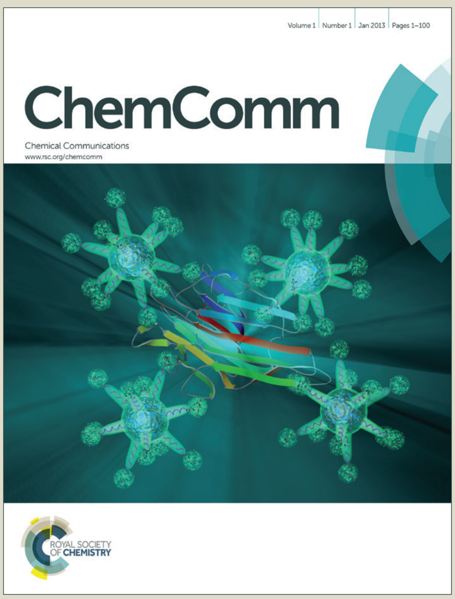
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ARTICLE TYPE

pH Responsive Polymersome Pickering Emulsion for Simple and Efficient Janus Polymersome Fabrication

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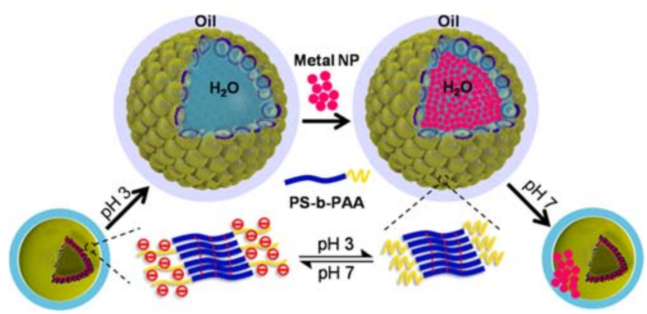
Crosslinked poly(acrylic acid)-b-polystyrene polymersomes were successfully employed to form a water-in-oil Pickering emulsion and enabled an easy and reversible disassembly due to the pH sensitivity. The side of the polymersomes exposed to the water phase was selectively modified with metal nanoparticles, allowing facile formation of anisotropically modified Janus polymersomes.

Polymersomes, self-assembled vesicles from amphiphilic block copolymers with a size range of 200–500 nm, have gained increasing interest due to their broad range of potential applications,[1] such as catalytic nanoreactors,[2] nanomotors[3] and drug delivery systems.[4] The structural stability of polymersomes surpasses that of conventional phospholipid-based liposomes under physical constraints, including extreme dilution. However, the polymersome hollow spherical shape with its subsequent isotropic surface properties sometimes hinders its usage in applications where particle anisotropy is required, for instance, in directed recognition and assembly processes, directional nanomotor movement and smart drug delivery systems. Therefore, scientists have recently focused on introducing anisotropic properties to the polymersome surface. These anisotropic polymersomes are called Janus polymersomes (JP),[5] which mostly have two different compositions and properties compartmentalized onto the same surface. Up to now, most successful attempts to fabricate Janus structures have been performed on solid particles,[5-6] such as silica, polystyrene beads and metal nanoparticles. A wide variety of methods has been utilized to synthesize these Janus particles, including self-assembly of block polymers,[7] toposelective modification,[8] microfluidic techniques,[9] electro-spinning,[10] controlled phase separation,[11] and Pickering emulsion interfacial synthesis.[12] However, most of the above-mentioned methods are not suitable for modifying soft hollow vesicle structures. Thus, examples of reported Janus capsules or polymersomes are rare. For instance, the only case in which platinum nanoparticles-modified Janus microcapsules were obtained was through an indirect way which consisted of first printing platinum nanoparticles (PtNP) onto a polymer multilayer-coated template, followed by removing the template.[13] Therefore, it is still a challenge to fabricate Janus polymersomes in a simple and efficient way.

