Multidomain Peptides as Single-Walled Carbon Nanotube Surfactants in Cell Culture

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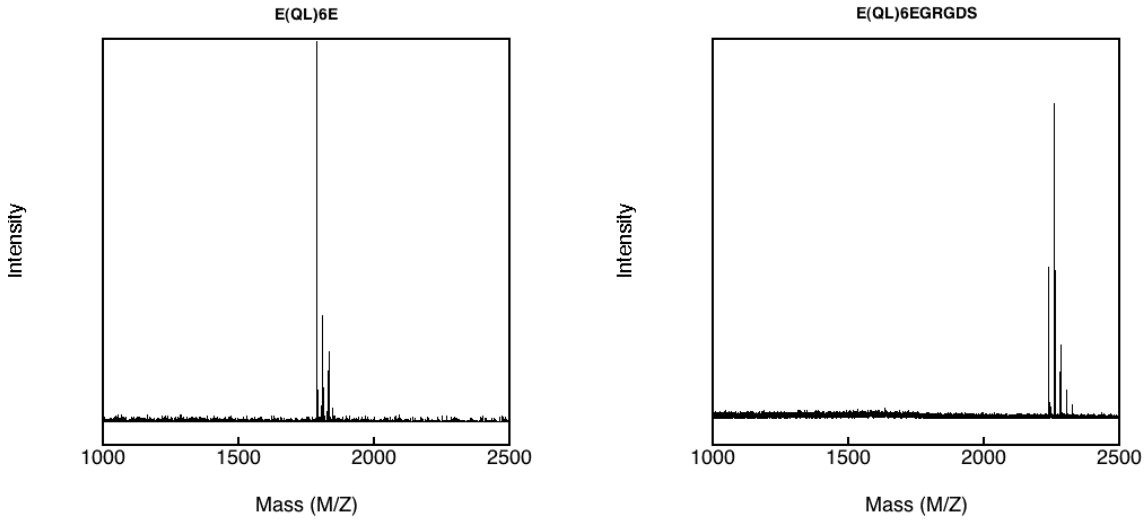
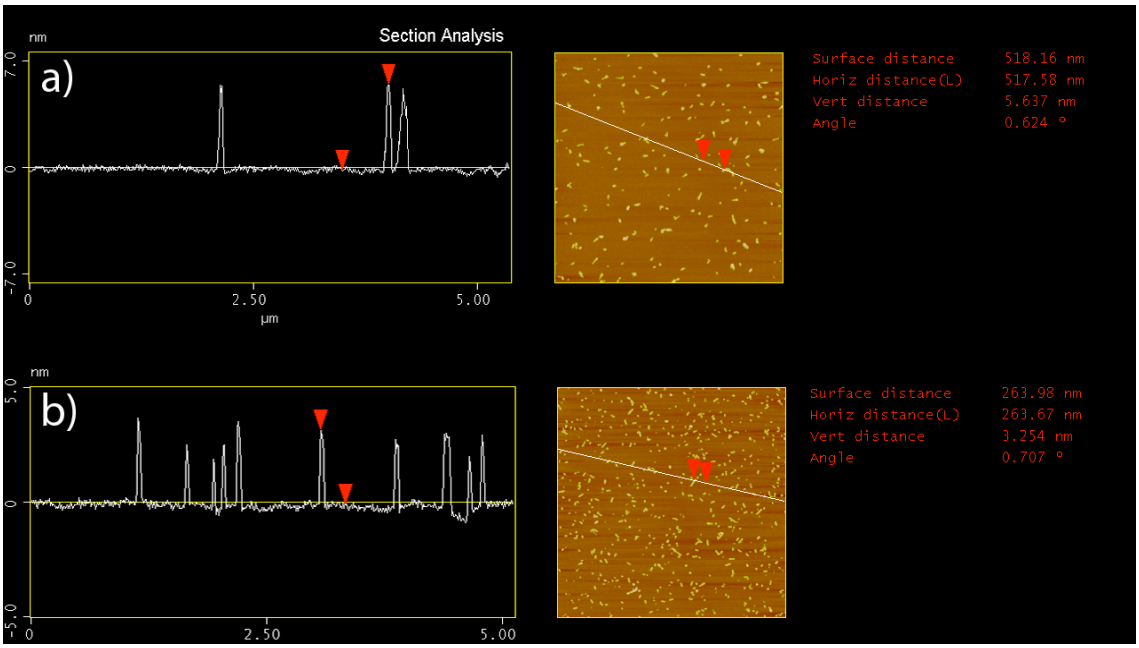
SUPPORTING INFORMATION

