Proton-regulated rectified ionic transport through solid-state conical nanopores modified with phosphate-bearing polymer brushes†

Basit Yameen,[a] Mubarak Ali,[b] Reinhard Neumann,[c] Wolfgang Ensinger,[b] Wolfgang Knoll[d] and Omar Azzaroni*[e]

We describe the use of polyprotic polymer brushes to construct robust signal-responsive chemical devices mimicking the transport properties of proton regulated biological channels.

Molecular design of nanopore architectures with functional features comparable to biological machineries is attracting widespread interest among "nanoscientists" worldwide.[1] Undoubtedly, the advent of track-etching techniques has given a decisive impetus to the development of this exciting area of nanotechnology.[2] Single conical nanopores are able to rectify the ion transport flowing through them, which is in close resemblance to voltage-gated biological channels.[3] However, controlling the internal architecture and the chemical features of synthetic nanopores, as precisely as nature does in biological channels, is a non-trivial task. One of the central features that determine the rectifying characteristics of the conical nanopores is the accurate control over the surface properties of the pore walls.[4] During recent years it has been demonstrated that the rectifying characteristics of solid-state nanopores emerge due to a synergy of the entropic driving force caused by the channel asymmetry and the electrostatic effects due to the fixed charges on the pore wall.[5] On the other hand, the efficiency by which many pore-forming proteins regulate the ionic transport through biological membranes using pH as a chemical trigger has been a source of inspiration for chemists to mimic such processes using solid-state nanopores.[6] For example, the M2 protein from influenza A, a well-known pH-gated ion channel, suffers conformational and electrostatic switches upon protonation of its histidine residues. The variation from uniprotonated to biprotonated states enables the modulation and fine tuning of the ion transport through the pore.[7] This example illustrates how nature controls the ion transport through membranes by tuning the electrostatic characteristics of the residues confined in the inner environment of the pore. With regard to solid-state nanopores, this poses challenging

situations that need to be faced to further expand the molecular design of fully "abiotic" nanopores. Of particular interest, and central to this work, is the quest for switchable nanopore machineries capable of generating multiple protonation states in such a way of tuning and controlling the transmembrane ion transport over a wide range of environmental pHs. From a chemical point of view, this would imply incorporating chemical groups displaying multiple pK_a values[8] in order to better regulate the protonation state of the pore walls. Recently, we have demonstrated that polymer brushes represent highly versatile building blocks to manipulate with nanoscale control the electrostatic environments of nanochannels and to significantly increase the surface density of functional groups.[9] Herein, we describe the construction of a solid-state nanopore-based chemical device whose permselectivity and rectifying properties are enhanced by using polyprotic polymer brushes as highly tuneable building blocks.

Single conical nanopores with tip diameter (d) ~ 16 nm and a base diameter (D) of ~ 290 nm, were fabricated by irradiation of polyethyleneterephthlate (PET) films with single swift heavy ions as described by Apel et al.[10] The single nanopore-containing membranes were modified with 4,4′-azobis(4-cyanopentanoic acid) as the surface-initiated polymerization initiator.[11] Then, the brush growth was accomplished by surface-initiated polymerization of the polyprotic monomer 2-(methacryloyloxy) ethyl phosphate (Fig. 1).[12] After a preset polymerization time the membranes were rinsed with deionized water and mounted in the conductivity cell. Considering the polyprotic nature of the monomer units (Fig. 2) we can infer that at strongly acidic pHs (pH $<$ pK_1) the dominant species in the brush are fully protonated MEP units (MEPH$_2$). As is well-known, the presence of rectification requires surface charges.[4] In fact, at pH $=$ 3 the conical nanopore displays no surface charges and consequently, no rectification is observed in the corresponding I–V curve (Fig. 3). The chemical richness of the polyprotic brush lies in the fact that the equilibrium of the monomer units involves a variety of charged states that are thermodynamically controlled by the pH value. So, proton concentration acts as an accurate chemical parameter responsible for setting well-defined electrostatic conditions in the pore. Hence, when pH is changed from 3 to 4, the population of "monovalent" charged units (MEPH$^-$) should grow at the expense of the neutral species (MEPH$_2$), thus giving origin to a charged pore (Fig. 2).[13]

