



HAL
open science

Noncovalent Stable Functionalization Makes Carbon Nanotubes Hydrophilic and Biocompatible

Friederike Ernst, Zhenghong Gao, Raul Arenal, Timm R Heek, Antonio Setaro, Rodrigo Fernandez-Pacheco, Rainer Haag, Laurent Cognet, Stephanie Reich

► **To cite this version:**

Friederike Ernst, Zhenghong Gao, Raul Arenal, Timm R Heek, Antonio Setaro, et al.. Noncovalent Stable Functionalization Makes Carbon Nanotubes Hydrophilic and Biocompatible. *Journal of Physical Chemistry C*, 2017, 121 (34), pp.18887-18891. 10.1021/acs.jpcc.7b03062 . hal-01630842

HAL Id: hal-01630842

<https://hal.science/hal-01630842>

Submitted on 8 Nov 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Non-Covalent Stable Functionalization Makes Carbon Nanotubes Hydrophilic and Biocompatible

Friederike Ernst^{a,}, Zhenghong Gao^b, Raúl Arenal^{c,d}, Timm Heek^e, Antonio Setaro^a, Rodrigo Fernandez-Pacheco^c, Rainer Haag^e, Laurent Cognet^b, and Stephanie Reich^a*

^a Dr. F. Ernst, Dr. A. Setaro, Prof. S. Reich, Institut für Experimentalphysik, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany

^b Dr. Z. Gao, Prof. L. Cognet, LP2N, University of Bordeaux, UMR 5298, 351 cours de la libération, F-33405 Talence, France; Institut d'Optique & CNRS, UMR 5298, F-33405 Talence, France

^c Dr. R. Arenal, Dr. R. Fernandez-Pacheco, Laboratorio Microscopias Avanzadas (LMA), Inst. Nanociencia Aragon (INA), Univ. Zaragoza, 50018 Zaragoza, Spain

^d Dr. R. Arenal, Fundacion ARAID, Zaragoza, 50018, Spain

^e Dr. T. Heek, Prof. R. Haag, Institut für Chemie und Biochemie, Freie Universität Berlin, Takustraße 3, 14195 Berlin, Germany

Semiconducting single wall carbon nanotubes possess an intrinsic photoluminescence in the near infrared region beyond 900 nm, the NIR-II window of biological imaging. Here, we introduce a modular molecule for non-covalent nanotube functionalization which renders carbon nanotubes

hydrophilic and fully biocompatible through a one-step process. We demonstrate through EELS spectroscopy that the non-covalent functionalization mechanism relies on tight and extremely robust pi-pi stacking, which survives an exchange of the solvent as well as drying. Furthermore, the modularity of the molecule design allows for the introduction of functional units into the molecule itself to modify the optical properties of the carbon nanotube, for instance to augment its excitation window through an excitation energy transfer, facilitating the excitation of most carbon nanotube chiralities at one single wavelength.

Introduction

Single wall carbon nanotubes are becoming popular for biomedical applications for several reasons: They are small, chemically stable, hollow and fillable, have a large absorption cross section, and lend themselves to chemical functionalization.^{1,2} Consequently, they can be used as cargo vessels to release drugs in a precise location in a cell or in the body,^{3,4} to locally convert electromagnetic radiation into heat in photothermal therapy,^{5,6} and can be functionalized with antibodies and a plethora of other attachments to be used as sensors.^{7,8} Carbon nanotubes also possess optoelectronic properties that make them a highly desirable candidate for biological imaging.^{9,10} The intrinsic luminescence emission of single wall carbon nanotubes lies in the near infrared region from 900nm – 1400nm, the second optical window of tissue. Biological tissues are relatively transparent between 650nm and 1350nm. However, in practice, the first optical window between 650nm and 950nm for which there are well established biocompatible fluorophores is obfuscated by tissue autofluorescence.¹¹ The second optical window between 1000nm and 1350nm has been demonstrated to exhibit 100-fold improved signal-to-noise ratios when imaging whole animals as compared to the first window, making carbon nanotube based systems with an intrinsic luminescence in the NIR highly desirable for biomedical imaging.¹²⁻¹⁴

Carbon nanotubes are intrinsically hydrophobic and have a propensity for bundling. For applications in biomedical imaging nanotubes require solubilization through non-covalent functionalization with amphiphilic surfactants. Using small molecules in carbon nanotube solubilization has the distinct advantage that the molecule's design is flexible and can easily be modified to incorporate additional functionalities. The biocompatibility of small surfactant-nanotube hybrids faces three challenges.

- (i) The surfactant has to suspend nanotubes in a wide range of pHs, reflecting the variable environments encountered in biological tissues.
- (ii) The surfactant ought not interact with the surrounding cellular environment itself and preclude interaction between nanotube and environment as well.
- (iii) The dynamic equilibrium between adsorbed and free surfactant must be strongly in favor of the adsorbed state. This stability is important not only for the sake of continued suspension, but also because the surfactant shields the environment against the nanotube.

