Nanotubes

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Single-Walled Nanotube/Amphiphile Hybrids for Efficacious Protein Delivery: Rational Modification of Dispersing Agents**

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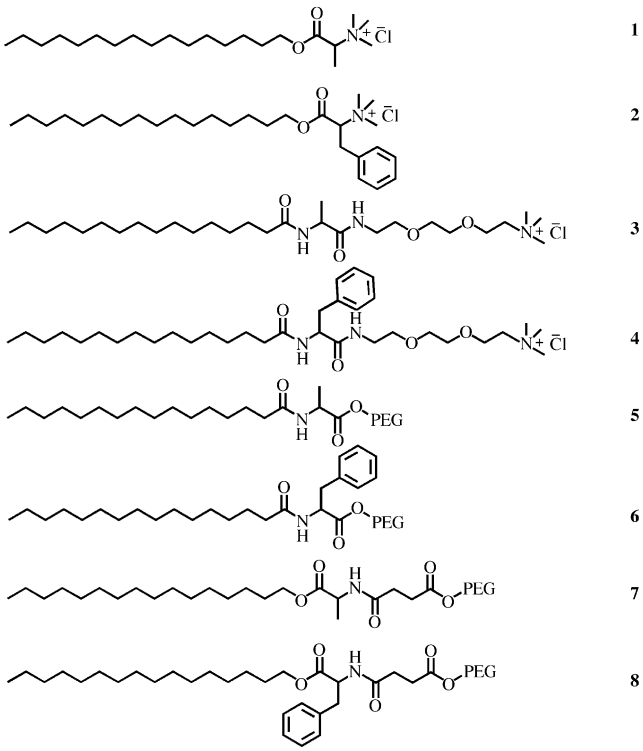
Carbon nanotubes (CNTs) represent a fascinating class of polyaromatic nanomaterials that have excellent optoelectronic properties, mechanical strength, and chemical stability.[1] The inherent amphiphobicity of these extraordinary nanomaterials renders them repellent to solvents of extreme polarity, thus hindering their exploitation in different branches of science, including biomedicine.[2] To counter this problem, exogenous dispersing agents have been used for the formation of soluble nanohybrids of single-walled or multi-walled carbon nanotubes (SWNT/MWNTs) by debundling of CNTs. In this regard, water-soluble CNT nanohybrids are rapidly emerging as promising materials in biology, primarily in the therapeutic and diagnostic fields, because of the chemical inertness of CNTs and their innate ability to penetrate membranes.[3] The dispersion and stabilization of CNTs in water is mainly regulated by the nature of the dispersing agents. Thus, the design and functionality of the dispersing agents are key in determining the properties of the nanohybrids, and can be instrumental in deciding the fate of dispersed CNTs as delivery vehicles.

To date, dispersion of CNTs by noncovalent interaction has been achieved mostly by using polymers, oligopeptides, biomolecules, and a few small molecules based on phospholipids, aromatic derivatives, common surfactants, and other species.[4] However, the importance of the structure of dispersing agents for the solubilization of CNTs in water, and consequently the utilization of these nanohybrids as biological transporters deserve due attention. In this context, a systematic study that correlates the influence of small molecules on the dispersion of CNTs in water, buffers, and cell culture media, and the biocompatibility of the resulting nanohybrids would be of great importance in developing efficient CNT-based transporters. To this end, we recently reported the dispersion of SWNTs in water by using amino acid based cationic amphiphiles,[5] however, several crucial factors need to be addressed before these dispersions can be utilized as delivery vectors across the cell membrane. Firstly, the nanohybrids need to be benign toward eukaryotic cells over a considerable period of time. Secondly, prolonged circulation must be ensured, as agglomeration of nanohybrids in the body fluid blocks the capillary vessels. Finally, the high protein content in the blood plasma could result in non-specific adsorption of proteins on the SWNT surface, thus leading to uptake of CNTs by macrophages of the logical reticuloendothelial system.[1a,3c,6] Herein, we report advancements in the rational design of amino acid based amphiphilic molecules that are simultaneously successful in the efficient dispersion of SWNTs in water, and also in improving the biocompatibility of nanohybrids. Stepwise modification in the amphiphile structure results in the development of biocompatible conjugates SWNT-**7** and SWNT-**8** (Scheme 1), which efficiently deliver proteins inside mammalian cells.



