000) to make NOVs biocompatible, stabilize the QSY21 adsorption, and to introduce carboxylic acid groups at the surface for covalent attachment of a Alexa 555-labelled secondary antibodies through amide bond formation. The *E. coli*-specific primary antibody (labelled with FITC for fluorescence validation) was then bound to the secondary antibody on IO-Au SERS NOVs to form the completed SERS nanotag. Before each experiment, these SERS nanotags were mixed with the bacteria sample to allow for the attachment of the nanotag to *E. coli* bacteria through specific immunochemistry. The structure of QSY21 and its typical Raman spectrum is shown at the center of Figure 3a. Raman bands at 1333, 1584 and 1641 cm^{-1} are from the xanthene ring stretching vibrations of the molecule. The strongest characteristic band is seen at 1496 cm^{-1} . The signal from the QSY21 attached to the NOV nanotag for this band demonstrates an enhancement factor of 4.9×10^4 over a 0.1 mM solution of QSY21 (26) and is used in this study for the quantitative measurement. Figure 3b and 3c shows TEM images of IO-Au SERS NOVs and those bound onto *E. coli*. On average, there are hundreds of NOVs bound to each *E. coli*, which gives a Raman signal sufficient to be detected at the single cell level. The confocal fluorescence microscopy image of Alexa 555 dye labelled secondary antibody on the *E. coli* cells (Figure 3d) clearly illustrates the uniform coating of NOVs on *E. coli* DH α 5 through specific immunochemical binding. Figure 3e and 3f are the schematic representation of the setup of the microfluidic device under Raman microscope and enlarged schematic view of DEP capture of the bacteria for the Raman detection with a portable Raman probe.

To demonstrate the potential capability of this method, both confocal (DXR, Thermo Fisher Scientific) and a portable system (ProRaman L, Enwave Optonics, Inc) was used. Similar studies were carried out with the two spectrophotometers for flow velocity and frequency optimization. Figure 4a shows the full Raman spectrum of QSY21 at different AC frequencies during capture of bacteria. The highest peak in the full spectra, 1496 cm^{-1} was used in further calculation and the higher capture was seen at the AC frequency of 100.0 kHz. The results between these two Raman systems were very consistent and correlate well with the fluorescence measurements, with the maximum capture at the flow velocity of 0.4 mm/sec (0.55 $\mu\text{l/s}$) (Figure 4b). However, the probe diameter at the focal point in the portable Raman system is about 100 μm (inset in Figure 4c), much larger than the 3.1 μm size in the confocal Raman microscope. This allows signals to be collected from many more bacteria and yields better statistics, but the laser intensity is lower as it is spread over a larger area. These two factors must be balanced for the optimum performance (19).

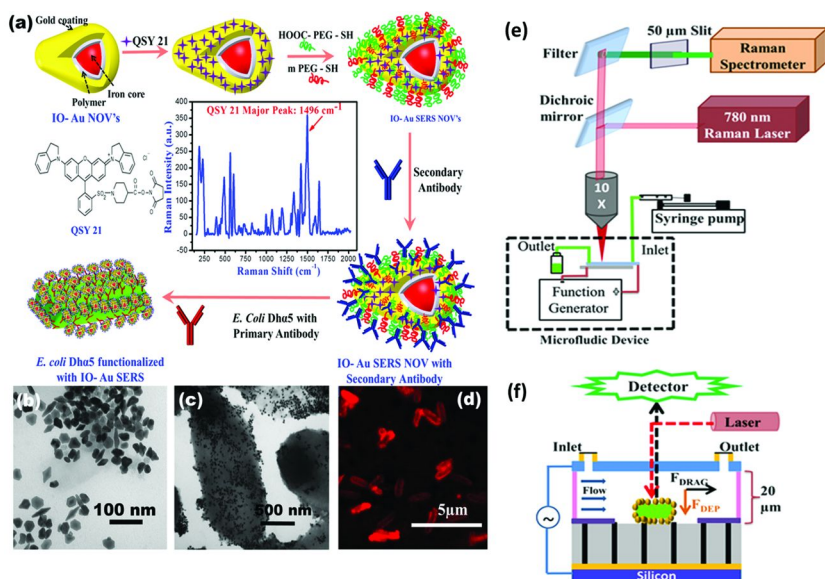


Figure 3. Schematic of the microfluidic dielectrophoretic device under a Raman microscope for bacteria detection. a) Schematic procedure for preparation of QSY 21 derivatized iron oxide-gold core-shell nano-ovals (IO-Au NOV's) as nanotags for SERS measurements and their attachment to *E. coli* bacterial cells through a FITC-labeled primary antibody and a Alexa 555 labeled secondary antibody. TEM images of (b) the starting IO-Au NOV's and (c) *E. coli* DHa5 bacterial cells attached with antibody-functionalized IO-Au NOV's. (d) Confocal fluorescence image of Alexa 555 in *E. coli* DHa5 bacterial cells attached with antibody-functionalized IO-Au NOV's. Alexa 555 was attached to the secondary antibody. (e) The overall experimental setup of a confocal Raman microscope equipped with a 780 nm laser and a 10X objective lens. (f) Enlarged schematic view of DEP capture of the bacteria bind with oval-shaped SERS nanotags for the Raman detection with a portable Raman probe. Adapted with permission from reference (19). Copyright 2015 Royal Society of Chemistry.

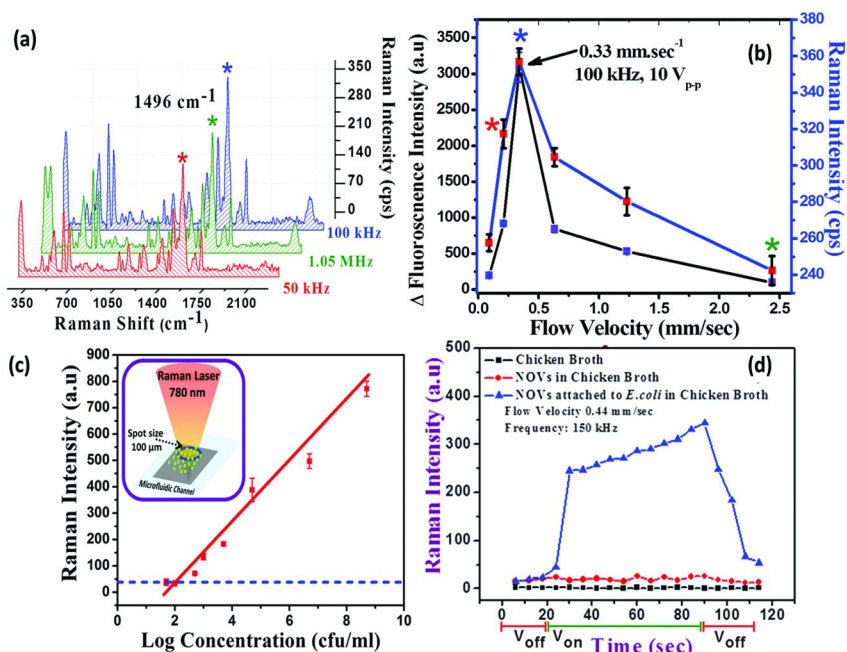


Figure 4. Assessing DEP capture of 5.3×10^5 CFU/mL *E. coli* cells with fluorescence and Raman measurements at various flow velocity and AC frequency. (a) The study of frequency of the AC current with fixed flow velocity of 0.33 mm/sec and voltage (10.0 V_{pp} with representative Raman spectra of QSY-21, showing 1496 cm^{-1} is the highest peak. (b) The study of *E. coli* cells at flow velocity of 0.21 mm/sec (red star), 0.33 mm/sec (blue star), and 2.43 mm/sec (green star) at fixed frequency (100 kHz) and voltage (10 V_{pp}). (c) The Raman intensity after 50 s of DEP capture from the bacteria solution with the concentration varying from 5 CFU/mL to 1.0×10^9 CFU/mL. The Raman measurements were carried out by focusing the laser beam within the $200 \mu\text{m} \times 200 \mu\text{m}$ active DEP area with a ProRaman L portable Raman system (Enwave Optronics). Inset shows that 100 μm diameter laser focal spot aligned with $200 \mu\text{m} \times 200 \mu\text{m}$ active DEP area. (d) Assessing DEP capture of *E. coli* cells with fluorescence and Raman measurements in different complex matrices. The kinetic curve of DEP capture of *E. coli* cells in a chicken solution at 10 V_{pp}, 0.44 mm/sec flow velocity, and 150 kHz AC frequency. Adapted with permission from reference (19). Copyright 2015 Royal Society of Chemistry.

