

Supporting information

**Activatable Hybrid Polyphosphazene-AuNP Nanoprobe for ROS Detection
by Bimodal PA/CT Imaging**

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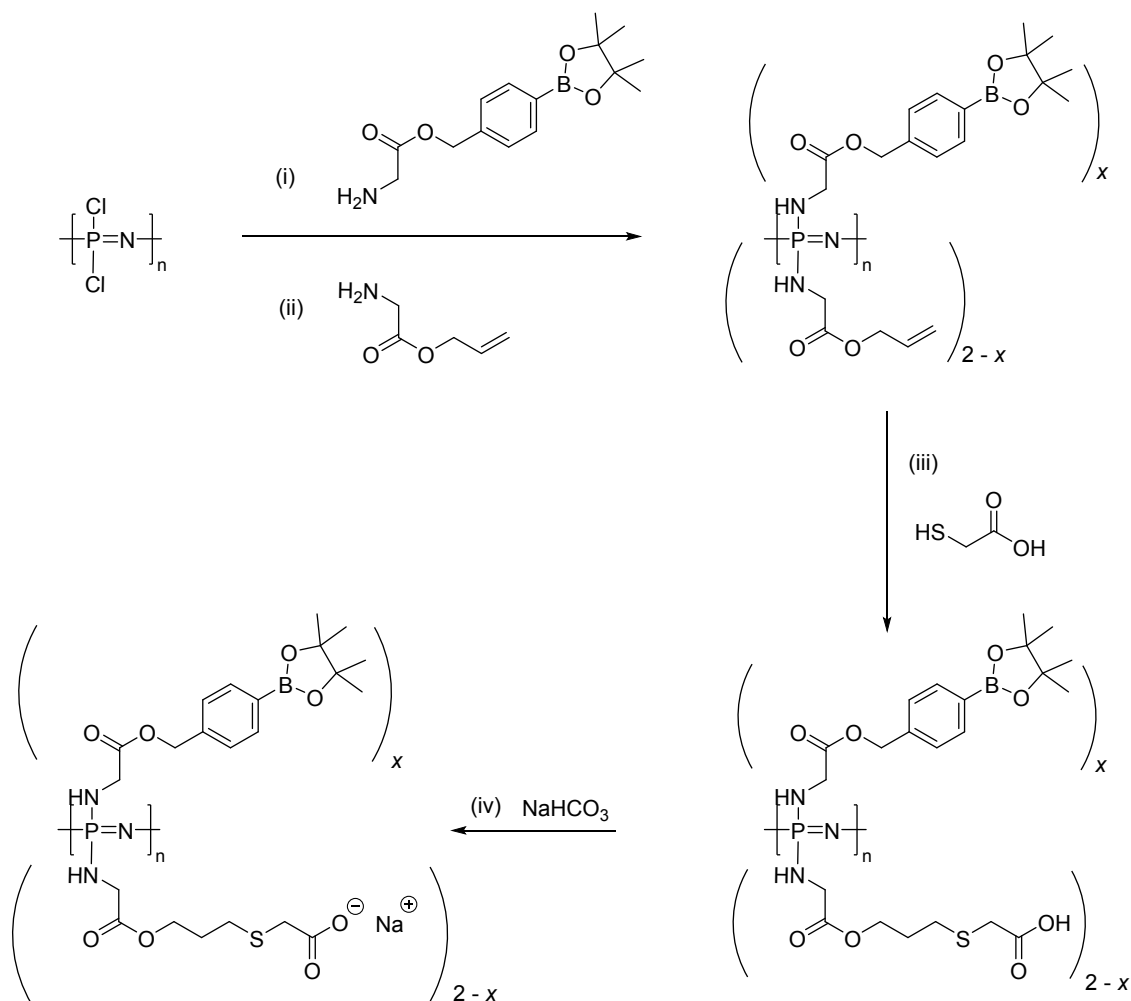
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1. Polymer synthesis experimental details



Scheme S1. Synthesis of the (arylboronate)polyphosphazene PPB in 4 steps. Reagents and conditions: (i) glycinate arylboronic acid pinacol ester, THF, Et_3N , rt, 24h; (ii) Excess of allyl glycinate, THF, Et_3N , rt, 24h; (iii) thioglycolic acid, 7 wt% DMPA, MeOH, 5 °C, 3h; (iv) NaHCO_3 solution. $X \approx 1$.