Scheme 1. Structure of amphiphiles. PEG = $-(CH_2CH_2O)_{12}CH_3$.

SWNT–amphiphile conjugates were prepared by sonication and subsequent centrifugation of SWNTs (see Experimental Section) in the presence of different amphiphiles (**1**–**8**; Scheme 1). The SWNT content in the dispersions was quantified by using a calibration plot in a similar manner as previously reported.[5,7] Amphiphile **1**, which comprises L-alanine modified with an ester-linked C_{16} alkyl chain and a quaternized N terminus, exhibited 92% SWNT dispersion

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(Figure 1), the resulting nanohybrid (50 μg mL^{-1}) showed 90% biocompatibility toward HepG2 cells when incubated for 3 h (determined by an MTT assay).[5] However, with

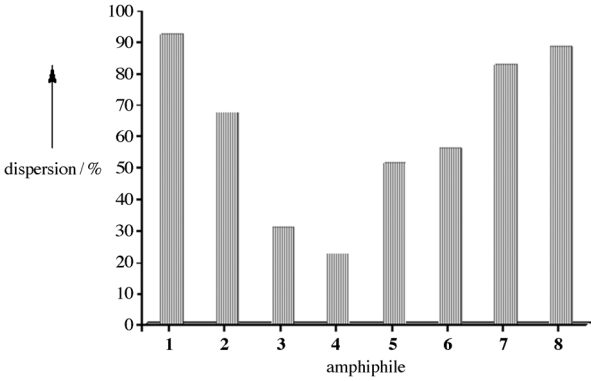

Figure 1. Percentage dispersions of SWNTs in the presence of **1**–**8**. Percent errors are within ±3% in triplicate experiments.

increasing time, biocompatibility progressively decreased at varying concentrations (5–50 μg mL^{-1}) of SWNT-**1** (Figure 2; see also Table S1 in the Supporting Information). Notably, the cell viability dropped to 31% after 24 h with 50 μg mL^{-1} of SWNT-**1**. The high positive charge density at the head of **1**

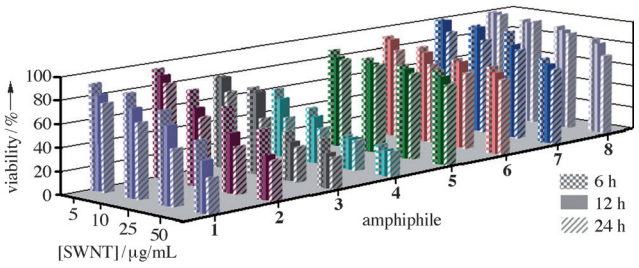

Figure 2. Viability of HepG2 cells treated with varying concentrations of SWNT-amphiphile nanohybrids at different time intervals. Percent errors are within ±5% in triplicate experiments.

arises from its small size, and might have enhanced the electrostatic interaction of SWNT-**1** with the cell membrane. Therefore, we used L-phenylalanine-based amphiphile **2**, which has a larger headgroup size, for further analysis. However, in contrast to **1**, amphiphile **2** exhibited poor dispersion ability (67%; Figure 1), and SWNT-**2** remained strongly cytotoxic, as its cell viability was comparable to that of SWNT-**1** (35% with 50 μg mL^{-1} after 24 h; Figure 2).

To induce more hydrophilicity and cell viability, the polar heads of **1** and **2** were modified by incorporating quaternized di(ethylene glycol) units (**3** and **4**).[6c] In contrast, the biocompatibility of the corresponding nanohybrids (SWNT-**3** and SWNT-**4**) was found to decline drastically at each investigated concentration (Figure 2). In particular, after 24 h, the cell viability was extremely low (ca. 27%) for both nanoconjugates at 50 μg mL^{-1}. Also, the CNT dispersion ability of **3** and **4** decreased to 31% and 22%, respectively (Figure 1); the positively charged amphiphiles within the nanohybrids possibly play a detrimental role by interacting strongly with the negatively charged mammalian cells.[8] The preceding observation also indicates that the presence of a cationic charge in the amphiphile is not mandatory to achieve efficient CNT dispersion, but reduces the cell viability. It is clear that these nanohybrids do not meet the above-mentioned essential criteria to be used as biological transporters.

