

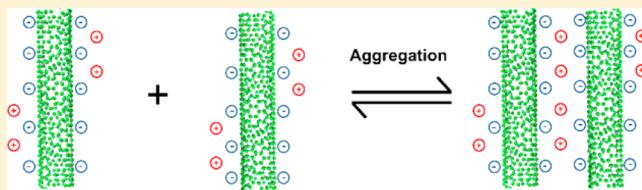
Mechanisms of Carbon Nanotube Aggregation and the Reversion of Carbon Nanotube Aggregates in Aqueous Medium

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Supporting Information

ABSTRACT: Single-walled carbon nanotubes (SWCNTs) dispersed in aqueous medium have many potential applications in chemistry, biology, and medicine. Reversible aggregation of SWCNTs dispersed in water has been frequently reported, but the mechanisms behind are not well understood. Here we show that SWCNTs dispersed into aqueous medium assisted by various charged molecules can be reversibly aggregated by a variety of electrolytes with two distinct mechanisms. Direct binding of counterions to SWCNTs leads to aggregation when the surface charge is neutralized from 74 to 86%. This aggregation is driven by electrostatic instead of van der Waals interactions, thus showing similarity to that of DNA condensation induced by multivalent cations. Sequestration of counterions by chelating reagents leads to the redispersion of SWCNT aggregates. In contrast to various metal ions, polyelectrolytes have the unique ability to induce SWCNT aggregation by bridging between individual SWCNTs. Aggregation through the latter mechanism can be engineered to be reversible by exploiting various mechanisms of chain breaking, including reduction of disulfide bond in the polymer chain, and the cleavage action of proteolytic enzymes. These findings clarify the mechanisms of SWCNT aggregation, and have broad implications in various applications of SWCNTs in water.



INTRODUCTION

Single-walled carbon nanotubes (SWCNTs) dispersed in aqueous media hold great promise for exciting applications in chemistry, biology, and nanomedicine.^{1–3} Central to these applications is the stability of these nanoscale dispersions in aqueous media.⁴ Reversible aggregation of dispersed SWCNTs in aqueous medium has been frequently reported. Various reagents or perturbations have been applied to control the aggregation status of SWCNTs, such as solution pH,^{5–9} light,^{10–12} oxidation and reduction of dispersants,^{13–16} temperature,^{5,8} and addition of salt.^{17–19} However, the molecular mechanisms that underlie SWCNT aggregation have not been explored systematically, and there have been inconsistent views on this very important issue.

The fundamental question that we concern is what the molecular interactions that drive this aggregation process are in aqueous medium. Although the surface of SWCNTs is hydrophobic in nature, which can in principle promote their self-association and aggregation through hydrophobic and van der Waals interactions, this is not true for SWCNTs that have been dispersed into aqueous media assisted by various charged dispersants. For these dispersed SWCNTs, their surface is coated by these dispersant molecules at various densities. The charge status of these dispersant molecules on SWCNT surface will have profound impact on the physical properties of SWCNTs in water. As demonstrated by Wang and Chen,⁵ SWCNTs dispersed by poly-L-lysine can undergo reversible aggregation that is dependent on the solution pH. When solution pH is close to the isoelectric point of lysine, dispersed SWCNTs become aggregated, suggesting that electrostatic

interactions may play a significant role in this aggregation and redispersion process. However, how electrostatic interactions may tune the interactions among individual SWCNTs remains largely unexplored. In several aspects, individual SWCNTs coated with charged dispersants on their surface share similarity with polyelectrolytes such as double-stranded DNA in solution. The sp² carbons of SWCNTs form the hydrophobic backbones, and resemble the aromatic base groups located at the center of double-helical DNA. The charged dispersant molecules are coated on this hydrophobic surface, very similar to the phosphate groups that lie on the exterior of double-stranded DNA. Therefore, mechanisms that operate in DNA condensation might work similarly in dispersed SWCNTs.

For SWCNTs dispersed with charged molecules, we hypothesize that their aggregation in aqueous medium is mediated by electrostatic instead of van der Waals interactions, and modulation of these electrostatic interactions can lead to reversion of SWCNT aggregates. Herein we test this hypothesis by systematically investigating SWCNT aggregation in aqueous medium mediated by a variety of dispersant molecules. We measure the surface charge status of SWCNTs during this aggregation process through measurement of the zeta potential, and correlate the extent of SWCNT aggregation with zeta potential measurement. These results allow us to clarify the mechanisms of SWCNT aggregation in aqueous medium. By further exploring these mechanisms, we demonstrate various

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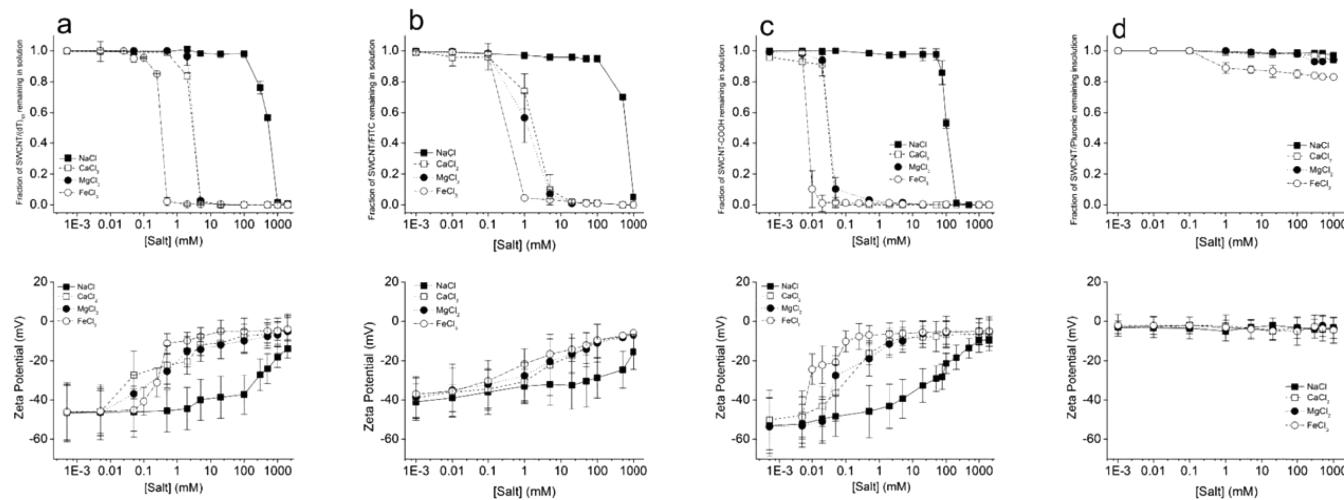


Figure 1. Fraction of individual (a) SWCNT/(dT)₃₀, (b) SWCNT/FITC, (c) SWCNT-COOH, and (d) SWCNT/Pluronic that remained in solution upon titration with various electrolytes (upper panel) and the zeta potential changes on SWCNTs associated with this process (lower panel). Error bars represent standard deviation from three independent repeats of the same experiments. Throughout this paper, aggregation and zeta potential profiles of AD SWCNTs are shown unless noted otherwise.

strategies that can be used to reverse the aggregation of SWCNTs in solution. These mechanisms that we identified also apply to typical cell culture conditions and are thus relevant to the potential biological applications of SWCNTs *in vivo*.

