Synthetic Bio-nanoreactor: Mechanical and Chemical Control of Polymersome Membrane Permeability

ARTICLE *in* ANGEWANDTE CHEMIE INTERNATIONAL EDITION · APRIL 2012

Impact Factor: 11.26 · DOI: 10.1002/anie.201108814 · Source: PubMed

5 AUTHORS, INCLUDING:

Jens Gaitzsch
University of Basel
15 PUBLICATIONS **136** CITATIONS
SEE PROFILE

Giuseppe Battaglia
University College London
119 PUBLICATIONS **3,017** CITATIONS
SEE PROFILE

Brigitte Voit
Leibniz Institute of Polymer Research Dres…
343 PUBLICATIONS **4,168** CITATIONS
SEE PROFILE

Nanoreactors

DOI: 10.1002/anie.201108814

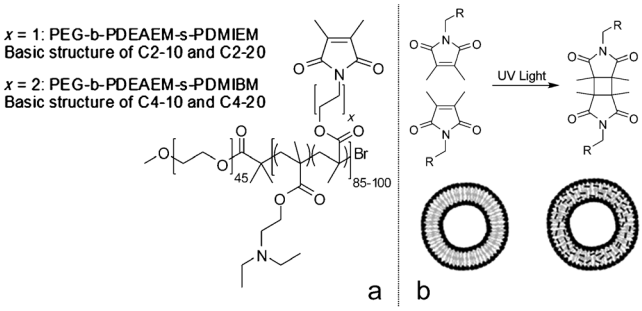
Synthetic Bio-nanoreactor: Mechanical and Chemical Control of Polymersome Membrane Permeability**

Jens Gaitzsch, Dietmar Appelhans, Linge Wang, Giuseppe Battaglia, and Brigitte Voit**

The combination of chemistry and biology has gained increased interest among scientists over the past decade. The goal of chemists has become to mimic biological structures by chemical means.[1] A good example of this is the development of wholly synthetic analogues of lipid vesicles. These are thought to be critical components in the evolution of life, since they would allow the necessary compartmentalization for complex biochemical processes to take place.[1,2] Within the last few years, in particular, a polymer-based equivalent, the polymersome, has been found to be a promising candidate for this.[3–6] Several reports have shown that polymersomes can be used to encapsulate DNA,[7] RNA,[8,9] as well as functional enzymes.[4,5,10–14] A great advantage of polymersomes over liposomes is their higher mechanical and chemical stability. The high flexibility in the chemical design of polymersomes allows the formation of membranes with tunable permeability.[15–19] This approach can be further enhanced by the incorporation of active molecular transporters such as transmembrane proteins.[5,20] However, it is not certain whether polymersome membranes can withstand high shear rates, which may occur during an industrial cleaning process.[16] Here, we present a new approach to modulate the permeability of polymersome membranes by using pH-sensitive photo-cross-linkable copolymers. We aimed to generate cross-linked polymersomes to tune the shear-rate-induced release of globular dendritic glycopolymers[21] of different sizes. The concept was then further expanded to an enzymatic reaction within polymersomes, with the aim of controlling transmembrane traffic by modulating the pH value.

Our amphiphilic block copolymer consists of well-known biocompatible and non-immunogenic poly(ethylene glycol) (PEG) as the hydrophilic part. The hydrophobic part is a statistical copolymer of the pH-sensitive diethyl amino ethyl methacrylate (DEAEM) and a photo-cross-linking unit of either 3,4-dimethyl maleic imidoethyl methacrylate (DMIEM, C2 polymers) or 3,4-dimethyl maleic imidobutyl methacrylate (DMIBM, C4 polymers) in 10 or 20 mol%, respectively (Scheme 1 and Table 1).