We recently introduced an amphiphilic molecule for functionalizing carbon nanotubes, PerPG. PerPG forms tightly bound complexes with carbon nanotubes, in effect serving as a surfactant in solution. Instead of a charged headgroup, PerPG possesses a hydrophilic polyglycerol (PG) dendron.¹⁵ PG dendrons are known to interact with proteins very weakly and are consequently popular as a protein repellent coating, for instance in anti-fouling surfaces.¹⁶⁻¹⁹ We combine the PG dendron with two parallel alkyl chains for nanotube individualization and a perylene diimide dye. The perylene-derived dye forms ultra-efficient energy transfer complexes with the nanotube.^{15,20} This energy transfer functionality enables the excitation of all nanotube chiralities

at one single wavelength, possibly potentiating their use in biomedical sensing, imaging and photothermal therapy applications.

Here, we present high-resolution transmission electron microscopy (HRTEM) and electron energy-loss spectroscopy (EELS) data confirming that. The nanotube-PerPG complexes are highly stable and robust against change of medium and drying. We assess PerPG's biocompatibility by performing a cell viability study and find that at relevant concentrations the small designer molecules are as biocompatible as conventionally used biocompatible poloxamers such as pluronic.²¹

Results and Discussion

Imaging of nanotube-PerPG hybrids

PerPG, shown in Fig. 1, solubilizes nanotubes efficiently in aqueous solution and forms energy transfer complexes with the carbon nanotubes.¹⁵ Its design is modular in nature, comprising alkyl chains for nanotube individualization (green), a pH independent PG dendron for water solubility (blue) and a functional perylene unit (red), which is the donor unit in the energy transfer complexes formed with the nanotubes. The combination of these three building blocks was previously systematically varied and optimized for both energy transfer and nanotube suspension capabilities.²² The suspension was further optimized for negligible free surfactant and stability through absorption and photoluminescence measurements.¹⁵ The energy transfer is FRET-like with an efficiency on the order of 99.99%. In conjunction with the spectral overlap between donor emission and acceptor absorption we calculate the distance between nanotube and dye in a point-dipole approximation to be 0.7nm.²⁰ However, the point dipole approximation overestimates the distance between donor and acceptor in this case, as an exciton in the nanotube

is not well described as a point-dipole. Instead, the dipole is oblong, decreasing the interaction with the point-dipole of the dye. Consequently, for a given interaction strength, the point-dipole approximation overestimates the distance between dye and nanotube.²³ Based on this, the upper limit for the distance between nanotube and dye unit is 0.7nm, only slightly more than for van der Waals bound systems. Hence we assumed that the extended π -orbitals of the carbon nanotube interact with the extended π -orbitals of the perylene derivative dye, resulting in an adsorption of the dye on the tube wall through a π - π stacking mechanism.^{20,23,24} The coverage rate depends slightly on the nanotube chirality and was previously estimated to be on the order of 0.1 through a detailed analysis of absorption, photoluminescence and photoluminescence excitation measurements of the carbon nanotube-PerPG complexes.²⁰

To confirm the adsorption of PerPG on the nanotube surface, we perform a high resolution TEM study. HRTEM micrographs show microcrystalline regions on top of the nanotubes, Fig. 2. These microcrystalline structures are shown to be PerPG in subsequent EELS measurements, Fig. 3. Spatially resolved EEL spectroscopy is an ideal technique to gain insight into the morphology of the nanotube-PerPG complexes, letting us directly visualize the organic biomolecules on top of the nanotube through the evaluation of the local chemical composition.^{25,26} Particular attention was paid to reducing the beam dose used to optimize the acquisition conditions, see Experimental Section. Figure 3B shows EEL spectrum-lines recorded on a bundle of functionalized carbon nanotubes. The locations of the spectral acquisitions are given in Fig. 3A, the corresponding high angular annular dark field (HAADF) scanning transmission electron microscopy (STEM) image. In the HAADF image we see a bright area of $\sim 5 \times 1.5 \text{ nm}^2$ in the middle of the nanotube bundle. Other regions like the one indicated are also

visible. These brighter appearing moieties are localized at the surface of the nanotubes as we have observed in the HRTEM micrographs, Fig. 2. The EEL spectrum line was acquired following the green line marked in Fig. 3A, which crosses this bright region. The sum of 5 EEL spectra was extracted from the two areas marked in the HAADF image, Fig. 3B. Both EEL spectra display the C-K edge with the characteristic peaks of sp^2 materials at 285 eV and 292 eV corresponding to the π^* and σ^* contributions, respectively.²⁵⁻³⁰ In the spectrum acquired over the bright region in the HAADF micrograph we additionally detect oxygen, see Fig. 3B: The O-K edge is clearly visible, as designated on the graph. The amount of oxygen in this region, obtained from the analysis of this EEL spectrum, is $\sim 14\%$. The combination of the HRTEM images and local analytical findings confirm that the perylene unit of PerPG π - π stacks on the nanotube wall, resulting in extremely tight binding between nanotube and the small amphiphilic molecule.

