Functionalized Nanopores

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Functionalization of Imprinted Nanopores in Nanometer-Thin Organic Materials**

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Recently, we described a method for creating nanometer-thin organic materials with nanopores of programmed size.[1] Control of pore geometry and mass transfer has been identified as key to advances in DNA-sequencing devices,[2] microreactors,[3] molecular electronics,[4] and drug-delivery devices.[5] Nanocapsules with selective permeability have gained considerable attention in biomedical applications.[6] Controlling the chemical environment of nanopores is critical for realizing the full potential of nanometer-thin porous materials.[7] Herein, we describe an efficient method for creating uniform nanopores with a programmed chemical environment and demonstrate the successful quantitative conversion of functional groups in the nanopores.

Using phospholipid bilayers as temporary self-assembled scaffolds, we directed the assembly of a nanometer-thin film of a cross-linked organic polymer, with embedded molecules of a pore-forming template (Figure 1). Previously, we used this method to create nanocapsules with nanopores of programmed size.[1] Modular construction of the template offers great versatility in varying the nanopore shape and size, as well as the nature and number of functional groups in the nanopores.

Although liposomes[8] made of dimyristoylphosphatidylcholine (DMPC) were used in this work to demonstrate the feasibility of our approach, we expect the method to be applicable to many other types of bilayers.[9] The pore-forming template **1** was synthesized in one step from commercially available materials. Coupling of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose with 4-vinylbenzoic acid was performed by a standard protocol and produced the desired product in 85% yield (Scheme 1).

At initial lipid (DMPC)/template (**1**) molar ratios of 34 or higher, virtually all the molecules of **1** are incorporated into the liposomes (Table 1, entries 1 and 2). Following the loading of *tert*-butylstyrene and divinylbenzene (1:1), and **1** into the DMPC liposomes and UV-initiated polymerization, methanol was added to precipitate the nanocapsules and to remove the lipids; the nanocapsules were washed with methanol, resuspended in benzene, and freeze-dried. The FTIR spectrum of

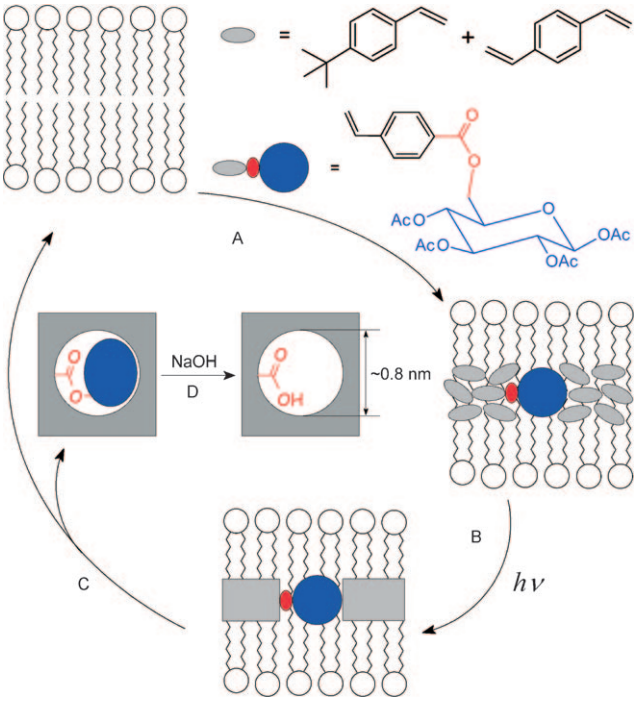
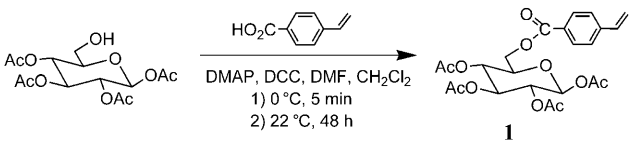


