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Supporting Information

A ratiometric solvent polarity sensing Schiff base molecule for estimating interfacial polarity of versatile amphiphilic self-assemblies

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Contents

Experimental

General experimental methods

Unless otherwise mentioned, all chemicals and solvents of purest grade were obtained from Sigma-Aldrich (USA) and were used without further purification. Milli-Q Millipore® 18.2 MΩ.cm water was used to prepare buffers and different solvent compositions contaning water for spectroscopic studies. The different buffer compositions were used to achive specific pH for the medium contaning aqueous buffer solution, pH 6.0–11.0: HEPES-NaOH for 6.0–7.5, tris-HCl for 7.5–9.0 and carbonate/bicarbonate for 9.0–11.0. The pH of the buffer was adjusted by an addition of required amount of either ~ 1.0 M NaOH or 1.0 M HCl solution, monitored with a Systronics digital pH meter (Model No. 335). For pH dependent studies, PMP was mixed with a required amount of different analytes in buffer solution, with adjustment of the final pH value with an addition of either 0.1 M NaOH or 0.1 M HCl if required. The medium dielectric constant and refractive indices for various solvent mixtures were estimated as reported previously.[1-4] ^{1}H- and ^{13}C-NMR spectra were performed in $CDCl_3$ with a Bruker 300-MHz NMR spectrophotometer using tetramethylsilane ($\delta = 0$) as an internal standard.The ESI-MS^{+} studies were performed with a Waters Qtof Micro YA263 mass spectrometer in the positive mode. IR measurements were performed with a Perkin-Elmer spectrum 2 spectrophotometer.

Synthesis of Tri-block copolymer (TBP)

The tri-block copolymer, containing poly(methylmethacrylate) central block (PMMA) and poly(2-dimethylaminoethyl) methacrylate end block (PDMA), with molecular weight of (M_n) ~ 28 kD and a polydispersity index (PDI) of 1.20 was synthesized by two step atom transfer radical polymerization as reported earlier.[5] The tertiary amino groups were subjected to quaternization by butyl bromide.[6] The structural analyses were performed by ^{1}H and ^{13}C-NMR measurements, where about complete quaternization was confirmed by the ^{1}H NMR spectra.[6] This TBP is abbreviated as QPDMA-*b*-PMMA-*b*-QPDMA.

Synthesis of Schiff-base molecule (PMP)

2-hydroxy-3-(hydroxymethyl)-5-methylbenzaldehyde (HHMB) was prepared according to the standard literature by staring with p-cresol.[7] An substantial amount of product yield (62% with respect to p-cresol) was obtained by recrystallizing the crude product from toluene-chloroform mixed solvents (8:2, *v/v*) followed by column chromatography and the purity was checked by NMR measurement. For synthesis of the Schiff base molecule, 2-((2-(pyridine-2-yl)ethylimino)methyl)-6-(hydroxymethyl)-4-methylphenol (PMP), to an methanolic solution of HHMB (0.166 g, 1 mmol), 2-(2-aminoethyl)-pyridine (AEP) (0.122g, 1 mmol) was added drop-wise at ambient temparature with constant stirring and 2 drops of AcOH were further added to it. The mixture was refluxed for 2 h at 40°C and then filtered. The filtrate was then evaporated under reduced pressure to get the crude product as gel. It was purified by column chromatography followed by rotary evaporation to obtain the pure product and dried over $CaCl_2$ under vacuum; yield: 85.8% with respect to HHMB. The structural analyses were performed by ESI-MS$^+$, ^{1}H and ^{13}C-NMR as well as IR measurements. ^{1}H NMR ($CDCl_3$, 300MHz): δ = 2.26 (s, 3H, Ar$\underline{CH_3}$), 3.17 (t, J = 6.9 Hz, 2H, $\underline{CH_2}$–CH_2), 4.02 (t, J = 6.9 Hz, 2H, CH_2–$\underline{CH_2}$), 4.7 (s, 2H, $\underline{CH_2}$), 6.92 (s, 1H, $\underline{\text{H-3}}$), 7.10-7.18 (4H, Ar$\underline{H}$), 7.26 (due to trace amount of $CHCl_3$ in the solvent $CDCl_3$), 7.57-7.62 (dd, J = 7.6 and 1.7 Hz, $\underline{\text{H-1}}$), 8.23 (s, 1H, imine-$\underline{H}$), 8.5 (s, 1H,-O$\underline{H}$) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 20.30, 39.38, 58.65, 61.85, 117.90, 121.57, 123.70, 127.21,128.43, 130.56, 132.29,136.49, 149.48, 157.78, 158.95, 165.37 ppm (Fig. S1). ESI-MS$^+$ for PMP in water: *m/z* Cal for $[PMP+H]^+$: 271.3425; Found: 271.3016 (Fig. S2). Selected IR in cm^{-1} (KBr): 3242 (br), 1632 (s), 1592 (s), 1462 (m), 1435 (s). 749(s).

