FEATURE ARTICLE

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Advances toward bioapplications of carbon nanotubes

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Bioapplications of carbon nanotubes have been predicted and explored ever since the discovery of these one-dimensional carbon allotropes. Indeed, carbon nanotubes have many interesting and unique properties potentially useful in a variety of biological and biomedical systems and devices. Significant progress has been made in the effort to overcome some of the fundamental and technical barriers toward bioapplications, especially on issues concerning the aqueous solubility and biocompatibility of carbon nanotubes and on the design and fabrication of prototype biosensors. In this article we take a comprehensive look at the advances in this fast-moving and exciting research field. We review the current status of available methodologies for the aqueous dispersion and solubilization of carbon nanotubes, discuss the results on modifications of carbon nanotubes with various biological and bioactive species, and highlight some of the recent achievements in the fabrication and evaluation of carbon nanotube-based bioanalytical devices.

Introduction

Carbon nanotubes are well-ordered, all-carbon hollow graphitic nanomaterials with a high aspect ratio, lengths from several hundred nanometers to several micrometers and diameters of 0.4–2 nm for single-walled (SWNT) and 2–100 nm for coaxial multiple-walled (MWNT) carbon nanotubes.[1] Conceptually the nanotubes are viewed as "rolled-up" structures of one or multiple layers of graphene sheets for SWNTs and MWNTs, respectively. These one-dimensional carbon allotropes are of high surface area, high mechanical strength but ultra-light weight, rich electronic properties, and excellent chemical and thermal stability.[2]

Even since the discovery of carbon nanotubes, researchers have been exploring their potential in bioapplications.[3–7] One focal point has been the development of nanoscale biosensor and bioreactor systems based on carbon nanotubes, which has been driven by the experimental evidence that biological species such as proteins and enzymes can be immobilized either in the hollow cavity or on the surface of carbon nanotubes.[3,4] Recently, hopes have been raised for the use of carbon nanotubes as superior biosensor materials in light of the successful fabrication of various electroanalytical nanotube devices, especially those modified by biological molecules.[8–11] These prototype devices, sometimes prepared as ordered arrays or single-nanotube transistors, have shown efficient electrical communications and promising sensitivities required for such applications as antigen recognition,[9] enzyme-catalyzed reactions[10] and DNA hybridizations.[11]

Another example for biorecognition is the scanning probe microscopy mapping of biofunctional receptors by using a carbon nanotube probe functionalized at the tip with biologically specific ligands.[5] There is also preliminary experimental evidence suggesting that carbon nanotubes may act as electromechanical actuators for artificial muscles[6] and that upon functionalization with suitable bioactive molecules carbon nanotubes may serve as feasible substrates for neuronal growth.[7]

For biocompatibility evaluations toward biological and biomedical applications, the lack of solubility of carbon nanotubes in aqueous media has been a major technical

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barrier. The recent bloom of chemical modification and functionalization methods has made it possible to solubilize and disperse carbon nanotubes in water, thus opening the path for their facile manipulation and processing in physiological environments. Equally important is the recent experimental demonstration that biological and bioactive species such as proteins, carbohydrates, and nucleic acids can be conjugated with carbon nanotubes. These nanotube bioconjugates will play a significant role in the research effort toward bioapplications of carbon nanotubes.

In this article we first review the current status of available methodologies for the aqueous dispersion and solubilization of carbon nanotubes, and then discuss the results on modifications of carbon nanotubes with various biological and bioactive species.

1. Dispersion upon oxidative acid treatments

As-produced SWNTs, regardless of the production procedure, usually contain amorphous carbon, carbon nanoparticles, and residues from the metal catalysts. For most applications, purification of as-produced materials is required. One of the most commonly used purification methods involves oxidative acid treatment steps, such as refluxing in dilute nitric acid or refluxing/sonication in a concentrated H_2SO_4/HNO_3 mixture.[12] An interesting phenomenon during such oxidative acid treatments is that after several washing/centrifugation cycles in the effort to remove excess acid, dark supernatant solution can be obtained.[13] Rinzler *et al.* attributed the composition of the dark solution to carboxylated carbonaceous materials, which were considered as the decomposed products of the impurities in the raw SWNT soot and were well solvated upon deprotonation as a consequence of favorable electric double-layer effects.[13] However, it became known that similar strong acid treatments, by generating surface defects and sometimes resulting in tube shortening, can also provide abundant carboxylated sites along the nanotube surface and shortened tube ends.[14,15] Some of the heavily oxidized SWNTs may then be stabilized in aqueous suspensions through a similar mechanism.[16–21]

Sano *et al.* found that the coagulation time constant for the shortened SWNT aqueous colloids (0.1–0.3 mg mL^{-1}) in 10 mM NaOH is around 20 days, much slower than those found in THF and $CHCl_3$ in which the nanotubes were essentially non-dispersible.[18] Some oxidative procedures may generate SWNT colloid dispersions with concentrations of up to $\sim$2 mg mL^{-1} at pH $\sim$ 3 and stable over a year,[17] or produce SWNT mats that are readily soluble in various aqueous buffers with a wide pH range from 3–12.[20]

Recently, Kovtyukhova, Mallouk and coworkers adopted techniques developed for graphite oxide synthesis to produce stable SWNT aqueous dispersions by using concentrated H_2SO_4 with $(NH_4)_2S_2O_8$ and P_2O_5, followed by treatment of H_2SO_4 and $KMnO_4$.[21] They found that after a brief settlement period, stable oxidized SWNT dispersions at concentrations above 0.3 wt% may form hydrogels due to the rich hydrogen bonding in between the exfoliated nanotubes. Similar hydrogel formation has also been observed for chemical vapor deposition (CVD)-produced MWNTs upon suitable oxidative acid treatments.[22,23]

Results from investigations on the Schulze–Hardy rule (interplay between van der Waals attraction and electric double-layer repulsion of colloidal particles) of such SWNT aqueous colloids suggest that the interactions between SWNTs in electrolyte solutions may be reduced to a simple solid sphere model when the ionic strength is close to the critical coagulation concentration (ccc).[19] Thus, practical applications of the SWNT colloid suspensions should be carried out below these critical ionic conditions to make use of the high surface areas of the nanotubes and prevent tube coagulation.

2. Non-covalent stabilization

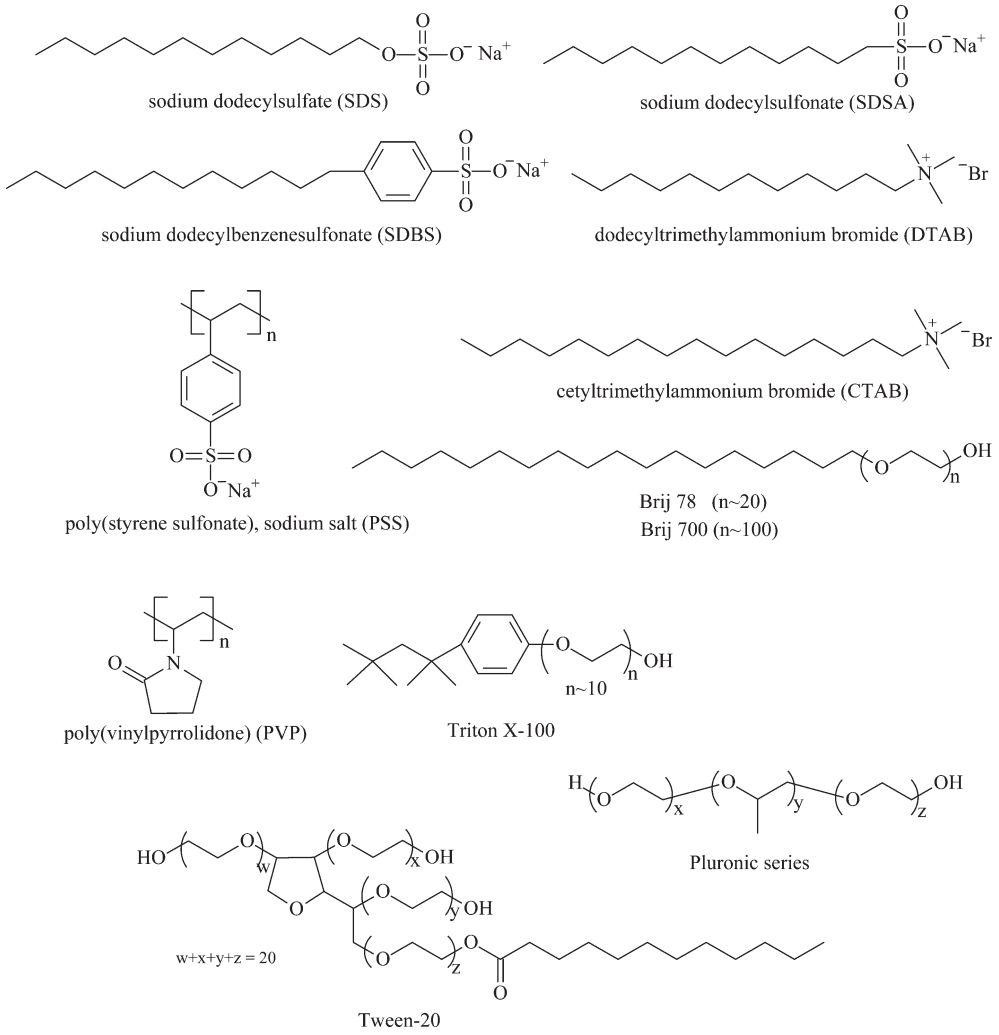
Many researchers have recommended the approach of non-covalent stabilization of carbon nanotubes in solution because the nanotube structures and properties can usually be preserved after the dispersion. In addition, the dispersion procedures are often straightforward, involving only ultrasonication and centrifugation or filtration. Hydrophobic interactions and favorable π–π interactions (or "π–π stacking") in water between absorbates and carbon nanotubes (sometimes with electrostatic interactions between ionic adsorbates) and supramolecular inclusion are the most frequently suggested mechanisms for non-covalent stabilization. Below, we discuss these non-covalent surfactant- and polymer-assisted aqueous nanotube dispersions. It should be noted, however, that there is no clear boundary between surfactants and polymers in such uses.