At this point, a poly(ethylene glycol) (PEG) moiety, which is well known to render molecules biocompatible and water-soluble,[6] was attached at the C terminus of amphiphiles based on L-alanine (**5**) and L-phenylalanine (**6**) with amide-linked C_{16} alkyl chains at the N terminus. This modification brought a remarkable change in the biocompatibility of SWNT-**5** and SWNT-**6** (Figure 2). The cell viability increased to 75–85% when a nanoconjugate concentration of 25 μg mL^{-1} was used over different time intervals. Encouragingly, the cell viability was still approximately 68%, even when the cells were incubated for 24 h with 50 μg mL^{-1} of the nanohybrids. However, a similar increase in the SWNT dispersion ability was not observed for **5** and **6**, possibly because of the lack of hydrophilic–lipophilic balance (HLB) required for efficient CNT dispersion. The dispersion was only about 51% and 56% for **5** and **6**, respectively (Figure 1), which is far less than that for **1** (92%). In the case of **1**, a high CNT dispersion was achieved by introducing hydrophilicity at the N terminus by quaternization of the primary amine of L-alanine. However, for **5** and **6**, the C terminus was made hydrophilic by the inclusion of a PEG moiety. Therefore, the hydrophilicity was relocated to the N terminus of L-alanine (with ester-linked C_{16} alkyl chains at the C terminus) through the incorporation of a PEG unit by using succinic acid as spacer (**7**). As a result, a striking increase in the SWNT-dispersing ability (82%) was observed for **7**. At the same time, the cytocompatibility also improved considerably to 85–95% at an SWNT-**7** concentration of up to 25 μg mL^{-1} and to 70% at 50 μg mL^{-1} of SWNT-**7** after 24 h. Similarly, the L-phenylalanine analogue of **7** with a PEG moiety at the N terminus (**8**) exhibited further improvement in the dispersion ability (90%, comparable to **1**) accompanied by a very high cell viability of about 90–99% at an SWNT-**8** concentration of up to 25 μg mL^{-1}. Even when the SWNT-**8** concentration was 50 μg mL^{-1}, the biocompatibility was approximately 75% after 24 h of incubation. The observed improvement in the cell viability may be attributed to the presence of an ester-linked PEG moiety and the absence of a cationic charge in the structure of amphiphiles.[9] The hydrophilic PEG moiety with a succinic acid spacer at the N terminus also provided the required HLB to increase the CNT dispersion. The biocompatibility of the nanohybrids of **7** and **8** was also tested by using the LIVE/DEAD viability kit for mammalian cells. The predominance of green cells clearly indicates that the majority of the cells were alive after 24 h of incubation with SWNT-**7** and SWNT-**8** at 25 μg mL^{-1} (see Figure S1 in the Supporting Information). These two nanoconjugates were used for further study, as they have the potential to fulfill the mentioned prerequisites needed for molecular transporters.

The superior SWNT dispersion obtained by using **7** and **8** was characterized by spectroscopic and microscopic inves-

tigations. It is known that the noncovalent mode of functionalization preserves the electronic properties of CNTs, and the quality of the dispersions can be examined by monitoring the absorption spectra of the nanohybrids.[5,7b] The exfoliation of the nanotube was evident from the well-resolved UV/Vis/NIR spectra of the aqueous dispersion of SWNT-**7** and SWNT-**8** (Figure 3a). In general, the distinctive peaks within the ranges 550–900 nm (S_{22} transition) and 800–1600 nm (S_{11} transition) arise from interband transitions between the mirror spikes in the density of states of the individualized SWNTs because of the superior quality of the dispersion.[7b]

