Supporting Information

Affinity Capillary Electrophoresis Method to Assess Carboxylation of Multiwalled Carbon Nanotubes

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Abstract:

This supporting information includes a stepwise description of the calculations of fractional binding, with the results of fractional binding calculations of a single affinity capillary electrophoresis binding analysis summarized in Table S-1. The results of reproducibility studies of sample stability and dried powder preparations are displayed as electropherograms and binding curves, with results summarized in Tables S-2 and S-3. Control runs demonstrating that complex formation is due to the combination of peptide and carbon nanotubes are provided in Figure S-3. Figures S4-6 depict electropherograms obtained from analysis of carbon nanotube acid treated at 0 ºC for 1, 3, and 6 hours.

Calculating K_D from the Increase in Migration Time (i.e. Shift):

The determination of the dissociation constant ($K_{D)}$ is accomplished in three steps. First, at each concentration of carbon nanotubes in the background electrolyte (i.e. 0 to 20 mg/L), the migration time for the peptide and the mesityl oxide neutral marker are collected at 214 nm and 254 nm, respectively. The migration time of the peptide reflects the apparent mobility (μ_{app}), while the migration time of the marker is used to calculate the electroosmotic flow (μ_{EOF}). Second, the electrophoretic mobility of the peptide in the free, partially bound, or bound state is calculated by subtracting the electroosmotic flow mobility from the apparent mobility. The electrophoretic mobility of the peptide in the absence of carbon nanotube (μ_{free}) is the starting position of the migration shift. The electrophoretic mobility of the peptide when fully bound to carbon nanotubes (μ_{max}) is the maximum migration shift of the peptide. The electrophoretic mobility of the peptide when partially bound to carbon nanotubes (μ_{mid}) is observed for migration shifts between the free and bound states of the peptide. Third, the fraction bound is then calculated by dividing the difference of the μ_{mid} and μ_{free} by the difference of μ_{Max} and μ_{free}. This represents the fraction of the peptide bound in between the free and complete complexed state. Therefore, at each carbon nanotube concentration used a fraction is determine using the μ_{free} and μ_{max}. The follow example shows how the fraction of peptide bound to carbon nanotube was determine at 1.5 mg/L carbon nanotube in the background electrolyte.

1. Calculation of apparent mobility and electroosmotic flow for 0, 1.5 and 20 mg/L is shown in the following example:
   At 0 mg/L the migration time of the peptide is 127.50 secs and neutral marker 156.00 secs

$$\mu_{app\_0}\left(\frac{cm^2}{V\ secs}\right) = \frac{Length\ Capillary\ (cm) * Length\ to\ Detector(cm)}{Voltage\ (V) * migration\ time\ (s)} = \frac{30.00\ \pm 0.03\ cm * 20.00\ \pm 0.03\ cm}{10000\ \pm 100\ V *\ 127.50\ \pm 0.25\ s}$$
$$= \mathbf{0.000471\ \pm\ 0.000005}$$

$$\mu_{EOF}\left(\frac{cm^2}{V\ secs}\right) = \frac{Length\ Capillary\ (cm) * Length\ to\ Detector(cm)}{Voltage\ (V) * migration\ time\ (s)} = \frac{30.00\ \pm 0.03\ cm * 20.00\ \pm 0.03\ cm}{10000\ \pm 100\ V *\ 156.00\ \pm 0.25\ s}$$
$$= \mathbf{0.000385\ \pm\ 0.000004}$$

At 1.5 mg/L the migration time of the peptide is 321.48 secs and neutral marker 160.02 secs

$$\mu_{app\_1.5}\left(\frac{cm^2}{V\ secs}\right) = \frac{30.00\ \pm 0.03\ cm * 20.00\ \pm 0.03\ cm}{10000\ \pm 100\ V *\ 321.48\ \pm 0.25\ s} = \mathbf{0.000187\ \pm\ 0.000002}$$

$$\mu_{EOF}\left(\frac{cm^2}{V\ secs}\right) = \frac{30.00\ \pm 0.03\ cm * 20.00\ \pm 0.03\ cm}{10000\ \pm 100\ V *\ 160.02\ \pm 0.25\ s} = \mathbf{0.000375\ \pm\ 0.000004}$$

