CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200500809

Recognition-Induced Polymersomes: Structure and Mechanism of Formation

Oktay Uzun,[a] Hao Xu,[a] Eunhee Jeoung,[b] Raymond J. Thibault,[a] and Vincent M. Rotello*[a]

Abstract: Random polystyrene copolymers grafted with complementary recognition elements were combined in chloroform producing vesicular aggregates, that is, recognition-induced polymersomes (RIPs). Reflection interference contrast microscopy (RICM) in solution, coupled with optical microscopy (OM) and atomic force microscopy (AFM) on solid substrates, were used to determine the wall thickness of the RIPs. Rather than a conventional mono- or bilayer structure (~10 or ~20 nm, respectively) the RIP membrane was 43 ± 7 nm thick. Structural arrangement of the polymer chains on the RIP wall were characterized by using angle-resolved X-ray photoelectron spectroscopy (AR-XPS). The interior portion of the vesicle membrane was found to be more polar, containing more recognition units, than the exterior part. This gradient suggests that a rapid self-sorting of polymers takes place during the formation of RIPs, providing the likely mechanism for vesicle self-assembly.

Keywords: hydrogen bonds · molecular recognition · nanotechnology · self-assembly · supramolecular chemistry

Introduction

Application of specific recognition processes to the controlled aggregation of synthetic macromolecules, such as polymers[1] and dendrimers,[2] is a versatile strategy due to the precise "lock-and-key" control over molecular-level interactions,[3] as well as the inherent reversibility and self-healing properties of the resulting supramolecular materials. The noncovalent interactions that define the self-assembly process are responsible for the highly ordered, diverse systems found in nature; providing inspiration for the creation of new self-assembled structures.[4]

Vesicles or liposomes are versatile supramolecular systems with unusual stability and great potential as functional materials. The closed bilayer structure inherent within vesicular systems provides an effective barrier between the fluid internal medium and the external bulk environment, while still allowing selective transport across the membrane and opening a variety of applications. As such, these systems have generated much interest for their broad utility in fields as diverse as drug delivery,[5] encapsulation of active agents,[6] microreactivity,[7] and biomodels.[8] Significant advances in synthetic polymer chemistry have allowed for the advent of highly analogous vesicular architectures composed of well-defined amphiphilic polymers, that is, polymer vesicles (referred to as "polymersomes")[9] and peptide–polymer conjugates, called "peptosomes".[10] In recent years, numerous researchers have contributed to this growing field with a variety of polymers.[11] Notably, Discher and co-workers, who coined the term polymersome,[12] originally constructed polymersomes from diblock copolymers of polyethyleneglycol–polyethylethylene[13] and polyethyleneglycol–polybutadiene[14] (the former employed to allow membrane crosslinking) and demonstrated the resulting polymersomes to be nearly an order of magnitude stronger owing to the larger membrane thickness (~8 nm) relative to liposomes (~3–4 nm). Additionally, Eisenberg and co-workers have invested considerable effort to demonstrate the thermodynamics of formation,[15] stabilization,[16] and size control;[17] the kinetics of fusion;[18] and other vesicle transformations.[19] Taken as a whole, these vesicular systems have broadened our capability to develop pragmatic devices for these applications.

In recent studies, we have reported the construction of giant vesicular aggregates, or recognition-induced polymer-

[a] O. Uzun, H. Xu, R. J. Thibault, Prof. V. M. Rotello
Department of Chemistry, University of Massachusetts
710 North Pleasant Street, Amherst, MA 01003 (USA)
Fax: (+1) 413-545-4490
E-mail: rotello@chem.umass.edu

[b] Dr. E. Jeoung
Department of Chemistry, Kangnung National University
123 Jibyeon-dong, Gangneung, Gangwondo, 210–702 (Korea)

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author. It contains synthesis, sample preparation, and optical microscopy images.

somes (RIPs), from spontaneous assembly of randomly substituted, complementary copolymers.[20] Upon combination in noncompetitive solvents, the covalently attached diamidopyridine (DAP) and thymine (Thy) recognition elements on random polystyrene copolymers (Figure 1) form a three-