2.1. Surfactant-assisted dispersion

Processing carbon nanotubes in aqueous media has been largely dependent on the use of surfactants because of their ready commercial availabilities, low costs, and relatively simple experimental procedures (Scheme 1). Islam *et al.* evaluated various SWNT aqueous suspensions assisted by a range of surfactants with optimum nanotube–surfactant ratios of 1 : 5 to 1 : 10 by weight.[24] In that study, the widely used surfactants sodium dodecyl sulfate (SDS) and Triton X-100 suspended SWNTs to 0.1 (SDS) and 0.5 mg mL^{-1} (Triton X-100) upon ultrasonication. However, the suspensions were stable for less than a week. Sodium dodecylbenzene sulfonate (SDBS, or NaDDBS), on the other hand, was found to stabilize SWNTs up to 20 mg mL^{-1}. According to atomic force microscopy (AFM) evaluations, the SDBS-assisted SWNT suspension was rich in individual SWNTs even at a relatively high concentration (10 mg mL^{-1}). Furthermore, it was stable over months without significant aggregation or bundling. The authors claimed that the strong interactions between SDBS and SWNTs are the combined effect of the relatively long lipid chain of SDBS and the π–π interactions between aromatic moieties on the surfactant molecule and the graphitic surface of nanotubes. Such hydrophobic and π–π interactions seemed to be more pronounced in another study, reported by Nakashima *et al.*,[25] in which SWNTs were solubilized in water by a pyrene-carrying ammonium ion upon mild bath sonication.

Mioskowski and coworkers found *via* extensive transmission electron microscopy (TEM) studies that SDS molecules, as well as many other synthetic amphiphilic molecules with long lipid chains, adsorb on carbon nanotubes, forming half-cylinder structures that are perpendicular to the tube axis or tilt by a small angle.[26] Triton X-100, however, did not form such organized structures on the nanotube surfaces, presumably because it employs the π-stacking mechanism instead of half-micelle-like adsorption.

The recent finding that individual SWNT dispersions could be achieved by using SDS under ultrasonication/ultracentrifugation conditions[27] has stimulated significant research interest, such as the nearly complete optical assignments of SWNTs[28] and further chemical manipulation of individual nanotubes.[29] In addition to SDS, various other surfactants, including anionic, cationic, and non-ionic ones, were also found to be able to suspend individual SWNTs at mass conversions on the order of $\sim$5% (starting from 300 mg mL^{-1} nanotube dispersion and 2 wt% surfactant).[30] Results from the molecular simulation study by O'Connell *et al.* suggest that the density of the SDS-stabilized individual SWNT micelle-like structure is 1.0 g cm^{-3},[27] which may explain the (kinetically) stable nature



Scheme 1

of the suspension. Based on kinetic Raman/fluorescence studies, Strano *et al.*[31] proposed that surfactant molecules adsorb and diffuse in between the "unzipped" gaps of a nanotube bundle during sonication and eventually separate the individual nanotubes from the bundle. Such an equilibrium process is dependent on the surfactant concentration because the nanotube suspension is unstable when below the critical micelle concentration (CMC) of the surfactants and gradually coagulates over a period of hours.[31] The equilibrium mechanism seems to be supported by the failure to observe the supramolecular organization of surfactants below CMC or after dialysis.[26] Important parameters associated with CMC, such as ionic strength and pH of the aqueous media, would therefore affect the adsorption of ionic surfactants.

The thermodynamic nature of such surfactant-assisted dispersions may have significant implications in practical applications. For example, there is evidence that the weak surfactant–nanotube interactions might be replaced by the much stronger non-specific adsorption of biological species, such as proteins, on the nanotube surface.[32] Separately, there was also concern over the potential denaturing effect of some surfactants on the biological species for the proposed use of surfactant-dispersed nanotubes in bioapplications.[33]

2.2. Polymer-assisted dispersion

Compared to common surfactants discussed above, polymers usually have more robust surface adsorption because of more involved interaction sites.[34] However, in the case of carbon nanotubes, where hydrophobic interactions may dominate the adsorption, the use of polymers seems to give no significant improvement in dispersion efficiency in comparison with the use of the surfactants.[30] Nevertheless, a variety of nonionic and ionic polymers were found to be able to disperse nanotubes.[30,35,36] For example, a Nafion polymer, with a polar side chain, was found to solubilize carbon nanotubes in phosphate buffer solution (and ethanol).[35] The Nafion-assisted carbon nanotube dispersions were used in the fabrication of highly sensitive and discriminative oxidase-based amperometric biosensors.[35] The sensors were based on glassy carbon electrodes with a Nafion–SWNT composite coating, taking advantage of the electrocatalytic properties of nanotubes toward hydrogen peroxide and NADH and the widely acknowledged antifouling, discriminative, and biocompatible nature of the composite coating.

In a dispersion with a series of nonionic polymers, such as the Pluronic series including poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) triblock polymers, the dispersion efficiency seems to be dependent considerably more on the molecular weight of hydrophilic portions than that of hydrophobic ones (Fig. 1).[30] There is little nanotube dispersion when these polymers are of low molecular weight.

O'Connell *et al.* used an SDS-stabilized SWNT dispersion as starting material to prepare nanotube dispersions stabilized by various ionic and nonionic polymers.[33] The procedure was rather straightforward, with simple mixing, incubation, filtration, and centrifugation. It was proposed that the physical length of high molecular weight linear polymers and the 1D structure of nanotubes provide a "wrapping" scheme for

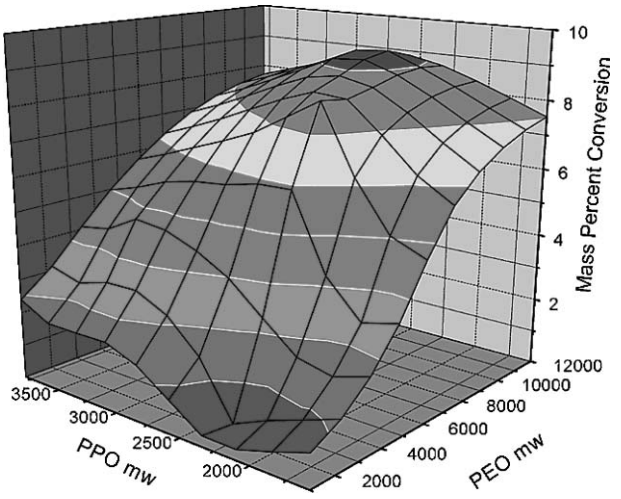


Fig. 1 Surface contour plots of PEO and PPO molecular weight effects within the Pluronic series of polymers against the mass percent conversion of individual nanotubes. (Reproduced from ref. 30 by permission. © Copyright 2003 American Chemical Society.)

hydrophobic interactions between polymers and nanotubes. In this scheme, the hydrophobic portion of the polymers, rather than coiling randomly, extends to cover the nanotube surface, thus forming nearly uniform monolayers. Multi-helical wrapping was suggested to be more plausible since it allows much higher nanotube surface area coverage and lower polymer backbone strain. Such wrapping seems quite robust. For example, polyvinyl pyrrolidone (PVP)-wrapped SWNTs can resist a cross-flow velocity of 0.5 mL min^{-1}.[33] While the wrapped PVP is NMR-silent, the signals can be recovered upon addition of an organic solvent, such as tetrahydrofuran (THF), suggesting that the polymer–nanotube interactions can be reversed by changing the solvent system. In the same work,[33] aqueous SWNT dispersions assisted by ionic polymers such as polystyrene sulfonate (PSS) were found to be sensitive to ionic strength. Somewhat surprising is the observation that the PVP-SWNTs were salted out, though at higher ionic strength. It was suggested that the carried charge from the nanotubes (similar to that in the SWNT colloid dispersions discussed in Section 1) is the role player.

Another interesting strategy to prepare stable polymer-assisted SWNT aqueous dispersion was inspired by the synthesis of empty cross-linked polymeric micelles.[37] In the reported work, Kang and Taton used an amphiphilic diblock polymer to stabilize SWNTs in polar solvents.[38] They then permanently cross-linked the hydrophilic outer shell of the micelle, poly(acrylic acid), by a diamine linker (Fig. 2). The micelle formation was controlled by gradual addition of water into dimethylformamide (DMF), a good solvent for both blocks of the polymer. Higher yields in the dispersion of SWNTs were achieved by crosslinking at high water/DMF ratios, which is consistent with the favorable hydrophobic interaction between polystyrene blocks and the nanotube surface under such conditions. The resulting sample of micelle-encapsulated SWNTs could be easily purified, isolated, and re-dispersed in water at over 0.5 mg mL^{-1} and was soluble even in some organic solvents.

Some of the natural and synthetic biomacromolecules, such as Gum Arabic,[39] amylose,[40,41] cyclodextrins,[42,43] DNA,[44,45] peptides,[46,47] and ferritin protein,[48] can also disperse SWNTs in water in a non-covalent fashion. These systems have more biological and biomedical implications, thus will be discussed in Section 4.

3. Functionalization with hydrophilic polymers/oligomers

The desired aqueous solubility of carbon nanotubes may be achieved in a more controllable fashion *via* sidewall- or defect-targeted functionalization of the nanotubes.[49–57] It is widely acknowledged that the solubility of the functionalized nanotubes is associated with that of the functional groups.[49–57] Although the mechanisms are much more complicated than they appear due to the distinct structural variations of carbon nanotubes themselves,[58,59] the linkages between functional groups and the nanotubes are often considered to be mostly covalent bonds, which are more robust during manipulation and processing than the non-covalent interactions in surfactant- or polymer-assisted dispersions.

Defect-targeted functionalization takes advantage of the nanotube-bound carboxylic acids,[15,60] which are generated during oxidative acid treatments as discussed in Section 1 above. Amidation and esterification chemistry, as well as ionic interaction schemes, are widely applied.[52,53] The nanotube electronic structure, notwithstanding the presence of the defect sites, is generally retained. Sidewall-targeted functionalization techniques, on the other hand, are mostly derived from the well-developed fullerene chemistry or graphite chemistry, in which the conjugated nanotube surface electronic structure is altered.[50] In either of the two types of functionalization schemes, the aqueous solubilization of carbon nanotubes can be realized by the mostly covalent attachment of hydrophilic polymeric or oligomeric species.

