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Anion-exchangeable polymer vesicles through the self-assembly of hyperbranched polymeric ionic liquids

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The work reports the self-assembly of anion-exchangeable vesicles from an amphiphilic hyperbranched polymeric ionic liquid (HBPIL). By a simple one-step anion exchange with methyl orange, the obtained HBPILs could self-assemble into pH-indicative and colorful vesicles in water with color changes directly visible to naked eyes in response to solution pH. In addition, by another step of anion exchange with bovine serum albumin (BSA), the BSA-coated vesicles could also be readily prepared.

In the realm of rapidly developing families of ionic liquids,[1] polymeric ionic liquids (PILs), including IL moieties either in their backbone or side chains, has drawn a great deal of interest in recent years.[2] Since they combine IL properties such as negligible vapor pressure, ionic conductivity and anion exchanging abilities with the improved mechanical durability and dimensional control of polymers,[3] PILs could be applied in many areas such as support for catalysts,[4] ion conductors,[5] construction of porous polymers[6] and so on.[7] However, it should be noted that the reported PILs are mainly based on one-dimensional linear polymers. Hyperbranched polymer is such a kind of three-dimensional polymer possessing lots of excellent properties such as low viscosity, large inner cavities and numbers of surface functionalities.[8] However, few hyperbranched PILs (HBPILs) have been reported up to now.[9]

In addition, recently, self-assembly of amphiphilic PILs have also been extensively studied owing to the tunable performance of the obtained nanostructures. However, most of these nanostructures are limited to micelles.[7a, 10] Although vesicles are very attractive and promising in drug delivery, nanoreactors, and artificial organelles, the PIL-based polymer vesicles have seldom been reported.[11,12]

Herein, we report the self-assembly of a novel amphiphilic HBPIL into vesicles. As illustrated in Scheme 1, the amphiphilic HBPIL was prepared through the graft of 1-methylimidazole (MIM) groups onto the terminal of HBPO-star-PEO (abbreviated as HsP) (HBPO = hyperbranched poly(3-ethyl-3-oxetanemethanol), PEO = poly(ethylene glycol)), following a procedure reported by Mülhaupt et al.[9a] At the beginning, MIM groups were complexated with OTs negative ions to form HsP-MIM/OTs. Subsequently, the OTs negative ions were exchanged by methyl orange (MO) ions, and the

obtained amphiphilic HsP-MIM/MOs could self-assemble into pH-responsive vesicles in water with many MIM$^+$/MO$^-$ ion pairs hanging on the surface of the vesicles. Benefiting from the excellent anion-exchanging capacities of ion liquids, the obtained polymer vesicles could further exchange with some specific anion-containing compounds to obtain new surface-modified vesicles, for example, the BSA-coated vesicles.

The amphiphilic hyperbranched multiarm copolymer HsP (Mn = 8,500 Da) was synthesized through cationic ring-opening polymerization (CROP) according to our previous method.[13] It consisted of a hydrophobic hyperbranched HBPO core (Mn= 4,300 Da, degree of branching (DB) = 43%) and many hydrophilic PEO arms (degree of polymerization (DP) = 2). Subsequently, partial hydroxyl groups of PEO arms were tosylated and then further reacted with MIMs to get amphiphilic HBPIL with MIMs as cationic parts and OTs as anionic parts, that is, HsP-MIM/OTs (Figs. S1 and S2, ESI†). The obtained HsP-MIM/OTs with a OTs percentage grafting of 16% are highly water soluble and completely dissolved in water. Thus, hydrophobicity must be increased to induce self-assembly behavior.
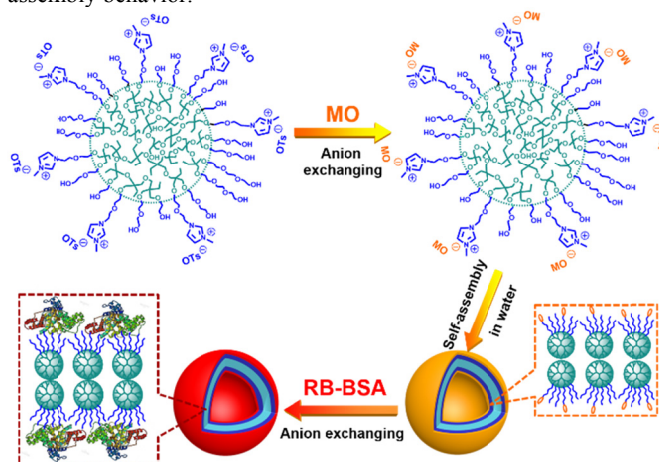
Scheme 1 Synthesis and self-assembly of HBPILs. The hydrophobic HBPO core is in light blue, the hydrophilic PEO arms and MIM$^+$/MO$^-$ ion pairs are in blue, the MO counterions are in orange and RB-BSAs are in red.
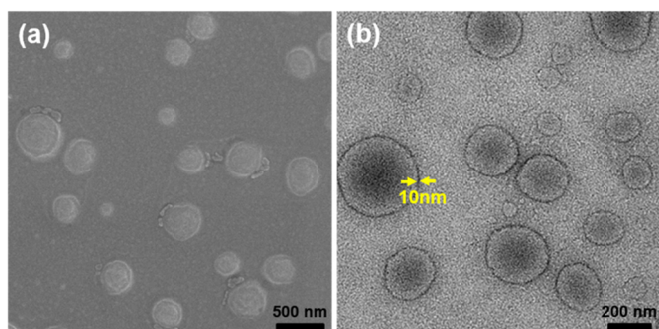
Fig. 1 Self-assemblies from HsP-MIM/MOs in water captured by SEM (a) and TEM (b).