At 20 mg/L the migration time of the peptide is 565.74 secs and neutral marker 152.76 secs

$$\mu_{app\_20}\left(\frac{cm^2}{V\ secs}\right) = \frac{30.00\ \pm 0.03\ cm * 20.00\ \pm 0.03\ cm}{10000\ \pm 100\ V * 565.74\ \pm 0.25\ s} = \mathbf{0.000106\ \pm\ 0.000001}$$

$$\mu_{EOF}\left(\frac{cm^2}{V\ secs}\right) = \frac{30.00\ \pm 0.03\ cm * 20.00\ \pm 0.03\ cm}{10000\ \pm 100\ V *\ 152.76\ \pm 0.25\ s} = \mathbf{0.000393\ \pm\ 0.000004}$$

^Initial error is instrumental and propagated within the calculation.

2. Calculation of electrophoretic mobility peptide in 0 (μ_{free}), 1.5 mg/L (μ_{mid}), 20 mg/L (μ_{max}) carbon nanotubes background electrolyte. The electrophoretic mobility is the difference of the apparent mobility of the peptide and the μ_{EOF} determined at each concentration and detailed above.

$$\mu_{free} = \mu_{app\_0} - \mu_{EOF} = 0.000471\ \pm 0.000005 - 0.000385\ \pm 0.000004 = \mathbf{0.000086\ \pm 0.00006}$$

$$\mu_{mid} = \mu_{app\_1.5} - \mu_{EOF} = 0.000187\ \pm 0.000002 - 0.000375\ \pm 0.000004 = \mathbf{-0.000188\ \pm 0.00004}$$

$$\mu_{max} = \mu_{app\_20} - \mu_{EOF} = 0.000106\ \pm 0.000002 - 0.000393\ \pm 0.000004 = \mathbf{-0.000287\ \pm 0.00004}$$

^Error is propagated for this calculation.

3. Calculation of fraction bound in 1.5 mg/L carbon nanotubes in background electrolyte, where μ_{free} is the electrophoretic mobility of the peptide with no carbon nanotube in the background electrolyte and μ_{max} (maximum bound) is the electrophoretic mobility of peptide when the peptide is fully bound to carbon nanotubes. The bound fraction for 0 mg/L is equal to 0 as no binding has occurred and is 1 when at maximum binding at 20 mg/L.

$$\frac{\mu_{mid} - \mu_{free}}{\mu_{max} - \mu_{free}} = \frac{-0.000188\ \pm 0.000004 - 0.000086\ \pm 0.000006}{-0.000287\ \pm 0.000004 - 0.000086\ \pm 0.000006} = \mathbf{0.74\ \pm 0.03}\ (\mathbf{3.5}\%)$$

^Error is propagated for this calculation.

Table S-1 Sample Calculation of Fractional Binding of each point in a single curve

[CNT] mg/L	**Peptide** t_m (s)	**μ_{app} ± SD** (cm^2/Vs) x10^6	**Mesityl Oxide** t_m (s)	**μ_{EOF} ± SD** (cm^2/Vs) x10^6	**μ_{EPH} ± SD** (cm^2/Vs) x10^6	**Numerator** ± SD (cm^2/Vs) x10^6	**Denominator** ± SD (cm^2/Vs) x10^6	**Fraction** ± SD (%RSD)
0	127.50	471 ± 5	156.00	385 ± 4	86 ± 6	0 ± 9	-373 ± 8	0 ± 0 (N/A)
0.5	138.78	432 ± 4	158.28	379 ± 4	53 ± 6	-54 ± 6	-373 ± 8	0.09 ± 0.02(22%)
1	202.98	296 ± 3	158.76	378 ± 4	82 ± 5	-82 ± 5	-373 ± 8	0.45 ± 0.02 (4%)
1.5	321.48	187 ± 2	160.02	375 ± 4	188 ± 4	-274 ± 8	-373 ± 8	0.74 ± 0.03 (4%)
2.5	358.50	167 ± 2	155.76	385 ± 4	218 ± 4	-304 ± 4	-373 ± 8	0.82 ± 0.03 (4%)
5	433.98	138 ± 1	156.00	385 ± 4	246 ± 4	-332 ± 4	-373 ± 8	0.89 ± 0.03 (3%)
10	452.76	133 ± 1	157.74	380 ± 4	248 ± 4	-334 ± 4	-373 ± 8	0.90 ± 0.03 (3%)
20	565.74	106 ± 1	152.76	393 ± 4	287 ± 4	-373 ± 4	-373 ± 8	1.0 ± 0.03 (3%)