3.1. Functionalization

Sun and co-workers prepared one of the first water-soluble carbon nanotube samples *via* the covalent attachment of an amphiphilic aminopolymer, poly(propionylethylenimine-*co*-ethylenimine) (PPEI-EI, molecular weights of 50000 and 200000, Scheme 2), to nanotubes, which followed the defect-targeted acylation–amidation reaction scheme.[61,62] The resulting PPEI-EI-functionalized carbon nanotubes were highly soluble in both chloroform and water, and strongly luminescent.[61] Directly heating the aminopolymer with nanotubes without the thionyl chloride treatment resulted in samples of similar solubility behavior.[63] This is consistent with the

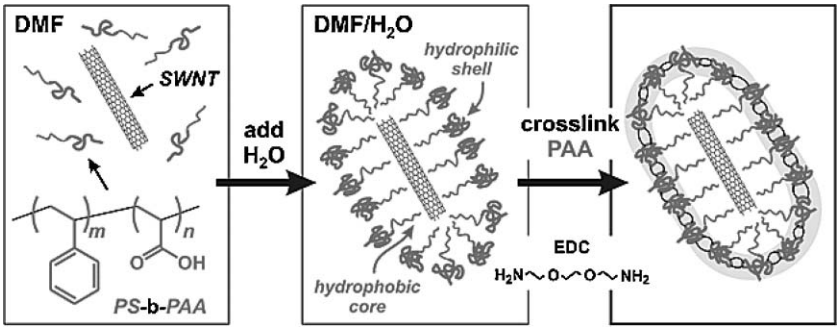
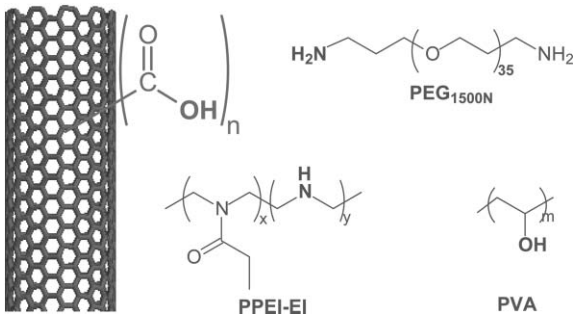


Fig. 2 The preparation of micelle-encapsulated SWNTs. (Reproduced from ref. 38 by permission. © Copyright 2003 American Chemical Society.)



Scheme 2

observation of Haddon and coworkers for octadecylamine-functionalized SWNTs prepared in a similar fashion, for which a zwitterion interaction mechanism was proposed.[64]

Alternatively, the amidation of nanotube-bound carboxylic acids by PPEI-EI can be achieved by carbodiimide activation—widely used in peptide synthesis—in room temperature water.[65,66] This kind of mild reaction condition has been proven to be quite useful, especially for the attachment of fragile biological species, such as proteins, to the nanotubes.[67] The use of bath sonication during the reaction significantly improves the nanotube solubilization, but the nanotubes are shortened if lengthy sonication (>12 h) is applied.[65,66]

According to high-resolution TEM analyses of the PPEI-EI-functionalized SWNTs,[66] the nanotube bundles are exfoliated into thinner ones and individual tubes with significant polymer coating (Fig. 3). Thus, the solubility of PPEI-EI-functionalized nanotubes in water and other solvents can be attributed to the nearly complete passivation of the nanotube surface. Scanning tunneling microscopy (STM) analyses of similar samples further revealed that the attached aminopolymers sometimes even crystallize along the chiralty of the nanotubes.[68] While this wrapping scheme seems similar to those found in the polymer-assisted dispersions of carbon nanotubes,[33,69] the binding here should be more robust because of the covalent linkages.

Huang *et al.* reported a more systematic comparison of different reaction routes in the functionalization of SWNTs with diamine-terminated oligomeric poly(ethylene glycol) (PEG_{1500N}, Schemes 2 and 3).[70] It is widely known that the PEG chain possesses many properties pertinent to biomedical

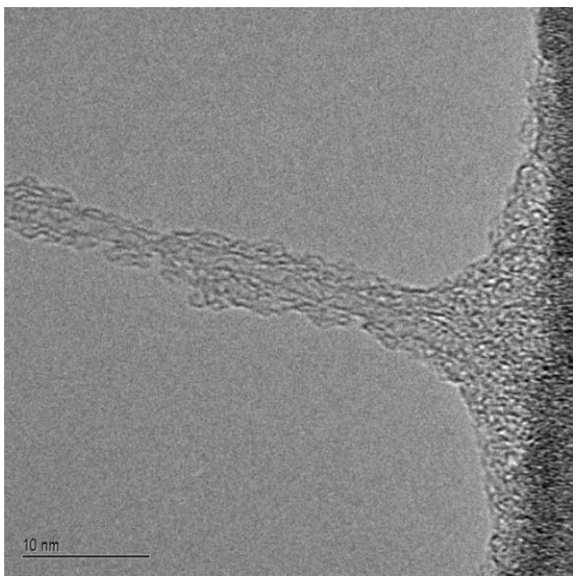
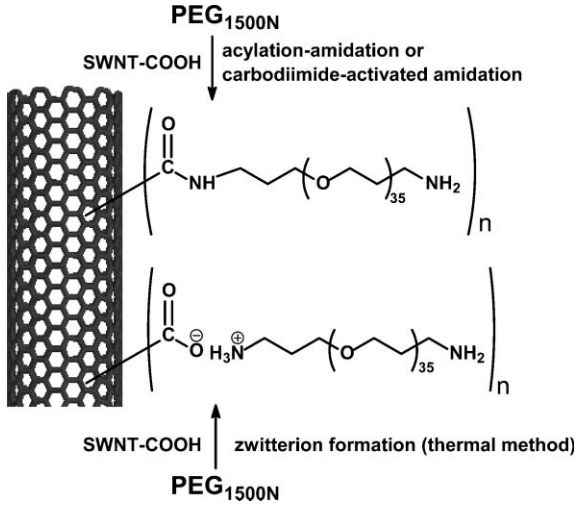


Fig. 3 High-resolution TEM image of an individual PPEI-EI-functionalized SWNT (scale bar = 10 nm). (Reproduced from ref. 66 by permission. © Copyright 2003 American Chemical Society.)



Scheme 3

and biotechnical applications.[71] The results from the comparison studies seem to suggest that the soluble sample from the carbodiimide activation scheme contains more bundled SWNTs.[70] However, the solubilization efficiencies of these reactions are generally similar, with higher temperature and longer reaction time facilitating the solubilization of more nanotubes. These soluble samples were also used to estimate and compare the absorptivities of the functionalized SWNTs.[72]

In the effort to investigate the selectivity in functionalization and solubilization toward nanotubes of different diameters and lengths, Huang *et al.* carried out repeated thermal reactions of PEG_{1500N} with purified SWNTs.[73] The residual insoluble SWNT sample from the first round of functionalization and solubilization was used as the starting material for the next round, and so on for five repetitions. The five soluble fractions were found to be similar, except that according to Raman spectroscopy results the earlier fractions contained more SWNTs of smaller diameters. This implies that the functionalization and solubilization are in favor of smaller SWNTs, with the final solid residue enriched with the nanotubes of larger diameters.[73] For MWNTs, no preferential functionalization and solubilization were observed in similar repeated reactions.[74]

Oligomeric and polymeric species containing PEG chains have been popular in the functionalization of carbon nanotubes in general.[18,75–81] For example, Sano *et al.*[18] prepared amine-terminated PEO-functionalized SWNTs soluble in water and organic solvents. Negra *et al.*[78] developed a procedure based on microwave heating to obtain amine-terminated PEG-functionalized SWNTs in a shorter reaction time (~ 1 h). Kahn *et al.*[82] derivatized SWNTs *via* reaction with 2-aminomethyl 18-crown-6 ether (CE) by using a room-temperature grinding method. The CE-SWNT sample was found to be soluble in various solvents, including water, with solubility up to ~ 1.1 mg mL^{-1}.

The water solubility of the PEG-functionalized SWNT samples also allowed further chemical manipulations and bioconjugations in an aqueous medium. For example, Fu *et al.*[83] reported that the nanotubes functionalized with oligomeric PEG and PEG-containing dendrons, both with ester linkages, could be used as starting materials in ester-to-amide transformation reactions in ambient water for the preparation of protein–nanotube conjugates.

Sidewall-targeted functionalization schemes, such as the reaction with aryl diazonium salts,[79] 1,3-dipolar cycloaddition of azomethine ylides[80] and (*R*)-oxycarbonyl nitrene addition,[81] were also used to attach molecules with PEG moieties to SWNTs.

Other polymers for the functionalization and aqueous solubilization of carbon nanotubes include poly(vinyl alcohol)

(PVA)[84] and poly(vinyl acetate-*co*-vinyl alcohol) (PVA-VA).[61] Here the relevance to potential bioapplications is due to the fact that PVA is recognized as one of the few water-soluble vinyl polymers susceptible to biodegradation under suitable conditions.[85]

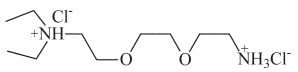
3.2. Aqueous solubilities

The aqueous solubility of modified carbon nanotubes depends on the functional groups used and the modification method applied because the nanotubes without modification are intrinsically water-insoluble. In other words, the water solubility of functional groups (or adsorbed species), the extent of functionalization or modification, and the strength of interactions between the functional groups and the nanotubes are the most important factors for the aqueous solubility. The strength of interactions is also the key to determine the stability of a specific nanotube dispersion.

Sun and coworkers recently carried out a systematic study of the aqueous solubility of SWNTs functionalized by different hydrophilic oligomers and polymers, including PEG_{1500N}, PPEI-EI and PVA.[86] The SWNT contents in the functionalized nanotube samples, required for the calculation of nanotube-equivalent solubility values, were carefully determined by averaging the results from thermogravimetric analysis (TGA) and quantitative NMR signal integration. The solubilities of SWNT equivalent in these samples are generally in the range of 10–50 mg mL^{-1}, which is about one order of magnitude higher than most numbers reported in the literature (Table 1). One of the PEG_{1500N}-SWNT samples has an even higher aqueous solubility of over 87 mg mL^{-1} SWNT equivalent, which is comparable to or higher than those of most water-soluble fullerene C_{60} derivatives.[87] The results suggest that attaching hydrophilic oligomers and polymers to SWNTs might be generally a more effective way for their aqueous dispersion and solubilization than most non-functionalization methods.