Figure 1. Directed assembly of a nanometer-thin polymer film with uniform functionalized nanopores. A) A self-assembled phospholipid bilayer is loaded with hydrophobic monomers (gray) and a pore-forming template (gray–red–blue). The template consists of three parts: a polymerizable moiety (gray) to covalently anchor the template to the polymer matrix, a degradable linker (red) to create a functionalized nanopore, and a bulky hydrophobic unit (blue) to define the pore size. B) Polymerization produces a nanometer-thin film with copolymerized template molecules in the bilayer interior. C) The phospholipids are removed with the help of a detergent or by solvent exchange. D) The bulky hydrophobic groups of the pore-forming template are removed by chemical degradation to yield nanometer-thin films with nanopores containing a single functional group.



Scheme 1. Synthesis of a polymerizable and degradable pore-forming template (**1**).

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Table 1: Incorporation of **1** into liposomes and nanocapsules.

Entry	Initial DMPC/**1**[a]	Incorporation of **1** into liposomes[b] [%]	Incorporation of **1** into nanocapsules[c] [%]
1	41	99 ± 2	98 ± 3
2	34	98 ± 2	96 ± 5
3	28	94 ± 4	84 ± 7
4	21	77 ± 5	67 ± 4
5	14	59 ± 2	56 ± 4
6	7	41 ± 4	36 ± 2

[a] Initial DMPC/**1** molar ratio. [b] Percentage of the initial amount of **1** found in the liposomes, measured by HPLC. [c] Percentage of the initial amount of **1** found in the nanocapsules, measured by FTIR.

the nanocapsules revealed a characteristic peak at 1759 cm^{-1} corresponding to the C=O stretching of the ester groups (Figure 2). Nearly all the molecules of **1** incorporated into the liposomes were found in the nanocapsules after the removal

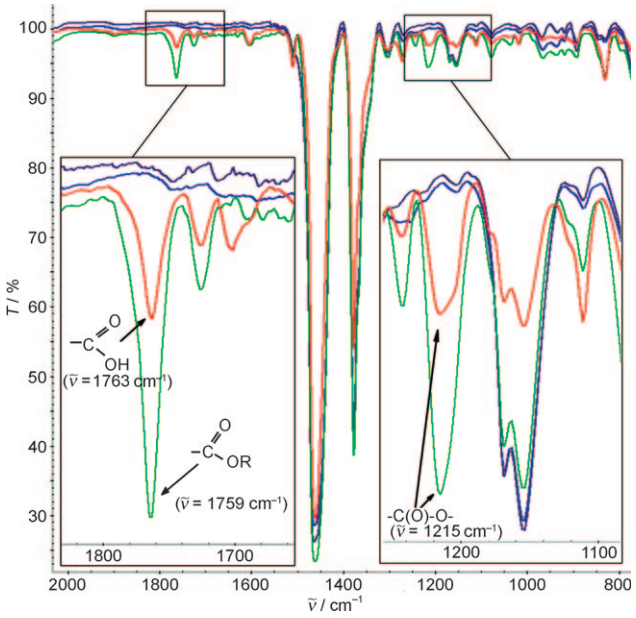

Figure 2. FTIR spectra of nanocapsules without a pore-forming template (blue); of nanocapsules with GPA, after hydrolysis (purple); and of nanocapsules with **1**, before (green) and after (red) hydrolysis.

of the lipids and the multiple methanol washings (Table 1). FTIR spectra of the nanocapsules revealed a shift of the band corresponding to the carbonyl group of **1** to higher wavenumbers (1759 cm^{-1} for **1** in the nanocapsules versus 1748 cm^{-1} for free **1**; see the Supporting Information), which is common for functional groups incorporated into a bulk polymer.[10] These data agree with the embedding of **1** into a nanometer-thin polymer film, as shown in Figure 1.

