A EUROPEAN JOURNAL

CHEMPHYSCHEM

OF CHEMICAL PHYSICS AND PHYSICAL CHEMISTRY

Accepted Article

Title: Separating extreme pH gradients using amphiphilic copolymer membranes.

Authors: Lorena Ruiz-Pérez, Claire Hurley, Salvador Tomás, and Giuseppe Battaglia

To be cited as: *ChemPhysChem* 10.1002/cphc.201800187

Link to VoR: http://dx.doi.org/10.1002/cphc.201800187

A Journal of ChemPubSoc Europe

WILEY-VCH

www.chemphyschem.org

10.1002/cphc.201800187

WILEY-VCH

Separating extreme pH gradients using amphiphilic copolymer membranes

Lorena Ruiz-Pérez*,[a] , Claire Hurley, [b] Salvador Tomas, [c] and Giuseppe Battaglia*,[a]

Abstract:

Polymeric vesicles, also called polymersomes, are highly efficient biomimetic systems. They can generate compartmentalized volumes at the nanoscale supported by synthetic amphiphilic membranes that closely mimic their biological counterparts. Membrane permeability and the ability to separate extreme pH gradients is a crucial condition a successful biomimetic system must meet. We show polymersomes formed by non ionic polybutadiene-b-polyethylene oxide (PBd-b-PEO) amphiphilic block copolymers engineer robust and stable membranes that are able to sustain pH gradients of 10 for a minimum of 8 days. Cells endo-lysomal compartments separate gradients between 3 and 1, while we generated a pH gradient of three folds as great. This feature clearly is of great importance for applications as nanoreactors and drug delivery systems where separating different aqueous volumes at nanoscale level is an essential requirement.

Amphiphilic block copolymers can self-assemble in water into well-ordered nanostructures.[1] These ordered nanostructures can be tuned over a wide variety of morphologies, ranging from discrete micelles and vesicles to continuous network structures.[2]

In particular, polymeric vesicles, also called polymersomes[3] have been gaining more attention lately as they reassemble those arrangements generated by biological membranes in cellular compartmentation.[4] The capability to generate compartmentalized volumes at the nanoscale is one of the essential motifs used by cells in synthesizing biomolecules and performing the biochemical reactions required for their function.[5] This motif has been recently mimicked using block copolymer vesicles as nanoreactors.[6] In addition, polymeric vesicles offer exceptional possibilities to devise nanocontainers with exciting applications in biomedicine, electronics, cosmetics and food science. [6-7]

[a] Dr, L, Ruiz-Pérez and Prof, G, Battaglia
Department of Chemistry
University College London
20 Gordon street, WC1H 0AJ London, UK
E-mail: l.ruiz-perez@ucl.ac.uk and g.battaglia@ucl.ac.uk
[b] Dr, C, Hurley
Department of Physics
University of Warwick
Coventry West Midlands CV4 7AL, United Kingdom
E-mail: c.r.hurley@warwick.ac.uk
[c] Dr, S, Tomás
Department of Biological Sciences
Birbeck,University of London
Malet Street, Bloomsbury London WC1E 7HX, United Kingdom
Email: s.tomas@bbk.ac.uk

Supporting information for this article is given via a link at the end of the document.

It has been reported that polymersomes can retain encapsulated molecules over a period of days to weeks.[8] Permeation through the vesicle membrane is the main effect that causes the loss of the encapsulated molecules. Consequently, evaluating the permeability of specific molecules is one of the most crucial measurements to fully characterize amphiphilic membranes.
Water and ion permeabilities have been widely studied by different techniques such as membrane potential measurements,[9] fluorescence quenching methods,[10] micropipette aspiration techniques,[11] and anti-Stokes Raman scattering.[12] The permeability of more complex and nonionic molecules has been measured by NMR techniques.[13] However, molecular exchange through amphiphilic membranes always takes place in an aqueous environment, and the permeating molecules undergo no great variation in their individual properties.
Battaglia *et al* reported that the permabilities of different polymeric membranes from a series of poly(ethylene oxide)- *co*-polybutylene oxide (EB) copolymers and egg yolk phosphatidylcholine depended on the thickness of the membranes as predicted by Fick's first law.[14] This lead to the conclusion that vesicle permeability can be tuned by selecting the appropriate composition of the amphiphilic membrane.