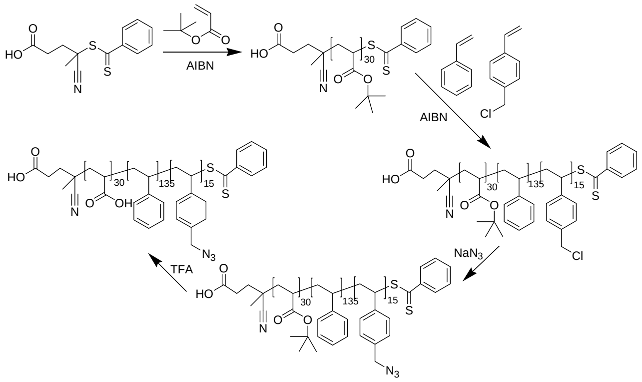


Scheme 1 Fabrication process of Janus Polymersomes (JP) by pH responsive Polymersome Pickering Emulsion (PPE) formation and disassembly.

Here we report a new strategy to fabricate JP by anisotropically modifying the surface of polymersomes that are employed in a polymersome Pickering emulsion (PPE). (Scheme 1). Water-in-oil Pickering emulsions can be conveniently stabilized by covalently crosslinked polymersomes, as was recently reported by the Armes[14] and our group.[15] As each of the polymersomes is in contact on one side with the oil and on the other side with the water phase, selective modification from either one of the phases is possible. Although there were some reports about fabrication of Janus solid particles using the Pickering emulsion method,[12] to our knowledge, this is the first example to modify Janus polymersomes through this technique. The advantages of the PPE method are that it creates spontaneously an anisotropic environment for modification and it can be conveniently applied to polymersomes without disrupting the spherical hollow structure.

To obtain a PPE of which the polymersomes can be effectively modified both covalently and non-covalently, we designed a polymer building block of which the hydrophilic domain consisted of poly(acrylic acid) (PAA). Furthermore, the pH sensitivity of the PAA corona enables the polymersomes to reversibly move away or toward the PPE interface upon changing pH, which is of great importance for obtaining single dispersed JPs after the anisotropic modification in the PPE state (Scheme 1).

As we reported earlier, due to the exposure of polymersomes to organic solvent in the PPE, it is necessary to stabilize the polymersomes by covalent coupling of the polymer components.[4c,14,15] Therefore, we combined the poly(acrylic acid) block with a hydrophobic domain containing azides as crosslinkable units. The desired poly(acrylic acid)-b-poly-(styrene-co-4-vinylbenzyl azide) (PAA-b-P(S-co-4-VBA)) polymer was prepared according to scheme 2. The first step involved the synthesis of the poly(*tert*-butyl acrylate)$_{30}$-chain



Scheme 2 Synthesis route toward block copolymer PAA$_{30}$-b-P(S$_{135}$-co-4VBA$_{15}$).

transfer agent (PtBA$_{30}$-CTA) via reversible addition–fragmentation chain-transfer polymerization (RAFT). Since azide moieties were not stable at elevated temperatures during polymerization, the second step was the extension of PtBA$_{30}$-CTA into a PtBA$_{30}$-b-poly-(styrene$_{135}$-co-4-vinylbenzyl chloride$_{15}$) (PtBA$_{30}$-b-P(S$_{135}$-co-4-VBC$_{15}$)) polymer by polymerizing styrene and 4-vinylbenzyl chloride (4-VBC) in a ratio of 90:10. Subsequent post-modification of the polymer with NaN_3 as the third step provided PtBA$_{30}$-b-P(S$_{135}$-co-4-vinylbenzyl azide$_{15}$) (PtBA$_{30}$-b-P(S$_{135}$-co-4-VBA$_{15}$)). The final step was the hydrolysis of *tert*-butyl acrylate to obtain PAA$_{30}$-b-P(S$_{135}$-co-4-VBA$_{15}$) with a number average molecular weight (Mn) of 20.8 kDa and a PDI of 1.25.