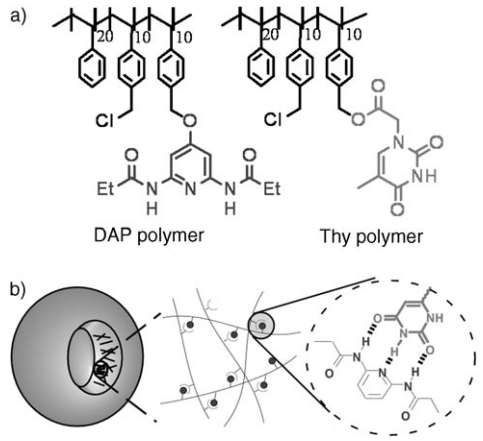

Figure 1. a) Random complementary DAP and Thy polymers. b) Schematic illustrating formation of vesicular aggregates.

point hydrogen bonding recognition dyad. This self-assembly is unprecedented, as there is no directionality inherent in the polymer components. While we have established that these specific three-point hydrogen bonds were necessary for the formation of vesicular structures,[21] the origin of the formation of vesicular structures from these random copolymers and the actual structure of the vesicle wall remained unknown. Understanding the mechanism of formation and the structure of the vesicle membrane will potentially lead to new technologies that incorporate a "lock-and-key" motif to control transport across the membrane and gain reversible control over the assembly process. Herein, we report our recent investigations on the structure of the RIP walls, providing insight into the vesicle wall structure, including thickness and the arrangement of the polymer chains in the RIP membrane. From the latter, we are able to provide a mechanism for RIP formation based on the self-sorting of the random polymer chains.

Results and Discussion

We performed freeze–fracture (FF) on a vitrified sample of RIPs to confirm the retention of the vesicular structure and visualized the replica with TEM (Figure 2). In recent years, researchers have performed FF-TEM in organic solvents;[22] however, to the best of our knowledge, this is the first account of freeze–fracture reported in chloroform. The micrographs in Figure 2 provide a definite confirmation of the vesicular morphology; however, the shadowing process obscured the quantitative determination of the vesicle membrane thickness.

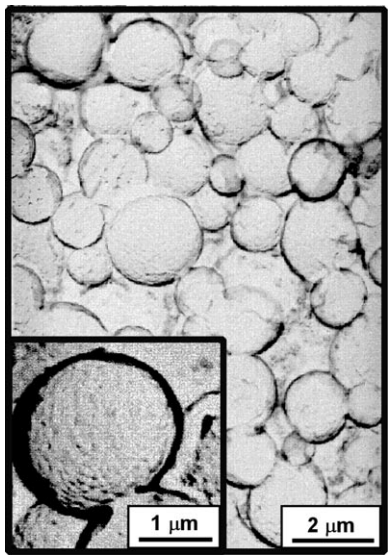

Figure 2. Freeze–fracture TEM images of polymersomes. Samples were vitrified in chloroform five minutes after combination of DAP and Thy polymers.

Our studies were focused on the quantitative characterization of the thickness of the polymersome membrane. Reflection interference contrast microscopy (RICM) was used to quantify the wall thickness in solution.[23] In the RICM technique, interference occurs between light reflected at the glass/vesicle interface and at the interior chloroform/membrane interface (Figure 3a). This interference between light

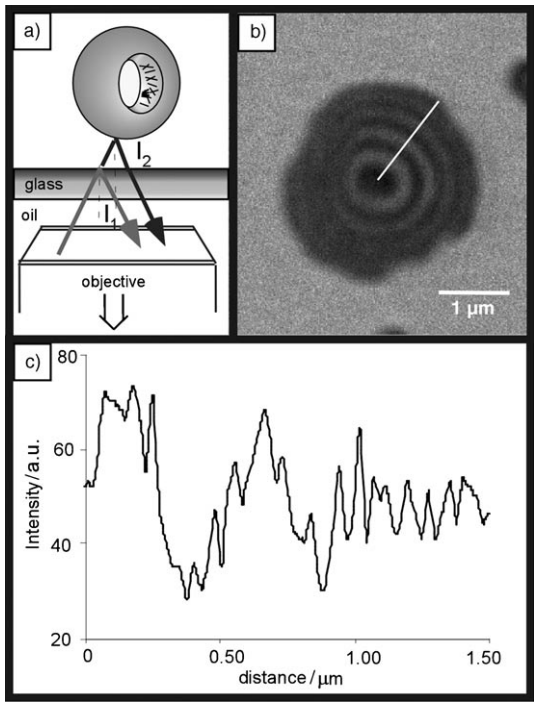

Figure 3. a) Schematic illustration of the RICM setup, b) RICM image, and c) intensity profile change with the radial distance from the center.

