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Biosensors |*Hot Paper*|

Polymer-Decorated Carbon Nanotubes as Transducers for Label-Free Photonic Biosensors

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Abstract: A biosensor taking advantage of the optical properties of sorted carbon nanotubes has been developed. A polyfluorene polymer bearing azido groups was synthesized and used for the selective extraction of semi-conducting nanotubes from the bulk population. The resulting polymer-decorated nanotubes were then conjugated by click-chemistry to a ligand unit (biotin), and the sensing properties of the biotinylated nanotubes were investigated by photoluminescence measurements, upon interaction with the streptavidin target.

Introduction

The selective detection of compounds of biological interest with high sensitivity is crucial as regards health issues. Indeed, early detection and diagnosis permit rapid implementation of an appropriate therapeutic treatment, which in turn maximizes the chances of a patient's recovery. Access to efficient systems for the detection of trace amounts of specific biomarkers thus appears of utmost importance and biosensors have been developed for this purpose. These devices usually consist of three components: 1) A sensing unit; 2) a physicochemical transducer, and 3) a signal processor. Two approaches currently exist for the detection of biomolecules, namely labeled assays that detect binding events using radioactive or fluorescent tags,[1] and label-free assays,[2] which do not require any tags. Label-free assays display some advantages such as direct measurements in complex samples, no alteration of the initial biological binding event, faster and accurate measurements, possible quantization, and no interference from the labels. In addition, label-free assays benefit from real-time readout and therefore faster analysis. Among the various label-free biosensors developed to date, carbon nanotube-based systems have recently emerged as innovative tools for the highly sensitive detection of biomolecules.[3] Thanks to their unique electronic/optical properties, combined with high specific surface area and device miniaturization, single-walled carbon nanotubes (SWCNTs) are appealing materials for such applications. Most biosensing platforms involving SWCNTs as transducers exploit either electronic or electrochemical detection in transistor-based or amperometric/voltametric set-ups, respectively.[4] Although convincing devices have been reported in the literature, biosensors taking advantage of the peculiar optical properties of SWCNTs have yet to be developed.

In this communication, we report the use of polymer-decorated single-walled carbon nanotubes as transducers for the detection of compounds of biological interest. Our approach relies on the design of an original polymer made of a polyfluorene backbone. The latter was used for the selection of semiconducting SWCNTs of specific chiralities/optical properties and for the introduction of functional groups onto the surface of the tubes. The polymer-coated nanotubes were then functionalized with ligand units, and evaluated for the detection of a target biomolecule, by monitoring the changes in the SWCNTs' optical properties. The use of fluorene-based polymers for the selective recognition/extraction of some semiconducting SWCNTs is known and well-documented.[5] The process involves preferential steric and electronic interactions of the polymer-backbone with nanotubes that are extracted depending on their chirality. For the development of our SWCNT-based biosensor, the design and synthesis of an original polymer was required to achieve two major goals: 1) The selective dispersion of individual SWCNTs with optical properties that are suitable to probe biomolecule interactions (i.e., semiconducting nanotubes of specific chirality) and 2) the introduction of reactive chemical groups on the tubes' surface to permit the grafting of target-specific ligands.

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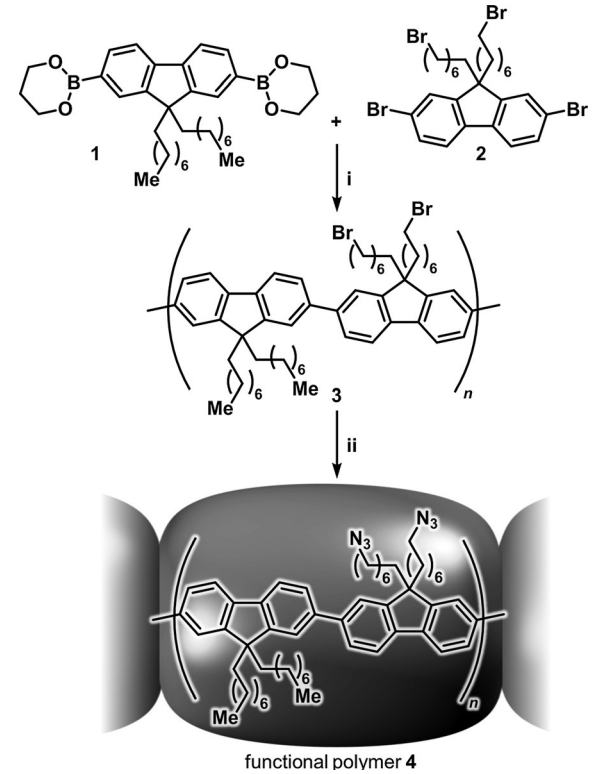
Results and Discussion