We would like to draw special attention to the fact that the nanotube-PerPG complexes were created in aqueous solution, resulting in well-individualized nanotubes as attested to by strong PL emission.¹⁵ The aqueous sample was subsequently dried and then re-dispersed in ethanol for these measurements, Fig. 4. This means the complexes are bound tightly enough to remain bundled throughout the drying process. Furthermore they are stable in air and highly robust with respect to change of the environment, indicating that this PerPG-nanotube complex may be bound tightly enough to satisfy challenges (i) and (iii) of achieving biocompatibility for small surfactant-nanotube hybrids.

Suitability for biomedical applications

We designed PerPG such that it is tailored for use in biomedical applications. Regarding the three challenges (i)-(iii) of biocompatibility discussed in the introduction,

challenge (i) pH independence and (ii) weak interaction with surrounding tissue, are met by the polyglycerol dendron as hydrophilic unit. The PG dendron's hydrophilicity is a result of its multiple oxygen bonds, not of the presence of a charged salt. All bonds are fully saturated and much less susceptible to deprotonation upon pH variation. PG dendrons have previously reported to not interact with proteins as necessary to meet challenge (ii).¹⁶⁻¹⁹ Challenge (iii), a strong preference towards the adsorbed over the free state, is tackled by the adsorption mechanism of PerPG as we verify from the absence of perylene luminescence. The emission of the perylene dye in the PerPG is quenched when it is in close contact with a nanotube. Residual dye emission is an excellent measure of free PerPG. The residual emission is quenched by a factor of 10^4 , corresponding to a ratio of adsorbed to free molecules of 10 000:1 or higher.¹⁵ This remarkably high ratio of adsorbed to free PerPG is attributed to the strong π - π stacking exhibited between the sp^2 bonded carbon network of the nanotube and the organic perylene moiety. This agrees with the HRTEM and EELS studies that highlighted the stability of the complexes with respect to drying and re-dispersal in another medium.

We assess the biocompatibility of PerPG suspended nanotubes by a cell viability study. To assess cell viability we conduct an MTT assay after exposure to the nanotube-PerPG hybrids.³¹ It measures the activity of cellular enzymes in metabolically active cells. The enzymes reduce yellow tetrazolium dyes into its insoluble, purple formazan that can be solubilized and quantified by spectrophotometric means. The MTT assay is widely accepted as a sensitive and reliable tool in assessing cytotoxicity.³¹

Carbon nanotubes suspended in PerPG are added to COS-7 cell cultures with a final nanotube concentration of $0.1\mu\text{g/ml}$, $0.1\mu\text{g/ml}$ being the highest concentration that may be used for

applications in single nanotube imaging and tracking. PerPG is benchmarked against Pluronic as a standard surfactant to make nanotubes biocompatible²¹ The relative cell viability, Fig.5A, demonstrated that PerPG is equally biocompatible as Pluronic. Figure 5B reports the cell viability of PerPG suspended nanotubes for different nanotube concentrations ranging from $1 \cdot 10^{-3}$ $\mu\text{g/ml}$ to $1 \mu\text{g/ml}$. The biocompatibility of the suspended nanotubes increases drastically with lowered concentration reaching 90% viability at $10^{-3} \mu\text{g/ml}$. PerPG thus reached the same performance as the polymeric biocompatible surfactant Pluronic, demonstrating that it is well suited as a biocompatible surfactant for nanotubes.

Experimental

Sample preparation 0.11g/l of HiPco SWNTs (Unidym, batch SP0295) were dispersed with $6 \cdot 10^{-5}$ M of PerPG or Pluronic through tip sonication with a Bandelin SonoPlus HD 2070 with 30% of the maximum power of 70W and a 3mm microtip. The sample was subsequently centrifuged at 30 000g and 23° for 90 min. Only the supernatant was used in the experiments. The synthesis of PerPG is published.¹⁵

HRTEM and EELS The transmission electron microscopy (TEM) samples were prepared by re-dispersing the dried nanotube-PerPG complexes in ethanol. The dispersions were ultrasonicated and subsequently deposited on holey carbon 3 mm copper grids. High resolution TEM was performed employing an imaging-side aberration-corrected FEI Titan-Cube microscope working at 80 keV, equipped with a Cs corrector (CETCOR from CEOS GmbH).

Spatially resolved electron energy loss spectroscopy (EELS) measurements were performed on probe-corrected scanning TEM (STEM) FEI Titan Low-Base 60-300 operating at 80 keV (fitted with a X-FEG® gun and Cs-probe corrector (CESCOR from CEOS GmbH)). EEL spectra were recorded using the spectrum-imaging (SPIM in 2D or spectrum-line (SPLI) in 1D) mode^{32,33} in a

Gatan GIF Tridiem ESR 865 spectrometer. The convergent semi-angle was 25 mrad and the collection semi-angle 80 mrad with an energy resolution ~ 1.2 eV.