Peptide Characterization. After synthesis by traditional solid-phase peptide synthesis and purification, multidomain peptides were subsequently characterized by MALDI-TOF mass spectrometry. $E(QL)_6E$, expected mass $[M+Na]^+$: 1788.0 Observed mass: 1788.1. $E(QL)_6EGRGDS$, expected mass $[M+Na]^+$: 2259.2 Observed mass: 2259.1. Additional peaks in MALDI spectra correspond to additional salt adducts.



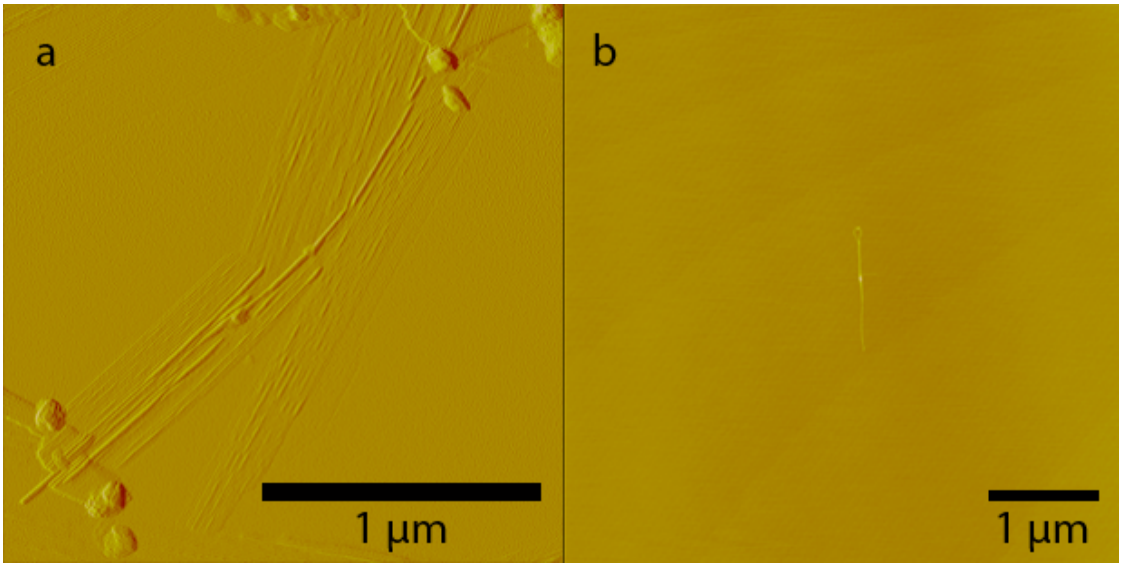
Supplementary Figure SI-1. MALDI-TOF mass spectrometry of peptides $E(QL)_6E$ and $E(QL)_6EGRGDS$.

Atomic Force Microscopy. SWCNT-peptide solutions were diluted 20 fold with ultra pure water and were dropped onto freshly cleaved mica while spinning on a Headway Research, Inc. Photo-resist spinner. The sample was rinsed with deionized water for 1-2 seconds and then spun for an additional 10 minutes. AFM images were collected in air, at ambient temperature, on a Digital Instruments Nanoscope IIIa atomic force microscope in tapping mode. Height Profiles were taken using Nanoscope software.



