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Supporting Information for

Charge variable PIB-based block copolymers as selective transmembrane ion transporters

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Experimental Details

Materials. *tert*-Butoxy carbonyl (Boc)-L-alanine (Boc-Ala-OH, 99%), 2-hydroxyethyl methacrylate (HEMA, 97%), dicyclohexylcarbodimide (DCC, 99%), pyrene, 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), Sephadex G-25, Triton X-100, 4-dimethylaminopyridine (DMAP, 99%), and anhydrous *N*,*N*-dimethylformamide (DMF, 99.9%) were procured from Sigma and used as obtained. Trifluoroacetic acid (TFA, 99.5%) was purchased from Sisco Research Laboratories (SRL) Pvt. Ltd, India. Metal salts (MCl where M = Li^+, Na^+, K^+, Rb^+, and Cs^+, and NaX where X = NO_2^-, Cl^-, Br^-, I^-, and ClO_4^-) and metal hydroxides (M = Li^+, Na^+, K^+, Rb^+, and Cs^+) were received either from SRL or Sigma. Polyethylene glycol methyl ether methacrylate (PEGMA, average molecular weight = 300 g/mol) and acrylic acid (AA) with the highest available purity were procured from Sigma and passed through a small plugged basic alumina column for purification before polymerization. The initiator 2,2'-azobisisobutyronitrile (AIBN, Sigma, 98%) was used after recrystallization from methanol (MeOH). The *tert*-butoxy carbonyl-L-alanylethyl methacrylate (Boc-Ala-HEMA) monomer was prepared from Boc-Ala-OH by coupling with HEMA using DCC and DMAP reagents as reported previously.[1] The chain transfer agent (CTA), 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid (CDP) was prepared according to the literature report.[2] Hydroxyl-terminated polyisobutylene (PIB-OH) (molecular weight of 1,000 g/mol) was utilized for the synthesis of PIB-CDP macro-CTA. The NMR solvents such as

chloroform-*d* ($CDCl_3$, 99.8% D), D_2O (99% D), and dimethyl sulphoxide-d_6 (DMSO-d_6, 99.8% D) were procured from Cambridge Isotope Laboratories, Inc., USA. Avanti mini-extruder was procured from Sigma for the preparation of the membrane using DOPC. The solvents like hexanes (mixture of isomers), tetrahydrofuran (THF), and dichloromethane (DCM) were purified by following the general purification procedure.[3]

Instrumentation. The ^1H NMR spectra was acquired in a Bruker Avance^{III} 500 MHz spectrometer. The number average molecular weight ($M_{n,SEC}$) of the polymers and their dispersity ($Đ$) were determined by size exclusion chromatography (SEC) using poly(methyl methacrylate) (PMMA) standard calibration in THF with 1.0 mL min^{-1} rate of flow at 30 ^oC. The instrument consists of one PolarGel-M guard column (50 × 7.5 mm), two PolarGel-M analytical columns (300 × 7.5 mm), and Waters 2414 refractive index (RI) detector. The UV-Vis spectroscopic measurements were conducted on a Perkin Elmer Lambda 35 spectrophotometer instrument with a 240 nm min^{-1} scanning rate. A fluorescence spectrometer (Horiba JobinYvon Fluoromax-3, Xe-150 W, 250-900 nm) was used to record fluorescence emission spectra. Hydrodynamic size distribution and zeta potential of the polymers were measured in a Malvern Nano Zetasizer dynamic light scattering (DLS) instrument, that was equipped with a helium-neon laser operational at a 633 nm wavelength and a detection angle of 173° at room temperature. Field emission scanning electron microscopy (FESEM) images were captured in a Carl Zeiss Sigma instrument. Transmission electron microscopy (TEM) images were taken from a JEOL JEM-2100F instrument operating at an accelerating voltage of 120 kV.

Synthesis of Block Copolymers using PIB-CDP macro-CTA. Reversible addition-fragmentation chain transfer (RAFT) polymerizations of methacrylate/acrylate-based monomers (AA, Boc-Ala-HEMA, and PEGMA) were conducted individually with PIB-CDP macro-CTA. A typical copolymerization procedure for preparing neutral PIB-based block copolymer (**PIB-*b*-PPEGMA**) was as follows: PEGMA (400 mg, 1.33 mmol), PIB-CDP (36.5 mg, 0.03 mmol), and AIBN (0.9 mg, 0.005 mmol; AIBN in THF was used as a stock solution) were dissolved in THF at [PEGMA]/[PIB-CDP]/[AIBN] = 50:1:0.2 in a 20 mL glass vial equipped with a magnetic stir bar. The vial was purged with dry N_2 in a cold condition for 10 min. Then, the vial was placed in a preheated polymerization block at 60 °C for 8 h. After the stipulated time the polymerization reaction was stopped by cooling the reaction vial on an ice-water bath and upon exposure to aerial oxygen. The polymerization

mixture was purified by reprecipitation in MeOH from the polymer solution in hexanes, and this process was repeated at least 5 times. Further, the obtained polymer was dried under a high vacuum at room temperature to obtain a yellowish compound. Similarly, **PIB-*b*-PAA**, and **PIB-*b*-P(Boc-Ala-HEMA)** block copolymers were prepared (Scheme S1). Homopolymer of PEGMA was prepared following similar method using CDP as a CTA.

Deprotection of Boc Group. To prepare PIB-based cationic copolymers, the Ala-containing polymer (**PIB-*b*-P(Boc-Ala-HEMA)**) was dissolved in DCM and reacted with TFA at room temperature. In a 20 mL glass vial containing 0.1 g of the polymer in 1.5 mL of DCM at room temperature, 1 mL of TFA was gradually added. The vial was vigorously agitated for 2 h. After that, the excess TFA along with DCM was removed using rota vapour. After repeated precipitation in diethyl ether, the deprotected polymer was dried under a high vacuum at 45 °C. The resultant polymer was named as **PIB-*b*-P(NH_3^+-Ala-HEMA)**.

Determination of Critical Aggregation Concentration (CAC). A specified quantity of pyrene in acetone was added to various concentrations of the block copolymer solutions, and it was then left undisturbed until all of the acetone had evaporated, resulting in a final concentration of pyrene in the solutions of 6.0×10^{-7} mol/L. The fluorescence intensities of each solution were measured at a wavelength of 339 nm. The ratio of the first and third vibrational peaks from the fluorescence intensities for pyrene emission, which are at 373 nm and 393 nm ($I_3/I_1 = I_{393}/I_{373}$) were plotted against the logarithm of polymer concentration (log C). The CAC value is obtained from the point of intersection from the two tangent lines that were created to connect the intensity ratio values from the plot of the intensity ratio I_{393}/I_{373} *versus* the log C.[4]

Preparation of Artificial Membrane. The artificial membrane was prepared using DOPC, according to the standard protocol from the literature report.[5] A thin film of HPTS loaded DOPC was prepared in a glass vial where the overall lipid and HPTS concentrations were maintained at 5 mM and 0.1 mM, respectively. The mixture was sonicated in a sonication bath for 30 min (twice) and then the film was prepared upon chloroform solution evaporation with the help of a N_2 purging which was kept overnight in a high vacuum for drying. After drying, 2 mL of HEPES buffer was added and sonicated until the suspension becomes a clear solution. The mixture was then freezed in liquid N_2 for 10 min and then heated at 45 °C for another 10 min. This process was repeated 5 times. A 10 mL LipexTM Thermobarrel

EXTRUDER (Northern Lipids Inc.) was used to extrude liposomes. 100 nm polycarbonate nucleopore Track-Etch Membranes from Whatman were used within the extruder. Finally, the dye-encapsulated liposomes were separated from the free dye on a Sephadex G-25 column using corresponding isotonic buffers as the eluting solution.