Figure 4c summarizes the SERS intensity of the captured NOV-labelled *E. coli* using the portable Raman setup while the *E. coli* concentration was varied from ~ 10 to 1×10^9 cells/mL. The intensity of QSY21 marker at the Raman shift of 1496 cm^{-1} could be noticeably separated from the carbon nanofiber signals at 1350 cm^{-1} (D-band) and 1600 cm^{-1} (G-band), respectively. The Raman intensity was a linear function of the logarithm of bacteria concentration when the concentration C is above ~ 100 cells/mL :

$$(RI)_{\text{portable}} = 108.8 \times \log C - 214.7 \quad (4)$$

where RI was the Raman intensity increase after 50 s of DEP capture. It is surprising that the RI signal is proportional to $\log C$ rather than directly proportional to C . There are two possibilities to consider: (1) the rapid decay of the electric field at positions further away from the VACNF tip may generate a highly non-uniform DEP force (proportional to $\tilde{N}E^2$) that does not act equally on all cells in the whole solution volume between the NEA and ITO electrodes; (2) the first captured bacteria may significantly screen the electric field and quickly lower the total DEP force on other cells in the solution. Overall, the larger size of the laser focal spot (100 μm in diameter) allowed the collection of Raman signals from a larger number of captured bacteria. But further increasing the Raman probe size to 300 μm gave a lower sensitivity, mainly due to lower excitation laser intensity as the power was spread out over a larger area.

For bacteria concentrations below the critical value $C_0 = \sim 100$ cells/mL, no measurable signal above the background, i.e. $(RI)_{\text{blank}} = \sim 36$ a.u., was detected. No captured bacterial cells were detected during the applied DEP period, which was limited by the slow mass transport of bacteria to the active area. But the Raman intensity increased as more bacteria were passed into the device at higher concentrations. The detection limit $\log C_{dl}$ was determined using calibration curve as follows:

$$\log C_{dl} = \log C_0 + 3\sigma_{\text{blank}}/m, \quad (5)$$

where σ_{blank} (~ 11.7) is the standard deviation of the Raman signal for bacteria concentration below C_0 and $m = 108.8$ is the slope of the calibration curve. The concentration detection limit was determined to be ~ 210 cells/mL.

To analyze the capture in complex samples; chicken broth, apple juice and soil solution were tested. One of representative plots with chicken broth is shown Figure 4d. The chicken broth and Mott's apple juice were obtained from the local store and soil samples were obtained from the lawn nearby. Chicken pieces, water and salt were label contents for chicken-in-water tin. Complex matrices solution was centrifuged at 14,000 rpm for 10 min and supernatant was collected. *E.coli* DH α 5 was added into the solution of processed chicken broth solution in concentration of 5×10^5 cells/mL. Complex matrices present different challenges due to inorganic and organic substance interactions, making it difficult to isolate the target to be tested. Sample preparation includes washing, centrifuge and filtration of the complex matrix. These steps become important to eliminate larger particles that can clog the micro-channels. The conductivity of bacteria in distilled water (pH 6.8) was 1.22×10^{-4} S/m. Conductivity of commercial chicken broth after sample processing, and addition of *E. coli* DH α 5 cells resulted in conductivity of 1.7×10^{-3} S/m. It can be noted that bacteria in complex matrices have a high Raman intensity at the frequency of 150.0 kHz and 100.0 kHz for chicken broth and soil solution respectively, as compared to the NOV in matrix (1.4×10^{10} NOV/mL). In apple juice, positive DEP capture of the bacteria was not observed. This can be either because of the denaturation of the antibodies due to acidic pH preventing NOV attachment to bacteria or the bacteria is experiencing n-DEP (19).

DEP Capture of Virus Particles

Bacteriophage T4r (Carolina Biological Supply Company, Burlington, NC) and T1 (ATCC, Manassas, VA) were chosen to demonstrate the capability to capture small virus particles using the nano-DEP device. The bacteriophage was co-cultured with *E. coli* as described in a previous report (18). The final solution was filtered with a 0.2 μm filter (Fisher, PA) to remove live bacteria or bacterial debris. Double layer agar method was used to determine the titer of the phages (16). Washing and labeling were carried out by centrifugation using Amicon® Ultra 0.5 centrifugal filter devices (Milipore, Billerica, MA). Labeling of phages was carried out using a 500X working solution of SYBR® Green I Nucleic Acid Gel Stain (Lonza, Rockland, ME) in TE buffer. The labeled and washed phages were dispensed in double DI water. From the discussion on CM factors, addition of 280 mM mannitol was necessary to enhance the efficiency of pDEP capture of virus particles (27, 28). The final concentration of the phages for the normal DEP experiments was $\sim 5 \times 10^9$ pfu/mL except in some concentration-dependent experiments.

The DEP experimental setup is similar to that for bacteria capture in the previous section. The flow of the labeled *Bacteriophage* T4r was first examined at low magnification (with a 10X objective lens) as shown in Figure 1b. The stretched lines represent the movement of individual bacteriophage particles carried by the hydrodynamic flow of the media during the exposure time. The figure indicates the distribution of the particles as they entered from the narrow straight channel (500 μm in width) into the larger circular microchamber (2.0 mm in diameter) and only a fraction of the bacteriophage particles passed above the active NEA area. Fluorescence videos over the 200 $\mu\text{m} \times 200 \mu\text{m}$ active NEA area was recorded with 50X objective lens as the labeled virus flowing through. The capture efficiency for bacteriophage was much higher than that of bacteria causing them to overlap after capture. Hence it was difficult to distinguish individual bacteriophages in many experiments. To overcome this, the integrated fluorescence intensity over the 200 $\mu\text{m} \times 200 \mu\text{m}$ active NEA area was used in place of counts of isolated bright spots (except in some later experiments) to quantify the capture efficiency during the kinetic DEP process (16).

As shown in Figure 5, the integrated fluorescence intensity rose to a saturated level in less than 10.0 sec as a 10 V_{pp} AC bias was applied on the DEP device while flowing 5×10^9 pfu/mL *Bacteriophage* T4r solution through the channel at the flow velocity v varying from 0.085 to 3.06 mm/sec while changing the frequency from 100 Hz to 1.0 MHz. Comparing DEP capture of *E. coli* cells (16, 18), DEP capture of *Bacteriophage* T4r requires lower frequency (from ~ 100.0 Hz to ~ 100.0 kHz with the maximum performance at 10 kHz).¹⁶ Considering that mannitol had to be added to adjust the permittivity and conductivity of the media (i.e. water) (27, 28), the small virus particles (*Bacteriophage* T4r, 80-200 nm in size) have very different CM factor as shown in Figure 5a.