It is worthwhile to note, however, that the standard for defining the nanotube "solubility" is probably different in different laboratories. Some start with a limited amount of SWNTs and directly achieve dispersion without sedimentation, while others separate insoluble nanotubes from soluble ones by means of filtration or centrifugation. Moreover, differences in the speed and duration of centrifugation may result in large variations in the solubility data. Generally speaking, centrifugation at higher speed and for longer times usually yields a supernatant solution containing thinner bundles and more individual nanotubes.[27,30] For example, Moore *et al.*[30] used high-shear homogenization, ultrasonication, and ultracentrifugation (122000 g for 4 h)[27] to prepare a dispersion containing largely individual SWNTs, but the nanotube concentration in such a dispersion was only 0.006–0.03 mg mL^{-1}. Therefore, the solubilities and solution concentrations of carbon nanotubes should generally be considered and used

Table 1 Aqueous solubility results of SWNTs

Functional group/dispersion method	SWNT equivalent solubility in water/mg mL^{-1}	SWNT type	Separation method	Ref.
Oxidative Treatments				
98% H_2SO_4/70% HNO_3 (3 : 1)	1.77 (pH = 3)	arc		17
98% H_2SO_4/30% H_2O_2 (9 : 1)	>0.15 (3 < pH < 12)	laser		20
98% H_2SO_4/$(NH_4)_2S_2O_8$/P_2O_5–$KMnO_4$–H_2SO_4	>0.65 wt% (pH = 3)	laser		21
98% H_2SO_4/70% HNO_3 (3 : 1)–98% H_2SO_4/30% H_2O_2 (4 : 1)	0.2–0.3 (pH = 12)	laser	centrifugation (3500 g)	18
Non-Covalent Stabilization				
SDBS	20	HiPco		24
SDS	⩽0.1	HiPco		24
SOBS	⩽8	HiPco		24
DTAB	<0.1	HiPco		24
Triton X-100	⩽0.5	HiPco		24
PVP	1.4	HiPco		33
PSS	4.1	HiPco		33
Nafion (0.5 wt% in phosphate buffer)	>0.5	arc		35
Dextrin	<0.05	HiPco		24
γ-Cyclodextrin	<0.2	HiPco		42
Starch	0.5	HiPco	centrifugation (3300 rpm)	40
Gum Arabic	⩽15 wt%	arc	centrifugation (4500 rpm)	39
"nano-1" (a synthetic amphiphilic peptide α-helix)	0.7	HiPco	centrifugation (14000 rpm)	46
poly(T) (30-mer)	4	HiPco	centrifugation (16000 g)	44
PSS–PAA (crosslinked micelle)	>0.5	HiPco	centrifugation (3030 g)	38
Defect-Targeted Functionalization				
$H_2N(CH_2)_2SO_3H$	1.3	arc	filtration	88
Glucosamine	~0.1	arc		89
2-Aminomethyl-18-crown-6 ether	1.1	HiPco	filtration	82
PEG_{1500N}		arc		86
Thermal reaction	>87		centrifugation (3000 g)	
Diimide coupling	38		centrifugation (3000 g)	
Acylation–amidation	>57		centrifugation (3000 g)	
PPEI-EI		arc		86
Thermal reaction	>20		centrifugation (3000 g)	
Diimide coupling	>15		centrifugation (3000 g)	
Acylation–amidation	>23		centrifugation (3000 g)	
PVA (*M*w ~20000 or 70000)		arc		86
Diimide coupling	7–8		centrifugation (3000 g)	
Sidewall-Targeted Functionalization				
	<20	HiPco		90
Others				
KOH	<3	laser		91

within the context of the specific experimental conditions and application requirements.

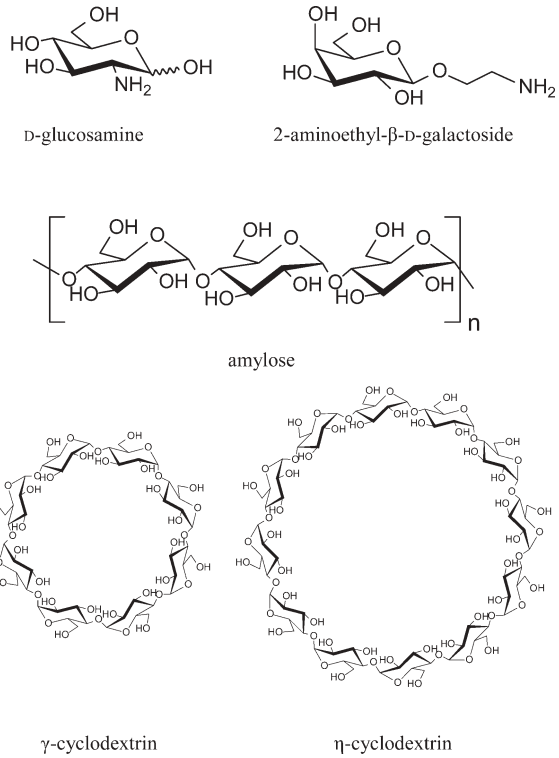
4. Modification with biological and bioactive species

Among the functional groups and stabilization agents for modification of carbon nanotubes, sometimes with aqueous solubilization, biological and bioactive species are obviously of special importance. Such modification enables evaluations of biocompatibility and more practical aspects of potential bioapplications of carbon nanotubes. Carbohydrates, nucleic acids and proteins are fundamental components in living organisms. Therefore, the development of controllable modification methods of carbon nanotubes by these molecules, as well as their analogs and precursors (such as oligosaccharides, oligonucleotides, amino acids, and peptides, *etc.*), represents another solid step toward the use of carbon nanotubes in biological and biomedical fields.

4.1. Carbohydrates

The role of carbohydrates in biological metabolism hints at interesting biomedical potentials of "sugar-coated" carbon nanotubes (Scheme 4). For example, Gu *et al.* have prepared several water-soluble monosaccharide-functionalized SWNT samples.[92] The bioactive sites (*i.e.*, monosaccharides) of these sugar-SWNTs are readily available and accessible because of the abundant carbohydrate functionalities along the high nanotube surface area in an aqueous environment. The nanotube-bound carbohydrate functionalities are being evaluated for immunological purposes, such as direct binding to pathogenic cells *via* specific adhesin–receptor interactions.[92] The sugar-SWNTs are also candidate biosensor materials for pathogenic detection or as inhibitors for the same pathogens.

Both non-covalent and covalent modification methods have been reported to modify carbon nanotubes with carbohydrates. Following an ancient Egyptian recipe for preparation of carbon-black ink, Bandyopadhyaya *et al.* used Gum Arabic (GA), a highly branched polysaccharide, to disperse SWNTs *via* sonication.[39]



Scheme 4

Star *et al.* used starch, composed of linear amylose and branched amylopectin, to form presumably supramolecular complexes with SWNTs in solution, and proposed a helical amylose-wrapping scheme.[40] The use of a starch–iodine complex instead of only starch in aqueous solution was required. In the same report, commercial amylose containing 10% butanol, which was considered to be "pre-complexed" in the helical structure, solubilized SWNTs in water directly, but an amylopectin–iodine complex did not dissolve SWNTs well.[40]

Kim *et al.* investigated the complexation of amylose with SWNTs from another angle and found that simply mixing a pre-sonicated aqueous dispersion of SWNTs and a DMSO solution of amylose resulted in a homogeneous colloid solution.[41] The solubilization efficiency of SWNTs dropped sharply when the content of DMSO was above ~50% in the mixed solvent system. Since it is known that the conformation of amylose in DMSO is helical and loosens up when water is added, the above finding suggests that while the helical amylose may be responsible for the solubilization, the presence of a pre-formed helical structure of amylose, as proposed by Star *et al.*,[40] may not be required.

Studies of the SWNT complexation with cyclodextrins (CD), which are macrocylic polysaccharides, have yielded some interesting results.[42,43,93] In particular, grinding γ-CD with HiPco SWNTs resulted in shortened nanotubes (or "soft cutting").[42] The observed aqueous solubility of γ-CD–SWNT (only in plastic containers) is unlikely due to encapsulation because the γ-CD ring is too small. Recently, Dodziuk *et al.*[43] reported aqueous solubilization of SWNTs with η-CD, which has a 12-membered ring structure with an inner cavity of ~1.8 nm in diameter, significantly larger than those in α-, β- and γ-CDs. Thus, the encapsulation of SWNT in the CD may be possible in this case.[43]

Synthetic amino-carbohydrates, such as *N*-saccharides and amino tethered *C*-saccharides, have been used in the covalent functionalization targeting nanotube-bound carboxylic acids.[92,94,95] For example, Pompeo and Resasco first reported the preparation of a water-soluble glucose–SWNT sample from the amidation reaction of nanotubes with glucosamine.[94] The aqueous solubility of the sample was ~0.1 mg mL^{-1} SWNT equivalent at room temperature and increased to ~0.4 mg mL^{-1} at temperatures close to the boiling point of water. Matsuura *et al.* reported that a β-galactoside (Gal)-functionalized SWNT sample, prepared by carbodiimide-activated amidation, was more difficult to disperse than the oxidatively shortened nanotube starting material.[95] This could be due to the strong self-aggregation of Gal. However, upon addition of a Gal-specific lectin, the turbid aqueous dispersion became clear. The Gal–lectin interaction probably resulted in the immobilization of larger-sized lectins on the Gal-functionalized nanotubes.