HPTS Assay. HPTS, a fluorescent pH indicator (pK_a 7.2), is trapped inside the water pool of the synthesized artificial membranes, i.e., the large unilamellar vesicles made by DOPC. The lipid suspension of the DOPC is prepared in HEPES buffer at pH 7. The ionophore is added to the DOPC in the HEPES buffer and after 50 s a base pulse is applied by the NaOH addition which generated a 0.6 units pH gradient between the bulk water and the liposome inner water pool. The basification of the inner water pool, which may result from H^+ efflux or OH^- influx is balanced by the opposite transport of ions of the same charge or by the symport of ions of the opposite charge, which is indicated by an increase in the HPTS fluorescence emission in response to the applied transmembrane pH gradient. Triton X-100 is used for the lysis of the vesicles after 250 s, which denoted the measurement of the maximum intensity of fluorescence, which is then used to normalize the data.[6]

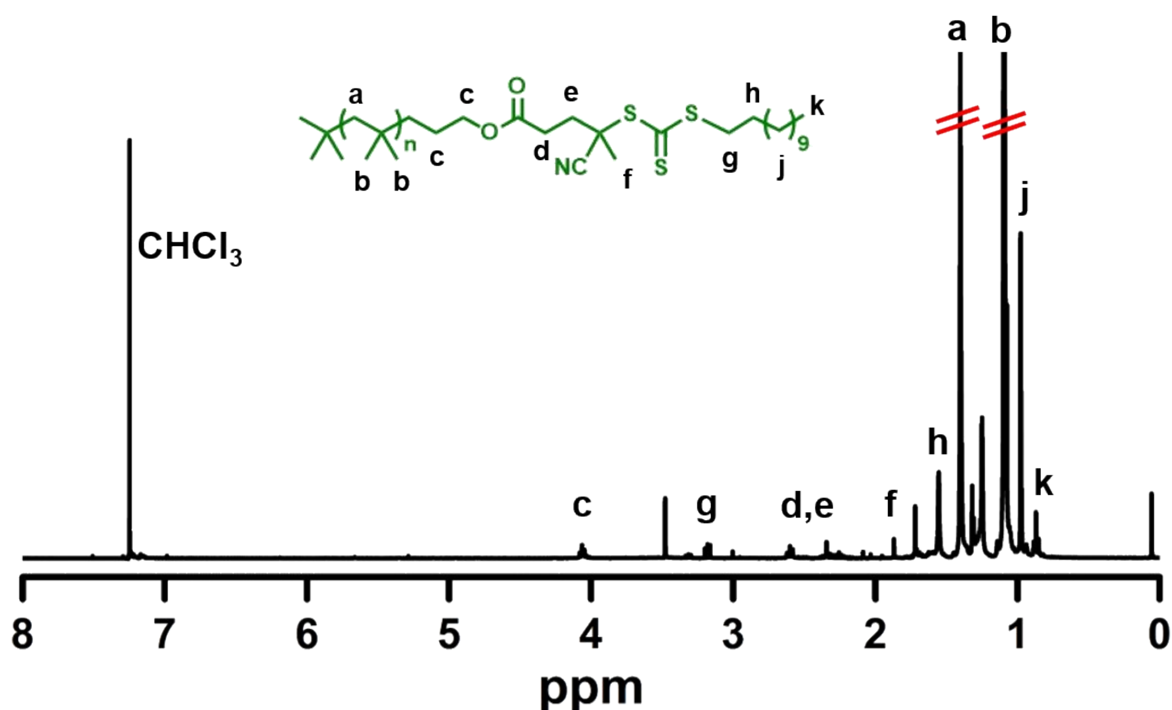
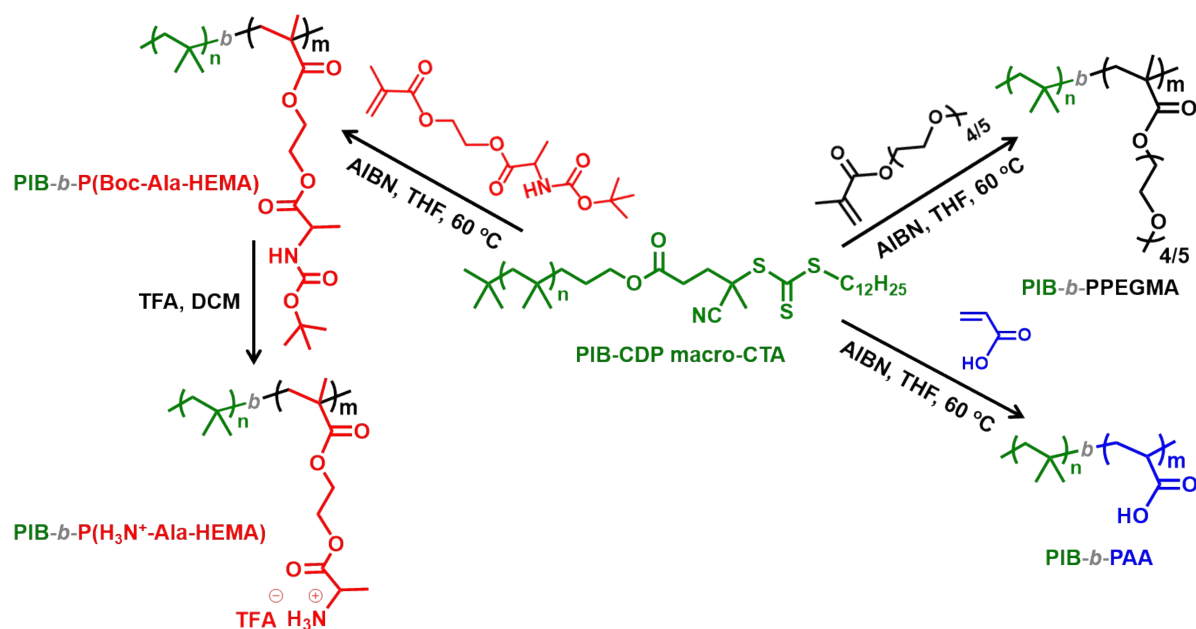


Fig. S1 ^{1}H NMR spectrum of PIB-CDP macro-CTA.



Scheme S1 Synthetic strategy of PIB-based amphiphilic block copolymers with charge variable pendants.