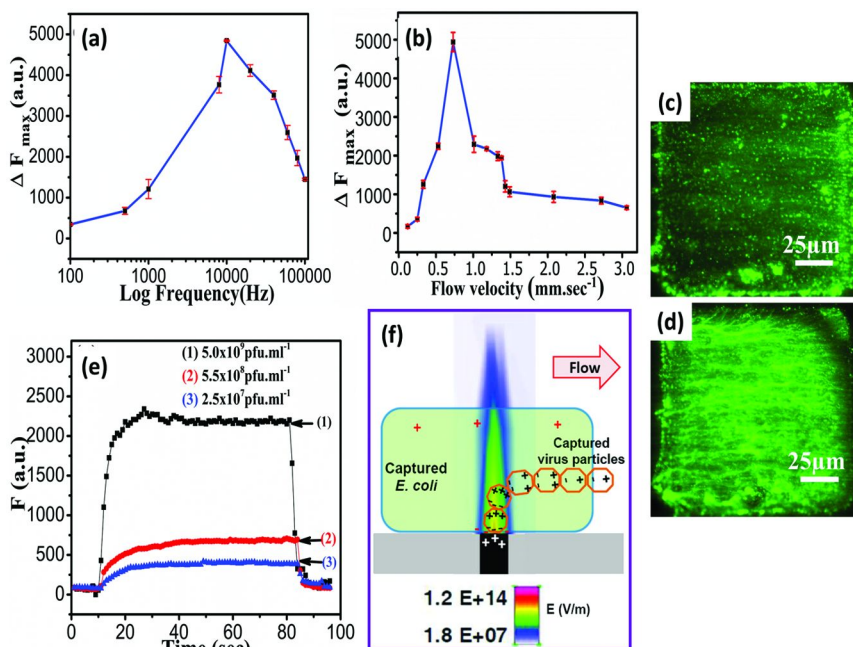


Figure 5. DEP capture of virus particles (Bacteriophage T4r) on VACNF in a microfluidic device. (a) The frequency dependence of DEP capture of 5×10^9 pfu/ml Bacteriophage T4r at a flow velocity of 0.73 mm/sec with the AC bias fixed at 10 V_{pp} . The maximum DEP integrated fluorescence intensity (ΔF_{max}) versus the applied AC frequency from 100 Hz to 1 MHz. The optimum capture was obtained with ~ 10 kHz AC voltage. (b) The quantity of DEP capture, represented by the maximum increase of the ΔF_{max} , versus the flow velocity, which is peaked at 0.73 mm/sec. (c) and (d) are the representative snapshots from the videos just before the AC voltage was turned off at flow velocity of 0.33, 0.73 and mm/sec, respectively. (e) DEP capture at different concentrations. The kinetic DEP capture curves when AC voltage is turned on and off with Bacteriophage T4r concentration at the normal concentration (5×10^9 pfu/ml) and two diluted concentrations (5.5×10^8 and 2.5×10^7 pfu/ml). (f) The schematic picture showing the difference in the polarization effect and capture profiles of bacteria (*E. coli* $\sim 1 \mu\text{m}$) and viruses on the FEM-simulated electric field profile on the tip of a nanoelectrode (200 nm in dia.). Adapted with permission from reference (22). Copyright 2013 Wiley-VCH.

Interestingly, a plot of the captured amount vs. the flow velocity showed a maximum at 0.73 mm/sec (see Figure 5b). This is in drastic contrast with the monotonic dependence on flow velocity in bacterial capture (see Figure 2b) (18). At $v < 0.73$ mm/sec, isolated bright spots were seen (Figure 5c), similar to bacteria capture. However, At $v \geq 0.73$ mm/sec, the snapshot images showed fractal-like

lightening patterns (Figure 5d). These patterns are called Lichtenberg figures and are commonly generated under conditions where a high electric field is produced at a sharp electrode surrounded by charged or polarizable materials as is the case here. The generation of such patterns requires a relatively high concentration of polarizable particles and so it was seen only when the particle flux was sufficiently high. Even though similar “pearl-chain-like” pattern was observed by Suehiro et al. in DEP trapping of *E. coli* cells between interdigitated microelectrodes (29), our previous DEP studies showed that only isolated *E. coli* cells were captured at the NEA (18, 30).

Most interestingly, the DEP kinetics dramatically changed when a very dilute solution of *Bacteriophage* T1 (8.7×10^4 pfu/mL) was passed through the nano-DEP device. At such a low concentration, the DEP capture was fully limited by mass transport. Figure 5e shows the different kinetic DEP capture curves when AC voltage is turned on and off with *Bacteriophage* T4r concentration at the normal concentration (5×10^9 pfu/ml) and two diluted concentrations (5.5×10^8 and 2.5×10^7 pfu/ml). At extremely low concentrations the captured virus particles can be precisely counted. From the experiment video at 0.87 mm/sec flow velocity, it was observed that 40 out of 67 particles were captured, giving a capture efficiency ~60%. This is very encouraging and can be further enhanced by fabricating elongated active NEA area across the full width of a straight microfluidic channel so all virus particles are forced to pass through the zone with strong electric field. With proper design, the NEA based DEP device may capture virus particles at concentrations potentially approaching 1-10 pfu/mL. By coupling with highly sensitive detection methods (such as surface enhanced Raman spectroscopy), it is very promising to develop an ultrasensitive portable microfluidic system for rapid viral pathogen detection. Figure 5f shows the differences in polarization between bacteria and viruses. Further, the detection of *Vaccinia* virus employed in nanostructured DEP device using impedance method. In-house stocks of *Vaccinia* virus (Copenhagen strain, VC-2) were amplified by standard viruses techniques of infecting HeLa cells knocked-down for an antiviral protein kinase, PKR (HeLa PKR-KD) followed by sucrose gradient centrifuge to achieve optimal yield of 2.0×10^8 pfu/mL. UV- inactivated vaccinia viruses were labeled with 50 μ M DiO lipophilic dye (Life Technologies, Carlsbad, CA) that stains the outer envelope of the virus by incubating the viruses at 37°C for 2 h. The nucleic acid (DNA) of the viruses was labeled with 50 μ l 20.0 μ M of Propidium Iodide (PI) aqueous solution. All the solutions were filtered and sterilized with 0.20 μ m at 121°C for 20 min.

The fluorescence detection of the *Vaccinia* viruses followed the same procedure as bacteriophage viruses except the fluorescence filter sets for Carl Zeiss FS plus upright fluorescence microscopes 485-20 nm excitation wavelength and an emission wavelength of 515-565 nm (filter set 17, Carl Zeiss) for DiO dye and an excitation wavelength of 640-20 nm and an emission wavelength of 690-50 nm (filter set 60, Carl Zeiss) for PI dye. The fluorescence videos were recorded at an exposure time set to 0.5 sec using multi-dimensional acquisition mode in the Axio-vision 4.7.1 release software (Carl Zeiss MicroImaging, Inc) for 85 sec. During which, no voltage (V_{off}) was applied in the initial ~16 sec, fixed AC voltage at different frequencies was applied (V_{on}) for ~54 sec, and no

voltage was applied (V_{off}) in the last ~ 15 sec. The response of *Vaccinia* Virus cells was monitored using a fluorescence microscope at different frequencies (f) of the sinusoidal AC voltage, at different flow velocities (v) and concentration of *Vaccinia* virus. The optimum flow velocity for *Vaccinia* virus was 0.40 mm/sec at the frequency of 1.0 Hz at the voltage of 8.0 V_{pp} . The difference between final impedance signal (Z_F) and the initial impedance signal (Z_O) and its ratio with initial impedance signal resulted in percentage change of impedance [$\% (Z_F - Z_O)/Z_O$]. To demonstrate the potential capability of the impedance method (Figure 6a), a concentration- dependent study from concentration $\sim 3 \times 10^3$ to 3×10^6 pfu/mL was employed to determine the limit of detection of $\sim 2.58 \times 10^3$ particles/ mL (20).

