Amphiphiles

DOI: 10.1002/ange.200602739

Grafting Short Peptides onto Polybutadiene-*block*-poly(ethylene oxide): A Platform for Self-Assembling Hybrid Amphiphiles**

Yan Geng, Dennis E. Discher, Justyna Justynska, and Helmut Schlaad**

The peptide in peptide-containing hybrid amphiphiles usually plays the role of a hydrated hydrophilic headgroup rather than that of a hydrophobic tail.[1–3] However, chiral peptide interactions within a dense hydrophobic core of an amphiphile assembly might better mimic the hydrophobic interactions that drive the collapse and ordering in protein folding and highly specific protein–protein associations.[4] The goal of this study was therefore to make self-assembling hybrid amphiphiles that integrate peptides within the hydrophobic core. More-practical reasons for attaching short peptides onto synthetic polymers involve biomedical applications: 1) amino acids chosen from those often found in protein cores (cysteine, phenylalanine, tryptophane, etc.) can help to tune the solubility of hydrophobic drugs, and 2) short peptides with less than six repeat units are less antigenic[5,6] than long polypeptides[7] or proteins.[8]

Purely synthetic block copolymer amphiphiles can self-assemble into various mesostructures (e.g. spherical micelles, wormlike micelles, and vesicles) in aqueous solutions[9–11] by similar principles to the hydrophobic effect operative for surfactants (i.e. minimization of the contact of the hydrophobic blocks with water).[12,13] Morphological phase diagrams mapped out for nonionic block copolymers of polybutadiene-*block*-poly(ethylene oxide) (PBD-*b*-PEO) show that their self-assembly structures in water are largely determined by the weight fraction of the hydrophilic PEO

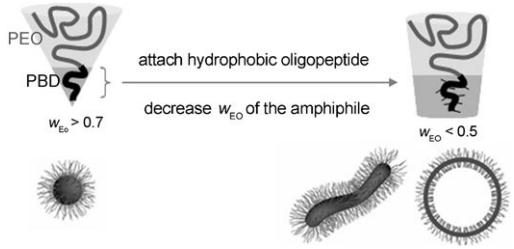
[*] Prof. Y. Geng
Department of Chemistry
University of Georgia
Athens, GA 30602 (USA)
Fax: (+1) 706-542-9454
E-mail: ygeng@chem.uga.edu
Homepage: http://www.chem.uga.edu/ygeng
J. Justynska, Dr. H. Schlaad
Max-Planck-Institut für Kolloid- und Grenzflächenforschung
Wissenschaftspark Golm, 14424 Potsdam (Germany)
Fax: (+49) 331-567-9502
E-mail: schlaad@mpikg-golm.mpg.de
Prof. D. E. Discher
University of Pennsylvania
Philadelphia, PA 19104-6315 (USA)

[**] We thank Reinhard Sigel, Hans Börner, and Markus Antonietti (MPI-KG) for their help and useful discussions. We also thank the Bates group at the University of Minnesota for cryo-TEM measurements. Grants were provided by the NSF, the Deutsche Forschungsgemeinschaft, and the Max-Planck-Gesellschaft.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