Chen *et al.* reported another interesting approach to the generation of surface functionalities amendable for carbohydrate immobilization.[96] They used appropriate plasma to treat CVD-grown carbon nanotubes perpendicularly aligned on a substrate. After the treatment with acetaldehyde plasma, the aligned nanotube substrate was immersed into an amino-dextran solution to form a Schiff-base, which was further reduced into a secondary amine by $NaBH_3CN$. The nanotube arrays after such sugar attachment became highly hydrophilic, and the contact angle of the arrays could not be measured because the water drops spread out instantaneously on the surface. When scraped off the substrate, the dextran-coated nanotubes could be dispersed into water.[96]

4.2. Nucleic acids

According to early electron microscopy observations,[97,98] platinated and iodinated helical double-stranded DNA might

be immobilized onto the surface and inside the opened cavity of MWNTs in a non-specific manner. In fact, there is both computational and experimental evidence for DNA insertion into or transport through the inner cavity of carbon nanotubes.[99,100]

Zheng *et al.* recently reported that a variety of single-stranded DNAs, short double-stranded DNAs, and some total RNAs can directly disperse individual SWNTs in water.[44] Simulation studies showed that the non-specific DNA–SWNT interactions in water are from the nucleic acid–base stacking on the nanotube surface, with the hydrophilic sugar–phosphate backbone pointing to the exterior to achieve the solubility in water. The mode of interaction could be helical wrapping or simple surface adsorption. Poly(A) and poly(C) strands, known for strongly self-stacking in solution, exhibited much lower dispersion efficiency toward SWNT than poly(T), which is consistent with the base-stacking mechanism. Also consistent is the fact that the interactions of SWNTs with single-stranded DNAs appear to be more favorable than with double-stranded ones.[44,45] Such DNA-assisted SWNT dispersion may be passed through an anion-exchange chromatography column, resulting in chromatographic separation with respect to both the lengths and the electronic properties of SWNTs (Fig. 4).[44] For the latter, the charge differences among the DNA–SWNT conjugates, which are associated with the negatively charged phosphate groups of DNA and the different electronic properties of SWNTs, have allowed post-production preparation of samples enriched in metallic and semiconducting SWNTs.

DNA molecules can also be attached to nanotubes by covalent linkages. Dwyer *et al.* carried out a ^{32}P radioisotope polyacrylamide gel electrophoresis (PAGE) study of the

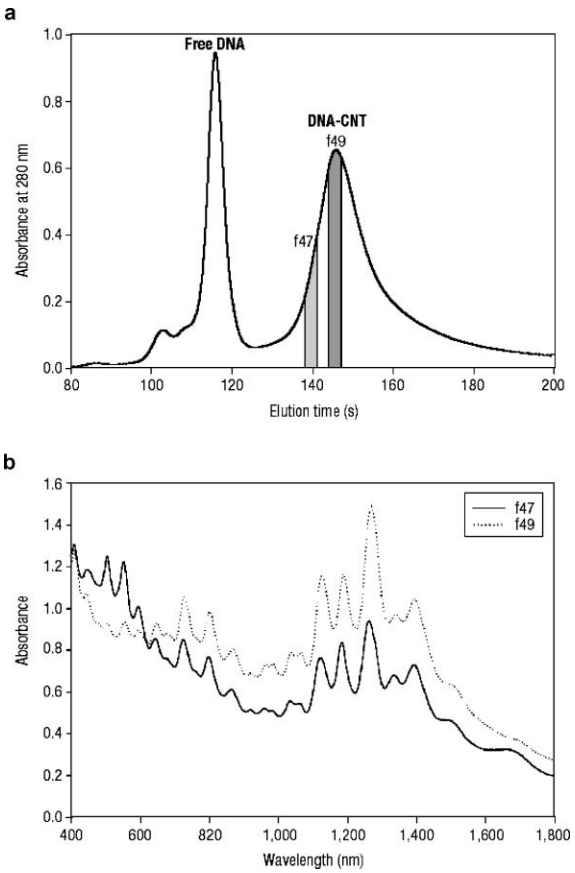


Fig. 4 Separation of DNA–SWNT by anion exchange chromatography. (a) The chromatogram showing fraction 47 (f47) and fraction 49 (f49). (b) Electronic absorption spectra of f47 and f49. (Reproduced from ref. 44 by permission. © Copyright 2003 Nature Publishing Group.)

amine-terminated oligonucleotide-functionalized SWNTs prepared from carbodiimide-activated amidation reaction.[101] They reported that some of the DNA molecules in the samples were essentially immobile in the gel. In the control experiments using carboxylic acid-terminated oligo(T) with no amine groups, there was hardly any material resistant toward the electrophoresis field. The reported results seem consistent with the assumed presence of amide linkages between the amine-terminated DNA and SWNTs.

The DNA end-attachment to the nanotube surface could allow further hybridization of the linked strands to their complementary sequences.[102,103] For example, the enhanced aqueous solubility of amine-terminated oligonucleotide-functionalized SWNTs has facilitated the convenient confocal fluorescence imaging of its hybridization toward a fluorescence dye-labeled complementary sequence.[103] Baker *et al.* covalently linked thiol-terminated oligonucleotides to SWNTs that were pre-functionalized with diamines by using a succinimide–maleimide linker, commonly used for thiol–amino coupling.[102] The hybridization of SWNT-attached DNA was apparently reversible upon denaturing the hybridized product, which could be used in a second-round hybridization.

DNA recognition may also be achieved by functionalization of carbon nanotubes with peptide nucleic acid (PNA), an uncharged DNA analog (Fig. 5).[104] When shortened nanotubes were used as starting material, the DNA hybridization occurred predominantly at or near the tube ends according to AFM images. This is consistent with the expected end-group chemistry and specific PNA–DNA hybridization.

Carbon nanotubes end-functionalized with selective DNA probes have been considered as promising ultrasensitive DNA sensors.[105–107] For example, Li *et al.* fabricated low-density aligned MWNT nanoelectrode arrays.[106] Upon the functionalization of the nanotube end-bound carboxylic acids with amine-terminated oligonucleotides, these arrays could be used as sensors to probe hybridized DNA targets of less than a few attomoles by signal amplification with $Ru(bpy)_3^{2+}$-mediated guanine oxidation, thus several orders of magnitude more sensitive than those with previous techniques.

4.3. Proteins

Direct modification of carbon nanotubes with proteins (including enzymes and antibodies) is in fact an earlier-explored but somewhat controversial topic because of the complexities in the structures and properties of both proteins and carbon nanotubes. Many proteins can be spontaneously adsorbed onto the nanotube surface, or be immobilized in a more controllable fashion *via* functionalization reactions. We will discuss below the different modes of protein–nanotube interactions, the related issues on biocompatibility and biorecognition, and the interaction and modification of carbon nanotubes with the building blocks of proteins—amino acids and peptides.

4.3.1. Non-specific adsorption. A variety of proteins can strongly bind to the MWNT exterior surface *via* non-specific adsorption.[3,4,108] When the ends of a MWNT are open as a result of oxidation treatment, smaller proteins can be inserted into the tubular channel (~5–10 nm in diameter).[3,4] An enzyme immobilized in this manner was shown to retain moderate bioactivity.[3,4] Balavoine *et al.* also reported an interesting observation that proteins such as streptavidin and HupR were adsorbed onto the MWNT surface to form helical crystallization structures (Fig. 6), resulting in ordered arrays of proteins on the nanotube surface.[108]

Recently, Boussaad *et al.* reported an *in situ* detection of non-specific adsorption of cytochrome c (cytc) onto a semiconducting SWNT transistor device by monitoring the conductance change in the nanotube (Fig. 7).[109] The sensitivity

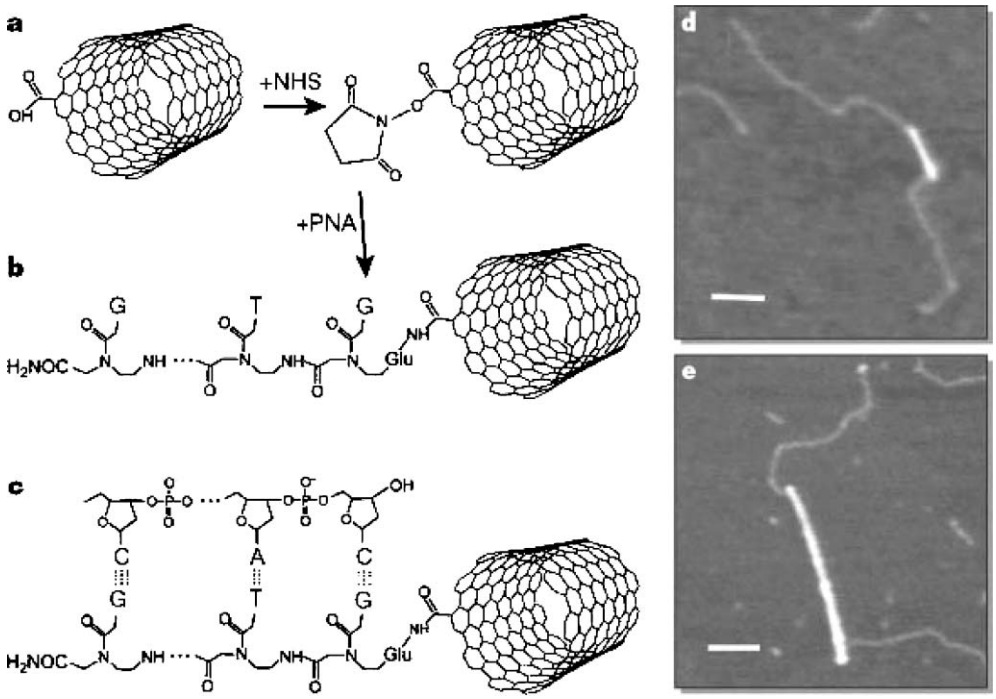


Fig. 5 (a,b) Attachment of PNA to SWNTs. (c) Hybridization of PNA–SWNT with a complementary DNA sequence. (d,e) AFM images of PNA–SWNTs (scale bars = 100 nm, SWNTs as bright lines and bound DNA as the paler strands). (Reproduced from ref. 104 by permission. © Copyright 2002 Nature Publishing Group.)

of the detection was high, around 20 protein molecules per nanotube. Metallic SWNTs, on the other hand, did not exhibit any observable conductivity change upon introduction of protein solution. The proposed mechanism was that the decrease in conductance is due to a reduction in the charge carriers of p-type semiconducting SWNTs by the positively charged cytc protein.[109] However, Chen *et al.* found that all the proteins under their investigation caused a decrease in the nanotube conductance, regardless of the proteins' net charge.[9] Nevertheless, the experiments on the adsorption behavior based on the transistor device characteristics and the quartz crystal microbalance (QCM) measurement were in good agreement.[9]

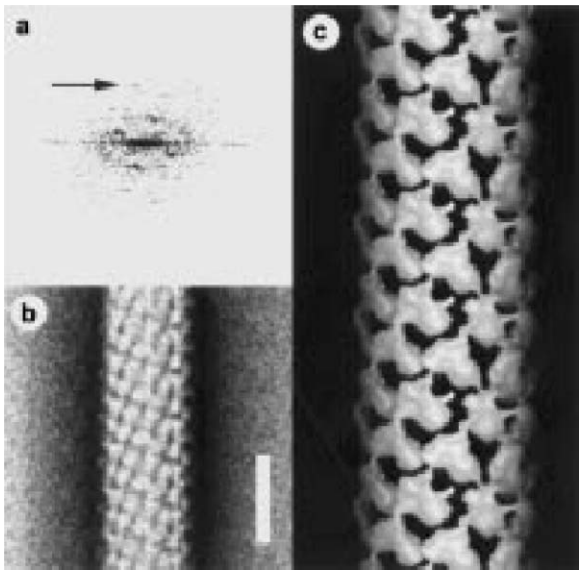


Fig. 6 Analysis of the streptavidin helices formed on MWNTs. (a) Computed power spectrum of the Fourier transform of a helical array of streptavidin molecules. (b) Noise-free view of the helical repeat obtained by correlation. (c) Three-dimensional model of streptavidin assemblies on MWNTs. (Reproduced from ref. 108 by permission. © Copyright WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1999.)