Table S-2. K_D values for a Single Preparation of Carbon Nanotubes (n = 3 curves)

Curve	K_D± SD (mg/L)
1	1.4 ± 0.3
2	1.2 ± 0.2
3	1.1 ± 0.2
Average	1.2 ± 0.2 (20%)

Individual curve fitting performed with [CNT] = 0, 0.5, 1.0, 1.5, 2.5, 5.0, 10, 20 mg/L with NanoLab 2 – 7 wt % COOH (15 ± 5 nm o.d., 1 -5 µm long) and using 25 µM WRWWWW at E = 333 V/cm in 25 mM MOPS buffered to pH 7

Figure S-1

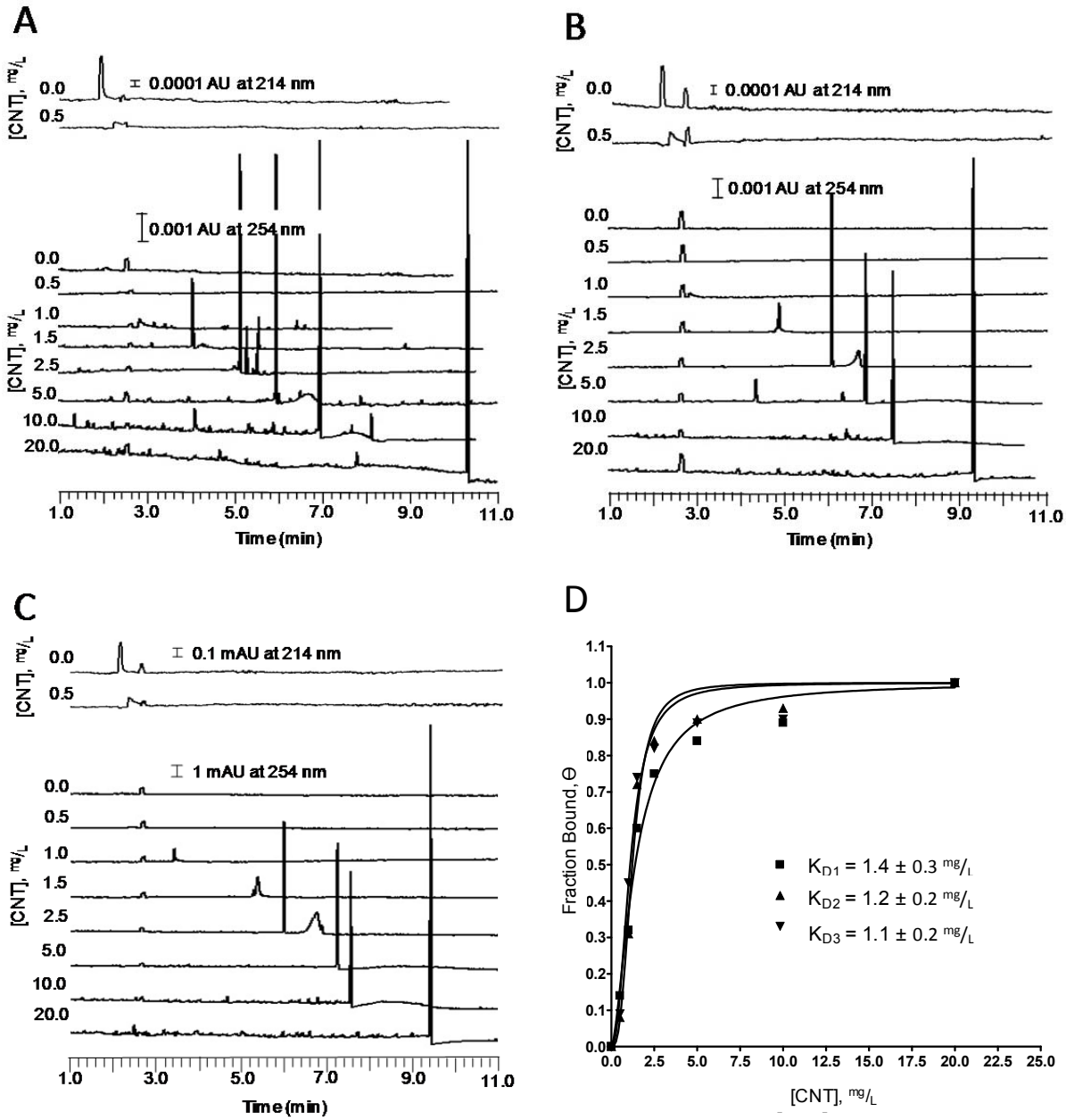

Figure S-1 Electropherograms (A to C) and resulting GraphPad fitted curves (D) obtained using 3 replicates of a carbon nanotube preparation made from a single powder stock. Each curve is performed with 7 different concentrations of carbon nanotubes for a total of 8 runs per curve to demonstrate solution stability.

Table S-3. K_D values for Three Powder Stock Preparations (*h* = 3 powder stocks, *n* = 9 curves)

Dried Powder Stock[1]	K_D± SD (mg/L)	ξ ± SD (mV)[3]
1	1.2 ± 0.2	-42 ± 1
2	0.9 ± 0.2	-43 ± 1
3	1.1 ± 0.2	-43 ± 1
Average[2]	1.1 ± 0.2 (20%)	-43 ± 1

[1] *n* = 3 individual curve fitting performed with 0, 0.5, 1.0, 1.5, 2.5, 5.0, 10, 20 mg/L [CNT] at E = 333 V/cm in 25 mM MOPS with a single preparation

[2] Data are the average and propagated error from curve fitting of three dried powder stocks (n = 9 curve fittings) using 25 μM WRWWWW peptide NanoLab carbon nanotubes, (2 – 7 wt % COOH, 15 ± 5 nm o.d., 1 -5 μm long).