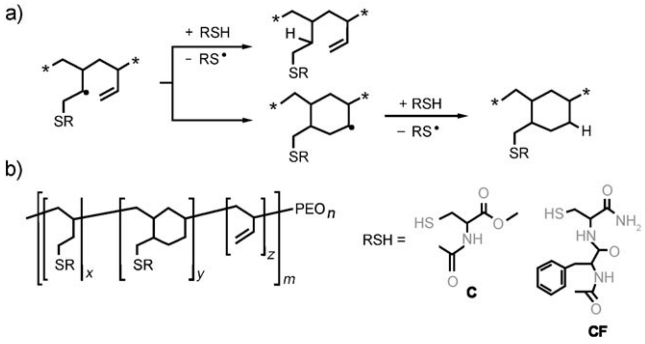
block (w_{EO}) and that decreasing w_{EO} disfavors spherical micelles and favors structures with lower mean curvature such as wormlike micelles and vesicles.[9] Although the structures of synthetic block copolymers can be tuned, the hybridization of synthetic copolymers with peptides leads to a further level of sophistication and functionality of the self-assembled structures.[1,2] Helical superstructures have recently been prepared from peptide hybrid amphiphiles, typically using long polypeptides as the hydrophilic block, such as polystyrene-*block*-poly(isocyanodipeptide)[14] and polybutadiene-*block*-poly(L-glutamate).[15,16] These peptide hybrids were generally synthesized by advanced techniques such as metal-catalyzed polymerization of isocyanopeptides or ring-opening polymerization of the α-amino acid *N*-carboxyanhydride.

Herein, we introduce a new type of self-assembling peptide hybrid amphiphiles by using synthetic PBD-*b*-PEO as the backbone and grafting cysteine-containing oligopeptides onto the hydrophobic PBD segment through a free-radical addition reaction. Modification with difluorocarbene was used in the solid-state to tune the self-assembly behavior of block copolymers;[17] in aqueous solutions, attaching short hydrophobic peptides decreases the w_{EO} value of the amphiphile and thus shifts the preferred self-assembly morphology towards those with lower curvatures, namely, from spherical micelles to wormlike micelles and vesicles (Scheme 1). Initial studies also suggest that chiral peptide interactions within the hydrophobic core foster the formation of helical superstructures.



Scheme 1. Change of the morphology towards lower curvature structures through the attachment of a hydrophobic peptide onto PBD-*b*-PEO.

Two PBD-*b*-PEO copolymer samples with similar number-average molecular weight (M_n) but different weight fractions of PEO were used: PBD_{14}-*b*-PEO_{93} (**1**, M_n = 4900 g mol^{-1}, w_{EO} = 0.83) and PBD_{25}-*b*-PEO_{75} (**2**, M_n = 4700, w_{EO} = 0.70). As reported earlier, PBD-*b*-PEO can be functionalized through free-radical addition of ω-functional mercaptans (RSH) without altering the molecular-weight distribution (MWD).[18,19] The number of functional groups attached to the PBD chain is usually lower than the number of converted double bonds owing to a side reaction of the radical species, namely, the formation of six-membered cyclic units (Scheme 2a). We have extended the radical addition route from mercaptans to chiral cysteine-containing oligopeptides. The route was first tested on an L-cysteine derivative (**C**) and then on the dipeptide (L,L)-cysteine-phenylalanine (**CF**) as a model oligopeptide to prove the applicability of this route (Scheme 2b).



Scheme 2. a) Possible reaction pathway of the intermediate radicals formed by the addition of RS· onto PBD. b) Chemical structure of **C**- and **CF**-grafted PBD-*b*-PEO.

Addition reactions of **C** onto **1** and **2** were performed in refluxing tetrahydrofuran (THF) using azoisobutyronitrile (AIBN) as the radical source ($[C{=}C]_0/[\mathbf{C}]_0/[AIBN]_0$ = 1:10:0.33). Conversion of PBD double bonds came to completion within 24 h and products **1**-**C** and **2**-**C** exhibited the same narrow MWDs as the precursors (PDI ≤ 1.05; see NMR spectroscopy and size-exclusion chromatography (SEC) in the Supporting Information).[18] In the case of **CF**, the molar ratio was chosen to be $[C{=}C]_0/[\mathbf{CF}]_0/[AIBN]$ = 1:2.5:0.33 for economic reasons. Further decreasing the amount of **CF** bears the risk that the PBD chains will undergo intermolecular cross-linking. Because of the poor solubility of **CF** in THF, the radical addition reaction was carried out in *N*-methylpyrrolidone (NMP) as the solvent. The ^{1}H NMR spectra (**1**-**CF** shown in Figure 1a, **2**-**CF** given in the Supporting Information) shows signals of the dipeptide at δ = 4.3, 4.5 (α-CH), 7.1–7.3 (phenyl), and 8.1 ppm (NH); the signals of the thioether linkage -CH_2SCH_2- arise at $\delta \approx 2.7$ and 2.9 ppm. Resonances at δ = 4.8–5.6 ppm indicate the presence of residual double bonds. Quantitative analysis of signal intensities relative to that of PEO ($\delta \approx 3.6$ ppm) suggested that both samples contain about 10 **CF** units and 2–3 unreacted butadiene units. SEC (with differential refractive