Cell viability study COS-7 cells are seeded in a 96 wells plate with 100 μ l of COS-7 solution ($2.5 \cdot 10^4$ cells/ml) in each well and cultured at 37°C and 5% CO₂ for 24h. 11 μ l of SWNT suspension (1 μ g/ml) is added to each well, resulting in a final concentration of 0.1 μ g/ml. Cultures are kept at 37°C and 5% CO₂ for further 24h. Subsequently, 10 μ l MTT kit is added to each well, followed by 3.5h incubation at 37°C in the dark for reaction. All medium is carefully removed and 150 μ l MTT solvent is added into each well, the plate is then wrapped with aluminum paper to isolate from light and put on a laboratory shaker (60 rpm) for thoroughly dissolving MTT reduction. Absorptions were measured with a plate reader (PLUOstar Omega) at 570nm, the fingerprint region for viable cells, and background is subtracted. The control culture was treated the same without adding SWNT suspension. All data were normalized by the control average.

Conclusions

In this paper, we show that a small functional molecule with a modular design, PerPG, is capable of suspending carbon nanotubes in biological surroundings. HRTEM imaging and EEL spectroscopy demonstrate that PerPG adsorbs to the nanotube with its perylene unit, confirming deductions made from earlier photoluminescence measurements. The HRTEM data also confirm the physical robustness of the nanotube-PerPG complexes to drying and solvent exchange and prove the complexes are stable in air, a result of the exceptionally strong binding interaction between nanotube and PerPG's perylene moiety. A polyglycerol dendron known for low levels of interaction with proteins was chosen for the hydrophilic part of PerPG to achieve biocompatibility of the nanotube complexes. Cell viability is demonstrated by an MTT assay. At

concentrations relevant for biotechnological applications the nanotube-PerPG complexes are equally biocompatible as nanotubes suspended in the biocompatible standard surfactant, pluronic F108. Until now, research into nanotube surfactants with a low cytotoxicity has concentrated on polymers. The modularity of the design of the small molecule surfactant is inherently flexible and could accommodate many other functionalities besides the energy transfer exhibited by PerPG in this manuscript. Tailored small functional molecules that efficiently act as surfactants have the potential to play a significant role in the solubilization of carbon nanotubes for biomedical applications.

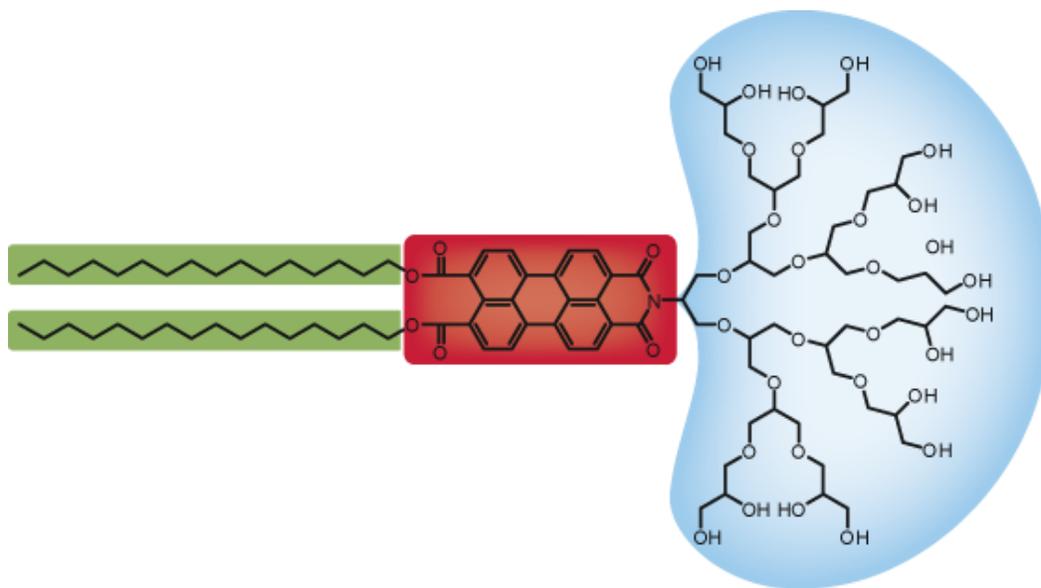


Figure 1. PerPG is a small amphiphilic molecule which serves as a biocompatible surfactant for carbon nanotubes. PerPG comprising three functional parts: Hydrophilic polyglycerol (PG)

dendron for water solubility (blue), perylene-derived dye core for attachment to the nanotube (red), and alkyl chains for nanotube individualization (green).