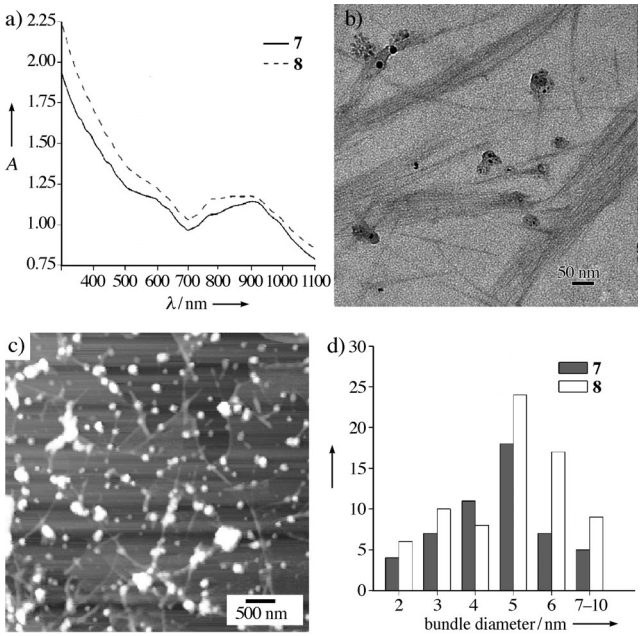

Figure 3. a) UV/Vis/NIR spectra of SWNT-**7** and SWNT-**8**. b) TEM images and c) AFM images of dispersed SWNT-**8**. d) Histogram of the bundle diameter obtained from the AFM images of SWNT-**7** and SWNT-**8**.

The high quality of the dispersion was further confirmed from the TEM images of SWNT-**7** (Figure S2) and SWNT-**8** (Figure 3b), which showed individual nanotubes that have an average diameter of 5 nm. The histograms drawn from the AFM images of SWNT-**7** and SWNT-**8** (representative images given in Figure 3c and Figure S3) also showed that the dispersed nanotubes have an average diameter of 5 nm (Figure 3d).[10] More importantly, in both cases, the average length of the SWNTs was found to be within the range of 400–600 nm (Figure S4), which is known to be the preferred length (< 1 μm) for cellular internalization.[11]

The stability of these biocompatible SWNT dispersions also needs to be examined in the presence of salt and protein before their utilization as biological transporters. High protein and salt content in the blood plasma could become a barrier for the stability of these aqueous SWNT dispersions. The stability of colloidal suspensions is based on the overall balance of the attractive van der Waals force and the repulsive electrostatic force that hinders aggregation.[12] The stability (quantified by the suspension stability index, see Experimental Section) of CNT nanohybrids of the most effective dispersing agents (**7**, **8**, as well as **1** for comparison) was investigated in the presence of increasing ionic concentrations of phosphate buffered saline (PBS). Interestingly, at a concentration of 50 μg mL^{-1}, both SWNT-**7** and SWNT-**8** were remarkably stable for 48 h in the presence of PBS at a concentration of up to 150 mM (Figure 4a). The stability of an SWNT-**8** suspension was also visually observed over varying concentrations of PBS (Figure 4b). On the other hand, the SWNT conjugate of **1** was found to precipitate when the concentration of PBS was above 75 mM, and the suspension

