ACCOUNTS
of chemical research

The Devil and Holy Water: Protein and Carbon Nanotube Hybrids

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RECEIVED ON DECEMBER 21, 2012

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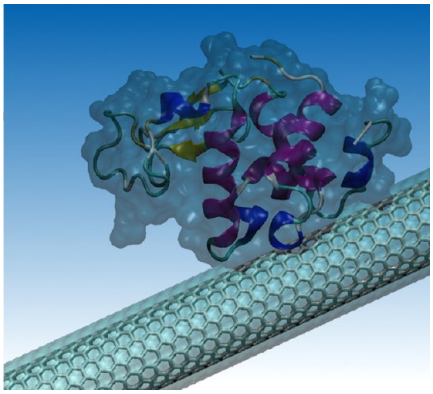

Integrating carbon nanotubes (CNTs) with biological systems to form hybrid functional assemblies is an innovative research area with great promise for medical, nanotechnology, and materials science applications. The specifics of molecular recognition and catalytic activity of proteins combined with the mechanical and electronic properties of CNTs provides opportunities for physicists, chemists, biologists, and materials scientists to understand and develop new nanomachines, sensors, or any of a number of other molecular assemblies. Researchers know relatively little about the structure, function, and spatial orientation of proteins noncovalently adsorbed on CNTs, yet because the interaction of CNTs with proteins depends strongly on the tridimensional structure of the proteins, many of these questions can be answered in simple terms.

In this Account, we describe recent research investigating the properties of CNT/protein hybrids. Proteins act to solvate CNTs and may sort them according to diameter or chirality. In turn, CNTs can support and immobilize enzymes, creating functional materials. Additional applications include proteins that assemble ordered hierarchical objects containing CNTs, and CNTs that act as protein carriers for vaccines, for example. Protein/CNT hybrids can form bioscaffolds and can serve as therapeutic and imaging materials.

Proteins can detect CNTs or coat them to make them biocompatible. One of the more challenging applications for protein/CNT hybrids is to make CNT substrates for cell growth and neural interfacing applications. The challenge arises from the structures' interactions with living cells, which poses questions surrounding the (nano)toxicology of CNTs and whether and how CNTs can detect biological processes or sense them as they occur.

The surface chemistry of CNTs and proteins, including interactions such as $\pi-\pi$ stacking interactions, hydrophobic interactions, surfactant-like interactions, and charge$-\pi$ interactions, governs the wealth of structures, processes, and functions that appear when such different types of molecules interact. Each residue stars in one of two main roles, and understanding which residues are best suited for which type of interaction can lead to the design of new hybrids. Nonlocally, the peptide or protein primary, secondary, and tertiary structures govern the binding of proteins by CNTs.

The conjugation of proteins with CNTs presents some serious difficulties both experimentally and culturally (such as bridging the "jargon barrier" across disciplines). The intersection of these fields lies between communities characterized by distinctly different approaches and methodologies. However, the examples of this Account illustrate that when this barrier is overcome, the exploitation of hybrid CNT–protein systems offers great potential.

Introduction

The conjugation of proteins with carbon nanotubes, CNTs, poses some serious difficulties both of experimental and of cultural nature. The two fields are often characterized by approaches and methodologies that are so distinctly different to make them akin to the devil, on the one side, and holy water, on the other side. It is not for authors to tell which is which. One can only draw the attention to the great potential for applications of merging proteins and CNTs. This Account illustrates some opportunities for the exploitation of these hybrid CNT–protein systems in nanotechnology, biology, medicine, and bioelectronics.

Published on the Web 07/05/2013 www.pubs.acs.org/accounts
10.1021/ar300347d

How Proteins and CNTs Interact

"Similis similia solvuntur" is an old Latin saying that means "similar dissolves similar". Proteins and CNTs are hardly similar. CNTs are geometrically simple objects made only of carbon atoms arranged in rigid cylinders. Proteins are truly "soft materials" that display an incredible variety of shapes and sizes. The driving forces for protein adsorption onto CNTs' surface are van der Waals (vdW) and hydrophobic interactions.[1−7] It is, in principle, possible to devise a strategy to develop an optimal peptide sequence to bind to a CNT. An interesting approach was based on random peptide libraries, such as phage display libraries.[1−3] The sequences that were identified suggested specific roles for individual amino acids in the direct peptide or protein interaction with CNTs.[1−3]

Surface Chemistry of CNTs and Proteins Control Their Binding

Four types of interactions control the binding of proteins to CNTs: (i) The vdW interaction, for a pair of atoms, is weak. However, the large number of protein−CNT contacts makes the total vdW force dominant. To assess the size of the interaction of residues with CNTs, it is possible to use the refractive index of the individual amino acids[8] (Figure 1a). Refraction is a function of polarizability, which is related to the vdW interaction. Unsurprisingly, aromatic residues show the highest values. Formation of $\pi-\pi$ stacks between these residues and the surface of the CNT play the most important role in the protein/CNT binding.[1,4−7,9] Experimentally, protein adsorption onto CNTs improves with the increase of the content of aromatic residues in the protein sequence.[1,7,9] Among the aromatic amino acids, tryptophan possesses the highest affinity for CNTs, followed by tyrosine, phenylalanine, and histidine.[10] Remarkably, there is a direct correlation between the polarizability and the binding strength of these amino acids. (ii) Hydrophobic interactions are another important contribution to protein−CNT interaction. Hydrophobicity originates from excluding the ordered water molecules from a nonpolar surface. Hydrophobic interactions tend to minimize the area of the nonpolar surface by clustering hydrophobic groups to avoid exposure to hydrophilic media. Amino acids with a hydrophobic side chain tend to bind to the hydrophobic surface of the CNT reducing the interfacial energy with the water interface (hydrophobic effect). It is possible to measure the hydrophobicity of individual amino acids and the tendency to bind to the CNT surface using the Wimley−White whole residue hydrophobicity scale (Figure 1b).[11] Other than the aromatic

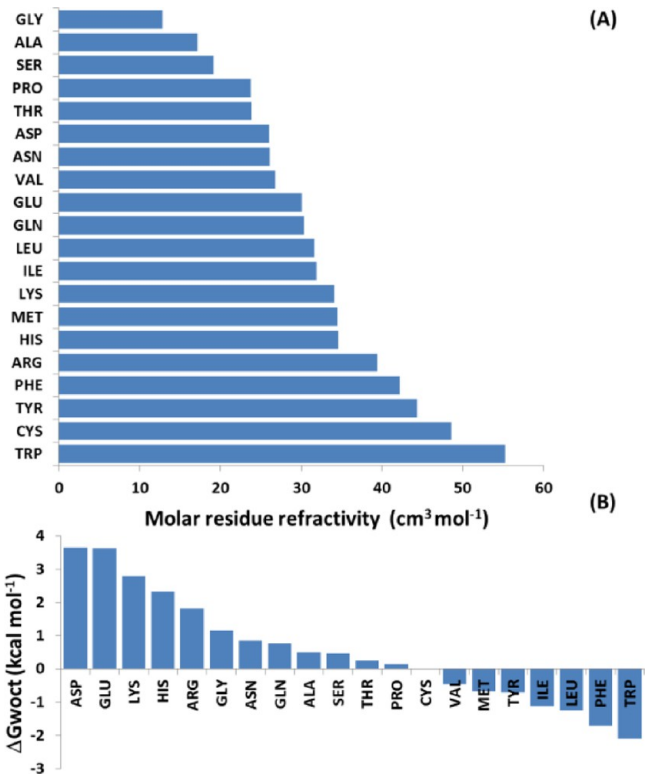

FIGURE 1. (A) Refractive indices[8] and (B) Wimley−White octanol hydrophobic scale[11] as a measure of the tendency of amino acids to interact with CNTs.