from the RIP membrane and light reflected by a planar substrate provides image contrast with spatial sensitivity of ~2 nm.[24] By plotting the intensity change with radial distance from the center of the micrograph, the thickness of the vesicle membrane was found to be 52 nm (see Supporting Information).

In further studies, we focused on the deposition of RIPs on the planar silica substrates to exploit quantitative surface characterization techniques on the vesicle membrane. RIPs were slowly deposited onto oxidized silicon substrate, by dipping into 3 mg mL^{-1} polymersome solution and removal. These surfaces were dried under high vacuum overnight. Initial optical microscopy studies confirmed that RIPs retained their shape (see Supporting Information).

The average mean diameter of the vesicles in solution was 3.3 ± 0.9 μm, after depositing them on silica substrate; the average mean diameter was 4.3 ± 1.3 μm. The diameters of RIPs placed on the surface were 1 μm larger than those in solution, and the overall size distribution profile was preserved in the deposition process (Figure 4).

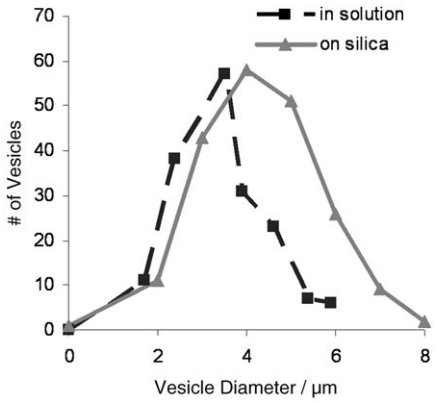

Figure 4. Size distributions of polymersomes in solution and after deposition on silica substrate.

Remarkably, optical microscopy images of the collapsed RIPs evidenced a consistent blue color indicating a uniform wall thickness corresponding to 70–100 nm. We used a more sensitive surface characterization method, atomic force microscopy (AFM), which has already been used as a complementary tool for determining the thickness of the vesicle walls[25] with <1 nm vertical resolution. Surface-deposited, collapsed spherical RIP membranes have a thickness equal to twice the wall thickness ($2d$) of the vesicles in solution. Half of this vertical distance from the AFM analysis provided the vesicle membrane thickness (Figure 5). We analyzed thirty different vesicles and the average thickness of the vesicle membrane is 43 ± 7 nm.

Taken together, these determinations of wall thickness on substrates and in solution indicate that the walls of our RIPs are of uniform thickness. Moreover, the RIP wall thickness changed relatively little upon drying, indicating that these walls are dense, as opposed to open gels that would be expected to exhibit greater shrinkage upon drying. Of particular interest is the fact that the wall thickness is not commensurate with either a monolayer or bilayer structure (~10 or 20 nm, respectively, for extended chains). The origin of this unanticipated yet highly uniform wall thickness provides an interesting goal for future structural and modeling studies that are currently being undertaken.

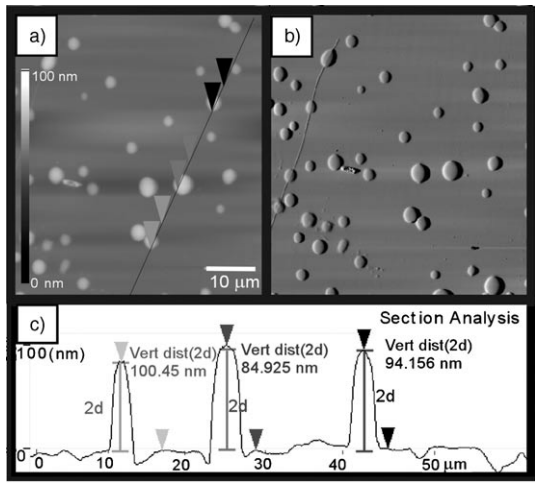

Figure 5. a) Atomic force microscopy (AFM) height image of RIPs on silicon substrate; the arrows indicate the position at which height measurements were made. b) AFM phase image and c) height profile of the AFM image: vertical distance at the center is equal to twice the wall thickness of the vesicle in solution.

