

Supporting Information for

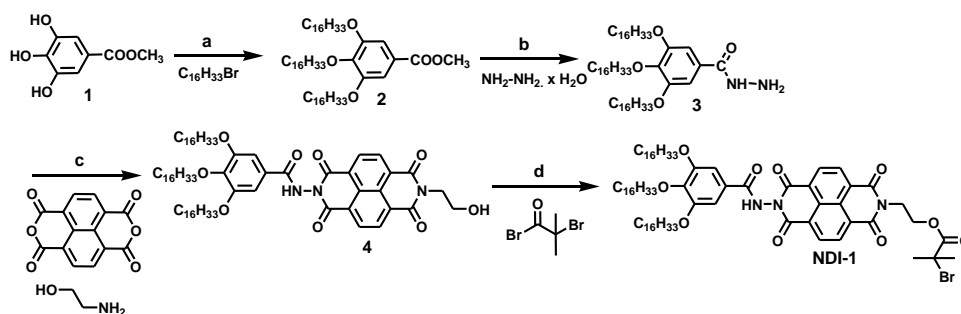
Luminescent Invertible Polymersome by Remarkably Stable Supramolecular-Assembly of Naphthalene-Diimide (NDI) π -System

Anindita Das and Suhrit Ghosh*

Polymer Science Unit
Indian Association for the Cultivation of Science
Jadavpur, Kolkata – 700 032, India.

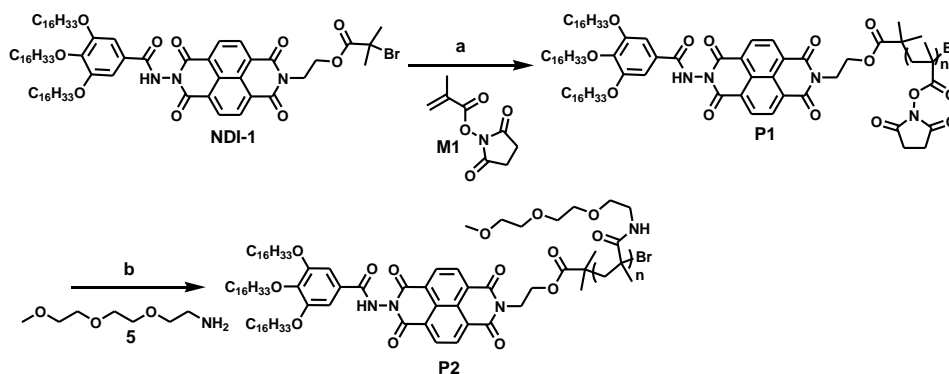
*Corresponding author; Email: psusg2@iacs.res.in

Synthesis and Characterization: Synthesis of initiator (**NDI-1**), parent polymer **P1** and the substituted polymer **P2** are depicted in Scheme S1 and Scheme S2, respectively. Synthesis of Compound **5** has been described by us elsewhere.¹



Reagents: a) 1-bromohexadecane, anhydrous K_2CO_3 , DMF, 85 °C, 48 h, 45 %; b) hydrazine hydrate, t-butanol, 80 °C, 24 h, 93 %; c) 1,4,5,8 naphthalenetetracarboxylic acid bis-anhydride, 2-aminoethanol, DMF, 140 °C, 18 h, 24 %; d) 2-bromoisobutyrylbromide, NEt_3 , DCM, 0 °C - rt, 18 h, 58 %

Scheme S1: Synthesis of Initiator



Reagents: a) PMDETA, CuBr, anisole, 90 °C, 55 min, 52 %; b) NEt_3 , DMF, 85 °C, 12 h, 50 %

Scheme S2: Synthesis of polymer P1 and P2

Methyl 3, 4, 5-tris(hexadecyloxy)benzoate² (2): Compound **1** (10 g, 54.31 mmol), 1-bromohexadecane (54.7 g, 179 mmol) and anhydrous K_2CO_3 (24.7 g, 179 mmol) were taken together in a 250 ml flask with dry DMF (60 ml) and the reaction mixture was stirred at 85 °C