Design and synthesis of the functional polymer

Previous reports have shown that among known polymers previously used for SWCNT isolation, commercially available poly[9,9-dioctylfluorenyl-2,7-diyl] (PFO, average MW of 53 kDa) gave the best results in terms of selectivity.[5a,6] We therefore reasoned that our functional polymer should have a chemical structure as close as possible to that of PFO. However, anchoring sites have to be introduced on the polymer for further chemical derivatization with a ligand unit. For this purpose, we selected azido groups, considering the versatility of the "click" conjugation reaction, and undertook the synthesis of an azido-containing PFO-polymer.[7] Thus, a new functional polymer was designed as a co-polymeric structure consisting of two different blocks, one of which was 9,9-dioctylfluorene-2,7-bis(trimethylborate) **1** (commercially available), whereas the other (**2**, bearing reactive functions) was derived from 2,7-dibromofluorene. The latter was bis-alkylated at the C-9 position under basic conditions in the presence of an excess of 1,7-dibromoheptane, leading to bis-brominated fluorene **2**.

Monomers **1** and **2** were then co-polymerized through a palladium-mediated Suzuki cross-coupling reaction to yield conjugated polymer **3**, which had an average molecular weight of 40 kDa, as determined by gel permeation chromatography. Treatment of **3** with NaN_3 in excess permitted the formation of the final functional polymer **4** bearing "clickable" azido groups (Scheme 1).[8] With polymer **4** in hand, we next evaluated its ability to extract semi-conducting species from a mixture of carbon nanotubes.



Scheme 1. Synthesis of functional polymer **4**. i) 1,7-Dibromoheptane (10 equiv), TBAB (0.2 equiv), aq. KOH (50 wt%), N_2 atm., 75 °C, 6 h; ii) **1** (1 equiv), **2** (1 equiv), $Pd(PPh_3)_4$ (0.1 equiv), TBAB (0.1 equiv), Na_2CO_3 (12 equiv), toluene/water 2:1, N_2 atm., 80 °C, 48 h; iii) NaN_3 (3 equiv), DMF, 60 °C, 18 h.

Selective extraction of semi-conducting nanotubes from bulk SWCNTs

Commercially available HiPCO SWCNTs were suspended in toluene and submitted to tip-ultrasonication in the presence of polymer **4**. This process enabled the polymer to disrupt nanotube bundles and adsorb at the surface of a sub-population of nanotubes. The polymer-decorated nanotubes were found to be dispersible in toluene and were separated from polymer-free SWCNTs (non-dispersible) by ultracentrifugation (1 h, 150 000*g*). At the end of the centrifugation cycle, the non-decorated nanotubes formed a pellet at the bottom of the centrifuge tube and were discarded, whereas those in interaction with **4** remained in suspension and were collected (Figure 1). Analysis of the supernatant by different spectroscopic techniques permitted us to determine the chirality of the selected nanotubes that were extracted in less than 1% yield (based on total mass of starting nanotubes).

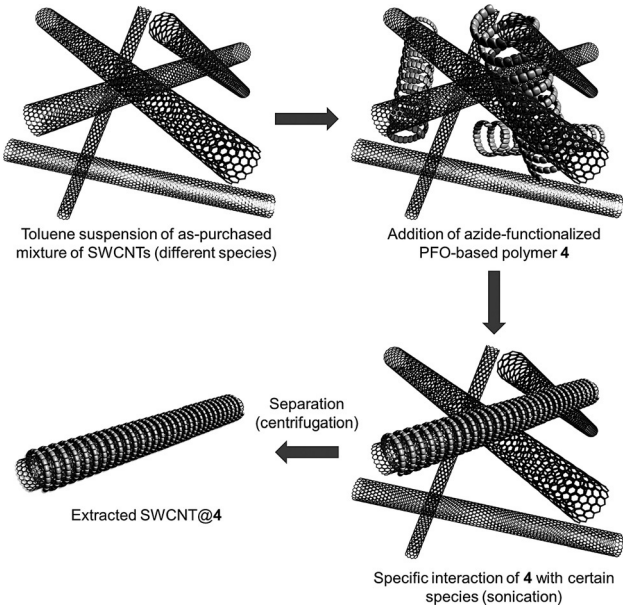


Figure 1. Extraction of semi-conducting species from a mixture of HiPco SWCNTs with functional polymer **4**.