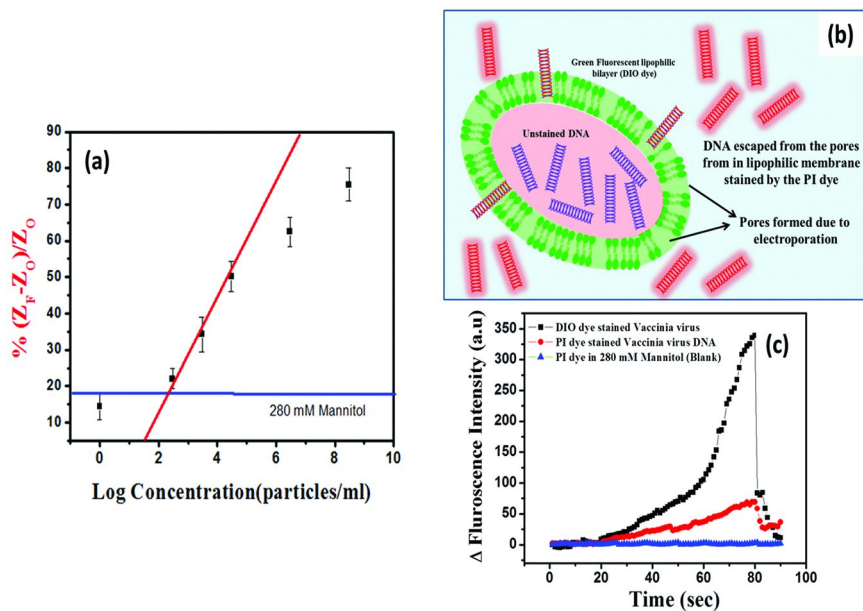


Figure 6. Impedance sensing of the *Vaccinia* viruses using VACNF NEA in a microfluidic device. (a) Calibration plot between the change in impedance after 54 sec at VACNF electrode and concentration variation from 3.0×10^8 pfu/mL to 3.0×10^3 pfu/mL. 250 mM Mannitol solution without viruses was a control sample. (b) The *Vaccinia* virus is stained with two fluorophores; DiO dye stains the outer lipophilic membrane and Propidium Iodide (PI) stains the DNA. (c) 3.0×10^6 pfu/mL under flow velocity of 0.401 mm/sec at 8.0 V_{pp} with 50.0 Hz, for 65 sec. During the high voltage application, the flow of the solution was stopped that assists in lysing the virus confirmed by the red fluorescence produced by Propidium Iodide (PI) dye intercalated with double-stranded DNA of the virus. 280 mM Mannitol was employed as a blank. Adapted with permission from reference (20).

Finally, electroporation of lipophilic membrane was observed when the viruses were exposed to high electric field on tips of VACNF. In electroporation experiments, Propidium Iodide (PI)--a membrane impermeable dye--stains the nucleic acid of *Vaccinia* virus. It was used with DiO dye that stains the lipophilic membrane of the virus. During the experiment, DiO dye-labeled viruses at a concentration of 3.010^6 pfu/mL in a solution containing PI dye were passed with a velocity of 0.401 mm/sec into a microfluidic chamber. The *Vaccinia* particles were captured in the active region when the voltage was turned to 8.0 V_{pp}, with the frequency of 50.0 Hz, and the flow velocity was reduced to 0.05 mm/sec for 60 sec. The high electric field assisted in breaking the lipophilic layer of the virus and extracting nucleic acid DNA into the solution. In Figure 6b, the green fluorescence from the DiO dye confirms the capture of the virus at 0.401 mm/sec, 8.0 V_{pp}. As the capture began, the extraction of DNA followed simultaneously, PI dye in solution intercalated with the extracted DNA increasing its fluorescence (~500 folds) giving out intense red fluorescence during the capture period. The control experiment of 50 μ l 20.0 μ M of Propidium Iodide (PI) in 280 mM mannitol solution showed absence of fluorescence in the microfluidic channel. When the bioparticles are exposed to the high electric field for longer periods of time, the pores become permanent, resulting in leaking of the nucleic acids. This is called irreversible electroporation and it is widely used for nucleic acid extraction (31–34).

Discussion

The DEP based on a VACNF NEA in microfluidic channel design acts as an effective and reversible electronic manipulation technique to rapidly concentrate bacteria and viruses into a micro-area from the solution flowing. We have seen clear differences in capture of bacteria and viruses due to the spatial distribution of the electrical field strength at the nanoelectrode tip as schematically illustrated in Figure 5f (16, 22). *Bacteriophage* T4r is similar in size as compared to the diameter of VACNF, causing virus to polarize to large extend. The captured virus acts as a extended tip attracting more viruses towards it. Bacteria *E.coli* has large size (~1 micron) causes the charges to dilute out within its structure. Hence no lightening patterns are seen with bacteria particles. A highly sensitive detection of bacteria, SERS nanotag based on QSY21 adsorbed on IO-Au NOV's provides greatly enhanced Raman signals and specific recognition to *E. coli* DH α 5 cell through highly selective immunochemical binding using two specific antibodies. The SERS signal measured with both of a confocal Raman microscope and a portable Raman system during DEP capture was fully validated with fluorescence measurements under all DEP conditions. This detection method yields a concentration detection limit of 210 CFU/mL using the portable Raman system. Finally, integration of nano-DEP system to electronically measure the electrical impedance of the viruses is efficient and cost-effective in the detection of virus concentrations in a sample. The high electric field at the tips of the VACNFs is capable to rupture the lipophilic membrane and extract the DNA. This device

with a dual function as a concentrator and DNA extractor can be a prospective device for future downstream processing and testing of biological samples.

Conclusion

A nano-DEP device based on a “points-and-lid” configuration of a nanoelectrode array against a macroscopic ITO counter electrode in a microfluidic channel has been designed, fabricated, and tested for pDEP capture of bacterial cells and viral particles. Reversible capture of both types of microbial particles was observed at high flow velocities. The device was successfully integrated with optical transducing techniques like fluorescence measurement, surface enhanced Raman spectroscopy, and electrochemical impedance sensing. Further application of nano-DEP device is to extract intracellular materials, such as DNA or proteins without lytic agent. All these studies revealed interesting interplay between the highly focused electric field at the nanoelectrode with bioparticles of comparable sizes. It is promising to develop such nano-DEP devices as an on-chip sample preparation module in a portable microfluidic system for rapid detection of microbes.

References

1. Fung, D. Y. C. Rapid Methods and Automation in Microbiology. *Compr. Rev. Food Sci. Food Saf.* **2002**, *1*, 3–22.
2. Lazcka, O.; Del Campo, F. J.; Munoz, F. X. Pathogen detection: A perspective of traditional methods and biosensors. *Biosens. Bioelectron.* **2007**, *22*, 1205–1217.
3. Hughes, M. P. Strategies for dielectrophoretic separation in laboratory-on-a-chip systems. *Electrophoresis* **2002**, *23*, 2569–2582.
4. Cabrera, C. R.; Yager, P. Continuous concentration of bacteria in a microfluidic flow cell using electrokinetic techniques. *Electrophoresis* **2001**, *22*, 355–362.
5. Sin, M. L. Y.; Mach, K. E.; Wong, P. K.; Liao, J. C. Advances and challenges in biosensor-based diagnosis of infectious diseases. *Expert Rev. Mol. Diagn.* **2014**, *14*, 225–244.
6. Pohl, H. A. *Dielectrophoresis: The Behavior of Neutral Matter in Non-uniform Electric Fields*; Cambridge University Press: New York, 1978.
7. Pohl, H. A. Nonuniform Field Effects in Poorly Conducting Media. *J. Electrochem. Soc.* **1960**, *107*, 386–390.
8. Markx, G. H.; Dyda, P. A.; Pethig, R. Dielectrophoretic separation of bacteria using a conductivity gradient. *J. Biotechnol.* **1996**, *51*, 175–180.
9. Voldman, J. Electrical forces for microscale cell manipulation. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 425–454.
10. Li, H.; Zheng, Y.; Akin, D.; Bashir, R. Characterization and Modeling of a microfluidic dielectrophoresis filter for biological species. *J. Microelectromech. Syst.* **2005**, *14*, 103–112.