amino acids, aliphatic residues such as leucine, isoleucine, methionine, and valine play a major role in hydrophobic interactions.[4−6] (iii) Amphiphilicity can also come into play. Experimentally some peptides whose residues are mostly hydrophilic can also strongly bind onto the CNTs.[2] MD simulations revealed that polar residues such as glutamine, asparagine, aspartic acid, glutamic acid, arginine, and lysine may provide significant contributions to the binding of proteins onto CNTs.[4−6] Inspection of their binding mode shows that the hydrophobic aliphatic chains of these residues interact with the tube, whereas the hydrophilic groups point out toward water, (in analogy to the hydrophilic head of a surfactant)[4−6] generating what were defined as surfactant-like interactions.[6,12] Amphiphilic residues behave similarly to surfactants in the binding to CNTs.[4,6,12] (iv) Electrostatic interactions are quantitatively smaller than the previous ones but can alter significantly the electronic properties of CNTs.[13,14] Positively charged residues (arginine and lysine, in particular) can interact with the CNT via the classic cation$-\pi$ interactions.[13−16] When a net charge is not present in the residue, a charge-transfer mechanism may still occur, for example, during physisorption of

aminic and amidic residues.[13] Although the amount of interaction is relatively small and can be reduced by the presence of counterions, the effect clearly appears when considering either the enrichment of metallic tubes in solution[15] or the gating effect by protein binding on semiconducting CNTs.[16]

Protein Binding by CNTs Is Governed by the Primary, Secondary, and Tertiary Structures of the Peptide or Protein

The presence of amino acids with a strong tendency to bind CNTs does not suffice to guarantee binding of peptides or proteins to CNTs. Proteins with similar contents of residues bind CNTs differently.[1−3,17] The presence of amphiphilicity in peptides or proteins is a prerequisite to disperse CNTs efficiently.[18] Screening of peptides demonstrated that only amphiphilic peptides with hydrophobic residues at the center or at one end of the sequence form stable dispersions of peptide/CNT conjugates.[19] The effect leverages the surfactant-like properties of the peptide. Proteins with similar percentages of hydrophobic, aromatic, and polar residues show different binding behaviors.[7,20] The reason is their different three-dimensional arrangement. The higher the content of interacting residues on the surface of the protein (and their clustering), the higher is the strength of protein binding to CNTs.[7,20] Recently, we highlighted the role of shape complementarity as the discriminating factor to control binding between nano-objects and proteins.[6,22] By measurement of shape complementarity[22] or the variation of the solvent accessible surface area[4,7] during the binding process, it is possible to estimate the total interaction energy between a protein and a CNT.

CNT−Protein Interaction in Nanotechnology

Dispersion of CNTs by Proteins. Formation of stable dispersions of debundled nanotubes is one of the most important prerequisites for the technological exploitation of CNTs.[23] Different techniques were employed to obtain debundled nanotubes: mechanical approaches, chemical functionalization of the CNT surface, and noncovalent approaches.[23] The noncovalent approaches are attractive because they preserves the most sought after properties of CNTs, while improving their dispersibility.[23] Amphiphilic molecules, surfactants, synthetic polymers, biopolymers, DNA, and short peptides were used to disperse CNTs.[23] In recent years, also proteins have become widely used as CNT-dispersing agents.[15,17,24,25] Proteins are naturally amphiphilic. Taking advantage of this feature may avoid complicated synthetic procedures or the use of organic solvents. Most proteins are also pH-responsive, which is an advantage for system manipulation.[25] Steric hindrance and electrostastic repulsion are two of the major mechanisms active in the dispersion of CNTs in protein solutions. The attractive van der Waals interactions between individual CNTs are short ranged and, within 5 nm of intertube distance, become less than $1k_BT$. If the radius of a protein adsorbed on the surface of a CNT is greater than 2.5 nm (as, for example, in the case of bovine serum albumin), the steric interaction suffices to provide a barrier to prevent CNT aggregation.[15] Alternatively, if the radius of a protein is smaller than 2.5 nm, it does not provide sufficient steric repulsion between adjacent CNTs to impart stabilization. A different mechanism can be invoked. Lysozyme, for instance, has a radius of gyration of 1.43 nm but efficiently disperses CNTs.[25] At pH values different from the isoelectric point (IEP), the charge distribution on the atoms of lysozyme makes the protein-stabilized CNTs repel each other.[25] Around the IEP, lysozyme becomes nearly neutral, which results in an attraction between the adducts and subsequent flocculation of the system.[25] Considering the enormous variety of existing proteins and the further possibility to design them, there is plenty of room for finding new ways of dispersing nanotubes under every possible condition.

Sorting CNTs. Protocols were developed to sort CNTs according to electronic properties, diameter, or chirality.[26] These methodologies are effective but have some important shortcomings, such as complex separation processes, special equipment requirements, and difficulties for achieving mass production.[7]

The preference of proteins for binding to metallic CNTs was demonstrated and explained by the tendency of charged residues to adsorb more strongly on metals.[13,15] Cyclic peptides with controllable pore diameters were synthesized to recognize different-diameter CNTs.[27,28] Lysozyme was shown to bind preferentially and to disperse CNTs with larger diameters, which, in turn, realized an efficient large-scale fractionalization and enrichment of large diameter CNTs, Figure 2.[29] It also allowed removal of SWNTs impurities from DWCNT−SWCNT mixtures.[29] A chirality-preferential dispersion of CNTs by phage-displayed peptides was obtained with enrichment of specific (*n*, *m*) tubes.[3]

CNTs as Support for Enzyme Immobilization. The high surface area of CNTs makes them ideal candidates to support enzymes.[30,31] These protein−CNT conjugates possess properties of soluble and immobilized enzymes, which

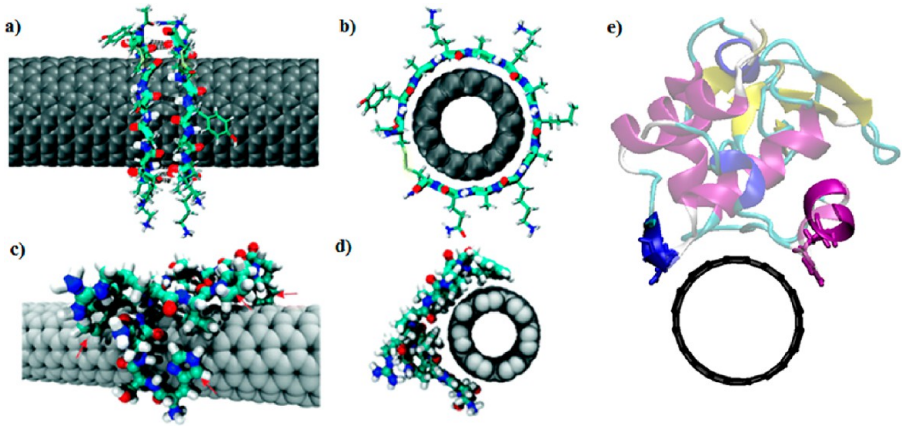

FIGURE 2. (a, b) Diameter-selective dispersion of CNTs by cyclic peptides. Adapted with permission from ref 28. Copyright 2010 American Chemical Society. (c, d) Chiral recognition of (7,6) CNTs by peptide HSNWRVPSPWQL. Adapted with permission from ref 3. Copyright 2012 The Royal Society of Chemistry. (e) Molecular tweezer recognition by lysozyme of a (10,10) CNT.