Characterization of the SWCNT-polymer assembly

The UV/Vis-NIR spectrum of the supernatant fraction (Figure 2a) showed characteristic adsorption bands of semi-conducting nanotubes in the 1000–1500 nm region but also an additional absorption band of the polymer **4** centered at approximately 560 nm (π–π^* transitions). From this spectrum, it appeared that polymer **4** was able to select carbon nanotubes with a chiral index (*n*,*m*) of (7,5), (7,6), (8,6), (8,7), and (9,7).

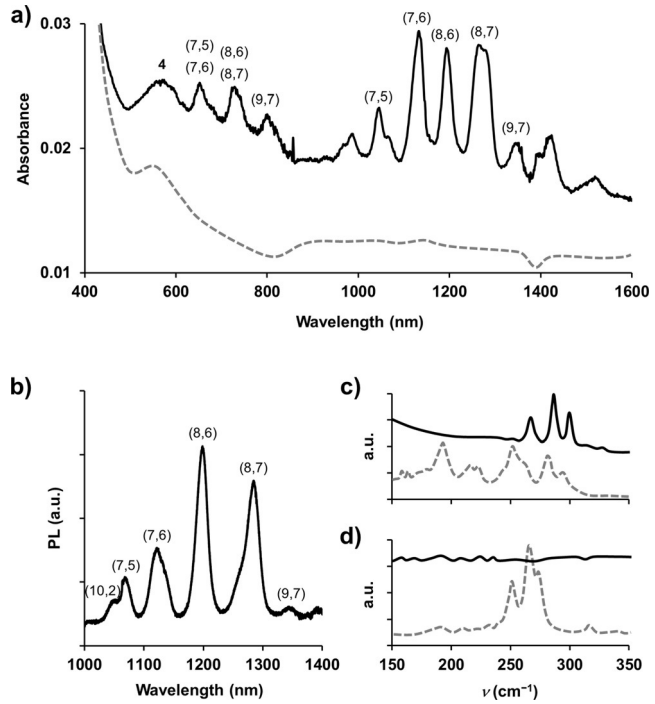


Figure 2. Characterization of the SWCNT@**4** assembly: a) UV/Vis-NIR spectra of **4** (dashed grey curve) and SWCNT@**4** (black curve); b) PL spectrum of SWCNT@**4** (exc. 734 nm); c) RBM bands on the Raman spectra of bulk SWCNTs (dashed grey curve) and SWCNT@**4** (black curve) excited at 660 nm; d) RBM bands on the Raman spectra of bulk SWCNTs (dashed grey curve) and SWCNT@**4** (black curve) excited at 514 nm.

These species were observed in the 600–800 nm (S_{22} transitions) and 1000–1500 nm (S_{11} transitions) regions of the UV/Vis-NIR spectrum.

The photoluminescence (PL) spectra of the SWCNT@**4** sample (excitation at 734 and 803 nm) confirmed the selection of semi-conducting nanotubes of chiralities that are consistent with previous reports.[5a,c,g,6] In fact, the selectivity of polymer **4** was close to that reported for commercial PFO, despite the presence of an additional band around 1075 nm that was assigned to (10,2)-tubes (Figure 2b). However, from the above analyses, it seemed difficult to rule-out the presence of metallic SWCNTs, which could impede the performance of the final optical biosensor.

To confirm that the polymer was fully selective of semi-conducting nanotubes, Raman spectroscopy of the extracted SWCNTs was recorded at two specific wavelengths (i.e., 514 and 660 nm) and the data were compared to those of bulk HiPCO tubes.