EXPERIMENTAL SECTION

Materials. One milligram of Arc-discharge (AD) SWCNTs (Helix Materials Solution, TX) were dispersed in 1 mL of distilled and deionized water (ddH₂O, Synergy UV, Millipore Corporation, Billerica, MA) with 1 h sonication in the presence of various dispersing reagents in a tip sonicator (Ultrasonic Processor S-4000, Misonix, Farmingdale, NY) as described previously to obtain singly dispersed tubes.^{19,20} This condition does not introduce defects to SWCNT sidewalls, as indicated by the intensity ratio between D and G bands from Raman spectrum measurements. The concentrations of dispersing reagents are 1 mg/mL for DNA oligo (dT)₃₀, fluorescein isothiocyanate (FITC), rhodamine B (RB), crystal violet (CV) and poly-L-lysine (PLL), and 40 mg/mL for Pluronic F 108. Ice was constantly added to the bath to prevent heating of the sample during sonication. P3-SWNTs (SWCNT-COOH, Carbon Solutions, Riverside, CA) were dispersed in ddH₂O with the same procedure described above. We adjusted concentration of dispersed SWCNTs in aqueous medium as ~0.02 µg/µL for consistency throughout the experiments. This concentration was determined based on the absorbance at 1023 nm, using an extinction coefficient of 11.9 (mg/mL)⁻¹cm⁻¹ for AD SWCNTs that we estimated previously.¹⁹ Dispersed SWCNTs were stable in aqueous medium for more than one month. (dT)₃₀ was from Integrated DNA Technologies (Coralville, IA). Pluronic F 108 was from BASF (Germany). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

Aggregation of SWCNTs by Various Electrolytes. Seven hundred microliters of individually dispersed SWCNTs was agitated in an orbital shaker (Excella E-24R incubator shakers, New Brunswick Scientific Co, Edison, NJ) in the presence of different concentrations of various electrolytes at 20 °C for 30 min at 200 rpm. All stock solutions of the electrolytes were made in ddH₂O. After agitation, 600 µL samples were used immediately for measurement of zeta potential in Zetasizer ZS90 (Malvern, UK). The rest of the samples were centrifuged for 30 min at 17 000g, and supernatants were collected for ultraviolet-visible-near-infrared (UV-vis-NIR) absorbance measurement (Shimadzu UV-1800, Kyoto, Japan) to determine the fraction of SWCNTs that remained in solution relative to the original quantity

of dispersed SWCNTs. Aggregated SWCNTs were not redispersed by themselves for greater than 2 weeks. Throughout this paper, aggregation and zeta potential profiles of AD SWCNTs are shown unless noted otherwise. All the starting materials for aggregation experiments are supernatants of SWCNTs after centrifugation to remove nondispersed SWCNTs.

Redispersion of Various SWCNT Aggregates. For ethylenediaminetetraacetic acid (EDTA)-, dithiothreitol (DTT)-, 2-mercaptoethanol-, or NaCl-mediated redispersion of various SWCNT aggregates, we first induced aggregation of SWCNTs with various electrolytes at designated concentrations by agitation at a speed of 200 rpm for 30 min at room temperature (~22 °C). We then titrated in EDTA, DTT, 2-mercaptoethanol, or NaCl, and agitated the sample at 20 °C for 30 min at 200 rpm. After agitation, 600 µL samples were used immediately for measurement of zeta potential. The rest of the samples were centrifuged for 30 min at 17 000g, and supernatants were collected for UV-vis-NIR absorbance measurement to determine the fraction of SWCNTs that were redispersed back to solution relative to the original quantity of dispersed SWCNTs. To determine the visible-NIR absorbance spectra before and after inducing aggregation and redispersion, AD SWCNTs, chemical vapor deposition (CVD) SWCNTs (SES Research, Richardson, TX) and high pressure carbon monoxide (HiPCO) SWCNTs (super purified grade, Unidym, Sunnyvale, CA) were dispersed in the presence of (dT)₃₀ and agitated for 30 min with 5 mM of CaCl₂ in order to induce aggregation. SWCNT samples were then agitated in the presence of 20 mM EDTA for redispersion. For side-by-side comparison, each untreated AD SWCNT/(dT)₃₀, CVD SWCNT/(dT)₃₀, and HiPCO SWCNT/(dT)₃₀ sample also went through the same procedures as described above, except that ddH₂O was used in lieu of either CaCl₂ or EDTA. UV-vis-NIR absorbance spectra were recorded and plotted in parallel for side-by-side comparison. Redispersed SWCNTs were stable for greater than 2 weeks in ambient conditions.

Enzymatic Reversion of Bridged SWCNT Aggregates. We first induced aggregation of negatively charged SWCNTs with 1 mM KA₈K and positively charged SWCNTs with 5 mM EA₈E (custom synthesized polypeptides, 95% purity, Pierce Protein, IL.). We then titrated in either protease (0.1% of trypsin and ~1 mg/mL Proteinase K), and agitated the sample at 20 °C for 30 min at 200 rpm. After agitation, the samples were centrifuged for 30 min at 17 000g, and supernatants were collected for UV-vis-NIR absorbance measurement to determine the fraction of SWCNTs that were redispersed back to solution relative to the original quantity of dispersed SWCNTs. For inhibition of protease activity as a control experiment,

SWCNT aggregates induced by polypeptides KA₈K or EA₈E were treated with either ~0.2 mg/mL trypsin inhibitor (from soybean) or 10 mM PMSF, respectively. Trypsin and proteinase K were then added to SWCNT aggregates to monitor the redispersion of SWCNTs as described above.

Aggregation of SWCNTs in Tissue Culture Media. One microgram of dispersed SWCNTs was agitated with various fractions of complete cell culture media {90% ATCC Dulbecco's modified Eagle medium (DMEM) + 10% fetal bovine serum (FBS)} in a total volume of 100 μ L. After agitation at 200 rpm for various durations at various temperatures as indicated, samples were centrifuged at 17 000g for 30 min (Legend Pro 17, Thermo Fisher Scientific, MA). Supernatants were collected after centrifugation, and the fraction of individual SWCNTs that remained in solution was measured with UV-vis-NIR spectrophotometer relative to the initial quantity of dispersed SWCNTs.

■ RESULTS AND DISCUSSION

Counterion-Mediated Aggregation of Dispersed SWCNTs. Previous studies on SWCNTs dispersed in H₂O using sodium dodecyl sulfate (SDS) showed that SWCNTs could undergo selective aggregation upon addition of salt.¹⁷ This phenomenon has been interpreted as aggregation mediated by van der Waals attractions. When the concentration of counterions was increased, the surface of individually dispersed SWCNTs could be completely neutralized, at which point the adjacent SWCNTs would come into contact due to van der Waals attractions. This leads to aggregation of SWCNTs upon addition of salt. To further explore this phenomenon in similar systems, we dispersed SWCNTs in H₂O using DNA oligos (dT)₃₀^{18,19} which carried negative charges under current experimental conditions. We then titrated SWCNT/(dT)₃₀ with various electrolytes, and monitored the surface charge status of SWCNTs by measuring the zeta potential of the SWCNTs after addition of the electrolytes at various concentrations. We then monitored the concentration of individually-dispersed SWCNT/(dT)₃₀ left in the aqueous medium after sedimentation.