Vesicle preparation

Adequate amount of 1,2-ditetradecanoyl-*sn*-glycero-3-phospo-(1'-rac-glycerol) (DMPG) was dissolved in 1 mL chloroform. Thin lipid film on the wall of a round bottom flask was prepared with a rotary evaporator by removing the chloroform solvent at room temperature. Residual amount of chloroform in the thin lipid film was completely removed *in vauco* for 3 h. Appropriate 20 mM buffer solution was added to the lipid film at 40°C for hydration of the film. The aqueous lipid solution was vortexed 2 min for complete dissolution of the lipids to form multilamellar vescicles (MLV). Typically small unilamellar vesicles (SUV) with diameters in the range of 20-60 nm as revelded from dynamic light scattering (DLS) measurement was genarated by disruption of MLV suspensions using sonic energy for 15 min by utilizing bath sonicator. For spectroscopic measurements, the resultant SUV solution was diluted with appropriate amount of buffer to obtain definite lipid concentration.

UV-Vis absorption and fluorescence studies

UV-Vis optical absorption and fluorescence measurement were performed in different solvent medium at 25°C with a UV-2450 spectrophotometer (Shimadzu, Japan) and Perkin Elmer LS-55 spectro-fluorimeter (Perkin Elmer, USA), respectively. Quartz cells with 1 cm path-lengths were used for absorption and fluorescence measurements, respectively. Spectro-fluorimeter equipped with an excitation and emission automatic polarizer was used for fluorescence anisotropic measurements. The solutions for analysis were passed through a 0.45 mm filter (Millex, Millipore) before the measurements. All measurements were repeated at least three times to check the reproducibility.

Theoretical calculations

Density functional theory (DFT) and time-dependent DFT (TD-DFT) calculations were performed using the Gaussian 09 program.[8] Geometry optimization based on the most probable structure for non-ionic (PMP^0) and zwitterionic form ($PMP^\pm$) was performed using B3LYP exchange-correlation function. The 6-31G++(d,p) basis set was selected for atoms and the geometries were optimized in gas phase. The global minima of all the structures were confirmed by the positive vibrational frequencies. To investigate the medium polarity dependent electronic absorption properties of the singlet excited state, TD-DFT calculation was applied using the optimized geometries of the ground states (S_0) for the respective species in different dielectric medium by adapting the conductor polarized continuum model (CPCM). Excitation energies, respective oscillator strengths (f_{cal}) and extinction coefficients (ε) of the optical absorption for the respective species were evaluated.

Table S1 Electronic excitation wavelength (nm), oscillator strengths (f_{cal}) and extinction coefficient (ε) of non-ionic (PMP^0) and zwitterionic ($PMP^{\pm}$) forms obtained by the TD-DFT/B3LYP/6-31G++(d,p) calculation on ground state geometries with CPCM different dielectric solvation. The experimentally obtained UV-Vis absorption (Obs/Abs) parameters for PMP are depicted for comparison.

	Form	**Solvent (κ)**	**λ (nm)**	**f_{cal}**	**$\varepsilon \times 10^{-4}$ ($M^{-1}\,cm^{-1}$)**
TD-DFT	PMP^0	Heptane (1.9)	324.63	0.109	0.46
		THF (7.4)	322.50	0.109	0.45
		MeOH (33.0)	321.45	0.104	0.43
		Water (78.5)	321.36	0.104	0.43
	$PMP^{\pm}$	Heptane (1.9)	393.08	0.169	0.66
		THF (7.4)	390.52	0.170	0.69
		MeOH (33.0)	388.52	0.164	0.66
		Water (78.5)	388.42	0.165	0.68
Obs/Abs	PMP	THF (7.4)	330.00 425.80	-	0.76 0.03
		MeOH (33.0)	329.00 423.25	-	0.56 0.32
		Water (78.5)	328.00 413.60	-	0.21 0.88