Here we demonstrate the ability of a polymersome membrane to sustain large pH gradients for a minimum period of eight days. For this investigation we used amphiphilic block copolymer formed by ethylene oxide (PEO) and butadiene (PBD) monomers. This judicious combination of hydrophilic and hydrophobic blocks was chosen for generating monodisperse unilamellar polymersomes that are non-sensitive to pH or ionic strength changes. This is a critical requirement for the polymersome membranes to remain intact under the conditions herein investigated. PBd-bPEO forms vesicles with a chemical composition similar to naturally occurring lipid bilayers which are known to be impermeable to ions. Polymersomes were loaded with a pH sensitive highly water soluble porphyrine dye in aqueous solution at pH2 and immersed in an alkaline supernatant at pH 12 for a period of eight days. The polymersome lumen pH was monitored via fluorescence measurement of the encapsulated dye. These were supported by measuring the supernatant pH upon polymersome osmolysis by sodium chloride addition.

Experimental Section

Polybutadiene-b-polyethylene oxide (PBd-b-PEO) block copolymer was synthesized via anionic polymerization using standard high vacuum techniques[15] and characterized *via* NMR and SEC. The PEO molar and

10.1002/cphc.201800187

weight fractions present in the block copolymer were found to be f_m=0.27 and f_{wt}=0.23. M_n PBd 5000; M_n PEO 1500. This PEO ratio was chosen for guaranteeing polymersome formation. The production of polymersomes with different membrane permeabilities can be achieved by changing the block-copolymer lengths while maintaining fixed their molar ratio in the mixture. We already reported this [16] however in the present work we assessed the temporal aspect of the permeability.

The synthesis is given in detail in the supporting information. Polymersomes have previously been formed in water from PBd-b-PEO of these characteristics.[3] Hereafter the term BDE1 will be used to refer to PBd (5kg/mol)-b-PEO (1.5Kg/mol). In order to monitor the polymersomes lumen pH, we encapsulated a porphyrine dye within the polymersomes. The fluorescence spectrum of this dye is sensitive to pH changes. The experimental procedure for encapsulation of porphyrine into BDE1 vesicles, fluorescence spectra and calibration of porphyrine, and porphyrine synthesis are provided in the supporting information. Trasmission electron microscope (TEM) images of porphyrine encapsulated BDE1 polymersomes and empty BDE1 polymersomes are supplied in the supportive information showing no change in polymersome morphology after porphyrine encapsulation. Polymersomes sizes were found to be circa 100nm as shown by TEM images and dynamic light scattering (DLS) measurements. We previously demonstrated that the preparation methods mentioned above lead to unilamellar vesicles[7c], and this is confirmed by the TEM micrographs herein supplied (SI).

Fig. 1 shows the peak ratios obtained from excitation spectra of pH 2 porphyrine loaded BDE1 polymersomes when immersed in a pH 12 solution for a period of 8 days. An example of the excitation spectra of porphyrine loaded BDE1 polymer vesicles in solution at a fixed pH 12 during the first and eighth day can be seen in Fig.2 (a) and (b) (black line).