Polymersomes were prepared by the cosolvent method. The block copolymer was dissolved in a mixture of THF and 1,4-dioxane (v/v 1:3),which is a good solvent combination for both segments. To induce self-assembly of the amphiphiles, ultrapure water, as a precipitant for polystyrene, was slowly added to the solvent mixture until a content of 50 vol% was reached. As crosslinking procedure Copper(I)-catalyzed Azide–Alkyne Cycloaddition (CuAAC) was used between the crosslinker 4,7,10,13,16-pentaoxanonadeca-1,18-diyne and azide groups in the polymersome membrane. The crosslinking process was monitored by Fourier transform infrared (FTIR) spectroscopy. After stirring for 1 day at room temperature, the azide peak at 2095 cm^{-1} completely disappeared which indicated full crosslinking of the polymersomes (Figure S1). After dialysis of the cloudy suspension against water, the crosslinked polymersomes were characterized by transmission electron microscopy (TEM; Figure 1a). The dried polymersomes show typical folds and creases which indicate the spherical hollow structure. The thickness of the polymersome bilayer was estimated to be 40–50 nm. The average diameters of the spherical objects as determined by dynamic light scattering (DLS) were in

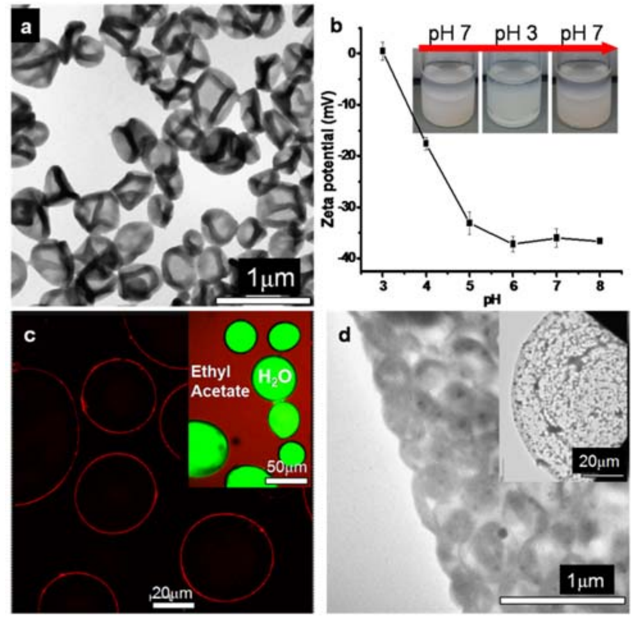


Fig. 1 a) TEM image of crosslinked polymersomes; b) zeta potential value of the polymersomes at pH 3-8, the inset photos show from left to right phase separation of the aqueous polymersome solution and ethyl acetate at pH 7, PPE formation at pH 3 and phase separation again at pH 7; c) CLSM images of PPE at pH 3 (water in ethyl acetate), the inset shows the PPE containing FITC-Dex (4.4 kDa) and Nile red dissolved in water and ethyl acetate, respectively; d) TEM images of polymersome Pickering emulsions, the inset is an overview image.

the range of 250–450 nm.

