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Supporting information

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

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1. Materials and methods

The HBPO-star-PEO copolymers were synthesized through cationic ring-opening polymerization (CROP) according to our previous method.[1,2] According to GPC, the number-average molecular weight (Mn) of the polymer is about 8500. Meanwhile, the polymer has a HBPO core with the Mn about 4,300 and degree of branching (DB) about 43%. In addition, the number-average degree of polymerization (DP_{arm}) of the PEO arm for the polymer is 2 according to ^{1}H NMR. 1-methylimidazole (J&K Chemical, 99%), *p*-toluenesulfonyl chloride (Sinopharm Chemical Reagent Co., Ltd, AR), rhodamine b (Sinopharm Chemical Reagent Co., Ltd, AR), methyl orange (J&K Chemical, AR), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, J&K Chemical, 99%), N-Hydroxysuccinimide (NHS, J&K Chemical, 98%) and bovine serum albumin (BSA, Sigma-Aldrich, 96%) were used as received. Chloroform ($CHCl_3$) and triethylamine (Sinopharm Chemical Reagent Co., Ltd, AR) were stored over calcium hydride and distilled under vacuum prior to use. All the other chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd and used as received.

The measurements such as ^{1}H nuclear magnetic resonance (^{1}H NMR), Fourier transform infrared (FT-IR), gel permeation chromatography (GPC), scanning electron microscope (SEM), transmission electron microscope (TEM), dynamic light scattering (DLS), fluorescent optical microscope and UV-vis absorption spectroscopy (UV-vis) were performed to characterize the constituents, structure, molecular weight, morphologies, absorption spectra and other properties of the polymers. ^{1}H NMR spectra with $CDCl_3$ or dimethylsulfoxide-d_6 (DMSO-d_6) as solvents were performed on a Varian Mercury Plus 400-MHz spectrometer at 298K. FT-IR spectra were measured as KBr pellets on a Perkin Elmer Paragon 1000 spectrophotometer in the range of 4000-450 cm^{-1}. The molecular weights of the products were measured by GPC on a HLC-8320GPC (TOSOH, EcoSEC GPC System) system at 40 ºC with N,N-dimethylformamide as mobile phase at a flow rate of 0.6 mL/min. SEM measurements were performed on Nova NanoSEM 450 (FEI), with an accelerating

voltage 5 kV. The samples for SEM observations were prepared by depositing several drops of the solution (1 mg/mL) onto the surfaces of clean silicon chips, and the samples were frozen in liquid nitrogen and freeze-dried in vacuum at -60 ºC for 24 hours. The samples were coated with a thin film of gold before measuring. TEM measurements were performed with a JEOL JEM-2100 instrument at a voltage of 200 kV. The samples were prepared by dropping solution (0.1 mg/mL) onto carbon-coated copper grids, and the grids were frozen in liquid nitrogen and freeze-dried in vacuum at -60 ºC. DLS measurements were performed with aqueous solutions on a Malvern Zetasizer Nano S (MalvernInstruments, Ltd.) equipped with a 4 mW He-Ne laser light operating at λ = 633 nm. All samples were kept at about 1 mg/mL and measured at 25 ºC with a scattering angle of 173º. For the fluorescent optical microscope observation (Leica DM4500 B), the aqueous solution (1 mg/mL) was dropped onto clean glass slide and then observed directly under the microscope at 25 ºC. UV-vis absorption spectra were performed on a Perkin Elmer Lambda 20 UV-Vis spectrometer. Samples with certain concentration were added to a 1cm quartz cuvette (Wavelength range: 200-800nm, Wavelength Accuracy: ±0.5 nm) for the measurements.

2. Synthesis and characterization of HsP-MIM/OTs

Herein, HBPO-star-PEO (HsP) with DP_{arm}=2 was synthesized and characterized previously.[1,2] There synthetic process included two steps to graft 1-methylimidazoles onto HsP. For the first step, partial hydroxyl groups of PEO arms of HsP were tosylated by *p*-toluenesulfonyl chloride, as shown in Figure S1. A typical synthetic process was as follows: HBPO-star-PEO (1.021 g, ca. 5 mmol OH group), dried triethylamine ($N(Et)_3$, 1.35 ml, 10 mmol) and 30 mL dried $CHCl_3$ were added into a 150 mL single-neck round-bottom flask. After stirred to the complete dissolution of the sample, *p*-toluenesulfonyl chloride (TsCl, 0.62 g, 3 mmol) dissolved in 20 mL dried $CHCl_3$ was added into the previous solution dropwise at 0 ºC and kept stirring at room temperature for 24 hours. Then the solvent was removed by rotary evaporation.