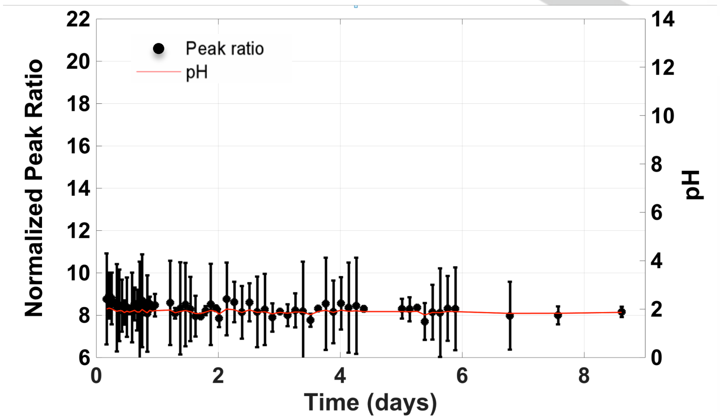


Figure 1. Peak ratios and polymersome internal pH obtained from excitation scans at a fixed emission λem=720nm of pH 2 loaded porphyrine BDE1 polymersomes at pH 12 for a period of eight days.

The polymersomes internal pH value could be calculated from the variations in the peak ratio values. The ratio (I_{peak1}-$I_{baseline}$)/(I_{peak3}-$I_{baseline}$) was plotted as a function of pH and fitted using the sigmoid function below

$$y = A_2 + (A_1 - A_2)/(1 + \exp((x - x_0)/D)) \quad (1)$$

where A_1, A_2, x_0 and D are constants given by the fit .The fit can be seen in Fig. S2.(b)). The constant values provided by the fit were A_1= 7.16, A_2 =21.27, x_0= 4.92 and D=1.21. The vesicles internal pH is also plotted in Fig.1. It can be observed that the pH inside the polymersomes remained constant, within the experimental error, at a value of pH~ 2 (Fig.1). Such phenomenon was observed for a period of eight days. Hence, since the supernatant pH was fixed at 12 the system under study efficiently preserved pH gradients ΔpH of an order of ΔpH~10. As mentioned above the amphiphilic blockcopolymers used here are not sensitive to pH changes hence both the polymersome morphology and membrane permeability were maintained during the environmental changes.

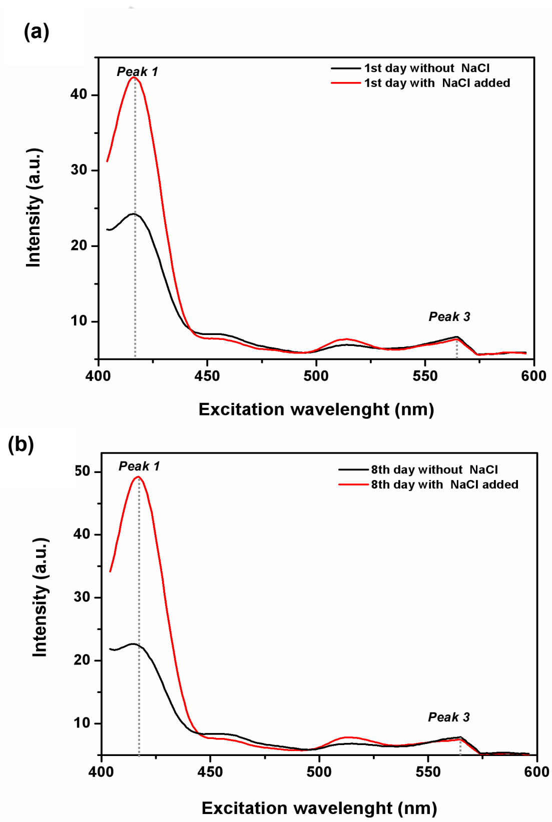


Figure. 2 Excitation spectra at a fixed emission λem=720nm for porphyrine loaded BDE1 vesicles at pH 12. The spectra before (black line) and after NaCl addition (red line) during the first and eighth day of measurements are shown in (a) and (b) respectively

10.1002/cphc.201800187

WILEY-VCH

Titration with NaCl solution was performed with the aim to match vesicle internal and supernatant pH. Approximately 60 mg/ml of NaCl were needed for the osmolysis of the polymersome membranes. Fig. 2 shows how the vesicle internal pH increased from approximately pH 2 to pH 7.7 upon addition of salt. The progressive raise in the polymersomes internal pH is caused by a progressive increase of the vesicle membrane's permeability to the supernatant.