for 48 h under N₂ atmosphere. The solution was allowed to cool to rt. The mixture was diluted with diethylether (80 ml) and washed with H₂O (2 x 50 ml) and brine (1 x 50 ml). The organic layer was dried over anhydrous Na₂SO₄ and the solvent was evaporated to get the crude product which was purified by column chromatography with basic alumina as the stationary phase and 5 % CH₂Cl₂ in petroleum ether as the eluent to get the pure product as a white solid (20 g, 45 %). Mp. 49 -51 °C; ¹H NMR (CDCl₃, 500 MHz): δ (ppm) = 7.25 (s, 2H), 4.03-3.99 (m, 6H), 3.88 (s, 3H), 1.82-1.72 (m, 6H), 1.48-1.44 (m, 6H), 1.31-1.26 (m, 72 H), 0.88 (t, *J* = 5.0 Hz, 9H); UV-visible (CH₂Cl₂): λ_{max} (ε) = 269 (9923 M⁻¹ cm⁻¹).

Methyl 3, 4, 5-tris(hexadecyloxy)benzohydrazide (3): Compound **2** (1.0 g, 1.167 mmol) and hydrazine hydrate (10 g, 555 mmol) were taken with t-butanol (30 ml) in a flask and refluxed at 80 °C for 24 h. Excess t-butanol was evaporated and the solution was diluted with CH₂Cl₂ (30 ml) and washed with water (2 x 30 ml). The organic extract was dried over anhydrous Na₂SO₄ and then CH₂Cl₂ was evaporated under reduced pressure to obtain the desired product as white solid (931 mg, 93 %). As the product was found to be pure from TLC and ¹H NMR, it was carried to the next step without further purification. Mp. 73 - 75 °C; ¹H NMR (CDCl₃, 500 MHz): δ (ppm) = 7.23 (b, 1H), 6.92 (s, 2H), 4.00 - 3.98 (m, 6H), 1.83 - 1.72 (m, 6H), 1.47-1.41 (m, 6H), 1.28 - 1.26 (m, 72H), 0.89 - 0.86 (m, 9H); FT-IR (wavenumber / cm⁻¹): 3319 (N-H, free amine), 3270 (N-H amide), 2955 (=C-H alkyl), 2917, 2848 (-CH alkyl), 1640, (hydrazide carbonyl) ; UV-visible (CH₂Cl₂): λ_{max} (ε) = 264 (9468 M⁻¹ cm⁻¹).

Compound 4: Compound **3** (922 mg, 1.075 mmol), 2-aminoethanol (98 mg, 1.613 mmol) and 1,4,5,8-naphthalenetetracarboxylic bis-anhydride (346 mg, 1.290 mmol) were taken together with dry DMF (15 ml) and heated at 140 °C for 18 h under N₂ atmosphere. The solution was cooled to rt and placed in the refrigerator for 2 h. The brown precipitate obtained was filtered and collected. The filtrate was added with MeOH (2 ml) to get a light brown precipitate which was mixed with the first crop and the combined solid obtained was purified by column chromatography using silica gel as stationary phase and 15 % ethylacetate in CH₂Cl₂ as eluent to obtain the desired product as light yellow solid (300 mg, 24 %). Mp. 117 - 120 °C; ¹H NMR (CDCl₃, 500 MHz): δ (ppm)= 8.81 (s, 4H), 8.25 (b, 1H), 7.18 (s, 2H), 4.49 (t, *J* = 5, 2H), 4.05 - 4.03 (m, 8H), 1.84 - 1.81 (m, 6H), 1.49 - 1.46 (m, 6H), 1.28 - 1.25 (m, 72H), 0.89 - 0.86 (m, 9H); FT-IR (wavenumber / cm⁻¹): 3487 (O-H), 3244 (N-H amide), 2956, 3082 (=C-H alkyl), 2917, 2849 (-CH alkyl), 1713 (imide carbonyl asymmetric stretching), 1697 (non H-bonded hydrazide carbonyl), 1667 (imide carbonyl symmetric stretching); UV-visible (CH₂Cl₂): λ_{max} (ε) = 279 (9595), 340 (9429), 358 (15599), 379 (19160 M⁻¹ cm⁻¹).

