[5] Hydrogen-bonded ferrocene-containing thermotropic liquid crystals: R. Deschenaux, F. Monnet, E. Serrano, F. Turpin, A.-M. Levelut, *Helv. Chim. Acta* **1998**, *81*, 2072.
[6] Reviews on low molar-mass ferrocene-containing thermotropic liquid crystals: a) R. Deschenaux, J.W. Goodby, in 'Ferrocenes: Homogeneous Catalysis, Organic Synthesis, Materials Science', Eds. A Togni, T. Hayashi, VCH, Weinheim, **1995**, Ch. 9, p. 471–495; b) T. Chuard, R. Deschenaux, *Chimia* **1998**, *52*, 547.
[7] T. Chuard, R. Deschenaux, *Helv. Chim. Acta* **1996**, *79*, 736; b) R. Deschenaux, M. Even, D. Guillon, *Chem. Commun.* **1998**, 537; c) B. Dardel, R. Deschenaux, M. Even, E. Serrano, *Macromolecules* **1999**, *32*, 5193; d) T. Chuard, R. Deschenaux, A. Hirsch, H. Schönberger, *Chem. Commun.* **1999**, 2103.
[8] T. Chuard, S.J. Cowling, M. Fernandez-Ciurleo, I. Jauslin, J.W. Goodby, R. Deschenaux, *Chem. Commun.* **2000**, 2109.
[9] a) R. Deschenaux, M. Schweissguth, A.-M. Levelut, *Chem. Commun.* **1996**, 1275; b) R. Deschenaux, M. Schweissguth, M.T. Vilches, A.-M. Levelut, D. Hautot, G.L. Long, D. Luneau, *Organometallics* **1999**, *18*, 5553.
[10] D.A. Tomalia, *Adv. Mater.* **1994**, *6*, 529.
[11] J.F.G.A. Jansen, E.M.M. de Brabander-van den Berg, E.W. Meijer, *Science* **1994**, *266*, 1226.
[12] a) J.W.J. Knapen, A.W. van der Made, J.C. de Wilde, P.W.N.M. van Leeuwen, P. Wijkens, D.M. Grove, G. van Koten, *Nature* **1994**, *372*, 659; b) C.C. Mak, H.-F. Chow, *Macromolecules* **1997**, *30*, 1228.
[13] A.W. Freeman, L.A.J. Chrisstoffels, J.M.J. Fréchet, *J. Org. Chem.* **2000**, *65*, 7612.
[14] E. Serrano, R. Deschenaux, unpublished results.
[15] The measurements were carried out under argon in CH_2Cl_2 + 0.1 M tetrabutylammonium perchlorate (TBAP); working electrode: platinum rotating disk electrode; reference electrode: silver wire; auxiliary electrode: platinum wire; internal reference: ferrocene.

Chimia 55 (2001) 142–146
© Neue Schweizerische Chemische Gesellschaft
ISSN 0009–4293

Polymerizable Amphiphilic Block Copolymers: From Nanostructured Hydrogels to Nanoreactors and Ultrathin Films

Corinne Nardin and Wolfgang Meier*

Abstract: Self-assembly of reactive amphiphilic block copolymers is used to prepare nanostructured hydrogels with exceptional permeability properties, vesicular structures and planar, freestanding membranes in aqueous solution. Although the underlying block copolymer membranes are two to three times thicker than conventional lipid bilayers they can be regarded as a mimetic of biological membranes and can be used as a matrix for membrane spanning proteins. Surprisingly the proteins remain functional despite the extreme thickness of the membranes and even after polymerization of the reactive block copolymers. The unique combination of block copolymers with membrane proteins allows the preparation of mechanically stable, defect-free membranes and nanocapsules that have highly selective permeability and/or specific recognition sites. This is documented by some representative examples.