60mg/ml of NaCl were added after the first and eighth day and excitation scans were taken, spectra are shown in Fig. 3 (a) and (b). After salt addition a significant increase in the *Peak 1* intensity at 416nm was observed for the first and eighth day respectively (Fig. 3 (a) and (b)). The peak ratios measured after salt addition for the first and eighth day could be translated into a polymersome internal pH of circa 7.6 according to the calibration graph and fit provided in the supporting information. Similarly, after salt addition the supernatant pH was measured during the first and eight day respectively and was found to be approximately 7.8 for both cases. Indeed at this condition the polymersome internal pH, measured by fluorescence, matched the external pH. The decrease of this latter is due the release of the protons from the polymersomes lumen. This is further confirmed by the calculations provided in the supporting information that lead to equation (2):

$$pH_{final} = pH_{lumen} - log\left(\frac{w_{copolymer}}{\rho_{PBD}}\right) \qquad (2)$$

Where pH_{final} is the supernatant pH after osmolysis of the polymersomes, pH_{lumen} is the original pH within the polymersomes, $w_{copolymer}$ is the concentration of the copolymer, and r_{PBD} is the density of polybutadiene.

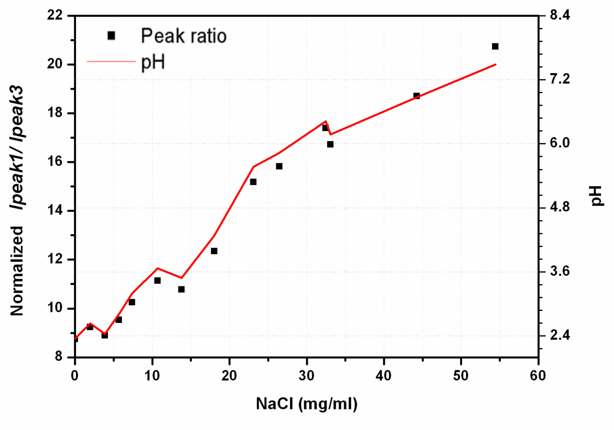


Figure 3. Peak ratios and polymersome internal pH obtained from excitation scans at a fixed emission λem=720nm of NaCl titrations on pH 2 loaded porphyrine BDE1 polymersomes at pH 12 for a period of eight days.

Separating extreme pH gradients is an essential condition used by cells within their endosomes and lysosomes to digest and metabolized any material that is internalised.[5] Here we demonstrated an even larger pH gradient can be sustained by employing a more robust and stable membrane. Indeed endolysomal compartments separate gradients between 3 and 1,[5] while we generated a pH gradient of 10. This feature clearly augurs well for applications such as nanoreactors and any other where separating different aqueous volume at the nanoscale is of asset.

Acknowledgements

We thank Reckitt Benckiser supporting this work. We thank EPSRC for paying part of GB salary.

Keywords: Polymersomes, pH gradient, Osmolysis, Polymeric membrane.