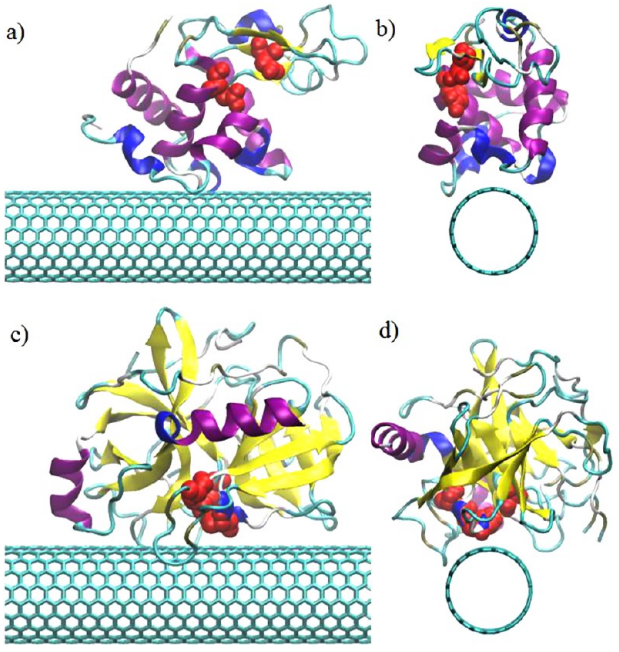

FIGURE 3. Interaction between (10,10) CNT and lysozyme (a, b) or α-chymotrypsin (c, d). In red are the catalytic residues of the proteins.

confer them useful attributes such as low mass transfer resistance, high activity and stability, and reusability.[30,31] In these systems, control of protein spatial orientation is achievable. The active site of the protein should be located away from the interaction area of the protein with the CNT as in lysozyme–CNT adducts where the protein remains active.[6] In contrast, adsorbed α-chymotrypsin retains only 1% of its native activity, because CNTs bind to the catalytic site, Figure 3.[30] Remarkably, conjugation of proteins with CNTs can increase both protein stability and activity,[31] even in strongly denaturing environments (high temperatures or organic solvents) to an extent greater than conventional flat supports.[31]

CNT–Enzyme Hybrids as Functional Materials. Highly active and stable polymer–nanotube–enzyme composites were prepared. Coatings with antimicrobial properties,[32] decontaminating microbicidal and sporicidal surfaces,[33] and self-cleaning and active antifouling films or paints[34] were realized. In these composites, enzymes both disperse CNTs in the matrix and perform their natural catalytic activity, while CNTs both support the enzyme in the polymeric matrices and improve the mechanical properties of the material.[32–34]

Proteins Assemble Ordered Hierarchical Objects. Proteins can integrate nanoscale objects in an ordered and hierarchical manner. Amphiphilic α-helical peptides were designed to control assembly of peptide-coated nanotubes into supramolecular structures.[18] Size and morphology of fibers was controlled by manipulating the solutions to affect peptide–peptide interactions.[18] Hierarchically controlled functionalization of the nanotube sidewalls with different nanoparticles (NPs) was also obtained by using proteins.[21,35] Hydrophobins, small and amphiphilic adhesion proteins found in filamentous fungi, were used for controlled functionalization of CNTs.[35] Self-assembly of CNTs and gold NPs, mediated by proteins, formed hybrid structures, where the one-dimensional regular array had a spacing of 2.6 nm, which implied a regular protein organization on the CNT sidewalls.[35] Peptide sequences were designed that organize themselves into a tubular structure surrounding the CNTs.[21] The geometrically defined, virus-like coating created by these peptides converts the smooth surfaces of CNTs into richly textured, multilayered surfaces with long-range order,

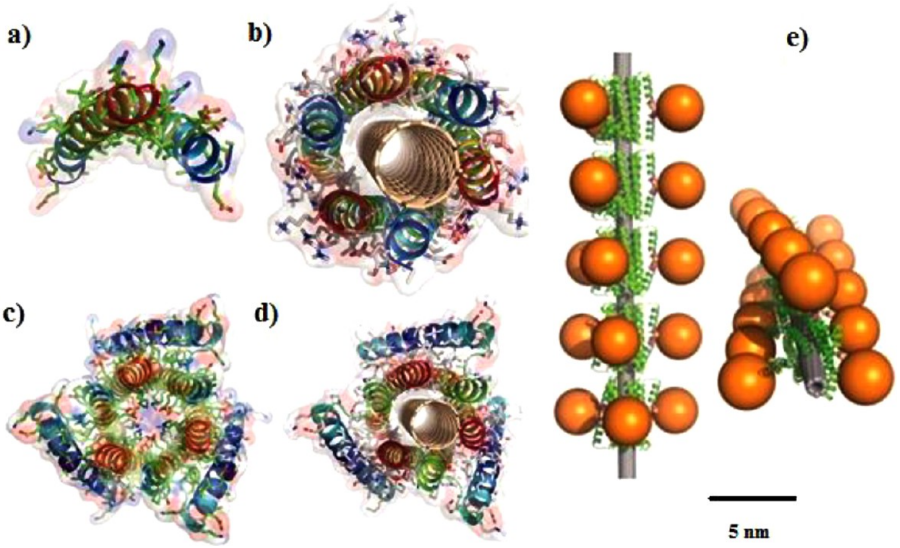

FIGURE 4. (a) Crystal structure of *de novo* designed helical assembly protein HexCoil-Ala (PDB 3S0R). (b) Model structure of HexCoil-Gly with a (3,8) CNT. (c) Crystal structure of the native inner ring of helices of a domain-swapped helical protein called DSD (PDB 1G6U). (d) Model of DSD-Ala with a (3,8) SWNT. (e) Computational model of gold nanoparticles grown on cysteine-modified DSD-Gly hexamers wrapped around individual CNT complex. Adapted with permission from ref 21. Copyright 2011 American Association for the Advancement of Science.

capable of directing the assembly of gold nanoparticles into helical arrays along the CNT axis, Figure 4.[21]

CNT–Protein Interaction in Biology and Medicine

Targeted Carriers for Therapeutic and Imaging Materials. CNTs' ability to pass cellular membranes can allow them to shuttle cargoes from the extracellular matrix into the cells[36,37] with great potential for drug delivery applications.[38–40] Among the pharmaceutically relevant entities that can be loaded inside CNTs are anticancer agents and antibiotics, genes encoding sequences for gene therapy (plasmid DNA, DNA, RNA), and small interfering RNA (siRNA) for RNA interference (RNAi) therapy.[38–40] CNTs can transport into cells also proteins,[41] adsorbed on the sidewalls or in their interior, and become agents of improved delivery efficiency or increased protein stability against proteases.

CNTs themselves are usable in cancer therapies including photothermal therapy, photoacoustic therapy, and radiofrequency ablation.[38–40] CNTs also show promise against a variety of pathogens for their strong antimicrobial activity.[42] Nonspecific cell targeting of CNTs remains a barrier to delivery applications. The CNT surface can be decorated with proteins or antibodies.[15,24,25] Besides enhancing CNT dispersibility in aqueous solutions, biomolecules provide appropriate selectivity either toward a loaded material or for the CNTs toward a therapeutic target.[38–40,43]

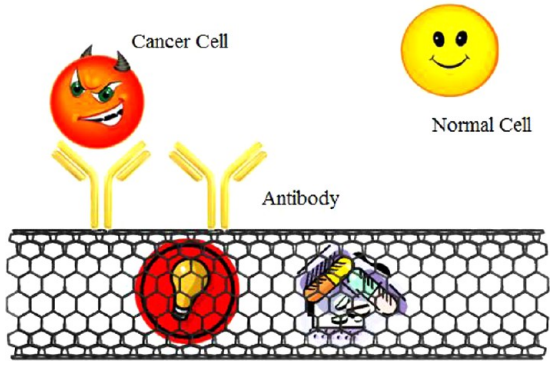

FIGURE 5. Tumor-cell targeting CNT constructs carrying drugs or imaging materials synthesized by coating CNTs with monoclonal antibodies.