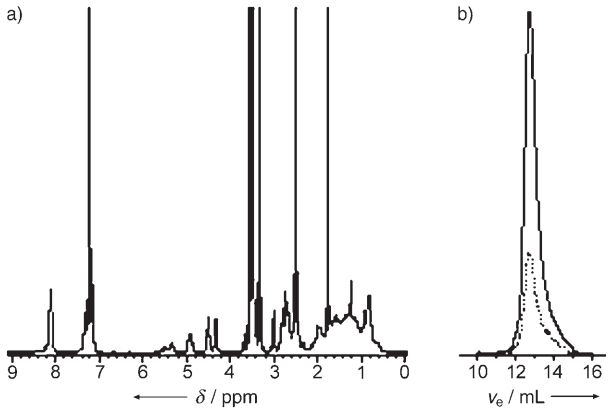


Figure 1. a) ^{1}H NMR spectrum (solvent: $[D_6]$DMSO) and b) SEC (eluent: NMP; solid line: RI; dashed line: UV, λ = 270 nm) of **1**-**CF**.

index (RI) and UV detectors) of **1**-**CF** (Figure 1b) confirmed the attachment of **CF** units onto the polymer (UV absorption at 270 nm) and the preservation of the narrow MWD (polydispersity index < 1.2).

As mentioned above, the morphology of the PBD-*b*-PEO self-assembled structures in water is dictated by the w_{EO} value. Spherical micelles predominate when $w_{EO} > 0.55$, whereas wormlike micelles form when $w_{EO} \approx 0.50$–0.55 and vesicles form when w_{EO} is well below 0.5.[9] The precursor PBD-*b*-PEO samples **1** ($w_{EO} = 0.83$) and **2** ($w_{EO} = 0.70$) form spherical micelles at 0.1% w/w in water, as visualized by cryogenic transmission electron microscopy (cryo-TEM; Figure 2). The dark spots observed in the micrographs correspond to the hydrophobic cores of the micelles, which are about 12 and 20 nm in diameter for **1** and **2**, respectively. The hydrodynamic diameters of the micelles determined by dynamic light scattering (DLS) are 27 and 45 nm, respectively.

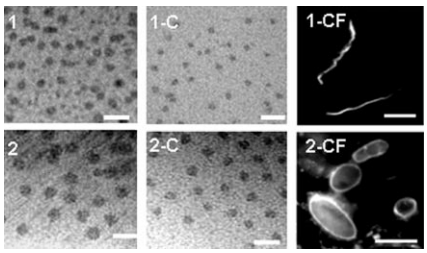


Figure 2. Visualization of self-assembled structures of PBD-*b*-PEO (**1**, **2**) and **C**-grafted hybrids (**1**-**C**, **2**-**C**) by cryo-TEM (scale bars = 50 nm) and **CF**-grafted hybrids (**1**-**CF**, **2**-**CF**) by FM (scale bars = 5 μm) in water.

Grafting **C** onto **1** and **2** had no effect on the shape of micelle cores, yet decreased their size by 10–30% (Figure 2). The hydrodynamic diameters of the micelles also decreased to about one third that of the precursor micelles. The formation of smaller-sized micelles can be attributed to the slight hydrophilic nature of cysteine, which increases the overall hydrophilic fraction of the hybrids and the corresponding interfacial curvature.