We showed previously[22] that our copolymers C2-10 and C2-20 (Scheme 1) are able to form polymersomes with a photo-cross-linkable and pH-sensitive moiety. Furthermore, the transmembrane traffic of dye molecules through cross-linked polymersomes could be triggered by changes in the pH value.[22] However, the cross-linking time of 80 minutes[22] was likely to destroy any functionality of a bioactive molecule eventually enclosed within. We are now able to reduce the time of the UV irradiation necessary to reach a cross-linked



Scheme 1. a) Chemical structure of amphiphilic block copolymers with an ethyl (C2) and butyl (C4) spacer in the cross-linking units DMIEM and DMIBM, which are used in 10 or 20 mol%, respectively (resulting in C2-10, C2-20, C4-10, and C4-20 nomenclature) and b) the cross-linking reaction occurring within the membrane.

Table 1: Properties of the polymers and polymersomes (Psome), including cross-linking time of the polymersomes.

	M_n[a] [kg mol^{-1}]	Cross-linker[b] (mol%)[c]	P'some size[d] [nm]	PDI[d]	t[e] [s]
C2-10	19.0	C2 (10)	125	0.2	180
C2-20	21.5	C2 (20)	120	0.2	120
C4-10	20.0	C4 (10)	100	0.2	120
C4-20	22.5	C4 (20)	125	0.2	30

[a] M_n is determined from signal intensities in the ^{1}H NMR spectra; the PDI of the copolymers is 1.3 (1.4 for C4-10). [b] Refers to the length of the carbon chain (Scheme 1 a). [c] With respect to the hydrophobic block length; determined from signal ratios in the ^{1}H NMR spectra. [d] Determined by DLS (diameter), PDI = polydispersity of polymersomes. [e] Cross-linking time.

[*] J. Gaitzsch, Dr. D. Appelhans, Prof. B. Voit
Leibniz-Institut für Polymerforschung Dresden e.V.
Hohe Strasse 6, 01069 Dresden (Germany)
J. Gaitzsch, Prof. B. Voit
Organische Chemie der Polymere
Fachrichtung Chemie und Lebensmittelchemie
Technische Universität Dresden
01062 Dresden (Germany)
E-mail: voit@ipfdd.de
Dr. L. Wang, Prof. G. Battaglia
Department of Biomedical Science, University of Sheffield
Firth Court, Western Bank, Sheffield S10 2TN (UK)
E-mail: g.battaglia@sheffield.ac.uk

[**] Financial Support from the Rosa-Luxemburg-Foundation and the Dresden International Graduate School for Biomedicine and Bioengineering (DIGS-BB) is gratefully acknowledged.

Supporting information for this article (experimental section and data on the polymersomes, release experiments, and enzymatic reaction) is available on the WWW under http://dx.doi.org/10.1002/anie.201108814.

state from over 60 minutes to only 30 seconds. This has been achieved by changing the UV source, and also by using a cross-linking unit with a longer spacer. The polymer series C2-10, C2-20, C4-10, and C4-20 were prepared, and the cross-linked polymersomes made up of the C4-20 polymer required only 30 seconds of UV irradiation (Table 1). The cross-linking unit in C4 polymers is attached to a butyl spacer (PDMIBM) instead of the ethyl spacer (PDMIEM) in the C2 polymers (Scheme 1). We postulate that less steric hindrance between the PDMIBM and neighboring PDEAEM molecules leads to a higher cross-linking efficiency. The C4-20 polymer was used for further studies because it required the shortest cross-linking time to form the polymersomes.

The next steps were to determine the stability of the cross-linked polymersomes at various pH values. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) studies showed that the cross-linking of the membrane hinders the disassembly of the polymersomes of the C4-20 polymer at acidic pH values (Figure 1 a,c). In contrast, in non-cross-linked polymersomes, C4-20 is fully hydrophilic at an acidic pH value and hence no longer able to form polymersomes (Figure 2). Furthermore, cross-linked polymersomes show a definite swelling/deswelling cycle upon switching of the pH value, which can be repeated at least five times (Figure 1 a, and Figure 4-SI in the Supporting Information). This cycling is entirely due to the physicochemical changes of the PDEAEM chains in going from an unprotonated, hydrophobic entangled state at a high pH value to a protonated, hydrated, hydrogel-like state at a low pH value. Such a change corresponds with an increase in the thickness of the polymersome membrane, as measured by TEM, that matches the increase in the vesicle diameter determined by DLS studies (Figure 1).