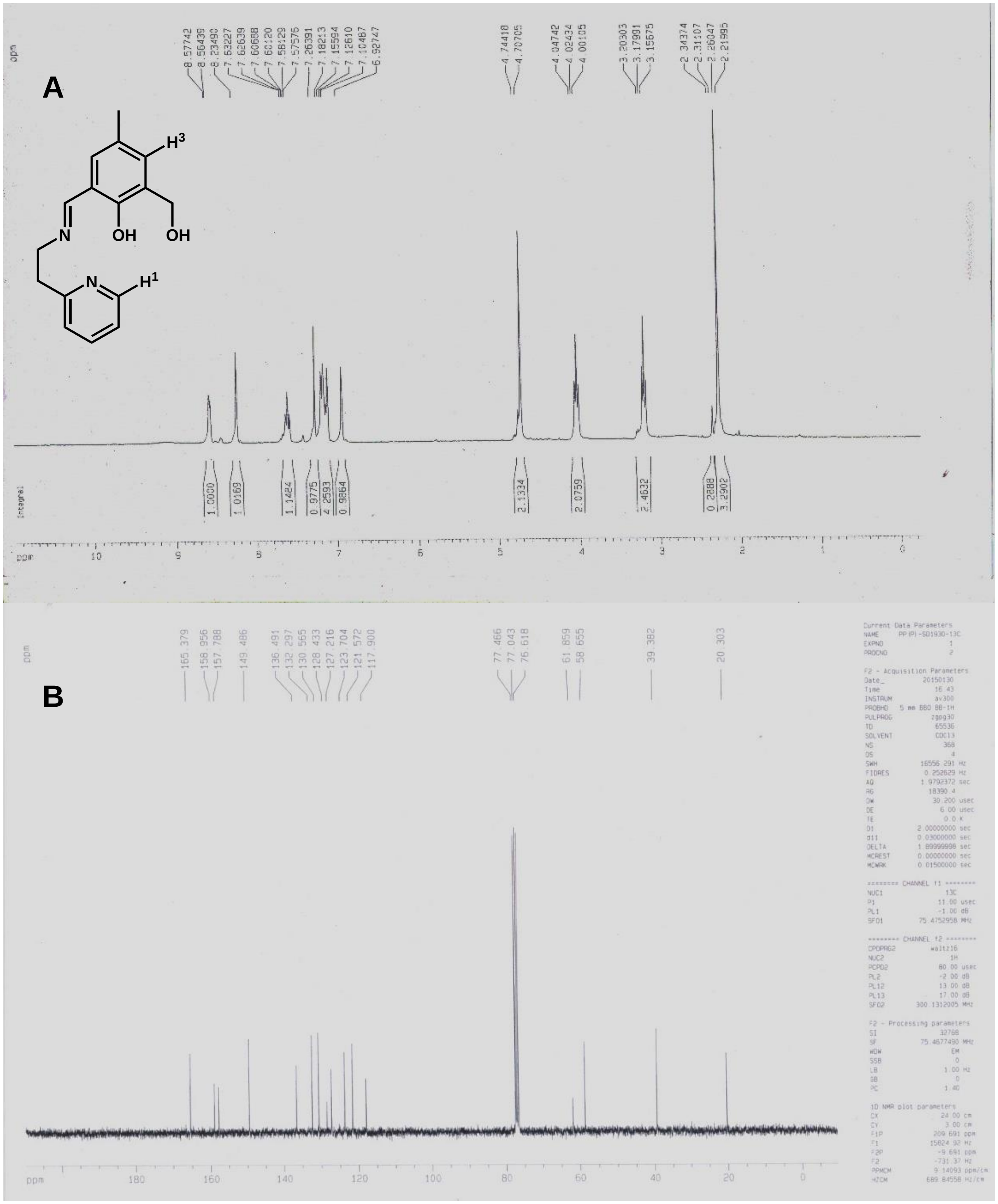

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Fig. S1 (A) ^{1}H-NMR and (B) ^{13}C-NMR spectra of Schiff base molecule PMP in $CDCl_3$.

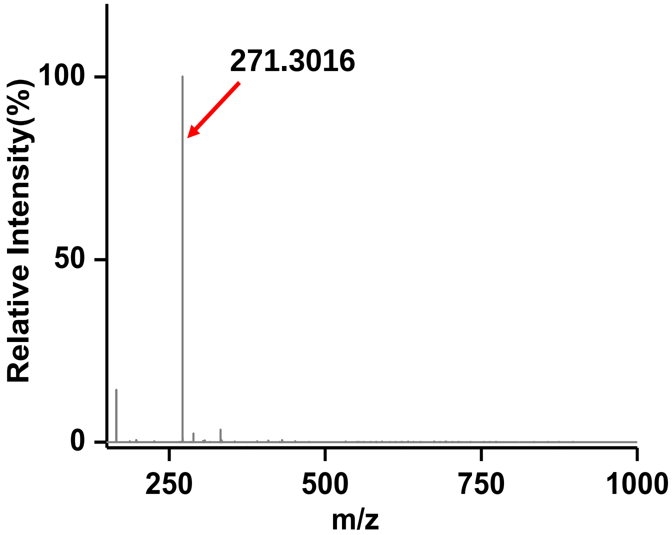

Fig. S2 ESI-MS$^+$ of Schiff base molecule PMP (*m/z* for [PMP+H]$^+$: obs'd – 271.3016 (cal'd – 271.3425)) in water.