Tumor-cell targeting CNT constructs were synthesized by coating CNTs with monoclonal antibodies, with the intent of delivering CNTs selectively to specific cellular sites.[43] Antibody-coated nanotubes attached themselves to the surface of the lymphoma cells. Upon exposure to near-infrared light, the tubes heated up and killed the cells, Figure 5. In a control group, with nanotubes coated by a different antibody, binding did not occur, and tumor cells were unharmed.[43]

CNTs can load in their hollow interior a variety of cargoes, including materials of medical interest for diagnostic such as Fe_2O_3 nanoparticles or Gd^{3+} ions for magnetic resonance imaging (MRI), I_2 molecules for X-ray computed tomography (CT) imaging, and radionuclides (^{86}Y, ^{211}At, and ^{225}Ac) for emission tomography (PET and SPECT) or

α-radioimmunotherapy.[38,39,44] The optical properties of CNTs may enable the development of new cell-specific diagnostic techniques based on near-infrared fluorescence, photoacoustics, and Raman imaging.[38,39,44] These hybrid constructs can provide the basis for theranostics, offering a platform for the development of candidates for simultaneous diagnosis and targeted delivery to specific sites in the body.

Immunization with Biofunctionalized CNT. CNTs are good multipresentation systems. They are able to present peptides attached to their surface with the correct conformation for recognition without perturbing the secondary structure of the peptide.[45,46] Peptide antigens are poorly immunogenic, and conjugation to protein carriers is usually necessary to improve antibody production. Protein carriers are intrinsically immunogenic, and the antibodies generated by them present low specificity. Decoration of CNTs with B and T cell peptide epitopes generated a multivalent system that induced strong immune responses.[45,46] The resulting antibodies neutralized viruses, demonstrating the potential of CNTs as components for synthetic vaccines.[45,46]

Protein/CNT Hybrid Bioscaffold. CNTs can be incorporated in proteic bioscaffolds, providing structural reinforcement as well as imparting novel properties, such as electrical conductivity,[38,47] in tissue engineering. Using CNTs for optical, magnetic resonance, and radiotracer contrast agents could provide better means for evaluating tissue formation. In addition, monitoring and altering intra- and intercellular processes with CNTs would be useful for designing improved engineered tissues.[38,47,48]

CNT as Substrates for Cell Growth and Neural Interfacing Applications. CNTs functionalized with cell adhesion peptides or proteins are promising candidates for next generation cell-growth substrates since they support cell adhesion, growth, and differentiation.[38,47,49] Functionalization of CNTs with extracellular matrix (ECM) proteins,[38,47,49,50] such as fibronectin or collagen,[38,47,49,50] produce effective platforms to control cell adhesion.[38,47,49,50] The adhesion sites of fibronectin–CNT hybrids induce selective cell growth. Fibronectin maintains its native structure and high activity on CNTs.[50] ECM proteins have a crucial role in cell–CNT interactions and provide an efficient strategy to control their growth.[38,47,49,50] Chemical and electrical properties of CNTs can guide and sense cell growth.[38,47,50]

In neuroscience, laminin-coated CNTs are substrates compatible with neurons and favor neuronal adhesion and neuron survival and growth. They also support neurite elongation.[51] These hybrids can serve as an extracellular scaffold to guide directed axonal growth.[51] They also have potential as nerve conduits in nerve tissue engineering and neuronal implants, Figure 6.[52,53]

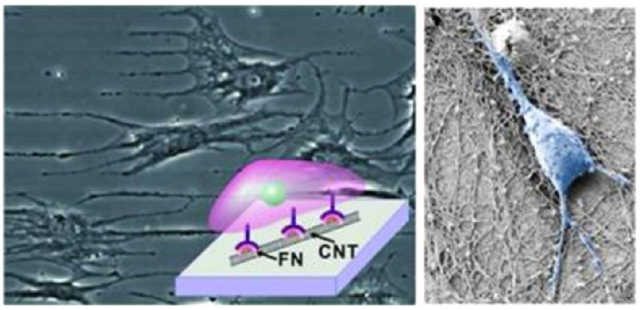

FIGURE 6. (a) Fibronectin–carbon nanotube (CNT) hybrid nanostructures to control the adhesion and growth of cells. Adapted with permission from ref 50. Copyright 2011 Wiley. (b) Scanning electron micrograph of a neuron (pseudocolored in blue) growing on a carbon nanotube layer. Adapted with permission from ref 53. Copyright 2009 Nature Publishing Group.

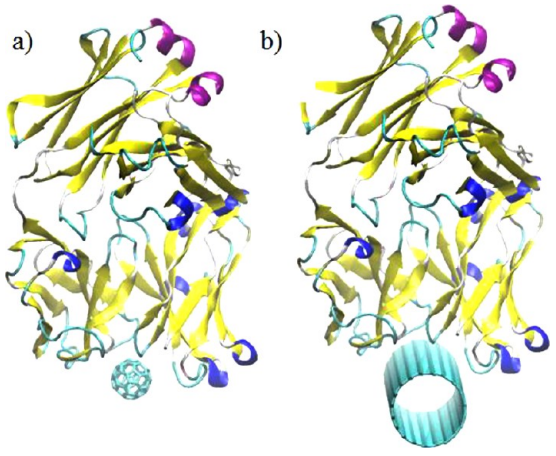

FIGURE 7. Docking models of the Fab fragment of the C_{60} antifullerene antibody (PDB 1EMT) interacting with (a) C_{60} and (b) (10,10) CNT.

Proteins That Detect CNTs. Erlanger and co-workers generated IgG antibodies in mice that bind specifically to C_{60}.[54] The same monoclonal antibody recognizes and binds specifically to CNTs.[55] X-ray crystallography of its Fab fragment showed that the binding cavity is a cluster of hydrophobic residues.[54] An induced fit mechanism binds fullerenes and explains how CNTs are recognized, Figure 7.[55] CNTs have a curved, hydrophobic, π-electron-rich surface analogous to that of C_{60}; the hydrophobic binding site of the antibody is sufficiently flexible to recognize both.[54] C_{60} antibodies on CNTs were directly imaged immunologically and by AFM.[55] Standard immunological techniques can be used in conjunction with these monoclonal antibodies to