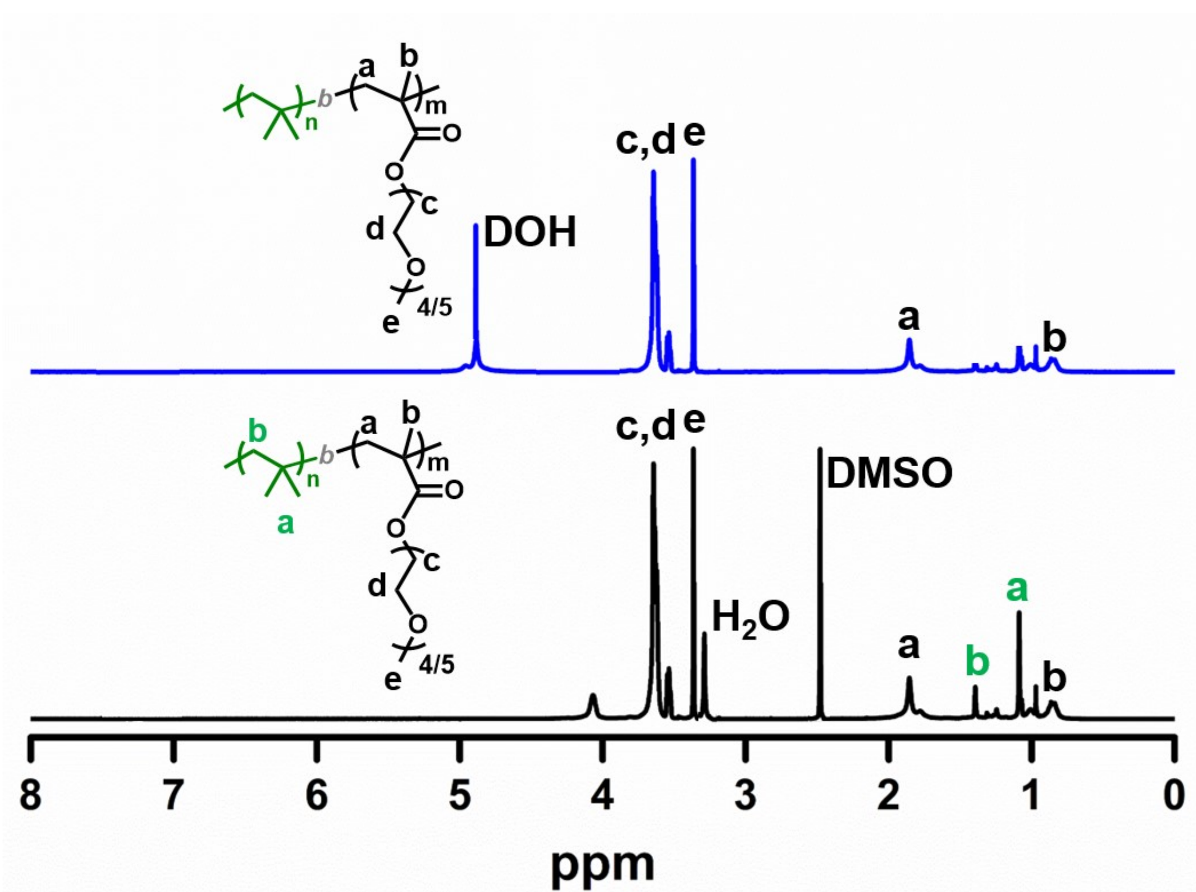


Fig. S2 ^{1}H NMR spectra of **PIB-*b*-PPEGMA** in DMSO-d_6 (black line) and D_2O (blue line).

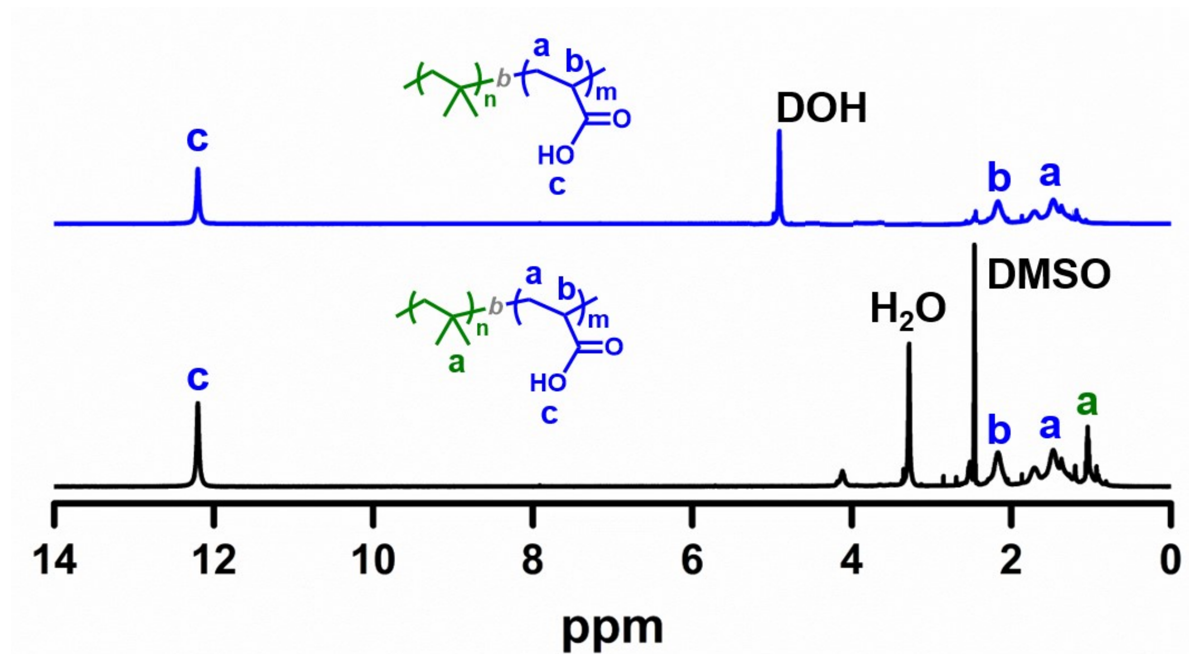


Fig. S3 ^{1}H NMR spectra of **PIB-*b*-PAA** in DMSO-d_6 (black line) and D_2O (blue line).

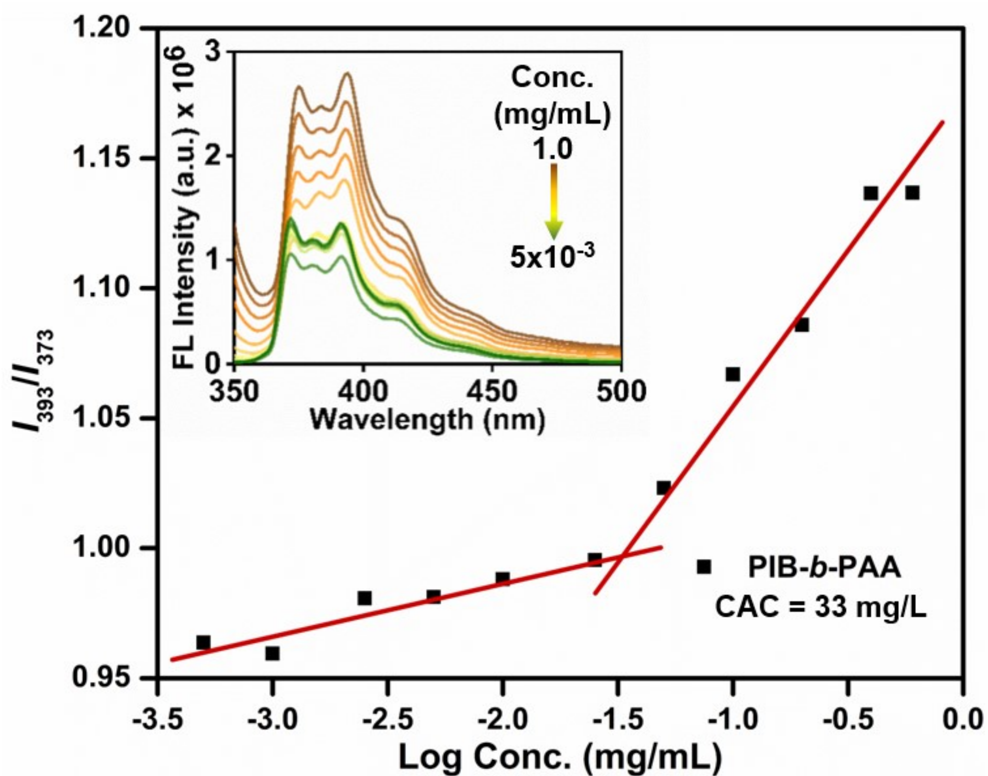


Fig. S4 Calculation of CAC for the **PIB-*b*-PAA** polymer; the inset displays the fluctuation in the encapsulated pyrene dye fluorescence emission.