As shown in Figure 1a, top panel, the fraction of dispersed SWCNTs that remained in solution did not change significantly until the concentration of the added electrolyte reached a certain threshold, which resulted in very sharp transitions in these curves as we progressively increased the concentrations of the electrolytes. This threshold behavior depends on the type of the electrolytes added, so that the higher the valence for the counterion, the lower the threshold concentration, which follows the order of Fe³⁺ < Mg²⁺ ≈ Ca²⁺ < Na⁺. In contrast to this threshold behavior, the zeta potential of the SWCNTs displayed a gradual instead of a sharp transition for all cases (Figure 1a, bottom panel). Upon addition of various electrolytes, the surface charge of SWCNTs diminished monotonically, suggesting either the binding of counterions to SWCNTs that lead to charge neutralization or perhaps, dissociation of (dT)₃₀ from SWCNT surface. Notably, this charge decrease occurred gradually and never reached 100% even when all the SWCNTs in solution were aggregated. Comparison between Figure 1a top and bottom panels suggests that, upon addition of electrolytes, aggregation of the SWCNTs did not occur until the overall surface charge was reduced below a certain threshold. Similar results were also observed for SWCNTs dispersed by FITC, another negatively charged dispersant for SWCNTs under these conditions that we described recently (Figure 1b).²⁰

In previous studies, the explanation offered for the aggregation of SWCNTs dispersed by SDS upon salt addition is that salt reduces the solubility of SDS in water,²¹ and the loss of SDS from SWCNT surface leads to charge depletion and aggregation. To test this type of mechanism, we repeated the above experiments using SWCNTs that carried covalent carboxylic functional moieties (SWCNT-COOH). The presence of COOH groups renders these SWCNTs readily dispersed in H₂O without addition of any external dispersant molecules. As shown in Figure 1c, the fraction of dispersed SWCNTs that remained in solution decreased sharply when the concentration of the added electrolyte reached a certain threshold. Similar to Figure 1a,b, the zeta potential of the SWCNT-COOH displayed a gradual instead of a sharp transition for all cases (Figure 1c, bottom panel). This apparent charge decrease never reached 100% even when all the SWCNTs in solution were aggregated. Because -COOH groups were covalently attached to SWCNTs, this result indicates that it is the binding of counterions to SWCNT surfaces that partially neutralizes surface charge, which leads to aggregation of SWCNTs when the surface charge is neutralized beyond a threshold. This threshold pattern follows the same order of Fe³⁺ < Mg²⁺ ≈ Ca²⁺ < Na⁺, as in Figure 1a,b, although the threshold concentrations of the electrolytes were different. For SWCNT-COOH, only 0.01 mM FeCl₃ is needed to aggregate more than 90% of SWCNTs, in contrast to 0.5 mM FeCl₃ that is needed to aggregate more than 90% of SWCNTs dispersed by (dT)₃₀. This difference is consistent with a higher surface charge density for SWCNT-COOH than SWCNT/(dT)₃₀, as revealed by the zeta potential measurement for these SWCNTs before addition of counterions (Figure 1c bottom panel). The higher surface charge may afford a tighter binding for the same counterions on SWCNT surface, which leads to the difference in threshold concentrations.

The above results suggest that it is the binding of counterions to SWCNTs instead of dissociation of charged dispersant molecules that triggers aggregation when the surface charge of SWCNT is neutralized beyond a threshold. To further test this hypothesis, we dispersed SWCNTs using uncharged molecules Pluronic F 108,⁴ and titrated the dispersed SWCNTs with various concentrations of NaCl, MgCl₂, CaCl₂, or FeCl₃. As shown in Figure 1d, none of these reagents induced aggregation of SWCNTs throughout the concentrations investigated, except FeCl₃, which induced 10% aggregation at 1 mM FeCl₃ but this aggregation was less than <20% even at 1 M FeCl₃ tested. Consistent with these observations, the surface charge of SWCNTs did not change over the range of salt concentrations investigated, indicating no binding of ions to SWCNT surfaces. Because Pluronic F 108 does not carry any charges, these results reinforce the idea that the aggregation phenomenon we observed in Figure 1a-c is due to electrostatic interactions between SWCNTs and counterions in solution. These results support the idea that direct binding of counterions to SWCNT due to electrostatic interactions triggers aggregation when the surface charge is neutralized beyond a threshold.

If the above hypothesis is true, it should also be applicable to SWCNTs that carry positive instead of negative charges, and negatively charged counterions can bind SWCNT surface that eventually leads to SWCNT aggregation. To test this directly, we dispersed SWCNTs using positively-charged molecules that we described recently.²⁰ Our prediction is that upon titration of negatively-charged counterions, these SWCNTs will undergo aggregation mediated by counterion binding. As expected, for

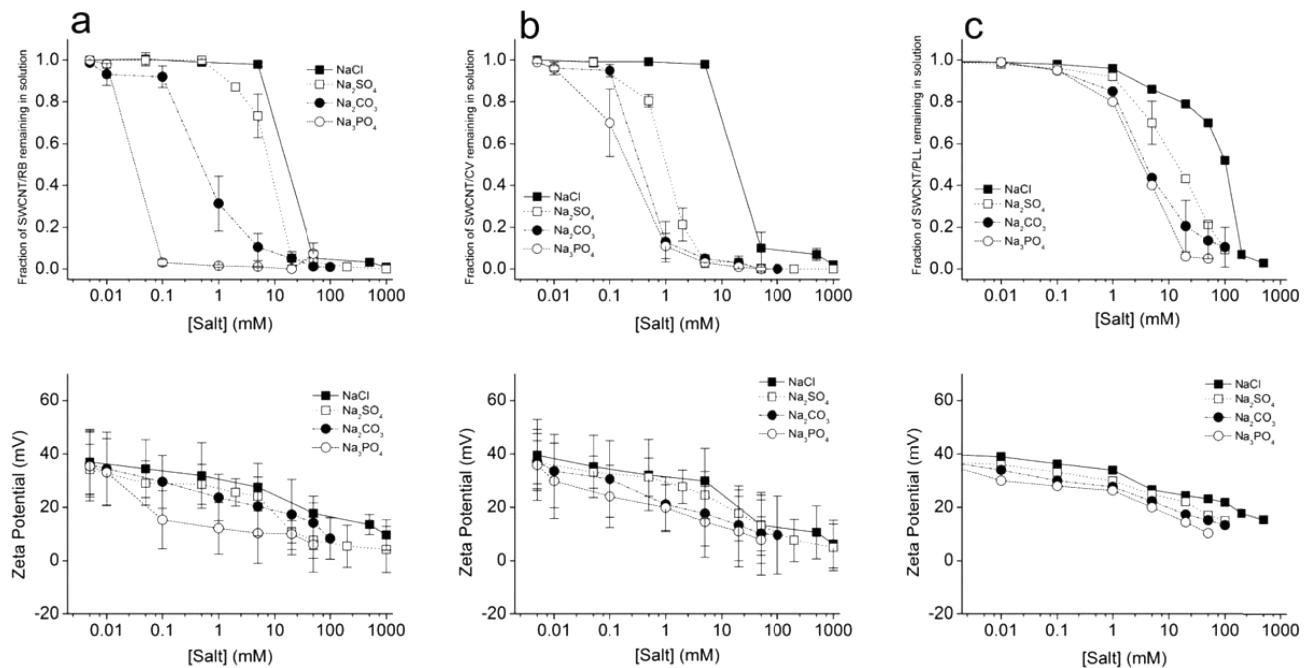


Figure 2. Aggregation of (a) SWCNT/RB (b) SWCNT/CV, and (c) SWCNT/PLL upon titration with various electrolytes and the zeta potential changes associated with the process. Error bars represent standard deviation from three independent repeats of the same experiments.