Keywords: Amphiphilic block copolymer · Hydrogel · Membrane · Membrane protein

*Correspondence: PD Dr. W. Meier
University of Basel
Institut für Physikalische Chemie
Klingelbergstrasse 80
CH–4056 Basel
Tel.: +41 61 267 38 35
Fax: +41 61 267 38 55
E-Mail: wolfgang.meier@unibas.ch

1. Nanostructured Hydrogels

A current topic in material sciences is the creation of nanometer-sized well-defined structures. One key step is to develop preparative procedures to control precisely the formation and the morphology of these structures. Self-assembled superstructures of surfactants and, especially those of amphiphilic block copolymers have proven to be valuable tools. Actually block copolymers consisting of hydrophilic and hydrophobic blocks behave in water like conventional surfactants: similar to the latter, they self-assemble in water into micelles of various shapes and at higher concentrations into lyotropic liquid crystalline phases. Their aggrega-

[5] Hydrogen-bonded ferrocene-containing thermotropic liquid crystals: R. Deschenaux, F. Monnet, E. Serrano, F. Turpin, A.-M. Levelut, *Helv. Chim. Acta* **1998**, *81*, 2072.
[6] Reviews on low molar-mass ferrocene-containing thermotropic liquid crystals: a) R. Deschenaux, J.W. Goodby, in 'Ferrocenes: Homogeneous Catalysis, Organic Synthesis, Materials Science', Eds. A Togni, T. Hayashi, VCH, Weinheim, **1995**, Ch. 9, p. 471–495; b) T. Chuard, R. Deschenaux, *Chimia* **1998**, *52*, 547.
[7] T. Chuard, R. Deschenaux, *Helv. Chim. Acta* **1996**, *79*, 736; b) R. Deschenaux, M. Even, D. Guillon, *Chem. Commun.* **1998**, 537; c) B. Dardel, R. Deschenaux, M. Even, E. Serrano, *Macromolecules* **1999**, *32*, 5193; d) T. Chuard, R. Deschenaux, A. Hirsch, H. Schönberger, *Chem. Commun.* **1999**, 2103.
[8] T. Chuard, S.J. Cowling, M. Fernandez-Ciurleo, I. Jauslin, J.W. Goodby, R. Deschenaux, *Chem. Commun.* **2000**, 2109.
[9] a) R. Deschenaux, M. Schweissguth, A.-M. Levelut, *Chem. Commun.* **1996**, 1275; b) R. Deschenaux, M. Schweissguth, M.T. Vilches, A.-M. Levelut, D. Hautot, G.L. Long, D. Luneau, *Organometallics* **1999**, *18*, 5553.
[10] D.A. Tomalia, *Adv. Mater.* **1994**, *6*, 529.
[11] J.F.G.A. Jansen, E.M.M. de Brabander-van den Berg, E.W. Meijer, *Science* **1994**, *266*, 1226.
[12] a) J.W.J. Knapen, A.W. van der Made, J.C. de Wilde, P.W.N.M. van Leeuwen, P. Wijkens, D.M. Grove, G. van Koten, *Nature* **1994**, *372*, 659; b) C.C. Mak, H.-F. Chow, *Macromolecules* **1997**, *30*, 1228.
[13] A.W. Freeman, L.A.J. Chrisstoffels, J.M.J. Fréchet, *J. Org. Chem.* **2000**, *65*, 7612.
[14] E. Serrano, R. Deschenaux, unpublished results.
[15] The measurements were carried out under argon in CH_2Cl_2 + 0.1 M tetrabutylammonium perchlorate (TBAP); working electrode: platinum rotating disk electrode; reference electrode: silver wire; auxiliary electrode: platinum wire; internal reference: ferrocene.

Chimia 55 (2001) 142–146
© Neue Schweizerische Chemische Gesellschaft
ISSN 0009–4293

Polymerizable Amphiphilic Block Copolymers: From Nanostructured Hydrogels to Nanoreactors and Ultrathin Films

Corinne Nardin and Wolfgang Meier*

Abstract: Self-assembly of reactive amphiphilic block copolymers is used to prepare nanostructured hydrogels with exceptional permeability properties, vesicular structures and planar, freestanding membranes in aqueous solution. Although the underlying block copolymer membranes are two to three times thicker than conventional lipid bilayers they can be regarded as a mimetic of biological membranes and can be used as a matrix for membrane spanning proteins. Surprisingly the proteins remain functional despite the extreme thickness of the membranes and even after polymerization of the reactive block copolymers. The unique combination of block copolymers with membrane proteins allows the preparation of mechanically stable, defect-free membranes and nanocapsules that have highly selective permeability and/or specific recognition sites. This is documented by some representative examples.