Alkaline hydrolysis of the template **1** produced nanopores with free carboxy groups (Figure 2). In control experiments, the FTIR spectra of nanocapsules made without pore-forming templates and of nanocapsules made with glucose pentaacetate (GPA; a structural analog of **1** without a polymerizable moiety) showed no signals corresponding to carbonyl groups after alkaline hydrolysis (Figure 2). Quantitative FTIR spectroscopic measurements, using the aromatic C–H stretching signal as an internal standard, indicated complete conversion of the ester groups of GPA into free carboxylic acids (see the Supporting Information). We conclude that all the molecule of **1** incorporated into the liposomes copolymerized with the *tert*-butylstyrene/divinylbenzene polymer matrix.

We converted the carboxy groups into acyl chloride groups by treatment with excess thionyl chloride and then formed two types of amides by reaction of the acid chloride with either benzylamine or 4-(aminomethyl)benzonitrile (Figure 3). We selected the latter to quantify the number of

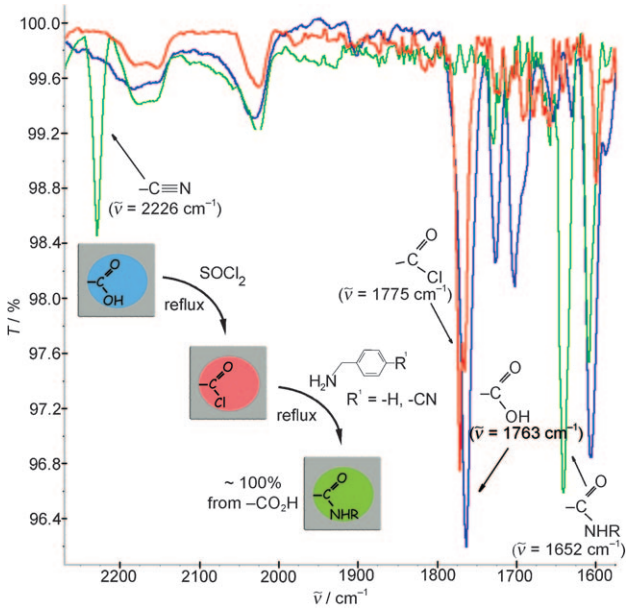

Figure 3. FTIR spectra of nanocapsules with carboxy groups (blue); of nanocapsules with acyl chloride groups, after reflux with thionyl chloride (red); and of nanocapsules with amide groups, after subsequent treatment with 4-(aminomethyl)-benzonitrile (green).

amide groups formed, by using the C≡N stretching band at 2226 cm^{-1} in the FTIR spectrum of the amide. The FTIR spectrum of the acid chloride shows a shift of the C=O stretching band to 1774 cm^{-1} from 1763 cm^{-1} for the carboxylic acid (Figure 3), as well as the absence of a band at 1215 cm^{-1} corresponding to C(O)–O stretching (see the Supporting Information). The FTIR spectrum of the amide shows a C=O stretching band at a much lower frequency (1650 cm^{-1}) than that of the carboxylic acid. On the basis of the intensity of the C≡N stretching band, we found that the carboxy groups were completely converted to amide groups. If a DMPC/**1** molar ratio of 34 is used, considering that the area of each DMPC molecule is 62 $Å^2$,[11] the resulting nanoporous material has an estimated pore density of 9.5×10^{16} pores m^{-2}, with an average distance between pore centers of 3.2 nm and with 3×10^3 pores per 100-nm nanocapsule.

Electron microscopy images demonstrated the preservation of the shape and integrity of the nanocapsules (Figure 4). Transmission electron microscopy (TEM) images show that the size and shape of liposomes loaded with **1** and the