The formation and disassembly of the PAA-b-P(S-co-4-VBA) polymersome Pickering emulsions was then studied under different pH conditions. The poly(acrylic acid) corona, known as a weak polyelectrolyte, is sensitive to pH changes. At neutral or basic pH, the corona was negatively charged (zeta potential: -36.8±2.6 mV, Figure1b) so that the surface of polymersomes was very hydrophilic. Therefore, the polymersomes preferred to stay in the water phase resulting in no PPE formation at pH 7 (inset photo in Figure 1b). Upon decreasing the pH, the corona gradually lost its negative charges (zeta potential: 0.8±2.8 mV at pH 3) and became relatively hydrophobic. After homogenizing, the PPE could therefore be formed at pH 3 (inset photo in Figure1b). The organic solvent used was ethyl acetate. For ease of characterization, the polymersomes were labeled with the dye rhodamine B. Thus, the polymersome Pickering emulsion was observed as round spherical droplets by confocal laser scanning microscopy (CLSM) (Figure 1c), showing a droplet size in the range of 20–50μm. In order to clearly distinguish the water and ethyl acetate phases in the Pickering emulsion, fluorescein isothiocyanate labeled dextran (FITC-Dex, 4.4 kDa) and Nile red (NR) were dissolved in water and ethyl acetate, respectively. With green emission (FITC-Dex) in the droplet and red emission (NR) outside of the droplet, the water-in-ethyl acetate Pickering emulsion could be clearly identified by the merged CLSM image (inset image in Figure 1c).TEM (Figure 1d) images revealed that the surface of the intact Pickering emulsion droplet consisted of closely packed polymersomes, which collapsed as a result of the drying process. The PPE could be stored unchanged for more than 6 months due to the stable attachment of the polymersomes at the water/ethyl acetate interface as long as the pH of the water phase did not change. However, when the pH increased again, the polymersomes became negatively charged, leading to disassembly of PPE and re-dispersion of the polymersomes in water (inset photo in Figure1b). The intactness of the re-dispersed polymersomes was checked by TEM (Figure S2), which indicated that the crosslinked polymersomes were stable against organic solvent. The reversible PPE formation and disassembly process could be repeated several times.

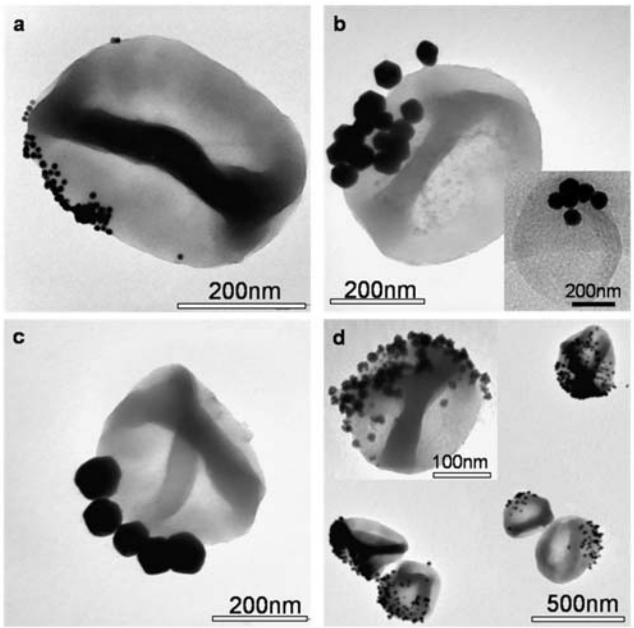


Fig. 2 a-c) TEM images of AuNP (10, 50, 80 nm) and d) PtNP modified JP. The inset image in b) is the Cryo-TEM image of AuNP (50 nm) modified JP, and the inset image of d) is the zoom-in TEM image of PtNP modified JP.