Phase segregation, and hence directionality, is a prerequisite for the formation of vesicular structures. The lack of well-defined head groups on our random copolymers makes the formation of vesicles in these systems unique. However, we believe that the randomness inherent within our polymers provides a "pseudo-blocky" structure (Figure 6). The

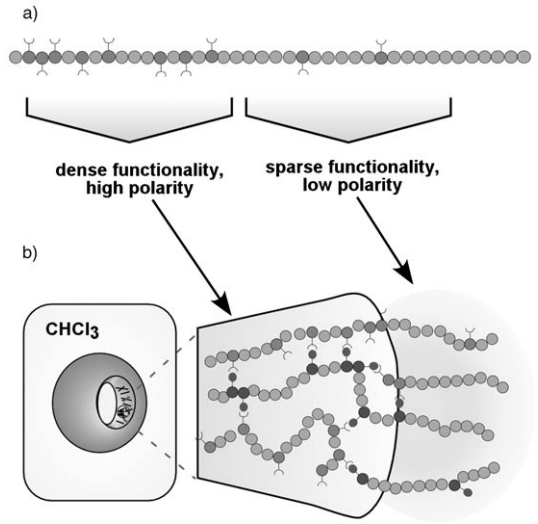

Figure 6. a) Schematic representation of random copolymer, in which one of four monomers is functional, and b) proposed self-sorting of random copolymers illustrating the "pseudo-blocky" structure of DAP and Thy polymers.

random dispersion of recognition elements creates regions of high and low polarity. It is these regions of high polarity that would self-assemble in such a way as to minimize contact with the nonpolar bulk medium (chloroform), effectively forming a wall. Curvature is then imparted to the system by the greater flexibility of the low polarity segments in to the bulk chloroform. While solvophobicity contributes to the microstructure, specific three-point hydrogen-bonding interactions are required for formation, as the unfunctionalized parent poly(styrene-*p*-(chloromethyl)styrene does not assemble; neither do the individual polymers assemble on the micron scale. This fashions a system in which specific molecular-recognition processes modulate nonspecific phase separation.

To validate the self-sorting hypothesis, angle-resolved X-ray photoelectron spectroscopy (AR-XPS) was performed on the vesicle membrane.[26] AR-XPS is based on acquisition of series of spectra at various values of the photoelectron takeoff angle (measured from the sample surface); at lower takeoff angles ($<15°$) sampling depth is 1–2 nm, as the takeoff angle increases ($>75°$) sampling depth reaches to 6–8 nm from the top layer of the surface.[27] We modified the RIP deposition process to obtain densely packed RIP surfaces, by dipping silica substrates into a more concentrated 4.5 mg mL^{-1} RIP solutions and slowly taking them out. These surfaces were then dried under high vacuum overnight. Optical microscopy images demonstrated that the densely packed RIP coverage on these surfaces retained the structure observed in our previous studies (see Supporting Information). XPS was performed and recorded with varying takeoff angles in the range 5–75° by rotating the sample on these surfaces (Figure 7).

The amount of nitrogen was used as a marker to link the density of DAP and Thy functional groups. The increase in nitrogen/carbon ratio from 3.958 to 6.361, by changing the takeoff angle from 15° to 75°, and the increase in the nitrogen amount from 2.18% to 5.49%, by changing the takeoff angle from 5° to 75°, verified that the interior part of the vesicle membrane is more polar; therefore it contains more recognition units than the exterior nonpolar part. In other words, there are more recognition-element-rich chain parts localized inside the vesicle wall, while the less-functionalized parts segregated to the exterior.