Keywords: Amphiphilic block copolymer · Hydrogel · Membrane · Membrane protein

*Correspondence: PD Dr. W. Meier
University of Basel
Institut für Physikalische Chemie
Klingelbergstrasse 80
CH–4056 Basel
Tel.: +41 61 267 38 35
Fax: +41 61 267 38 55
E-Mail: wolfgang.meier@unibas.ch

1. Nanostructured Hydrogels

A current topic in material sciences is the creation of nanometer-sized well-defined structures. One key step is to develop preparative procedures to control precisely the formation and the morphology of these structures. Self-assembled superstructures of surfactants and, especially those of amphiphilic block copolymers have proven to be valuable tools. Actually block copolymers consisting of hydrophilic and hydrophobic blocks behave in water like conventional surfactants: similar to the latter, they self-assemble in water into micelles of various shapes and at higher concentrations into lyotropic liquid crystalline phases. Their aggrega-

tion is controlled by hydrophobic interactions and their lyotropic phase behavior by packing constraints of hard sphere objects. However, it is the macromolecular nature of the block copolymers that makes up their special features. Their phase behavior in water is controlled by their chemical constitution (*e.g.* nature and sequence of the repeat units), the length and structure of the different blocks and the molecular architecture of the whole polymer (*e.g.* block, graft, star, multiblock copolymers). Interestingly also the molecular weight distribution of the individual blocks has significant influence on the phase behavior of such systems [1]. In this context we introduced recently a new type of an amphiphilic ABA triblock copolymer [2]. The polymer consists of a flexible, hydrophobic poly (dimethylsiloxane) (PDMS) middle block and two water-soluble poly (2-methyloxazoline) (PMOXA) side blocks. Additionally, the ends of this PMOXA-PDMS-PMOXA triblock copolymer carry methacrylate groups, which allow a crosslinking polymerization, *i.e.* to 'freeze in' the self-assembled superstructure of the system. We could show that with increasing polydispersity of these polymers bicontinuous cubic phases with a complex structure of mutually interwoven hydrophobic and water-swollen hydrophilic channels are stabilized at the cost of the more classical spherical, hexagonal or lamellar mesophases which possess interfaces of nearly constant curvature (see Fig. 1). This phenomenon can be explained by the fact that the polymers of higher polydispersity can better accommodate to the associated curvature variations in the topologically complex superstructures of the bicontinuous phase. In this case the polymer molecules can simply distribute themselves over the regions of variable curvature according to their respective chain length. It has to be emphasized that generally a subsequent polymerization of the reactive end groups of the polymers did not lead to any measurable changes in the structure or the phase behavior of these systems. This is due to rather low dynamics of the block copolymer molecules with respect to phase transition or separation in comparison to the rate of polymerization. As a result the covalently crosslinked nanostructured hydrogels have at the same time high permeability for hydrophilic and hydrophobic substances which could be interesting for applications as membranes for separation processes or as biomaterials [3].

2. Block Copolymer Vesicles and Ultrathin Films

For a given composition of the PMOXA-PDMS-PMOXA triblock copolymer ($M_{n,\ PMOXA}$ = 1800 gmol^{-1}, $M_{n,\ PDMS}$ = 5400 gmol^{-1}; M_w/M_n = 1.7) the phase behavior in water is similar to that of typical bilayer-forming lipids like lecithin [4][5]. Over the whole composition range the basic morphological unit of this PMOXA-PDMS-PMOXA triblock copolymer are lamellae with a hydrophobic PDMS core and a hydrated PMOXA corona. Similar to lecithin it may form vesicular structures in dilute aqueous solution, which consist of spherically closed triblock copolymer membranes [2][4]. Depending on the applied preparation method, their size can be controlled in the range from about 50 nm up to 50 μm. Similar to conventional lipid vesicles, small unilamellar triblock copolymer vesicles can be prepared, for example, by a combined injection and extrusion method [2]. Giant vesicles can be obtained by electroformation [6] (Fig. 2). In this case a thin film of polymer deposited on adjacent electrodes or conductive glasses is phoresed by alternating current into the aqueous solution.

The formation of vesicular aggregates from the triblock copolymer is generally a result of non-covalent interactions and, hence, is reversible (even though block copolymer aggregates may be significantly more stable than those formed from low molecular weight amphiphiles). This is, for example, directly reflected in the occurrence of a critical aggregation concentration (cac) below which the vesicles begin to disintegrate and dissolve as individual triblock copolymer molecules [2]. After a crosslinking polymerization of the methacrylate end groups of the underlying polymers, the particles are additionally held together by a covalently crosslinked polymer network structure. As a consequence, the cac vanishes upon polymerization and the resulting nano- and microcapsules possess solid-state properties such as shape persistence. Therefore they are able to preserve their hollow sphere morphology even after their isolation from the aqueous solution [2]. In contrast to their fluid-like, non-polymerized precursors a shear deformation of the polymerized vesicles may lead to cracks characteristic for solid materials. This is directly demonstrated in the reflection intensity contrast micrographs of Fig. 2 that show a giant polymerized triblock copolymer vesicle before and after shear-induced rupture.