NDI-1: A solution of 2-bromoisobutyrylbromide (0.26 ml, 2.164 mmol) in 2 ml dry CH₂Cl₂ was added dropwise to an ice-cold solution of compound **4** (280 mg, 0.216 mmol) and Et₃N

(0.3 ml, 2.164 mmol) in dry CH₂Cl₂ (15 ml) for 15 min. The reaction mixture was stirred in the ice-bath for 1 h and then at rt for 18 h. CH₂Cl₂ was evaporated under reduced pressure and the yellow crude was purified by column chromatography using silica gel column and 4 % EtOAc in CH₂Cl₂ as eluent to obtain the desired product as cream colored solid (180 mg, 58 %). Mp. 68 - 70 °C; ¹H NMR (CDCl₃, 500 MHz): δ 8.82 (dd, *J* = 15.5, 7.5, 4H), 8.30 (b, 1H), 7.18 (s, 2H), 4.61 - 4.58 (m, 4H), 4.04 (t, *J* = 6.5, 6H), 1.93 (s, 6H), 1.87 - 1.73 (m, 6H), 1.49 - 1.43 (m, 6H), 1.28 - 1.26 (m, 72H), 0.89 - 0.84 (m, 9H); FT-IR (wavenumber / cm⁻¹): 3432 (N-H amide), 2956 (=C-H alkyl), 2919, 2848 (-CH alkyl), 1741 (ester carbonyl), 1709 (imide carbonyl asymmetric stretching), 1695 (non H-bonded hydrazide carbonyl), 1655 (imide carbonyl symmetric stretching); UV-visible (CH₂Cl₂): λ_{max} (ε) = 273 (8775), 339 (8616), 358 (15260), 378 (19442 M⁻¹ cm⁻¹).

Polymer P1: Cu(I)Br (2.3 mg, 0.016 mmol) was taken in a 10 ml glass ampule equipped with a septa and a gas inlet/outlet. The ampule was degassed with argon for 5 min. A stock solution of N,N,N',N',N''-pentamethyl diethylenetriamine (PMDETA) in dry and degassed anisole (10 μl in 100 μl) was made and from this 69 μl (0.033 mmol) was added to the polymerization vessel and the reaction mixture was stirred for another 5 minutes. To this a homogenous solution of the monomer **M1** (150 mg, 0.819 mmol) and initiator (**NDI-1**, 23.6 mg 0.0164 mmol) in 0.25 ml degassed anisole was added and was stirred for another 2 minutes and the flask was transferred to a preheated oil bath at 90 °C and polymerization was carried out under argon atmosphere for 55 minutes while the reaction mixture solidified. The reaction was stopped and the green solid was dissolved in 0.1 ml DMF and precipitated from excess acetone. The precipitate obtained was centrifuged and dried in vacuum for 4 h to obtain the desired polymer as white solid (60 mg, 52 %). ¹H NMR (500 MHz, DMSO-D₆): δ (ppm) = 8.77 (broad peak, 4H), 7.32 (broad peak, 2H), 4.39 (broad peak, 4H), 4.04-3.94 (m, 6H), 2.78 (broad peak, 174H), 1.45 -1.44 (broad peak, 132H) 1.23 (s, 132H,), 0.85 (t, *J* = 7, 9H); FT-IR (wavenumber / cm⁻¹): 3511 (N-H amide), 2996 (=C-H alkyl), 2925, 2853 (-CH alkyl), 1812 (ester C=O), 1783 (imide C=O, asymmetric stretching), 1741 (imide C=O, symmetric stretching).

Polymer P2: Polymer **P1** (50 mg, 0.273 mmol), **5** (178 mg, 1.092 mmol) and Et₃N (0.152 ml, 1.092 mmol) were dissolved in 0.2 mL dry DMF and the solution was stirred at 85 °C for 12 h under N₂ atmosphere. The solution was cooled to rt and then poured in excess diethylether to obtain a sticky brown precipitate which was dissolved in water and purified by micro-centrifugation using a Amicon Ultracel-3K (MWCO: 3 KD) centrifugation tube to remove water soluble impurities. The centrifuged solution was freeze dried to obtain the pure polymer as off white solid (30 mg, 50 %). Molecular weight was obtained from GPC; M_n= 9900, PDI= 1.32; ¹H NMR (500 MHz, DMSO-D₆): δ (ppm) = 8.77 (broad peak, 4H), 7.25 (broad peak, 2H), 3.52 - 3.20 (several broad peaks overlapping with DMSO peak), 1.96-0.85 (broad peak);

FT-IR (wavenumber / cm^{-1}): 3433 (N-H amide), 2922 (=CH alkyl), 2853 (-CH alkyl), 1725 (ester C=O), 1650 (amide C=O).