As expected, at pH $=$ 4 the I–V curve displays a well-defined rectification behaviour according to the permselective

[a] Max-Planck-Institut für Polymerforschung, Mainz, Germany
[b] Technische Universität Darmstadt, Darmstadt, Germany
[c] GSI Helmholtzzentrum für Schwerionenforschung, Darmstadt, Germany
[d] Austrian Institute of Technology, Vienna, Austria
[e] Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA) – Universidad Nacional de La Plata – CONICET, CC. 16 Suc. 4, 1900 La Plata, Argentina.
E-mail: azzaroni@inifta.unlp.edu.ar; Fax: +54 221 425 4642; Tel: +54 221 425 7430; Web: http//softmatter.quimica.unlp.edu.ar
† Electronic supplementary information (ESI) available: Experimental details of the preparation and characterization of the brush-modified nanopores. See DOI: 10.1039/b920870d
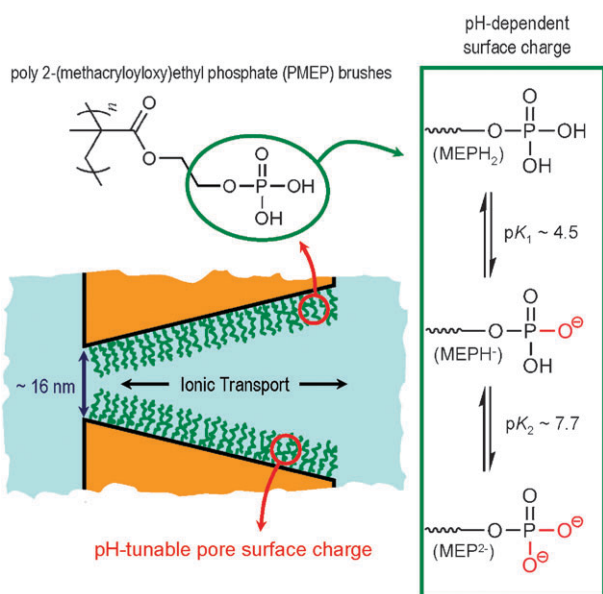
Fig. 1 Schematic depiction corresponding to the PMEP brush-modified conical nanochannel. The chemical structure of the polymer brush and the equilibrium associated to the pH-dependent behaviour of the polyprotic monomer units are also indicated. The pK values were taken from the literature (ref. 8).
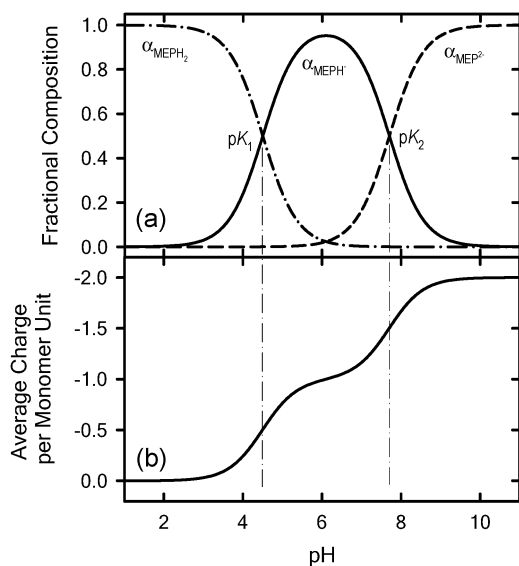

Fig. 2 (a) Fractional composition diagram for MEP monomer units. (b) Average charge per MEP monomer unit as a function of pH. The distribution of unprotonated (dashed trace), monoprotonated (solid trace) and biprotonated (dashed-dotted trace) species was calculated considering $pK_1 = 4.5$ and $pK_2 = 7.7$.