For this purpose, HsP-MIM/OTs were exchanged with MO anions through a dialysis method. The ^{1}H NMR and FTIR spectra proved that all OTs anions had been exchanged by MO anions after dialysis (Figs. S3-S5, ESI†). The obtained HsP-MIM/MOs are no longer water soluble and will form a turbid solution in water, indicating the formation of aggregates. The scanning electron microscope (SEM) measurement shows the aggregates are spherical particles collapsing on the substrate (Fig. 1a). The transmission electron microscope (TEM) image shows the particles are vesicles since there is a clear contrast difference between the boundary and the inner pool of each particle (Fig. 1b).[13] The average diameter of HsP-MIM/MO vesicles is about 320 nm through the statistical analysis of 30 vesicles from the TEM images. Although the vesicle size is polydisperse according to the TEM results, the wall thickness of vesicles is uniform and around 10 nm, which is equal to the length of two HsP molecules.[13,14] Hence, we deduced that HsP-MIM/MO vesicles might possess a bilayer structure as shown in Scheme 1.

The HsP-MIM/MO vesicles show an interesting pH chromism phenomenon. The vesicle solution changed its color from red to yellow, and became more and more turbid when the solution pH was increased from 1.50 to 9.08 (Fig. 2a). In the UV-vis absorption spectra, the wavelength of maximum absorbance (λ_{max}) of the vesicle solution shows a reverse sigmoid dependence on the solution pH: it does not change a lot at the beginning, then decreases dramatically and finally levels off again. Meanwhile, the size of HsP-MIM/MO vesicles also shows a pronounced dependence on solution pH according to dynamic light scattering (DLS) measurements (Figs. 2b and 2c). At the solution pH=1.50 or 2.08, only small particles with the average hydrodynamic diameter (D_h) below 5 nm were observed, indicating the disassembly of the HsP-MIM/MO vesicles into unimers at these strong acidic conditions. Then, the D_h of particles increases from 100 nm to 400 nm with the increase of solution pH from 2.87 to 5.17 and then keeps constant, indicating the reformation of polymer vesicles. The D_h variation interval is from pH=2 to 5, which is similar to that of the UV-vis experiment (Fig. S6, ESI†).