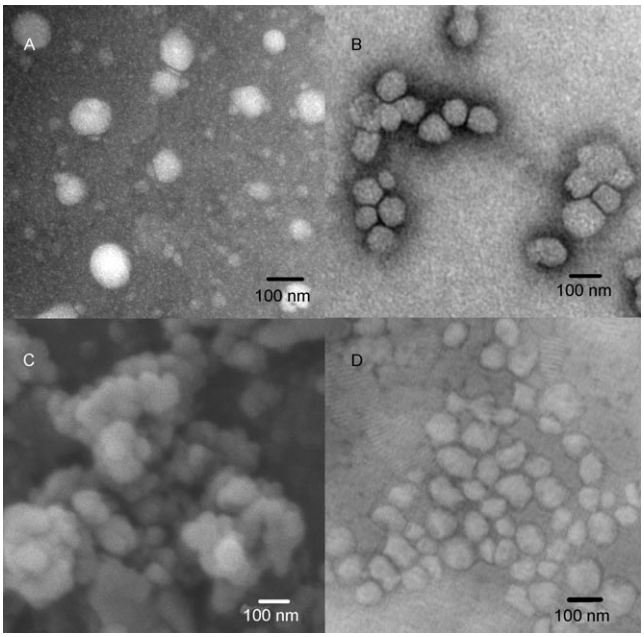

Figure 4. Electron microscopy images of nanocapsules. TEM images of A) liposomes loaded with **1** and the monomers; and of B) liposomes containing polymer nanocapsules, after polymerization. C) SEM image of polymer nanocapsules, after lipid removal, hydrolysis, precipitation, multiple washings, resuspension, and freeze-drying. D) TEM image of polymer nanocapsules, after lipid removal.

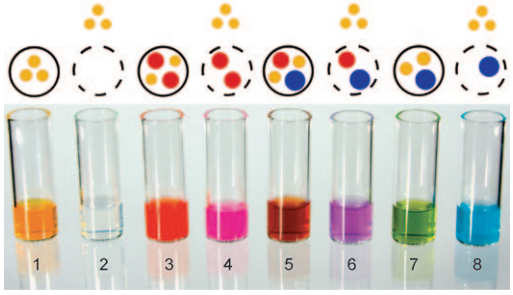

Figure 5. Colored size-probe retention assay. Nanocapsules with encapsulated colored size-probe mixtures were prepared and separated on a size-exclusion column to remove released probes. Photograph of the nanocapsule fractions: with encapsulated 0.6-nm (yellow) probes, 1) before template removal, and 2) after template removal, demonstrating complete release of the 0.6-nm probes; with encapsulated 0.6-nm and 1.1-nm (red) probes, 3) before template removal, and 4) after template removal, demonstrating complete release of the 0.6-nm probes and retention of the 1.1-nm probes; with encapsulated 0.6-nm, 1.1-nm, and 1.6-nm (blue) probes, 5) before template removal, and 6) after template removal, demonstrating release of the 0.6-nm probes and retention of the 1.1-nm and 1.6-nm probes; with encapsulated 0.6-nm and 1.6-nm probes, 7) before template removal, and 8) after template removal, demonstrating release of the 0.6-nm probes and retention of the 1.6-nm probes.

monomers (Figure 4A) are similar to those of liposomes containing polymer nanocapsules (Figure 4B). In agreement with previous reports,[18g] the detergent-assisted lipid removal did not affect the size distribution of the nanocapsules (Figure 4D). Remarkably, a scanning electron microscopy (SEM) image shows clusters of nanocapsules with the same size after lipid removal, hydrolysis, precipitation in methanol, multiple washings, resuspension in benzene, and freeze-drying (Figure 4C). These results suggest that the nanocapsules with nanometer-thin walls are stable under regular handling conditions, such as solvent exchange.

We used the previously described colored size-probe retention assay to demonstrate the successful formation of nanopores with a narrow size distribution.[1] We encapsulated mixtures of molecules with different colors and sizes in liposomes, carried out the polymerization, and separated the nanocapsules from released probes on a size-exclusion column. We used a yellow 0.6-nm probe (methyl orange), a red 1.1-nm probe (Procion Red), and a blue 1.6-nm probe (1:1 β-cyclodextrin–Reactive Blue conjugate) to gauge the pore size.[1] All probes were retained in the nanocapsules prior to template removal. After opening the nanopores, the 0.6-nm probes were completely released, and the 1.1-nm and 1.6-nm probes were retained (Figure 5), suggesting a pore size of (0.8 ± 0.2) nm. Considering that size-probe release from 100-nm nanocapsules would occur faster than chromatographic separation, quantitative retention of the 1.1-nm and 1.6-nm probes allows us to conclude that very few nanocapsules, if any, contain pinholes or pores larger than 1.1 nm. The pore size of the nanocapsules is preserved even after they are freeze-dried and resuspended in water. When porous nanocapsules containing 1.1-nm probes were dried, solubilized in 2% Triton X-100 solution, and passed through a size-exclusion column, no release of the encapsulated probes was observed. Combined with SEM images (Figure 4), this information provides strong evidence that the materials preserve both their structure and function upon solvent exchange and drying.