transport of cationic species through the negatively charged nanopore, i.e.: cation channel. In this case, the degree of rectification (f_{rec}), defined as the ratio between currents measured at voltages of the same amplitude but opposite polarities, was 6. These results are comparable to f_{rec}s obtained in single conical nanopores modified with monolayer assemblies.[4,14]

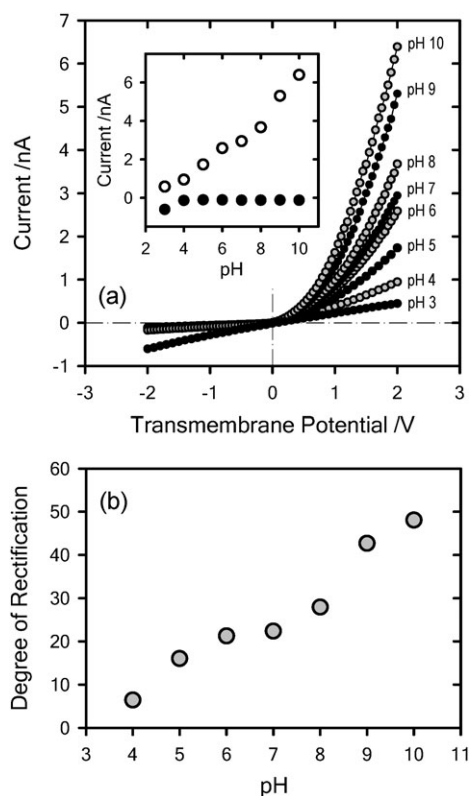The use of polyprotic brushes, instead of monolayer assemblies, provides a unique tool to increase the density of

Fig. 3 (a) I–V curves corresponding to a single conical nanopore modified with poly 2-(methacryloyloxy)ethyl phosphate brushes measured at different pH values (using 0.1 M KCl as electrolyte). The inset describes the changes in the transmembrane currents measured at 2 V (○) and −2 V (●). (b) Representation of the rectification efficiency (f_{rec}) of the PMEP-modified nanopore as a function of pH.

functional groups (brush-type architecture) and finely tune the surface charge of the pore walls (polyprotic equilibrium).[15] Recent theoretical work by Hänggi and co-workers demonstrated that the rectification efficiency in conical nanopores is strongly dependent on their surface charge.[5b] In this context, dressing the inner walls of the nanochannel with a polymer brush could introduce a facile chemical route to boost the f_{rec} values and further enhance the permselective properties of the pore.

The fractional composition diagram for MEP (Fig. 2) evidences that the average charge per monomer unit can be adjusted via regulation of the environmental pH (Fig. 2). This fact indicates that the polyprotic brush would enable the tuning of the rectified permselective transport in close resemblance to a pH-actuated "electrostatic nanovalve". Fig. 3a reveals that increasing the pH above 4 resulted in a significant increase of the rectification behaviour displayed by the pore. This increase was not solely reflected by the magnitude of the measured transmembrane currents (Fig. 3a), f_{rec} values also evidenced a quantitative enhancement. Changing pH from 3 to 4 (Fig. 3a) led to a decrease in the absolute values of the transmembrane currents measured at −2 V from 660 to 140 pA, whereas the ionic currents measured at the reversed bias, i.e: +2 V, increased from 409 to 950 pA. This experimental observation reflects that the strong cation permselective properties readily emerge upon the right