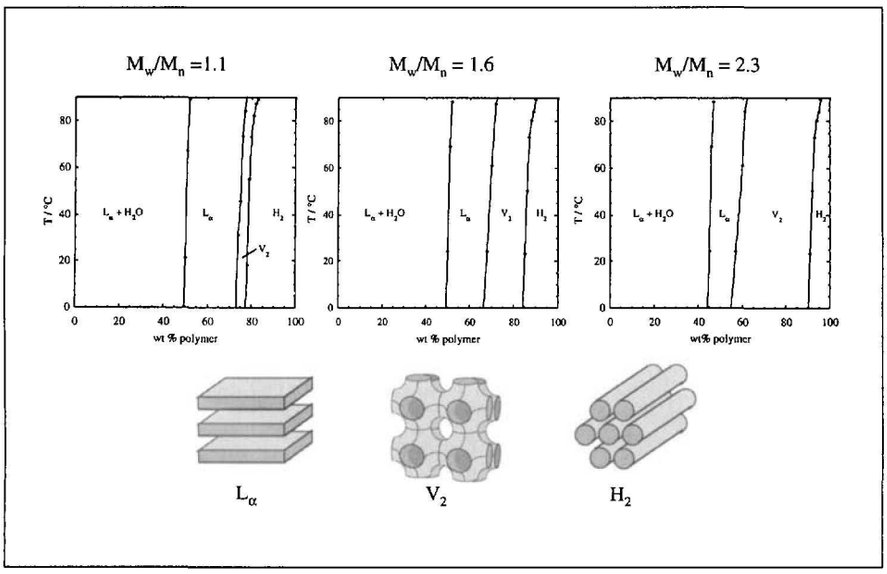

Fig. 1. Phase diagrams of poly (2-methyloxazoline)-block-poly (dimethylsiloxane)-block-poly (2-methyloxazoline), PMOXA-PDMS-PMOXA triblock copolymers in water: influence of the polydispersity of the hydrophilic blocks ($M_{n,\ PDMS}$=5400 g mol^{-1}, $M_{n,\ PMOXA}$ =2100 g mol^{-1}). Below: schematic representation of the structure of the lamellar phase (L_α), the bicontinuous cubic phase (V_2) and the inverse hexagonal phase (H_2) formed by the polymer.

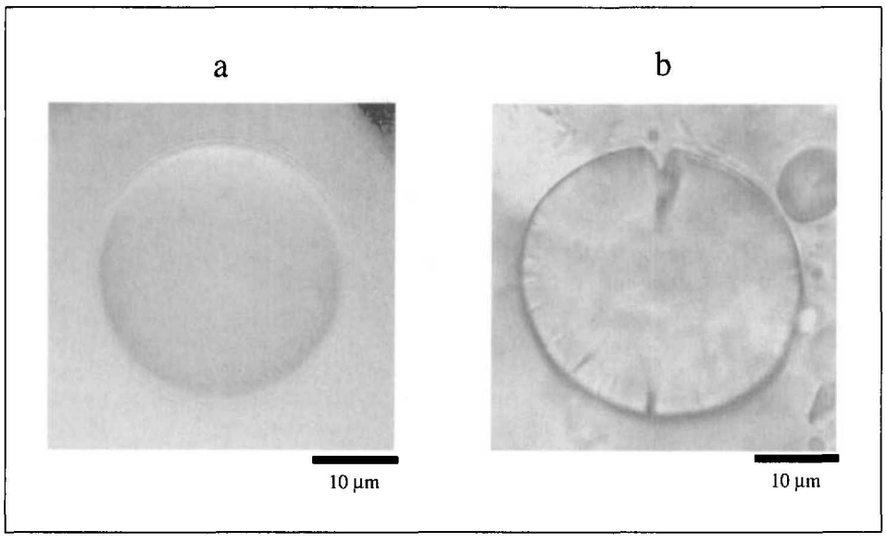

Fig. 2. Reflection intensity contrast micrograph of a giant, polymerized poly (2-methyloxazoline)-block-poly (dimethylsiloxane)-block-poly (2-methyloxazoline), PMOXA-PDMS-PMOXA triblock copolymer vesicle; a: before shear induced rupture; b: after shear induced rupture.