In contrast, dipeptide **CF** is hydrophobic, and grafting **CF** onto **1** and **2** leads to a decrease of w_{EO} and shifts the morphology towards those with smaller curvatures, that is, wormlike micelles and vesicles. Fluorescence microscopy (FM) revealed giant wormlike micelles with contour lengths of 10–15 μm for **1**-**CF** ($w_{EO} = 0.54$) and vesicles with diameters of 2–5 μm for **2**-**CF** ($w_{EO} = 0.43$) (Figure 2). FM is widely used to study cellular structures and dynamics, but has recently been established also as a reliable and convenient technique for visualizing micrometer-sized wormlike micelles and vesicles.[20–22] Unlike cryo-TEM, FM allows direct observation of samples in aqueous solutions without requiring fixation and constraint in thin films, and thus provides more equilibrated and convenient access to length and dynamic measurements of soft objects. The w_{EO} values of the **CF**-grafted PBD-*b*-PEO that correspond to wormlike micelles and vesicles are quantitatively comparable to the PBD-*b*-PEO morphological phase diagram,[9] thus suggesting that the self-assembly of such hybrids in water is likewise dictated by the hydrophobic–hydrophilic balances.

Integrating the dipeptide **CF** into PBD-*b*-PEO not only changed the hydrophobic–hydrophilic ratio of the amphiphile, but also introduced hydrogen-bonding, π–π, and chiral interactions into the hydrophobic core of the assembly, which might induce the formation of helices. Helices often emerge in nature from such interactions, for example, in proteins and DNA. As helices are generally more observable in fibrous structures, we then examined the giant **1**-**CF** wormlike micelles in more detail. FM revealed the existence of both right- and left-handed defected helical structures with supermolecular pitches of a few micrometers (Figure 3a). Sequen-

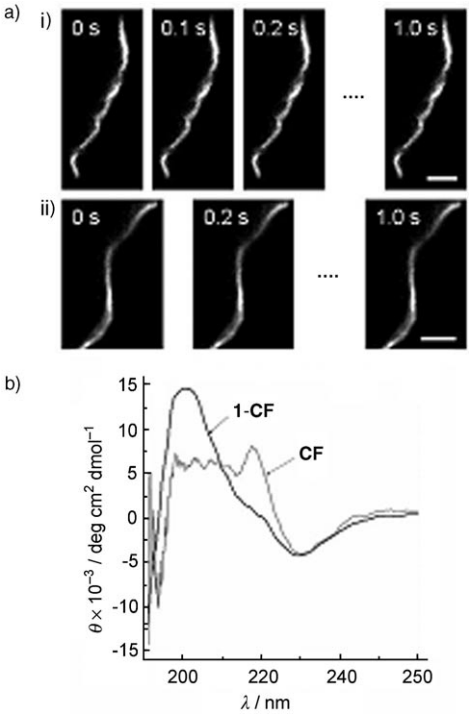


Figure 3. a) FM snapshots of i) right-handed and ii) left-handed distorted helical structures (scale bars = 2 μm); and b) CD spectrum of **1**-**CF** wormlike micelles in water.

tial snapshots of individual **1**-**CF** wormlike micelles confirm that their helical curvature is rigid, whereas pristine PBD-*b*-PEO wormlike micelles are extremely soft and flexible and exhibit large thermal fluctuations.[23] Analysis of the circular dichroism (CD) spectrum of an aqueous solution of **1**-**CF** wormlike micelles (Figure 3b) indicates a near racemic mixture of helices with right-handed and left-handed screw senses (see the Supporting Information). Although investigations continue, the capability of such amphiphilic hybrids that contain hydrophobic peptides to self-assemble into helical superstructures is evident.