1.1 Materials

All synthetic procedures were carried out air-free either under nitrogen using Schlenk line techniques or under argon in a glovebox (MBRAUN). The glassware was dried in an oven at 120°C prior use. The monomer trichlorophosphoranimine ($\text{Cl}_3\text{P}=\text{NSiMe}_3$) was synthesized as

reported previously.¹ Boc-gly-arylboronic acid pinacol ester was synthesized according to previous work.² Boc-gly-allyl was synthesized by previously reported method.³ Dichlorotriphenylphosphorane, lithium bis(trimethylsilyl)amide, phosphorus trichloride, sulfonyl chloride, allyl bromide, potassium carbonate, N,N'-dicyclohexylcarbodiimide, 4-(dimethylamino)pyridine (DMAP), 2,2-dimethoxy-2-phenylacetophenone (DMPA), and thioglycolic acid were purchased from Sigma (Vienna, AT). N-(tert-Butoxycarbonyl)glycine, calcium chloride dihydrate and trimethylamine (Et₃N) were acquired from Merck, 4-(Hydroxymethyl)phenylboronic acid pinacol ester from Flurochem and trifluoroacetic acid (CF₃CO₂H) from TCI Europe. All solvents were obtained from VWR Europe. The photochemical reaction was carried out in a glass vial in a Rayonet Chamber Reactor with UV lamp from Camag centered at 254 nm and the solvent was purged with N₂ for at least 30 min prior use.

Poly(dichloro)phosphazene (Scheme S1) was synthesized solvent-free similar to previously reported procedure.¹ Dichlorotriphenylphosphorane (4.00 mg, 12.00 mmol, 1 eq.) was mixed with Cl₃P=NSiMe₃ (1.50 g, 6.67 mmol, 556 eq.) and stirred for 24 h until a viscous solution was obtained and a single peak in the ³¹P NMR spectroscopy at -18.2 ppm was observed. Meantime, boc-gly-arylboronic acid pinacol ester (2.61 g, 6.67 mmol, 1 eq.) was deprotected in CF₃CO₂H/DCM (1:3) overnight at room temperature. The solvent was removed under vacuum and toluene was added three times to remove the excess of CF₃CO₂H by coevaporation. The glycinate arylboronic acid pinacol ester was obtained as a white solid and used without further purification in the post-polymerization step. In the glove box, it was redissolved in 50 mL anhydrous THF and an excess of Et₃N (~1.5 mL, 1.6 eq.) was added to neutralize possible CF₃CO₂H residues. Then the poly(dichloro)phosphazene (6.67 mmol, 1 eq.) solution was transferred to the previous solution and stirred at room temperature for 24 h. Meanwhile, boc-gly-allyl was deprotected following the same procedure. Excess of boc-gly-

allyl (2.59 g, 12.03 mmol, 1.8 eq.) was deprotected in $\text{CF}_3\text{CO}_2\text{H}/\text{DCM}$ (1:3) overnight at room temperature. The solvent was removed under vacuum and toluene was added three times to remove the excess of $\text{CF}_3\text{CO}_2\text{H}$. The allyl glycinolate was obtained as a white solid and re-dissolved in anhydrous THF (~50 mL) and excess of Et_3N (~2 mL, 2.1 eq.). The allyl glycinolate solution was added dropwise to the partially substituted polymer and stirred at room temperature for further 24 h. Then, the suspension was filtered to remove the insoluble triethylammonium chloride salt and the solvent was concentrated under vacuum. The polymer was purified by dialysis (3.5 kDa cut-off) in H_2O for 30 min and then in EtOH overnight. The solvent was then removed under vacuum to yield an orange sticky product. Yield = 1.70 g (63%). ^1H NMR (300 MHz, CD_3OD , δ): 7.67 (br, 2H, Ar-H), 7.30 (br, 2H, Ar-H), 5.86 (br, 1H, $\text{CH}_2\text{-CH}=\text{CH}_2$), 5.17 (br, 4H, $\text{CH}_2\text{-CH}=\text{CH}_2$, $\text{COO-CH}_2\text{-Ar}$), 4.54 (br, 2H, $\text{OOC-CH}_2\text{-CH}=\text{CH}_2$), 3.83 (br, 4H, $\text{OOC-CH}_2\text{-NH}$), 1.29 (br, 12H, O-C-CH_3) ppm. ^{31}P NMR (121 MHz, CD_3OD , δ): 2.10 ppm. GPC $M_w = 340\,000$, $M_n = 100\,600$.