references

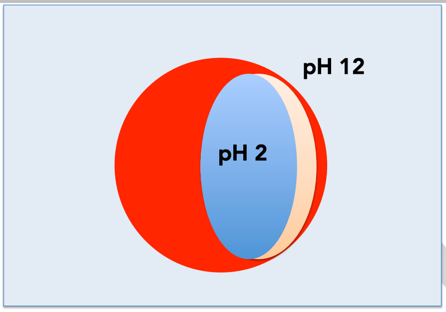
[1] M. Massignani, H. Lomas, S. G. Battaglia,, *Polymersomes: A synthetic biological approach to encapsulation and delivery*, Springer, pp. 115–154, 229 pp., New York, **2010**.
[2] G. Battaglia, A. J. Ryan, *Nature Materials* **2005**, *4*, 869-876.
[3] B. M. Discher, Y. Y. Won, D. S. Ege, J. C.-M. Lee, F. S. Bates, D. E. Discher, D. A. Hammer, *Science* **1999**, *284*, 1143-1146.
[4] aG. Battaglia, A. J. Ryan, *J. Am. Chem. Soc* **2005**, *127*, 8757-8764; bL. Ruiz-Perez, J. Madsen, E. Themistou, J. Gaitzsch, L. Messager, S. P. Armes, G. Battaglia, *Polym. Chem* **2015**, *6*, 2065-2068.
[5] B. Alberts, A. Johnson, P. Walter, J. Lewis, M. Raff, K. Roberts, *Molecular Biology of the Cell*, Fifth ed., Garland Science, Taylor & Francis Group, New York ,USA, **2008**.
[6] T. Smart, H. Lomas, M. Massignani, M. V. Flores-Merino, L. R. Perez, G. Battaglia, *Nanotoday* **2008**, *3*, 38-46.
[7] aC. LoPresti, H. Lomas, M. Massignani, T. Smart, G. Battaglia, *J. Mater. Chem.* **2009**, *19*, 3576 - 3590; bL. Ruiz-Pérez, L. Messager, J. Gaitzsch, A. Joseph, L. Sutto, F. L. Gervasio, G. Battaglia, *Science Advances* **2016**, *2, e1500948* ; cJ. Bain, L. Ruiz-Pérez, A. J. Kennerley, S. P. Muench, R. Thompson, G. Battaglia, S. S. Staniland, *Scientific Reports* **2015**, *5*; dA. Joseph, C. Contini, D. Cecchin, S. Nyberg, L. Ruiz-Perez, J. Gaitzsch1, G. Fullstone, X. Tian, J. Azizi, J. Preston, G. Volpe, G. Battaglia, *Science Advances* **2017**, *3: e1700362*, 1-12.
[8] M. Antonietti, S. Forster, *Adv. Mater.* **2003**, *15*, 1323-1333.
[9] J. M. Ward, *Plant Physiol.* **1997**, *114*, 1151-1159.
[10] J. C. Mathai, G. D. Sprott, M. L. Zeidel, *J. Biol. Chem* **2001**, *276*, 27266-27271.
[11] K. Olbrich, W. Rawicz, D. Needham, E. Evans, *Biophys. J.* **2009**, *79*.
[12] E. O. Potma, W. P. d. Boeij, P. J. M. V. Haastert, D. A. Wiersma, *Proc. Natl. Acad. Sci.* **2001**, *98*, 1577-1582.
[13] A. Rumplecker, S. Forster, M. Zahres, C. Mayer, *J. Chem. Phys* **2004**, *120*, 8740-8747.
[14] G. Battaglia, A. J. Ryan, S. Tomas, *Langmuir* **2006**, *22*, 4910-4913.
[15] N. Hadjichristidis, H. Iatrou, S. Pispas, M. Pitsikalis, *J. Polym. Sci.,PartA: Polym. Chem* **2000**, *38*, 3211-3234.
[16] G. Battaglia, A. J. Ryan, S. Tomas, *Langmuir* **2006**, *22*, 4910-4913.

Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Polymeric vesicles, also called polymersomes, are highly efficient biomimetic systems. Membrane permeability and the ability to separate extreme pH gradients is a crucial condition a successful biomimetic system must meet. We show polymersomes formed by polybutadiene-b-polyethylene oxide (PBd-b-PEO) amphiphilic block copolymers engineer robust and stable membranes that are able to sustain pH gradients of 10 for a minimum of 8 days.



Lorena Ruiz-Pérez, Claire Hurley, Salvador Tomas, and Giuseppe Battaglia**

Page No. – Page No.

Separating extreme pH gradients using amphiphilic copolymer membranes