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Functionalization of Self-Assembled Hexa-*peri*-hexabenzocoronene Fibers with Peptides for Bioprobing

Meizhen Yin,*,†,‡ Jie Shen,§ Wojciech Pisula,‡ Minghui Liang,|| Linjie Zhi,|| and Klaus Müllen*,‡

State Key Laboratory of Chemical Resource Engineering, College of Materials Science and Engineering, Beijing University of Chemical Technology, 100029 Beijing, China, Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany, Department of Entomology, College of Agriculture and Biotechnology, China Agricultural University, 100094 Beijing, China, and National Center for Nanoscience and Technology, 100190 Beijing, China

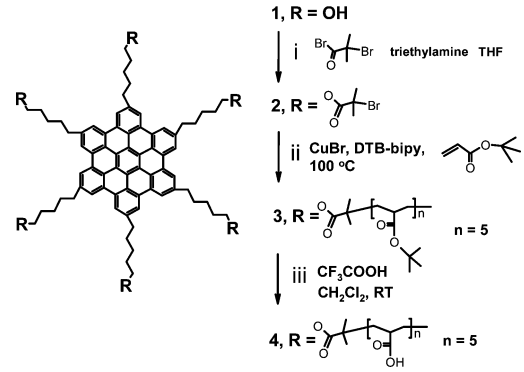
Received July 15, 2009; E-mail: yinmz@yahoo.com; muellen@mpip-mainz.mpg.de

Molecular self-assembly of macromolecules and nanoreactors is widely observed in nature[1] but is also used in device fabrication, where the deposition of organic or biological molecules on substrates is controlled by the interplay of intra- and intermolecular as well as interfacial interactions.[2] Hydrogen bonding and π stacking are particularly important driving forces for the self-assembly process and have yielded various complex structures, including columnar organizations.[3] The columnar stacking of hexa-*peri*-hexabenzocoronenes (HBCs) under mesophase or fiber formation has been intensively studied and shown to have enormous potential for the fabrication of organic electronic devices.[4] Thereby, the introduction of alkyl side chains or functional groups and the mode of processing allow one to control the self-assembly and fiber growth.[5]

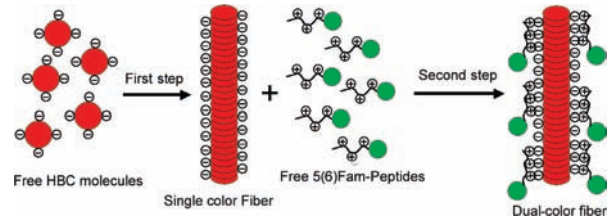
The use of conjugated supramolecular architectures to dictate the organization of biomolecules is a topic of current interest.[6] The only case of HBC assembly possessing a protein element is an amphiphilic HBC complexed with poly(ethylene oxide)-*block*-poly(L-lysine) for bioinspired columnar structures.[7] For direct biological applications utilizing HBC, it is necessary to design water-soluble HBC derivatives for interaction with biological molecules such as proteins and DNA in physiological solution. Carbon nanotubes and carbon fibers have been further functionalized for biological applications through the binding of proteins and nucleic acids.[8] An analogous yet previously elusive functionalization of HBC fibers with such biomolecules would require the use of HBC-derived water-soluble nanotubes or fibers. Herein, we introduce a novel water-soluble HBC derivative (**4**, Scheme 1) with peripheral functional groups, which facilitates a two-step assembly process in water that includes fiber formation via π stacking and subsequent peptide probing via electrostatic interactions (Scheme 2). In the first step, the HBC derivative self-assembles into water-soluble fibers that serve as templates for further functionalization with the biomolecules. In the second step, the peripheral functional groups bind peptides, leading to the formation of well-defined second-step fibers. Of great importance for the hybrid design is the well-studied fluorescence of HBC chromophores,[4,5a] which enables one to monitor the assembly process by fluorescence microscopy.