[3] Data collected from single 5 mg/L carbon nanotube sample

Figure S-2

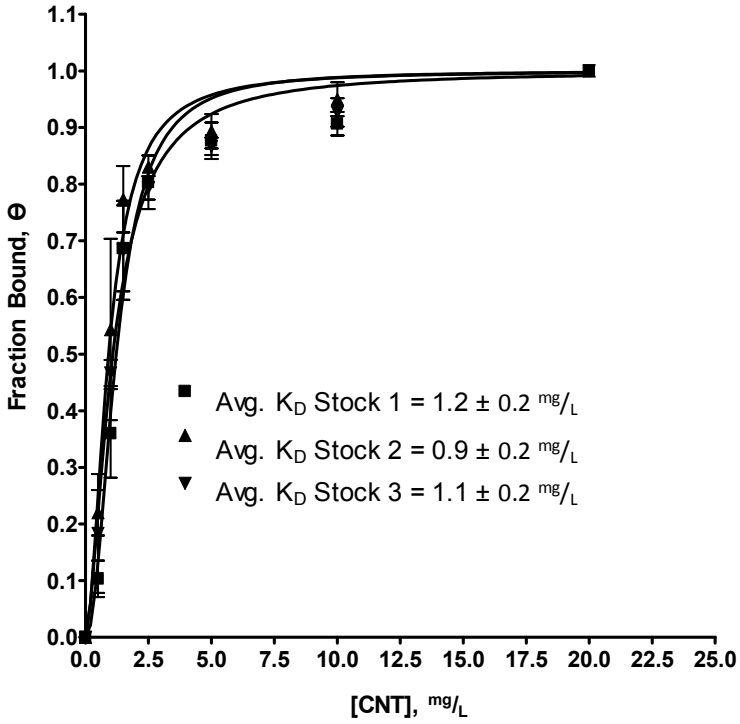

Figure S-2. Contains binding curves from replicate analysis from three independent dried powder stocks of NanoLab carbon nanotubes, (2 – 7 wt % COOH, 15 ± 5 nm o.d., 1 -5 µm long). All data points are an average of triplicate analyses of the diluted stocks with error bars representing the standard deviation of the average.

Figure S-3

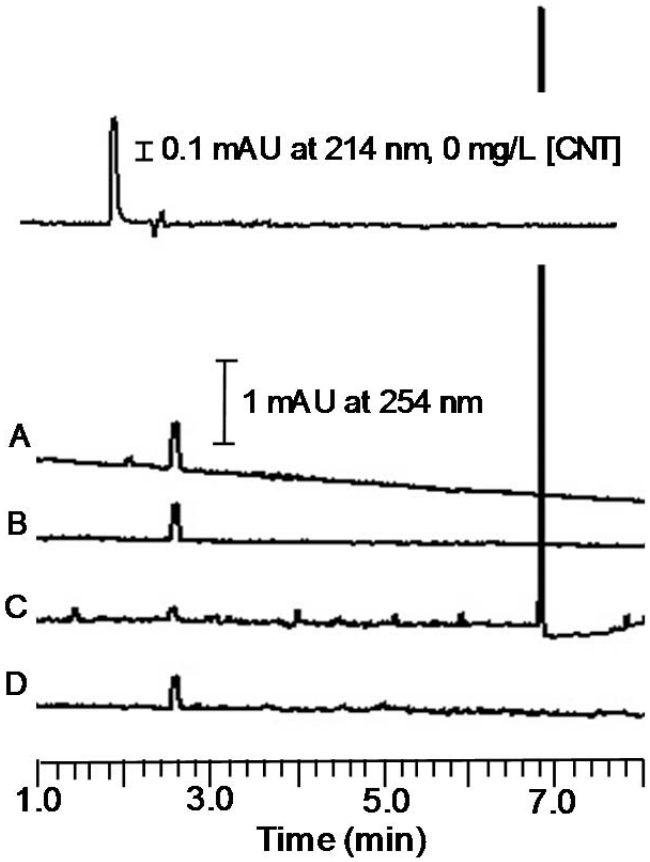

Figure S-3. Depicts the control electropherograms for affinity capillary electrophoresis analysis demonstrating that the complex