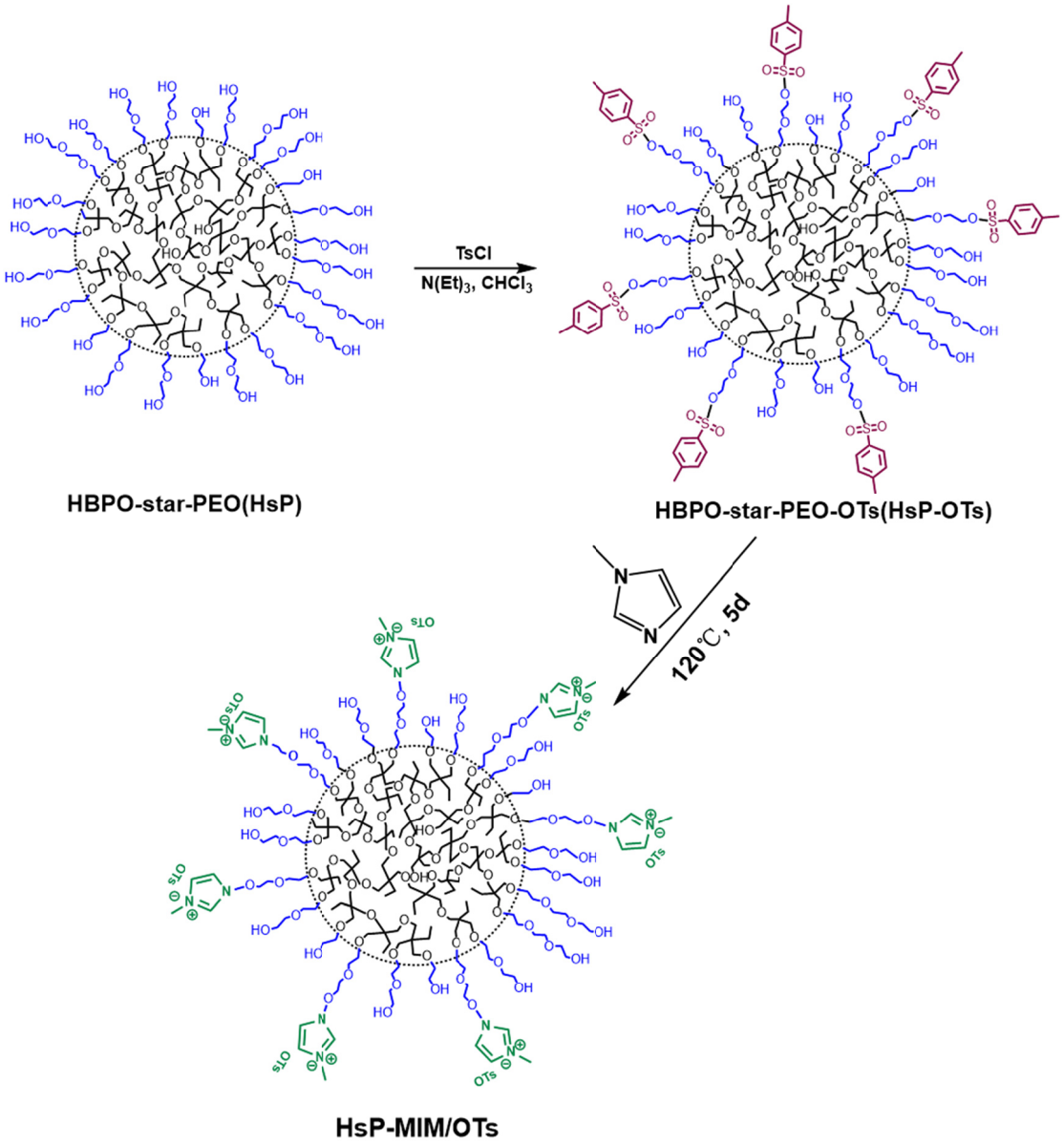

Figure S1. Synthetic scheme of HsP-MIM/OTs. The HBPO core is in black, the PEO arms are in blue, the grafted tosylates are in purple, and the MIM/OTs ion pairs are in green.