In summary, we have demonstrated an efficient method for controlling the chemical environment of molecular-size pores in nanometer-thin organic materials. This method combines the use of temporary self-assembled scaffolds with molecular imprinting, which has been widely used to fabricate functional materials.[12] We created uniformly sized nanopores with a single carboxy functional group, and quantitatively converted the carboxy group into an acyl chloride group and subsequently into an amide group. These transformations open opportunities for further functionalization for controlling mass transfer across the nanopore, for example, with stimuli-responsive moieties, or for creating arrays of functional groups that may potentially act as molecular-recognition sites.

Experimental Section

1: 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (522 mg, 1.5 mmol), 4-dimethylaminopyridine (DMAP; 37 mg, 0.3 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (DCC; 310 mg, 1.5 mmol) were added to a stirred solution of 4-vinylbenzoic acid (222 mg, 1.5 mmol) in an anhydrous CH_2Cl_2 (5 mL)/*N*,*N*-dimethylformamide (DMF; 4 mL) mixture at 0 °C. The reaction mixture was stirred for 5 min at 0 °C and then for 48 h at ambient temperature. The reaction was monitored by thin-layer chromatography (TLC; 1:1 hexane/ethyl acetate). The precipitated urea was filtered off, and the filtrate was evaporated under vacuum. The residue was taken up in CH_2Cl_2, and the solution

was washed with 3% aqueous $NaHCO_3$ solution and dried over molecular sieves. The solvent was evaporated to yield the product as a clear oil, which crystallized within 15 min. Yield 85%; mp 121–122°C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 7.6$–7.4 (m, 2H), 8.1–7.9 (m, 2H), 6.9–6.6 (m, 2H), 6.00–5.80 (m, 2H), 5.80–5.70 (m, 2H), 5.35–5.10 (m, 2H), 4.60–4.45 (m, 2H), 4.40–4.20 (m, 2H), 4.15–3.80 (m, H), 1.96 (s, 3H), 2.01 (s, 3H), 2.05 (s, 3H), 2.10 ppm (s, 3H); ^{13}C NMR (270 MHz, $CDCl_3$): $\delta = 20.6$, 20.8, 20.9, 21.1 (CH_3), 62.2 (d, $J_{C,C} = 44.2$ Hz, 1C, C6), 68.2, 69.3, 72.8, 73.0 (C2, C3, C4, C5), 91.8 (d, $J_{C,C} = 44.2$ Hz, 1C, C1), 116.7, 136.1 (C=C), 128.3, 130.2, 133.7, 142.3 (Ph), 166.8, 169.0, 169.5, 169.6, 170.2 ppm (C=O). Elemental analysis (%) calcd for $C_{23}H_{26}O_{11}$: C 57.74, H 5.48; found: C 57.64, H 5.61.