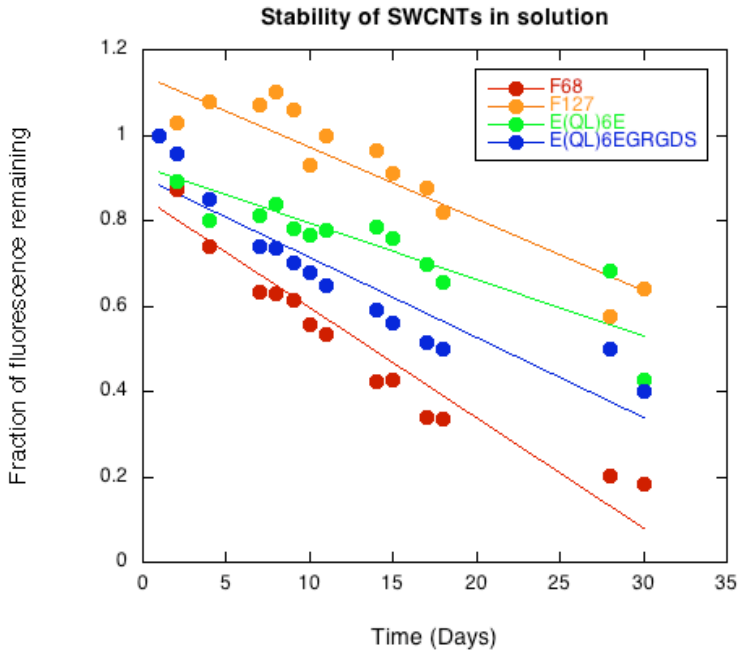
Supplementary Figure SI-2. AFM height profiles of SWCNT suspensions of (a) $E(QL)_6E$ and (b) $E(QL)_6EGRGDS$.

Length Distribution of SWCNTs in Peptide and SDBS-Suspended Samples. While the AFM height profiles of peptide-suspended SWCNTs were consistent with calculated heights of SWCNTs with a multidomain peptide coating, it was observed that peptide-SWCNTs suspensions showed a large number of short carbon nanotubes when observed by AFM compared with what would be expected for HiPCO SWCNTs. To confirm this, SWCNT suspensions were made under identical suspension conditions, with SDBS as the suspending agent. These SDBS-suspended samples were spin-coated onto fresh mica and AFM was subsequently performed. The resulting AFM images show SWCNTs that are highly bundled, but longer SWCNTs do appear to be present. This suggests that multidomain peptide surfactants may preferentially suspend shorter SWCNTs.



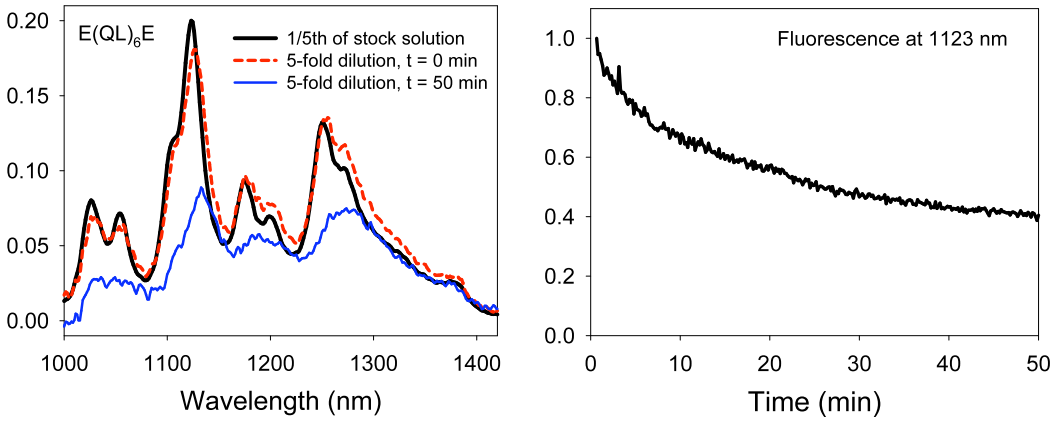
Supplementary Figure SI-3. AFM of SDBS-suspended SWCNTs. (a) shows a 2 x 2 µm image and (b) shows a 5 x 5 µm image.