The XPS data indicate that a self-sorting process occurs during the formation of RIPs. This process provides the likely mechanism for phase segregation, hence vesicle formation. What is surprising is the rapidity of the self-sorting event: vesicle formation is instantaneous upon mixing of the solutions of the two complementary copolymers. Clearly these systems are highly dynamic, an area we will explore in future studies.

Conclusion

In summary, RICM in solution, and optical microscopy and AFM on solid substrates demonstrate that RIP walls have a uniform thickness of ~50 nm. Additionally, we have shown that the statistical distribution of recognition units on the polymers generated varying degrees of "pseudo-blocky" structure, and hence directionality for the vesicle formation. Angle-resolved XPS studies show that there are more recognition-element-rich chain parts localized within the vesicle wall, while the less-functionalized parts segregated to the exterior. Further research on gelation properties, membrane mechanical strength, and functional nanoparticle–polymersome conjugates are currently underway and will be reported in due course.

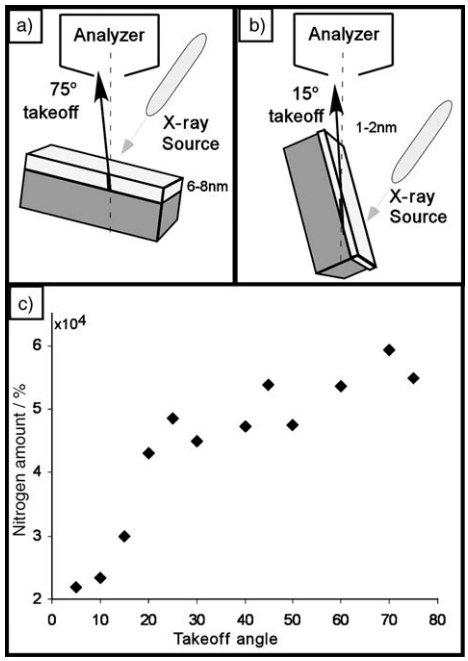

Figure 7. a) and b) Schematic illustration of the decrease in sampling depth by changing the takeoff angle from 75° to 15° in angle-resolved XPS. c) XPS atomic concentration (nitrogen) for polymersomes on silica substrate, recorded with takeoff angles from 5° to 75°.

Experimental Section

For details of the experimental procedures, please see the Supporting Information.

Acknowledgements

O.U. is indebted to Yao Lin for helpful discussions. Financial support from the NSF [CHE-0518487 (V.R.)] and MRSEC (DMR 0213695) are gratefully acknowledged.

[1] a) S. Forster, M. Konrad, *J. Mater. Chem.* **2003**, *13*, 2671–2688; b) B. W. Messmore, J. F. Hulvat, E. D. Sone, S. I. Stupp, *J. Am. Chem. Soc.* **2004**, *126*, 14452–14458; c) M. Muthukumar, C. K. Ober, E. L. Thomas, *Science* **1997**, *277*, 1225–1232; d) S. H. M. Sontjens, R. P. Sijbesma, M. H. P. van Genderen, E. W. Meijer, *Macromolecules* **2001**, *34*, 3815–3818; e) R. P. Sijbesma, F. H. Beijer, L.

Brunsveld, B. J. B. Folmer, J. H. K. Hirschberg, R. F. M. Lange, J. K. L. Lowe, E. W. Meijer, *Science* **1997**, *278*, 1601–1604; f) V. Berl, M. Schmutz, M. J. Krische, R. G. Khoury, J. M. Lehn, *Chem. Eur. J.* **2002**, *8*, 1227–1244; g) J. M. Lehn, *Polym. Int.* **2002**, *51*, 825–839.

[2] a) S. C. Zimmerman, L. J. Lawless, *Supramolecular Chemistry of Dendrimers, Vol. 217*, Springer, Berlin, **2001**, pp. 95–120; b) H. W. Gibson, L. Hamilton, N. Yamaguchi, *Polym. Adv. Technol.* **2000**, *11*, 791–797; c) E. R. Gillies, J. M. J. Frechet, *J. Org. Chem.* **2004**, *69*, 46–53; d) V. Percec, M. Glodde, G. Johansson, V. S. K. Balagurusamy, P. A. Heiney, *Angew. Chem.* **2003**, *115*, 4474–4478; *Angew. Chem. Int. Ed.* **2003**, *42*, 4338–4342; e) C. Kim, S. J. Lee, I. H. Lee, K. T. Kim, *Chem. Mater.* **2003**, *15*, 3638–3642; f) G. M. Dykes, D. K. Smith, *Tetrahedron* **2003**, *59*, 3999–4009.