Nanocapsules with functionalized nanopores: *tert*-Butylstyrene (17.64 μL, 9.63×10^{-5} mol), *p*-divinylbenzene (13.70 μL, 9.62×10^{-5} mol), and 2,2-dimethoxy-2-phenylacetophenone (UV initiator; 3 mg, 0.117×10^{-5} mol) were added to a solution of DMPC (5.9×10^{-5} mol, 20 mg mL^{-1} in $CHCl_3$) and **1** (0.87×10^{-5} mol, 2.08 mg mL^{-1} in $CHCl_3$). The monomers were purified on a column of neutral alumina prior to addition. The $CHCl_3$ was evaporated using a stream of purified argon to form a lipid–monomer film on the wall of a culture tube. The film was further dried under vacuum for 30 min to remove traces of $CHCl_3$. GC and UV/Vis analyses confirmed that the concentration of monomers after drying remained the same. The dried film was hydrated with deionized water to give a dispersion of multilamellar vesicles, which was then extruded at 32°C through a polycarbonate Nucleopore track-etch membrane (Whatman) with 0.1-μm pore size using a Lipex stainless steel extruder (Northern Lipids). Prior to polymerization, oxygen was removed by passing purified nitrogen or argon through the solution. The sample was irradiated ($\lambda = 254$ nm) in a photochemical reactor equipped with a stirrer (10 lamps of 32 W each; 10-cm distance between the lamps and the sample) for 60 min. UV and GC analyses confirmed that greater than 90% of the monomers were polymerized. Triton X-100 (0.5 mL, 2%) and NaOH (0.5 mL, 1M) were added, and the mixture was stirred for 1 h at ambient temperature. Methanol (10 mL) was added, and the precipitate was washed 3–5 times with methanol, resuspended in benzene, and freeze-dried. The nanocapsules with carboxy groups (10 mg) were suspended in toluene (3 mL), mixed with thionyl chloride (5 mL), and refluxed for 12 h. The mixture was evaporated to dryness and washed with toluene to yield nanocapsules with acyl chloride groups. These nanocapsules were suspended in toluene (3 mL), then triethylamine and either 4-(aminomethyl)benzonitrile (0.2 g) or benzylamine (3 mL) were added, and the reaction mixture was heated under reflux overnight to produce nanocapsules with amide groups.

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[1] D. C. Danila, L. T. Banner, E. J. Karimova, L. Tsurkan, X. Wang, E. Pinkhassik, *Angew. Chem.* **2008**, *120*, 7144–7147; *Angew. Chem. Int. Ed.* **2008**, *47*, 7036–7039.

[2] a) H. Bayley, C. R. Martin, *Chem. Rev.* **2000**, *100*, 2575–2594; b) J. J. Kasianowicz, E. Brandin, D. Branton, D. W. Deamer, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13770–13773; c) P. Chen, J. Gu, E. Brandin, Y. R. Kim, Q. Wang, D. Branton, *Nano Lett.* **2004**, *4*, 2293–2298.

[3] a) D. M. Vriezema, M. C. Aragones, J. A. A. W. Elemans, J. J. L. M. Cornelissen, A. E. Rowan, R. J. M. Nolte, *Chem. Rev.* **2005**, *105*, 1445–1489; b) C. Nardin, S. Thoeni, J. Widmer, M. Winterhalter, W. Meier, *Chem. Commun.* **2000**, 1433–1434.

[4] a) Z. Lin, D. H. Kim, X. Wu, L. Boosahda, D. Stone, L. LaRose, T. P. Russell, *Adv. Mater.* **2002**, *14*, 1373–1376; b) U. Jeong, D. Y. Ryu, J. K. Kim, D. H. Kim, X. Wu, T. P. Russell, *Macromolecules* **2003**, *36*, 10126–10129.

[5] a) Y. Wei, K.-Y. Qiu in *Nanoporous Materials: Science and Engineering (Series on Chemical Engineering.* (Eds., G. Q. Lu, X. S. Zhao), Imperial College, London, **2004**, pp. 873–892; b) T. A. Desai, D. J. Hansford, L. Kulinsky, A. H. Nashat, G. Rasi, J. Tu, Y. Wang, M. Zhang, M. Ferrari, *Biomed. Microdevices* **1999**, *2*, 11–40.

[6] a) G. B. Sukhorukov, A. L. Rogach, M. Garstka, S. Springer, W. J. Parak, A. Muñoz-Javier, O. Kreft, A. G. Skirtach, A. S. Susha, Y. Ramaye, R. Palankar, M. Winterhalter, *Small* **2007**, *3*, 944–955; b) K. Kostarelos, A. D. Miller, *Chem. Soc. Rev.* **2005**, *34*, 970–994.