The synthetic strategy for the water-soluble HBC **4** is described in Scheme 1. The starting hexahydroxy HBC derivative **1** was esterified with commercially available 2-bromo-2-methylpropionyl bromide to yield the desired macroinitiator **2**. This macroinitiator contains six arms of 2-bromo-2-methylpropionic ester groups serving as initiator sites for the further construction of star molecule **3** (Figure S1 and Table S1 in the Supporting Information) by a grafting-from protocol using

Scheme 1. Synthesis of Negatively Charged HBC Derivative **4**



Scheme 2. Schematic Drawing To Illustrate the Two-Step Template Strategy[a]



[a] In the first step, the negatively charged HBC **4** aggregates into a red fluorescent fiber. In the second step, green fluorescein positively charged 5(6)-Fam-conjugated peptides bind to the negatively charged red fiber by electrostatic interactions, resulting in the formation of a dual-color fiber in double-fluorescence imaging.

atom-transfer radical polymerization (ATRP).[9] Subsequent deprotection of the *tert*-butyl groups under acidic conditions provided the desired negatively charged product **4** bearing carboxylic acid groups that introduce water solubility and biomolecular interactions.[10] The characterization of the compounds by IR and ^{1}H NMR spectroscopy is shown in Figures S2 and S3.

The absorption and fluorescence spectra of **4** in water are shown in Figure S4. To demonstrate the ability to grow fibers of **4**, its aqueous solution was first drop-cast onto a SiO_2 wafer surface. Remarkably, well-organized microfibers with diameters ranging from 500 nm to 2 μm and lengths up to 200 μm were formed, as revealed by scanning electron microscopy (SEM) (Figure 1A), fluorescence microscopy (Figure 1B), and transmission electron microscopy (TEM) (Figure 2A). High-resolution TEM disclosed that the fiber was composed of highly ordered columnar structures (Figure 2B) well-aligned along the fiber axis. Fast Fourier transform analysis (Figure 2C) confirmed the pronounced columnar orientation

† Beijing University of Chemical Technology.
‡ Max Planck Institute for Polymer Research.
§ China Agricultural University.
|| National Center for Nanoscience and Technology.

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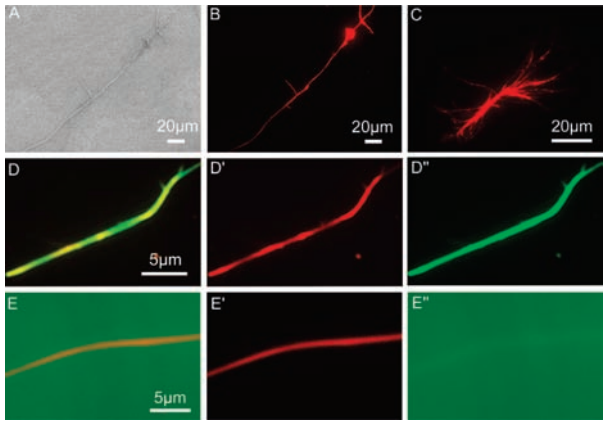

Figure 1. (A) SEM and (B−E) fluorescence images of (A−D) negatively charged HBC **4** fibers and (E) a neutral HBC-C12 fiber. Green-fluorescent, positively charged 5(6)-Fam-conjugated peptides bind to the negatively charged fiber and form a dual-color fiber in double-fluorescence imaging (D) but do not bind to the neutral fiber and thus are evenly distributed (E). Separate channels are shown in D′ (red, HBC **4** fiber), D″ (green, fiber of 5(6)-Fam), E′(red, HBC-C12 fiber), and E″ (green, evenly distributed 5(6)-Fam).

by the distinct reflections, which are only observed for HBCs with intensive π-stacking interactions.[11]

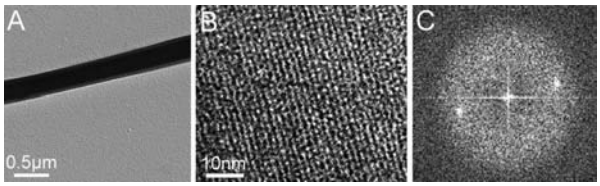

Figure 2. (A) TEM image of the microfiber formed when **4** was drop-cast from aqueous solution (1×10^{-5} M) onto a copper grid. (B) High-resolution TEM image of the fiber, displaying its columnar structures. (C) Fast Fourier transform analysis.