The shape persistence of the polymerized vesicles could be particularly interesting in context of possible applications. It allows, for example, preformed capsules to be loaded with guest molecules in an organic solvent, the loaded polymer shells to be isolated and subsequently the encapsulated material to be released in an aqueous medium [2].

Generally the triblock copolymer vesicles are (even without polymerization) stable over a period of more than one year, *i.e.* the underlying triblock copolymer membranes are considerably more stable than conventional lipid bilayers.

A well-established technique to quantify viscoelastic properties of planar lipid membranes (so-called 'Black Lipid Membranes') is to apply controlled forces *e.g. via* a short electric field pulse [7]. Adapting the preparation protocol of conventional low molecular weight lipid membranes we recently succeeded in preparing analog planar freestanding films, so-called 'Black Polymer Membranes' ('BPM') from our triblock copolymer [8][9]. Electric field pulses were used to charge the membrane causing an electric stress inside the film. Above a critical voltage, rupture of the membrane is induced and a fast discharge across the defect can be observed. An analysis of the time course of the electric voltage during such experiments gives information about the thickness of the membranes, the energy barrier of the membrane against rupture and the kinetics of defect widening and the underlying physical forces. Interestingly the energy barrier for the formation of a defect in the triblock copolymer membranes was found to be higher by a factor of 4 compared to conventional lipid membranes [8][10]. This enhanced mechanical stability is partly a result of the increased thickness of the triblock copolymer membranes. In good agreement with cryogenic transmission electron microscopy (cryo-TEM) investigations [5][11] the experiments yielded a hydrophobic thickness (*i.e.* the PDMS part) of the triblock copolymer membranes of about 10 nm, *i.e.* about two to three times the thickness of a conventional lipid bilayer [12][13]. As was to be expected the crosslinking polymerization of the reactive triblock copolymer molecules within these films further enhances their mechanical stability and the energy barrier for pore formation increases by an additional factor of 2.5 [8].

3. Reconstitution of Membrane Proteins in Block Copolymer Membranes

Another interesting aspect of the freestanding films is that one has direct access to both sides of the membranes, which allows investigation of transmembrane transport processes. In fact conventional black lipid membranes are frequently employed as model systems to reconstitute membrane proteins (Fig. 3) responsible for translocation [7][14]. It is obvious that the tendency of the triblock copolymer to form membrane-like superstructures in water closely resembles the behavior of lipid molecules. Hence the block copolymer membranes can be regarded as a mimetic of biological membranes and we found it quite tempting to try to reconstitute such membrane proteins also in our BPMs. We expected the resulting new combination of the broadness of polymer science with the richness of natural or genetically modified proteins to create new types of hybrid materials with unique properties.

For the reconstitution experiments we used well-characterized bacterial porins as model systems [14–17]. Porins are transmembrane proteins, which form trimeric channels in the outer membrane of Gram-negative bacteria. These water-filled channels allow passive diffusion of small solutes like ions, nutrients or antibiotics across the membrane. Therefore, the incorporation of the channels into planar freestanding films could directly be monitored using conductivity measurements [7][14]. For example, the protein maltoporin forms very narrow channels of about 150 pS at 1M KCl. Additionally it possesses stereo-specific binding sites for maltooligosaccharides inside the aqueous channels, which enhance the diffusion of the sugars across membranes [14–16][18][19]. Therefore, not only the incorporation of maltoporin into the polymer membranes but also its binding to maltooligosaccharides could be monitored. Upon titration the sugar is driven into the channels in a concentration dependent manner and causes closure of the channels. Hence the binding constants between sugars and the proteins can be calculated from the concentration dependence of conductivity data. Interestingly the sugar affinity constants for maltoporin within the triblock copolymer membranes were the same and in good agreement with previous investigations on maltoporin in conventional lipid membranes [7][14]. This indicates that the proteins remain fully functional de-

spite the fact that their hydrophobic–hydrophilic pattern is naturally optimized with respect to the thinner biological membranes and the block copolymer membranes are considerably thicker than conventional lipid bilayers due to the larger size of the underlying block copolymer molecules [5][11][20]. It seems, however, that the high flexibility and the conformational freedom of the polymer molecules allow a block copolymer membrane to adapt to the specific geometric and dynamic requirements of membrane proteins without considerable loss of free energy. Since the polymerizable groups of the macromonomers are attached to the very ends of the hydrophilic blocks, the hydrophobic PDMS middle block preserves a certain mobility within the membrane even after the crosslinking reaction [8]. As a result the binding affinity for the sugars remains unchanged even after polymerization of the block copolymer-protein hybrid membranes. Obviously, the conformation of the protein is not affected by the artificial surrounding within such a polymerized triblock copolymer membrane and its functionality is fully preserved.