Additional Figures:

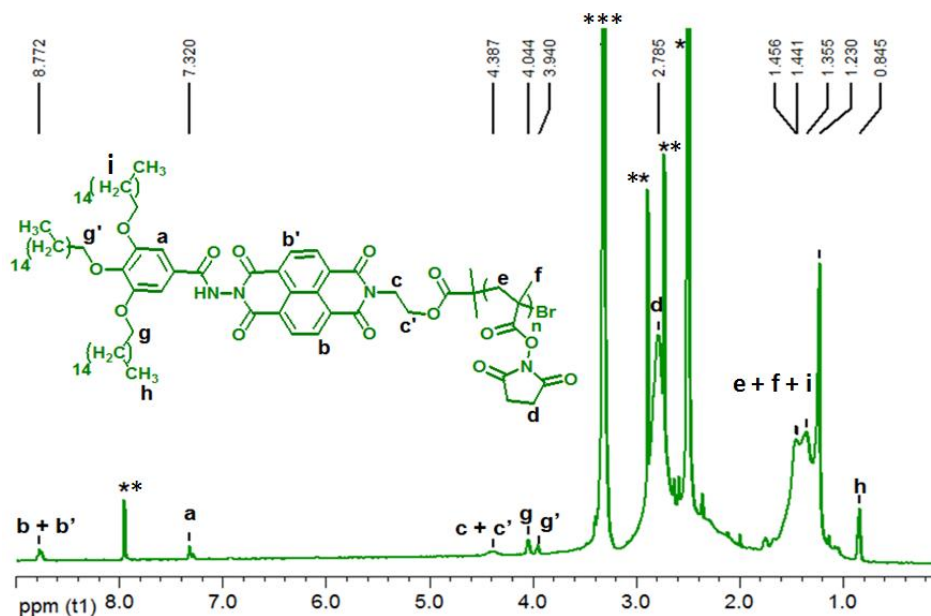


Figure S1: ^1H NMR of **P1** in DMSO-d_6 ; * indicates peak from DMSO , ** indicates peaks from DMF and *** indicates peak from water in DMSO .

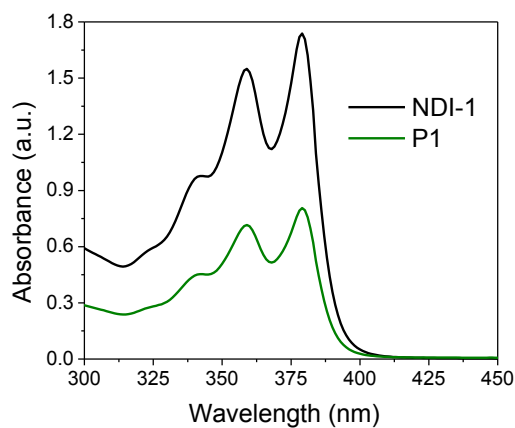


Figure S2: UV/vis spectra of **P1** (0.5 mg / ml) and **NDI-1** (0.25 mg / ml) in DMF ; $l = 1.0$ cm, $T = 25$ $^\circ\text{C}$