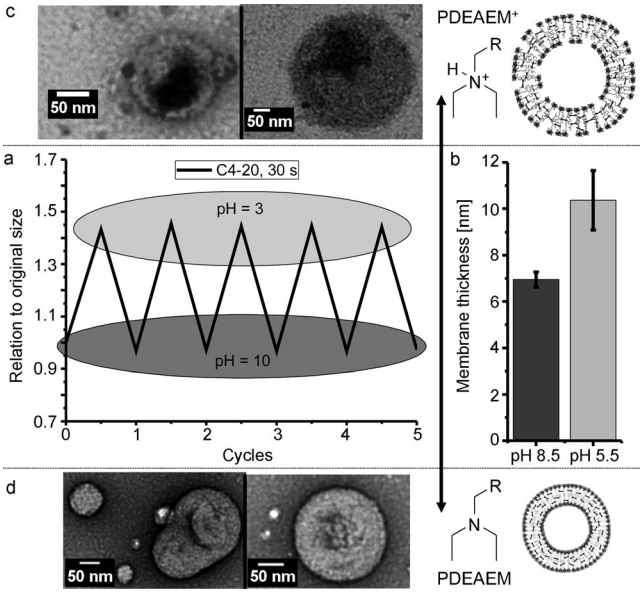


Figure 1. Development of the polymersomes upon changes in the pH value. a) Reversible change in the diameter of polymersomes between pH 3 and pH 10 of the C4-20 polymer, as measured by DLS. b) The membrane thickness changes in the same proportion as the vesicles. c,d) Vesicles (sketches shown) could be visualized at pH 3 (c) and pH 10 (d) by using TEM.

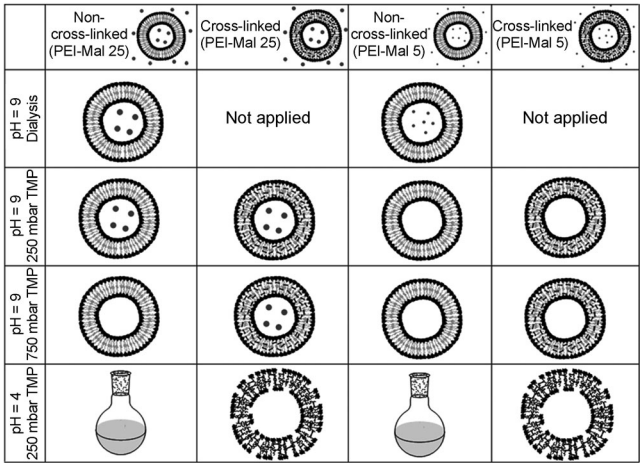


Figure 2. Pressure-dependent release of fluorescein-labeled PEI-Mal 25 and PEI-Mal 5 from cross-linked and non-cross-linked polymersomes at pH 4 and 9. The retention of the nanoparticles within the polymersomes was observed by the absorption maxima of fluorescein at 500 nm. TMP = transmembrane pressure.

To determine the mechanical stability and permeability of our pH-sensitive polymersomes in a cross-linked and non-cross-linked state (Figure 2) we studied the effect of the shear rate on polymersomes containing maltose-decorated hyperbranched polyethyleneimine (PEI-Mal) nanoparticles.[21] PEI cores of 5000 Da (PEI-Mal 5, diameter 4 nm) and 25 000 Da (PEI-Mal 25, diameter 10 nm) were used to study their release at various pH values and shear rates. These readily available fluorescein-labeled organic nanoparticles are protein-sized and softer than traditional silica or gold nanoparticles, thus making them ideal test molecules. They were enclosed within the polymersomes during the self-assembly of C4-20, and then the vesicles were exposed to different conditions (Figure 2).