The pH responsibility of the HsP-MIM/MO vesicles should be originated from MO moieties since only MO can cause color changes in the vesicles. As a direct evidence, the λ_{max} in the UV-vis absorption spectra of pure MO dyes displays a similar dependence on solution pH with a pH-jump ranging from 2 to 5 when compared to that of HsP-MIM/MO vesicles (Fig. S7b, ESI†). Thus, the pH-dependent size changes of HsP-MIM/MO vesicles can be easily understood according to the ionization balance of MO. At the strong acidic condition, MO is in a more hydrophilic state with a new positive-charged nitrogen atom (state B in Fig. 2d), and thus the HsP-MIM/MO vesicles were disassembled into unimers at pH=1.50 or 2.08 probably due to strong electrostatic repulsion among unimers; With the increase of solution pH, MO takes a more hydrophobic state (state A in Fig. 2d), and thus the HsP-MIM/MO vesicles were
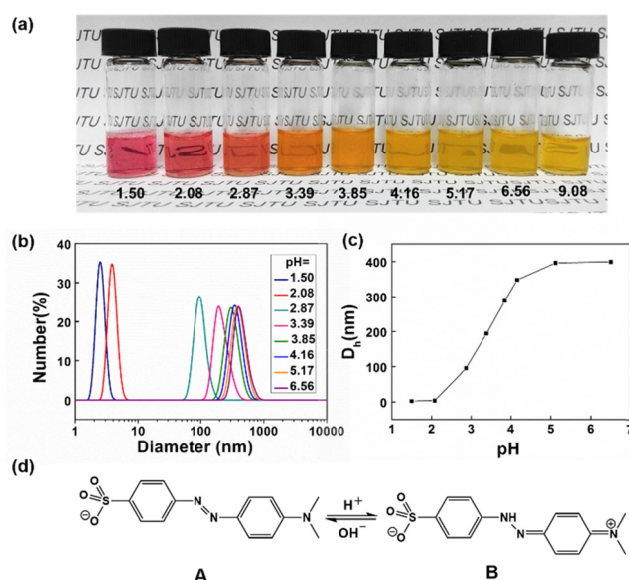

Fig. 2 pH chromism characterizations. Digital photos (a) and number-averaged size distributions (b) of HsP-MIM/MO vesicle solutions at various pH. (c) The plot of D_h versus solution pH. (d) pH responsive mechanism of a MO molecule.

reformed due to increased hydrophobic interaction. The continuous increase of vesicle size might be induced by the fusion of vesicles driven by the increased hydrophobicity of vesicles originating from MO moieties.

It should be noted that there are still some differences in the pH responsibility of HsP-MIM/MO vesicles and pure MOs. The λ_{max} jump of the HsP-MIM/MO vesicles in the λ_{max} vs pH plot is sharper than that of pure MO, and there is also an evident blue-shift (Fig. S7b, ESI†) in the HsP-MIM/MO vesicles. These differences might be related to the crowded MO groups on the surface of the vesicles, which leads to locally high concentration of MO groups for the quick response and a certain extent of π-π stacking for the blue-shift. In addition, the functionality of hyperbranched PILs is of great importance to determine the self-assembly behaviour. It was found that HsP-MIM/OTs with 5% OTs groups directly self-assembled into vesicles in water. After anion exchange with MO, the HsP-MIM/MO vesicles did not show obvious pH-responsibility. Besides, the HsP-MIM/OTs containing 35% OTs groups are totally water soluble. After anion exchange with MO, the obtained HsP-MIM/MOs are still too hydrophilic to self-assemble in water probably. Only the HsP-MIM/OTs with 16% OTs groups show an interesting pH-responsive self-assembly behaviour.

pH-indicative vesicles with color changes visible to naked eyes are very challenging to be constructed.[15] However, herein, we can used a simple anion exchange method to fulfill it. Furthermore, the obtained HsP-MIM/MO vesicles can be further functionalized through another step of anion exchange, for example the vesicles functionalized by proteins. There are abundant carboxyl anions in a single BSA protein, so it can exchange with the MO anions in HsP-MIM/MO vesicles to get BSA-coated vesicle, a so-called HsP-MIM/BSA vesicles.

As proof-of-principle experiments, an aqueous solution of rhodamine-B (RB) labelled BSA (RB-BSA) (2.0 mg/mL) was added into the HsP-MIM/MO vesicle solution (0.5 mg/mL), and the mixed solution was stirred for 30 min followed by dialysis against water for one day (MWCO: 3,500 Da) and (MWCO: 100,000 Da) for two days, respectively. Then the final pink and turbid solution in dialysis bag was collected and characterized by optical and fluorescent microscopes as well as UV-Vis spectrometer, separately. According

to the optical micrograph, giant particles around 10 μm were obtained (Fig. 3a). The fluorescent micrograph indicates these particles are giant vesicles, and the vesicle structure can be discerned from the contrast difference between bright red periphery and dark inner pool (Fig. 3b). The strong red fluorescence in each vesicle supports the RB-BSA had already been coated onto the vesicles.