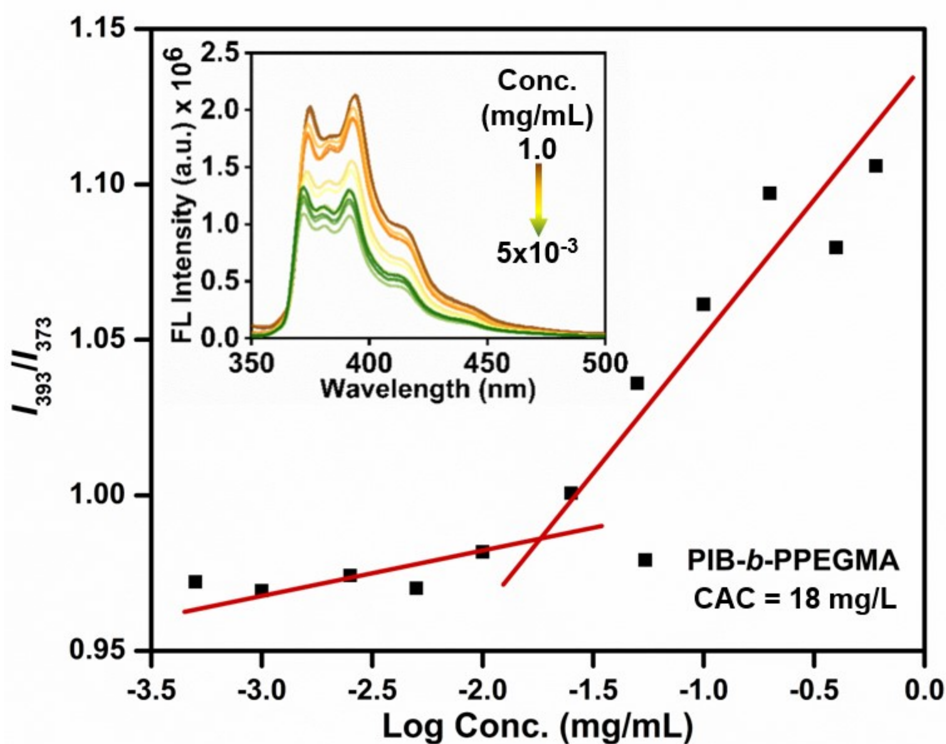


Fig. S5 Calculation of CAC for the **PIB-*b*-PPEGMA** polymer; the inset displays the fluctuation in the encapsulated pyrene dye fluorescence emission.

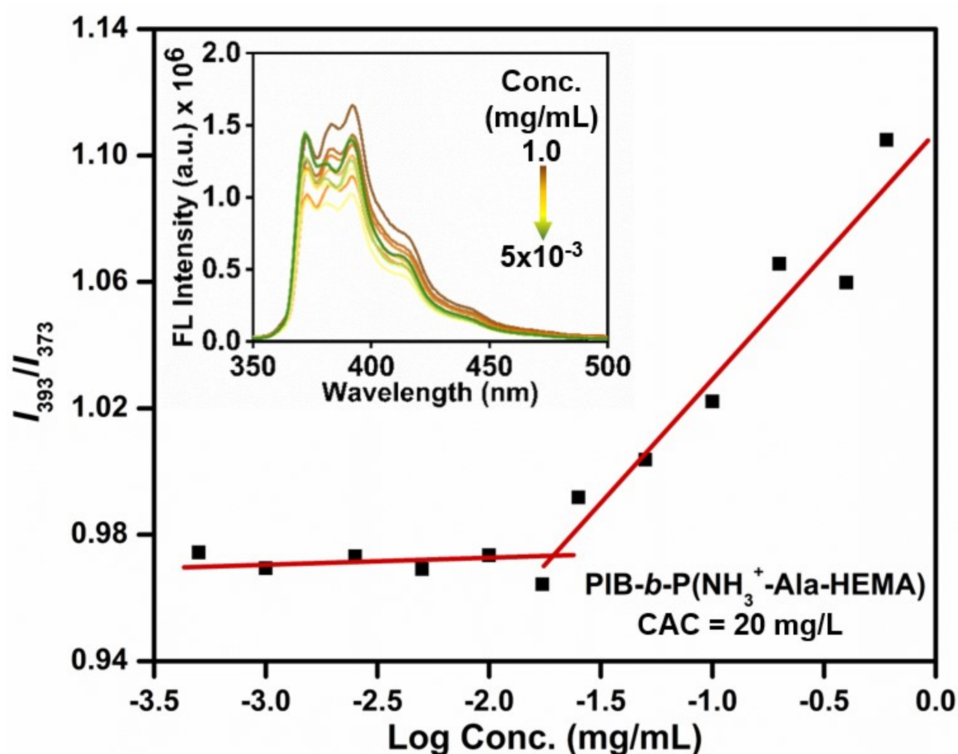


Fig. S6 Calculation of CAC for the **PIB-*b*-P(NH$_3^+$-Ala-HEMA)** polymer; the inset displays the fluctuation in the encapsulated pyrene dye fluorescence emission.

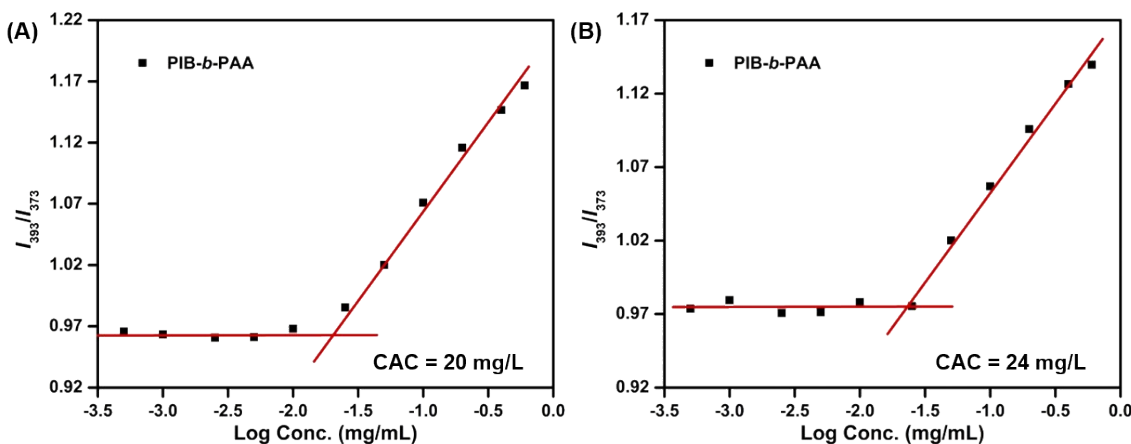


Fig. S7 Calculation of CAC for the **PIB-*b*-PAA** polymer in (A) HEPES buffer and (B) HEPES buffer in the presence of 100 mM NaCl.

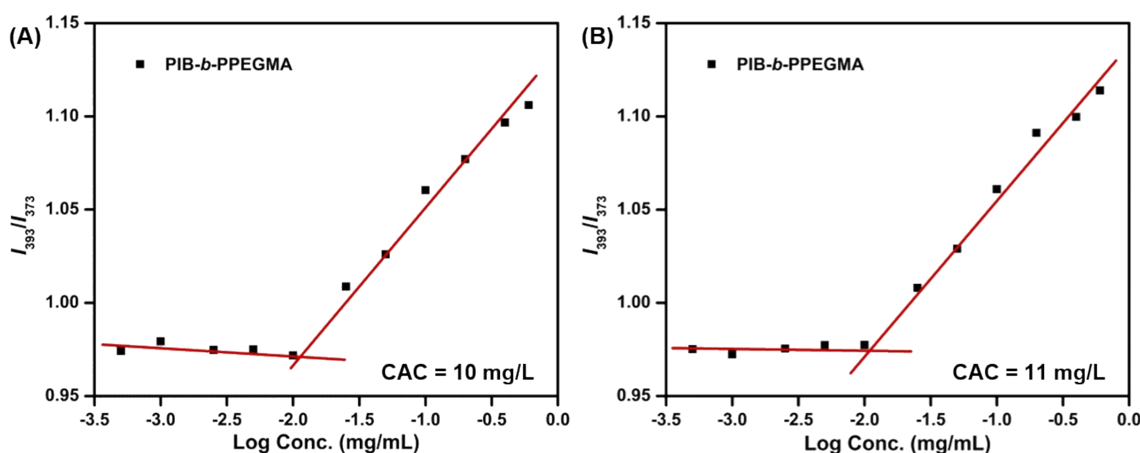


Fig. S8 Calculation of CAC for the **PIB-*b*-PPEGMA** polymer in (A) HEPES buffer and (B) HEPES buffer in the presence of 100 mM NaCl.