[3] a) J. M. Lehn, *Makromol. Chem. Macromol. Symp.* **1993**, *69*, 1–17; b) L. J. Prins, D. N. Reinhoudt, P. Timmerman, *Angew. Chem.* **2001**, *113*, 2446–2492; *Angew. Chem. Int. Ed.* **2001**, *40*, 2383–2426; c) D. C. Sherrington, K. A. Taskinen, *Chem. Soc. Rev.* **2001**, *30*, 83–93; d) R. P. Sijbesma, E. W. Meijer, *Chem. Commun.* **2003**, 5–16.

[4] a) F. R. Kersey, G. Lee, P. Marszalek, S. L. Craig, *J. Am. Chem. Soc.* **2004**, *126*, 3038–3039; b) F. Hof, S. L. Craig, C. Nuckolls, J. Rebek, *Angew. Chem.* **2002**, *114*, 1556–1578; *Angew. Chem. Int. Ed.* **2002**, *41*, 1488–1508; c) F. Hof, J. Rebek, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4775–4777; d) J. D. Hartgerink, E. Beniash, S. I. Stupp, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5133–5138; e) E. R. Zubarev, M. U. Pralle, L. M. Li, S. I. Stupp, *Science* **1999**, *283*, 523–526; f) T. L. Breen, J. Tien, S. R. J. Oliver, T. Hadzic, G. M. Whitesides, *Science* **1999**, *284*, 948–951; g) M. C. T. Fyfe, J. F. Stoddart, *Acc. Chem. Res.* **1997**, *30*, 393–401; h) K. Yamauchi, J. R. Lizotte, D. M. Hercules, M. J. Vergne, T. E. Long, *J. Am. Chem. Soc.* **2002**, *124*, 8599–8604; i) E. A. Archer, N. T. Goldberg, V. Lynch, M. J. Krische, *J. Am. Chem. Soc.* **2000**, *122*, 5006–5007; j) K. M. Chen, X. P. Jiang, L. C. Kimerling, P. T. Hammond, *Langmuir* **2000**, *16*, 7825–7834; k) J. M. Pollino, M. Weck, *Org. Lett.* **2002**, *4*, 753–756.

[5] a) J. A. Zasadzinski, *Curr. Opin. Solid State Mater. Sci.* **1997**, *2*, 345–349; b) I. F. Uchegbu, S. P. Vyas, *Int. J. Pharm.* **1998**, *172*, 33–70; c) M. D. Brown, A. Schatzlein, A. Brownlie, V. Jack, W. Wang, L. Tetley, A. I. Gray, I. F. Uchegbu, *Bioconjugate Chem.* **2000**, *11*, 880–891; d) I. M. Hafez, P. R. Cullis, *Adv. Drug Delivery Rev.* **2001**, *47*, 139–148.

[6] a) P. Dalhaimer, H. Bermudez, D. E. Discher, *J. Polym. Sci. Part B* **2004**, *42*, 168–176; b) P. Walde, S. Ichikawa, *Biomol. Eng.* **2001**, *18*, 143–177; c) L. D. Mayer, J. Reamer, M. B. Bally, *J. Pharm. Sci.* **1999**, *88*, 96–102; d) S. A. Walker, M. T. Kennedy, J. A. Zasadzinski, *Nature* **1997**, *387*, 61–64; e) M. *J.* Gradzielski, *Eur. Phys J. B* **2003**, *15*, R655–R697.