environmental conditions. Furthermore, increasing pH from 4 to 10 led to a continuous increase in rectified transmembrane currents (Fig. 3b), thus reflecting the ability of the MEP brush-modified nanopore to act as a selective "electrostatic nanovalve" precluding and boosting the transmembrane anionic and cationic transport, respectively (Fig. 3a, inset). The remarkable resemblance between the increasing variation of the average charge of MEP monomers (Fig. 2b) and the rectification efficiency (Fig. 3b) as a function of pH clearly illustrates the critical role of the surface charges in enabling highly permselective nanopores. The use of polyprotic brushes provided a facile route for building-up nanofluidic devices with different transport regimes. Below pK_1 (pH = 3) the nanopore displayed a low-conductance non-permselective state. Conversely, above pK_1 the nanopore reflected well-defined tunable rectifying properties in agreement with the pH-dependence of the average charge of the MEP units. Increasing the environmental pH from 3 (non-rectifying regime) to 10 (rectifying regime) led to a 16-fold increase in transmembrane ionic currents, from 409 pA to 6.42 nA. In a similar way, f_{rec} evidenced an order of magnitude increase upon changing the pH from 4 to 10. At pH > pK_2 the brushes were fully deprotonated, thus leading to the maximum surface charge confined in the pore. In these cases, f_{rec} reached values close to 50, one order of magnitude higher than the typical values obtained from conical pores modified with monolayer assemblies.

In summary, the modification of single conical nanopores with polyprotic macromolecular building blocks led to a nanodevice whose electronic readout, derived from the transmembrane ion current, can be finely tuned over a wide range of pH. The electrostatic characteristics of the thermo-dynamically controlled environments arising from the multiple protonation states of phosphate groups are responsible for tuning the transmembrane ionic transport and the rectification properties of the pore. This work illustrates the potential and versatility of polyprotic pH-responsive macromolecular building blocks as a key enabler to achieve integrated functional systems and addressable chemical nanodevices mimicking the transport properties and gating functions of specific biological channels.

Notes and references

1 (a) L. A. Baker and S. P. Bird, *Nat. Nanotechnol.*, 2008, **3**, 73; (b) C. R. Martin and Z. Siwy, *Science*, 2007, **317**, 331; (c) S. Howorka and Z. Siwy, *Chem. Soc. Rev.*, 2009, **38**, 2360; (d) C. Dekker, *Nat. Nanotechnol.*, 2007, **2**, 209; Fu, H. Tokuhisa and L. A. Baker, *Chem. Commun.*, 2009, 4877; (e) M. Wanunu and A. Meller, *Nano Lett.*, 2007, **7**, 1580; (f) I. Vlassiouk and Z. S. Siwy, *Nano Lett.*, 2007, **7**, 552; (g) X. Hou, W. Guo, F. Xia, F.-Q. Nie, H. Dong, Y. Tian, L. Wen, L. Wang, L. Cao, Y. Yang, J. Xue, Y. Song, Y. Wang, D. Liu and L. Jiang, *J. Am. Chem. Soc.*, 2009, **131**, 7800; M. Ali, B. Yameen, R. Neumann, W. Ensinger, W. Knoll and O. Azzaroni, *J. Am. Chem. Soc.*, 2008, **130**, 16351.

2 (a) A. A. Lev, Y. E. Korchev, T. K. Rostovtseva, C. L. Bashford, D. T. Edmonds and C. Pasternak, *Proc. R. Soc. London, Ser. B*, 1993, **252**, 187; (b) C. A. Pasternak, G. M. Alder, P. Y. Apel, C. L. Bashford, Y. E. Korchev, A. A. Lev, T. K. Rostovtseva and N. I. Zhitariuk, *Nucl. Instrum. Methods Phys. Res., Sect. B*, 1995, **105**, 332; (c) C. L. Bashford, *Biosci. Rep.*, 1995, **15**, 553.

3 (a) L. T. Sexton, L. P. Horne and C. R. Martin, *Mol. BioSyst.*, 2007, **3**, 667; (b) Z. Siwy, *Adv. Funct. Mater.*, 2006, **16**, 735; (c) C. Wei, A. J. Bard and S. W. Feldberg, *Anal. Chem.*, 1997, **69**, 4627; (d) Z. Siwy and A. Fuliński, *Phys. Rev. Lett.*, 2002, **89**, 198103; (e) Z. Siwy, Y. Gu, H. A. Spohr, D. Baur, A. Wolf-Reber, R. Spohr, P. Apel and Y. E. Korchev, *Europhys. Lett.*, 2002, **60**, 349.