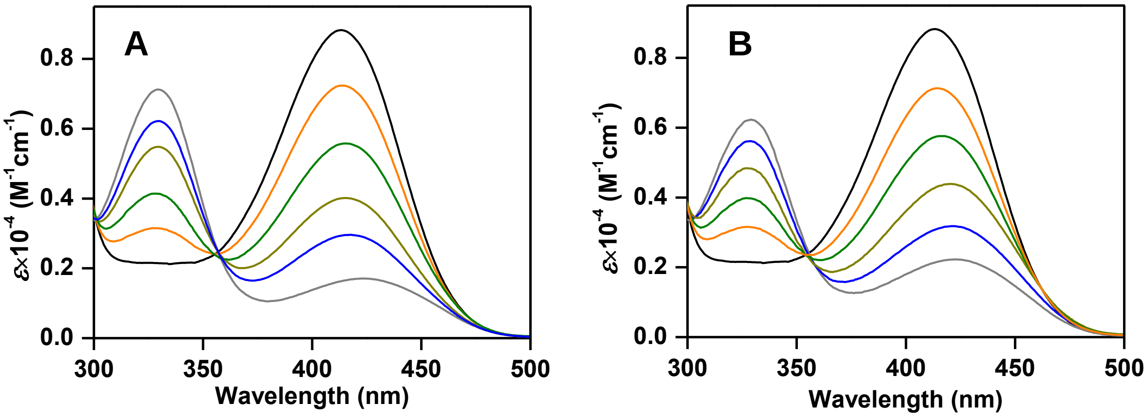

Fig. S3 UV-Vis absorption spectra of PMP (5 μM) in (A) acetone/water and (B) ethanol/ water mixed medium at different dielectric constants (wt % of acetone or ethanol in the mixtures are depicted in bracket): (A) gray, 19.1 (1.0); blue, 29.6 (0.8); dark yellow, 41.8 (0.6); green, 54.6 (0.4); orange, 67.0 (0.2); and black, 78.5 (0.0): (B) gray, 24.3 (1.0); blue, 32.9 (0.8); dark yellow, 43.5 (0.6); green, 55.1 (0.4); orange, 67.3.0 (0.2); and black, 78.5 (0.0).

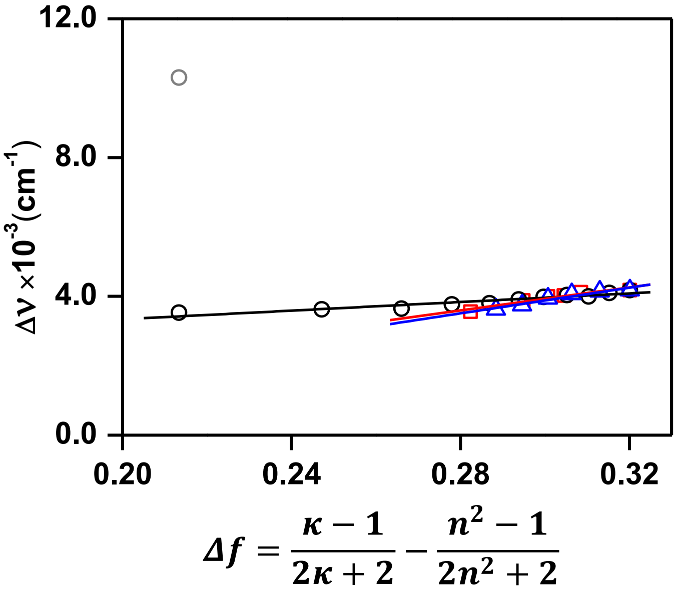

Fig. S4 Lippert plot for PMP in (black) THF/water, (red) acetone/water and (blue) ethanol/water mixtures: Δν, stokes' shift for ~ 500 nm fluorescence intensity; Δ*f*, orientation polarizability containing dielectric constant (*κ*) and refractive index (*n*). The energy difference (cm^{-1}) between the absorption intensity maxima around 330 nm and the fluorescence intensity maxima around 500 nm for PMP in THF medium is depicted by gray point for comparison.