In order to demonstrate the possibility to use this method for the formation of JPs, we chose to modify the surface of the polymersomes with metal nanoparticles. We first employed gold nanoparticles (AuNP) to create JPs because of the strong coordination between AuNPs and poly(acrylic acid) and also AuNP's physico-chemical stability at different pHs. The polymersomes first formed a PPE at pH 3 as described before. The concentrated AuNP solution was then introduced to the water phase. After a quick homogenization step, AuNPs became attached to the part of the polymersome surface facing the water phase. Then the PPE was disassembled by increasing the pH to 7 and the AuNP modified polymersomes were re-dispersed in the water phase. After removing the ethyl acetate, the AuNP modified JP aqueous solution was stable for more than 6 months. Three differently sized AuNPs (diameter 10 nm, 50 nm and 80 nm) were selected to fabricate the JPs. As shown in the TEM images (Figures 2a-c, overview images in Figures S3-5), all three sizes of AuNPs were successfully attached to the polymersome surface, and they were all packed tightly on a small area of the polymersomes, which indicates the coordination between AuNPs and the poly(acrylic acid) polymersome corona only occurred on the surface area exposed to the water phase. The inset Cryo-TEM image of 50 nm AuNP modified JPs represents the in-situ anisotropic state of objects (inset image in Figure 2b). In case of the 10 nm AuNPs a polymersome decoration efficiency of around 50% was observed. This is a result of the fact that these small particles tend to cluster on the polymersome surface, and the concentration of AuNPs (10 times compared to the concentration of polymersomes) is not sufficient to reach a higher level of coverage. In the case of the other, bigger particles, clustering is less severe and the coverage is much higher, as indicated by TEM. The control experiments with a homogeneous mixture of polymersomes and AuNPs only led to the random attachment of AuNPs on the polymersomes, as shown in figure S6. In order to exclude the possibility that also AuNPs could act as colloidal stabilizing agents of Pickering emulsions, aqueous AuNP (50 nm) solutions with ethyl acetate were homogenized together. However, no AuNP-stabilized Pickering emulsion was obtained because of the high hydrophilicity of the AuNPs surface (citrate as ligands, Figure S7). In order to show the versatility of the procedure, PtNP-modified JPs were obtained following the same method (Figure 2d). The PtNPs were synthesized with polyvinylpyrrolidone (PVP) as capping agent, which enabled strong hydrogen bonding with poly(acrylic acid) especially at acidic conditions. Thus, the coverage of PtNPs on the polymersome surface was larger than that of AuNPs. The PtNP-modified JPs could possibly be applied as nanomotor due to the catalytic decomposition of hydrogen peroxide solutions in presence of Pt.[3]

In conclusion, the first simple and efficient preparation of Janus Polymersomes (JP) through a Polymersome Pickering Emulsion (PPE) route has been demonstrated and verified. pH-sensitive crosslinked PAA-b-P(S-co-4-VBA) polymersomes were shown to form and stabilize water/oil Pickering emulsions and disassemble to the dispersed state in aqueous solution at acidic and neutral pH, respectively. When the polymersomes were positioned at the water/oil interface in the PPE, the surface of the polymersomes exposed to the water phase was successfully modified by metal nanoparticles. This versatile and easy approach to Janus Polymersomes opens up many perspectives for the use of these structures in applications where anisotropic properties are desired.

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Notes and references

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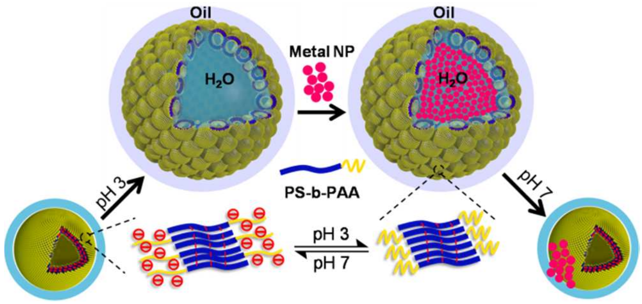
1. D. E. Discher, A. Eisenberg, *Science*, 2002, **297**, 967.
2. (a) S. F. M.van Dongen, H. P. M. de Hoog, R. Peters, M. Nallani, R. J. M. Nolte, J. C. M. van Hest, *Chem. Rev.*, 2009, **109**, 6212; (b) D. M. Vriezema, P. M. L. Garcia, N. S. Oltra, N. S. Hatzakis, S. M. Kuiper, R. J. M. Nolte, A. E. Rowan, J. C. M. van Hest, *Angew. Chem., Int. Ed.*, 2007, **46**, 7378; (c) P. Broz, S. Driamov, J. Ziegler, N. Ben-Haim, S. Marsch, W. Meier, P. Hunziker, *NanoLett.*, 2006, **6**,

2349; (d) J. Gaitzsch, D. Appelhans, L. Wang, G. Battaglia, B. Voit, *Angew. Chem., Int. Ed.*, 2012, **51**, 4448.