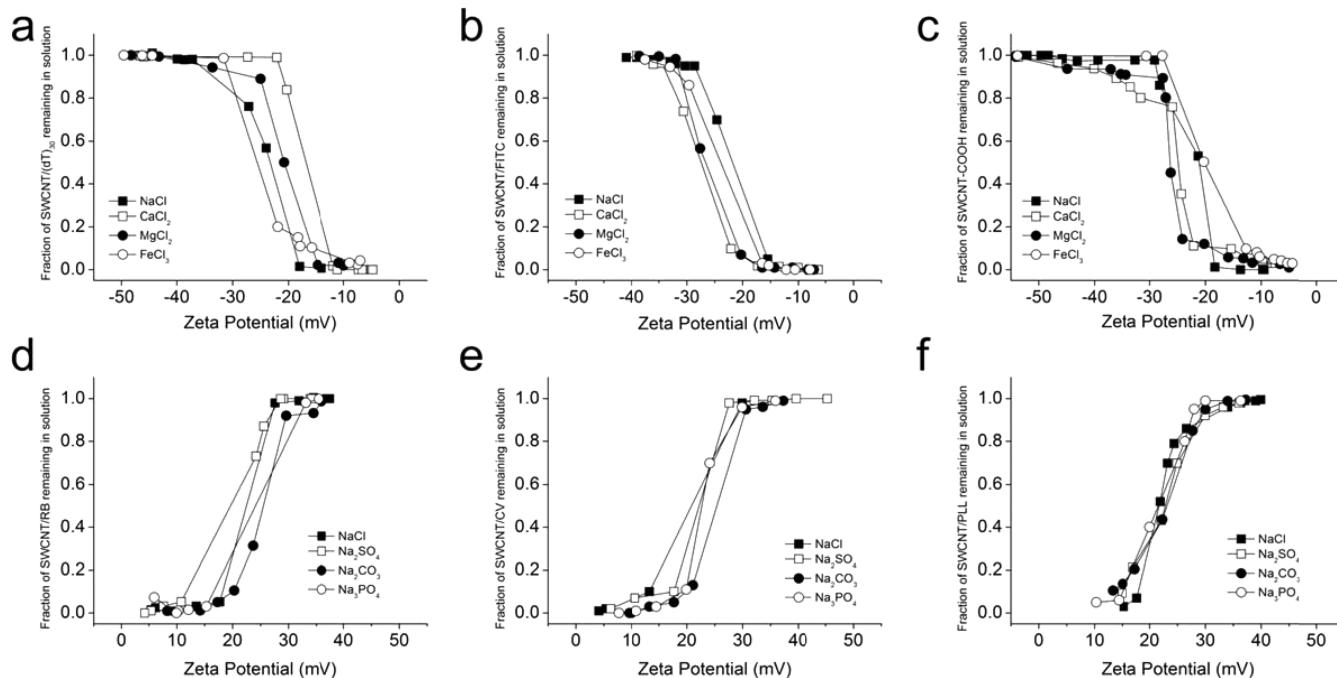


Figure 3. Fraction of individually dispersed (a) SWCNT/(dT)₃₀, (b) SWCNT/FITC, (c) SWCNT-CH₂COOH, (d) SWCNT/RB, (e) SWCNT/CV, and (f) SWCNT/PLL that remained in solution as a function of zeta potential on the SWCNT surface.

SWCNTs dispersed by RB, CV or PLL, titration of NaCl, Na₂SO₄, Na₂CO₃, and Na₃PO₄ induced aggregation of SWCNTs. As shown in Figure 2, the fraction of dispersed SWCNTs that remained in solution did not change significantly until the concentration of the added electrolyte reached a certain threshold, which resulted in sharp transitions in these curves as we increased the concentrations of the electrolytes. This threshold behavior depends on the type of the electrolytes added, which roughly follows the order of PO₄³⁻ < CO₃²⁻ < SO₄²⁻ < Cl⁻. In contrast to this threshold behavior, the zeta

potential of the SWCNTs displayed a gradual instead of sharp decrease for all cases (Figure 2, bottom panels), suggesting the direct binding of counterions to SWCNT surface. Comparison between Figure 2 top and bottom panels further indicates that aggregation of the SWCNTs does not occur until the surface charge is neutralized beyond a certain threshold. Moreover, this charge neutralization never reached 100%, even when all the SWCNTs in solution were aggregated. All these observations were consistent with our expectations and thus further support our hypothesis that counterion binding induces partial charge

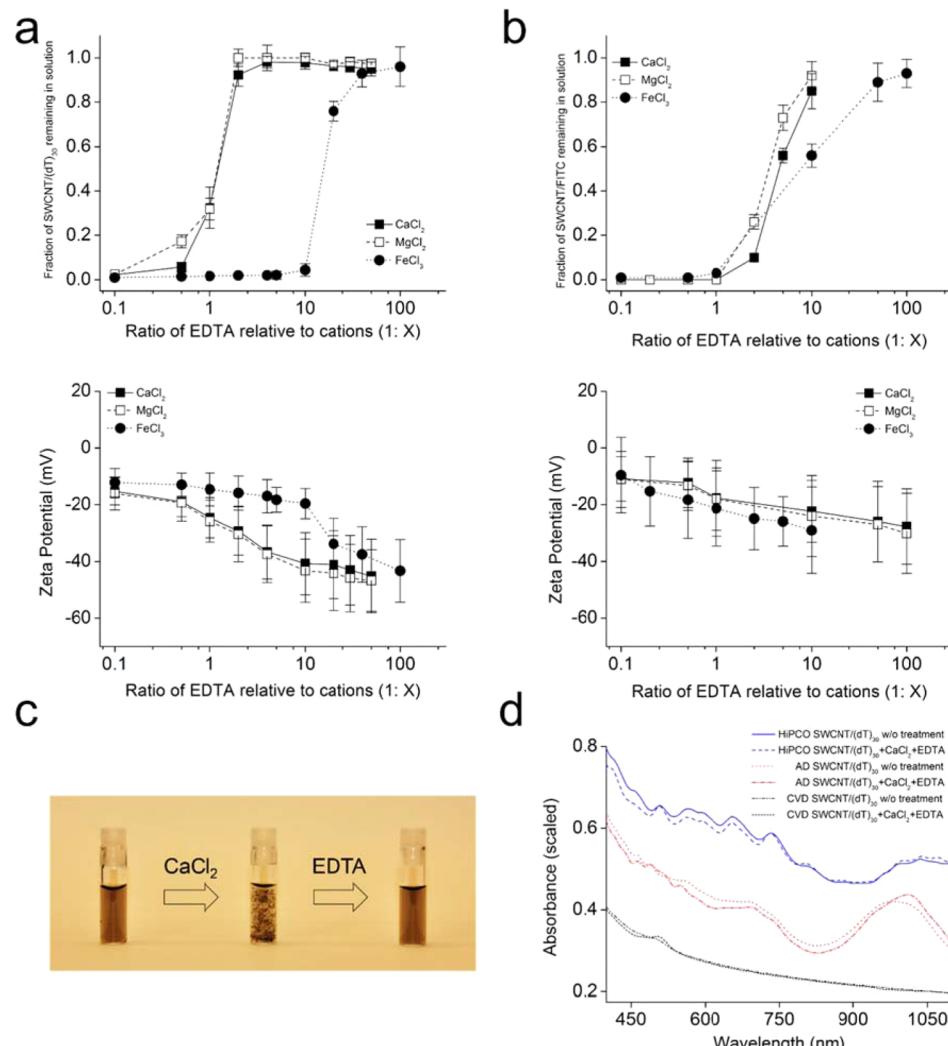


Figure 4. EDTA-mediated redispersion of aggregated (a) SWCNT/(dT)₃₀ and (b) SWCNT/FITC. The concentrations of metal ions used to induce aggregation are 0.5 mM FeCl₃, 5 mM CaCl₂, and 5 mM MgCl₂ for SWCNT/(dT)₃₀ and 1 mM FeCl₃, 5 mM CaCl₂, and 5 mM MgCl₂ for SWCNT/FITC. (c) CaCl₂- and EDTA-mediated aggregation and redispersion of SWCNT/(dT)₃₀. (d) Visible–NIR absorbance spectra of various dispersed SWCNTs before and after aggregation and redispersion. Error bars represent standard deviation from three independent repeats of the same experiments.

neutralization on SWCNT surface, which leads to SWCNT aggregation.