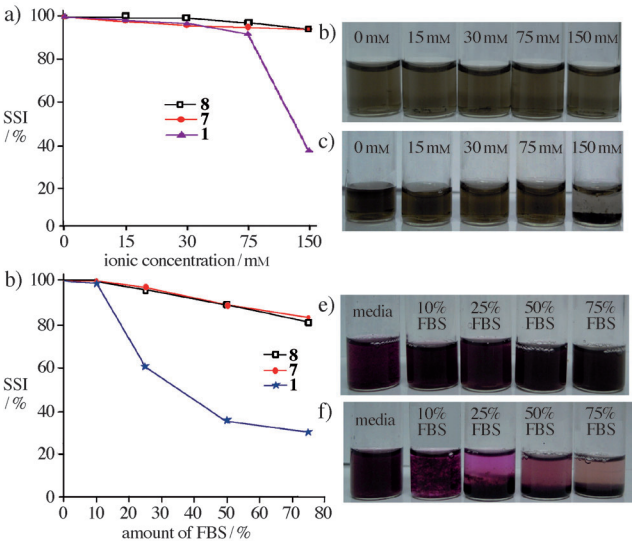

Figure 4. a) Suspension stability index (SSI) of **1**, **7**, and **8** at 50 μg mL^{-1} with varying concentrations of PBS. Corresponding photographs of the vials containing b) SWNT-**8** and c) SWNT-**1** after 48 h. d) Suspension stability index of **1**, **7**, and **8** with varying FBS concentrations in DMEM and corresponding photographs of e) SWNT-**8** and f) SWNT-**1** after 48 h.

stability index decreased to 38% at 150 mM PBS. Precipitation of nanotubes at high concentration of PBS was clearly visible from the respective images (Figure 4c). Upon increasing the ionic strength, electrical double-layer formation takes place on the surface of the colloidal particles, which reduces the repulsive forces and leads to a loss of colloidal stability, thus resulting in aggregation of particles. The superior suspension stability of the SWNT nanohybrids of **7** and **8** confirms their eligibility to be used in biological media.

The stability of SWNT dispersions was subsequently tested in cell culture media (DMEM) comprising varying concentration of fetal bovine serum (FBS) protein. It has been observed that the high protein content in the blood plasma adversely affects the colloidal stability, particularly for a noncovalent CNT dispersion.[3c] Replacement of the dispersing agent from the surface of the nanotube by a plasma protein leads to the agglomeration and consequently the precipitation of CNT in biological milieu.[6] Encouragingly, almost no precipitation was observed when SWNT-**7** and SWNT-**8** were incubated in FBS-containing DMEM media (0–75%; Figure 4d). Even at 75% FBS, both nanohybrids of **7**

and **8** exhibited more than 80% stability after 48 h (Figure 4e). However, SWNT-**1** showed a considerable decrease in the suspension stability index with the increasing FBS concentration, and the precipitation of CNTs is evident from the respective images (Figure 4d,f). The PEG moiety in **7** and **8** might be the key factor for the stabilization of SWNT dispersions in the presence of proteins and salts. The uncharged hydrophilic PEG unit easily avoids the electrostatic interaction with proteins and salts, and is also known to create a hydration layer around itself, thus preventing unwanted interactions with different components of biological media.[6b] Considering the necessity of prolonged circulation of nanoconjugates in body fluids, the stability of SWNT-**8** was also tested in 10% FBS-DMEM over several days. An SWNT dispersion with PEG-functionalized **8** was found to be stable for the entire period of time, and the suspension stability index was higher than 85%, even after six days (Figure S5); in fact, the suspensions were stable for several weeks. Thus, superior SWNT dispersion in water, high biocompatibility, and considerable buffer and serum stability of the SWNT conjugates were achieved by rational structural modification of the dispersing agent.