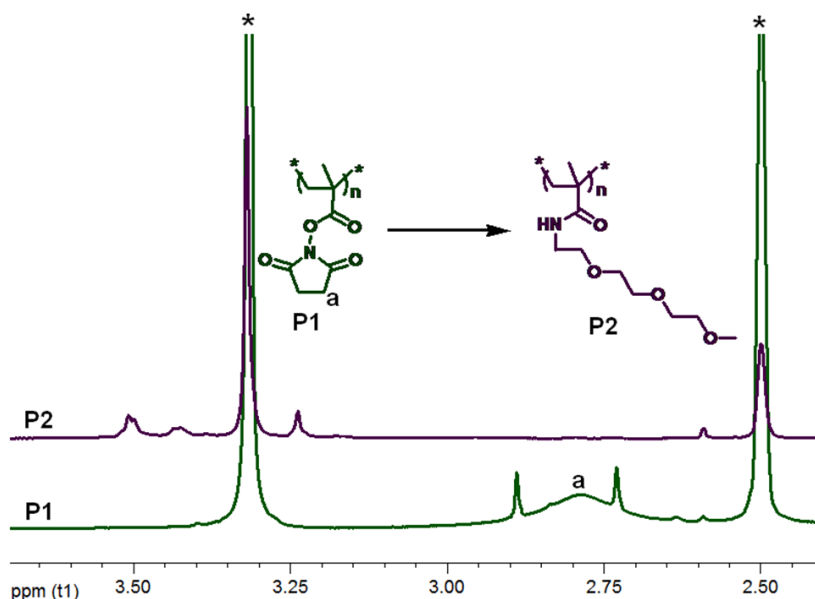


Figure S3: ^1H NMR spectra (selected region) of **P1** and **P2** in DMSO-d_6 ; * indicates peaks from DMSO and water. NHS-proton peak (a) was completely absent in **P2** spectrum clearly suggesting complete substitution. Further new peaks in the region of 3.2-3.6 ppm (which also overlaps with DMSO peaks) suggests incorporation of oligooxyethylene moiety¹

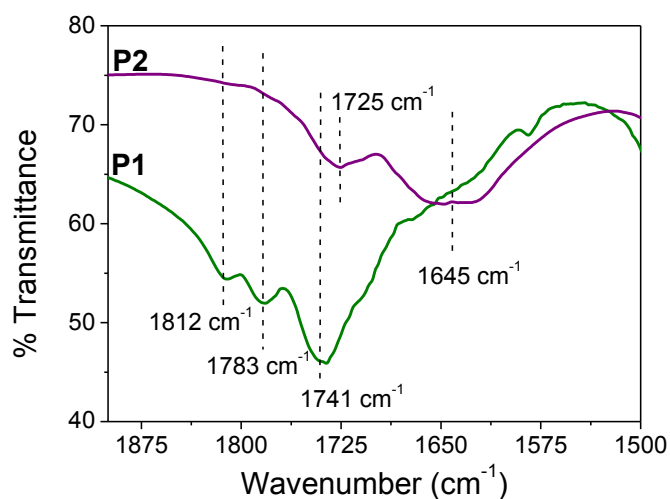


Figure S4: FT-IR spectra of **P1** and **P2** in KBR pellet. Peaks at 1812, 1783 and 1741 cm^{-1} are absent in **P2** suggesting complete removal of the NHS-ester group. In **P2** spectrum, peaks at 1725 and 1645 cm^{-1} (broad peak) are due to the ester + imide and amide carbonyls, respectively^{1,3}

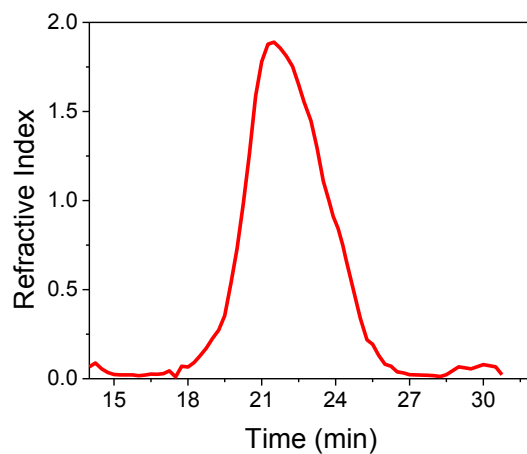


Figure S5: GPC chromatogram of **P2** in THF

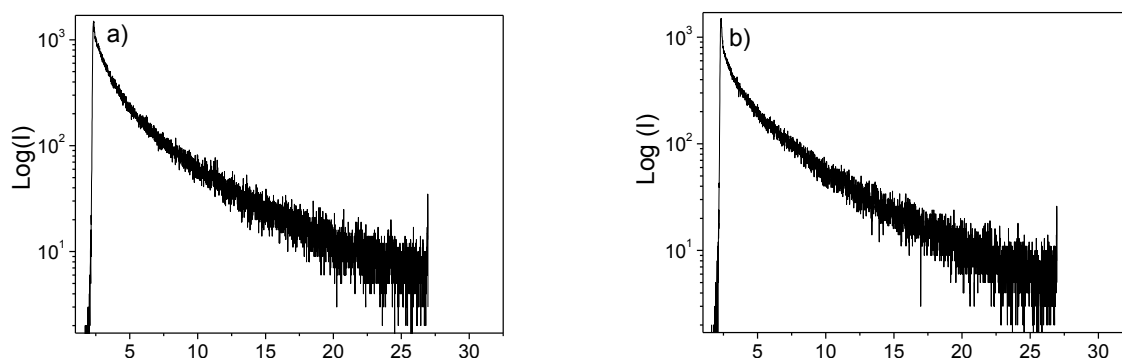


Figure S6: Fluorescence decay profile of NDI functionalized **P2** in THF (a) and H₂O (b); [P2] = 2.5 mg / ml, λ_{ex} = 340 nm