The UV-Vis absorption spectra can provide further evidence to prove the BSA functionalization on vesicles. HsP-MIM/MO vesicles had an adsorption peak at 451 nm in neutral water assigned to MO
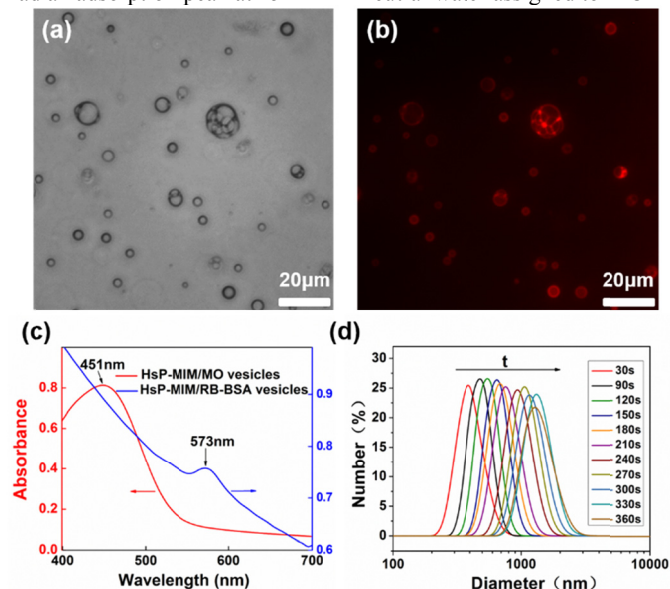

Fig. 3 Characterizations on protein-coated vesicles. Optical microscope (a) and fluorescent micrographs (b) of HsP-MIM/RB-BSA vesicles. (c) UV-Vis absorption spectra of HsP-MIM/MO solution before and after anion exchanging with RB-BSA. (d) Real-time number-averaged size distributions of HsP-MIM/MO solution (1 mL, 0.5 mg/mL) after the addition of 300 μL RB-BSA solution (2.0 mg/mL).

moieties, however, this peak disappeared after exchanged with BSAs, and a new adsorption peak at 573 nm appeared (Fig. 3c). This new peak should be attributed to the absorption peak of RB-BSA moieties (Fig. S8, ESI†). So the UV-Vis spectra further indicated that the MO anions in the HsP-MIM/MO vesicles have been totally exchanged by BSAs to obtain HsP-MIM/BSA vesicles.

During the experiments, we also noted a big size difference between the HsP-MIM/MO and HsP-MIM/BSA vesicles. The HsP-MIM/MO vesicles are around 300 nm in water, however, the HsP-MIM/BSA vesicles are around 10 μm. What is the mechanism under it? We supposed the large size of HsP-MIM/BSA vesicles was induced by vesicle fusion. There are two evidence to support this hypothesis. Firstly, some intermediates of fusing vesicles, the aggregated vesicles, can be directly seen in the optical/fluorescent vesicle image (Figs. 3a and 3b). Secondly, the size of HsP-MIM/MO vesicles after the addition of BSAs was continuously tracked by DLS, and it showed a continuous increase of size from 300 nm to micron size after incubated for 330s. Vesicle fusion will lead to the mixing of vesicle content and the molecular rearrangements in the vesicle membrane, which can explain why all the MO moieties, either in the outside or inside surface of the HsP-MIM/MO vesicles, were exchanged by BSAs. Since the anions in BSAs (e. g. carboxyl groups) formed ion pairs with MIM cations and the BSAs became more restricted in conformation after BSAs were coated onto HsP-MIM/BSA vesicles, the hydrophobicity of the coated BSAs as well as the vesicles was greatly enhanced. Thus, the aggregation and

fusion of the vesicles might be driven by the enhanced intervesicular hydrophobic interactions originating from coated BSAs.

In conclusion, a pH-responsive and colorful polymer vesicles have been successfully prepared through the aqueous self-assembly of an amphiphilic hyperbranched polymeric ionic liquid with methyl orange as the counteranions. In addition, the vesicles can be further functionalized with proteins through another step of anion exchange. In fact, this type of anion-exchangeable vesicles can be functionalized with all kinds of anions through a simple anion exchange process. The advantage of facile functionalization makes this type of vesicles quite unique when compared with other polymer vesicles as well as in subsequent applications.

Notes and references

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1. (a) R. D. Rogers and K. R. Seddon, *Science*, 2003, **302**, 792; (b) N. V. Plechkova and K. R. Seddon, *Chem. Soc. Rev.*, 2008, **37**, 123; (c) T. Welton, *Chem. Rev.*, 1999, **99**, 2071; (d) R. Sheldon, *Chem. Commun.*, 2001, 2399; (e) Z. Bai and T. P. Lodge, *J. Am. Chem. Soc.*, 2010, **132**, 16265; (f) Y. Y. He, Z. B. Li, P. Simone and T. P. Lodge, *J. Am. Chem. Soc.*, 2006, **128**, 2745; (g) S. So and T. P. Lodge, *J. Phys. Chem. C*, 2014, **118**, 21140.