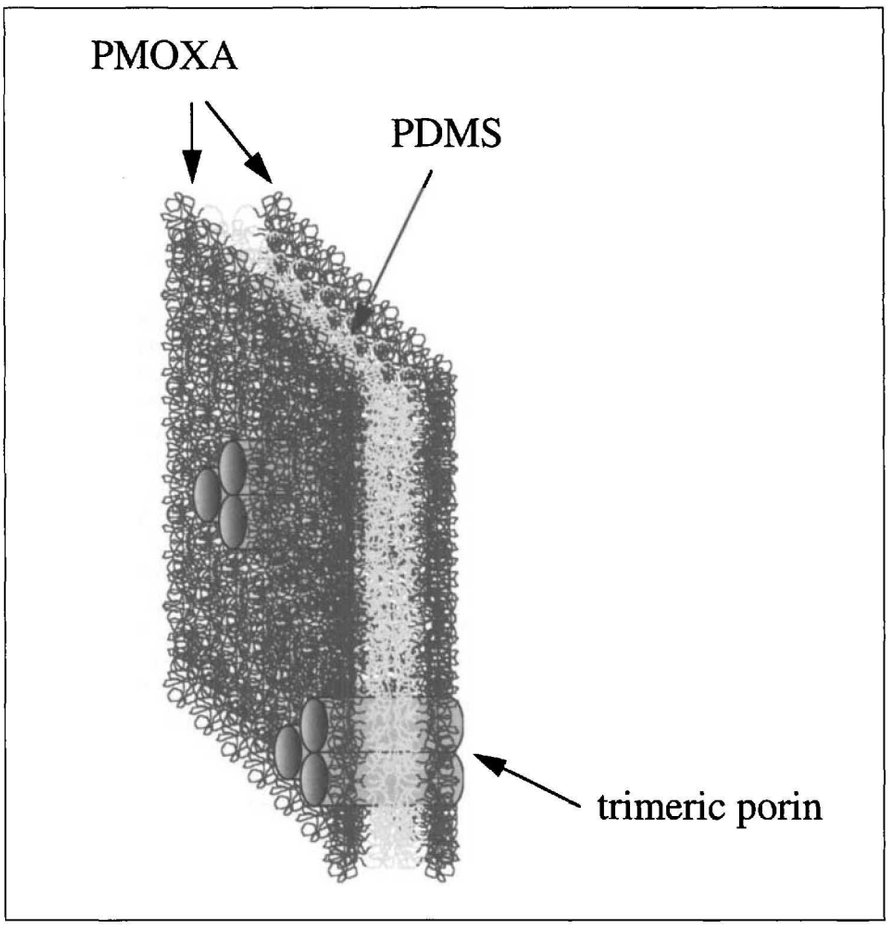

Fig. 3. Schematic representation of a channel protein-containing (polymerized) poly (2-methyloxazoline)-block-poly(dimethylsiloxane)-block-poly(2-methyloxazoline), PMOXA-PDMS-PMOXA triblock copolymer freestanding film.

4. The Nanoreactor

Generally nature provides a wide range of such membrane proteins allowing translocation of specific substances. Recently we made use of the resulting block copolymer-protein hybrid membranes to prepare a new type of stable nanoreactor with controlled permeability [11]. Incorporation of membrane proteins into the shell of (polymerized) triblock copolymer vesicles allows the selective harvest or separation of specific molecules and also their release on demand. The shell can protect encapsulated enzymes against a hostile environment and the channels in the shell can be used for 'pre-filtering' the substrates to enhance the sensitivity of the enzyme [11].