11. Ermolina, I.; Milner, J.; Morgan, H. Dielectrophoretic investigation of plant virus particles: Cow Pea Mosaic Virus and Tobacco Mosaic Virus. *Electrophoresis* **2006**, *27*, 3939–3948.
12. Morgan, H.; Green, N. G. Dielectrophoretic manipulation of rod-shaped viral particles. *J. Electrostat.* **1997**, *42*, 279–293.
13. Akin, D.; Li, H.; Bashir, R. Real-Time Virus Trapping and Fluorescent Imaging in Microfluidic Devices. *Nano Lett.* **2003**, *4*, 257–259.
14. Li, J.; Ng, H. T.; Cassell, A.; Fan, W.; Chen, H.; Ye, Q.; Koehne, J.; Han, J.; Meyyappan, M. Carbon Nanotube Nanoelectrode Array for Ultrasensitive DNA Detection. *Nano Lett.* **2003**, *3*, 597–602.
15. Li, J.; Madiyar, F. R.; Swisher, L. In *Carbon Nanomaterials for Biomedical Applications*; Zhang, M., Naik, R. R., Dai, L., Eds.; Springer Series in Biomaterials Science and Engineering; Springer International Publishing: New York, 2016; Vol. 5, Chapter 4, pp 113–143.
16. Madiyar, F. R.; Syed, L. U.; Culbertson, C. T.; Li, J. Manipulation of Bacteriophages with Dielectrophoresis on Carbon Nanofiber Nanoelectrode Arrays. *Electrophoresis* **2013**, *34*, 1123–1130.
17. Arumugam, P. U.; Chen, H.; Siddiqui, S.; Weinrich, J. A. P.; Jejelowo, A.; Li, J.; Meyyappan, M. Wafer-scale fabrication of patterned carbon nanofiber nanoelectrode arrays: A route for development of multiplexed, ultrasensitive disposable biosensors. *Biosens. Bioelectron.* **2009**, *24*, 2818–2824.
18. Syed, L. U.; Liu, J.; Price, A. K.; Li, Y.-f.; Culbertson, C. T.; Li, J. Dielectrophoretic capture of *E. coli* cells at micropatterned nanoelectrode arrays. *Electrophoresis* **2011**, *32*, 2358–2365.
19. Madiyar, F. R.; Bhana, S.; Swisher, L. Z.; Culbertson, C. T.; Huang, X.; Li, J. Integration of a nanostructured dielectrophoretic device and a surface-enhanced Raman probe for highly sensitive rapid bacteria detection. *Nanoscale* **2015**, *7*, 3726–3736.
20. Madiyar, F. R. Nanoelectrode based devices for rapid pathogen detection and identification. Ph.D. Thesis, 2015.
21. Pohl, H. A. *Dielectrophoresis: The Behavior of Neutral Matter in Non-uniform Electric Fields*; 1978.
22. Madiyar, F. R.; Syed, L. U.; Arumugam P. U.; Li, J. In *Advances in Applied Nanotechnology for Agriculture*; Bosoon, P., Appell, M., Eds.; ACS Symposium Series 1143; American Chemical Society: Washington, DC, 2013; Chapter 6, pp 109–124.
23. Gray, D. S.; Tan, J. L.; Voldman, J.; Chen, C. S. Dielectrophoretic registration of living cells to a microelectrode array. *Biosens. Bioelectron.* **2004**, *19*, 1765–1774.
24. Bhana, S.; Rai, B. K.; Mishra, S. R.; Wang, Y.; Huang, X. Synthesis and properties of near infrared-absorbing magnetic-optical nanopins. *Nanoscale* **2012**, *4*, 4939–4942.
25. Bhana, S.; Chaffin, E.; Wang, Y.; Mishra, S. R.; Huang, X. Capture and detection of cancer cells in whole blood with magnetic–optical nanoovals. *Nanomedicine* **2013**, *9*, 593–606.

26. Dreaden, E. C.; Alkilany, A. M.; Huang, X.; Murphy, C. J.; El-Sayed, M. A. The golden age: gold nanoparticles for biomedicine. *Chem. Soc. Rev.* **2012**, *41*, 2740–2779.
27. Hughes, M. P.; Morgan, H.; Rixon, F. J. Dielectrophoretic manipulation and characterization of herpes simplex virus-1 capsids. *Eur. Biophys. J.* **2001**, *30*, 268–272.
28. Hughes, M. P.; Morgan, H.; Rixon, F. J.; Burt, J. P. H.; Pethig, R. Manipulation of herpes simplex virus type 1 by dielectrophoresis. *Biochim. Biophys. Acta, Gen. Subj.* **1998**, *1425*, 119–126.
29. Suehiro, J.; Yatsunami, R.; Hamada, R.; Hara, M. Quantitative estimation of biological cell concentration suspended in aqueous medium by using dielectrophoretic impedance measurement method. *J. Phys. D: Appl. Phys.* **1999**, *32*, 2814–2820.
30. Arumugam, P. U.; Chen, H.; Cassell, A. M.; Li, J. Dielectrophoretic Trapping of Single Bacteria at Carbon Nanofiber Nanoelectrode Arrays. *J. Phys. Chem. A* **2007**, *111*, 12772–12777.
31. Pakhomov, A. G.; Miklavcic, D.; Markov, M. S. *Advanced Electroporation Techniques in Biology and Medicine*; CRC Press: New York, 2010.
32. Chen, D.; Mauk, M.; Qiu, X.; Liu, C.; Kim, J.; Ramprasad, S.; Ongagna, S.; Abrams, W. R.; Malamud, D.; Corstjens, P.; Bau, H. H. An integrated, self-contained microfluidic cassette for isolation, amplification, and detection of nucleic acids. *Biomed Microdevices* **2010**, *12*, 705–719.
33. Bahi, M. M.; Tsaloglou, M. N.; Mowlem, M.; Morgan, H. Electroporation and lysis of marine microalga *Karenia brevis* for RNA extraction and amplification. *J. R. Soc. Interface.* **2011**, *8*, 601–608.
34. Kim, J.; Johnson, M.; Hill, P.; Gale, B. K. Microfluidic sample preparation: cell lysis and nucleic acid purification. *Integr. Biol.* **2009**, *1*, 574–586.

Chapter 12

Nanotechnology in Agriculture

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An overview is given of the application of nanotechnology to agriculture. This is an active field of R&D, where a large number of findings and innovations have been reported. For example, in soil management, applications reported include nanofertilizers, soil binders, water retention aids, and nutrient monitors. In plants, nanotechnology methods have been found to deliver DNA to plant cells, enhance nutrient absorption, detect plant pathogens, regulate plant hormones, and many other applications. In animal husbandry, nanocapsules have been devised to deliver vaccines and improve delivery of nutrients. Numerous postharvest applications have been reported, including the generation of nanocellulose from agriculture wastes, nanocomposites, silk, biochar, and nanosilver, among many others. It may be noted that most of the reported work in agricultural nanotechnology are in the developmental stages and not yet commercialized. Nonetheless, because of potential benefits, further progress in this field is expected in the future.

Introduction

Nanotechnology is a promising new development that has stimulated a lot of new ideas and innovations (1, 2). The key characteristics of this technology include the small size (1-100 nm) and the large surface-to-volume ratio of the nano-materials. It may be noted that a virus has a size of about 20-400 nm; a

globular protein with molecular weight of about 20-50 kDa has a minimum size of about 2 nm; the bond length of a C-C bond is about 0.15 nm. Thus, nanotechnology permits us to access very small dimensions, and in the process numerous new developments and applications have been found.

Throughout history, agriculture has always benefitted from innovations (3, 4). As the world's population continues to increase, the global production of food must keep up in order to obviate food shortages. Climate change, increased energy use, need for clean water, and environmental issues all contribute to the challenges. Thus, there is incentive to develop high-yielding, drought- and pest-resistant crops, and consumers are increasingly paying attention to nutrition and food safety. It is natural then to turn to nanotechnology to seek the means to improve farming or add value to agricultural products. Indeed, in the past 15 years, there have been a lot of R&D activities in agricultural nanotechnology. A large number of recent review articles are available (e.g., (5–12)).

An overview is provided in this article on some of the advances. Because of the wide scope of this topic, only an overview is given, with selective examples given to illustrate the potential of nanotechnology in agriculture. Because of space limitations, nanotechnology in food areas is not covered here.

Schemes for the Applications of Nanotechnology to Agriculture

For convenience, a schematic diagram is shown in Figure 1 that attempts to organize some of the applications of agricultural nanotechnology. In a coarse-grained analysis, one can categorize applications into five areas: 1) applications in soil management, 2) applications related to plant growth, health, and identification, 3) applications relating to animal husbandry, 4) postharvest applications, and 5) related areas, such as nanotechnology relating to water, energy, and food.