To quantitatively examine the dependence of SWCNT aggregation on their surface charge status, we plotted the fraction of SWCNTs that remained in solution as a function of the measured zeta potential on SWCNT surfaces for each charged dispersant molecule that we have investigated (Figure 3). Within each panel, the responses from the addition of various electrolytes were plotted for the same dispersant molecules. Notably, these plots all clustered closely to each other despite the differences in the counterions added. This result indicates that regardless of the type of counterions, SWCNTs undergo aggregation when their surface charge is neutralized beyond a threshold. The apparent plateaus in these plots permit quantitation of the zeta potential at which SWCNTs are fully aggregated. As shown in Supporting Information Table S1, for various charged dispersant molecules or groups, SWCNTs are fully aggregated when the surface charge was neutralized to an average of 80%, varying from 74 to 86%. This result is similar to DNA condensation induced by multivalent counterions, which occurs when 89–90% of the

DNA phosphate charges were neutralized by condensed counterions.^{21,22} This apparent attraction instead of repulsion between DNA molecules of the same charge is due to electrostatic correlation between screening counterions^{23–26} instead of van der Waals attractions. Thus, our results reveal the similarity between DNA condensation and SWCNTs aggregation, and SWCNTs dispersed by various charged dispersant molecules simply behave as polyelectrolytes. This property was conferred by the charges on the dispersant molecules (Figure S1). The essential feature in this model is that the aggregation of SWCNTs dispersed by these charged dispersants is driven by electrostatic interactions, instead of van der Waals or hydrophobic interactions among SWCNTs. Electrostatic attractions between individual SWCNTs of the same charge can develop as a result of correlation between screening counterions. These electrostatic interactions as mediated by the charged dispersant and counterions lead to SWCNT aggregation in solution.

Reversion of SWCNT Aggregates Induced by Metal Ions. The above model of SWCNTs aggregation indicates that interaction of counterions with dispersant groups on SWCNT

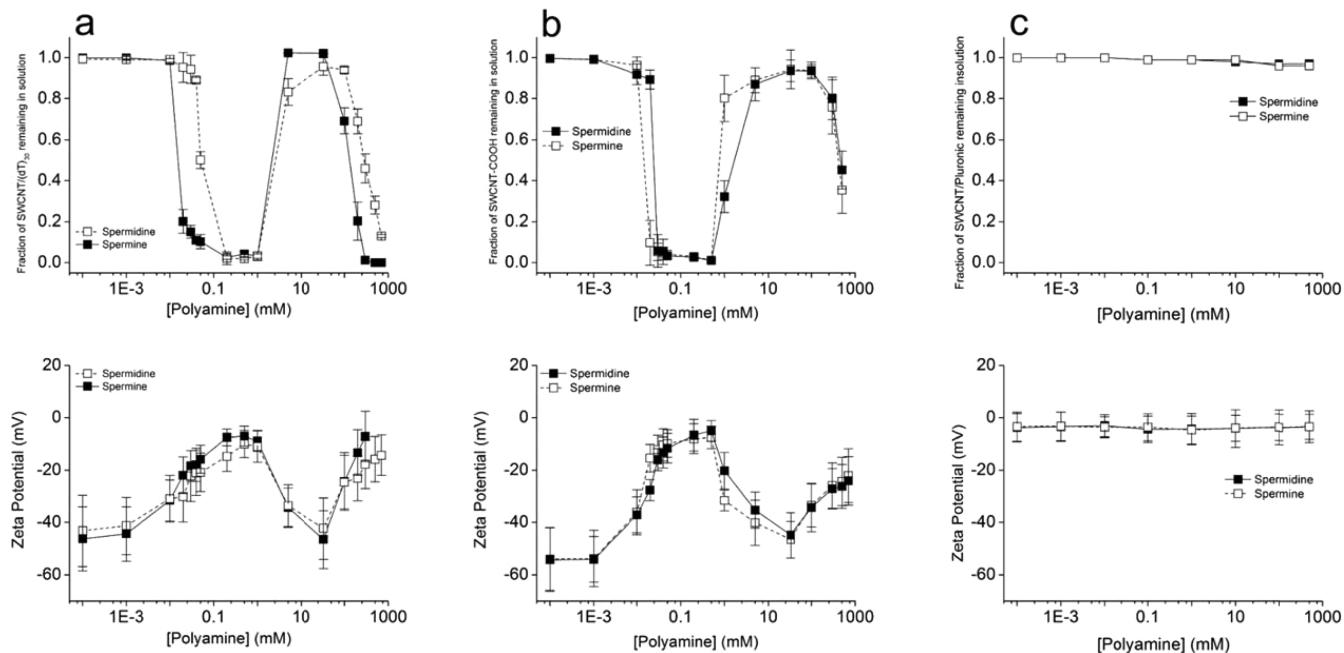


Figure 5. Spermine and spermidine concentration-dependent aggregation and redispersion of (a) SWCNT/(dT)₃₀, (b) SWCNT-COOH, and (c) SWCNT/Pluronic together with the zeta potential changes associated with the process. Error bars represent standard deviation from three independent repeats of the same experiments.

surfaces eventually leads to SWCNT aggregation. The model implies that removal of bound counterions from aggregated SWCNTs surface may lead to redispersion of SWCNTs. To test this hypothesis, we induced aggregation of SWCNTs dispersed with negatively charged dispersant molecules ((dT)₃₀ and FITC) through addition of Mg²⁺, Ca²⁺, or Fe³⁺, and then titrated in EDTA that can chelate these metal ions. As shown in Figure 4a,b, we plotted the fraction of SWCNTs that remained in solution as a function of added EDTA expressed as the ratio between [EDTA] and the concentration of corresponding metal ions. All the aggregates can be redispersed into solution upon addition of EDTA above certain threshold, consistent with the threshold phenomenon observed in aggregation experiments and suggest that this process might be fully reversible under these conditions. A camera shot for this process is shown in Figure 4c, where the middle test tube showed the formation of SWCNT aggregates, which instantaneously disappeared upon addition of EDTA above a threshold level. Examination of the UV-vis-NIR absorbance spectra of the dispersed HiPCO SWCNTs before aggregation and after redispersion revealed only small changes in peak position and very similar features for AD and CVD SWCNTs we have compared. The peak features of these spectra closely resemble those we published previously,¹⁹ suggesting that the above aggregation and redispersion process are reversible (Figure 4d). We confirmed that our dispersion procedure did not induce oxidation of SWCNTs through Raman spectra measurement.²⁰ Thus, the E₁₁ peaks displayed for HiPCO (blue spectra) may be related to the quality of the SWCNTs from the manufacturer. Control experiments using aggregates of SWCNT/RB or SWCNT/CV induced by addition of CaCl₂ (aggregation due to Cl⁻ binding to positively charged SWCNT surface) showed that addition of EDTA did not induce any redispersion of these aggregates, consistent with our expectation that only chelation of bound counterions on the surface of SWCNTs resulted in the dispersion of SWCNTs aggregates (Figure S2). We note