To demonstrate this we incorporated the porin OmpF into the membranes of triblock copolymer vesicles to control the permeability of their shells. It is known that molecules with a molecular weight above 400 $gmol^{-1}$ are sterically excluded from these channels [15]. As a representative example we encapsulated the enzyme β-lactamase (MW 50000 $gmol^{-1}$) in the aqueous core domain of the nanocapsules (see Fig. 4). β-Lactamase hydrolyzes β-lactam antibiotics like ampicillin (MW: 349 $gmol^{-1}$). In contrast to ampicillin, the product of the hydrolysis, the

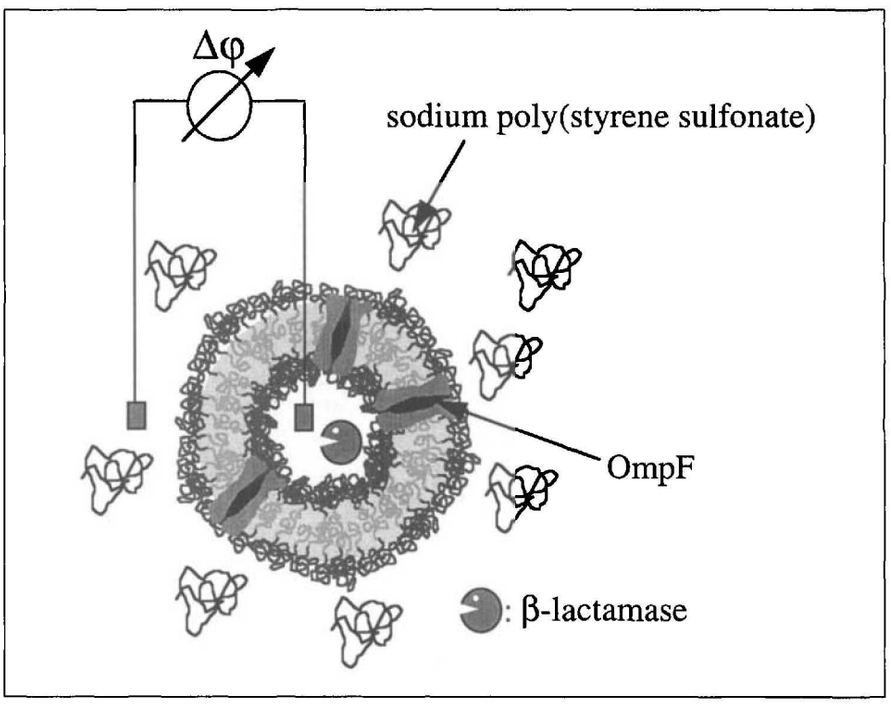

Fig. 4. Schematic view of a PMOXA-PDMS-PMOXA nanoreactor with encapsulated β-lactamase and of the Donnan potential induced by polyelectrolyte present in the external solution.

ampicillinoic acid, can reduce iodine to iodide. Therefore, the activity of the enzyme could readily be monitored by iodometry, *i.e. via* the decolorization of a starch-iodine complex [21][22]. It has to be emphasized that a subsequent polymerization of the nanoreactors did not change their activity within experimental error.

Interestingly, for a given ampicillin concentration outside the nanoreactors a steady state is rapidly established at which the rate of antibiotic diffusion through the OmpF channels and the β-lactam hydrolysis are equal thus resulting in a constant ampicillinoic acid release [23]. This makes the nanoreactors interesting as drug delivery systems. In such systems often a drug release is required which is constant over an extended period of time.

Furthermore, the protein OmpF has the interesting property of being closed if a transmembrane voltage above a critical threshold voltage of about 100 mV is applied [6][24]. It seems that the cells from which the protein has been isolated have evolved this mechanism to protect themselves against drastic changes of their environment. Recently we could show that this gating transition could be used to switch on or off the nanoreactors *via* external stimuli [23]. The possibility to trigger the activation (or deactivation) of such systems is highly interesting for applications since it allows local and temporal control on the uptake and the release of substrate. The block copolymer-protein hybrid shells of the nanoreactors can be regarded as a semi-permeable membrane separating their internal volume from the external solution. Large molecules above 400 $gmol^{-1}$ are excluded. This property opens a convenient approach to trigger the gating transition of OmpF. Large polyelectrolyte ions, like poly (styrene sulfonate), do not permeate and therefore their sodium counterions must be distributed inside and outside the nanocapsules according to a Donnan equilibrium, *i.e.* their concentration should be different on both sides of the nanocapsules wall giving rise to a Donnan potential. If this potential exceeds the critical one necessary for closure of OmpF the substrates can no longer enter the interior of the nanoreactors, *i.e.* the reactors are deactivated [23]. The closing is a reversible process and decreasing the potential below 100 mV reactivates the nanoreactors. This could be done by diluting the system with buffer or by increasing the Na^+-concentration in the system. In both cases the nanoreactors regain the full activity after reactivation.