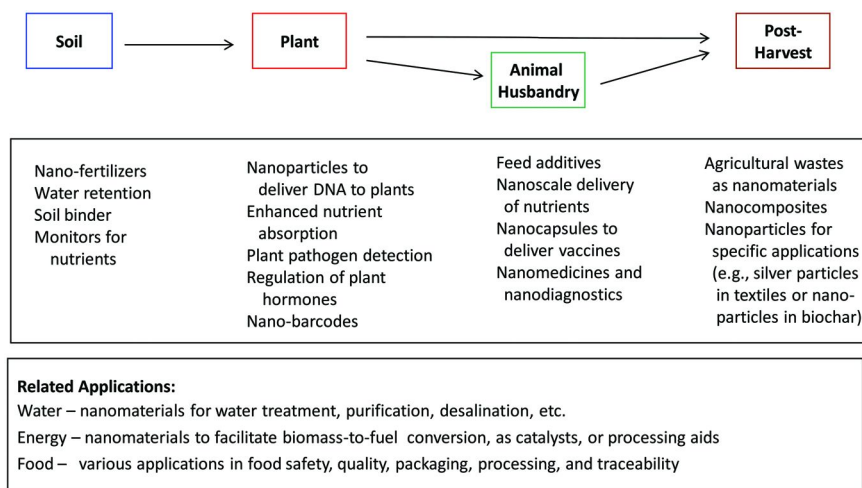


Figure 1. Simplified scheme for the application of nanotechnology to agriculture.

Table 1. Technology Types and Modes of Formation/Action for Selected Applications of Nanotechnology in Agriculture

1. Chemistry	
Nanoparticles (used as is, or in formulations)	- Gold nanoparticles (nanomedicines, nanodiagnostics) - Silver nanoparticles (antimicrobial, flame retardant) - Carbon nanotubes (fertilizer, agrochemicals) - Nanoclay (antimicrobial, filler for composites) - Metal oxides (e.g., TiO₂, ZrO, Al₂O₃, SiO₂ for disease control, fertilizer, agrochemical applications) - Biochar (sorption, filtration media, soil amendment) - Globular proteins (variety of uses)
Nanocapsules and nanoencapsulation	- improved delivery, increased stability, and/or controlled release of agrochemicals fertilizers, pheromones, and plant growth regulators
Nano-emulsions and suspensions	- increased solubility, potency, accessibility, and/or controlled release of active ingredient
Nano-gels	- encapsulation of active ingredient
2. Materials	
Nanocomposites used in ag/foods	- Nanoclay as fillers for food packaging film - Metal oxide fillers as antimicrobials
Use of agri-based materials in nanocomposites	- Starch and cellulosic derivatives as polymeric matrix - Nanocellulose as filler - Agri-based materials as compatibilizing agents
3. Nanobiotechnology	
Genetic engineering	- Identification of genes important to crop productivity, disease-related mutations, etc. - Targeted delivery of DNA, nanomodifiers, and phytomedicines to live cells
4. Nano-sensor Technology	
- Detection of agrochemicals, pathogens, moisture, pH, etc. (e.g., for precision agriculture)	
- Detection of environmental pollutants	
5. Information Technology	
- Nano-barcodes	
- sensing/global positioning for tracking	
- smart cards for plants and animals for identification and tracking	

A different analysis can be made considering the types of technologies involved and the different modes of formation or actions of the nanomaterials used in agriculture. This is summarized in Table 1. At least five technology areas can be discerned: 1) chemistry, 2) materials, 3) nanobiotechnology, 4) nanosensor technology, 5) information technology. Despite the categorization, the applications in agriculture are highly interdisciplinary and need the expertise of people in multiple disciplines in order to successfully bring them to the marketplace.

Selected Applications of Nanotechnology in Agriculture

In order to illustrate the potential of nanotechnology in agriculture, selected examples of applications are given in the following sections.

1. Bionanotechnology

There has been a lot of effort to map the whole genomes of plants (13–15). Through genetic engineering, the genes important to crop productivity, drought resistance and disease resistance can be identified and reengineered at the genetic and cellular levels (11).

Examples include the work of Torney et al. (16) who used 3-nm SiO₂ nanoparticles to deliver DNA and other chemicals into isolated tobacco and corn cells. Silica serves as a carrier for the DNA and the genes are inserted and activated without undesirable side effects. Other engineered silica techniques have also been devised to deliver DNA to tobacco and corn plants (17, 18). Kovalchuk et al. (19) used DNA/protein nano-complexes to deliver DNA to target monocot plant cell.

2. Nanosensors and Nano-Barcodes

Precision farming (20, 21) refers to the systems whereby the crop yield and usage of agrochemicals can be monitored through geospatial techniques and sensors. In this way, plant development, environmental conditions, fertilizer and agrochemical usage, and conditions involving water, soil and agro waste can be studied and optimized. The goal is to increase agricultural productivity with the use of these systems.

Nano-sensors can monitor crop growth and soil conditions, pathogens on crops and animals, usage and soil penetration of fertilizers, and environmental pollution (5, 22, 23). Biosensor development is part of the U.S. National Nanotechnology Initiative (24).

Nano-barcodes have been developed that can tag many pathogens that are detected through fluorescence (25). With this system an infection in a farm can be diagnosed and nano-barcodes can be used to trace the bacteria in a compost.

3. Enhanced Delivery of Active Ingredients to Plants

Nanotechnology can be employed to microencapsulate, emulsify, or coat agrochemicals or other active ingredients for enhanced delivery to the soil or to the plant (5, 6, 10, 26–28). The active ingredients may include herbicides, insecticides, fungicides, fertilizers, pheromones, and plant growth regulators. Nanocapsule formation or nanoencapsulation is particularly useful if the active ingredient is hydrophobic or requires controlled release.

4. Nanotechnology for Animals

As in the case of plants, similar delivery systems can be applied to treat infection or nutrient deficiency in livestock. Nanotechnology platforms have been incorporated into vaccine development. Different carriers, including liposomes, emulsions, polymer-based particles, and carbon-based nanomaterials have been attempted (29, 30). Theragnostics, which combine therapy and diagnostics through nanotechnology, may improve disease detection and treatment (31). Nanoscale delivery of nutrients in feed has been shown to improve the nutritional profiles of feed and feed efficiency (11).

In chickens, coatings have been made to incorporate TiO₂. With light and humidity, TiO₂ oxidizes, kills the bacteria in the feed, and thereby cleans the coating surface (32). Another application is the use of modified nanoclays to mitigate the effects of aflatoxin on chickens (33).

5. Water/Liquid Retention or Purification

In farming a desirable goal is to retain water in the soil. Zeolite is a natural wetting agent and it assists in keeping water in sandy soils and increase porosity in clay soils (34). This is especially useful in drought-prone areas.

Water purification can be achieved through the use of nanomaterials, which can remove toxins and other undesirable chemicals (35–38). Examples of nanomaterials used include nanoscale metal oxides, zeolites, carbon nanotubes, and biochar. Undesirable chemicals include agrochemical residues, metallic ions, arsenic, viruses, bacteria, and protozoan cysts.

6. Postharvest Applications

Post-harvest applications represent a huge opportunity for nanotechnology in agriculture. The research in these areas is diverse and often requires detailed expertise in the application areas being considered. For example, there is a lot of interest in converting agricultural byproducts and waste materials into value-added products. The reason for this interest includes sustainability and the desire to decrease the dependence on fossil derived raw materials. A good example in the context of this article is nanocellulose, which can be produced from a variety of agri-based sources (39–41). Nanocellulose has been found to be useful as high-performance filters, aerogels, thickeners, flavor carriers and suspension stabilizers, among many other applications.