As a starting point, we demonstrated that both dendritic nanoparticles can be encapsulated within non-cross-linked polymersomes and retained after purification by dialysis at pH 9 (Figure 2). However, dialysis with classical dialysis tubes is a time-consuming process that takes several days. Moreover, this technique has the drawback that no shear rate can be applied during the polymersome cleaning process. The application of a pressure to the solution would speed up various processes of interest by placing polymersomes under different shear rates.

Consequently, we tested the purification process using a KrosFlo Research IIi system (also known as a hollow fiber filtration (HFF) system) and applying transmembrane pressures (TMP) of 250 and 750 mbar (Figure 2). The time for the purification of the polymersomes could be shortened to 1 hour at 250 mbar TMP and to only 30 min at 750 mbar TMP. In a first experiment, non-cross-linked polymersomes hosting the two different nanoparticles PEI-Mal 5 and PEI-Mal 25 were processed at 250 mbar and pH 9. Here, only the large nanoparticles (PEI-Mal 25) could be retained, but not PEI-

Mal 5. This result indicates that some polymersomes rupture as a consequence of the shear rate and undergo cargo loss. We assume this rupture occurs through the formation of small pores (i.e. poration) within the membrane that heal over after the shear rate is removed. Furthermore, this membrane poration is accompanied by a polymersome squeezing/deforming process when pressure is applied. DLS studies carried out after each shear-rate experiment confirmed that the polymersomes are still detectable (see the Supporting Information).

The finding that neither type of PEI-Mal nanoparticles are retained at a TMP of 750 mbar at pH 9 when using non-cross-linked polymersomes indicates that the pore size depends on the shear rate (see Figure 2 and the Supporting Information). We performed the same process at a TMP of 750 mbar and pH 9 with cross-linked polymersomes. While the small PEI-Mal 5 nanoparticles still exit the polymersomes, the larger PEI-Mal 25 nanoparticles could now be detected after the procedure (Figure 2). This observation suggests that membrane cross-linking increases the resistance to shear-rate-induced poration compared to non-cross-linked membranes. The polymersome membrane still retains its characteristic ability to form pores and self-heal (see the Supporting Information). Neither nanoparticles are retained when swollen, cross-linked polymersomes are processed, for example, in acidic media (pH 4) and at a low TMP of 250 mbar. Hence, we can conclude that polymersomes (whether their membrane is cross-linked or not) respond to an applied shear rate by forming controllable, definite-sized pores (see Figure 2 and the Supporting Information). The use of such an approach would thus enable us to design polymersomes whose permeability could be finely controlled by the shear rate as well as the cross-linking state and the solution pH value.

To further validate the responsive nature of the cross-linked polymersomes we created a bionanoreactor by incorporating myoglobin as a model enzyme. This is a very well characterized enzyme[23] with an iron-containing central complex. Myoglobin is able to catalyze oxidative reactions, for example, the well-known reaction of guaiacol with hydrogen peroxide. An advantage of this enzymatically controlled reaction is that the final product (oxidised guaiacol) has an absorption maximum at 470 nm and thus UV/Vis spectroscopy can be used to monitor the reaction.[23] We first tested the ability of myoglobin to withstand the UV irradiation necessary for cross-linking the polymersomes. In the absence of polymersomes, the enzyme still shows high catalytic activity after irradiation (see the Supporting Information). After incorporating myoglobin within the polymersomes and applying our shear-rate-induced purification step (200 mbar TMP), we cross-linked the polymersomes and then checked the activity of the enclosed enzyme under various conditions (Figure 3).