that the threshold concentration of EDTA required to fully disperse the aggregates vary with the type of metal ions and dispersant molecules. For example, for SWCNT/(dT)₃₀, almost stoichiometric amount of EDTA is sufficient to redisperse all the aggregates induced by addition of Ca²⁺ or Mg²⁺, but almost 10-fold higher concentration is needed to redisperse the aggregates induced by addition of Fe³⁺. These concentrations are not consistent with the binding constants for EDTA with these metal ions.²⁷ Rather, it suggests that the potential packing structures of these aggregates and the resulting accessibility of metal ions in these aggregates may partially determine the concentration of EDTA needed to redisperse them. Consistent with this view, the aggregates of SWCNT/FITC in general require a higher concentration of EDTA to redisperse than SWCNT/(dT)₃₀ (Figure 4b), even for the aggregates induced by the same metal ions. This result suggests that the overall structure of SWCNT/FITC aggregates may be more compact than that of SWCNT/(dT)₃₀. These experiments, altogether, further support our model that the aggregation is due to binding of counterions to SWCNT surface, and sequestration of the bound counterions can lead to the complete redispersion of SWCNTs in solution. Previous study has revealed that SWCNTs dispersed by PLL can be aggregated and redispersed depending on the pH, which is resulted from the change in the charge status of PLL in response to pH.⁵ Although pH is a different trigger for SWCNTs aggregation and redispersion compared to counterions in current studies, both employ similar mechanisms of electrostatic interactions to control the aggregation status of SWCNTs.

Aggregation and Redispersion of SWCNTs Induced by Polyelectrolytes. In all the above studies, the counterions we studied were monomeric in their chemical structures, i.e., all the charges were carried in a single functional group within the molecule. Under physiological conditions, electrolytes that carry multiple charged groups in a single molecule exist, such as polyamines. To examine whether these polyamines can induce

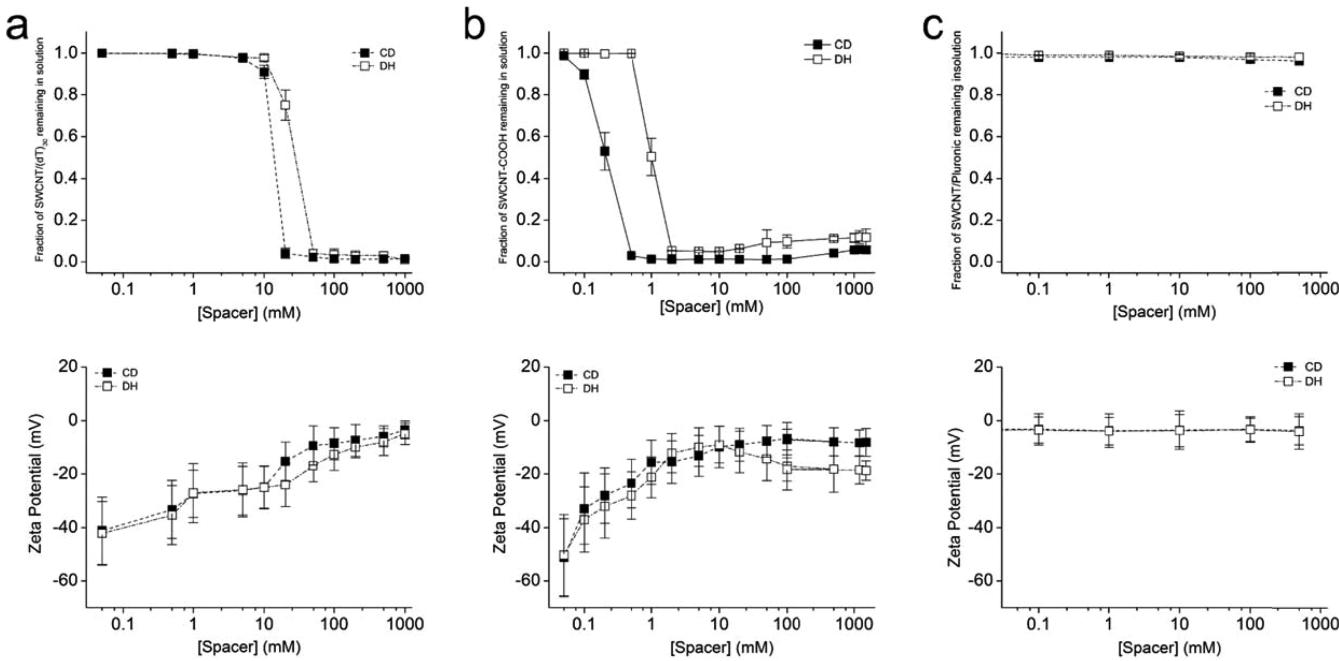


Figure 6. Cystamine dihydrochloride (CD) and diaminohexane (DH) mediated aggregation of (a) SWCNT/(dT)₃₀, (b) SWCNT-COOH, and (c) SWCNT/Pluronic. Error bars represent standard deviation from three independent repeats of the same experiments.

aggregation of SWCNTs through similar mechanisms as we demonstrated above, we prepared either SWCNT/(dT)₃₀ or SWCNT-COOH dispersion, and then tested spermidine, spermine, together with 1,6-diaminohexane (DH) and cystamine dihydrochloride (CD) for their effects on dispersed SWCNTs. As shown in Figure 5a for SWCNT/(dT)₃₀, as we titrated spermidine or spermine, SWCNTs underwent aggregation as we expected; however, as we further increase the concentration of these polyamines above 1 mM, the aggregates transiently disappeared. Further addition of polyamines led to aggregation again, so that at 1 M polyamine, almost all SWCNTs were aggregated. This phenomenon was highly reproducible for SWCNT/(dT)₃₀ (Figure 5a) or SWCNT-COOH (Figure 5b). Notably, the zeta potential we measured for SWCNTs also varied with the concentration of polyamine in phase: surface charge was neutralized to about 80% when SWCNTs were fully aggregated; the zeta potential recovered to original values when aggregates became redispersed. Control experiments using SWCNTs dispersed by Pluronic F 108 did not yield any aggregation or redispersion (Figure 5c), suggesting once again that this complex phenomenon of aggregation, redispersion, and reaggregation was caused by polyamine binding, dissociation, and rebinding.