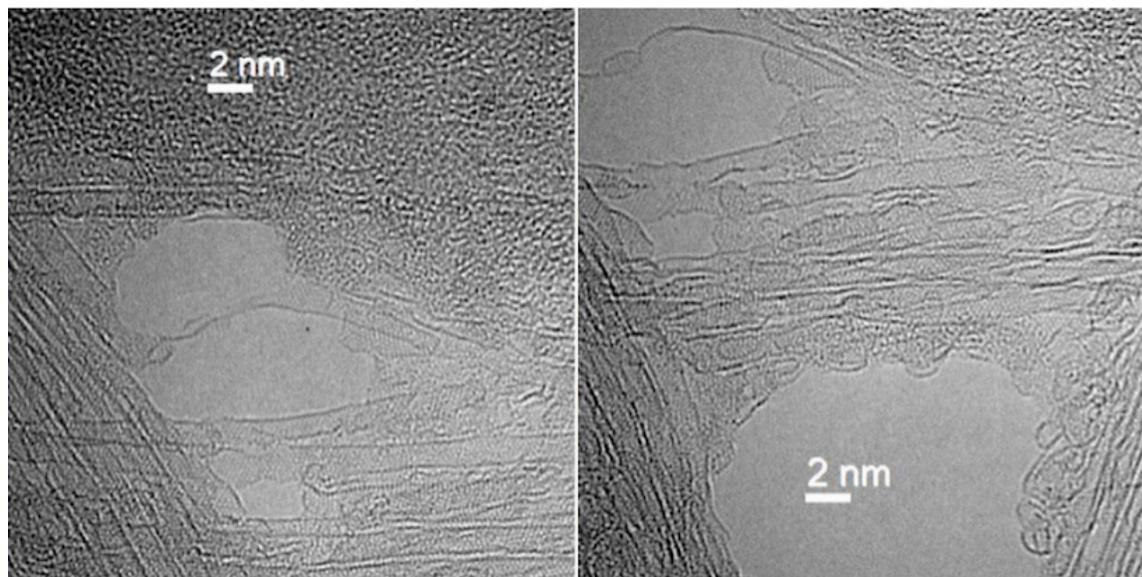


Figure 2. High resolution transmission electron micrographs of nanotube-PerPG hybrids. The PerPG molecules can be seen as faint microcrystalline regions superimposed on the nanotubes as confirmed by EELS measurements, see Fig. 3.

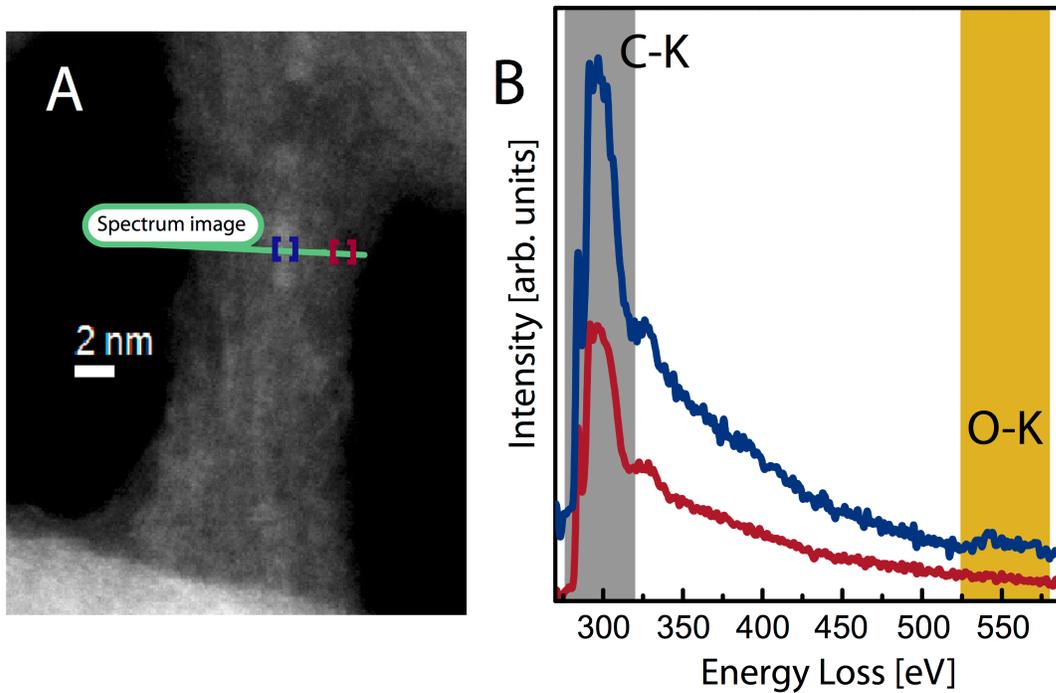


Figure 3. A HAADF image of a bundle of functionalized carbon nanotubes. EELS data were acquired along the green line. B Sums of 5 EEL spectra collected from two different areas, marked in blue and red. While the C-K edge is visible in both spectra, the O-K edge is only visible in the blue (top) EEL spectrum acquired in the bright area of the HAADF image.