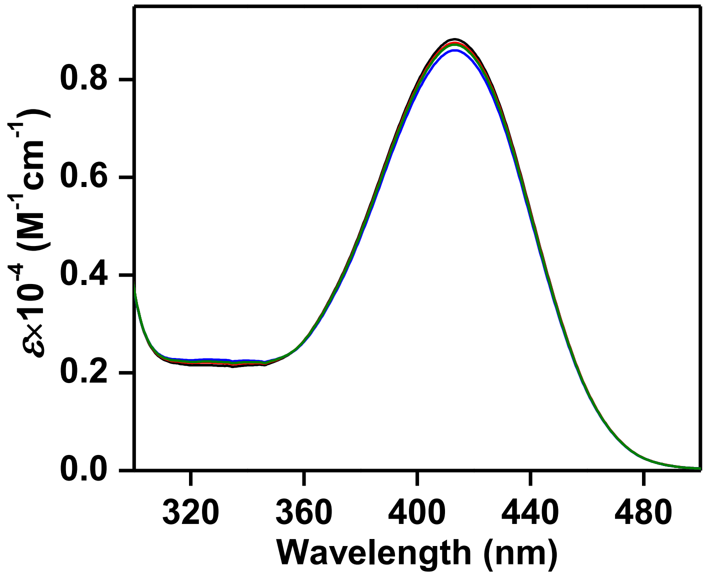

Fig. S5 UV-Vis absorption spectra of Schiff base molecule PMP (5 μM) in buffer at different pH: black, 6.0; red, 7.0; blue, 9.0; green, 11.0.

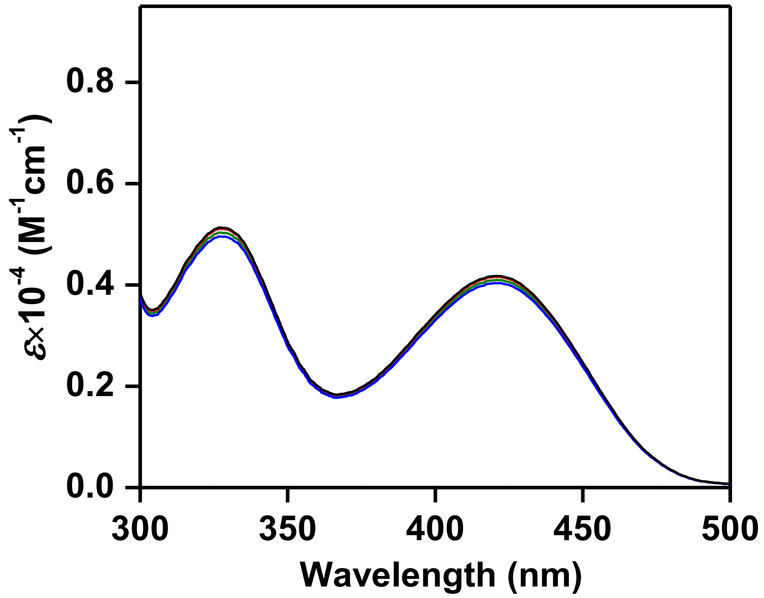

Fig. S6 UV-Vis absorption spectra of Schiff base molecule PMP (5 μM) in 1:1 THF/buffer solution at different pH: black, 6.0; red, 7.0; blue, 9.0; green, 11.0.

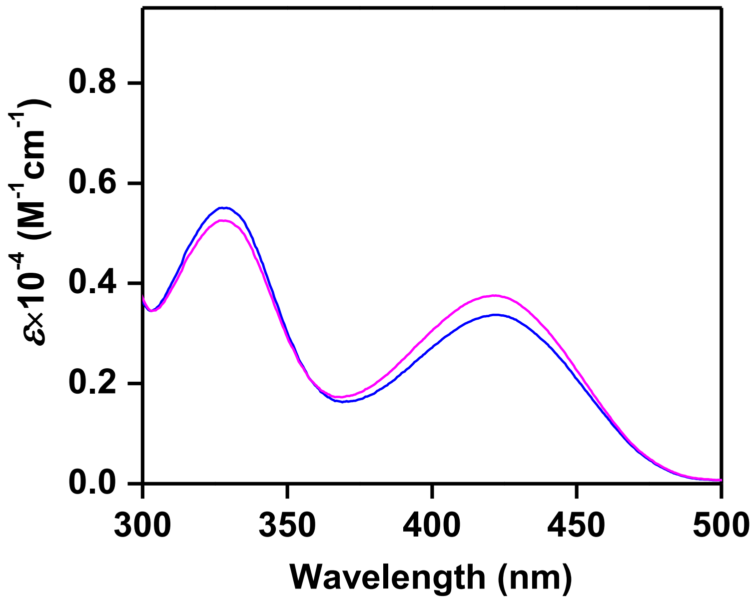

Fig. S7 Solvent dependent UV-Vis absorption spectra of PMP (5 μM) under identical dielectric constant (37.5): pink, THF/water mixture (52.5% (*w/w*) THF in the mixture); blue, ACN.

