Nanotubes

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Integration of a Self-Assembling Protein Scaffold with Water-Soluble Single-Walled Carbon Nanotubes**

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Self-assembling proteins offer exciting opportunities as scaffolds that can position functional components with nanometer resolution.[1] In particular, this concept has been demonstrated through the use of viral capsids[2] to template the growth of inorganic materials[3] and to orient numerous molecular building blocks[3d,4–7] with well-defined spatial relationships. For device integration, a crucial next step is the development of strategies to interface these components with electronic materials. This is a challenging endeavor because of the differences in solubility between inorganic materials and proteins, as well as the limited range of processing conditions that are tolerated by biological components. Viral capsids have been attached onto metal surfaces through cysteine bioconjugation to maleimide-terminated monolayers,[7b] histidine/Ni^{2+} interactions,[7c] and dip-pen nanolithography.[7d] As an alternative platform, we sought to integrate the rich electronic properties of single-walled carbon nanotubes (SWCNTs)[8] with tobacco mosaic virus (TMV) capsids by using well-defined chemical linkages. As we have recently reported the efficient self-assembly of light-harvesting systems with chromophore-labeled TMV coat proteins,[7a] this attachment strategy could lead to a new class of hybrid photonic materials.

The cylindrical protein coat of TMV is 18 nm in diameter and 300 nm in length. The capsids can be obtained in low polydispersity (Figure S2 in the Supporting Information) through propagation in tobacco plant hosts and are thermally stable in aqueous buffer up to 75 °C. As we have previously shown,[4c,7a] S123C TMV mutants can be functionalized in three different locations by using chemoselective reactions to provide the attachment sites needed to prepare multicomponent nanoscale materials. A diazonium coupling reaction on the exterior of the cylinder was used to introduce thousands of ketone functional groups,[4c] allowing further bioconjugation through oxime formation with alkoxyamines (see Figure 2). This modular two-step strategy provides a convenient means to control the surface interactions of the capsids and was therefore used for SWCNT attachment.

A number of strategies have become available to allow SWCNTs to be dissolved in water.[9,11] Surfactants,[9,11c] polymer wrapping,[12] bifunctional polymers,[13] biomaterials,[14] and biomimics[15] have all yielded unbundled SWCNTs in aqueous solution. In particular, pyrene-bearing molecules have been proven effective for binding SWCNT sidewalls through noncovalent interactions. The binding energy of pyrene to the surface of a SWCNT has been calculated to be 0.42 eV (ca. 9.7 kcal mol^{-1}), which suggests an equilibrium constant for adsorption of approximately 1×10^7 M^{-1}.[16a] As such, pyrenes bearing hydrophilic groups (such as ammonium ions,[16b,c] carboxylates,[17] and block co-polymer components[18]) can give rise to tubes with aqueous solubility. Similarly, reactive pyrene derivatives have been used to elaborate the sidewalls of surface-grown SWCNTs to create biomolecular sensors.[19] In this manner, porphyrins,[13a,20] enzymes,[21] nanoparticles,[22] and fullerenes[17] have been associated with SWCNTs on surfaces.

Guided by these successes, we report the design and synthesis of a multifunctional pyrene-anchored polymeric surfactant that can transfer SWCNTs to aqueous solution and participate in the attachment of TMV capsids. This new surfactant compares well with other methods[9] for nanotube solubilization in water and provides an alkoxyamine handle for attachment to proteins modified by the growing set of site-selective bioconjugation reactions that are capable of introducing ketone groups.[10]

Synthesis of *O*-pyrenyl-*O′*-aminooxypoly(ethylene glycol) (**3a**, PPAA) proceeded by treating poly(ethylene glycol) ($M_r = 1900$–2200) under Mitsunobu conditions with *N*-hydroxyphthalimide, followed by liberation of the alkoxyamine group via hydrazinolysis (Scheme 1). One equivalent of pyrenecarboxaldehyde was then coupled to the bis(alkoxyamine) by oxime formation. The monopyrene conjugate was purified by reversed-phase chromatography on C-18 resin. The resulting polymer was observed to degrade over the course of one week in aqueous solution and therefore was used immediately after purification or stored under N_2 as a dry solid. Synthesis of *O*-pyrenyl-*O′*-methylpoly(ethylene glycol) (**3b**, PPME), a control surfactant that does not react with ketones, proceeded with similar efficiency.