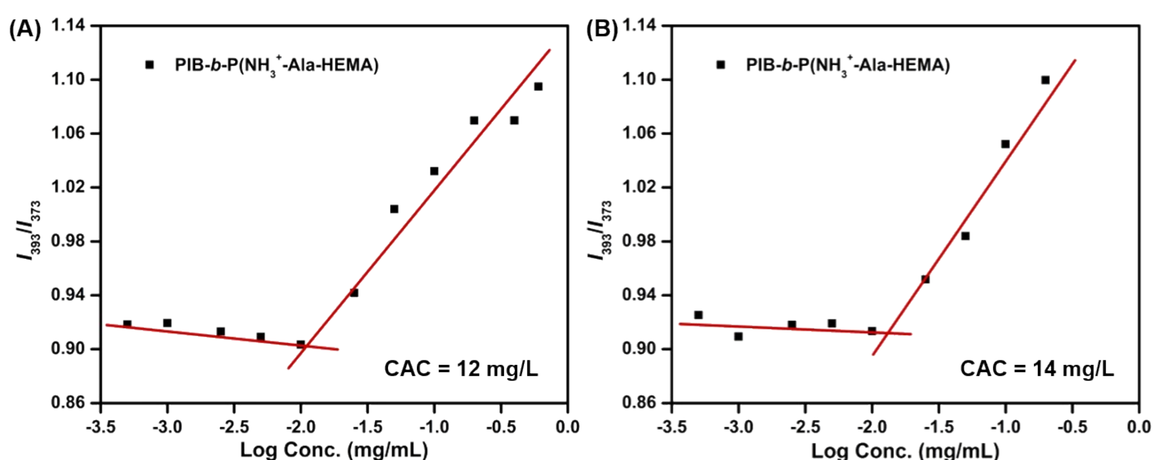


Fig. S9 Calculation of CAC for the **PIB-*b*-P(NH_3^+-Ala-HEMA)** polymer in (A) HEPES buffer and (B) HEPES buffer in the presence of 100 mM NaCl.

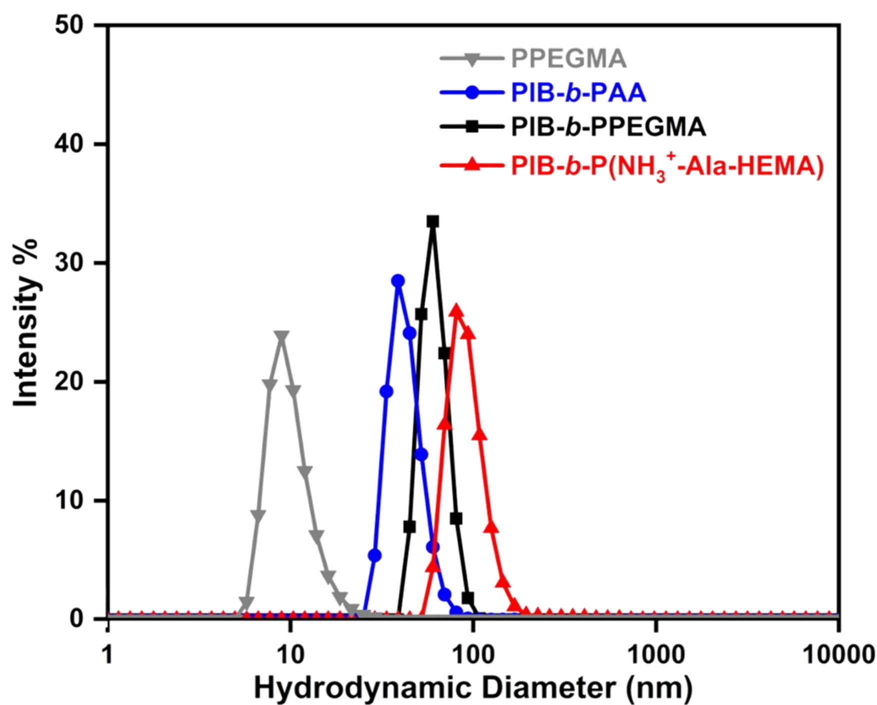


Fig. S10 DLS measurement of **PPEGMA**, **PIB-*b*-PAA**, **PIB-*b*-PPEGMA**, and **PIB-*b*-P(NH_3^+-Ala-HEMA)** in water.

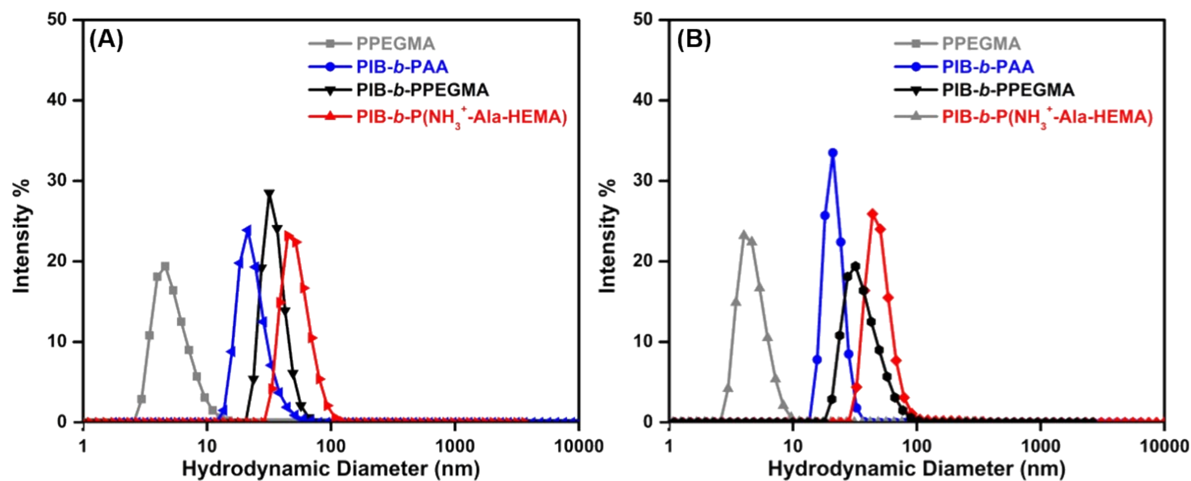


Fig. S11 DLS measurement of **PPEGMA**, **PIB-*b*-PAA**, **PIB-*b*-PPEGMA**, and **PIB-*b*-P(NH_3^+-Ala-HEMA)** in (A) HEPES buffer and (B) HEPES buffer in the presence of 100 mM NaCl.