Dai and coworkers reported that incubating the CVD-produced SWNTs (directly grown on a TEM grid) in aqueous ferritin solution resulted in no observable adsorption.[110] In contrast, both Azamian *et al.*[32] and Lin *et al.*[48] found strong adsorption of ferritin onto SWNTs in an aqueous environment. The ferritin adsorption was in fact so significant that it resulted in the solubilization of the SWNTs in water.[48] The adsorbed ferritin could be largely removed from the nanotube surface *via* dialysis against pure water according to TEM analyses of the sample before and after the dialysis (Fig. 8).

Mechanistically, the non-specific adsorption of proteins onto the nanotube surface may be more complicated than the widely attributed hydrophobic interactions.[9,48,108,110,111] For example, Shim *et al.* observed that streptavidin is readily adsorbed onto as-grown SWNTs, but not fibrinogen, despite the well known affinity of fibrinogen to hydrophobic surfaces.[111] For the non-specific adsorption of proteins on other surfaces, electrostatic interactions, hydrogen bonding, and other mechanisms are generally considered.[112] However, Azamian *et al.* found that the adsorption of proteins on SWNTs is insensitive to the protein isoelectric point (pI), with both positively and negatively charged proteins showing strong adsorption,[32] thus inconsistent with an electrostatic interaction mechanism. It is quite possible that the observed substantial protein adsorption is at least in part associated with the amino affinity of carbon nanotubes,[113] since there are abundant surface amino groups in the proteins under consideration.[48]

The peptide–nanotube interactions may serve as a model for protein–nanotube interactions. Wang *et al.* employed a phage display technique to determine if there is selective affinity for different peptide sequences toward carbon nanotubes under conditions for non-covalent interactions.[47] They found that the histidine (H) unit and especially the tryptophan (W) unit contribute significantly to the peptide interaction with the nanotube surface. Their suggestion was that the aromatic ring structures in these amino acid residues contribute to the observed affinity of the peptides to carbon nanotubes. In addition, they found that amphiphilic peptides and the more flexible peptide sequences in solution are more favorable in binding to the nanotubes. The former is understandable for

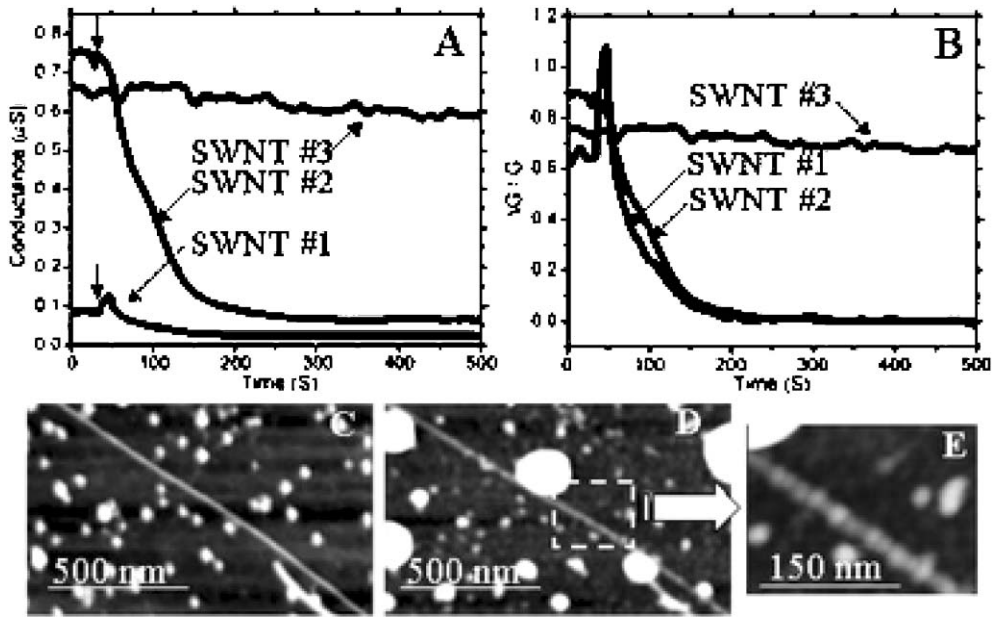


Fig. 7 (A) The conductance of p-type semiconducting (#1,#2) and metallic (#3) SWNTs simultaneously recorded as a function of time during the adsorption of the protein cytc. The arrow points to the start of the introduction of cytc into the buffer solution. (B) Plots of the relative change of conductance *vs.* time. Tapping mode AFM images of a SWNT before (C) and after (D) the introduction of the protein solution. (E) shows the region outlined with a dashed rectangle in (D). (Reproduced from ref. 109 by permission. © Copyright 2003 Royal Society of Chemistry.)

their surfactant-like structures, which could efficiently disperse SWNTs into water.

Dieckmann and coworkers synthesized a peptide α-helix with 29 amino acid residues (called "nano-1") according to an amphiphilic design from computation.[46] The peptide, with a spring-like secondary structure, can solubilize SWNTs into water up to 0.7 mg mL^{-1}, thus acting much like surfactants. Nano-1 can self-assemble in aqueous solution due to charge–charge interactions. This leads to the favorable precipitation of the peptide-dispersed SWNTs to form fiber-like assemblies with controlled sizes upon addition of aqueous NaCl in different concentrations. Amphiphilic molecules such as DMF can promote the self-assembly of the peptide-dispersed SWNTs as well, mimicking the self-association of proteins under similar conditions.

It is clear from these studies that a comprehensive understanding of the non-specific protein–carbon nanotube interactions requires more investigations at the molecular level. For example, different amino acid residue compositions and sequences, as well as the secondary (and maybe tertiary and quaternary) structures, of the proteins may significantly affect the non-specific interactions and related properties. The same molecular level investigation is also required for an improved understanding of the role of the nanotube electronic structures in the interactions. Nevertheless, the current knowledge on non-specific protein–nanotube interactions has already been applied to the development of biosensors[8,114–121] and biocatalytic devices.[3,4,122] For example, Rege *et al.* reported that leaching-stable biocatalytic films with a high enzyme stability could be prepared by incorporating SWNTs into an enzyme-polymeric composite system.[122] Such stability was attributed primarily to the strong non-specific enzyme–nanotube interactions in the polymer matrix during water transport.

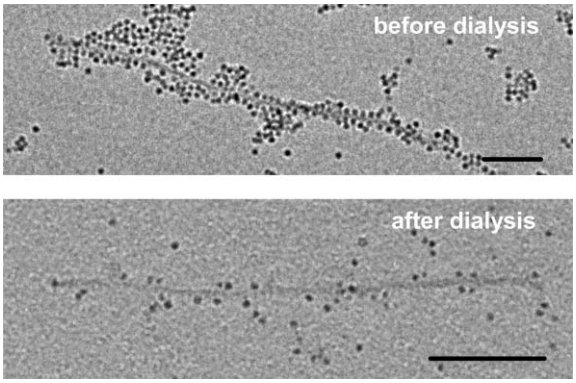


Fig. 8 TEM images of ferritin–SWNT conjugates obtained in aqueous solution without the carbodiimide coupling agent before (top) and after (bottom) dialysis (scale bars = 100 nm).

4.3.2. Protein resistance and biorecognition. The non-specific adsorption of proteins on the nanotube surface is not always desirable, and in fact must be avoided in some cases, which requires the alleviation or elimination of such adsorption. There are already a wealth of molecules reported in the literature that are commonly used for protecting various surfaces from proteins in mechanisms such as steric repulsion, hydration, and solvent structuring.[71,112,123] Among these protein-resistant molecular species, PEG is often considered as a benchmark material.[71,112,123] Thus, the coating of nanotube surface with PEG functionalities is a strategy to prevent the non-specific protein adsorption when necessary. However, the non-covalent coating requires additional effort because PEG by itself does not adsorb well on SWNTs.[24,33,111] For example, Shim *et al.* pre-adsorbed Triton molecules on as-grown SWNTs on mica to facilitate the subsequent PEG adsorption.[111]

The functionalization of carbon nanotubes with PEG moieties is a more effective and unambiguous approach to achieve the desired protein resistance. Lin *et al.*[48] studied the interactions between PEG_{1500N}-functionalized SWNTs and ferritin in an aqueous solution, and found that these water-soluble SWNTs with covalent PEG coatings are indeed strongly resistant toward ferritin adsorption (Fig. 9). Lin *et al.* also observed similar protein resistant behavior in PPEI-EI- and PVA-functionalized SWNTs.[48] An obvious common feature among the three functional groups (PEG, PPEI-EI, and PVA) is their hydrophilicity.