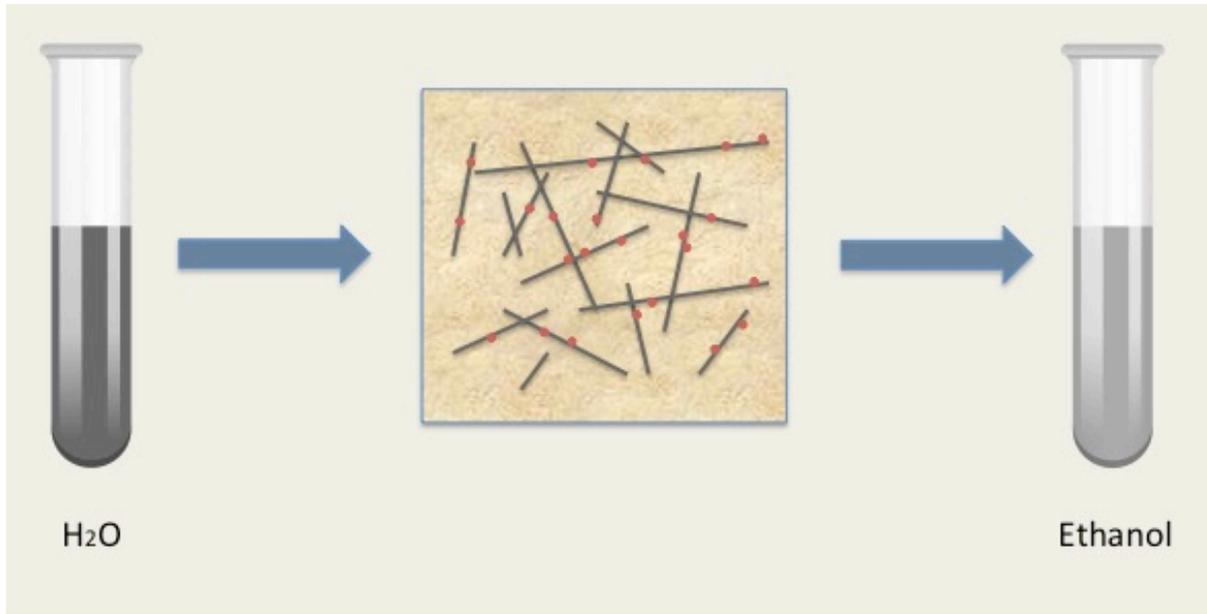


Figure 4. The carbon nanotube-PerPG complexes created in aqueous solution remain stable through the process of drying and redispersion in ethanol.

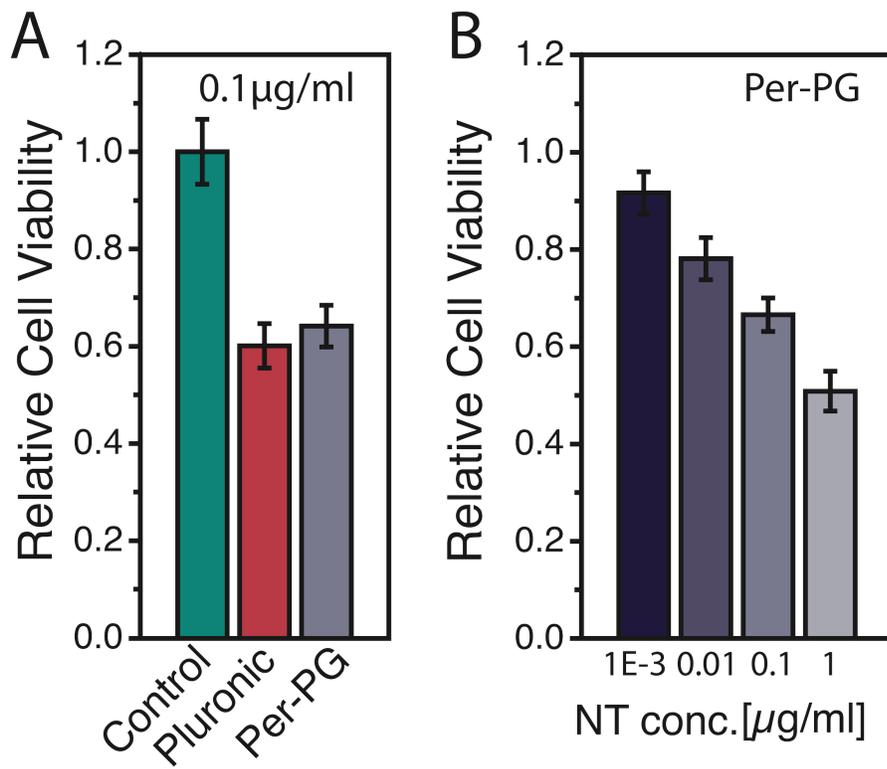


Figure 5. Relative cell viability as measured in an MTT assay for different surfactants and NT concentrations A Relative cell viability after exposure to nanotubes suspended with Pluronic and PerPG at a nanotube concentration of 0.1 μ g/ml. The control group was subjected to the same treatments without addition of nanotubes. B Relative cell viability for different concentrations of PerPG suspended nanotubes.