Finally, conjugates SWNT-**7** and SWNT-**8** were used as cellular delivery vehicles because of their fulfillment of the essential criteria for biological transporters. Fluorescence-labeled proteins bovine serum albumin/fluorescein isothiocyanate (BSA-FITC, excitation wavelength 494 nm, emission at 518 nm, 66 kDa) and streptavidin/Alexa Fluor 546 (excitation wavelength 556 nm, emission at 573 nm, 53 kDa) were noncovalently loaded onto the surface of dispersed nanotubes by stirring the proteins (3.0 μM) in an aqueous dispersion of SWNTs (10 and 25 $\mu g\,mL^{-1}$) at room temperature in the dark for 4 h. The loading of the BSA-FITC was confirmed from the AFM image of the protein-loaded SWNTs (Figure S6a,b), and also by using fluorescence spectroscopy. The protein-incubated solution was centrifuged, and the SWNT-containing pellet was repeatedly washed with water until the supernatant was free from unbound BSA-FITC. The pellet was redispersed by sonication to release the adsorbed protein, the dispersion was further centrifuged, and the fluorescence spectrum of the supernatant showed a signal that confirmed the binding of the protein on the SWNT surface (Figure S6c). The internalization of a protein-loaded nanotube was investigated in both normal Chinese hamster ovary (CHO) cells, as well as in human hepatocellular liver carcinoma (HepG2) cells. Most promisingly, the successful delivery of proteins into the cells was distinctly visible from the highly intense green fluorescence of BSA-FITC (Figure 5b,d,f) and red fluorescence of streptavidin/Alexa Fluor 546 (Figure 5h) after 6 h of incubation. The nature of live healthy mammalian cells was confirmed from their spindle shape in the respective bright field images, as well as from the presence of a large number of green cells in fluorescence images (in the absence of fluorescence-labeled protein) by using the LIVE/DEAD viability kit (Figure S7). The inability of self-internalization of BSA-FITC alone (in the absence of SWNTs) was established from the very poor intensity (generic) of the green fluorescence (Figure S8) in the corresponding control experiment. Internalization of BSA-FITC in the presence of surfactant alone in its micellar form was investigated. Critical micellar concentrations (CMCs) of **7** and **8** were 31 and 18 $\mu g\,mL^{-1}$, respectively (Figure S9). Thus, the surfactant concentration was varied from 50 to 200 $\mu g\,mL^{-1}$ (above the CMCs) for both **7** and **8**. Under identical experimental conditions (including the same protein concentrations), no internalization of BSA-FITC was observed in the absence of SWNTs (Figure S10 and S11). Internalization of SWNTs was confirmed from the TEM image of Triton X treated ruptured cells after protein delivery (Figure S12, see also experimental details in the Supporting Information). Hence, the SWNT conjugates of **7** and **8** efficiently transport the biomolecules into the eukaryotic cells.

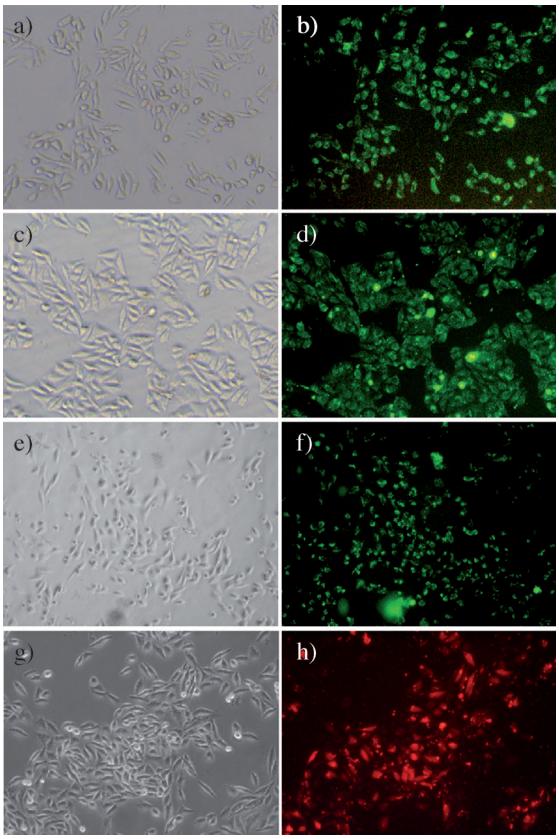

Figure 5. Microscopy images (a, c, e, g: bright field; b, d, f, h: fluorescence) of HepG2 cells with a, b) BSA-FITC loaded SWNT-**7** and c, d) BSA-FITC loaded SWNT-**8**, and of CHO cells with e, f) BSA-FITC loaded SWNT-**8** and g, h) streptavidin/Alexa Fluor 532 loaded SWNT-**8** (all after 6 h incubation). Typical concentrations of nanohybrid = 10 $\mu g\,mL^{-1}$ and protein = 3 μM.

In summary, a systematic structure–property correlation led to the development of a new class of amphiphilic biocompatible nanohybrids that showed excellent dispersing ability and remarkable stability in simulated biological milieu, and most importantly transported proteins across cellular membranes. The present investigation allows the exploitation of different molecular structures in increasing the potential of CNTs in therapeutics and diagnostics.