This (arylboronate)polyphosphazene polymer (228 mg, 0.51 mmol, 1 eq.), thioglycolic acid (93.56 mg, 70 mL, 1.01 mmol, 2 eq. per allyl group), and DMPA (22.66 mg, 7 wt%) were dissolved in MeOH, which was purged with N_2 previously for at least 30 min. The reaction was carried out under UV light at 5°C for 3 h. After the reaction, the polymer was purified by precipitation in EtOAc (to remove DMPA). Yield = 121 mg (44%). ^1H NMR (300 MHz, CD_3OD , δ): 7.69 (br, 2H, Ar-H), 7.23 (br, 2H, Ar-H), 5.04 (br, 2H, $\text{COO-CH}_2\text{-Ar}$), 4.11-3.86 (br, 6H, $\text{COO-CH}_2\text{-CH}_2\text{-CH}_2\text{-S}$, 2x $\text{OOC-CH}_2\text{-NH}$), 3.15 (br, 2H, $\text{CH}_2\text{-S-CH}_2\text{-COOH}$), 2.65 (br, 2H, $\text{COO-CH}_2\text{-CH}_2\text{-CH}_2\text{-S}$), 1.86 (br, 2H, $\text{COO-CH}_2\text{-CH}_2\text{-CH}_2\text{-S}$), 1.28 (br, 12H, (O-C-CH_3)₂) ppm.

121 mg (0.22 mmol, 1 eq.) of the carboxylic acid functionalized polymer was suspended in NaHCO₃ solution (19 mg, 0.22 mmol, 1 eq., 5 mL H₂O). The suspension was rigorously stirred until its total dissolution and then the polymer was lyophilised. Yield = quantitative. ¹H NMR (300 MHz, D₂O, δ): 7.62 (br, 2H, Ar-H), 7.14 (br, 2H, Ar-H), ~4.93 (br^{*a}, COO-CH₂-Ar), 3.99-3.60 (br, 6H, COO-CH₂-CH₂-CH₂-S, 2x OOC-CH₂-NH₂), 3.09 (br, 2H, CH₂-S-CH₂-COOH), 2.43 (br, 2H, COO-CH₂-CH₂-CH₂-S), 1.72 (br, 2H, COO-CH₂-CH₂-CH₂-S), 1.18 (br, 1H^{*b}, (O C-(CH₃)₂)₂) ppm. ³¹P NMR (121 MHz, D₂O, δ): 2.60 ppm. ^{*a} Due to the overlapping of the D₂O signal with this region, there is some uncertainty in the integration. For this reason, it has been measured also in CD₃OD. ¹H NMR (300 MHz, CD₃OD, δ): 7.64 (br, 2H, Ar-H), 7.29 (br, 2H, Ar-H), 5.10 (br, 2H, COO-CH₂-Ar), 4.14-3.81 (br, 6H, COO-CH₂-CH₂-CH₂-S, 2x OOC-CH₂-NH₂), 3.13 (br, 2H, CH₂-S-CH₂-COOH), 2.60 (br, 2H, COO-CH₂-CH₂-CH₂-S), 1.86 (br, 2H, COO-CH₂-CH₂-CH₂-S), 1.29 (br, 1H^{*b}, (O C-(CH₃)₂)₂) ppm. ³¹P NMR (121 MHz, CD₃OD, δ): 2.29 ppm. ^{*b} Due to the hydrolysis of the boronic acid ester in water to cleave the pinacol group.