Table S1: Fluorescence lifetime data for **P2** in THF and Water. Emission was monitored at 440 nm

P2	Component Life time (ps) ^a			
	t_1	t_2	t_3	t_{avg}
THF	50 (67 %)	1000 (26 %)	4600 (7 %)	616
Water	50 (81 %)	980 (13 %)	4600 (6 %)	444

^aValues in parenthesis shows the decay contribution corresponding to each lifetime. Average lifetimes (t_{avg}) were calculated using the following equation: $t_{avg} = a_1t_1 + a_2t_2 + a_3t_3$

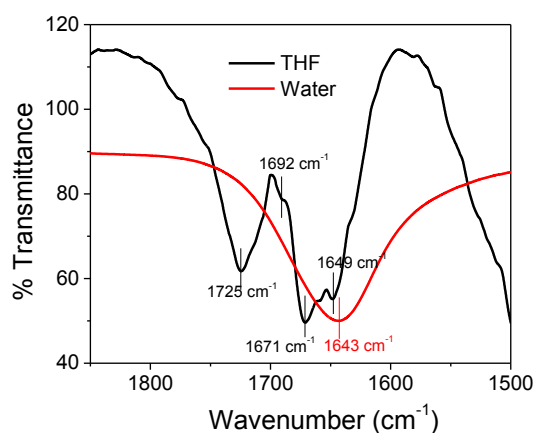


Figure S7: FT-IR spectra of **P2** in THF (black) and H₂O (red). Peaks at 1725 and 1671 cm⁻¹ corresponds to antisymmetric and symmetric stretching of imide carbonyl. Whereas peaks at 1692 and 1649 cm⁻¹ are due to the hydrazide and amide carbonyls, respectively, in THF. In H₂O, a broad peak appears at 1643 cm⁻¹; [**P2**] = 2.5 mg / ml

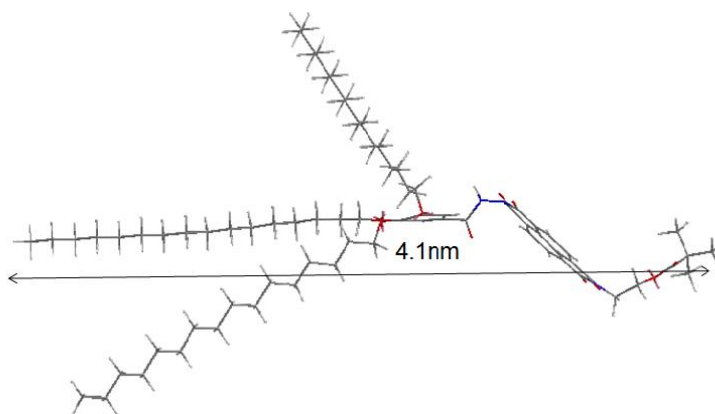


Figure S8: Energy minimized structure of the hydrophobic wedge-linked NDI-segment. Molecular modeling was done in Chem3D Ultra 8.0 using MM2 for energy minimization.