Nanocomposites constitute another large application area (42). One approach is to use a biopolymer and add nanofillers(s) in order to improve its end-use properties (43, 44). The biopolymer may include carbohydrates, proteins, and polymers from microbial fermentation or synthesis. The fillers may include clay, silicates, metal, metal oxide, or other biopolymers. The target end-point may be improved mechanical, barrier, antimicrobial, or other properties. Another approach is to use agri-based materials as fillers. Notable examples are cellulose, starch and chitosan nanoparticles (45–47). Nanofibers from wheat straw and soy hulls have also been produced to make bio-nanocomposites (48). Agri-based materials, sometimes with modification, can also be used as compatibilizing agents in composites (49, 50).

Biochar is the carbonaceous material obtained by heating in a limited oxygen environment an agri-based raw material. It can be used as catalyst, composite filler, and filtration, water treatment, or soil conditioning agent (51–53). Biochar was shown to contain carbon nanoparticles, and they seemed to enhance the growth rate of wheat plants (54). Biochar nanoparticles were also obtained from rice peel via the hydrothermal carbonization process (55). The structure of biochar have been shown to contain nano-pores that may aid in the storage of hydrogen (56).

For safety purposes, many articles of clothing need the addition of flame retardants. Silver nanoparticles have been shown to be effective flame retardants for cotton and wool (57, 58).

Another good example of an agri-based natural material is silk. In a series of detailed studies, Asakura et al (59, 60) have characterized the chemical and physical structures of silk, and genetically improved its fiber properties. Nanofibers have been produced which show promise as useful biomaterials in a number of medical and surgical applications (61, 62).

Issues in Commercialization

From the above examples, it is obvious that there are many opportunities for nanotechnology in agriculture. There has been an increasing number of patents in this area. However, relative to food science and other areas, most of the nanotechnologies in agriculture are still in the research and development stage (11, 63). The major issue seems to be the perceived cost-versus-benefit of the nano products. These products tend to require high production costs, which can only be economically viable when produced in high volumes. Coupled with uncertainties in legislative actions and public opinion, there are distinct commercialization challenges (63).

Another major issue is the safety aspect of nano products. As indicated in the chapter by Philbert Martin (64) and others (7–12, 65), nanoparticles may infiltrate the human body and perhaps evade the body's defense mechanisms because of their size. The impact of nanomaterials on the environment also cannot be taken for granted (12, 65). It is important then to pay attention to the potential toxicity of nanoparticles and the regulatory requirements in the process of commercialization.

Conclusions

Nanotechnology poses both opportunities and challenges for agriculture. One of the goals of agricultural nanotechnology is to decrease the levels of active ingredients used in agrochemical products and applications (e.g., through improved delivery, stability, or controlled release of the ingredients), optimize fertilizer use, and increase crop yield through genetic engineering and/or improved water and nutrient intake. For livestock, nanotechnology permits enhanced feed formulations, improved vaccines, diagnostics, and medications. The opportunities for post-harvest uses are plentiful and diverse; many of the applications are specialized and require detailed knowledge and expertise in the topics under consideration.

The full potential of nanotechnology in agriculture remains to be seen as most of the technologies are not yet commercialized. Cost of production, safety, consumer acceptance, government regulations and intellectual property are issues to be considered. Nevertheless, this is a very promising field, and in view of continuing interest and activities, further progress is expected in the future.

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References

1. Ramsden, J. *Nanotechnology: An Introduction*; Elsevier: Oxford, U.K., 2011.
2. Sparks, S. *Nanotechnology: Business Applications and Commercialization*; CRC Press: Boca Raton, FL, 2012.
3. The Robinson Library. *A Timeline of Agricultural Development*; 2015; <http://www.robinsonlibrary.com/agriculture/agriculture/history/timeline.htm> (accessed on 1/2/15).
4. Clancy, H. *Eleven innovations to fight food and water scarcity*; 2014; <http://www.greenbiz.com/blog/2014/02/25/new-report-emerging-agriculture-technology> (accessed on 1/2/15).
5. Sekhon, B. S. *Nanotechnol. Sci. Appl.* **2014**, 4, 31–53.
6. Kumari, A.; Yadav, S. K. *Crit. Rev. Food Sci. Nutr.* **2014**, 54, 975–984.
7. Mukhopadhyay, S. S. *Nanotechnol. Sci. Appl.* **2014**, 7, 63–71.
8. Prasad, R.; Kumar, V.; Prasad, K. S. *Afr. J. Biotechnol.* **2014**, 13, 705–713.
9. Misra, A. N.; Misra, M.; Singh, R. *Int. J. Pure Appl. Sci. Technol.* **2013**, 16, 1–9.
10. Rai, M.; Ingle, A. *Appl. Microbiol. Biotechnol.* **2012**, 94, 287–293.
11. Chen, H.; Yada, R. *Trends Food Sci. Technol.* **2011**, 22, 585–594.

12. Hong, J.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. *ACS Symp. Ser.* **2013**, *1124*, 73–90.
13. Appels, R.; Nystrom-Persson, J.; Keeble-Gagnere, G. *Funct. Integr. Genomics* **2014**, *14*, 1–9.
14. Michael, T. P.; Jackson, S. *Plant Genome* **2013**, *6*, 1–7.
15. Young, N. D. *Phytopathology* **1996**, *34*, 479–501.
16. Torney, F.; Trewyn, B. G.; Lin, V. S. Y.; Wang, K. *Nat. Nanotechnol.* **2007**, *2*, 295–300.
17. Park, I. Y.; Kim, I. Y.; Yoo, M. K.; Choi, Y. J.; Cho, M. H.; Cho, C. S. *Int. J. Pharm.* **2008**, *359*, 280–287.
18. Galbraith, D. W. *Nat. Nanotechnol.* **2007**, *2*, 272–273.
19. Kovalchuk, I.; Ziemienowicz, A.; Eudes, F. U.S. Patent Application 20120070900 A1. Mar 22, 2012.
20. Krishna, K. R. *Precision Farming: Soil Fertility and Productivity Aspects*; CRC Press: Boca Raton, FL, 2013.
21. Zhang, C.; Kovacs, J. M. *Precis. Agric.* **2012**, *13*, 693–712.
22. Rai, V.; Acharya, S.; Dey, N. *J. Biomater. Nanobiotechnol.* **2012**, *3*, 315–324.
23. Vamvakiki, V.; Chaniotakis, N. A. *Biosens. Bioelectron.* **2007**, *22*, 2848–2853.
24. Fadel, T. R.; Meador, M. A. The Role of Chemical Sciences in the National Nanotechnology Initiative: Accomplishments and Future Direction. *Nanotechnology: Delivering on the Promise Volume 1*; ACS Symposium Series 1220; American Chemical Society: Washington, DC, 2016; Chapter 3.
25. Li, Y.; Cu, Y. T.; Luo, D. *Nat. Biotechnol.* **2005**, *23*, 885–889.
26. Sasson, Y.; Levy-Ruso, G.; Toledano, O.; Ishaaya, I. Nanosuspensions: emerging novel agrochemical formulations. In *Insecticides Design Using Advanced Technologies*; Ishaaya, I., Nauen, R., Horowitz, A. R., Eds.; Springer-Verlag: Berlin & Heidelberg, 2007; pp 1–39.
27. Johnston, C. T. *Clay Miner.* **2010**, *45*, 245–279.
28. Das, M.; Saxena, N.; Dwivedi, P. D. *Nanotoxicology* **2009**, *3*, 10–18.
29. Kim, M.; Park, J. Y.; Shon, Y.; Kim, G.; Shim, G.; Oh, Y. *Asian J. Pharm. Sci.* **2014**, *9*, 227–235.
30. Nasir, A. *J. Invest. Dermatol.* **2009**, *129*, 1055–1059.
31. Morris, K. *Lancet Infect. Dis.* **2009**, *9*, 215.
32. Clements, M. Pullet production gets silver lining; *Poultry International*, April 2009.
33. Shi, Y. H.; Xu, Z. R.; Feng, J. L.; Wang, C. Z. *Anim. Feed Sci. Technol.* **2006**, *129*, 138–148.
34. Geohumus, GmbH. *Think and invent global, produce and act local*; 2016; <http://www.geohumus.com/en/> (accessed 1/5/16).
35. McMurray, T. A.; Dunlop, P. S. M.; Byrne, J. A. *J. Photochem. Photobiol., A* **2006**, *182*, 43–51.
36. Li, Q.; Wu, P.; Shang, J. K. In *Nanotechnology Applications for Clean Water*; Savage, N., Diallo, M., Duncan, J., Street, A., Sustich, R., Eds.; Elsevier: New York, 2009; pp 17–37.