[7] a) E. Harth, B. Van Horn, D. S. Germack, C. P. Gonzales, R. D. Miller, C. J. Hawker, *J. Am. Chem. Soc.* **2002**, *124*, 8653–8660; b) C. Nardin, J. Widmer, M. Winterhalter, W. Meier, *Eur. Phys. J. E* **2001**, *4*, 403–410; c) D. A. Rudkevich, *Chem. Bull.* **2002**, *75*, 393–413; d) A. Fischer, A. Franco, T. Oberholzer, *Chembiochem* **2002**, *3*, 409–417; e) K. Morigaki, T. Baumgart, U. Jonas, A. Offenhausser, W. Knoll, *Langmuir* **2002**, *18*, 4082–4089; f) M. Moffitt, A. Eisenberg, *Macromolecules* **1997**, *30*, 4363–4373.

[8] a) M. Lindgren, M. Hallbrink, A. Prochiantz, U. Langel, *Trends Pharmacol. Sci.* **2000**, *21*, 99–103; b) J. Gariepy, K. Kawamura, *Trends Biotechnol.* **2001**, *19*, 21–28; c) D. Singh, S. K. Bisland, K. Kawamura, J. Gariepy, *Bioconjugate Chem.* **1999**, *10*, 745–754; d) M. C. Morris, L. Chaloin, F. Heitz, G. Divita, *Curr. Opin. Biotechnol.* **2000**, *11*, 461–466.

[9] a) H. G. Dobereiner, *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 256–263; b) B. M. Discher, D. A. Hammer, F. S. Bates, D. E. Discher, *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 125–131.

[10] a) F. Checot, S. Lecommandoux, H. A. Klok, Y. Gnanou, *Eur. Phys. J. E* **2003**, *10*, 25–35; b) S. Kimura, D. H. Kim, J. Sugiyama, Y. Imanishi, *Langmuir* **1999**, *15*, 4461–4463; c) H. Kukula, H. Schlaad, M. Antonietti, S. Forster, *J. Am. Chem. Soc.* **2002**, *124*, 1658–1663.

[11] a) D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967–973; b) H. Bermudez, A. K. Brannan, D. A. Hammer, F. S. Bates, D. E. Discher, *Macromolecules* **2002**, *35*, 8203–8208; c) R. Dimova, U. Seifert, B. Pouligny, S. Forster, H. G. Dobereiner, *Eur. Phys. J. E* **2002**, *7*, 241–250; d) C. K. Haluska, W. T. Gozdz, H. G. Dobereiner, S. Forster, G. Gompper, *Phys. Rev. Lett.* **2002**, *89*, 238302; e) F. H. Meng, C. Hiemstra, G. H. M. Engbers, J. Feijen, *Macromolecules* **2003**, *36*, 3004–3006; f) F. Najafi, M. N. Sarbolouki, *Biomaterials* **2003**, *24*, 1175–1182; g) V. Pata, N. Dan, *Biophys. J.* **2003**, *85*, 2111–2118; h) A. A. Reinecke, H. G. Dobereiner, *Langmuir* **2003**, *19*, 605–608; i) P. J. Photos, L. Bacakova, B. Discher, F. S. Bates, D. E. Discher, *J. Controlled Release* **2003**, *90*, 323–334; j) J. K. Harris, G. D. Rose, M. L. Bruening, *Langmuir* **2002**, *18*, 5337–5342.

[12] 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.

[13] J. C. M. Lee, H. Bermudez, B. M. Discher, M. A. Sheehan, Y. Y. Won, F. S. Bates, D. E. Discher, *Biotechnol. Bioeng.* **2001**, *73*, 135–145.

[14] a) B. M. Discher, H. Bermudez, D. A. Hammer, D. E. Discher, Y. Y. Won, F. S. Bates, *J. Phys. Chem. B* **2002**, *106*, 2848–2854; b) H. Aranda-Espinoza, H. Bermudez, F. S. Bates, D. E. Discher, *Phys. Rev. Lett.* **2001**, *87*, 208301.