2. Physico-chemical characterization of PPB NP and PCPP NP

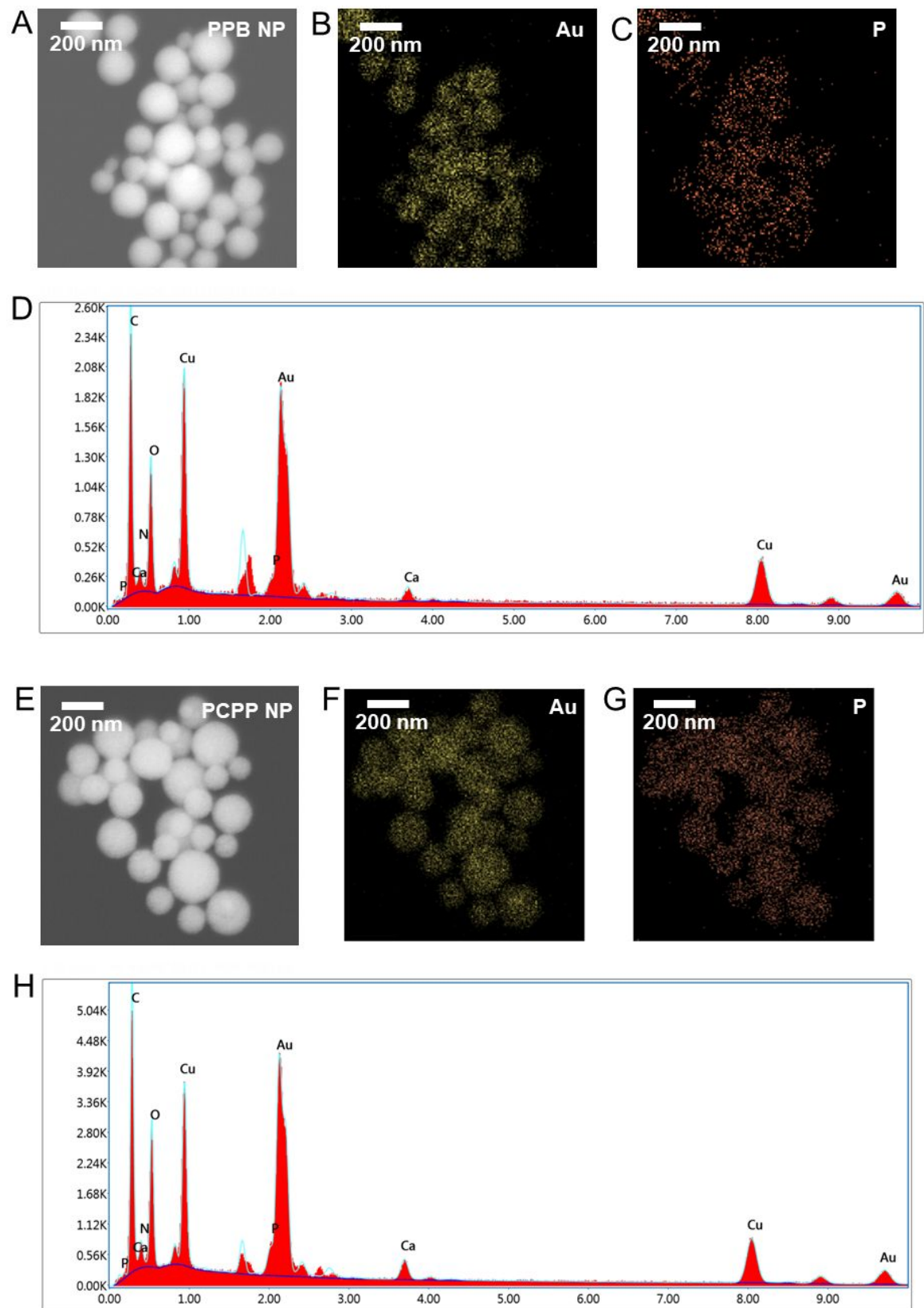


Figure S1. Characterization of PPB NP by A) SEM, B-C) elemental mapping of Au (*green*) and P (*orange*) respectively, D) EDX. Characterization of PCPP NP by E) SEM, F-G) elemental mapping of Au (*green*) and P (*orange*) respectively, H) EDX.