37. Farmen, L. In *Nanotechnology Applications for Clean Water*; Savage, N., Diallo, M., Duncan, J., Street, A., Sustich, R., Eds.; Elsevier: New York, 2009; pp 115–130.
38. Diallo, M. In *Nanotechnology Applications for Clean Water*; Savage, N., Diallo, M., Duncan, J., Street, A., Sustich, R., Eds.; Elsevier: New York, 2009; pp 143–155.
39. Habibi, Y.; Lucia, L. A.; Rojas, O. J. *Chem. Rev.* **2010**, *110*, 3479–3500.
40. Brinchi, L.; Cotana, F.; Fortunati, E.; Kenny, J. M. *Carbohydr. Polym.* **2013**, *94*, 154–169.
41. Li, M. C.; Wu, Q.; Song, K.; Qing, Y.; Wu, Y. *ACS Appl. Mater. Interfaces* **2015**, *7*, 5006–5016.
42. *Eco-friendly Polymer Nanocomposites: Processing and Properties*; Thakur, V. K., Thakur, M. K., Eds.; Springer: New Delhi, 2015.
43. Othman, S. H. *Agric. Agric. Sci. Procedia* **2014**, *2*, 296–303.
44. Mihindukulasuriya, S. D. F.; Lim, L. *Trends Food Sci. Technol.* **2014**, *40*, 149–167.
45. Khalil, H. P. S. A.; Bhat, A. H.; Yusra, A. F. I. *Carbohydr. Polym.* **2012**, *87*, 963–979.
46. Rhim, J.-W.; Hong, S.-I.; Park, H.-M.; Ng, P. K. W. *J. Agric. Food Chem.* **2006**, *54*, 5814.
47. Lin, N.; Huang, J.; Chang, P. R.; Anderson, D. P.; Yu, J. J. *Nanomater.* **2011**, Article ID 573687; <http://dx.doi.org/10.1155/2011/573687> (accessed 1/6/16).
48. Alemdar, A.; Sain, M. *Bioresour. Technol.* **2008**, *99*, 1664–1671.
49. Imre, B.; Pukánszky, B. *Eur. Polym. J.* **2013**, *49*, 1215–1233.
50. Islam, M. T.; Alam, M. M.; Zoccola, M. *Int. J. Innovative Res. Sci. Eng. Technol.* **2013**, *2*, 5444–5451.
51. Peterson, S. C.; Jackson, M. A.; Appell, M. *ACS Symp. Ser.* **2013**, *1143*, 193–205.
52. Klasson, K. T.; Boihem, L. M., Jr.; Uchimiya, M.; Lima, I. M. *Fuel Process. Technol.* **2014**, *123*, 27–33.
53. Cheng, H. N.; Wartelle, L. H.; Klasson, K. T.; Edwards, J. C. *Carbon* **2010**, *48*, 2455–2469.
54. Saxena, M.; Maitya, S.; Sarkar, S. *RSC Adv.* **2014**, *4*, 39948–39954.
55. Bradl, H. B.; Bottlinger, M. Abstract in 3rd International Conference on Industrial and Hazardous Waste Management (Crete 2012); <http://www.srcosmos.gr/srcosmos/showpub.aspx?aa=16540> (accessed 1/5/15).
56. Klasson, K. T.; Uchimiya, M.; Lima, I. M. *Ind. Crops Prod.* **2015**, *67*, 33–40.
57. Osório, I.; Igreja, R.; Franco, R.; Cortez, J. *Mater. Lett.* **2012**, *75*, 200–203.
58. Nam, S.; Condon, B. D. *Cellulose* **2014**, *21*, 2963–2972.
59. Zhu, Z.; Kikuchi, Y.; Kojima, K.; Tamura, T.; Kuwabara, N.; Nakamura, T.; Asakura, T. *J. Biomater. Sci. Polym. Ed.* **2010**, *21*, 395–411.
60. Ohgo, K.; Zhao, C.; Kobayashi, M.; Asakura, T. *Polymer* **2003**, *44*, 841–846.
61. Miyamoto, S.; Koyanagi, R.; Nakazawa, Y.; Nagano, A.; Abiko, Y.; Inada, M.; Miyaura, C.; Asakura, T. *J. Biosci. Bioeng.* **2013**, *115*, 575–578.
62. Yagi, T.; Sato, M.; Nakazawa, Y.; Tanaka, K.; Sata, M.; Itoh, K.; Takagi, Y.; Asakura, T. *J. Artif. Organs* **2011**, *14*, 89–99.

63. Parisi, C.; Vigani, M.; Rodriguez-Cerezo, E. *Nano Today* **2015**, *10*, 124–127.
64. Philbert, M. A. Nanomaterials: Promise in Balance with Safety. *Nanotechnology: Delivering on the Promise Volume 1*; ACS Symposium Series 1220; American Chemical Society: Washington, DC, 2016; Chapter 10.
65. Maysinger, D. *Org. Biomol. Chem.* **2007**, *5*, 2335–2342.

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Charles L. Geraci, (Ph.D., Michigan State University) is Associate Director for Nanotechnology, National Institute for Occupational Safety and Health (NIOSH). He has 40 years of Industrial Hygiene practice experience, including Director of a regional laboratory for DataChem Laboratories; Associate Director of HS&E at the Procter & Gamble Company; and Director of Industrial Hygiene for two consulting firms. He is certified by the American Board of Industrial Hygiene in both the Comprehensive Practice and the Chemical Aspects of Industrial Hygiene and is a Fellow of the American Industrial Hygiene Association. He provides overall coordination and strategic guidance to the nanotechnology research program at NIOSH and collaborates internationally with other country programs on various aspects of nanotechnology workplace safety and health. He directly manages a number of NIOSH nanotechnology projects that focus on the development and dissemination of workplace risk management guidelines, including the document “Approaches to Safe Nanotechnology.” Dr. Geraci sponsors the NIOSH nanotechnology field team that is conducting visits to nanomaterial producers and users to characterize exposures, evaluate controls, and develop best practices.

Diane Grob Schmidt

Diane Grob Schmidt (Ph.D., University of Cincinnati), the 2015 ACS President, was an Executive at The Procter & Gamble Company, where she served as a R&D Section Head for 17 years. Her P&G career spanned 1981-2014 during which she played key roles in such brands as Tide®, Head & Shoulders®, Pert Plus® and Safeguard®. Dr. Schmidt had responsibility for health, safety and environment, and regulatory affairs before retiring from P&G in 2014. She is currently an Adjunct Professor in the Department of Chemistry at the University of Cincinnati. She is the inventor or co-inventor on myriad patents, in addition to author or co-author on chemistry publications in refereed journals. She has received many awards, including ACS Fellow, Fellow of the Division of Chemical Health & Safety, Henry Hill Award, and Distinguished Scientist of Cincinnati (first woman so honored). She has served on the editorial boards of the Journal of the Society of Cosmetic Chemists and the Journal of Chemical Health & Safety. She has been an ACS member since 1968 and held a wide variety of leadership positions, including three consecutive terms on the Board of Directors. As 2015 ACS President, her Presidential theme was “Inspiring and Innovating for Tomorrow”. Her legacy as ACS President includes: U.S. and Global Grand Challenges via impactful programming (Nanotechnology, Energy, BRAIN Initiative/Chemical Measurements/Chemical Neurotransmission), championing the establishment of the American Association of Chemistry Teachers (AACT), advocacy on behalf of ACS members and focus on industry and industrial ACS members.

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