formation is only observed in the presence of WRWWWW peptide. Trace A is of 25 μM WRWWWW and 30 μM mesityl oxide in 0 mg/L carbon nanotubes in background electrolyte. Trace B is 30 μM mesityl oxide in 0 mg/L carbon nanotubes in background electrolyte. Trace C is of 25 μM WRWWWW and 30 μM mesityl oxide in 2.5 mg/L carbon nanotubes in background electrolyte. Trace D is of 30 μM mesityl oxide in 2.5 mg/L carbon nanotubes in background electrolyte.

Figure S-4

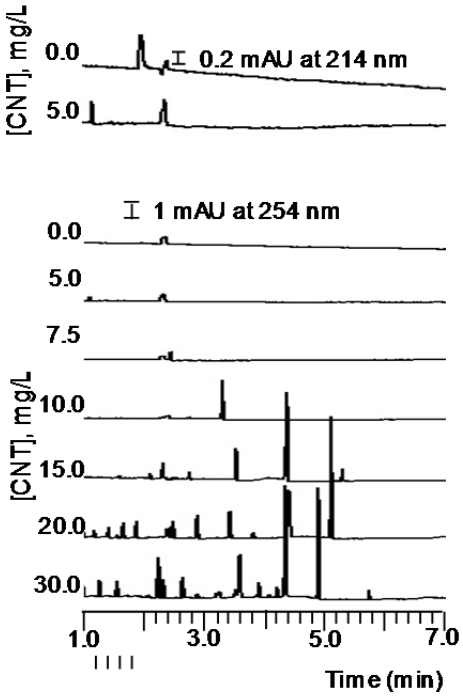

Figure S-4. Depicts electropherograms obtained from a single analysis of carbon nanotube acid treated at 0 ºC for 1 hour.