The crude product was dissolved in 10 mL $CHCl_3$ and dialyzed against (MWCO: 3500 Da) ethanol for three days. After removal of the solvents and being dried in vacuum at 50 ºC for two days, some transparent viscous pure product named as HBPO-star-PEO-OTs (HsP-OTs) was obtained.

For the second step, HsP-OTs were reacted with 1-methylimidazoles to produce HsP-MIM/OTs with imidazoles as cationic parts and tosylates (OTs) as anionic parts as shown in Figure S1. In a typical experiment, HsP-OTs (1.22 g, *ca.* 1 mmol OTs) and 1-methylimidazole (5 mL, 60 mmol) were added into a 25 mL single-neck round-bottom flask. The mixture was stirred at 120 ºC for five days. Then unreacted 1-methylimidazoles were removed by rotary evaporation. The crude product was dissolved in 10 mL ethanol and dialyzed against (MWCO: 3500 Da) ethanol for three days. After removal of the solvents and being dried in vacuum at 100 ºC for three days, the final product HsP-MIM/OTs of a viscous light yellow liquid was obtained with a final yield of 85%.

The intermediate products HsP, HsP-OTs and final product HsP-MIM/OTs were characterized by ^{1}H NMR (Figure S2). Comparing with the ^{1}H NMR spectrum of HsP, the ^{1}H NMR spectrum of HsP-OTs showed new signals appeared at 2.42 ppm, 7.32 ppm and 7.77 ppm attributed to the protons from tosylate groups, which confirmed the obtaining of HsP-OTs. The conversion ratio from hydroxyl groups to tosylate groups could be calculated by comparing the integral of peaks H with the integral of peaks A ($3S_H/2S_A$, Figure S2a), and the result was about 16%.

Similarly, new signals located at 3.84 ppm, 7.68 ppm and 9.07 ppm, attributing to the protons from 1-methylimidazoles, appeared at the ^{1}H NMR spectrum of HsP-MIM/OTs, which indicated the successful preparation of amphiphilic hyperbranched multiarm polymeric ionic liquids. The grafting ratio of ionic liquids was about 16% according to the ^{1}H NMR spectra ($3S_{G'}/S_A$, Figure S2b), indicating that all OTs anions had formed ionic complex with MIM cationic ions.

