Supporting Information

A simple interfacial pH detection method for cationic amphiphilic self-assemblies utilizing a Schiff-base molecule

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Synthesis of QPDMA-b-PMMA-b-QPDMA block co-polymer (TBP):

The triblock copolymer containing central poly(methyl methacrylate) block (PMMA) and poly(2-dimethyl aminoethyl)methacrylate end block (PDMA) with molecular weight $(M_n) \sim 28$ kD and a polydispersity index (PDI) 1.20 was synthesized by two step atom transfer radical polymerization. A difunctional Br-PMMA-Br was synthesized at 35°C using CuCl/bpy as the catalyst and 1,2-bis(bromoisobutyryloxy)ethane as the initiator with the following recipe: MMA (7 g, 0.07 mol), acetone (4.2 mL), CuCl (0.1 g, 0.00094 mol), bpy (0.3 g, 0.0019 mol) and 1,2-bis(bromoisobutyryloxy)ethane (0.34 g, 0.00094 mol). After 12 h, the conversion was 80% and the M_n and PDI values were 6300 g/mol and 1.30 respectively. In the next step, the dried and purified Cl-PMMA-Cl macroinitiator was used to polymerize DMA. The recipe was as follows: DMA (4.66 g, 0.03 mol), acetone (4.2 mL), CuCl (0.016 g, 0.00016 mol), bpy (0.05 g, 0.00032 mol) and Cl-PMMA-Cl (1.1 g, 0.00017 mol) (Mn = 6300 and PDI = 1.30). After 12 h the conversion was 78%. The polymer was purified by passing its solution through a silica gel column using toluene as an eluent. The copper free solution was concentrated by rotary evaporator and precipitated in petroleum ether. The polymer was dissolved in acetone and precipitated in petroleum ether again. The precipitated mass was dried in air for 12 h and then in vacuum oven at 60°C for 48 h. For quaternization, PDMA-PMMA-PDMA copolymer (poly-TBP) (1.0 g) was dissolved in DMF (10 mL). Excess n-butyl bromide (1.0 g) was added into the copolymer solution. Next, the mixture was stirred at 50°C for 24 h. The DMF was removed by rotary evaporator, and the polymer was dissolved in THF and precipitated in hexane. This process was repeated twice to remove any unreacted butyl bromide. The structural analyses were performed by ¹H and ¹³C-NMR measurements, where about complete quaternization was confirmed by the ¹H NMR spectra (Fig. S10). This TBP is abbreviated as QPDMA-b-PMMA-b-QPDMA. ¹H NMR (DMSO-d₆, 300MHz): $\delta = 3.33$ (s, 8H, +N(CH₃)₂, nBu), 3.98 (broad, 4H, -CH2-N+-CH2), 3.54 (s, 3H, -O-COCH3 of PMMA) and broad 4.37 (2H, -COOCH₂-) for quaternized PDMA unit (Fig. S10).

System	pН	Anisotropy	
AH	6.0	0.010	
	10.0	0.012	
AH/CTAB	6.0	0.068	
	10.0	0.070	
AH/TBP	6.0	0.135	
	10.0	0.134	

Table S1. Steady state fluorescence anisotropies of AH in the presence and absence of different amphiphilic self-assembled molecules.

Excitation/Emission wavelength	Medium	рН	Lifetime	Fraction
(nm)			(ns)	(%)
450/550	buffer	7.0	3.88	-
		9.0	0.31 3.90	78 22
		12.0	0.30 3.90	87 13
	CTAB/ buffer	7.0	4.17	-
		9.0	3.7	-
	TBP/buffer	7.0	4.32	-
		9.0	3.9	-
370/550	buffer	7.0	0.41 3.84	92 08
		9.0	0.35 3.3	95 05
		12.0	0.31 3.15	97 03
	CTAB/	7.0	3.32	-
	buller	9.0	3.30	-
	BP/buffer	7.0	3.42	-
		9.0	3.39	-

Table S2. Fluorescence transient decay parameters of AH.



Fig. S1. (A) ¹H-NMR and (B) ¹³C-NMR spectra of AH in DMSO-D₆.



Fig. S2. ESI-MS⁺ spectrum of [AH+H]⁺ (m/z: obs – 233.0276, cal – 233.2420) in water.