The first experiments showed that guaiacol does not react in the absence of hydrogen peroxide, as determined by measuring the myoglobin activity at pH 8. At this pH value, the PDEAEM polymersome membrane is unprotonated and, therefore, fully hydrophobic. Consequently, no enzymatic activity was observed after adding hydrogen peroxide to the bionanoreactor solution at pH 8 (Figure 3, "b" state). This

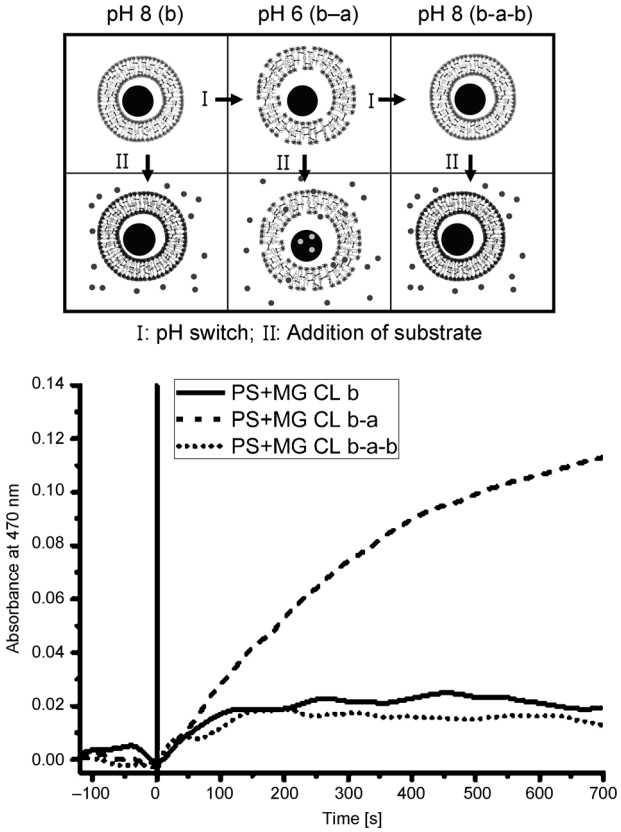


Figure 3. Schematic representation of the reaction of guaiacol and hydrogen peroxide in myoglobin-filled polymersomes: First at a basic (b), then at an acidic (b-a), and again at a basic (b-a-b) pH value. Hydrogen peroxide is added 120 s after the addition of guaiacol. The graph shows the normalized time-dependent absorption at 470 nm.

finding clearly indicates that the reagents cannot diffuse into the polymersome lumen where the enzyme is localized. In contrast, a higher and longer increase in the absorption of the reaction product, indicating a catalyzed reaction between guaiacol and hydrogen peroxide within the bionanoreactor, was evident at pH 6 (Figure 3, "b-a" state). The polymersome membrane is now in a swollen state and totally hydrophilic. Transmembrane diffusion is thus possible, thereby allowing the guaiacol and hydrogen peroxide to reach the myoglobin, which catalyzes the reaction. As the pH value is increased again to 8, the reaction is stopped because the nonpermeable polymersome membrane reforms (Figure 3, "b-a-b" state; for further details see the Supporting Information). Thus, the myoglobin inside the polymersomes is no longer accessible to the reagents from outside. This also indicates that myoglobin cannot diffuse out of the polymersomes if no shear-rate conditions are applied.

In conclusion, we have successfully demonstrated that polymersomes undergo selective shear-rate-induced release of two different nanometer-sized dendritic glycopolymers through controlled poration of the membrane. These membrane pores can be controlled by the shear rate, cross-linking, and the pH value. This specific approach for shear-rate-induced release is only possible with our pH-sensitive, photo-cross-linkable polymersomes. We showed the simultaneous

encapsulation of guest macromolecules during the self-assembly of pH-sensitive, amphiphilic block copolymers followed by photo-cross-linking. On the basis of these findings, we were able to develop a bionanoreactor without the need to use a transmembrane protein to control the feed of an enzyme into the polymersome lumen. The pore size and transmembrane trafficking of the reagents can be controlled in the case of enzymatic reactions inside polymersomes by pH-induced swelling and shrinking of the pH-stable polymersomes.