In conclusion, we report a new type of peptide hybrid amphiphiles by directly grafting cysteine-containing oligopeptides onto synthetic PBD-*b*-PEO copolymers. The morphologies (spherical micelles, wormlike micelles, and vesicles) of such self-assembled hybrid amphiphiles may be tuned by

controlling the hydrophobic–hydrophilic ratios. Helical superstructures also arise from the chiral peptide interactions inside the assembly core. This new class of hybrids allows the integration of functionalities into the assembly core for various applications. Future investigations will likely include exploring the attachment of different functional oligopeptides, studying their self-assembly and superstructures, and pursuing their biomedical applications.

Received: July 10, 2006
Published online: October 20, 2006

Keywords: amphiphiles · helical structures · micelles · self-assembly · vesicles

[1] J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, *294*, 1684 – 1688.
[2] H. Schlaad, M. Antonietti, *Eur. Phys. J. E* **2003**, *10*, 17 – 23.
[3] H. Schlaad, *Adv. Polym. Sci.* **2006**, *202*, 53 – 74.
[4] J. Miao, J. Klein-Seetharaman, H. Meirovitch, *J. Mol. Biol.* **2004**, *344*, 797 – 811.
[5] A. Y. Rudensky, P. Preston-Hurlburt, S.-C. Hong, A. Barlow, C. A. Janeway, *Nature* **1991**, *353*, 622 – 627.
[6] J. W. Yewdell, J. R. Bennink, *Curr. Opin. Immunol.* **2001**, *13*, 13.
[7] T. J. Deming, *Adv. Drug Delivery Rev.* **2002**, *54*, 1145 – 1155.
[8] K. Velonia, A. E. Rowan, R. J. M. Nolte, *J. Am. Chem. Soc.* **2002**, *124*, 4224 – 4225.
[9] S. Jain, F. S. Bates, *Science* **2003**, *300*, 460 – 464.
[10] S. Förster, M. Zisenis, E. Wenz, M. Antonietti, *J. Chem. Phys.* **1996**, *104*, 9956 – 9970.
[11] D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967 – 973.
[12] J. N. Israelachvili, *Intermolecular and Surface Forces*, Academic Press, London, **1985**.
[13] P. Alexandridis, B. Lindman, *Amphiphilic Block Copolymers: Self-assembly and Applications*, Elsevier, New York, **2000**.
[14] J. J. L. M. Cornelissen, M. Fischer, N. A. J. M. Sommerdijk, R. J. M. Nolte, *Science* **1998**, *280*, 1427 – 1430.
[15] H. Kukula, H. Schlaad, M. Antonietti, S. Förster, *J. Am. Chem. Soc.* **2002**, *124*, 1658 – 1663.
[16] F. Chécot, S. Lecommandoux, Y. Gnanou, H.-A. Klok, *Angew. Chem.* **2002**, *114*, 1395 – 1399; *Angew. Chem. Int. Ed.* **2002**, *41*, 1339 – 1343.
[17] Y. Ren, T. P. Lodge, M. A. Hillmyer, *Macromolecules* **2000**, *33*, 866 – 876.
[18] J. Justynska, Z. Hordyjewicz, H. Schlaad, *Polymer* **2005**, *46*, 12057 – 12064.
[19] J. Justynska, H. Schlaad, *Macromol. Rapid Commun.* **2004**, *25*, 1478 – 1481.
[20] B. M. Discher, Y.-Y. Won, D. S. Ege, J. C.-M. Lee, F. S. Bates, D. E. Discher, D. A. Hammer, *Science* **1999**, *284*, 1143 – 1146.
[21] P. Dalhaimer, H. Bermudez, D. E. Discher, *J. Polym. Sci. Part B* **2004**, *42*, 168 – 176.
[22] Y. Geng, D. E. Discher, *J. Am. Chem. Soc.* **2005**, *127*, 12780 – 12781.
[23] P. Dalhaimer, F. S. Bates, D. E. Discher, *Macromolecules* **2003**, *36*, 6873 – 6877.