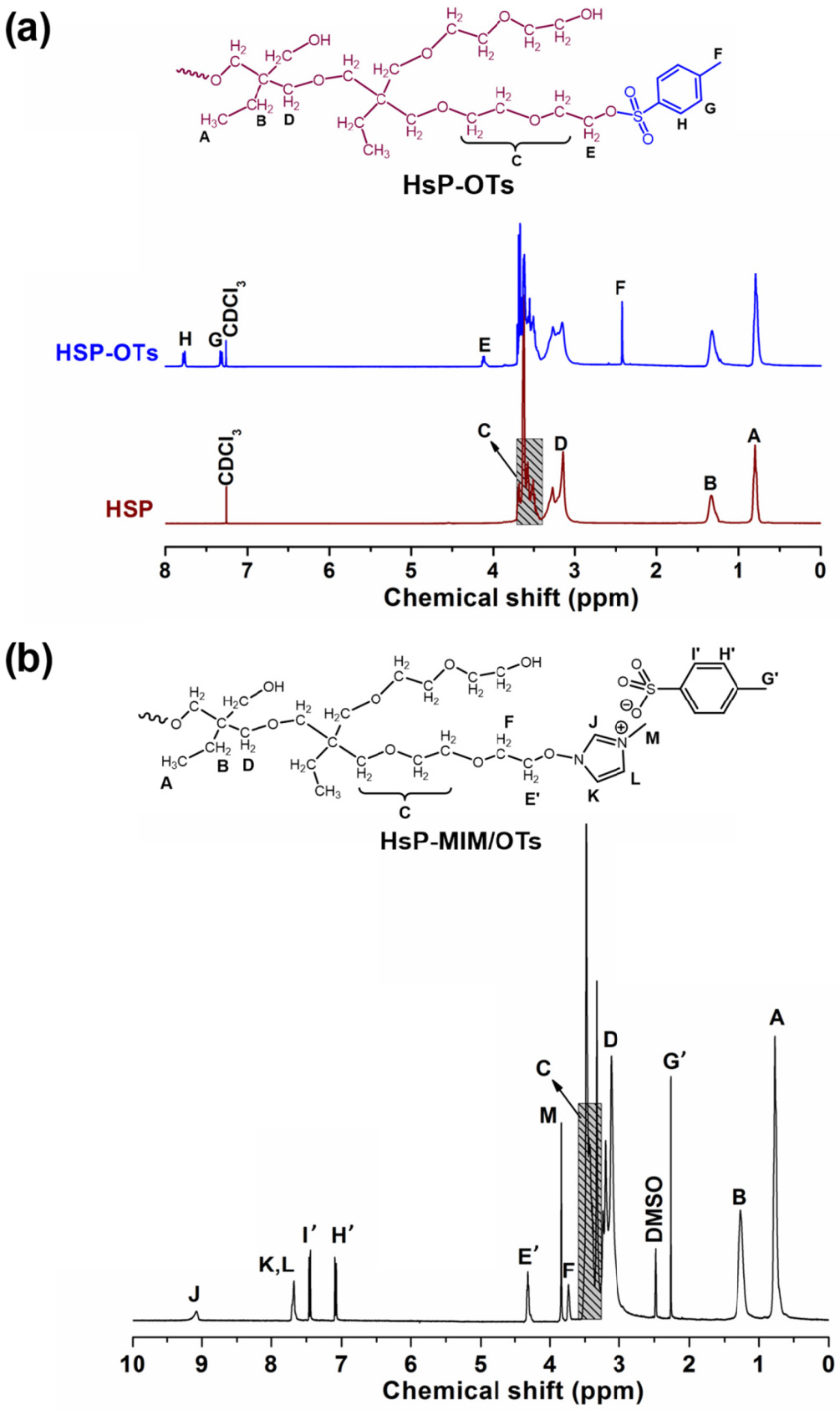

Figure S2. ^{1}H NMR spectra of HsP, HsP-OTs and HsP-MIM/OTs.

3. Synthesis and characterization of HsP-MIM/MO

For the synthesis, HsP-MIM/OTs (0.61 g, 0.45 mmol) were dissolved in 10 mL deionized water, followed by the addition of excess pure methyl orange (MO) aqueous solution (0.5 mg/mL). After stirring 2 h, the obtained turbid solution was dialyzed against (MWCO: 3,500 Da) deionized water for a week to remove unreacted MO and exchanged OTs groups. After removal of the solvents and being dried in vacuum at 50 ºC for three days, the final salmon product HsP-MIM/MO was obtained. The synthetic scheme is shown in Figure S3.

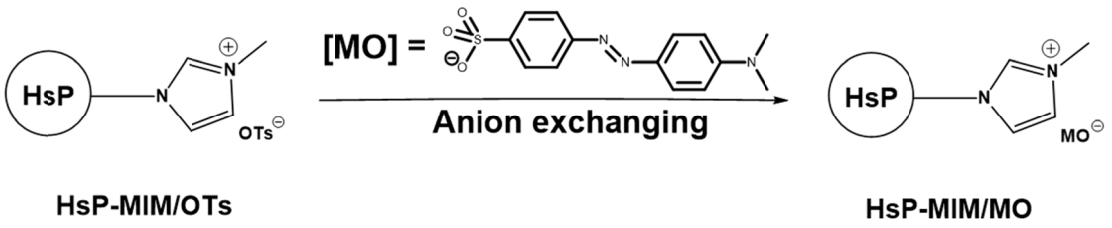

Figure S3. Synthetic scheme of HsP-MIM/MO.

^{1}H NMR was used to characterize HsP-MIM/MO. From the ^{1}H NMR spectrum (Figure S4), we could see that all the signals attributed to protons of HsP-MIM/MO. Specifically, the signal peaks at 3.07 ppm, 6.84 ppm, 7.73 ppm and 7.80 ppm were attributed to MO groups and original signals attributed to OTs groups disappeared completely, which indicated the successful preparation of HsP-MIM/MO. The conversion ratio from hydroxyl groups to MO groups could be calculated by comparing the integral of peaks M with the integral of peaks A ($3S_M/2S_A$, Figure S4) and the result was about 16%. The ratio value was the same with that of OTs groups on HsP-MIM/OTs, further indicating that all OTs anions had been exchanged by MO anions.