Figure S-5

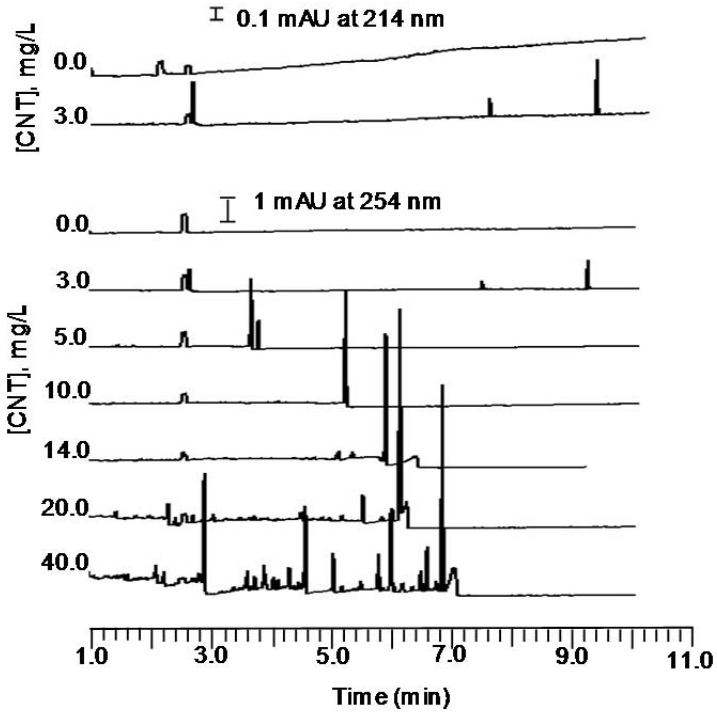

Figure S-5. Depicts electropherograms obtained from a single analysis of carbon nanotube acid treated at 0 ºC for 3 hours.

Figure S-6

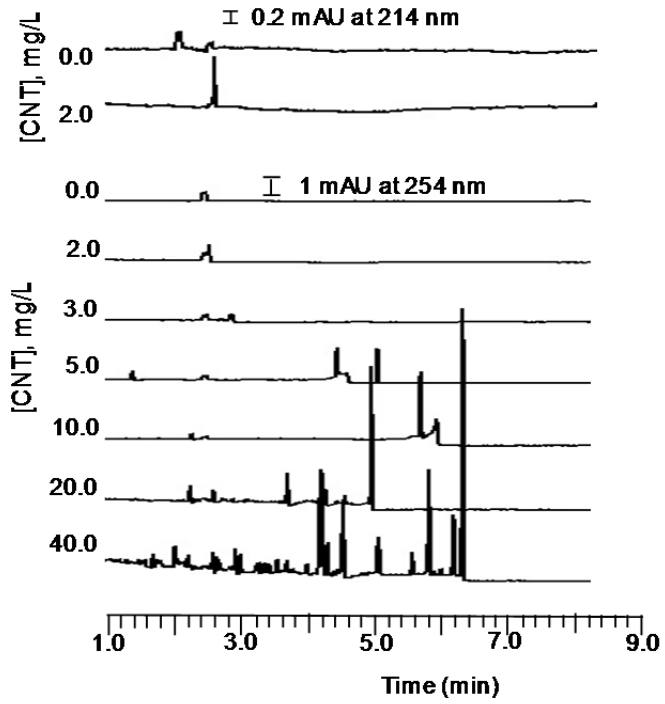

Figure S-6. Depicts electropherograms obtained from a single analysis of carbon nanotube acid treated at 0 ºC for 6 hours.