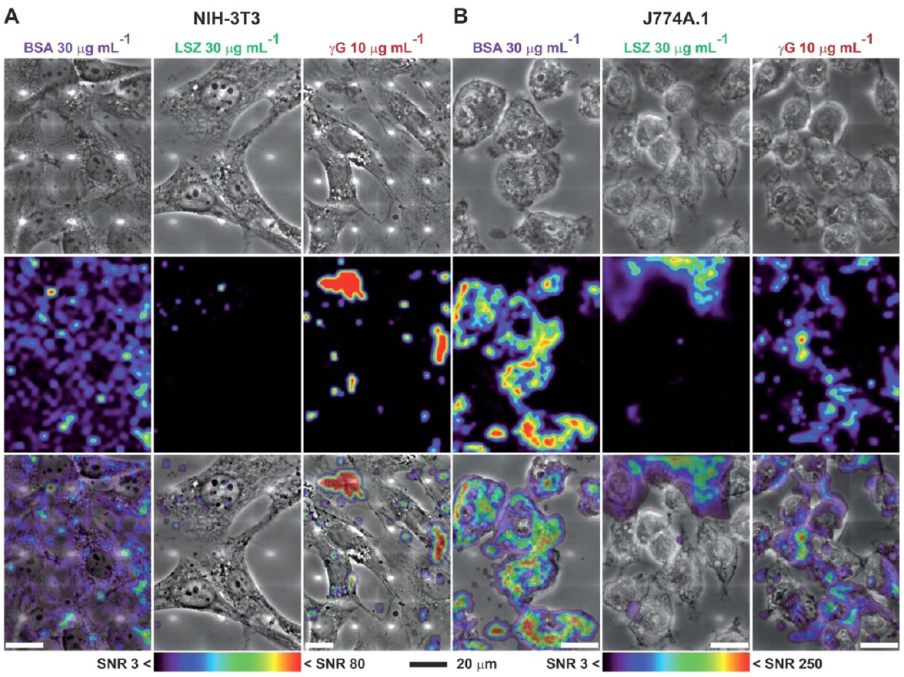

FIGURE 8. (A) Raman maps of NIH-3T3 murine fibroblasts exposed to CNT–protein show different levels of heterogeneous CNT delivery and subcellular localization. (B) Similar imaging for the macrophage-like cell line J774A.1 that shows more homogeneous uptake of CNT–γG and highlights the aggregation of CNT–LSZ. Reprinted with permission from ref 59. Copyright 2012 The Royal Society of Chemistry.

detect, monitor, and quantify CNTs. Specific antibodies able to recognize specific nanotubes can be developed.

CNT Alteration of Protein Functioning. A toxicological profile of CNTs has emerged during the last years.[4,5] One of the main aspects involves CNT interaction with proteins. CNTs may interfere directly with protein functions.[4,5] Park et al. reported that CNTs block ion channel proteins.[56] They speculated that nanotubes fit into the pore and either hinder ion movement or prevent conformational changes. Islam et al.[57] demonstrated the ability of CNTs to reorganize actin structures in cells and *ex vivo*. Cell proliferation was greatly reduced in CNT-treated cells with an increase in actin-related division defects.

Protein Biocompatible CNT Coating. CNT toxicity can be completely alleviated by chemical functionalization;[58] in the same way, encapsulation by protein may control and decrease the overall CNT cytotoxicity.[4,5,7] Well-dispersed CNTs are less toxic than their agglomerates.[7] Protein binding also alters the cellular interaction pathways of CNTs.[7] Binding of blood proteins, in particular albumins, to the CNT surface results in reduced cytotoxicity of these protein-coated CNTs.[7] The coating of CNTs with proteins can confer on them a new biological "identity", defining their recognition by cells of the innate immune system, which may affect CNT cellular uptake and recovery (Figure 8),[59,60] biodistribution,[61] and toxicity.[4,5,7]

CNT–Protein Interaction for Sensors

Electrochemical Sensors. Hybrid systems obtained by conjugating redox proteins with CNTs have potential in bioelectronics and biosensing and for biofuel cells.[62–65] Redox proteins produce processable signals in response to biological events, environment modifications, or optical absorption. CNTs can act as efficient transducers of tiny signals.[62] Protein–nanotube conjugates can provide appropriate molecular orientation, flexibility, and efficient, reproducible electrical conduction. CNTs can increase protein loading and assist electron transfer to the electrode. The possibility of direct electron transfer between a CNT and glucose oxidase allowed the construction of amperometric glucose biosensors.[65,66] Several proteins, in particular heme proteins, were electrochemically contacted with the use of CNTs to produce biosensors, Figure 9.[65]

Carbon Nanotube Field-Effect Transistors (CNTFET). Semiconducting CNTs can be used for field-effect transistors (FETs).[67] The conductivity of a CNT is sensitive to its environment and varies in the presence of nearby charges and with the adsorption of molecules.[13] CNTFET sensors are small, their response is fast, and the active detection area is scalable to the size of individual proteins. These sensors are extremely sensitive since all the current passes through the detection point.[67]

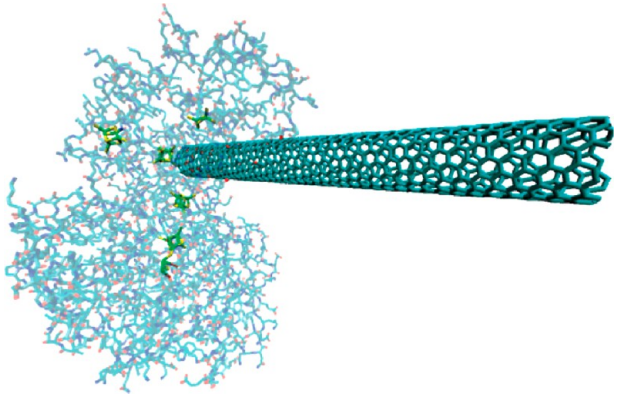

FIGURE 9. Wiring-up proteins to electrodes by CNT. Reprinted with permission from ref 63. Copyright 2007 American Chemical Society.

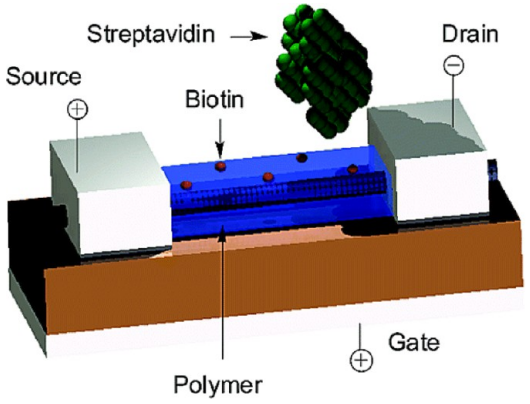

FIGURE 10. Biotin–streptavidin binding is detected by changes in device characteristics. The polymer layer avoids nonspecific protein binding. Reprinted with permission from ref 70. Copyright 2003 American Chemical Society.

Detection of Proteins and Biorecognition Events. Proteins carry charged groups that, upon adsorption, can electrostatically gate a semiconducting CNT.[13] The gating was exploited for protein detection in solution with direct electronic readout.[13,68] In a streptavidin sensor, a gradual shift in threshold voltage was observed as a result of the slow accumulation of a monolayer of adsorbed proteins.[13] The conductivity changes were assigned to charge injection from the amine groups of the protein.[13] Cytochrome *c* adsorption onto an individual CNTFET was detected by monitoring the decrease of transport in the CNTFET device.[69] The negative shift in conductivity allowed estimating the number of adsorbed proteins.[69]

CNTFETs detected biotin–streptavidin binding.[70] The source–drain current dependence on gate voltage of the CNTFET showed a significant change upon streptavidin binding to the biotin-functionalized carbon nanotube, Figure 10.[70]

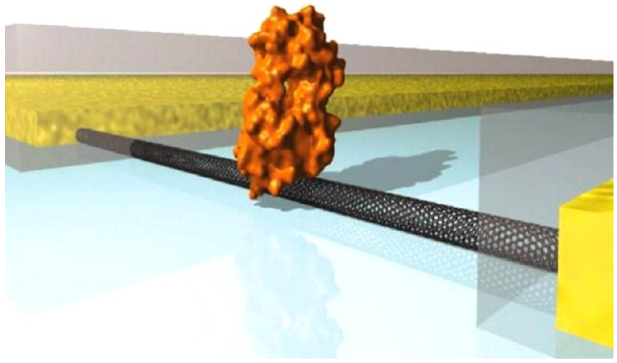

FIGURE 11. Tethering a single lysozyme molecule to a CNTFET produces a stable, high-bandwidth transducer for protein motion. Adapted with permission from ref 72. Copyright 2012 American Association for the Advancement of Science.