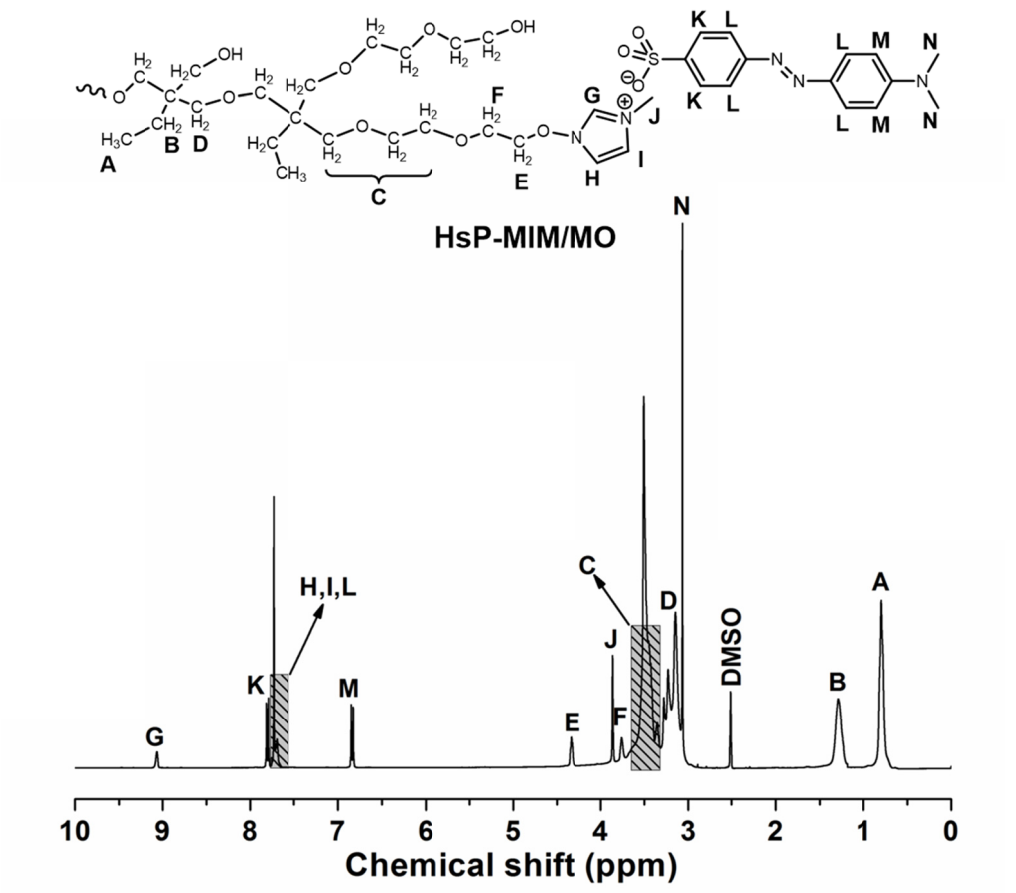

Figure S4. ^{1}H NMR spectrum of HsP-MIM/MOs.

4. Synthesis of rhodamine b labelled bovine serum albumin (RB-BSA)

RB-BSA was prepared through esterification between bovine serum albumin and rhodamine b. Briefly, rhodamine b (0.096 g, 0.2 mmol COOH group) were dissolved in 2 mL deionized water. Then EDC (0.038 g, 0.2 mmol) and NHS (0.023 g, 0.2 mmol) were added and the mixture was stirred for 5 h. After the white precipitate was removed by centrifugation, the supernatant was collected and BSA (0.68 g) in 15mL Phosphate Buffered Saline (PBS) buffer (pH 8.0) was added dropwise. The mixture was kept in dark and stirred at 4 ºC for 12 h, and then dialyzed against (MWCO: 3500 Da) PBS buffer (pH 8.0) for five days to remove the unreacted agents. Then the RB-BSA solution inside the dialysis bag was collected, subpackaged and stored under -20 ºC.

5. FT-IR characterization

FT-IR spectra were used to characterize hyperbranched polymeric ionic liquids. As shown in Figure S5, when compared with HsP, the FT-IR spectrum of HsP-OTs showed new absorption bands at 1160 cm^{-1} and 1355 cm^{-1} ascribed to the characteristic absorption peaks of -SO_2- bonds in OTs groups. The FT-IR spectrum of HsP-MIM/OTs showed new absorption bands appeared at 1009 cm^{-1} and 1035 cm^{-1} ascribed to the characteristic absorption peaks of SO_3^- bonds, and another new absorption appeared at 1571 cm^{-1} attributed to C=N bonds of MIM groups, supporting the formation of MIM/OTs ionic pairs in the obtained polymers. In the spectrum of HsP-MIM/MO, two new absorption bands from MO moieties, one at 1608 cm^{-1} ascribed to N=N bonds and the other at 1191 cm^{-1} ascribed to C-N groups, appeared, indicating the successful preparation of HsP-MIM/MO.