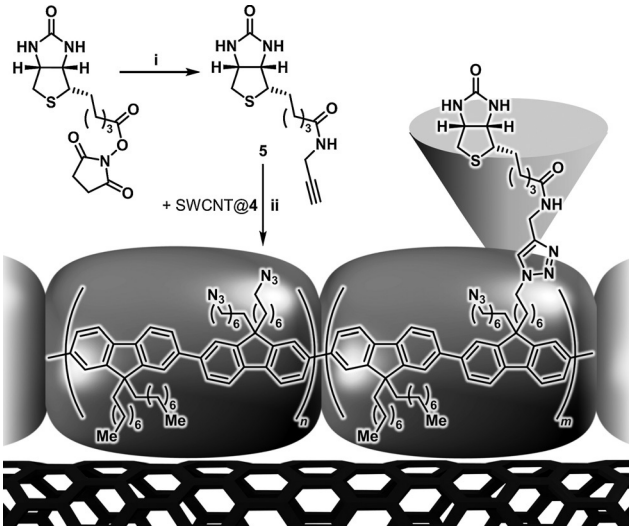
As Raman spectroscopy is a resonant process, the allowed optical transitions can be reported on a plot as a function of the diameter of the nanotube. Also known as a Kataura plot, it anticipates that both metallic and semiconducting HiPCO nanotubes are simultaneously probed at 514 and 660 nm.[9] Figure 2c and d display the Raman spectra in the radial breathing mode (RBM) range (ν below 300 cm^{-1}) of pristine and of polymer-decorated SWNTs. At 660 nm (Figure 2c), higher frequency RBMs (225–300 cm^{-1}) are attributed to semi-conducting nanotubes and lower frequency RBMs (150–225 cm^{-1}) to metallic ones. Comparison of the Raman spectra of the extracted and bulk SWCNT population showed the disappearance of RBM bands in the 150–225 nm regions in the extracted sample. On the contrary, at 514 nm (Figure 2d), higher frequency RBMs (225–300 cm^{-1}) are attributed to metallic nanotubes and lower frequency RBMs (150–225 cm^{-1}) to semiconducting ones. Again, comparison of the Raman spectra of the extracted and bulk SWCNT population showed the disappearance of higher frequency RBM bands in the extracted sample. In addition, the Raman spectra of the bulk CNT in the tangential mode ($1400 < \nu_{TM} < 1600$ cm^{-1}) is broader and more asymmetric (Breit–Wigner–Fano line shape) than that of the extracted samples (Figure S1 in the Supporting Information). This is also in accordance with the selective extraction of semi-conducting CNTs by polymer **4**. These results thus confirmed the absence of metallic SWCNTs in the extracted sample, which contained only semi-conducting SWCNTs.[6c,10]

Grafting of the ligand units on the polymer-decorated SWCNTs

Semi-conducting SWCNTs decorated with the azido-polymer constitute a versatile platform for the immobilization of a variety of either ligands or receptors. Indeed, azido groups are able to selectively react with alkynes through 1,3-dipolar cycloaddition reactions. Also known as the Huisgen cycloaddition or "click" reaction, the process can efficiently be catalyzed by cuprous salts, to afford triazole groups connecting the azido and alkyne partners. As a matter of fact, most biomolecules can be derivatized with an alkyne moiety capable of chemoselectively reacting with the azido groups borne by polymer **4**. To demonstrate the usefulness of our SWCNT@**4** construct in the detection of biomolecules, the streptavidin-biotin couple was chosen as a model target-ligand system. Streptavidin is a bacterial protein that has very high affinity towards biotin with a dissociation constant in the order of 10^{-14} mol L^{-1}.[11,12]

First, biotin succinimidyl ester was reacted with propargylamine to provide access to the alkyne-biotin derivative **5**.[13] The latter was then submitted to the "click" reaction with SWCNT@**4** in a mixture of toluene/water and in the presence of CuBr, PMDETA (*N*,*N*,*N*′,*N*′′,*N*′′-pentamethyldiethylenetriamine), and sodium ascorbate, under nitrogen at room temperature. After 24 h of reaction, biotinylated construct SWCNT@**4-biotin** (Scheme 2) was produced and characterized by various spectroscopic techniques. First, the UV/Vis-NIR spectrum of SWCNT@**4-biotin** was recorded and indicated an overall decrease in intensity of absorption bands in the 1000–1500 nm region, although no significant shifts were detected when compared to SWCNT@**4** (Figure S2 in the Supporting Information).