4 Z. Siwy, E. Heins, C. C. Harrell, P. Kohli and C. R. Martin, *J. Am. Chem. Soc.*, 2004, **126**, 10850.

5 (a) J. Cervera, B. Schiedt, R. Neumann, S. Mafé and P. Ramírez, *J. Chem. Phys.*, 2006, **124**, 104706; (b) I. D. Kosińska, I. Goychuk, M. Kostur, G. Schmid and P. Hänggi, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2008, **77**, 031131.

6 (a) Y. Ito, Y. S. Park and Y. Imanishi, *J. Am. Chem. Soc.*, 1997, **119**, 2739; (b) Y. Ito and Y. S. Park, *Polym. Adv. Technol.*, 2000, **11**, 136.

7 (a) L. H. Pinto, L. J. Holsinger and R. A. Lamb, *Cell*, 1992, **69**, 517; (b) I. Kass and I. T. Arkin, *Structure*, 2005, **13**, 1789; (c) A. Okada, T. Miura and H. Takeuchi, *Biochemistry*, 2001, **40**, 6053; (d) H. Takeuchi, A. Okada and T. Miura, *FEBS Lett.*, 2003, **552**, 35.

8 J. Zhang, J. Kirkham, C. Robinson, M. L. Wallwork, D. A. Smith, A. Marsh and M. Wong, *Anal. Chem.*, 2000, **72**, 1973.

9 (a) B. Yameen, M. Ali, R. Neumann, W. Ensinger, W. Knoll and O. Azzaroni, *J. Am. Chem. Soc.*, 2009, **131**, 2070; (b) B. Yameen, M. Ali, R. Neumann, W. Ensinger, W. Knoll and O. Azzaroni, *Nano Lett.*, 2009, **9**, 2788; (c) B. Yameen, M. Ali, R. Neumann, W. Ensinger, W. Knoll and O. Azzaroni, *Small*, 2009, **5**, 1287; (d) A. Calvo, B. Yameen, F. J. Williams, G. J. A. A. Soler-Illia and O. Azzaroni, *J. Am. Chem. Soc.*, 2009, **131**, 10866; (e) A. Calvo, B. Yameen, F. J. Williams, O. Azzaroni and G. J. A. A. Soler-Illia, *Chem. Commun.*, 2009, 2553.

10 P. Y. Apel, Y. E. Korchev, Z. Siwy, R. Spohr and M. Yoshida, *Nucl. Instrum. Methods Phys. Res., Sect. B*, 2001, **184**, 337.

11 (a) W. Huang, G. Skanth, G. L. Baker and M. L. Bruening, *Langmuir*, 2001, **17**, 1731; (b) O. Prucker and J. Ruhe, *Langmuir*, 1998, **14**, 6893.

12 F. Zhou and W. T. S. Huck, *Chem. Commun.*, 2005, 5999.

13 D. C. Harris, *Quantitative Chemical Analysis*, W.H. Freeman & Company, New York, 6th edn, 2002, p. 16.

14 (a) M. Ali, P. Ramírez, S. Mafé, R. Neumann and W. Ensinger, *ACS Nano*, 2009, **3**, 603; (b) M. Ali, B. Schiedt, K. Healy, R. Neumann and W. Ensinger, *Nanotechnology*, 2008, **19**, 08713.

15 (a) R. Advincula, in *Surface-Initiated Polymerization I*, ed. R. Jordan, Springer-Verlag, Heidelberg, 2006, p. 107; (b) O. Azzaroni, A. A. Brown and W. T. S. Huck, *Adv. Mater.*, 2007, **19**, 151–154; (c) O. Azzaroni, A. A. Brown and W. T. S. Huck, *Angew. Chem., Int. Ed.*, 2006, **45**, 1770; (d) A. A. Brown, O. Azzaroni and W. T. S. Huck, *Langmuir*, 2009, **25**, 1744; (e) J. Rühe, W. Knoll, in *Supramolecular Polymers*, ed. A. Ciferri, Marcel Dekker, New York, 2000, ch. 13, p. 565.