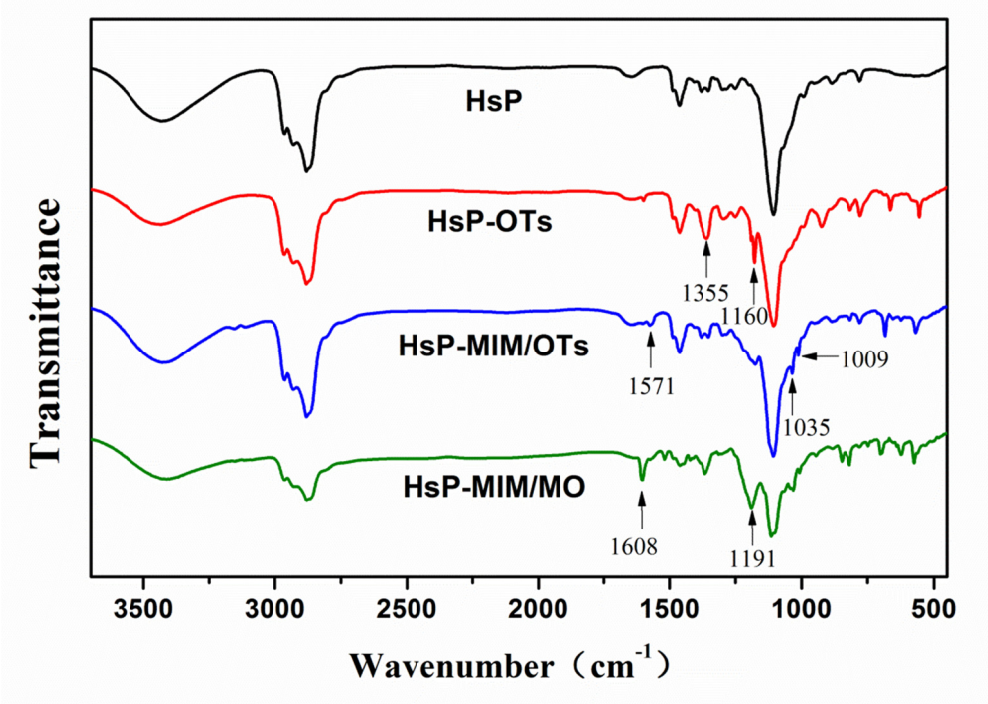

Figure S5. The FTIR spectra of hyperbranched polymeric ionic liquids.

6. Preparation of HsP-MIM/MO vesicles

HsP-MIM/MO vesicles were obtained by the direct self-assembly of HsP-MIM/MOs in deionized water. In a typical experiment, HsP-MIM/MOs (20 mg,

0.09 mmol) was added into a 100 mL single-neck round-bottom flask, followed by the slow addition of 40 mL water controlled by syringe pump within 8 hrs. The obtained solution had yellow turbidity, which indicated the formation of self-assemblies (0.5 mg/ml).