The PL spectrum of SWCNT@**4-biotin** (Figure 3b) was also similar to that of SWCNT@**4** with, however, some minor shifts. The relative intensities of the different bands were also affected as the ratio of the PL intensity of the (8,6) and (8,7) SWCNTs ($I_{(8,6)}/I_{(8,7)}$) dropped from 1.2 to 0.95 after the conjugation to biotin (Figure 3a). Taken together, these subtle changes in the UV/Vis-NIR and PL spectra suggest effective covalent attach-



Scheme 2. Grafting of biotin units on SWCNT@**4**. i) Propargylamine (1.5 equiv), NEt_3 (2 equiv), DMF, room temp., 24 h; ii) SWCNT@**4** (1 equiv), **5** (2 equiv), CuBr (1 equiv), PMDETA (1 equiv), sodium ascorbate (2 equiv), toluene/H_2O, N_2 atm., room temp., 24 h.

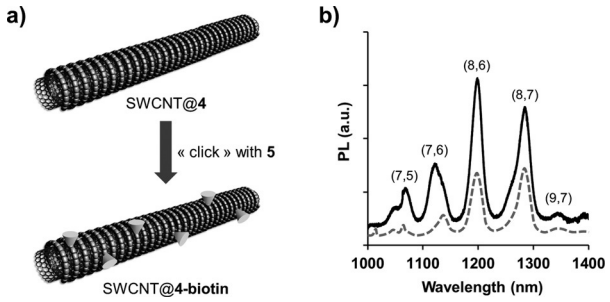


Figure 3. a) Decoration of the SWCNT@**4** assembly with biotin through "click" chemistry; b) PL spectra of SWCNT@**4** (black curve) and SWCNT@**4-biotin** (grey dashed curve).

ment of the biotin unit to the polymer-coated nanotubes. The SWCNT@**4-biotin** platform was thereafter evaluated for the selective sensing of streptavidin.

Selective detection of streptavidin using the SWCNT@4-biotin biosensor

The SWCNT@**4-biotin** assembly was studied in interaction with streptavidin. First, a thin film was obtained by drop casting the SWCNT@**4-biotin** suspension on a glass surface before a solution of streptavidin (10 μM in water) was deposited. After a couple of minutes, streptavidin in excess was washed with water (5×60 μL), and the PL spectrum was recorded. Comparison of the optical properties of the SWCNT@**4-biotin** film before and after addition of streptavidin was performed to determine whether the target-ligand (i.e., streptavidin–biotin) interaction could be detected. As shown in Figure 4a, obvious shifts were observed in the PL spectrum of the sample that interacted with streptavidin, as the band corresponding to (8,6)-SWCNTs underwent a marked 10 nm redshift, whereas that of

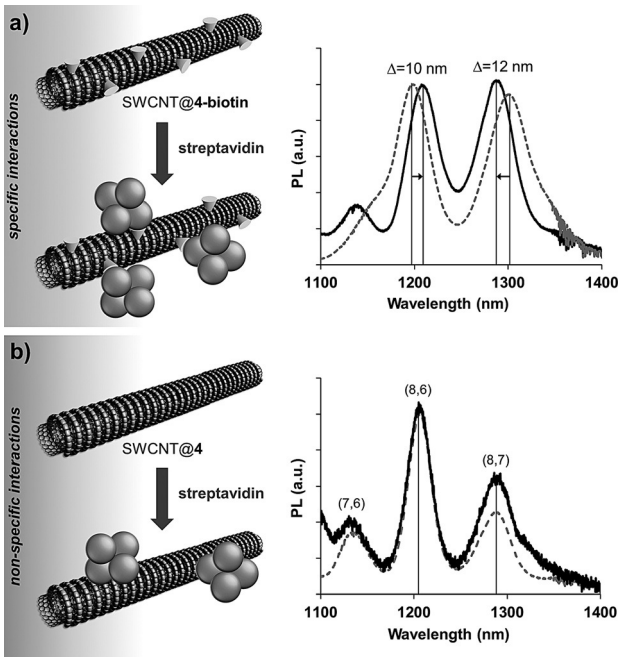


Figure 4. a) Specific interactions monitored by comparison of the PL spectra of SWCNT@**4-biotin** before (grey dashed curve) and after (black curve) addition of streptavidin; b) Non-specific interactions monitored by comparison of the PL spectra of SWCNT@**4** before (grey dashed curve) and after (black curve) addition of streptavidin.

(8,7)-SWCNTs was blueshifted by 12 nm. For affinity-based systems with lower association constants than the biotin–streptavidin couple, it is likely that higher concentrations of the target will be required to saturate the low-affinity interactions.