Stability of Peptide-SWCNT Suspensions. The stability of peptide-SWCNT suspensions has been monitored in two ways. First, the stability of these suspensions in isolation was investigated. Peptide-SWCNT suspensions were prepared as described and allowed to sit for one month at room temperature. The near-IR fluorescence of each suspension was measured daily. It was found that for suspensions in all surfactants, fluorescence decreases over time.



Supplementary Figure SI-4. Near-IR fluorescence at 1034 nm of isolated SWCNT suspensions in various surfactants.

Second, we monitored the near-IR SWCNT fluorescence of peptide-coated SWCNT suspensions to assess stability in the presence of cell culture medium. A stable 5.6 mM aqueous suspension of SWCNTs coated by peptide $E(QL)_6E$ was diluted by a factor of 5 with the cell culture medium described in the text, after which near-IR fluorescence spectra were recorded at selected intervals. Figure SI-5 (a) compares spectra of the suspension before exposure to the culture medium, just after 5-fold dilution with the medium, and 50 min later. During this observation period, peaks of the emission spectrum become broadened, red-shifted, and less intense. The kinetics of this process are displayed in Fig. SI-5 (b), which plots emission intensity at the 1123 nm peak as a function of time after dilution. These data can be fit by a biexponential transformation model with characteristic lifetimes of approximately 2 and 20 min and a final intensity of 35% of the initial value. We suggest that this process involves the displacement of the peptide coating by proteins in the culture medium, or co-adsorption of proteins onto the peptide-coated SWCNTs.



Supplementary Figure SI-5. (a) Fluorescence spectra of the SWCNT suspension in peptide $E(QL)_6E$ before addition of the cell culture medium (scaled down by a factor of 5), immediately after 5-fold dilution in the culture medium, and 50 min after the dilution. (b) Kinetics of fluorescence emission at 1123 nm after 5-fold dilution by cell culture medium.

1 and 2-way ANOVA. Analysis of variance (ANOVA) was performed using Prism software. 2-way ANOVA was performed to determine whether the interaction of SWCNTs with the surfactant had an impact on the solution toxicity, while 1-way ANOVA and Tukey tests were used to compare each condition with every other condition.

2-way ANOVA

150 μM

Source of Variation	% of total variation	P value
Interaction	8.04	0.548
Surfactant	39.79	0.017
SWNTs	1.02	0.5342

300 μM

Source of Variation	% of total variation	P value
Interaction	0.38	0.9027
Surfactant	92.22	<0.0001
SWCNTs	0	0.9501

1 mM

Source of Variation	% of total variation	P value
Interaction	4.25	0.0438
Surfactant	88.4	<0.0001
SWNTs	0.23	0.4345

1-way ANOVA
NIH 3T3 cells, no SWNTs, 150 μM

ANOVA comparison	P value
SDBS vs Pluronic F-68	> 0.05
SDBS vs Pluronic F-127	> 0.05
SDBS vs $E(QL)_6E$	> 0.05
SDBS vs $E(QL)_6EGRGDS$	> 0.05
Pluronic F-68 vs Pluronic F-127	> 0.05
Pluronic F-68 vs $E(QL)_6E$	> 0.05
Pluronic F-68 vs $E(QL)_6EGRGDS$	> 0.05
Pluronic F-127 vs $E(QL)_6E$	> 0.05
Pluronic F-127 vs $E(QL)_6EGRGDS$	> 0.05