Because of the peripheral side arms of **4** with multiple −COOH groups, the HBC fibers from **4** are negatively charged in water.[10a] Therefore, we hypothesized that the assembled fibers may act as templates for binding of positively charged peptides by electrostatic interactions. We rationalized that if the bound peptides were fluorescein-labeled, the final fibers should be visualized as a dual-color assembly in double-fluorescence imaging (Scheme 2).

In an initial spectral analysis, a nonfluorescent, positively charged peptide (Arg-Lys-Arg-Lys-Arg-Arg) formed by the enrichment of the amine groups was used to study the polyelectrolyte−polyelectrolyte interactions in water. As shown in Figure S4, both the UV−vis absorbance and the photoluminescence intensity of a mixture of **4** and the peptide exhibited an enhancement relative to that of the isolated compounds, indicating a strong electrostatic interaction. The peptide is nonfluorescent and is not visible in a fluorescence microscope. Therefore, it was conjugated to a green carboxyfluorescein, 5(6)-Fam.[12] The green color of the fluorescein-conjugated peptide is complementary to the red fluorescence of the HBC fibers and can be used in double-fluorescence imaging, giving the final proof of the second-step assembly (Scheme 2). To perform the second assembly step, a water solution of the green fluorescein-conjugated peptide was dropped directly onto the HBC fibers from **4** at the surface of a glass slide (Figure 1C). After a 5 min incubation, the sample was scanned in the fluorescence microscope to monitor the second-step assembly. Interestingly, the clustered bunches of fibers on the glass slide (Figure 1C) were dispersed into individual fibers in water (Figure 1D,D′ and Figure S7). This increased dispersion can be ascribed to the new electrostatic interaction. Excitingly, the peptides aggregated as fibers by electrostatic interactions with the HBC fibers and emitted green fluorescence (Figure 1D,D″). Thus, the red HBC fibers were coated with green fluorescein-labeled peptides to form the second-step fibers; the latter were detectable in dual-channel (green and red) when scanned in a fluorescence microscope (Figure 1D). In the total emission spectra, the second-step fibers (Figure 1D) appeared as two peaks corresponding to HBC fibers and 5(6)-Fam (Figure S5). In a negative control, the green fluorescein-conjugated peptide was also dropped onto neutral nonfunctionalized hexadodecyl HBC (HBC-C12)[5c] fibers (Figure 1E′ and Figure S6), which did not show binding, as evidenced by the even distribution of 5(6)-Fam peptide in the double-fluorescence images (Figure 1E,E″). Through these fluorescence measurements, we have proven the functionalization of the negatively charged HBC fibers by positively charged biological molecules.

In summary, we have described a novel water-soluble HBC derivative with peripheral negatively charged side arms that self-assembles into micro- and nanofibers via π stacking. Furthermore, the negative charges of the HBC fiber periphery act as anchors to recruit positively charged biomolecules via electrostatic interactions. The two-step assembly process presented here is the first example of a fiber-forming HBC derivative that can serve as a template for further functionalization with biomolecules. Our strategy opens a new way to design different HBC analogues with special functional groups for use as specific biological sensors.