AUTHOR INFORMATION

Corresponding Author

* Correspondence should be addressed to f.ernst@fu-berlin.de

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

We gratefully acknowledge the financial support of the European Research Council (grant number 210642) and the German Research Foundation (DFG via SFB 658, subprojects A6 and B7). ZG and LC thank J. Varuela and L. Groc for the use of the absorption reader for MTT assay and acknowledge financial support from CNRS, Agence Nationale de la Recherche (ANR-14-OHRI-0001-01), IdEx Bordeaux (ANR-10-IDEX-03-02) and the France-BioImaging national infrastructure (ANR-10-INBS-04-01). The microscopy works were conducted in the Laboratorio de Microscopias Avanzadas at the Instituto de Nanociencia de Aragon - Universidad de Zaragoza (Spain). R.A. gratefully acknowledges the support from the Spanish Ministry of

Economy and Competitiveness (MINECO) through project grant FIS2013-46159-C3-3-P and MAT2016-79776-P (AEI/FEDER, UE). The Government of Aragon, and the European Social Fund are gratefully acknowledged. R.A. gratefully acknowledges the project “Construyendo Europa desde Aragon” 2014-2020 (grant number E/26).

REFERENCES

- 1 Z. Liu, S. Tabakman, K. Welsher and H. Dai, Carbon Nanotubes in Biology and Medicine: In vitro and in vivo Detection, Imaging and Drug Delivery. *Nano Res.*, 2009, **2**, 85–120.
- 2 S. Kruss, A. J. Hilmer, J. Zhang, N. F. Reuel, B. Mu and M. S. Strano, Carbon nanotubes as optical biomedical sensors. *Adv. Drug Deliv. Rev.*, 2013.
- 3 G. Bartholomeusz, P. Cherukuri, J. Kingston, L. Cognet, R. Lemos, T. K. Leeuw, L. Gumbiner-Russo, R. B. Weisman and G. Powis, In Vivo Therapeutic Silencing of Hypoxia-Inducible Factor 1 Alpha (HIF-1 α) Using Single-Walled Carbon Nanotubes Noncovalently Coated with siRNA. *Nano Res.*, 2009, **2**, 279–291.
- 4 A. Bianco, K. Kostarelos and M. Prato, Applications of carbon nanotubes in drug delivery. *Curr. Opin. Chem. Biol.*, 2005, **9**, 674–9.
- 5 P. Chakravarty, R. Marches, N. S. Zimmerman, A. D.-E. Swafford, P. Bajaj, I. H. Musselman, P. Pantano, R. K. Draper and E. S. Vitetta, Thermal ablation of tumor cells with antibody-functionalized single-walled carbon nanotubes. *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 8697–702.

- 6 N. W. S. Kam, M. O'Connell, J. A. Wisdom and H. Dai, Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 11600–5.
- 7 P. W. Barone, S. Baik, D. a Heller and M. S. Strano, Near-infrared optical sensors based on single-walled carbon nanotubes. *Nat. Mater.*, 2005, **4**, 86–92.
- 8 Z. Chen, S. M. Tabakman, A. P. Goodwin, M. G. Kattah, D. Daranciang, X. Wang, G. Zhang, X. Li, Z. Liu, P. J. Utz, K. Jiang, S. Fan and H. Dai, Protein microarrays with carbon nanotubes as multicolor Raman labels. *Nat. Biotechnol.*, 2008, **26**, 1285–92.
- 9 K. Welsher, Z. Liu, D. Daranciang and H. Dai, Selective probing and imaging of cells with single walled carbon nanotubes as near-infrared fluorescent molecules., *Nano Lett.*, 2008, **8**, 586–90.
- 10 A. G. Godin, J. A. Varela, Z. Gao, N. Danné, J. P. Dupuis, B. Lounis, L. Groc and L. Cognet, Single-nanotube tracking reveals the nanoscale organization of the extracellular space in the live brain, *Nat. Nanotechnol.*, 2017, **12**, 238-243
- 11 A. M. Smith, M. C. Mancini and S. Nie, Bioimaging: second window for in vivo imaging. *Nat. Nanotechnol.*, 2009, **4**, 710–1.
- 12 Y. T. Lim, S. Kim, A. Nakayama, N. E. Stott, M. G. Bawendi and J. V Frangioni, Selection of Quantum Dot Wavelengths for Biomedical Assays and Imaging, *Mol. Imaging*, 2003, **2**, 50–64.