Experimental Section

Details on the synthesis and characterization of amphiphiles are given in the Supporting Information. Preparation of SWNT–amphiphile conjugates and determination of dispersions (%): SWNTs (1 mg) were added to an aqueous solution (4 mL) of the respective surfactants (2.5 mg mL^{-1}). The solutions were tip-sonicated for 30 min followed by bath sonication for 2 h and subsequent tip sonication for 30 min. The solutions were then subjected to centrifugation at 25000 *g* for 30 min to precipitate the heavy bundles. The amount of the dispersed SWNTs in the supernatant was calculated from the observed absorbance value at 550 nm that was derived from the linear plot of absorbance versus concentration prepared in a similar manner to that previously reported.[5,7] The percentage dispersion was determined as the ratio of the amount of SWNTs in the dispersion to the amount of SWNTs initially added. Details of spectroscopic and microscopic characterization are given in the Supporting Information.

Preparation of nanoconjugates for cell culture experiments: The dispersion was ultracentrifuged at 375000 *g* for 15 min and the supernatant was discarded. The residue was redispersed in water for further use.

Suspension stability index of SWNT–amphiphile conjugates: The redispersed aqueous SWNT–amphiphile suspension was added to solutions of PBS (0–150 mM) and FBS (0–75%) in DMEM so that the final concentration of the nanoconjugate remained 50 μg mL^{-1}. The solutions were left undisturbed for 48 h. Finally, the absorbance of the supernatant was recorded at 550 nm for the different suspensions, in order to calculate the suspension stability index ($=A_t/A_0\times100$, where A_t = absorbance of the supernatant after 48 h at 550 nm, and A_0 = initial absorbance of the solution at 550 nm)

Details of the cell viability experiment using the MTT assay and LIVE/DEAD viability kit for mammalian cells are given in the Supporting Information.

Preparation of protein–SWNT–amphiphile conjugates and their cellular uptake (details given in the Supporting Information): Aqueous SWNT–amphiphile hybrids (100 μg mL^{-1}) were mixed with a solution of the fluorophore-tagged protein (30 μM). The solutions were stirred at room temperature in the dark for 4 h in order to allow adsorption of proteins onto the nanotube surface. The HepG2 and CHO cells were seeded into 24-well chambered plates 24 h prior to incubation with a protein–SWNT–amphiphile suspension. The cells were typically mixed with a protein-loaded SWNT–amphiphile conjugate and incubated at 37°C for 6 h in a 5% CO_2 atmosphere in DMEM cell growth medium. The concentration of SWNTs in the incubated solution was varied between 10 to 25 μg mL^{-1} to maintain a protein concentration of 3 μM. The cells were then washed with PBS in order to ensure complete removal of the excess conjugate. The internalization was monitored by fluorescence microscopy.

Supporting information for this article including details of the synthesis and characterization of amphiphiles, dispersions, spectroscopic and microscopic experiments, protein binding, and a cytotoxicity assay is available on the WWW under http://www.angewandte.org.