5. Conclusions

Reactive amphiphilic block copolymers provide a convenient basis for the preparation of a wide variety of nanostructured polymeric materials ranging from hydrogels to nanoreactors and biomimetic membranes.

Especially the novel block copolymer-protein hybrid materials open a whole new area, taking advantage of the many possibilities that polymer chemistry offers, *e.g.* using electrically neutral polymers as a matrix for membrane proteins. Moreover the unique combination of block copolymers with membrane proteins allows preparation of mechanically stable, defect-free membranes and nanocapsules that have highly selective permeability and/or specific recognition sites. The proteins remained fully active even after crosslinking polymerization of the underlying reactive block copolymers which led to a considerable mechanical stabilization of the whole system.

It is important to note that despite their intriguing properties, membrane proteins were in the past not accessible as material components since they were difficult to produce and to purify on a large scale. Only recently larger quantities of, for example, porins can be produced (some grams as compared to a few micrograms), so that our approach can be regarded as one example of their multitude of possible future applications in polymer and materials science.

Acknowledgements

Financial support of the Swiss National Science Foundation is gratefully acknowledged.

Received: January 15, 2001

[1] C. Nardin, W. Meier, manuscript in preparation.
[2] C. Nardin, T. Hirt, J. Leukel, W. Meier, *Langmuir* **2000**, *16*, 1035.
[3] T. Hirt, R.C. Baron, D. Lohmann, W. Meier, WO 99/12059.
[4] D.D. Lasic, 'Liposomes: From Physics to Applications', Elsevier Science Publications B. V. Amsterdam, **1993.**
[5] C. Nardin, M. Winterhalter, W. Meier, *Angew. Chem.* **2000**, *112*, 4747.
[6] C. Nardin, E. Mendez, W. Meier, manuscript in preparation.
[7] R. Benz, K. Bauer, *Eur. J. Biochem.* **1988**, *11*, 76.
[8] C. Nardin, M. Winterhalter, W. Meier, *Langmuir* **2000**, *16*, 7708.
[9] C. Wilhelm, M. Winterhalter, U. Zimmermann, R. Benz, *Biophys. J.* **1993**, *64*, 121.
[10] P. Mueller, D.P. Rudi, H.T. Tien, W.C. Wescott, *J. Phys. Chem.* **1963**, *67*, 534.
[11] C. Nardin, S. Thoeni, J. Widmer, M. Winterhalter, W. Meier, *Chem. Commun* **2000**, 1433.
[12] R. Benz, K. Janko, *Biochem. Biophys. Acta* **1976**, *455*, 721.
[13] J.P. Dilger, R. Benz, *J. Membr. Biol.* **1985**, *85*, 181.
[14] M. Winterhalter, *Colloids and Surfaces A* **1999**, *149*, 547.
[15] H. Nikaido, *Molecular Microbiology* **1992**, *6 (4)*, 435.
[16] T. Schirmer, T.A. Keller, Y-F. Wang, J.P. Rosenbusch, *Science* **1995**, *267*, 512.
[17] B. Eisenberg, *Acc. Chem. Res.* **1998**, *31*, 117.
[18] Y-F. Wang, R. Dutzler, P.J. Rizkallah, J.P. Rosenbuch, T. Schirmer, *J. Mol. Biol.* **1997**, *272*, 56.
[19] R. Dutzler, Y-F Wang, P.J. Rizkallah, J.P. Rosenbusch, T. Schirmer, *Structure* **1996**, 4.
[20] B.M. Disher, Y-Y. Won, D.S. Ege, J.C.M. Lee, F.S. Bates, D.E. Disher, D.A. Hammer, *Science* **1999**, *284*, 1143.
[21] W. Zimmermann, A. Rosselet, *Antimicrobial Agents and Chemotherapy* **1977**, *12*, 368.
[22] R.P. Novick, *Biochem. J.* **1962**, *83*, 236
[23] C. Nardin, J. Widmer, M. Winterhalter, W. Meier, *Eur. Phys. J. E*, in print.
[24] P. Van Gelder, F. Dumas, M. Winterhalter, *Biophys. Chem* **2000**, *85*, 153.