To confirm that the observed shifts were due to specific interactions between the biotin ligand and the streptavidin target and not to non-specific interactions with the nanotube surface, a control experiment was performed. A film of the non-biotinylated SWCNT@**4** was deposited on the glass surface and, as above, streptavidin was added. In this case, no spectral shift of the associated PL spectrum (Figure 4b) was detected upon addition of streptavidin, demonstrating that specific biotin–streptavidin interactions were responsible for the modifications of the optical properties of the SWCNT@**4-biotin** assembly.

Conclusions

An original polyfluorene-based polymer bearing azido groups, which allow easy conjugation of a wide array of biologically relevant ligands, was designed. The polymer was used to extract specific semi-conducting species (excluding metallic nanotubes) from a commercial mixture of single-walled carbon nanotubes with excellent selectivity. The polymer-decorated tubes were then further modified by conjugation of a model ligand (biotin) and drop-casted on a glass surface. The sensing properties of the system were investigated by photoluminescence studies. Upon specific interaction with the streptavidin target, the PL of the nanotubes exhibited marked shifts. Taken together, these results demonstrate the concept of a new type

of biosensor relying on the optical properties of carbon nanotubes, rather than on transport properties and resistivity modifications.[14] The azide-functionalized platform can accommodate virtually any ligand or receptor and thus be adapted to the specific detection of a variety of disease markers or other analytes of interest. The integration of nanotube-based transducers into silicon-based photonic structures could offer, in the near future, the possibility of simultaneous and real-time detection of multiple target molecules.[15]

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Keywords: biosensor · carbon nanotubes · click · extracting polymer · photoluminescence