In contrast to these observations in Figure 5a,b, experiments using either DH or CD produced simpler results. As shown in Figure 6a,b, SWCNTs underwent aggregation as we titrated either DH or CD into SWCNT dispersion. Almost all SWCNTs were aggregated at 100 mM DH or CD, and no transient redispersion was observed throughout this process. Control experiments using SWCNT/Pluronic showed no aggregation throughout the concentrations of DH or CD tested (Figure 6c). Because DH and spermidine only differ slightly in their chemical structures: spermidine has one extra $-\text{CH}_2-$ and one extra $-\text{NH}-$ group that carried one more positive charge than DH under current solution conditions (Chart S1),^{28,29} we reasoned that the above process of aggregation started with the direct binding of the positively

charged amino groups at the ends of these molecules to SWCNT surfaces. This is possible because these positive charges serve as counterions for the negative charges on the surface of SWCNTs, either due to the DNA phosphate backbone or $-\text{COOH}$ group that was covalently attached to the SWCNT surface. However, more importantly, these molecules can induce SWCNT aggregation through bridges mediated by the charged groups at both ends of these chain molecules. This bridging effect is favored by entropy, as these chain molecules have more degrees of freedom upon bridging between two individual SWCNTs, in contrast to the binding of both ends of the molecule to the same tube.^{30,31} For both spermidine and spermine but not DH or CD, due to the presence of additional positive charges, the increasing concentration of polyamines in solution increases the ionic strength of the solution, so that these entropic bridge interactions are weakened and collapsed (as shown in Figure S3), consistent with the recovery of surface charge monitored by zeta potential (Figure 5a,b bottom panel). This collapse of entropic bridges leads to the transient redispersion of SWCNT aggregates. This collapse does not occur to either DH or CD due to their lower charge status as compared to either spermidine or spermine.

To directly test this model of SWCNT aggregation induced by addition of polyelectrolytes, we first focused on the bridging effect. We used CD and DH to induce aggregation of either SWCNT/(dT)₃₀ or SWCNT-COOH. We then titrated the aggregates with either DTT or 2-mercaptoethanol. If the bridging effect is responsible for SWCNT aggregation, we would expect a redispersion of SWCNT aggregates formed with CD upon addition of either DTT or 2-mercaptoethanol, because both reducing agents can reduce CD and thus break the molecule into two separate parts. In contrast, aggregates formed with DH should remain intact because DH contains no disulfide bonds that can be reduced. As we expected, more than 80% of the aggregates formed with CD could be redispersed upon addition of either DTT or 2-mercaptoethanol. This was

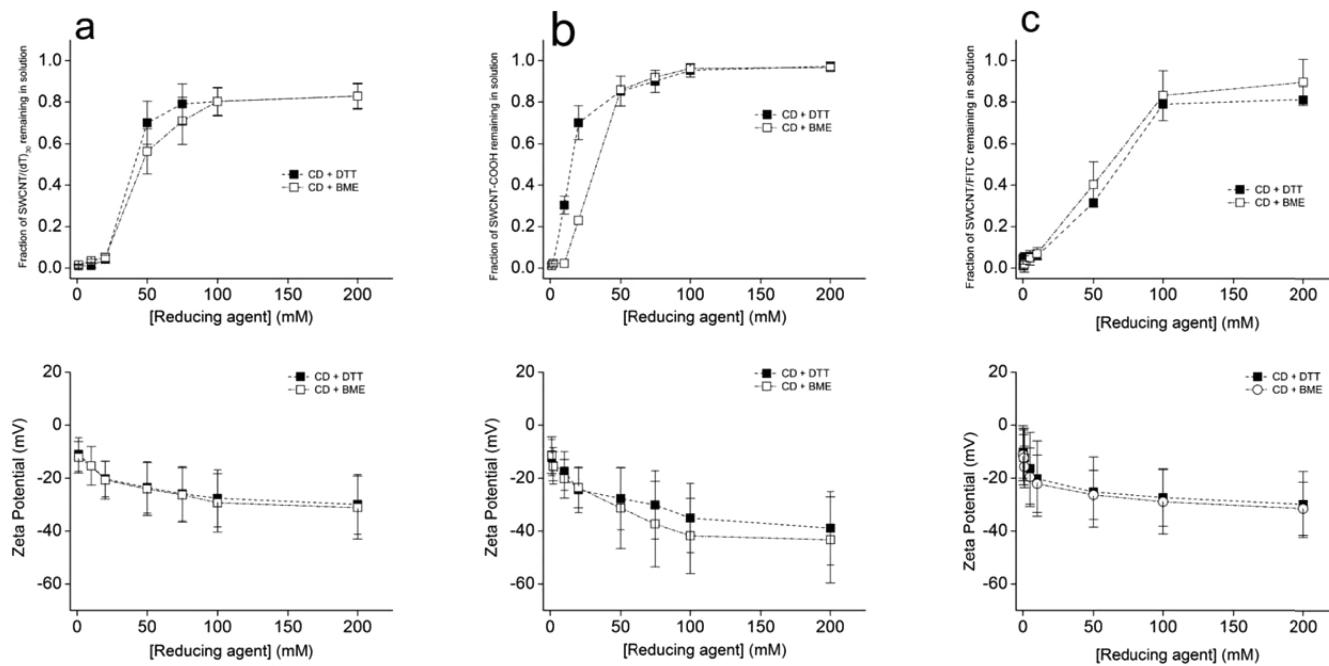


Figure 7. Disulfide bond reducing agents, DTT or 2-mercaptoethanol (BME), mediated redispersion of (a) SWCNT/(dT)₃₀, (b) SWCNT-COOH, and (c) SWCNT/FITC aggregates induced by addition of CD. The corresponding zeta potential changes associated with the process were shown in bottom panels. Error bars represent standard deviation from three independent repeats of the same experiments.

true for both SWCNT/(dT)₃₀ and SWCNT-COOH (Figure 7). In contrast, the aggregates formed with DH remain intact throughout the concentrations of both reducing agents used (Figure S4). These results directly support our hypothesis that the initial SWCNT aggregates formed in the presence of these polyelectrolytes are mediated by bridging interactions.

We then focused on the transient redispersion of SWCNT aggregates upon addition of medium concentrations of spermidine or spermine, which was absent for either CD or DH. To directly examine the transient nature of this redispersion, we first induced SWCNT/(dT)₃₀ aggregates using polyamine at concentrations of 0.5 mM. We then titrated the mixture with increasing concentrations of NaCl ([NaCl]). Interestingly, increasing [NaCl] first reduced aggregation. At 200 mM NaCl, almost all SWCNT aggregates were redispersed (Figure 8). Further addition of NaCl induced aggregation again. These observations were very similar to the redispersion and aggregation of SWCNTs induced by either spermidine or spermine as shown in Figure 5 and suggests that the transient redispersion is due to increased ionic strength in solution that weakened the entropic bridge interactions. The corresponding zeta potential measurement for this process further support this notion. The zeta potential first decreased upon addition of NaCl, indicating dissociation of bound polyamine molecules. This was then followed by a steady increase in zeta potential, which resulted from the reassociation of counterions to SWCNTs at high concentrations and induced reaggregation of SWCNTs.

This bridging effect as we observed for polyelectrolytes suggests a possible application of these molecules to induce reversible aggregation and redispersion of SWCNTs that can be controlled through chain breaking. To test this possibility, we synthesized oligopeptides that are flanked by either two positive lysine residues or two negative glutamic acid residues on the two ends of the peptides (Chart S1). As expected, the positively charged peptide KA₈K can induce aggregation of either

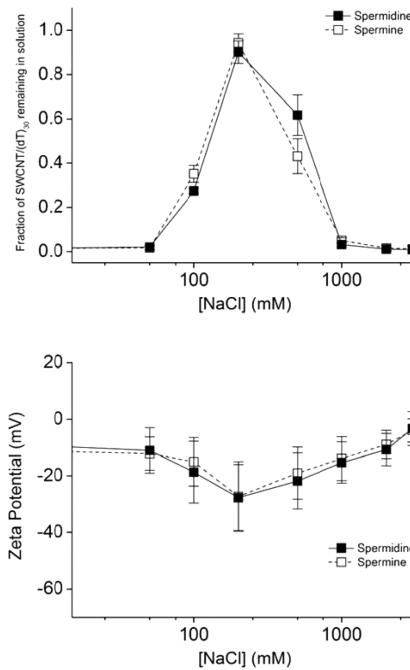


Figure 8. Redispersion of SWCNT/(dT)₃₀ aggregates induced by polyamines upon titration with NaCl. Error bars represent standard deviation from three independent repeats of the same experiments.