2. (a) C. M. LangmuirStancik, A. R. Lavoie, P. A. Achurra, R. M. Waymouth and A. P. Gast, *Langmuir*, 2004, **20**, 8975; (b) Y. J. Men, D. Kuzmicz and J. Y. Yuan, *Curr. Opin. Colloid Interface Sci.*, 2014, **19**, 76; (c) M. Q. Cai, X. Q. Wei, C. H. Du, X. M. Ma and M. C. Jin, *J. Chromatogr. A*, 2014, **1349**, 24; (d) L. Y. Chang, C. P. Lee, C. T. Li, M. H. Yeh, K. C. Ho and J. J. Lin, *J. Mater. Chem. A*, 2014, **2**, 20814; (e) D. D. Bendejacq and V. Ponsinet, *J. Phys. Chem. B*, 2008, **112**, 7996; (f) S. Amajjahe and H. Ritter, *Macromolecules*, 2008, **41**, 3250; (g) M. A. Ayoubi, K. Z. Zhu, B. Nystrom, K. Almdal, U. Olsson and L. Piculell, *Soft Matter*, 2013, **9**, 1540; (h) E. Karjalainen, N. Chenna, P. Laurinmaki, S. J. Butcher and H. Tenhu, *Polym. Chem.*, 2013, **4**, 1014.

3. (a) F. Gu, H. Dong, Y. Li, Z. Sun and F. Yan, *Macromolecules*, 2014, **47**, 6740; (b) B. Lin, L. Qiu, B. Qiu, Y. Peng and F. Yan, *Macromolecules*, 2011, **44**, 9642; (c) B. Lin, H. Dong, Y. Li, Z. Si, F. Gu and F. Yan, *Chem. Mater.*, 2013, **25**, 1858.

4. (a) R. N. Biboum, F. Doungmene, B. Keita, P. de Oliveira, L. Nadjo, B. Lepoittevin, P. Roger, F. Brisset, P. Mialane, A. Dolbecq, I. M. Mbomekalle, C. Pichon, P. C. Yin, T. B. Liu and R. Contant, *J. Mater. Chem*, 2012, **22**, 319; (b) Q. Zhao, P. F. Zhang, M. Antonietti and J. Y. Yuan, *J. Am. Chem. Soc.*, 2012, **134**, 11852.

5. (a) T. K. Carlisle, W. M. McDanel, M. G. Cowan, R. D. Noble and D. L. Gin, *Chem. Mater.*, 2014, **26**, 1294; (b) A. S. Shaplov, D. O. Ponkratov, P.H. Aubert, E. I. Lozinskaya, C. Plesse, F. Vidal and Y. S. Vygodskii, *Chem. Commun.*, 2014, **50**, 3191.

6. (a) S. Soll, Q. Zhao, J. Weber and J. Yuan, *Chem. Mater.*, 2013, **25**, 3003; (b) A. Wilke, J. Yuan, M. Antonietti and J. Weber, *ACS Macro Letters*, 2012, **1**, 1028; (c) Z. B. Zhang, Q. Zhao, J. Y. Yuan, M. Antonietti and F. H. Huang, *Chem. Commun.*, 2014, **50**, 2595; (d) Q. Zhao, M. J. Yin, A. P. Zhang, S. Prescher, M. Antonietti and J. Y. Yuan, *J. Am. Chem. Soc.*, 2013, **135**, 5549.