[15] a) K. Yu, C. Bartels, A. Eisenberg, *Macromolecules* **1998**, *31*, 9399–9402; b) A. V. Kabanov, T. K. Bronich, V. A. Kabanov, K. Yu, A. Eisenberg, *J. Am. Chem. Soc.* **1998**, *120*, 9941–9942; c) S. Burke, A. Eisenberg, *High Perform. Polym.* **2000**, *12*, 535–542; d) L. B. Luo, A. Eisenberg, *Angew. Chem.* **2002**, *114*, 1043–1046; *Angew. Chem. Int. Ed.* **2002**, *41*, 1001–1004; e) L. F. Zhang, A. Eisenberg, *Polym. Adv. Technol.* **1998**, *9*, 677–699; f) H. W. Shen, A. Eisenberg, *Angew. Chem.* **2000**, *112*, 3448–3450; *Angew. Chem. Int. Ed.* **2000**, *39*, 3310–3312.

[16] L. B. Luo, A. Eisenberg, *J. Am. Chem. Soc.* **2001**, *123*, 1012–1013.

[17] L. B. Luo, A. Eisenberg, *Langmuir* **2001**, *17*, 6804–6811.

[18] A. A. Choucair, A. H. Kycia, A. Eisenberg, *Langmuir* **2003**, *19*, 1001–1008.

[19] a) F. T. Liu, A. Eisenberg, *J. Am. Chem. Soc.* **2003**, *125*, 15059–15064; b) S. E. Burke, A. Eisenberg, *Polymer* **2001**, *42*, 9111–9120.

[20] a) F. Ilhan, T. H. Galow, M. Gray, G. Clavier, V. M. Rotello, *J. Am. Chem. Soc.* **2000**, *122*, 5895–5896; b) U. Drechsler, R. J. Thibault, V. M. Rotello, *Macromolecules* **2002**, *35*, 9621–9623.

[21] O. Uzun, A. Sanyal, H. Nakade, R. J. Thibault, V. M. Rotello, *J. Am. Chem. Soc.* **2004**, *126*, 14773–14777.

[22] C. Boettcher, B. Schade, J. H. Fuhrhop, *Langmuir* **2001**, *17*, 873–877.

[23] a) I. Weber, *Methods Enzymol.* **2003**, *361*, 34–47; b) C. Picart, K. Sengupta, J. Schilling, G. Maurstad, G. Ladam, A. R. Bausch, E. Sackmann, *J. Phys. Chem. B* **2004**, *108*, 7196–7205; c) F. Dubreuil, N. Elsner, A. Fery, *Eur. Phys. J. E* **2003**, *12*, 215–221; d) N. Fang, V. Chan, K. T. Wan, H. Q. Mao, K. W. Leong, *Colloids Surf. B* **2002**, *25*, 347–362.

[24] a) R. Parthasarathy, J. T. Groves, *Cell Biochem. Biophys.* **2004**, *41*, 391–414; b) H. Verschueren, *J. Cell Sci.* **1985**, *75*, 279–301; c) N. Fang, V. Chan, K. T. Wan, H. Q. Mao, K. W. Leong, *Colloids Surf. B* **2002**, *25*, 347–362; d) G. Wiegand, K. R. Neumaier, E. Sackmann, *Appl. Opt.* **1998**, *37*, 6892–6905.

[25] a) S. L. Li, A. F. Palmer, *Langmuir* **2004**, *20*, 7917–7925; b) I. Reviakine, A. Brisson, *Langmuir* **2000**, *16*, 1806–1815; c) H. Egawa, K. Furusawa, *Langmuir* **1999**, *15*, 1660–1666.

[26] a) L. Andruzzi, A. Hexemer, X. Li, C. K. Ober, E. J. Kramer, G. Galli, E. Chiellini, D. A. Fischer, *Langmuir* **2004**, *20*, 10498–10506; b) C. Chan, *Polymer Surface Modification and Characterization*; Hanser/Gardner, Cincinnati, **1994**, pp. 121–125; c) K. B. Walters, D. W. Schwark, D. E. Hirt, *Langmuir* **2003**, *19*, 5851–5860; d) S. Netcheva, P. Bertrand, *J. Polym. Sci. Part B* **2001**, *39*, 314–325.

[27] J. M. Solletti, M. Botreau, F. Sommer, W. L. Brunat, S. Kasas, T. M. Duc, M. R. Celio, *Langmuir* **1996**, *12*, 5379–5386.

Received: July 13, 2005
Published online: September 27, 2005