3. Degradability of PPB NP by ROS species

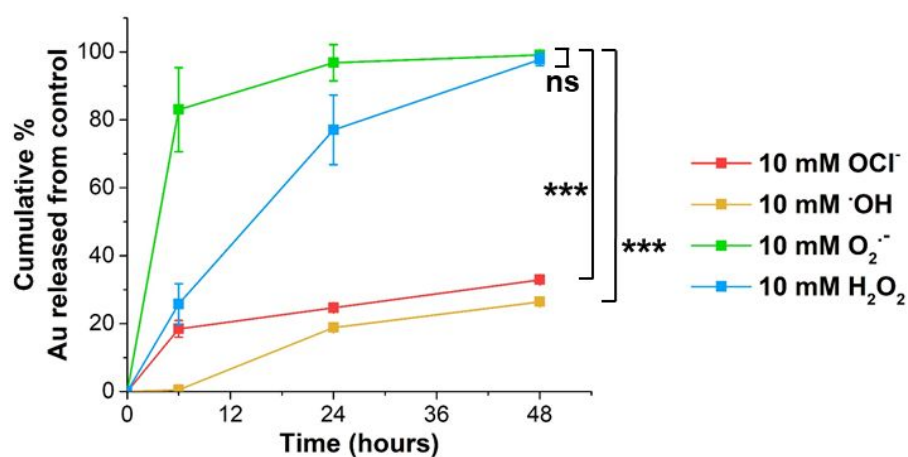


Figure S2. Effect of different ROS species of PPB NP degradation determined by cumulative gold released. *** and 'ns' indicate either a statistically significant difference at $p < 0.001$ or non-statistically significant differences, respectively.