The use of functionalized CNTFETs can be extended also to antigen–antibody[68] or virus recognition.[71]

These devices can detect, with high specificity, clinically important biomolecules associated with human diseases.[67] The same approach can be used in the synthesis and fabrication of CNT microarrays for proteomics applications aimed at detecting large numbers of different proteins. These arrays are attractive because no labeling is required and all aspects of the assay can be carried out in solution.[67,68]

CNT/Protein Hybrid To Sense Biological Processes. Catalytic activity of enzymes can be monitored.[72] Choi et al.[72] attached a single T4 lysozyme molecule to a CNTFET device. In their single-molecule enzymatic reaction assay, lysozyme dynamics was monitored revealing the conformational motions of the enzyme active site during enzymatic reaction turnover, Figure 11.[72] Molecular motions created changes in electrostatic potentials that were converted into dynamically changing electron fluxes, identifying the hydrolytic events.[72]

CNT/Protein Hybrid To Sense Molecules. Biorecognition activity of a protein attached to a CNT transistor can be used to sense molecules.[73] These devices eavesdrop on what proteins do, converting the information of the binding into electrical signals.[73] Johnson and co-workers coupled olfactory receptor proteins (ORs) with CNT transistors, Figure 12, making it possible to transduce signals associated with odorants binding to the ORs.[73]

Conclusion

CNTs and proteins differ greatly. CNTs are simple objects made of carbon atoms arranged cylindrically, characterized by outstanding mechanical and electrical properties.

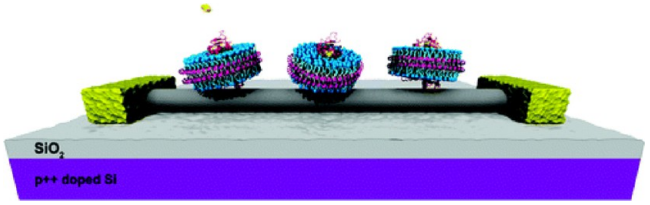

FIGURE 12. Coupling olfactory receptor proteins (ORs) with a CNTFET produced a device able to transduce signals associated with molecule binding to ORs. Reprinted with permission from ref 73. Copyright 2011 American Chemical Society.

Proteins are "soft materials", with a huge assortment of chemical–physical features and offering an incredible variety of shapes and sizes. They are "nanomachines" able to "sense" the presence of molecules or to assemble or disassemble molecular size components with incredible efficiency or to be structural or "active mechanical" components of an organism. The development of hybrid CNT–protein systems potentially combines CNT and protein properties and may drive fundamental technological advancements in different fields such as nanotechnology, medicine, materials science, and biology.

BIOGRAPHICAL INFORMATION

Matteo Calvaresi was born in 1979 and received his Ph.D. in 2008. In 2011, he became Assistant Professor of Organic Chemistry at the University of Bologna. He was Visiting Researcher at Peking University and Visiting Professor at University of Pune. His current research interests focus on the study of interactions between proteins and carbon nanomaterials, both experimentally and computationally.

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FOOTNOTES

The authors declare no competing financial interest.
Dedicated to Prof. Maurizio Prato on the occasion of his C 60th birthday.

REFERENCES

1 Wang, S.; Humphreys, E. S.; Chung, S.-Y.; Delduco, D. F.; Lustig, S. R.; Wang, H.; Parker, K. N.; Rizzo, N. W.; Subramoney, S.; Chiang, Y. M.; Jagota, A. Peptides with Selective Affinity for Carbon Nanotubes. *Nat. Mater.* **2003**, *2*, 196–200.

2 Brown, S.; Jespersen, T. S.; Nygard, J. A Genetic Analysis of Carbon-Nanotube-Binding Proteins. *Small* **2008**, *4*, 416–420.

3 Yu, T.; Gong, Y.; Lu, T.; Wei, L.; Li, Y.; Mu, Y.; Chen, Y.; Liao, K. Recognition of Carbon Nanotube Chirality by Phage Display. *RSC Adv.* **2012**, *2*, 1466–1476.

4 Zuo, G.; Kang, S.-G; Xiu, P.; Zhao, Y.; Zhou, R. Interactions Between Proteins and Carbon-Based Nanoparticles: Exploring the Origin of Nanotoxicity at the Molecular Level. *Small* **2013**, *9*, 1546–1556.

5 Yang, S.-T.; Liu, y.; Wang, Y.-W.; Cao, A. Biosafety and Bioapplication of Nanomaterials by Designing Protein–Nanoparticle Interactions. *Small* **2013**, *9*, 1635–1653.

6 Calvaresi, M.; Hoefinger, S.; Zerbetto, F. Probing the Structure of Lysozyme-Carbon-Nanotube Hybrids with Molecular Dynamics. *Chem.—Eur. J.* **2012**, *18*, 4308–4313.

7 Gea, C.; Dua, J.; Zhaoa, L.; Wanga, L.; Liua, Y.; Lia, D.; Yanga, Y.; Zhoub, R.; Zhaoa, Y.; Chaid, Z.; Chena, C. Binding of Blood Proteins to Carbon Nanotubes Reduces Cytotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 16968–16973.

8 McMeekin, T. L.; Wilensky, M.; Groves, M. L. Refractive Indices of Proteins in Relation to Amino Acid Composition and Specific Volume. *Biochem. Biophys. Res. Commun.* **1962**, *7*, 151–156.

9 Zorbas, V.; Smith, A. L.; Xie, H.; Ortiz-Acevedo, A.; Dalton, A. B.; Dieckmann, G. R.; Draper, R. K.; Baughman, R. H.; Musselman, I. H. Importance of Aromatic Content for Peptide/Single-Walled Carbon Nanotube Interactions. *J. Am. Chem. Soc.* **2005**, *127*, 12323–12328.

10 Xie, H.; Becraft, E. J.; Baughman, R. H.; Dalton, A. B.; Dieckmann, G. R. Ranking the Affinity of Aromatic Residues for Carbon Nanotubes by Using Designed Surfactant Peptides. *J. Pept. Sci.* **2008**, *14*, 139–151.

11 White, S. H.; Wimley, W. C. Membrane Protein Folding and Stability: Physical Principles. *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 319–365.

12 Li, J.; Garg, M.; Shah, D.; Rajagopalan, R. Solubilization of Aromatic and Hydrophobic Moieties by Arginine in Aqueous Solutions. *J. Chem. Phys.* **2010**, *133*, No. 054902.

13 Bradley, K.; Briman, M.; Star, A.; Gruner, G. Charge Transfer from Adsorbed Proteins. *Nano Lett.* **2004**, *4*, 253–256.

14 Samarajeewa, D. R.; Dieckmann, G. R.; Nielsen, S. O.; Musselman, I. H. Modifying the Electronic Properties of Single-Walled Carbon Nanotubes Using Designed Surfactant Peptides. *Nanoscale* **2012**, *4*, 4544–4554.

15 Nepal, D.; Geckeler, K. E. Proteins and Carbon Nanotubes: Close Encounter in Water. *Small* **2007**, *3*, 1259–1265.

16 Star, A.; Gabriel, J.-C. P.; Bradley, K.; Grüner, G. Electronic Detection of Specific Protein Binding Using Nanotube FET Devices. *Nano Lett.* **2003**, *3*, 459–463.

17 Matsuura, K.; Saito, T.; Okazaki, T.; Ohshima, S.; Yumura, M.; Iijima, S. Selectivity of Water-Soluble Proteins in Single-Walled Carbon Nanotube Dispersions. *Chem. Phys. Lett.* **2006**, *429*, 497–502.

18 Dieckmann, G. R.; Dalton, A. B.; Johnson, P. A.; Razal, J.; Chen, J.; Giordano, G. M.; Muñoz, E.; Musselman, I. H.; Baughman, R. H.; Draper, R. K. Controlled Assembly of Carbon Nanotubes by Designed Amphiphilic Peptide Helices. *J. Am. Chem. Soc.* **2003**, *125*, 1770–1777.