Fig. S3. UV-Vis absorption spectra of Schiff-base molecule AH (5 μ M). (A) The spectra in the presence of different concentrations of CTAB (0–5.0mM) in 20 mM tris-HCl buffer, pH 7.3. (B) The spectra in the absence (solid) and presence (broken) of deviation-saturated concentration of CTAB (5.0 mM) at different bulk pH: red, 6.0; black, 7.0; blue, 8.0. Spectrum in the absence of CTAB is shown in black in (A). The increase and decrease in intensities with increase in CTAB concentration are indicated in arrows.



Fig. S4. UV-Vis absorption spectra of Schiff-base molecule AH (5 μ M) in the presence of (red) SDS (5 mM) or (purple) TX-100 (5 mM) or (dark yellow) tetra-n-butylammonium cation (5.0 mM) in 20 mM tris-HCl buffer, pH 7.3. The spectrum in the absence of SDS, TX-100 and tetra-n-butylammonium cation is shown in black.



Fig. S5. Changes in the UV-Vis absorption spectra of Schiff-base molecule AH (5 μ M) by an addition of CTAB and/or NaCl in 20 mM tris-HCl buffer, pH 7.3: (black) without CTAB; (pink) with CTAB (5.0 mM); (blue) with CTAB (5 mM) and NaCl (750 mM).



Fig. S6. UV-Vis absorption spectra of Schiff-base molecule AH (5 μ M) with different concentration of the lipid mixture of LUV (d = 100 nm) in 20 mM tris-HCl buffer, pH 7.3: (A) 100% DDAB (lipid, 0–2.0 mM) (B) DDAB:DOPC (2:1) (total lipid, 0–2.2 mM) and (C) DDAB:DOPC (1:1) (total lipid, 0–2.5 mM). The increase and decrease in intensities with increase in lipid concentration are indicated in arrows.



Fig. S7. UV-Vis absorption spectra of Schiff-base molecule AH (5 μ M) in the presence of different diameters of 100% DDAB-LUV (lipid, 2.5 mM) in 20 mM tris-HCl, pH 7.3. Diameter of LUV: red, 50 nm; orange, 100 nm; blue, 200 nm. The spectrum in the absence of LUV is shown in black.



Fig. S8. UV-Vis absorption spectra of Schiff-base molecule AH (5 μ M) in the absence (solid) and presence (broken) of 100% DDAB-LUV of (d =100 nm; lipid, 2.5 mM) in different bulk pH: red, 20 mM cacodylate buffer, pH 6.0; black, 20 mM tris-HCl buffer, pH 7.0; blue, 20 mM tris-HCl buffer, pH 8.0.



Fig. S9. UV-Vis absorption spectra of Schiff-base molecule AH (5 μ M) (solid) in the presence of different deviation-saturated lipid mixtures of LUV, and (broken) its filtrated solution: red, 100% DDAB-LUV at pH 6.0; blue, DDAB-LUV at pH 8.0; dark cyan, 1:1 DDAB-LUV at pH 8.0. The filtrated solution was obtained using a 100K MW cut-off filter.



Fig. S10. (A) ¹H-NMR and (B) ¹³C-NMR spectrum of TBP polymer in DMSO-D₆.



Fig. S11. Normalized fluorescence excitation spectra of Schiff base molecule AH (5 μ M) for 550 nm emission at two different pH values: black, 20 mM tris-HCl buffer, pH 7.0; blue, 20 mM carbonate/bicarbonate, pH 12.0.



Fig. S12. Ground state optimized geometries of neutral AH and its two possible anion forms A⁻-I and A⁻-II.



Fig. S13. UV-Vis absorption spectra of Schiff-base molecule AH (5 μ M) in 20 mM carbonate/bicarbonate, pH 9.1, containing different extent of ethanol (v/v) (50–90%). The spectrum without ethanol at pH 9.1 is depicted in blue. The increase and decrease in intensities with increase of ethanol are indicated in arrows.



Fig. S14. pH dependent UV-Vis absorption spectral change of AH (5 μ M). The pH was increased from 6.0 (black) to 12.0 (blue) by an addition of 0.1 M NaOH and subsequently decreased to 6.0 (red broken) by an addition of 0.1 M HCl in 20 mM tris-HCl buffer.