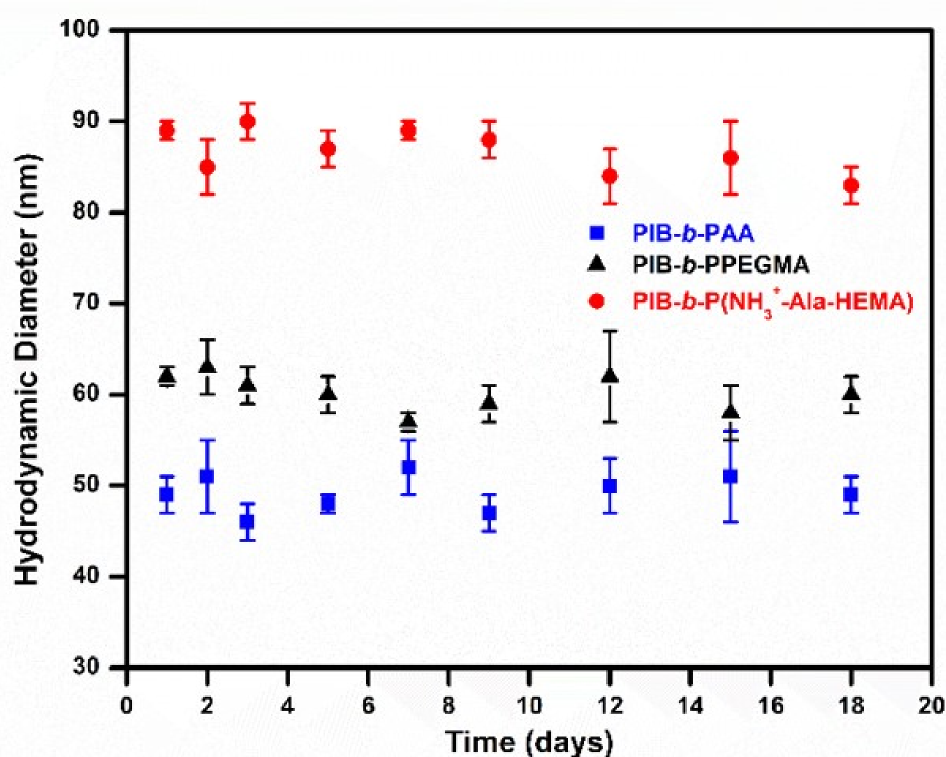


Fig. S12 The stability of the water-soluble aggregates of **PIB-*b*-PAA**, **PIB-*b*-P(NH_3^+-Ala-HEMA)**, and **PIB-*b*-PPEGMA**. The hydrodynamic diameter of the polymer solution (1 mg/mL) was determined on a regular basis while it was kept at 25 °C. Error bars represent the standard deviation of 3 measurements.

Microscopic images were captured to explore the morphology of the block copolymer aggregates in an aqueous solution. The **PIB-*b*-PAA**, **PIB-*b*-P(NH_3^+-Ala-HEMA)**, and **PIB-*b*-PPEGMA** copolymers showed micellar aggregates of size 40 nm, 85 nm, and 60 nm, respectively (Fig. S9A-C) in an aqueous medium, which is comparable with the size obtained from the DLS measurement. FESEM study further confirmed the morphological structures acquired from TEM, which revealed a comparable-sized micellar structure for the copolymers in an aqueous media (Fig. S9D-F). The PIB-based block copolymers formed micellar types aggregates with PIB segments in the inner hydrophobic pockets which were confirmed by the reduced signal intensities of PIB protons in the ^1H NMR spectra of the block copolymers in D_2O (Fig. S2-S3, blue line and Fig. S10, blue line).

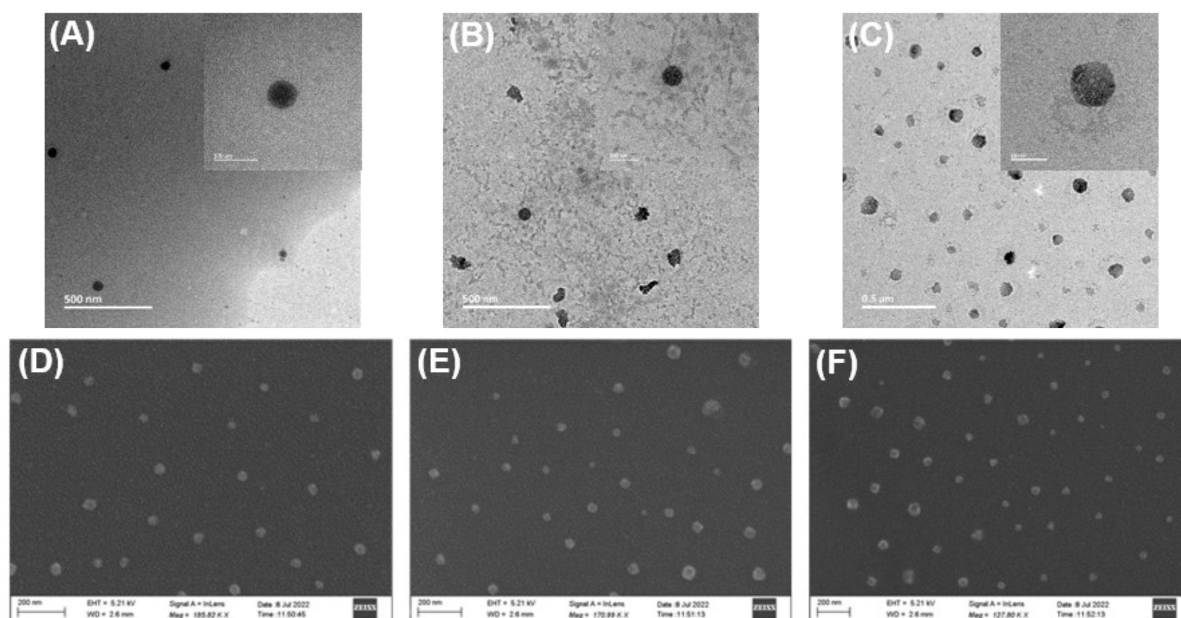


Fig. S13 TEM images (scale bar 500 nm) of (A) **PIB-*b*-PPEGMA**, (B) **PIB-*b*-PAA**, and (C) **PIB-*b*-P(NH_3^+-Ala-HEMA)**; FESEM images (scale bar 200 nm) of (D) **PIB-*b*-PPEGMA**, (E) **PIB-*b*-PAA**, and (F) **PIB-*b*-P(NH_3^+-Ala-HEMA)**.