The adsorption of Triton molecules on the nanotube surface is insufficient to impart protein resistant properties, despite the presence of a short PEG chain in the molecular structure (on average of ~10 PEG units in Triton X-100, see

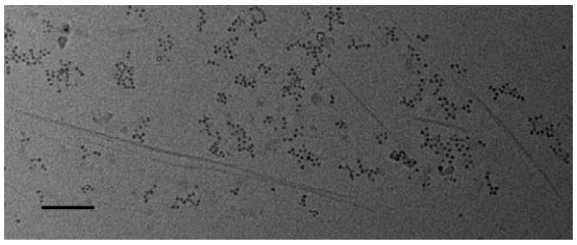


Fig. 9 A TEM image of the specimen from the aqueous solution containing ferritin and PEG_{1500N}–SWNT (scale bar = 100 nm).

Scheme 1).[9,32,111,124] Chen *et al.* adsorbed SWNTs, as-grown on quartz substrates, with Tween-20 and Pluronic P103, both with many more PEG units per molecule (Scheme 1).[9] They found that the modified SWNTs were resistant toward proteins such as streptavidin, avidin, bovine serum albumin (BSA), α-glucosidase and staphylococcal protein A (SpA). For example, a Tween-coated semiconducting nanotube device did not show any changes in conductance upon exposure to various types and concentrations of protein solutions. It was suggested that these surfactants formed a nearly uniform layer on the nanotube surface by favorable hydrophobic interactions, with the PEG segments extending into the aqueous solution to provide the observed protein resistance. It was further hypothesized that such a non-covalent protein resistant coating might require amphiphilic molecules with a large linear hydrophobic segment, such as the aliphatic part in Tween-20 and the poly(propylene oxide) block in Pluronic P103.[9]

Erlanger *et al.* reported that the anti-fullerene IgG monoclonal antibody (mAb) could still bind to SWNTs in a colloidal suspension even in the presence of Tween-20 for preventing non-specific adsorption.[125] It was suggested that the structural similarity between SWNT and fullerene C_{60} allowed the antibody to recognize both. Separately, according to Azamian *et al.*, the non-specific adsorption on SWNTs by some proteins, such as cytochrome c and ferritin, could not be alleviated by the presence of either Triton X-100 or Tween-20.[32]

The control of non-specific protein adsorption is important to the use of carbon nanotubes in specific protein-binding or biorecognition. For example, Star *et al.* co-adsorbed poly(ethylene imine) (PEI) and PEG on an SWNT field effect transistor (FET) device, followed by the biotinylation of PEI for specific streptavidin recognition.[126] The modified device exhibited different device characteristics from those with direct non-specific adsorption. Similarly, the modification of carbon nanotubes with the adsorption of biotinylated Tween-20 allowed streptavidin recognition by the specific biotin–streptavidin interaction, but provided resistance toward other protein adsorptions.[9]

The protein-resistant molecular species may be covalently conjugated to specific antigens to allow sensitive detection of antibodies, or *vice versa*.[9] Chen *et al.* covalently linked U1A (a protein involved in the splicing of mRNA) to Tween-20, and then non-covalently coated the modified surfactant onto a SWNT device.[9] The device was capable of detecting the binding of 10E3 (a specific antibody for recognition of U1A) at concentrations no more than 1 nM (Fig. 10), which compares favorably to standard fluorescence-based detection with immobilized antigens on planar arrays.

4.3.3. Specific binding in functionalization. The non-specific adsorption of proteins on carbon nanotubes is an interesting phenomenon, but represents a relatively less controllable mode of protein–nanotube interactions. More robust and predictable conjugation may be achieved *via* covalent functionalization.

Dai and coworkers reported the use of a bifunctional molecule, 1-pyrenebutanoic acid succinimide ester, as a linker to immobilize proteins on SWNT surfaces.[110] The assumption

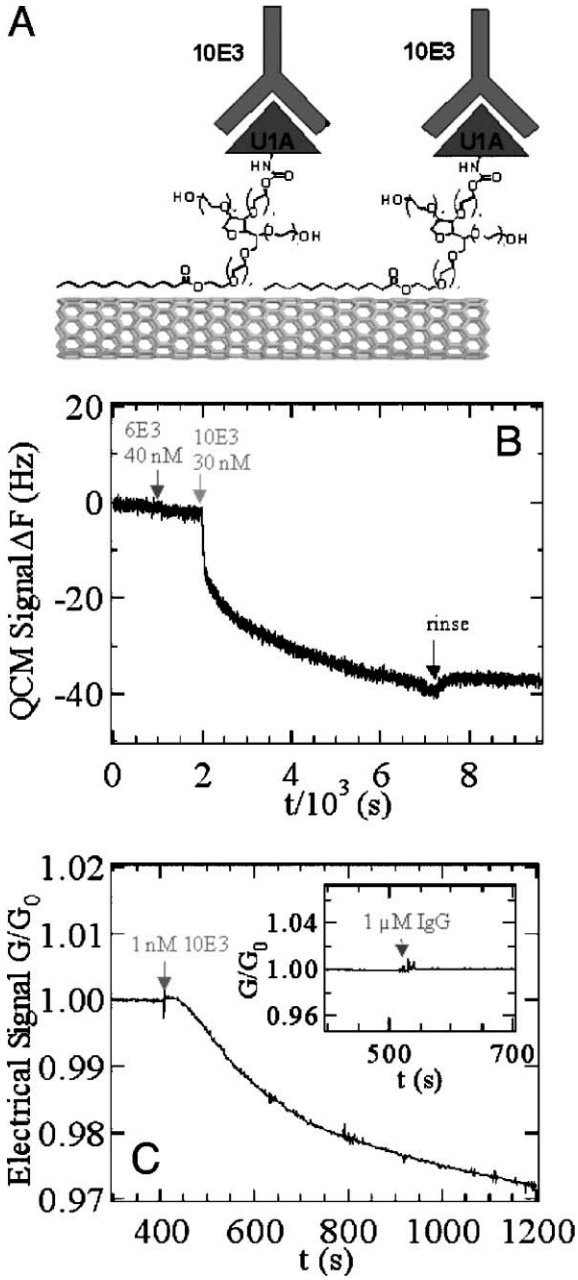


Fig. 10 Specific detection of monoclonal antibodies (mAbs) binding to a recombinant human autoantigen. (A) Scheme for specific recognition of 10E3 mAb with a nanotube device coated with a U1A antigen–Tween conjugate. (B) QCM frequency shift *vs.* time curve showing selective detection of 10E3 while rejecting the non-U1A specific antibody. (C) Conductance *vs.* time curve showing specific response to 1 nM 10E3 while rejecting polyclonal IgG at a much greater concentration of 1 M (inset). (Reproduced from ref. 9 by permission. © Copyright 2003 National Academy of Sciences, USA.)

was that the pyrene moiety in the bifunctional molecule would be π–π stacking with the graphitic nanotube sidewall so that the succinimidyl ester group at the other end of the bifunctional molecule would be available for reaction with primary and secondary amines in the proteins.[110] Besteman *et al.* adopted the same concept to immobilize glucose oxidase (GOx) on semiconducting SWNTs that were as-grown on a silicon wafer.[127] They used the GOx-decorated SWNT as a sensor device to measure both enzyme activity and pH changes down to 0.1 in the range of 4–5.5. However, while the intended protein immobilization was successful in both studies, the need for a bifunctional molecule as a linker is questionable, especially in light of the overwhelming experimental evidence for the natural affinity of proteins toward carbon nanotubes.

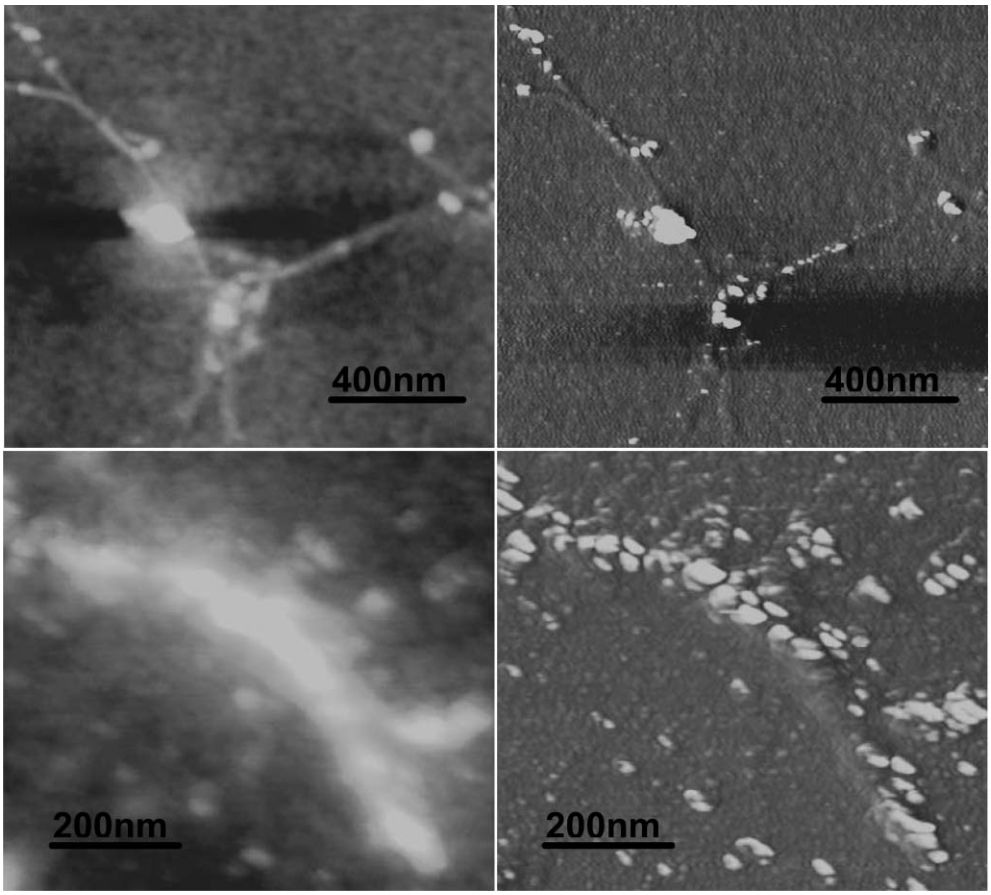


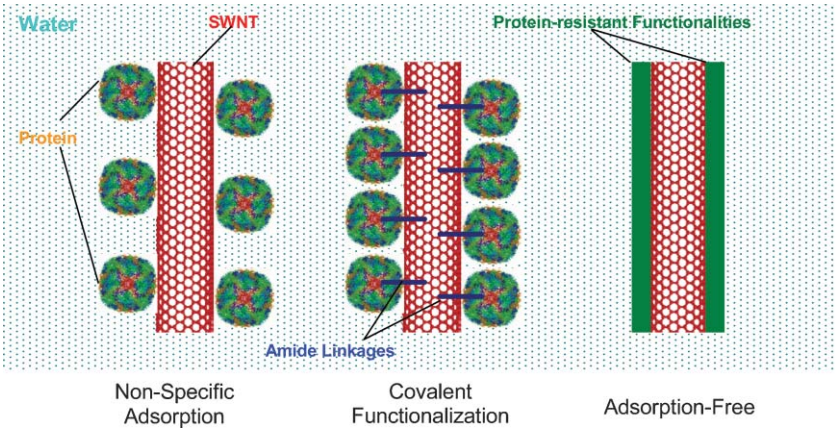
Fig. 11 Height (left) and phase (right) images from the AFM analyses of the BSA–SWNT conjugate (top) and BSA–MWNT conjugate (bottom) samples on mica substrates. (Reproduced from ref. 67 by permission. © Copyright 2002 American Chemical Society.)