19 Deshpande, M. S.; Mazumdar, S. Sequence Specific Association of Tryptic Peptides with Multiwalled Carbon Nanotubes: Effect of Localization of Hydrophobic Residues. *Biomacromolecules* **2012**, *13*, 1410–1419.

20 Matsuura, K.; Saito, T.; Okazaki, T.; Ohshima, S.; Yumura, M.; Iijima, S. Selectivity of Water-Soluble Proteins in Single-Walled Carbon Nanotube Dispersions. *Chem. Phys. Lett.* **2006**, *429*, 497–502.

21 Grigoryan, G.; Kim, Y. H.; Acharya, R.; Axelrod, K.; Jain, R. M.; Willis, L.; Drndic, M.; Kikkawa, J. M.; DeGrado, W. F. Computational Design of Virus-Like Protein Assemblies on Carbon Nanotube Surfaces. *Science* **2011**, *332*, 1071–1076.

22 Calvaresi, M.; Zerbetto, F. Baiting Proteins with C_{60}. *ACS Nano* **2010**, *4*, 2283–2299.

23 Premkumar, T.; Mezzenga, R.; Geckeler, K. E. Carbon Nanotubes in the Liquid Phase: Addressing the Issue of Dispersion. *Small* **2012**, *8*, 1299–1313.

24 Karajanagi, S. S.; Yang, H.; Asuri, P.; Sellitto, E.; Dordick, J. S.; Kane, R. S. Protein-Assisted Solubilization of Single-Walled Carbon Nanotubes. *Langmuir* **2006**, *22*, 1392–1395.

25 Nepal, D.; Geckeler, K. E. pH-Sensitive Dispersion and Debundling of Single-Walled Carbon Nanotubes: Lysozyme as a Tool. *Small* **2006**, *2*, 406–412.

26 Hersam, M. C. Progress Towards Monodisperse Single-Walled Carbon Nanotubes. *Nat. Nanotechnol.* **2008**, *3*, 387–394.

27 Ortiz-Acevedo, A.; Xie, H.; Zorbas, V.; Sampson, W. M.; Dalton, A. B.; Baughman, R. H.; Draper, R. K.; Musselman, I. H.; Dieckmann, G. R. Diameter-Selective Solubilization of Single-Walled Carbon Nanotubes by Reversible Cyclic Peptides. *J. Am. Chem. Soc.* **2005**, *127*, 9512–9517.

28 Chiu, C.-C.; Maher, M. C.; Dieckmann, G. R.; Nielsen, S. O. Molecular Dynamics Study of a Carbon Nanotube Binding Reversible Cyclic Peptide. *ACS Nano* **2010**, *4*, 2539–2546.

29 Nie, H.; Wang, H.; Cao, A.; Shi, Z.; Yang, S.-T.; Yuan, Y.; Liu, Y. Diameter-Selective Dispersion of Double-Walled Carbon Nanotubes by Lysozyme. *Nanoscale* **2011**, *3*, 970–973.

30 Karajanagi, S. S.; Vertegel, A.; Ravi, A.; Kane, S.; Dordick, J. S. Structure and Function of Enzymes Adsorbed onto Single-Walled Carbon Nanotubes. *Langmuir* **2004**, *20*, 11594–11599.

31 Asuri, P.; Karajanagi, S. S.; Yang, H.; Yim, T. J.; Kane, R. S.; Dordick, J. S. Increasing Protein Stability Through Control of the Nanoscale Environment. *Langmuir* **2006**, *22*, 5833–5836.

32 Pangule, R. C.; Brooks, S. J.; Dinu, C. Z.; Bale, S. S.; Salmon, S. L.; Zhu, G. Z.; Metzger, D. W.; Kane, R. S.; Dordick, J. S. Antistaphylococcal Nanocomposite Films Based on Enzyme−Nanotube Conjugates. *ACS Nano* **2010**, *4*, 3993–4000.

33 Dinu, C. Z.; Zhu, G.; Bale, S. S.; Anand, G.; Reeder, P. J.; Sanford, K.; Whited, G.; Kane, R. S.; Dordick, J. S. Enzyme-Based Nanoscale Composites for Use as Active Decontamination Surfaces. *Adv. Funct. Mater.* **2010**, *20*, 392–398.

34 Asuri, P.; Karajanagi, S. S.; Kane, R. S.; Dordick, J. S. Polymer-Nanotube-Enzyme Composites as Active Antifouling Films. *Small* **2006**, *3*, 50–53.

35 Kurppa, K.; Jiang, H.; Szilvay, G. R.; Nasibulin, A. G.; Kauppinen, E. I.; Linder, M. B. Controlled Hybrid Nanostructures through Protein-Mediated Noncovalent Functionalization of Carbon Nanotubes. *Angew. Chem., Int. Ed.* **2007**, *46*, 6446–6449.

36 Lacerda, L.; Raffa, V.; Prato, M.; Bianco, A.; Kostarelos, K. Cell Penetrating Carbon Nanotubes in the Delivery of Therapeutics. *Nano Today* **2007**, *2*, 38–43.

37 Höfinger, S.; Melle-Franco, M.; Gallo, T.; Cantelli, A.; Calvaresi, M.; Gomes, J. A.; Zerbetto, F. A Computational Analysis of the Insertion of Carbon Nanotubes into Cellular Membranes. *Biomaterials* **2011**, *32*, 7079–7085.

38 Heister, E.; Brunner, E. W.; Dieckmann, G. R.; Jurewicz, I.; Dalton, A. B. Are Carbon Nanotubes a Natural Solution? Applications in Biology and Medicine. *ACS Appl. Mater. Interfaces* **2013**, *5*, 1870–1891.

39 Kostarelos, K.; Bianco, A.; Prato, M. Promises, Facts and Challenges for Carbon Nanotubes in Imaging and Therapeutics. *Nat. Nanotechnol.* **2009**, *4*, 627–633.

40 Liu, Z.; Robinson, J. T.; Tabakman, S. M.; Yang, K.; Dai, K. Carbon Materials for Drug Delivery & Cancer Therapy. *Mater. Today* **2011**, *14*, 316–323.

41 Kam, N. W.; Dai, H. Carbon Nanotubes as Intracellular Protein Transporters: Generality and Biological Functionality. *J. Am. Chem. Soc.* **2005**, *127*, 6021–6026.

42 Kang, S.; Pinault, M.; Pfefferle, L. D.; Elimelech, M. Single-Walled Carbon Nanotubes Exhibit Strong Antimicrobial Activity. *Langmuir* **2007**, *23*, 8670–8673.

43 Chakravarty, P.; Marches, R.; Zimmerman, N. S.; Swafford, A. D.-E.; Bajaj, P.; Musselman, I. H.; Pantano, P.; Draper, R. K.; Vitetta, L. S. Thermal Ablation of Tumor Cells with Antibody-Functionalized Single-Walled Carbon Nanotubes. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 8697–8702.

44 Liu, Z.; Yang, K.; Lee, S.-T. Single-Walled Carbon Nanotubes in Biomedical Imaging. *J. Mater. Chem.* **2011**, *21*, 586–598.

45 Pantarotto, D.; Hoebeke, J.; Graff, R.; Partidos, C. D.; Briand, J.-P.; Prato, M.; Bianco, A. Synthesis, Structural Characterization and Immunological Properties of Carbon Nanotubes Functionalized with Peptides. *J. Am. Chem. Soc.* **2003**, *125*, 6160–6164.