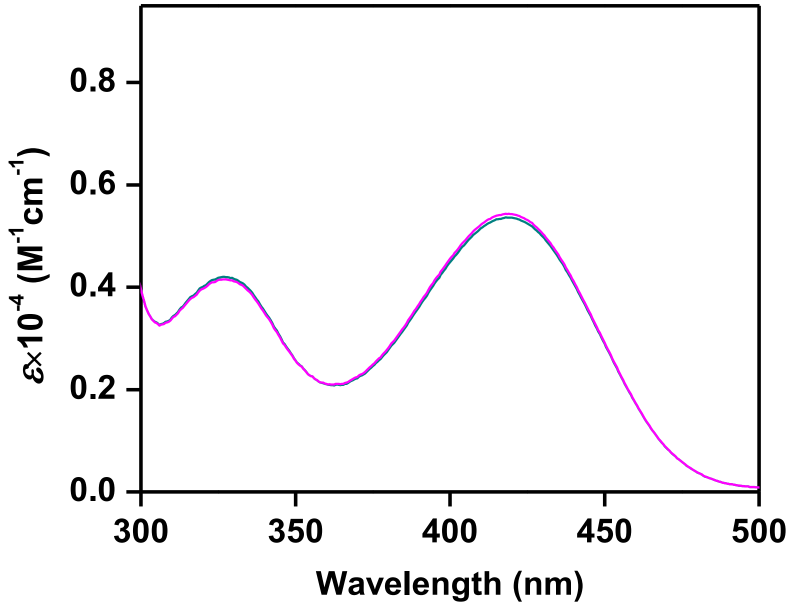

Fig. S8 UV-Vis absorption spectra of PMP (5 μM) at different medium viscosity under identical dielectric constant (51.0): pink, water/THF mixed medium (37.5% (*w/w*) THF in the mixture); cyan, water/glycerol mixed medium (80% (*w/w*) glycerol in the mixture).

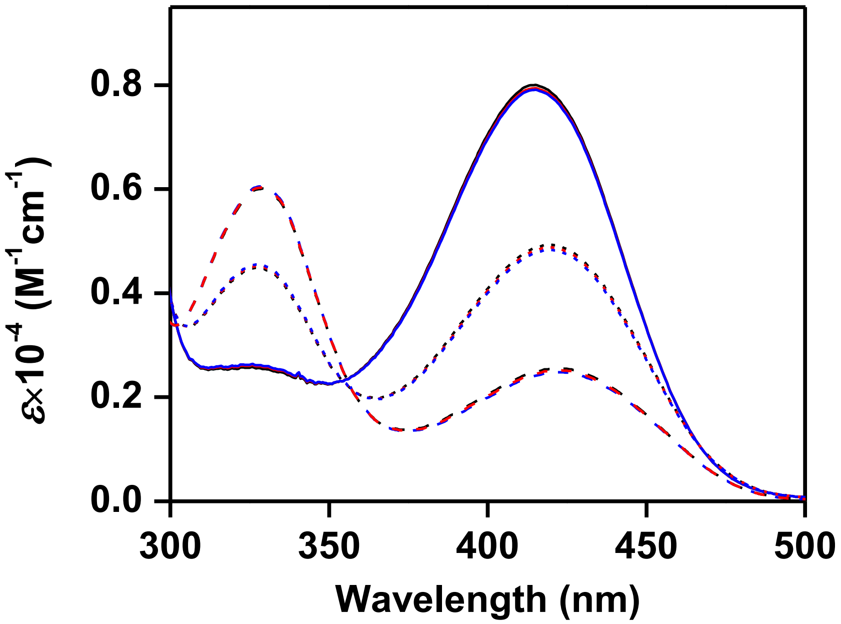

Fig. S9 UV-Vis absorption spectra of PMP (5 μM) in buffer of different pH (6.0, black; 7.0, red; 8.0, blue) and dielectric constants (solid, 72.0; short dash, 48.0; broken, 25.0) of the medium.