Huang *et al.* used BSA as a model protein to study covalent conjugation with carbon nanotubes.[67] The work took advantage of the wealth of pendant amino groups on the protein surface to carry out amidation reactions with the nanotube-bound carboxylic acid groups. The amidation was based on carbodiimide activation at room temperature, similar to that in the functionalization of carbon nanotubes with the aminopolymer PPEI-EI discussed earlier.[65,66] According to images from AFM analyses, the BSA species are intimately associated with individual or thin bundles of carbon nanotubes. For MWNTs produced from the CVD method,[128] which are known to contain abundant surface defects, the nanotube is essentially covered by the proteins (Fig. 11). The results from the total protein analysis based on the modified Lowry procedure indicate that the majority of the protein in the conjugate sample remain bioactive.[67]

The role of the coupling agent for amidation has been debated.[32,48] Azamian *et al.* suggested that the extent of protein–nanotube conjugation is similar with or without the presence of coupling reagents.[32] More recently, Lin *et al.* compared ferritin–SWNT conjugation in water under both non-specific adsorption and covalent functionalization conditions.[48] Indeed, strong conjugation, resulting in the solubilization of the conjugated SWNTs, was observed under both conditions, but more nanotubes were solubilized under the covalent functionalization conditions in the presence of a carbodiimide coupling agent. A more important difference was the fact that the non-covalently adsorbed ferritin species were more removable in vigorous dialysis (Fig. 8). It seems that non-specific adsorption may also contribute significantly to covalent functionalization, with the coupling reagent "locking" the adsorbed proteins in place to yield more stable protein–SWNT conjugates. The various modes of protein conjugation (or no conjugation) with carbon nanotubes are summarized in Scheme 5.

There is an even milder route for the conjugation of carbon nanotubes with proteins, for which a water-soluble functionalized nanotube sample is used as the starting material in room-temperature ester-to-amide transformation reactions in a homogeneous aqueous environment.[83] According to Fu *et al.*, SWNTs were first functionalized with hydrophilic oligomeric species *via* the esterification of the nanotube-bound carboxylic acids to achieve water solubility.[83] The nanotube sample was then mixed with BSA protein in an aqueous solution for the room-temperature reaction that transforms the esters in the starting material to more stable amide linkages with BSA species. The entire conjugation procedure does not subject the protein to any physiologically damaging experimental conditions. Thus, the method may be valuable for the preparation of conjugates involving more fragile biological species.

Conjugation *via* functionalization has been applied to the fabrication of biosensors and bioelectronic devices based on enzyme–nanotube or antibody–nanotube conjugates.[10,129–132] For example, Gooding *et al.* immobilized a microperoxidase, MP-11, onto a perpendicularly aligned SWNT arrays on a cysteamine-modified gold substrate electrode *via* carbodiimide-activated amidation chemistry (Fig. 12).[10] They observed efficient electrical communication between the underlying substrate electrode and the redox proteins through the SWNTs, similar to that found in the absence of nanotubes. Wohlstadter *et al.* used strong acid to etch a polymer–nanotube composite film to obtain a densely packed nanotube sheet with available surface carboxylic acid groups.[132] They immobilized streptavidin on the nanotube sheet by carbodiimide-activated coupling, and then attached a biotinylated mAb for α-fetoprotein (AFP) *via* the specific biotin–streptavidin



Scheme 5

interaction. The device with electrochemiluminescence detection was sensitive at AFP concentrations as low as 0.1 nM.

Amino acids and peptides are building blocks of proteins, so that studies on the modification and functionalization of carbon nanotubes with amino acids and peptides are important for the bottom-up design of immuno-nanotubes and in the pursuit of understanding of carbon nanotube–protein interactions. In addition to the examples provided earlier for the non-covalent modification of carbon nanotubes with peptides,[46,47] there has also been the covalent attachment of amino acids and peptides to carbon nanotubes. For example, Prato, Bianco and coworkers prepared amino acid-[90] and peptide-functionalized SWNTs[133–135] *via* a two-step procedure. The first step was the preparation of the functionalized nanotubes with an amino-ethylene glycol linker, which became highly soluble in water upon acidification. For the amino acid attachment in the second step, Fmoc-protected amino acids were directly added to the free amino groups on the nanotubes by an amidation reaction.[90] Alternatively, oligo- and polypeptide could also be covalently linked to the amino-carbon nanotubes *via* either amidation with fully protected peptides or a succinimide–maleimido bridge.[133]

Immunological studies *in vitro* by these researchers showed that an antigenic epitope from the foot-and-mouth disease virus (FMDV) upon attachment to SWNTs retained the active secondary structure for spatial interaction with specific antibodies.[133] The potential for such systems in vaccine delivery applications has been explored and discussed.[134,135]

Concluding remarks

The methodology development for the aqueous dispersion and solubilization of carbon nanotubes has reached the point of enabling studies of the nanotubes in physiological environments. Among the available methods, oxidative acid treatment disperses a large quantity of carbon nanotubes, but the quality of the dispersion may not be as high as those with other methods. Non-covalent stabilization offers variety in the

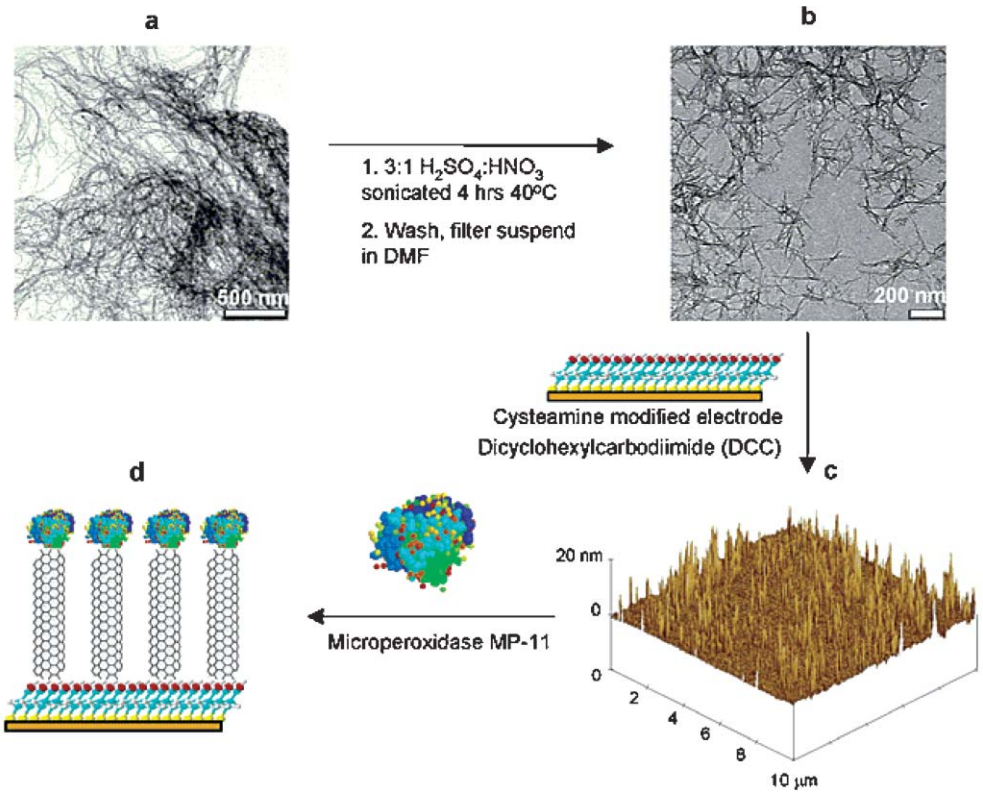


Fig. 12 The steps involved in the fabrication of aligned shortened SWNT arrays for direct electron transfer with enzymes such as microperoxidase MP-11. (Reproduced from ref. 10 by permission. © Copyright 2003 American Chemical Society.)

degree of dispersion, which may be further developed and optimized on a system by system basis for specific uses. The chemical modification and functionalization method produces soluble carbon nanotube samples, thus amenable to ultimate dispersion, but the method is intrusive to the nanotube structure and also mechanistically complex. The effects of the modification and functionalization on the intrinsic properties of carbon nanotubes are far from being fully understood. Nevertheless, solubilization *via* chemical modification and functionalization is effective in the imparting of biocompatibility into carbon nanotubes, especially for the stable conjugation of carbon nanotubes with a variety of biological and bioactive molecules and species (proteins, carbohydrates, DNA, *etc.*). These bioconjugated carbon nanotubes and their beginning to be used in exploratory biosensors and other devices represent solid progress toward the predicted and desired bioapplications. Further advances in continuing investigations may depend on an improved understanding of the physico-chemical and biological (such as toxicity[136]) properties of carbon nanotubes, better control of the bioconjugation of carbon nanotubes based on mechanistic elucidations of the conjugation process and conjugate structures, and a broader exploration of other bioapplication opportunities beyond those that have been identified.[137]

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