7. pH-responsibilities of MO and HsP-MIM/MO aqueous solutions

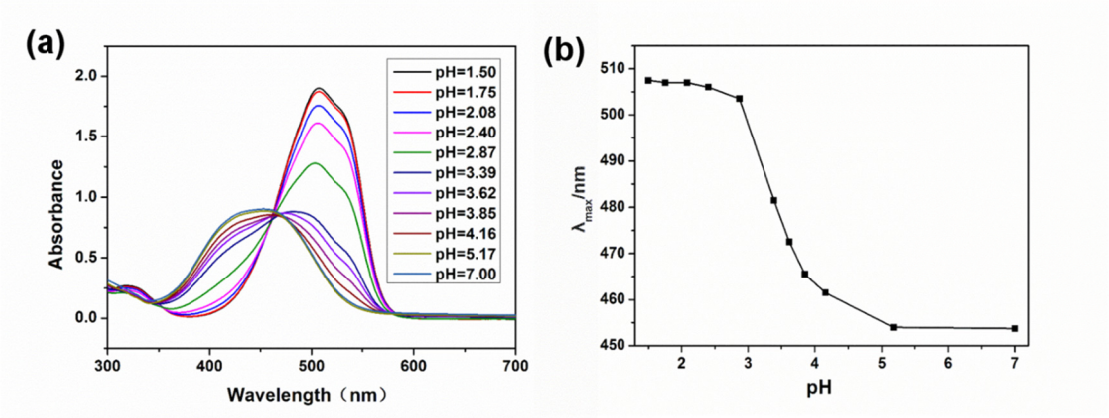

Figure S6. (a) The UV-Vis spectra of HsP-MIM/MO aqueous solution at varied pH. (b) Variation of the wavelength of maximum absorbance (λ_{max}) of HsP-MIM/MO aqueous solution with pH.

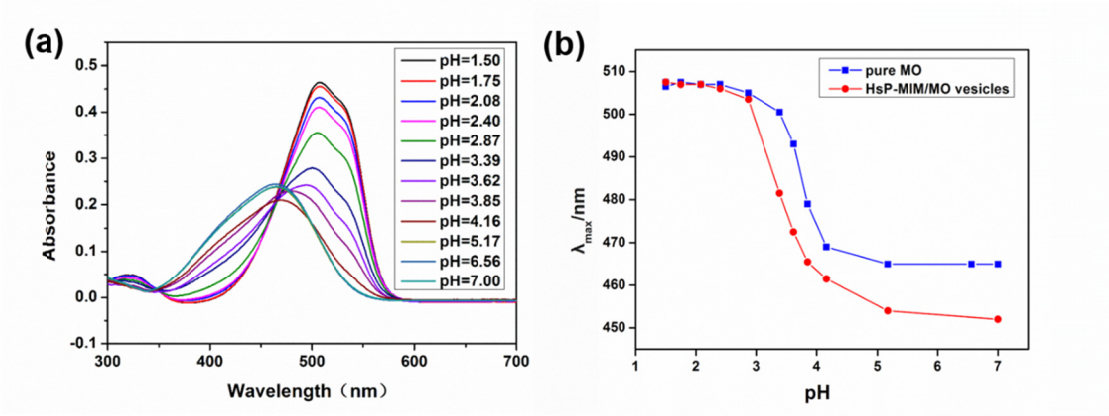

Figure S7. (a) The UV-Vis spectra of MO aqueous solution at varied pH. (b) A comparison of pH-responsibility of λ_{max} between MO and HsP-MIM/MO aqueous solutions.

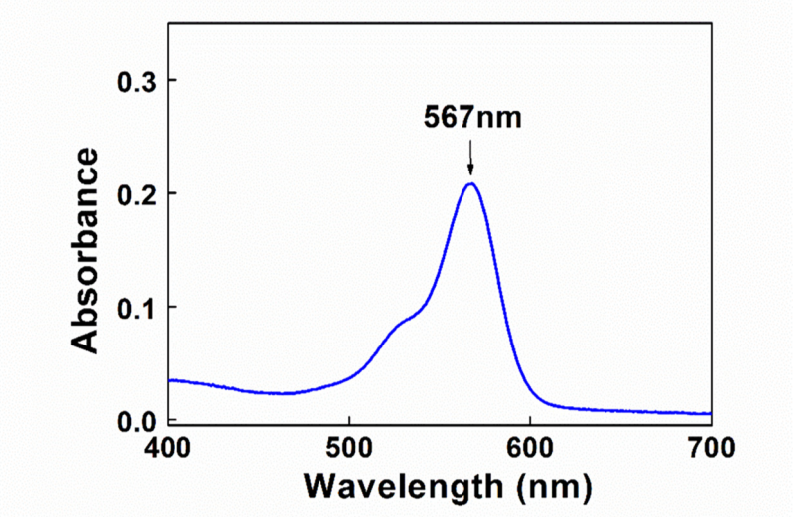

Figure S8. UV-Vis absorption spectra of pure RB-BSA aqueous solution.

Notes and references

1. Y. Zhou, D. Yan, W. Dong and Y. Tian, *J. Phys. Chem. B*, 2007, **111**, 1262.

2. Y. F. Zhou and D. Y. Yan, *Angew. Chem. Int. Ed.*, 2004, **43**, 4896.