4. Contrast enhancement in CT and PA imaging

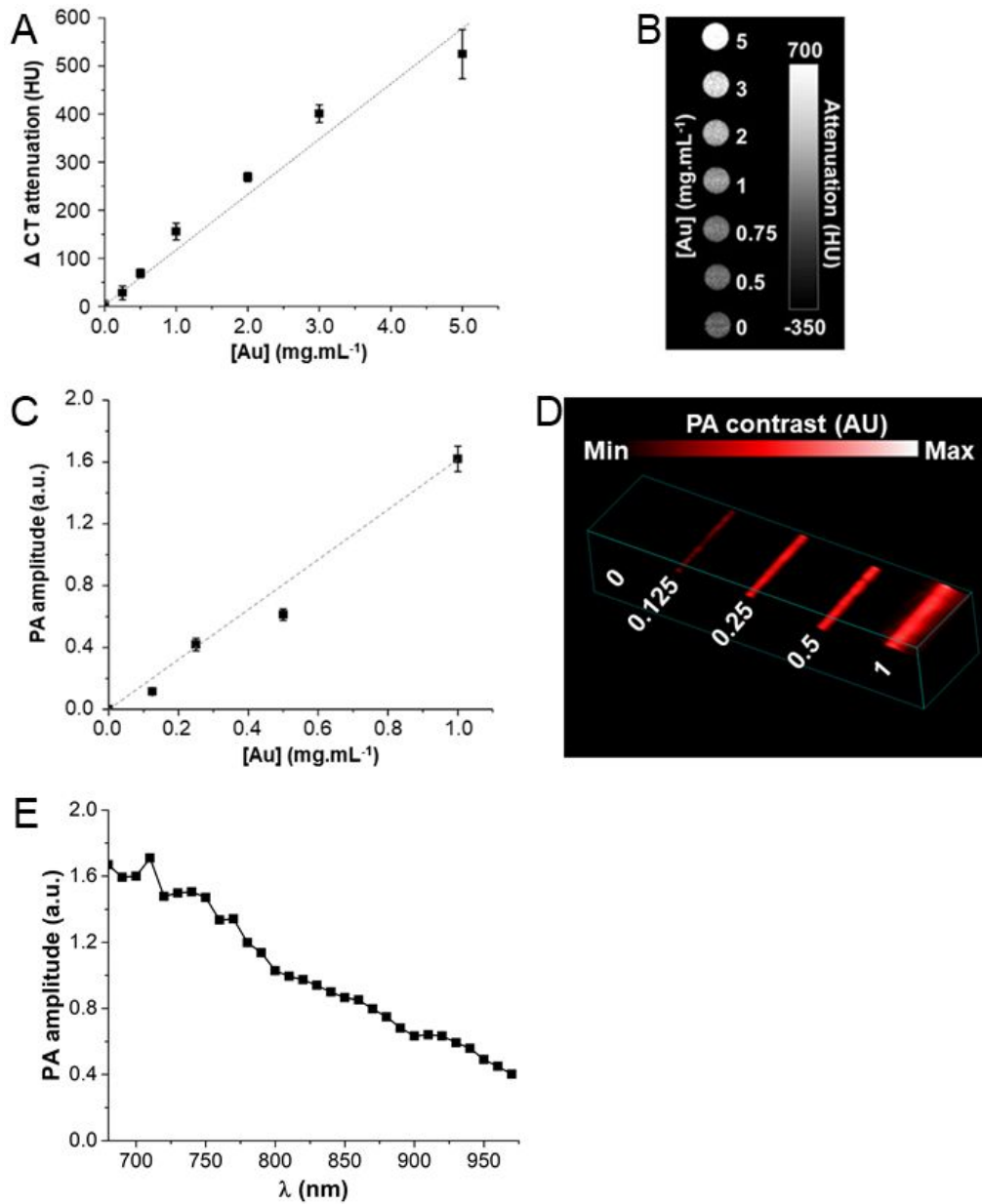


Figure S3. Contrast enhancement provided by the ROS sensitive nanoparticles at various gold concentrations in A-B) CT and C-D) PA imaging. E) PA signal at laser wavelengths from 680 to 970 nm.

5. H₂O₂ dose response of PPB NP

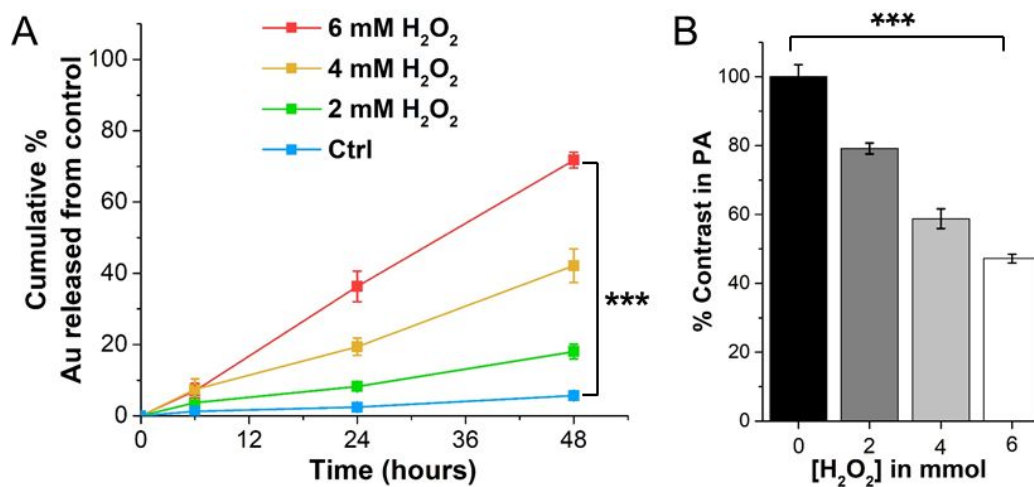


Figure S4. A) Cumulative gold released determined by ICP-OES showing the H₂O₂ dose dependent degradation of PPB NP. B) Percentage of contrast in PA depending on the dose of H₂O₂. Experiments done at a gold concentration of 1 mg.mL⁻¹. *** indicates a statistically significant difference at $p < 0.001$.

6. Effect of PPB NP on cell viability

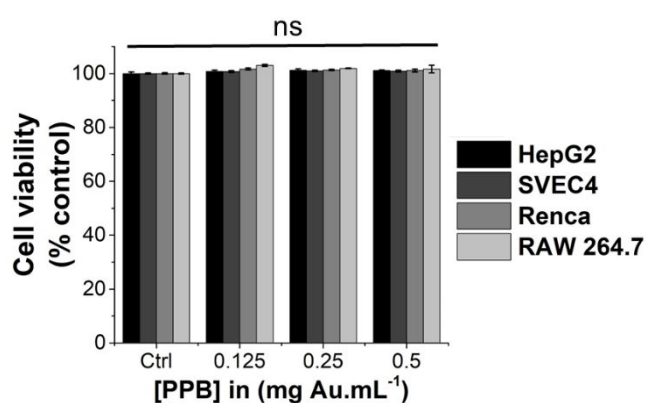


Figure S5. Viability of 4 cell lines incubated for 8 hours with the nanoparticles suspended in cell medium, determined by LIVE-DEAD assay. 'ns' indicates non-statistically significant differences.