Solubilized SWCNTs were prepared by adapting previously reported procedures.[11a,c] HiPco SWCNTs (CNI, Inc.) were dissolved at 1 mg mL^{-1} in 5 mM PPAA or PPME in water by subjecting them to bath sonication at room temperature

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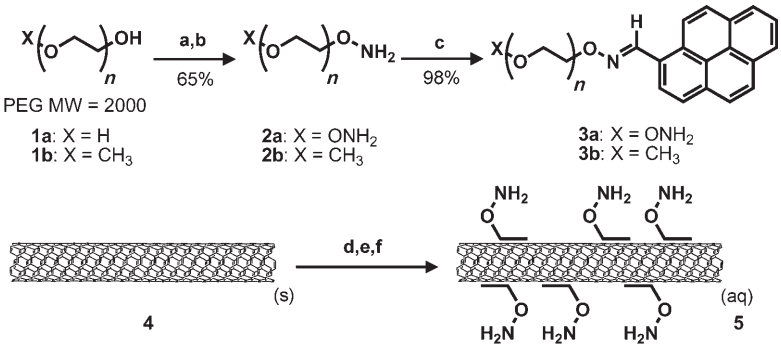


Scheme 1. Synthesis of ketone-reactive pyrene surfactants and generation of water-soluble, ketone-reactive SWCNTs: a) Two iterations of *N*-hydroxyphthalimide, DIAD, PPh_3, CH_2Cl_2; b) aqueous H_2NNH_2, CH_2Cl_2; c) pyrenecarboxaldehyde, 10 mol% TFA, CH_2Cl_2; monopyrene conjugate **3a** was purified by reversed-phase chromatography on C-18 resin; d) 5 mM **3a** or **3b** in deionized H_2O, sonication (100 W, 42 kHz, 1 h); e) centrifugation (rcf 57000 *g*, 1 h), isolation of top 80% of supernatant; f) when required, removal of excess surfactant by gel filtration was achieved by using sephadex S-1000 SF resin preequilibrated with either deionized H_2O or 100 μM **3a** to yield reactive SWCNT **5**. DIAD = diisopropylazodicarboxylate; TFA = trifluoroacetic acid.

for 1 h. The use of lower concentrations of **3a** or **3b** did not lead to appreciable nanotube solubilization (data not shown). Following sonication, the sample was centrifuged at a relative centrifugal force (rcf) of 57000 *g* for 1 h to remove the bundled tubes. Vis/NIR characterization of the upper 80% of the resulting nanotube solutions indicated similar spectral properties to SWCNTs solubilized with sodium dodecylbenzene sulfonate (SDBS) (Figure S5 in the Supporting Information). Exposure of SWCNTs to the individual components that make up PPAA and PPME showed no solubilization of the nanotubes in water, highlighting the unique character of the monopyrene conjugates (Figure S4 in the Supporting Information). Nanotubes dissolved in this fashion were stable toward aggregation over the course of weeks.

Purified SWCNTs dissolved in **3a** or **3b** were solubilized primarily as single tubes and small bundles, on the basis of atomic force microscopy (AFM) and transmission electron microscopy (TEM) analysis (Figure 1). TEM of the nanotubes (negatively stained with uranyl acetate) showed characteristic tube lengths in the range 500–2000 nm. AFM revealed tube diameters in the range 2.0–5.5 nm. The increased diameter is in part attributed to the polymeric coating on the tube surface, as has been observed previously for peptide surfactants.[14c]

A simple protocol was developed for the removal of unbound **3a**, which resulted from the use of excess polymer in the tube solubilization process. A size-exclusion column with a high molecular-weight cutoff resin (1000 kDa) was prepared. Early eluting fractions of SWCNT samples in deionized water contained minimal free polymer, as observed by the loss of around 98% of the pyrene fluorescence of the solution (measured at 410 nm and normalized to SWCNT absorbance at 742 nm; Figure S6 in the Supporting Information). Nanotube samples prepared in this fashion were stable toward aggregation for up to two days in water. For applications requiring buffered aqueous solution (up to 100 mM), improved stability was observed by eluting the nanotubes from columns preequilibrated with 100 μM surfactant.

Tyrosine 139 of the TMV capsid was modified with the diazonium salt of *p*-aminoacetophenone in greater than 90% yield, giving rise to approximately 2000 exterior ketones (k-TMV; Scheme 2).[4c] Exposure of these capsids to PPAA resulted in an SDS-PAGE shift (after disassembly; SDS-PAGE = sodium dodecylsulfate polyacryl-

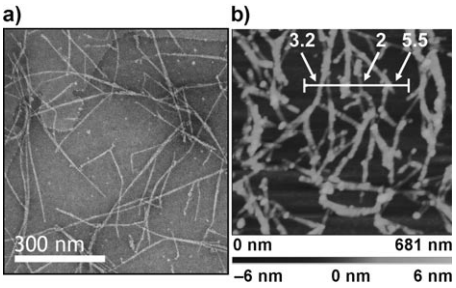
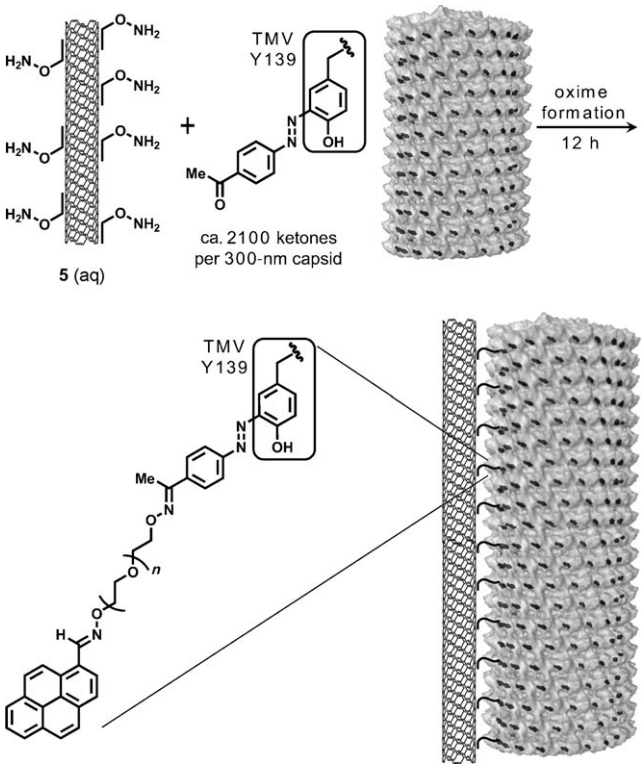