- 13 K. Welsher, Z. Liu, S. P. Sherlock, J. T. Robinson, Z. Chen, D. Daranciang and H. Dai, A route to brightly fluorescent carbon nanotubes for near-infrared imaging in mice. *Nat. Nanotechnol.*, 2009, **4**, 773–80.
- 14 K. Welsher, S. P. Sherlock and H. Dai, Deep-tissue anatomical imaging of mice using carbon nanotube fluorophores in the second near-infrared window. *Proc. Natl. Acad. Sci.*, 2011, **108**, 8943–8948.
- 15 F. Ernst, T. Heek, A. Setaro, R. Haag and S. Reich, Energy Transfer in Nanotube-Perylene Complexes. *Adv. Funct. Mater.*, 2012, **22**, 3921–3926.
- 16 H. Frey and R. Haag, Dendritic polyglycerol: a new versatile biocompatible material. *Rev. Mol. Biotechnol.*, 2002, **90**, 257–267.
- 17 M. Wyszogrodzka and R. Haag, A convergent approach to biocompatible polyglycerol "click" dendrons for the synthesis of modular core-shell architectures and their transport behavior. *Chemistry*, 2008, **14**, 9202–14.
- 18 M. Wyszogrodzka and R. Haag, Study of single protein adsorption onto monoamino oligoglycerol derivatives: a structure-activity relationship. *Langmuir*, 2009, **25**, 5703–12.
- 19 M. Wyszogrodzka and R. Haag, Synthesis and characterization of glycerol dendrons, self-assembled monolayers on gold: a detailed study of their protein resistance. *Biomacromolecules*, 2009, **10**, 1043–54.
- 20 F. Ernst, T. Heek, A. Setaro, R. Haag and S. Reich, Excitation characteristics of different energy transfer in nanotube-perylene complexes. *Appl. Phys. Lett.*, 2013, **102**, 233105.

- 21 S. Ghosh, S. M. Bachilo, R. A. Simonette, K. M. Beckingham and R. B. Weisman, Oxygen Doping Modifies Near-Infrared Band Gaps in Fluorescent Single-Walled Carbon Nanotubes, *Science*, 2010, **330**, 1656–1660.
- 22 F. Ernst, T. Heek, A. Setaro, R. Haag and S. Reich, Functional Surfactants for Carbon Nanotubes: Effects of Design, *J. Phys. Chem. C*, 2013, **117**, 1157–1162.
- 23 C. Y. Wong, C. Curutchet, S. Tretiak and G. D. Scholes, Ideal dipole approximation fails to predict electronic coupling and energy transfer between semiconducting single-wall carbon nanotubes. *J. Chem. Phys.*, 2009, **130**, 81104.
- 24 P. G. Wu and L. Brand, Resonance Energy Transfer: Methods and Applications. *Anal. Biochem.*, 1994, **218**, 1–13.
- 25 A. Setaro, M. Adeli, M. Glaeske, D. Przyrembel, T. Bisswanger, G. Gordeev, F. Maschietto, A. Faghani, B. Paulus, M. Weinelt, R. Arenal, R. Haag and S. Reich, Preserving π -conjugation in covalently functionalized carbon nanotubes for optoelectronic applications. *Nat. Commun.*, 2017, **8**, 14281.
- 26 L. Alvarez, F. Fall, A. Belhboub, R. Le Parc, Y. Almadori, R. Arenal, R. Aznar, P. Dieudonné-George, P. Hermet, A. Rahmani, B. Jousset, S. Campidelli, J. Cambedouzou, T. Saito and J.-L. Bantignies, One-Dimensional Molecular Crystal of Phthalocyanine Confined into Single-Walled Carbon Nanotubes. *J. Phys. Chem. C*, 2015, **119**, 5203–5210.
- 27 P. Ayala, R. Arenal, A. Loiseau, A. Rubio and T. Pichler, The physical and chemical properties of heteronanotubes. *Rev. Mod. Phys.*, 2010, **82**, 1843–1885.

- 28 R. Arenal, X. Blase and A. Loiseau, Boron-nitride and boron-carbonitride nanotubes: synthesis, characterization and theory. *Adv. Phys.*, 2010, **59**, 101–179.
- 29 L. Alvarez, Y. Almadori, R. Arenal, R. Babaa, T. Michel, R. Le Parc, J.-L. Bantignies, B. Joussetme, S. Palacin, P. Hermet and J.-L. Sauvajol, Charge Transfer Evidence between Carbon Nanotubes and Encapsulated Conjugated Oligomers. *J. Phys. Chem. C*, 2011, **115**, 11898–11905.
- 30 R. Arenal, L. De Matteis, L. Custardoy, A. Mayoral, M. Tence, V. Grazu, J. M. De La Fuente, C. Marquina and M. R. Ibarra, Spatially-resolved EELS analysis of antibody distribution on biofunctionalized magnetic nanoparticles. *ACS Nano*, 2013, **7**, 4006–13.
- 31 A. P. Wilson, Cytotoxicity and viability. In *Animal Cell Culture: A Practical Approach*, ed. J. R. W. Masters, Oxford University Press, Oxford, 3rd edn., 2000, p. 190.
- 32 C. Jeanguillaume and C. Colliex, Spectrum-image: The next step in EELS digital acquisition and processing. *Ultramicroscopy*, 1989, **28**, 252–257.
- 33 R. Arenal, F. de la Peña, O. Stéphan, M. Walls, M. Tencé, A. Loiseau and C. Colliex, Extending the analysis of EELS spectrum-imaging data, from elemental to bond mapping in complex nanostructures. *Ultramicroscopy*, 2008, **109**, 32–8.

TOC

Ultra-stable functionalization of carbon nanotubes based on a pi-pi stacking mechanism yields nanotube-surfactant complexes that are fully biocompatible as well as resistant to change of medium and drying.