7. ROS and TNF- α production in macrophages

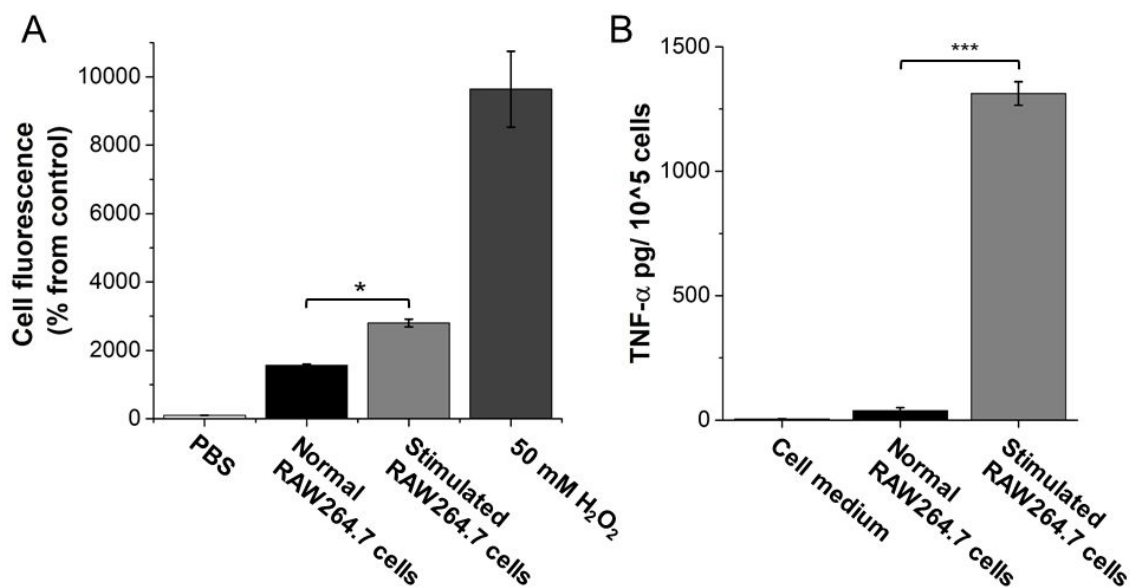


Figure S6. A) ROS quantification with H₂DCFDA fluorescence assay in macrophages with (grey) or without (black) inflammation stimulation. B) TNF- α release from macrophages determined using ELISA assay with (grey) or without (black) inflammatory stimulation; * and *** indicate a statistically significant difference at $p < 0.05$ or $p < 0.001$, respectively.

References

- ¹ Iturmendi, A.; Theis, S.; Maderegger, D.; Monkowius, U.; Teasdale I. Coumarin-Caged Polyphosphazenes with a Visible-Light Driven On-Demand Degradation *Macromol. Commun.* **2018**, *39* (18), 1800377.
- ² Iturmendi, A.; Monkowius, U.; Teasdale, I. Oxidation Responsive Polymers with a Triggered Degradation via Arylboronate Self-Immulative Motifs on a Polyphosphazene Backbone *ACS Macro Letters* **2017**, *6* (2), 150-154.

³ Rothmund, S.; Aigner, T. B.; Iturmendi, A.; Rigau, M.; Husar, B.; Hildner, F.; Oberbauer, E.; Prambauer, M.; Olawale, G.; Forstner, R.; Liska, R.; Schroder, K. R.; Bruggemann, O.; Teasdale I. Degradable Glycine-Based Photo-Polymerizable Polyphosphazenes for Use as Scaffolds for Tissue Regeneration *Macromol. Biosci.* **2015**, *15* (3), 351-363.