3. D. A. Wilson, R. J. M. Nolte, J. C. M. van Hest, *Nat. Chem.* 2012, **4**, 268.
4. (a) F. Ahmeda, R. I. Pakunlub, A. Brannanc, F. Batesc, T. Minkob, D. E. Discher, *Journal of Controlled Release,* 2006, **116**, 150; (b) D. E. Dischera, V. Ortizb, G. Srinivasb, M. L. Kleinb, Y. Kima, D. Christiana, S. Caia, P. Photosa, F. Ahmed, *Progress in Polymer Science,* 2007, **32**, 838; (c) M. C. M. van Oers, L. K. E. A. Abdelmohsen, F. P. J. T. Rutjes, J. C. M. van Hest, *Chem. Commun.*, 2014, **50**, 4040.
5. A. Walther, A. H. E. Müller, *Chem. Rev.*, 2013, **113**, 5194; A. Perro,S. Reculusa, S. Ravaine,E. Bourgeat-Lamic, E. Duguet, *J. Mater. Chem.*, 2005,**15**, 3745.
6. S. Jiang, Q. Chen, M. Tripathy, E. Luijten, K. S. Schweizer, S. Granick, *Adv.Mater.* 2010,**22**, 1060.
7. (a) L. Nie, S. Liu, W. Shen, D. Chen, M. Jiang, *Angew. Chem.,Int. Ed.* 2007, **46**, 6321; (b) F. Wurm, H. M. König, S. Hilf, A. F. M. Kilbinger, *J. Am. Chem. Soc.* 2008, **130**, 5876.
8. (a) Y. Lu, H. Xiong, X. Jiang, Y. Xia, M. Prentiss, G. M. Whitesides, *J. Am. Chem. Soc.* 2003, **125**, 12724; (b) H. Y. Koo, D. K. Yi, S. J. Yoo, D. Y. Kim, *Adv. Mater.* 2004, **16**, 274.
9. (a) D. Dendukuri, D. C. Pregibon, J. Collins, T. A. Hatton, P. S. Doyle, *Nat. Mater.* 2006, **5**, 365; (b) T. Nisisako, T. Torii, *Adv.Mater.* 2007, **19**, 1489.
10. K. H. Roh, D. C. Martin, J. Lahann, *Nat. Mater.* 2005, **4**, 759.
11. (a) C. L. Zhang, B. Liu, C. Tang, J. G. Liu, X. Z. Qu, J. L. Li, Z. Z. Yang, *Chem. Commun.* 2010, **46**, 4610; (b) C. Tang, C. L. Zhang, J. G. Liu, X. Z. Qu, J. L. Li, Z. Z. Yang, *Macromolecules* 2010, **43**, 5114.
12. (a) B. Liu, W. Wei, X. Qu, Z. Yang, *Angew. Chem. Int. Ed.,* 2008, **47**, 3973; (b) B. Liu, C. L. Zhang, J. G. Liu, X. Z. Qu, Z. Z.Yang, *Chem. Commun.* 2009, **45**, 387. (c) L. Petit, E. Sellier, E. Duguet, S. Ravaine, C. Mingotaud, *J. Mater. Chem.,* 2000, **10**, 253. (d) D. Suzuki, S. Tsuji, H. Kawaguchi, *J. Am. Chem. Soc.* 2007, **129**, 8088.
13. Y. Wu, Z. Wu, X. Lin, Q. He, J. Li, *ACS Nano*, 2012, **6**, 10910.
14. K. L. Thompson, P. Chambon, R. Verber, S. P. Armes, *J. Am.Chem. Soc.* 2012, **134,** 12450.
15. Z. Wang, M. C. M. van Oers, F. P. J. T. Rutjes, J. C. M. van Hest, *Angew. Chem. Int. Ed.,* 2012, **51**, 10746.



A versatile and easy to use method is reported for the preparation of Janus polymersomes via a Pickering emulsion strategy