7. (a) J. Texter, *Macromol. Rapid Commun.*, 2012, **33**, 1996; (b) T.-P. Fellinger, A. Thomas, J. Yuan and M. Antonietti, *Adv. Mater.*, 2013, **25**, 5838; (c) J. Guo, L. Qiu, Z. Deng and F. Yan, *Polym. Chem.*, 2013, **4**, 1309; (d) J. M. Lu, F. Yan and J. Texter, *Prog. Polym. Sci.*, 2009, **34**, 431; (e) D. Mecerreyes, *Prog. Polym. Sci.*, 2011, **36**, 1629; (f) J. B. Tang, H. D. Tang, W. L. Sun, H. Plancher, M. Radosz and Y. Q. Shen, *Chem. Commun.*, 2005, 3325; (g) J. Y. Yuan and M. Antonietti, *Polymer*, 2011, **52**, 1469; (h) J. Y. Yuan, D. Mecerreyes and M. Antonietti, *Prog. Polym. Sci.*, 2013, **38**, 1009; (i) P. F. Zhang, J. Y. Yuan, T. P. Fellinger, M. Antonietti, H. R. Li and Y. Wang, *Angew. Chem. Int. Ed.*, 2013, **52**, 6028.
8. Y. Zhou and D. Yan, *Chem. Commun.*, 2009, 1172.
9. (a) F. Schuler, B. Kerscher, F. Beckert, R. Thomann and R. Mulhaupt, *Angew. Chem. Int. Ed.*, 2013, **52**, 455; (b) J.F. Huang, H. Luo, C. Liang, I. W. Sun, G. A. Baker and S. Dai, *J. Am. Chem. Soc.*, 2005, **127**, 12784.
10. (a) X. Li, X. Ni, Z. Liang and Z. Shen, *J. Polym. Sci., Part A: Polym. Chem.*, 2012, **50**, 2037; (b) J. Guo, Y. Zhou, L. Qiu, C. Yuan and F. Yan, *Polym. Chem.*, 2013, **4**, 4004; (c) M. Koebe, M. Drechsler, J. Weber and J. Yuan, *Macromol. Rapid Commun.*, 2012, **33**, 646; (d) P. M. Carrasco, A. Ruiz de Luzuriaga, M. Constantinou, P. Georgopanos, S. Rangou, A. Avgeropoulos, N. E. Zafeiropoulos, H.J. Grande, G. n. Cabañero, D. Mecerreyes and I. Garcia, *Macromolecules*, 2011, **44**, 4936; (e) C. Jin, T. Zhang, F. Liu, L. Wang, Q. Yin and D. Xiao, *RSC Advances*, 2014, **4**, 8216.
11. (a) R. P. Brinkhuis, F. P. J. T. Rutjes and J. C. M. van Hest, *Polym. Chem.*, 2011, **2**, 1449; (b) M. C. M. van Oers, F. P. J. T. Rutjes and J. C. M. van Hest, *Curr. Opin. Biotechnol.*, 2014, **28**,10; (c) C. Nardin, S. Thoeni, J. Widmer, M. Winterhalter and W. Meier, *Chem. Commun.*, 2000, 1433; (d)J. S. Lee and J. Feijen, *J. Control Release*, 2012, **161**, 473; (e) F. Meng, Z. Zhong and J. Feijen, *Biomacromolecules*, 2009, **10**, 197; (f) D. E. Discher and F. Ahmed, *Annu. Rev. Biomed. Eng.*, 2006, **8**, 323; (g) P. Tanner, P. Baumann, R. Enea, O. Onaca, C. Palivan and W. Meier, *Acc. Chem. Res.*, 2011, **44**, 1039.
12. (a) P. Coupillaud, M. Fevre, A. L. Wirotius, K. Aissou, G. Fleury, A. Debuigne, C. Detrembleur, D. Mecerreyes, J. Vignolle and D. Taton, *Macromol. Rapid Commun.*, 2014, **35**, 422; (b) K. Vijayakrishna, D. Mecerreyes, Y. Gnanou and D. Taton, *Macromolecules*, 2009, **42**, 5167; (c) J. Yuan, S. Soll, M. Drechsler, A. H. Muller and M. Antonietti, *J. Am. Chem. Soc.*, 2011, **133**, 17556. (d) M. Marguet, C. Bonduelle and S. Lecommandoux, *Chem. Soc. Rev.*, 2013, **42**, 512; (e) A, Carlsen and S. Lecommandoux, *Curr. Opin. Colloid Interface Sci.*, 2009, **14**, 329.
13. (a) Y. F. Zhou and D. Y. Yan, *Angew. Chem. Int. Ed.*, 2004, **43**, 4896; (b) Y. Zhou, D. Yan, W. Dong and Y. Tian, *J. Phys. Chem. B*, 2007, **111**, 1262.
14. W. Jiang, Y. Zhou and D. Yan, *Chem. Soc. Rev.*, 2015, DOI: 10.1039/c4cs00274a.
15. X. Zhang, S. Rehm, M. M. Safont-Sempere and F. Wurthner, *Nat. Chem.*, 2009, **1**, 623.

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