[7] a) Z. Wang, S. Cohen, *Angew. Chem.* **2008**, *120*, 4777–4780; *Angew. Chem. Int. Ed.* **2008**, *47*, 4699–4702; b) E. N. Savariar, K. Krishnamoorthy, S. Thayumanavan, *Nat. Nanotechnol.* **2008**, *3*, 112–117; c) V. Gorteau, G. Bollot, J. Mareda, D. Pasini, D.-H. Tran, A. Lazar, A. W. Coleman, N. Sakai, S. Matile, *Bioorg. Med. Chem.* **2005**, *13*, 5171–5180; d) R. J. Brea, L. Castedo, J. R. Granja, *Chem. Commun.* **2007**, 3267–3269; e) S. L. Cockroft, J. Chu, M. Amorin, M. R. Ghadiri, *J. Am. Chem. Soc.* **2008**, *130*, 818–820.

[8] a) N. Poulain, E. Nakache, A. Pina, G. J. Levesque, *J. Polym. Sci.* **1996**, *34*, 729–737; b) J. Hotz, W. Meier, *Langmuir* **1998**, *14*, 1031–1036; c) C. Nardin, T. Hirt, J. Leukel, W. Meier, *Langmuir* **2000**, *16*, 1035–1041; d) J. Kurja, R. J. M. Noelte, I. A. Maxwell, A. I. German, *Polymer* **1993**, *34*, 2045–2049; e) C. A. McKelvey, E. W. Kaler, J. A. Zasadzinski, B. Coldren, H. T. Jung, *Langmuir* **2000**, *16*, 8285–8290; f) M. Jung, D. H. W. Huber, P. H. H. Bomans, P. M. Frederic, J. Meuldijk, A. M. van Herk, H. Fischer, A. I. German, *Langmuir* **1997**, *13*, 6877–6880; g) J. F. P. da Silva Gomes, A. F.-P. Sonnen, A. Kronenberger, J. Fritz, M. A. N. Coelho, D. Fournier, C. Fournier-Noël, M. Mauzac, M. Winterhalter, *Langmuir* **2006**, *22*, 7755–7759.

[9] a) J. C. Stendahl, M. S. Rao, M. O. Guler, S. I. Stupp, *Adv. Funct. Mater.* **2006**, *16*, 499–508; b) M. J. Boerakker, N. E. Botterhuis, P. H. H. Bomans, P. M. Frederik, E. M. Meijer, R. J. M. Nolte, N. A. J. M. Sonunerdijk, *Chem. Eur. J.* **2006**, *12*, 6071–6080; c) *Liposomes: A Practical Approach* (Eds: V. Torchilin, V. Weissig) Oxford University Press, Oxford, **2003**; d) E. T. Castellana, P. S. Cremer, *Surf. Sci. Rep.* **2006**, *61*, 429–444; e) D. Berti, *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 74–78; f) E. Sackmann, *Science* **1996**, *271*, 43–48.

[10] a) J.-R. Sarasua, N. L. Rogriguez, A. L. Arrazia, E. Meaurio, *Macromolecules* **2005**, *38*, 8362–8371; b) Y.-K. Han, E. M. Pierce, T. K. Kwei, *Macromolecules* **2000**, *33*, 1321–1329; c) J. He, J. Liu, *Polymer* **1999**, *40*, 959–969.

[11] G. Cevc, D. Marsh, *Phospholipid Bilayers*, Wiley-Interscience, New York, **1987**.

[12] a) W. Li, S. Li, *Adv. Polym. Sci.* **2007**, *206*, 191–210; b) J. D. Matry, M. Mauzac, *Adv. Polym. Sci.* **2005**, *172*, 1–35; K. Haupt, K. Mosbach, *Chem. Rev.* **2000**, *100*, 2495–2504.