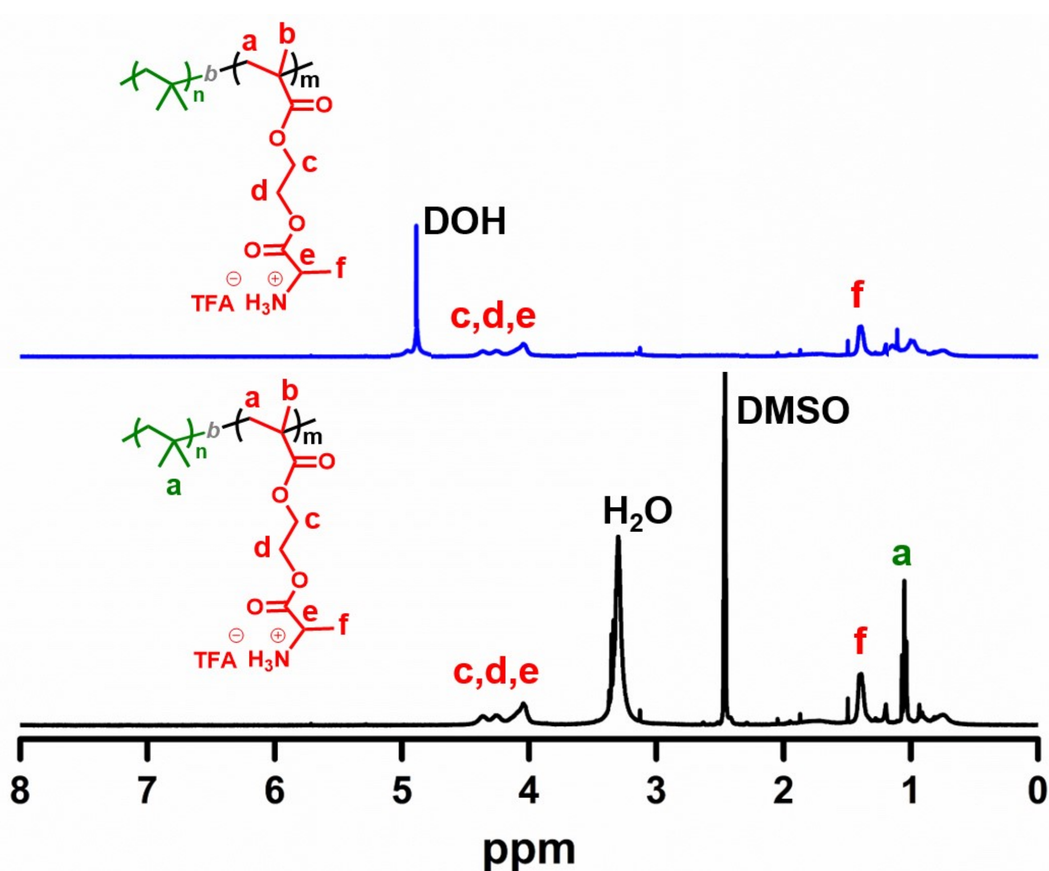


Fig. S14 ^{1}H NMR spectra of **PIB-*b*-P(NH_3^+-Ala-HEMA)** in DMSO-d_6 (black line) and D_2O (blue line).

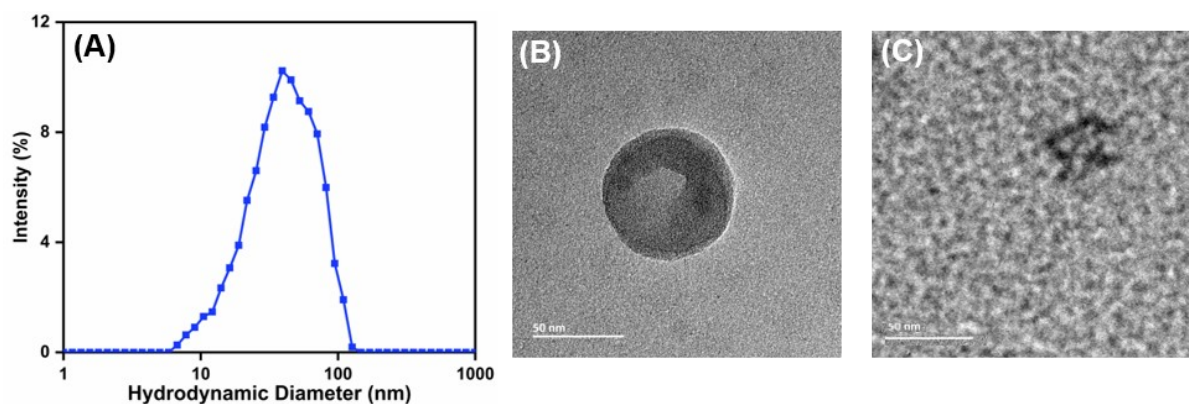


Fig. S15 DLS measurement of liposomes (A), TEM images of liposome before (B) and after (C) polymer treatment.

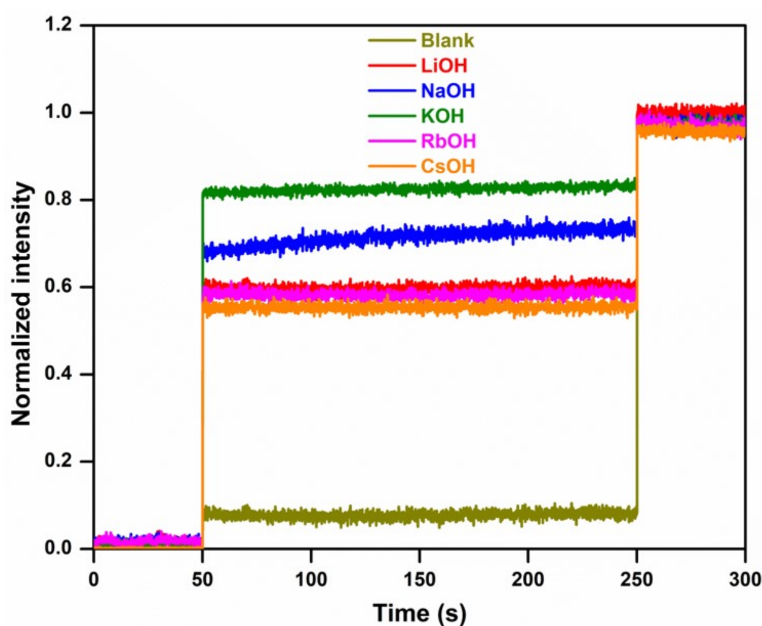


Fig. S16 Normalized fluorescence time course of HPTS emission after the addition of MOH (M = Li^+, Na^+, K^+, Rb^+ and Cs^+) (time 50 s, 25 μL of 0.1 M) to DOPC large unilamellar vesicles (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.25 mM lipid concentration, 25 mM HEPES, 100 mM MCl, pH 7.0; total volume: 3 mL) in the presence of **PIB-*b*-PAA** at 5 μg/mL concentration.

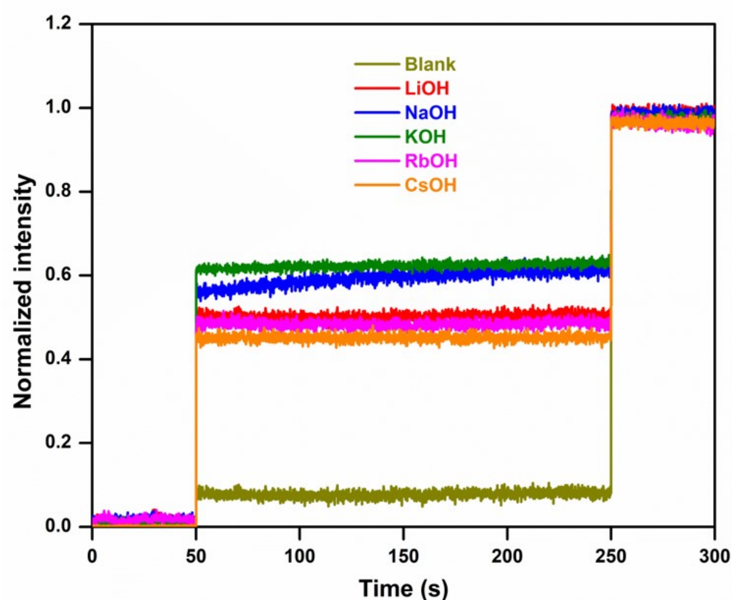


Fig. S17 Normalized fluorescence time course of HPTS emission after the addition of MOH ($M = Li^+$, Na^+, K^+, Rb^+ and Cs^+) (time 50 s, 25 µL of 0.1 M) to DOPC large unilamellar vesicles (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.25 mM lipid concentration, 25 mM HEPES, 100 mM MCl, pH 7.0; total volume: 3 mL) in the presence of **PIB-*b*-PPEGMA** at 5 µg/mL concentration.