SWCNT/(dT)₃₀ or SWCNT-COOH, while the negatively charged peptide EA₈E can induce aggregation of either SWCNT-CV or SWCNT-PLL, as shown in Figure 9 together with the zeta potential measurement results for this titration process. Under conditions where we induced full aggregation of SWCNTs, we treated the SWCNT aggregates with either trypsin that can cleave the KA₈K peptide or protease K that can cleave the EA₈E peptide. As shown in Figure 10, greater than 50% of SWCNT aggregates could be redispersed upon addition

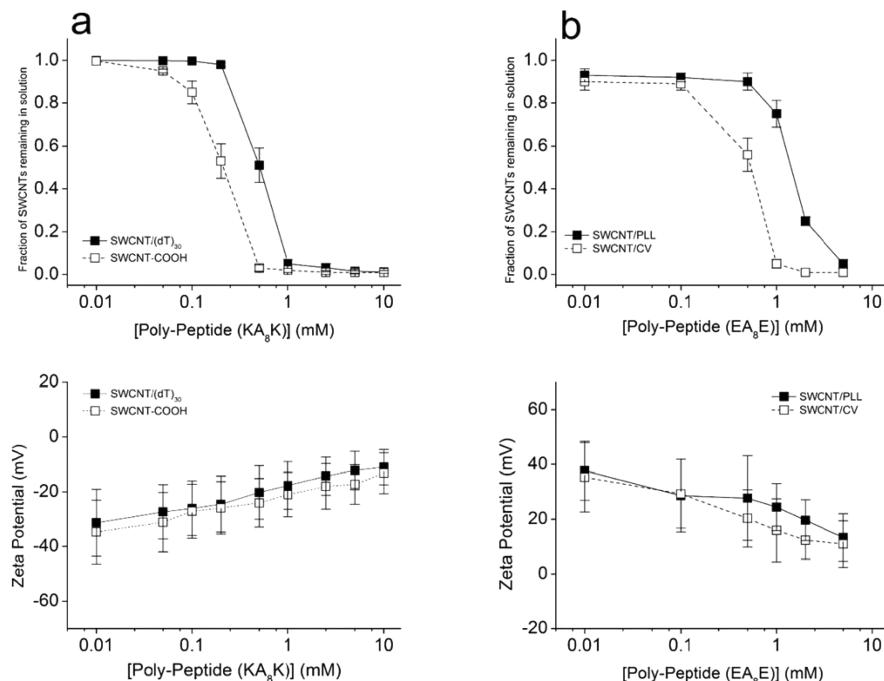


Figure 9. Fraction of individual (a) negatively charged SWCNTs and (b) positively charged SWCNTs that remained in solution after addition of polypeptides (KA_8K for negatively charged and EA_8E for positively charged SWCNTs). Error bars represent standard deviation from three independent repeats of the same experiments.

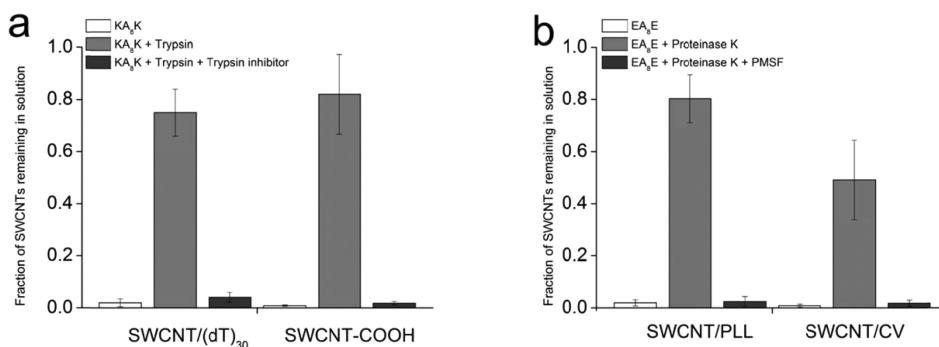


Figure 10. Enzyme mediated redispersion of (a) SWCNT/(dT)₃₀ and SWCNT-COOH aggregated by polypeptide (KA_8K) and (b) SWCNT/PLL and SWCNT/CV aggregated by polypeptide (EA_8E) and the inhibition of this redispersion by enzyme inhibitors. Error bars represent standard deviation from three independent repeats of the same experiments.

of these protease enzymes for SWCNTs initially dispersed with various molecules. This redispersion was due to protease action because inclusion of either trypsin inhibitor or PMSF (which inhibits protease K) completely blocked the redispersion of SWCNT aggregates (Figure 10).

Aggregation of SWCNTs in Cell Culture Media. The above results on SWCNT aggregation, as we observed for both monomeric electrolyte and polyelectrolyte molecules, bear direct relevance for the potential applications of SWCNTs as delivery vehicles for genes into the cells.^{32–34} When SWCNTs are conjugated with nucleic acids, they can undergo aggregation and redispersion as a result of interactions with their counterions. Notably, the concentrations of counterions that are required to induce aggregation of SWCNTs under current experimental conditions are close to the concentrations of these ions *in vivo*.^{35,36} Thus, SWCNTs conjugated with DNA or other charged molecules may well undergo aggregation *in vivo* that could lead to toxicity in cells and tissues.⁴ To test this aggregation, we incubated dispersed SWCNT/(dT)₃₀ or

SWCNT-COOH with varying fractions of tissue culture media under various conditions. As expected, majorities of SWCNTs precipitated out of the solution with 50% culture media in the solution (Figure S5). In contrast, controls using SWCNTs dispersed with Pluronic F 108 underwent little aggregation even when the solution contains 80% of culture media. Thus, our results suggest that the application of SWCNTs in tissue culture experiments has to consider the potential aggregation due to interactions with counterions.

CONCLUSIONS

Here we have investigated the properties of aggregation and redispersion of SWCNTs in aqueous medium. For SWCNTs that are dispersed into aqueous medium assisted by charged molecules, addition of electrolytes can induce their aggregation that is reversible under certain conditions. Electrolyte molecules act through direct binding to the charged SWCNT surface as counterions. Neutralization of the SWCNT surface charge by 74 to 86% leads to aggregation of SWCNTs. This aggregation

is driven by electrostatic attractions instead of van der Waals or hydrophobic interactions due to correlations between screening counterions, similar to the mechanisms of DNA condensation induced by multivalent cations. Polyelectrolyte can induce SWCNT aggregation through molecular bridging, which can be utilized to engineer the aggregation and redispersion of SWCNTs in solution by exploiting various chain breaking mechanisms. Our data suggest that SWCNTs can be aggregated during *in vitro* as well as *in vivo* applications as gene delivery vehicles, which may lead to toxicity of these nanomaterials *in vivo*. Our method of redispersing aggregated SWCNTs could be potentially used to control the aggregation status of SWCNTs within biological systems. The mechanisms that we identified for SWCNT aggregation have broad implications on various applications of SWCNTs in water.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary chart and supplementary figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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