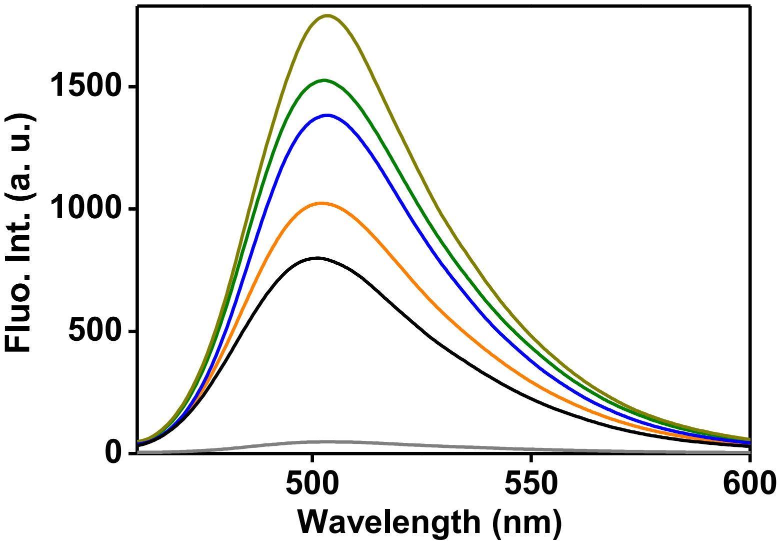

Fig. S10 Fluorescence spectra of PMP (5 μM) in water/THF mixed medium at different dielectric constants (wt % of THF in the mixtures are depicted in bracket): gray, 7.4 (1.0); blue, 18.3 (0.8); dark yellow, 32.0 (0.6); green, 48.2 (0.4); orange, 64.6 (0.2); and black, 78.5 (0.0).

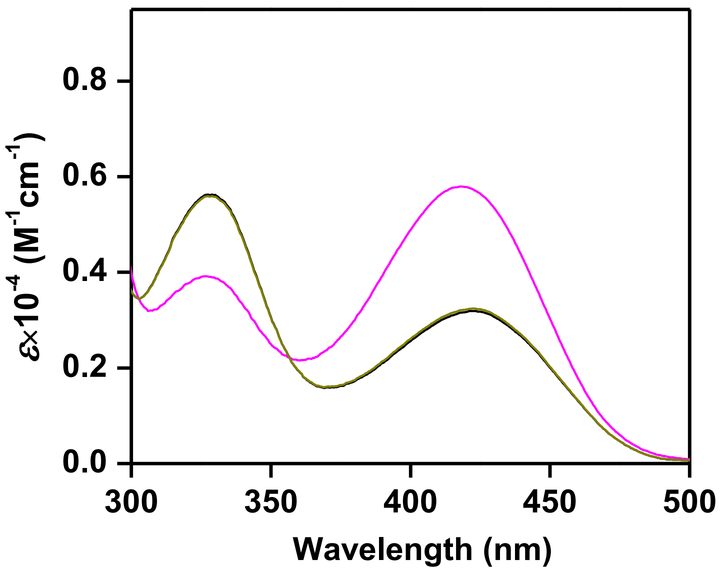

Fig. S11 Polarity dependent UV-Vis absorption spectral change of PMP (5 µM). The polarity was increased for the MeOH medium from 33.0 (black) to 55 (pink) by an addition of water (50% (*w/w*) of water in the MeOH/water mixture) and subsequently decreased to 33.0 by addition of THF (48 and 26% (*w/w*) of THF and water in THF/water/MeOH mixture, respectively).