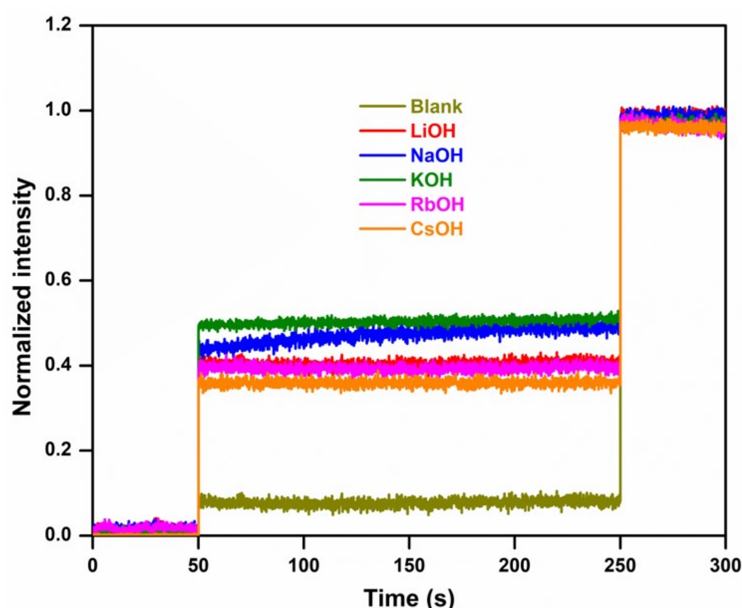


Fig. S18 Normalized fluorescence time course of HPTS emission after the addition of MOH ($M = Li^+$, Na^+, K^+, Rb^+ and Cs^+) (time 50 s, 25 µL of 0.1 M) to DOPC large unilamellar vesicles (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.25 mM lipid concentration, 25 mM HEPES, 100 mM MCl, pH 7.0; total volume: 3 mL) in the presence of **PIB-*b*-P(NH_3^+-Ala-HEMA)** at 5 µg/mL concentration.

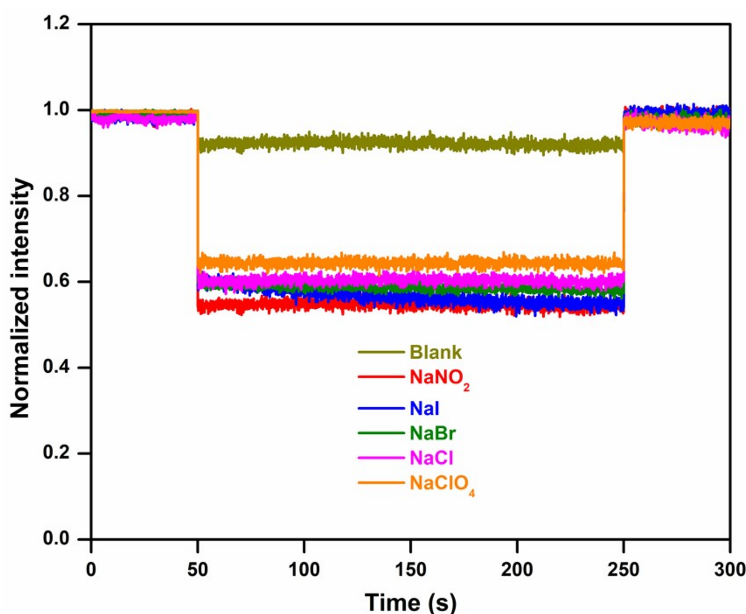


Fig. S19 Normalized fluorescence time course of HPTS emission after the addition of NaOH (time 50 s, 25 μL of 0.1 M) to DOPC large unilamellar vesicles (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.25 mM lipid concentration, 25 mM HEPES, 100 mM NaX where X = NO_2^-, Cl^-, Br^-, I^-, ClO_4^-, pH 7.0; total volume: 3 mL) in the presence of **PIB-*b*-PAA** at 5 μg/mL concentration.

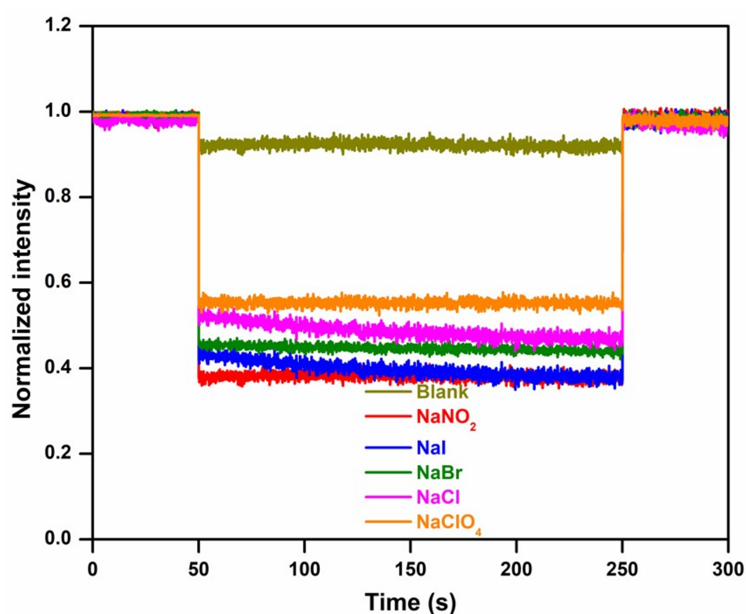


Fig. S20 Normalized fluorescence time course of HPTS emission after the addition of NaOH (time 50 s, 25 μL of 0.1 M) to DOPC large unilamellar vesicles (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.25 mM lipid concentration, 25 mM HEPES, 100 mM NaX where X = NO_2^-, Cl^-, Br^-, I^-, ClO_4^-, pH 7.0; total volume: 3 mL) in the presence of **PIB-*b*-PPEGMA** at 5 μg/mL concentration.

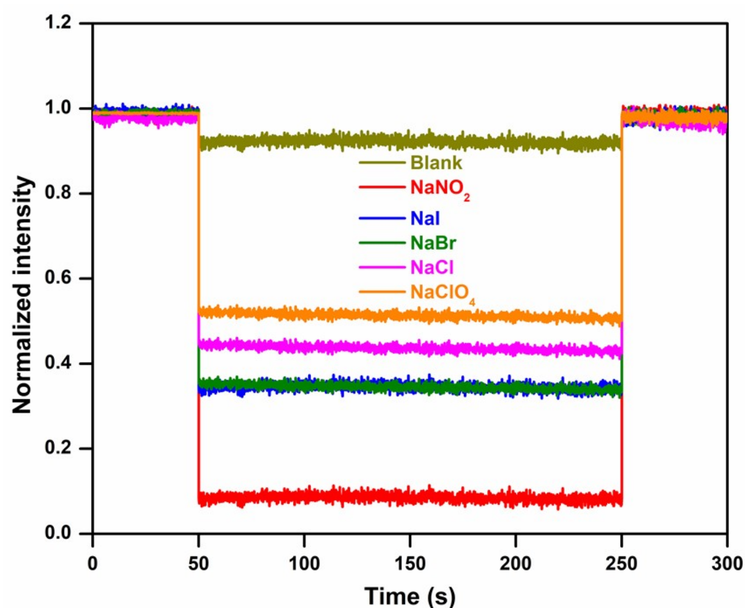


Fig. S21 Normalized fluorescence time course of HPTS emission after the addition of NaOH (time 50 s, 25 μL of 0.1 M) to DOPC large unilamellar vesicles (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.25 mM lipid concentration, 25 mM HEPES, 100 mM NaX where X = NO_2^-, Cl^-, Br^-, I^-, ClO_4^-, pH 7.0; total volume: 3 mL) in the presence of **PIB-*b*-P(NH_3^+-Ala-HEMA)** at 5 μg/mL concentration.

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