46 Pantarotto, D.; Partidos, C. D.; Hoebeke, J.; Brown, F.; Kramer, E.; Briand, J. P.; Muller, S.; Prato, M.; Bianco, A. Immunization with Peptide-Functionalized Carbon Nanotubes Enhances Virus-Specific Neutralizing Antibody Responses. *Chem. Biol.* **2003**, *10*, 961–966.

47 Harrison, B. S.; Atala, A. Carbon Nanotube Applications for Tissue Engineering. *Biomaterials* **2007**, *28*, 344–353.

48 Menard-Moyon, C.; Kostarelos, K.; Prato, M.; Bianco, A. Functionalized Carbon Nanotubes for Probing and Modulating Molecular Functions. *Chem. Biol.* **2010**, *17*, 107–115.

49 Cai, N.; Wong, C. C.; Gong, Y. X.; Tan, S. C. W.; Chan, V.; Liao, K. Modulating Cell Adhesion Dynamics on Carbon Nanotube Monolayer Engineered with Extracellular Matrix Proteins. *ACS Appl. Mater. Interfaces* **2010**, *2*, 1038–1047.

50 Namgung, S.; Kim, T.; Baik, K. Y.; Lee, M.; Nam, J.-M.; Hong, S. Fibronectin−Carbon-Nanotube Hybrid Nanostructures for Controlled Cell Growth. *Small* **2011**, *7*, 56–61.

51 Park, S. Y.; Choi, D. S.; Jin, H. J.; Park, J.; Byun, K.-E.; Lee, K.-B.; Hong, S. Polarization-Controlled Differentiation of Human Neural Stem Cells Using Synergistic Cues from the Patterns of Carbon Nanotube Monolayer Coating. *ACS Nano* **2011**, *5*, 4704–4711.

52 Fabbro, A.; Bosi, S.; Ballerini, L.; Prato, M. Carbon Nanotubes: Artificial Nanomaterials to Engineer Single Neurons and Neuronal Networks. *ACS Chem. Neurosci.* **2012**, *3*, 611–618.

53 Silva, G. A. Nanomedicine: Shorting Neurons with Nanotubes. *Nat. Nanotechnol.* **2009**, *4*, 82–83.

54 Braden, B. C.; Goldbaum, F. A.; Chen, B.-X.; Kirschner, A. N.; Wilson, S. R.; Erlanger, B. F. X-ray Crystal Structure of an anti-Buckminsterfullerene Antibody Fab Fragment: Biomolecular recognition of C_{60}. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12193–12197.

55 Erlanger, B. F.; Chen, B.-X.; Zhu, M.; Brus, L. Binding of an Anti-Fullerene IgG Monoclonal Antibody to Single Wall Carbon Nanotubes. *Nano Lett.* **2001**, *1*, 465–467.

56 Park, K. H.; Chhowalla, M.; Iqbal, Z.; Sesti, F. Single-Walled Carbon Nanotubes Are a New Class of Ion Channel Blockers. *J. Biol. Chem.* **2003**, *278*, 50212–50216.

57 Holt, B. D.; Short, P. A.; Rape, A. D.; Wang, Y.-L.; Islam, M. F.; Dahl, K. L. Carbon Nanotubes Reorganize Actin Structures in Cells and ex Vivo. *ACS Nano* **2010**, *4*, 4872–4878.

58 Ali-Boucetta, H.; Nunes, A.; Sainz, R.; Herrero, R.; Tian, B.; Prato, M.; Bianco, A.; Kostarelos, K. Asbestos-like Pathogenicity of Long Carbon Nanotubes Alleviated by Chemical Functionalization. *Angew. Chem., Int. Ed.* **2013**, *52*, 2274–2278.

59 Holt, B. D.; McCorry, M. C.; Boyer, P. D.; Dahl, K. N.; Islam, M. F. Not All Protein-Mediated Single-Wall Carbon Nanotube Dispersions Are Equally Bioactive. *Nanoscale* **2012**, *4*, 7425–7434.

60 Holt, B. D.; Dahl, K. N.; Islam, M. F. Cells Take up and Recover from Protein-Stabilized Single-Wall Carbon Nanotubes with Two Distinct Rates. *ACS Nano* **2012**, *6*, 3481–3490.

61 Wang, H.; Yang, S.-T.; Cao, A.; Liu, Y. Quantification of Carbon Nanomaterials in Vivo. *Acc. Chem. Res.* **2013**, *46*, 750–760.

62 Kim, S. N.; Rusling, J. F.; Papadimitrakopoulos, F. Carbon Nanotubes for Electronic and Electrochemical Detection of Biomolecules. *Adv. Mater.* **2007**, *19*, 3214–3228.

63 McDonald, T. J.; Svedruzic, D.; Kim, Y.-H.; Blackburn, J. L.; Zhang, S. B.; King, P. W.; Heben, M. J. Wiring-Up Hydrogenase with Single-Walled Carbon Nanotubes. *Nano Lett.* **2007**, *7*, 3528–3534.

64 Azamian, B. R.; Davis, J. J.; Coleman, K. S.; Bagshaw, C. B.; Green, M. L. H. Bioelectrochemical Single-Walled Carbon Nanotubes. *J. Am. Chem. Soc.* **2002**, *124*, 12664–12665.

65 Wang, J. Carbon-Nanotube Based Electrochemical Biosensors: A Review. *Electroanalysis* **2005**, *17*, 7–14.

66 Guiseppi-Elie, A.; Lei, C.; Baughman, R. H. Direct Electron Transfer of Glucose Oxidase on Carbon Nanotubes. *Nanotechnology* **2002**, *13*, 559–564.

67 Allen, B. L.; Kichambare, P. D.; Star, A. Carbon Nanotube Field-Effect-Transistor-Based Biosensors. *Adv. Mater.* **2007**, *19*, 1439–1451.

68 Chen, R. J.; Bangsaruntip, S.; Drouvalakis, K. A.; Wong Shi Kam, N.; Shim, M.; Li, Y.; Kim, W.; Utz, P. J.; Dai, H. Noncovalent Functionalization of Carbon Nanotubes for Highly Specific Electronic Biosensors. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4984–4989.

69 Boussaad, S.; Tao, N. J.; Zhang, R.; Hopson, T.; Nagahara, L. A. In Situ Detection of Cytochrome c Adsorption with Single Walled Carbon Nanotube Device. *Chem. Commun.* **2003**, 1502–1503.

70 Star, A.; Gabriel, J.-C. P.; Bradley, K.; Grüner, G. Electronic Detection of Specific Protein Binding Using Nanotube FET Devices. *Nano Lett.* **2003**, *3*, 459–463.

71 Mandal, H. S.; Su, Z.; Ward, A.; Tang, X. S. Carbon Nanotube Thin Film Biosensors for Sensitive and Reproducible Whole Virus Detection. *Theranostics* **2012**, *2*, 251–257.

72 Choi, Y.; Moody, I. S.; Sims, P. C.; Hunt, S. R.; Corso, B. L.; Perez, I.; Weiss, G. A.; Collins, P. G. Single-Molecule Lysozyme Dynamics Monitored by an Electronic Circuit. *Science* **2012**, *335*, 319–324.

73 Goldsmith, B. R.; Mitala, J. J., Jr.; Josue, J.; Castro, A.; Lerner, M. B.; Bayburt, T. H.; Khamis, S. M.; Jones, R. A.; Brand, J. G.; Sligar, S. G.; Luetje, C. W.; Gelperin, A.; Rhodes, P. A.; Discher, B. M.; Johnson, A. T. C. Biomimetic Chemical Sensors Using Nanoelectronic Readout of Olfactory Receptor Proteins. *ACS Nano* **2011**, *5*, 5408–5416.