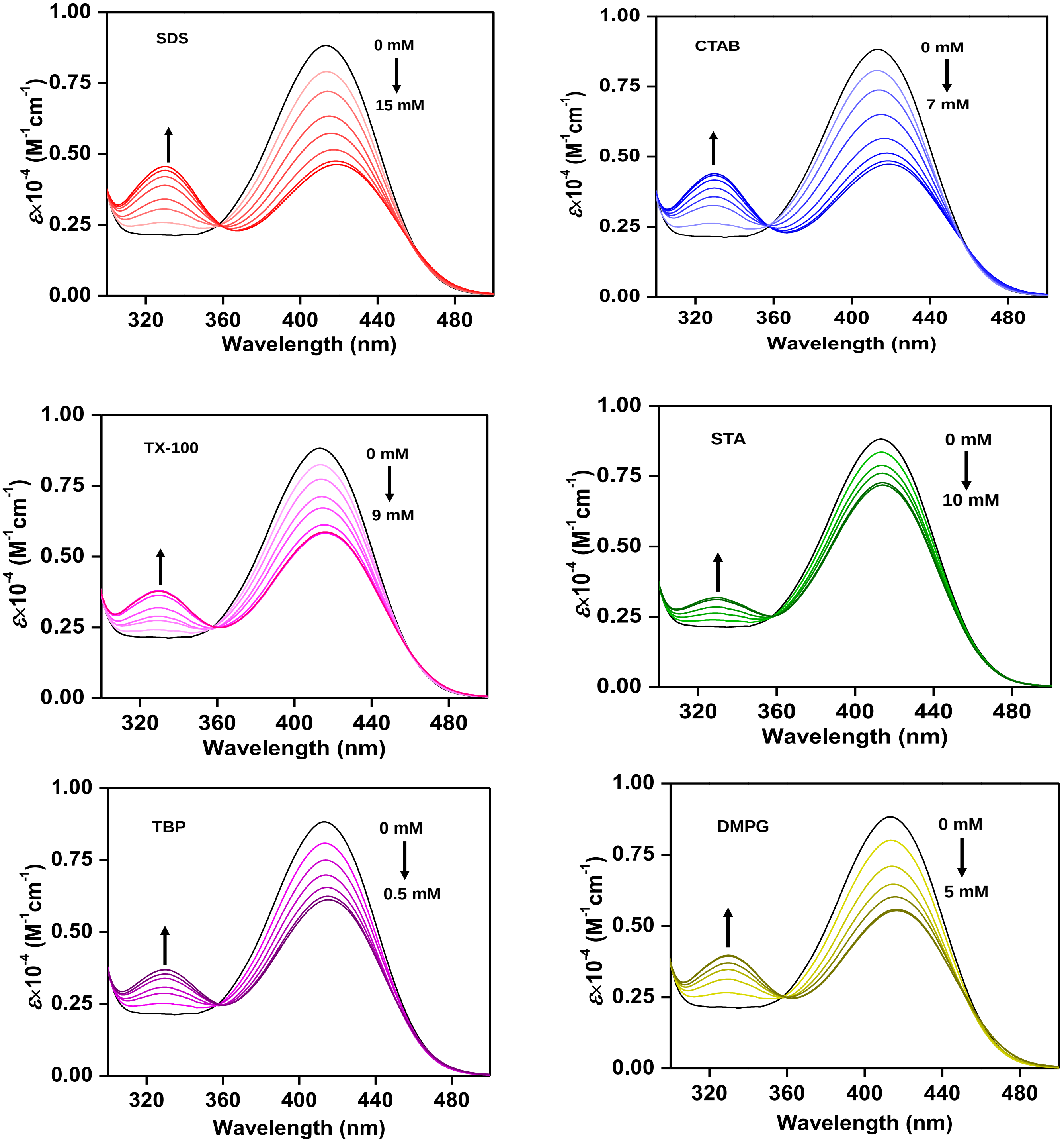

Fig. S12 UV-Vis absorption spectra of PMP (5 μM) in presence of increasing concentration of different self-assembled systems in 20 mM HEPES-NaOH, pH 7.3.

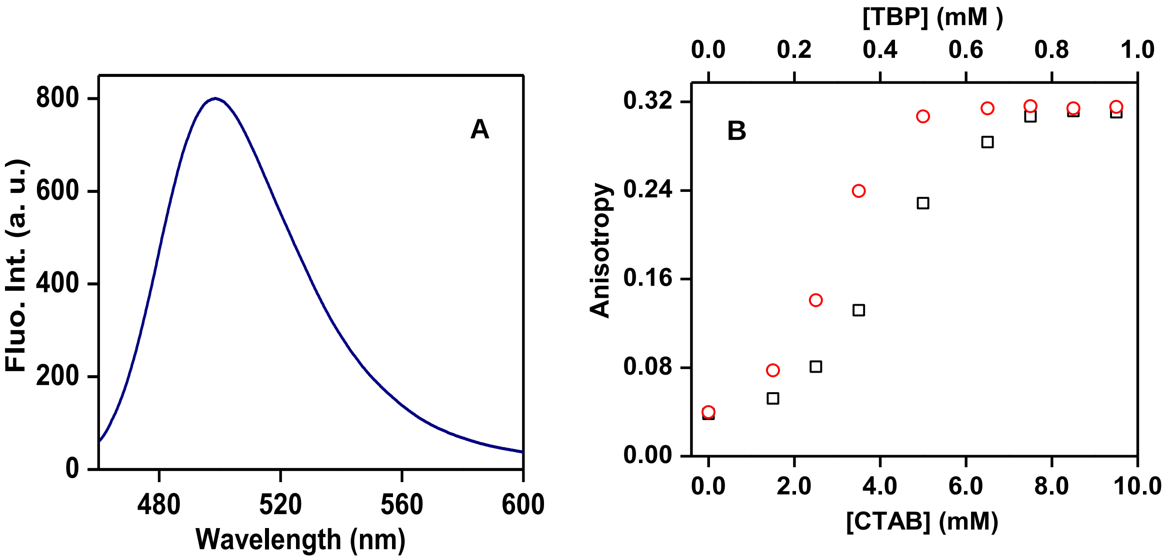

Fig. S13 (A) Steady state fluorescence spectra of PMP (5.0 µM) in buffer, pH 7.3. (B) Change in fluorescence anisotropy values with concentration of different amphiphilic systems in buffer, pH 7.3: (squire) CTAB (lower x-axis), (circle) polymer TBP (upper x-axis). (A,B) Excitation and (B) emission wavelength were 440 and 500 nm, respectively.

Reference:

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