[1] J. Shen, Y. Li, H. Gu, F. Xia, X. Zuo, *Chem. Rev.* **2014**, *114*, 7631 – 7677.
[2] a) X. Luo, J. J. Davis, *Chem. Soc. Rev.* **2013**, *42*, 5944 – 5962; b) L. Wu, X. Qu, *Chem. Soc. Rev.* **2015**, *44*, 2963 – 2997.
[3] a) N. Yang, X. Chen, T. Ren, P. Zhang, D. Yang, *Sens. Actuators B* **2015**, *207*, 690 – 715; b) Z. Wang, Z. Dai, *Nanoscale* **2015**, *7*, 6420 – 6431; c) K. Balasubramanian, M. Burghard, *Anal. Bioanal. Chem.* **2006**, *385*, 452 – 468; d) S. Liu, X. Guo, *NPG Asia Mater.* **2012**, *4*, e23.
[4] a) J. Wang, *Electroanalysis* **2005**, *17*, 7 – 14; b) R. J. Chen, S. Bangsaruntip, K. A. Drouvalakis, N. Wong Shi Kam, M. Shim, Y. Li, W. Kim, P. J. Utz, H. Dai, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4984 – 4989; c) K. Maehashi, K. Matsumoto, *Sensors* **2009**, *9*, 5368 – 5378; d) X. Pang, P. Imin, I. Zhitomirsky, A. Adronov, *Macromolecules* **2010**, *43*, 10376 – 10381; e) S.N. Kim, J.F. Rusling, F. Papadimitrakopoulos, *Adv. Mater.* **2007**, *19*, 3214 – 3228; f) D. Fu, H. Okimoto, C. W. Lee, T. Takenobu, Y. Iwasa, H. Kataura, L.-J. Li, *Adv. Mater.* **2010**, *22*, 4867 – 4871.
[5] a) A. Nish, J. Y. Hwang, J. Doig, R. J. Nicholas, *Nat. Nanotechnol.* **2007**, *2*, 640; b) J. Gao, M. Kwak, J. Wildeman, A. Herrmann, M. A. Loi, *Carbon* **2011**, *49*, 333 – 398; c) F. Chen, B. Wang, Y. Chen, L.-J. Li, *Nano Lett.* **2007**, *7*, 3013 – 3017; d) M. Tange, T. Okazaki, S. Iijima, *ACS Appl. Mater. Interfaces* **2012**, *4*, 6458 – 6462; e) F. Jakubka, S. P. Schießl, S. Martin, J. M. Englert, F. Hauke, A. Hirsch, J. Zaumseil, *ACS Macro Lett.* **2012**, *1*, 815 – 819; f) K. S. Mistry, B. A. Larsen, J. L. Blackburn, *ACS Nano* **2013**, *7*, 2231 – 2239; g) H. Ozawa, T. Fujigaya, Y. Niidome, N. Hotta, M. Fujiki, N. Nakashima, *J. Am. Chem. Soc.* **2011**, *133*, 2651 – 2657; h) F. Lemasson, N. Berton, J. Tittmann, F. Hennrich, M. M. Kappes, M. Mayor, *Macromolecules* **2012**, *45*, 713 – 722; i) N. Berton, F. Lemasson, J. Tittmann, N. Stürzl, F. Heinrich, M. M. Kappes, M. Mayor, *Chem. Mater.* **2011**, *23*, 2237.
[6] a) J. Gao, A. Loi, E. J. Figuerido de Carnavalho, M. C. dos Santos, *ACS Nano* **2011**, *5*, 3993 – 3999; b) J.-Y. Hwang, A. Nish, J. Doig, S. Douven, C.-W. Chen, L.-C. Chen, R. J. Nicholas, *J. Am. Chem. Soc.* **2008**, *130*, 3543 – 3553; c) N. Izard, S. Kazaoui, K. Mata, T. Okazaki, T. Saido, S. Ijima, N. Minami, *Appl. Phys. Lett.* **2008**, *92*, 243112.
[7] a) W. H. Binder, R. Sachsenhofer, *Macromol. Rapid Commun.* **2007**, *28*, 15 – 54; b) W. Xi, T. F. Scott, C. J. Kloxin, C. N. Bowman, *Adv. Funct. Mater.* **2014**, *24*, 2572 – 2590; c) M. Meldal, C. Wenzel Tornøe, *Chem. Rev.* **2008**, *108*, 2952 – 3012.
[8] I. Ozge Huyal, U. Koldemir, T. Ozel, H. Volkan Demir, D. Tuncel, *J. Mater. Chem.* **2008**, *18*, 3568 – 3574.
[9] H. Kataura, Y. Kumazawa, Y. Maniwa, I. Umezu, S. Suzuki, Y. Ohtsuka, Y. Achiba, *Synth. Met.* **1999**, *103*, 2555 – 2558.
[10] S. Kazaoui, S. Cook, N. Izard, Y. Murakami, S. Maruyama, N. Minami, *J. Phys. Chem. C* **2014**, *118*, 18059 – 18063.
[11] a) P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski, F. R. Salemme, *Science* **1989**, *243*, 85 – 88; b) L. Chaiet, F. J. Wolf, *Arch. Biochem. Biophys.* **1964**, *106*, 1 – 5; c) N. M. Green, *Methods Enzymol.* **1990**, *184*, 51 – 67; d) M. Wilchek, E. A. Bayer, O. Livnah, *Immunol. Lett.* **2006**, *103*, 27 – 32; e) M. Holzinger, A. Le Goff, S. Cosnier, *New J. Chem.* **2014**, *38*, 5173 – 5180.
[12] a) E. A. Bayer, H. Ben-Hur, G. Gitlin, M. Wilchek, *J. Biochem. Biophys. Methods* **1986**, *13*, 103 – 112; b) E. A. Bayer, H. Ben-Hur, M. Wilchek, *Anal. Biochem.* **1986**, *154*, 367 – 370.
[13] a) P.-C. Lin, S.-H. Ueng, S.-C. Yu, M.-D. Jan, A. A. Kumar, C.-C. Yu, C.-C. Li, *Org. Lett.* **2007**, *9*, 2131 – 2134; b) C. C. Cosner, J. T. Markiewicz, P. Bourbon, C. J. Mariani, O. Wiest, M. Rujoi, A. I. Rosenbaum, A. Y. Huang, F. R. Maxfield, P. Helquist, *J. Med. Chem.* **2009**, *52*, 6494 – 6498.
[14] F. R. Baptista, S. A. Belhout, S. Giordani, S. J. Quinn, *Chem. Soc. Rev.* **2015**, *44*, 4433 – 4453.
[15] a) E. Gaufrès, N. Izard, A. Noury, X. Le Roux, G. Rasigade, A. Beck, L. Vivien, *ACS Nano* **2012**, *6*, 3813 – 3819; b) A. Noury, X. Le Roux, L. Vivien, N. Izard, *Nanotechnology* **2014**, *25*, 215201.

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