Received: December 14, 2011
Published online: March 21, 2012

Keywords: bio-nanoreactors · membranes · polymersomes · poration · shear force

[1] J. W. Szostak, D. P. Bartel, P. L. Luisi, *Nature* **2001**, *409*, 387.
[2] V. Noireaux, A. Laibacher, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 17669.
[3] C. LoPresti, H. Lomas, M. Massignani, T. Smart, G. Battaglia, *J. Mater. Chem.* **2009**, *19*, 3576.
[4] J. C. M. van Hest, K. T. Kim, S. A. Meeuwissen, R. J. M. Nolte, *Nanoscale* **2010**, *2*, 844.
[5] K. Renggli, P. Baumann, K. Langowska, O. Onaca, N. Bruns, W. Meier, *Adv. Funct. Mater.* **2011**, *21*, 1241.
[6] D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967.
[7] H. Lomas, I. Canton, S. MacNeil, J. Du, S. P. Armes, A. J. Ryan, A. L. Lewis, G. Battaglia, *Adv. Mater.* **2007**, *19*, 4238.
[8] Y. H. Kim, M. Tewari, J. D. Pajerowski, S. S. Cai, S. Sen, J. Williams, S. Sirsi, G. Lutz, D. E. Discher, *J. Controlled Release* **2009**, *134*, 132.
[9] A. Lewis, G. Battaglia, I. Canton, P. Stratford, A61 K 47/48 ed., Biocompatibles UK Ltd., UK, **2009**.
[10] D. E. Discher, B. M. Discher, Y. Y. Won, D. S. Ege, J. C. M. Lee, F. S. Bates, D. A. Hammer, *Science* **1999**, *284*, 1143.
[11] W. Meier, P. Tanner, S. Egli, V. Balasubramanian, O. Onaca, C. G. Palivan, *FEBS J.* **2011**, *278*, 32.
[12] P. Broz, S. Driamov, J. Ziegler, N. Ben-Haim, S. Marsch, W. Meier, P. Hunziker, *Nano Lett.* **2006**, *6*, 2349.
[13] S. F. M. van Dongen, M. Nallani, J. L. L. M. Cornelissen, R. J. M. Nolte, J. C. M. van Hest, *Chem. Eur. J.* **2009**, *15*, 1107.
[14] S. F. M. van Dongen, W. P. R. Verdurmen, R. J. R. W. Peters, R. J. M. Nolte, R. Brock, J. C. M. van Hest, *Angew. Chem.* **2010**, *122*, 7371; *Angew. Chem. Int. Ed.* **2010**, *49*, 7213.
[15] G. Battaglia, A. J. Ryan, S. Tomas, *Langmuir* **2006**, *22*, 4910.
[16] J. F. Le Meins, O. Sandre, S. Lecommandoux, *Eur. Phys. J. E* **2011**, *34*, 1.
[17] O. Onaca, P. Sarkar, D. Roccatano, T. Friedrich, B. Hauer, M. Grzelakowski, A. Guven, M. Fioroni, U. Schwaneberg, *Angew. Chem.* **2008**, *120*, 7137; *Angew. Chem. Int. Ed.* **2008**, *47*, 7029.
[18] J. C. M. van Hest, K. T. Kim, J. H. Zhu, S. A. Meeuwissen, J. J. L. M. Cornelissen, D. J. Pochan, R. J. M. Nolte, *J. Am. Chem. Soc.* **2010**, *132*, 12522.
[19] J. F. Le Meins, A. C. Carlsen, A. N. Glaser, S. Lecommandoux, *Langmuir* **2011**, *27*, 4884.
[20] J. C. M. van Hest, R. P. Brinkhuis, F. P. J. T. Rutjes, *Polym. Chem.* **2011**, *2*, 1449.
[21] D. Appelhans, H. Komber, M. A. Quadir, S. Richter, S. Schwarz, J. van der Vlist, A. Aigner, M. Müller, K. Loos, J. Seidel, K. F. Arndt, R. Haag, B. Voit, *Biomacromolecules* **2009**, *10*, 1114.
[22] J. Gaitzsch, D. Appelhans, D. Grafe, P. Schwille, B. Voit, *Chem. Commun.* **2011**, *47*, 3466.
[23] T. Hayashi, T. Matsuo, D. Murata, Y. Hisaeda, H. Hori, *J. Am. Chem. Soc.* **2007**, *129*, 12906.