Figure 1. Characterization of HiPco SWCNTs solubilized by **3a**: a) TEM image of the SWCNTs in water (stained with $UO_2(OAc)_2$) showing an even dispersion of tubes; b) AFM height image of SWCNTs in **3a**. Arrows indicate the location of measured heights.



Scheme 2. Modification of SWCNTs with ketone-modified tobacco mosaic virus (k-TMV) capsids. Structures were formed by treating **5** with intact k-TMV capsids (80 μM in deionized H_2O) overnight in deionized H_2O or 100 μM **3a**.

amide gel electrophoresis) that indicated 70% conversion to the monoconjugate, as measured by densitometry (Figure S3 in the Supporting Information).

In a similar fashion, the ketone groups on the capsids were attached to alkoxyamines on the solubilized SWCNTs through oxime formation. TEM analysis of the resulting reaction solution indicated a substantial fraction of TMV–SWCNT hybrids in close contact with parallel alignment (Figure 2a), with some unconjugated capsids remaining in solution. In addition to the expected 300-nm rods, a significant number of the SWCNT-bound capsids were oriented in a

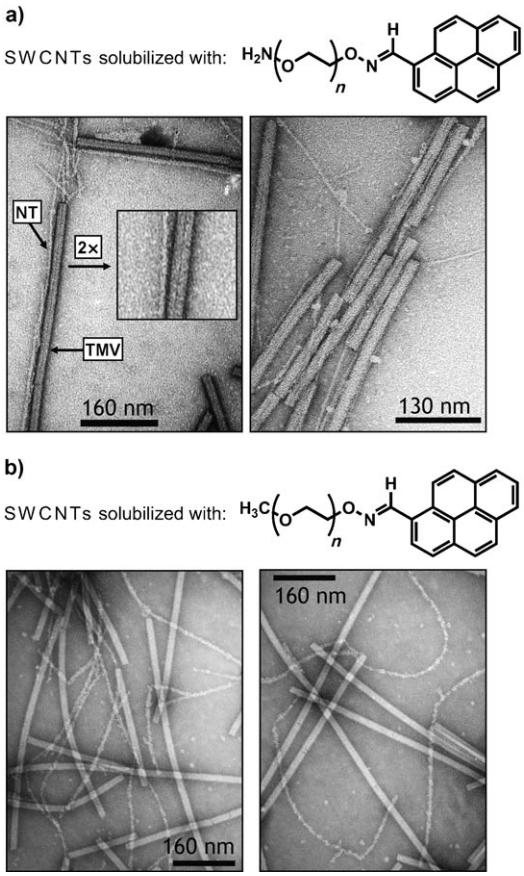


Figure 2. Alignment of k-TMV capsids and SWCNTs. a) The parallel alignment in PPAA highlights the specificity of the oxime formation. b) Control experiments in which SWCNTs were dissolved in PPME show no linearity between the TMV capsids and the nanotubes.

contiguous end-to-end fashion. This aggregation behavior has also been reported in the presence of divalent cations.[23] The resulting SWCNT–TMV conjugates were observed to retain their water solubility. Interestingly, equivalent alignment was observed even when free **3a** was present in solution (up to 5 mM). Although the free polymer would be expected to compete for binding to TMV ketones, the numerous alkoxyamines displayed by the SWCNTs are believed to bind more efficiently due to cooperative effects. Control experiments using surfactant **3b** demonstrated the necessity of the alkoxyamine–ketone pair, as only nonspecific deposition of both components was apparent on the surface of the TEM grid (Figure 2b). Similarly, binding was not observed for PPAA-decorated tubes upon exposure to TMV capsids lacking ketone groups (data not shown).

These new polymeric surfactants provide a convenient means to attach ketone-labeled biomolecular platforms to electroactive components through well-defined chemical linkages. We are currently attaching light-harvesting TMV capsids[7a] to SWCNTs that bridge electrodes with the goal of preparing hybrid photovoltaic materials.[21,24] We are also exploring the generality of this approach by attaching proteins to SWCNTs through the use of other site-selective bioconjugation reactions that introduce ketone groups.

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