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Supporting Information Available: Experimental procedures, characterization of the macromolecules, supporting figures and text, and complete ref 6a. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Vriezema, D. M.; Aragones, M. C.; Elemans, J. A. A. W.; Cornelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M. *Chem. Rev.* **2005**, *105*, 1445. (b) Philp, D.; Stoddart, J. F. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1154. (c) Percec, V.; Dulcey, A.; Balagurusamy, V. S. K.; Miura, Y.; Smidrkal, J.; Peterca, M.; Nummelin, S.; Edlund, U.; Hudson, S. D.; Heiney, P. A.; Duan, H.; Magonov, S. N.; Vinogradov, S. A. *Nature* **2004**, *430*, 764.
(2) (a) Rabe, J. P.; Buchholz, S. *Science* **1991**, *253*, 424. (b) Samori, P. *Chem. Soc. Rev.* **2005**, *34*, 551. (c) Mamdouh, W.; Uji-i, H.; Ladislaw, J. S.; Dulcey, A. E.; Percec, V.; De Schryver, F. C.; De Feyter, S. *J. Am. Chem. Soc.* **2006**, *128*, 317.
(3) (a) Lehn, J. M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH: Weinheim, Germany, 1995. (b) Hoeben, F. J. M.; Jonkheijm, P.; Meijer, E. W.; Schenning, A. P. H. J. *Chem. Rev.* **2005**, *105*, 1491.
(4) Wu, J.; Pisula, W.; Müllen, K. *Chem. Rev.* **2007**, *107*, 718.
(5) (a) Hill, J. P.; Jin, W.; Kosaka, A.; Fukushima, T.; Ichihara, H.; Shimomura, T.; Ito, K.; Hashizume, T.; Ishii, N.; Aida, T. *Science* **2004**, *304*, 1481. (b) Kastler, M.; Pisula, W.; Wasserfallen, D.; Pakula, T.; Müllen, K. *J. Am. Chem. Soc.* **2005**, *127*, 4286.
(6) (a) Janssen, P. G.; et al. *J. Am. Chem. Soc.* **2009**, *131*, 1222. (b) Chi, C.; Mikhailovsky, A.; Bazan, G. C. *J. Am. Chem. Soc.* **2007**, *129*, 11134. (c) Liu, B.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 589.
(7) Thünemann, A. F.; Kubowicz, S.; Burger, C.; Watson, M. D.; Tchebotareva, N.; Müllen, K. *J. Am. Chem. Soc.* **2003**, *125*, 352.
(8) (a) Sun, Y.; Fu, K.; Lin, Y.; Huang, W. *Acc. Chem. Res.* **2002**, *35*, 1096. (b) Lee, C. S.; Baker, S. E.; Marcus, M. S.; Yang, W.; Eriksson, M. A.; Hamers, R. J. *Nano Lett.* **2004**, *4*, 1713. (c) Pastine, S. J.; Okawa, D.; Kessler, B.; Rolandi, M.; Llorente, M.; Zettl, A.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2008**, *130*, 4238.
(9) (a) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921. (b) Yin, M.; Habicher, W. D.; Voit, B. *Polymer* **2005**, *46*, 3215. (c) Yin, M.; Bauer, R.; Klapper, M.; Müllen, K. *Macromol. Chem. Phys.* **2007**, *208*, 1646. (d) Yin, M.; Cheng, Y.; Liu, M.; Gutmann, J. S.; Müllen, K. *Angew. Chem., Int. Ed.* **2008**, *47*, 8400. (e) Yin, M.; Shen, J.; Pflugfelder, G. O.; Müllen, K. *J. Am. Chem. Soc.* **2008**, *130*, 7806.
(10) (a) Yin, M.; Shen, J.; Gropeanu, R.; Pflugfelder, G. O.; Weil, T.; Müllen, K. *Small* **2008**, *4*, 894. (b) McRae, R. L.; Phillips, R. L.; Kim, I.-B.; Bunz, U. H. F.; Fahrni, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 7851.
(11) Feng, X.; Pisula, W.; Kudernac, T.; Wu, D.; Zhi, L.; De Feyter, S.; Müllen, K. *J. Am. Chem. Soc.* **2009**, *131*, 4439.
(12) Weston, S. A.; Parish, C. R. *J. Immunol. Methods* **1990**, *133*, 87.

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