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[1] a) Z. Liu, K. Yang, S.-T. Lee, *J. Mater. Chem.* **2011**, *21*, 586; b) F. Lu, L. Gu, M. J. Meziani, X. Wang, P. G. Luo, L. M. Veca, L. Cao, Y. P. Sun, *Adv. Mater.* **2009**, *21*, 139.
[2] D. Das, P. K. Das, *Langmuir* **2009**, *25*, 4421.
[3] a) D. Pantarotto, R. Singh, D. M. Carthy, M. Erhardt, J. P. Briand, M. Prato, K. Kostarelos, A. Bianco, *Angew. Chem.* **2004**, *116*, 5354; *Angew. Chem. Int. Ed.* **2004**, *43*, 5242; b) J. Chen, S. Chen, X. Zhao, L. V. Kuznetsova, S. S. Wong, I. Ojima, *J. Am. Chem. Soc.* **2008**, *130*, 16778; c) A. P. Goodwin, S. M. Tabakman, K. Welsher, S. P. Sherlock, G. Prencipe, H. Dai, *J. Am. Chem. Soc.* **2009**, *131*, 289; d) S. Dhar, Z. Liu, J. Thomale, H. Dai, S. J. Lippard, *J. Am. Chem. Soc.* **2008**, *130*, 11467; e) N. Nakayama-Ratchford, S. Bangsaruntip, X. Sun, K. Welsher, H. Dai, *J. Am. Chem. Soc.* **2007**, *129*, 2448; f) M. Benincasa, S. Pacor, W. Wu, M. Prato, A. Bianco, R. Gennaro, *ACS Nano* **2011**, *5*, 199; C. G. Salzmann, M. A. H. Ward, R. M. J. Jacobs, G. Tobias, M. L. H. Green, *J. Phys. Chem. C* **2007**, *111*, 18520.
[4] a) N. W. S. Kam, H. Dai, *J. Am. Chem. Soc.* **2005**, *127*, 6021; b) B. G. Cousins, A. K. Das, R. Sharma, Y. Li, J. P. McNamara, I. H. Hillier, I. A. Kinloch, R. V. Ulijn, *Small* **2009**, *5*, 587; c) Y. Li, B. G. Cousins, R. V. Ulijn, I. A. Kinloch, *Langmuir* **2009**, *25*, 11760; d) C. Backes, C. D. Schmidt, K. Rosenlehner, F. Hauke, J. N. Coleman, A. Hirsch, *Adv. Mater.* **2010**, *22*, 788; e) E. Schopf, R. Broyer, L. Tao, Y. Chen, H. D. Maynard, *Chem. Commun.* **2009**, 4818; f) L. Shao, G. Tobias, C. G. Salzmann, B. Ballesteros, S. Y. Hong, A. Crossley, B. G. Davis, M. L. H. Green, *Chem. Commun.* **2007**, 5090; g) M. A. Herrero, J. Guerra, V. S. Myers, M. V. Gomez, R. M. Crooks, M. Prato, *ACS Nano* **2010**, *4*, 905; h) V. Georgakilas, K. Kordatos, M. Prato, D. M. Guldi, M. Holzinger, A. Hirsch, *J. Am. Chem. Soc.* **2002**, *124*, 760.
[5] S. Brahmachari, D. Das, P. K. Das, *Chem. Commun.* **2010**, *46*, 8386.
[6] a) S. Park, H. S. Yang, D. Kim, K. Job, S. Jon, *Chem. Commun.* **2008**, 2876; b) S.-T. Yang, K. A. S. Fernando, J.-H. Liu, J. Wang, H.-F. Sun, Y. Liu, M. Chen, Y. Huang, X. Wang, H. Wang, Y.-P. Sun, *Small* **2008**, *4*, 940; c) X. Li, K. L. Liu, M. Wang, S. Y. Wong, W. C. Tjiu, C. B. He, S. H. Goh, J. Li, *Acta Biomater.* **2009**, *5*, 2002.
[7] a) S. Attal, R. Thiruvengadathan, O. Regev, *Anal. Chem.* **2006**, *78*, 8098; b) A. Di Crescenzo, D. Demurtas, A. Renzetti, G. Siani, P. D. Maria, M. Meneghetti, M. Prato, A. Fontana, *Soft Matter* **2009**, *5*, 62.
[8] A. Makovitzki, Y. Shai, *Biochemistry* **2005**, *44*, 9775.
[9] A. M. Aberle, F. Tablin, J. Zhu, N. J. Walker, D. C. Gruenert, M. H. Nantz, *Biochemistry* **1998**, *37*, 6533.
[10] C. Backes, C. D. Schmidt, F. Hauke, C. Bottcher, A. Hirsch, *J. Am. Chem. Soc.* **2009**, *131*, 2172.
[11] N. W. S. Kam, Z. Liu, H. Dai, *Angew. Chem.* **2006**, *118*, 591; *Angew. Chem. Int. Ed.* **2006**, *45*, 577.
[12] X. Wang, T. Xia, S. A. Ntim, Z. Ji, S. George, H. Meng, H. Zhang, V. Castranova, S. Mitra, A. E. Nel, *ACS Nano* **2010**, *4*, 7241.