$E(QL)_6E$ vs $E(QL)_6EGRGDS$	> 0.05

NIH 3T3 cells, with SWNTs, 150 μM

ANOVA comparison	P value
SDBS vs Pluronic F-68	> 0.05
SDBS vs Pluronic F-127	> 0.05
SDBS vs $E(QL)_6E$	> 0.05
SDBS vs $E(QL)_6EGRGDS$	> 0.05
Pluronic F-68 vs Pluronic F-127	> 0.05
Pluronic F-68 vs $E(QL)_6E$	> 0.05
Pluronic F-68 vs $E(QL)_6EGRGDS$	> 0.05
Pluronic F-127 vs $E(QL)_6E$	> 0.05
Pluronic F-127 vs $E(QL)_6EGRGDS$	> 0.05
$E(QL)_6E$ vs $E(QL)_6EGRGDS$	> 0.05

NIH 3T3 cells, no SWNTs, 300 μM

ANOVA comparison	P value
SDBS vs Pluronic F-68	< 0.001
SDBS vs Pluronic F-127	< 0.001
SDBS vs $E(QL)_6E$	< 0.001
SDBS vs E(QL)6EGRGDS	< 0.001
Pluronic F-68 vs Pluronic F-127	> 0.05
Pluronic F-68 vs $E(QL)_6E$	> 0.05
Pluronic F-68 vs $E(QL)_6EGRGDS$	> 0.05
Pluronic F-127 vs $E(QL)_6E$	> 0.05
Pluronic F-127 vs $E(QL)_6EGRGDS$	> 0.05
$E(QL)_6E$ vs $E(QL)_6EGRGDS$	> 0.05

NIH 3T3 cells, with SWNTs, 300 μM

ANOVA comparison	P value
SDBS vs Pluronic F-68	< 0.001
SDBS vs Pluronic F-127	< 0.001
SDBS vs $E(QL)_6E$	< 0.001
SDBS vs $E(QL)_6EGRGDS$	< 0.001
Pluronic F-68 vs Pluronic F-127	> 0.05
Pluronic F-68 vs $E(QL)_6E$	> 0.05
Pluronic F-68 vs $E(QL)_6EGRGDS$	> 0.05
Pluronic F-127 vs $E(QL)_6E$	> 0.05
Pluronic F-127 vs $E(QL)_6EGRGDS$	> 0.05
$E(QL)_6E$ vs $E(QL)_6EGRGDS$	> 0.05

NIH 3T3 cells, no SWNTs, 1 mM

ANOVA comparison	P value
SDBS vs Pluronic F-68	< 0.001
SDBS vs Pluronic F-127	< 0.001
SDBS vs $E(QL)_6E$	< 0.01
SDBS vs $E(QL)_6EGRGDS$	< 0.01
Pluronic F-68 vs Pluronic F-127	> 0.05
Pluronic F-68 vs $E(QL)_6E$	> 0.05
Pluronic F-68 vs $E(QL)_6EGRGDS$	> 0.05
Pluronic F-127 vs $E(QL)_6E$	> 0.05
Pluronic F-127 vs $E(QL)_6EGRGDS$	> 0.05
$E(QL)_6E$ vs $E(QL)_6EGRGDS$	> 0.05

NIH 3T3 cells, with SWNTs, 1 mM

ANOVA comparison	P value
SDBS vs Pluronic F-68	< 0.001
SDBS vs Pluronic F-127	< 0.001
SDBS vs $E(QL)_6E$	< 0.001
SDBS vs $E(QL)_6EGRGDS$	< 0.05
Pluronic F-68 vs Pluronic F-127	> 0.05
Pluronic F-68 vs $E(QL)_6E$	< 0.01
Pluronic F-68 vs $E(QL)_6EGRGDS$	< 0.001
Pluronic F-127 vs $E(QL)_6E$	< 0.05
Pluronic F-127 vs $E(QL)_6EGRGDS$	< 0.001
$E(QL)_6E$ vs $E(QL)_6EGRGDS$	< 0.01

Note: Prism software did not return specific P values for 1-way ANOVA. Instead, the report generated indicated the level of statistical significance, for example $P < 0.01$ or $P > 0.05$.