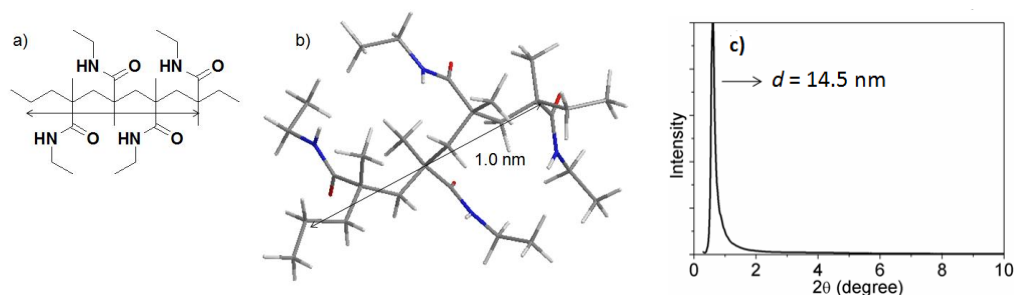


Figure S9: Energy minimized structure (b) of four repeat units (as shown in a) of the hydrophilic polymer chain. Oligooxyethylene side chain has been omitted for simplicity. Considering total number of repeat unit = 40, total length of the hydrophilic polymer chain = (1 × 10) nm = 10 nm. (c) Powder XRD pattern of air dried aqueous solution of **P2**. To corroborate the *d* value, we roughly estimated the length of the hydrophilic polymer chain and NDI segment (energy minimized structure is shown in Figure S8) to be ~ 10 nm and 4.1 nm, respectively. Thus the *d* spacing corresponding to the bilayer membrane should have been {2

$\times (10 \times 4.1) \} = 28.2 \text{ nm}$. But much lesser value from XRD suggests the hydrophilic polymer chains were far from being fully extended which is expected because in dry state a flexible polymer chain such as this has no motivation to remain fully extended, particularly when in contact with air which is known to be hydrophobic in nature.

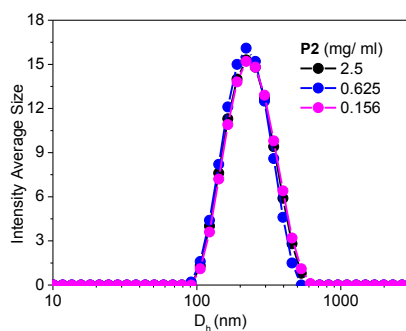


Figure S10: (a) Intensity averaged size distribution from DLS measurements of aqueous solution **P2** at various concentrations. $T = 25 \text{ }^\circ\text{C}$.

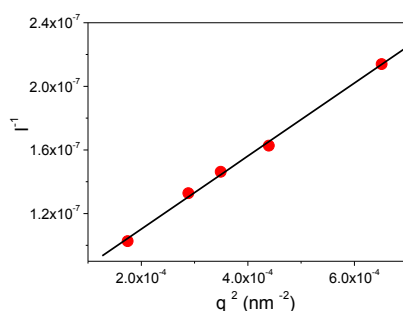


Figure S11: Partial Zimm plot obtained for **P2** in water (2.5 mg / ml). R_g was determined from the slope using the equation described before

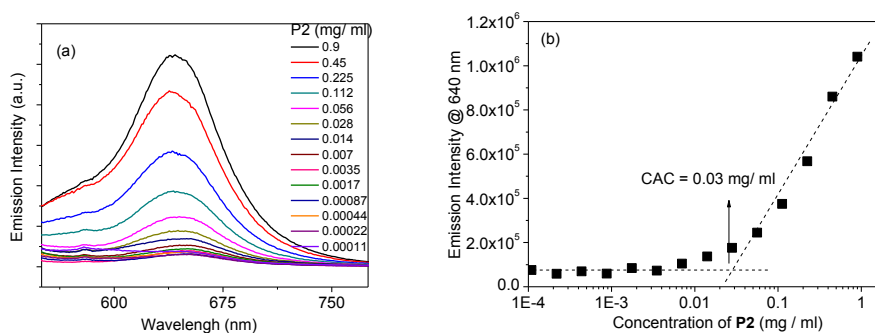


Figure S12: Emission spectra of Nile red ($\lambda_{ex} = 530 \text{ nm}$) in presence of various amount of **P2** in water. (b) Plot of emission intensity at 640 nm as a function of concentration of **P2**.

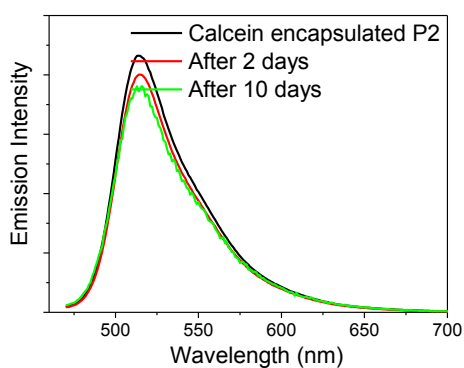


Figure S13: Emission spectra of Calcein encapsulated **P2** as a function of time. [Calcein] = 5×10^{-5} M, [**P2**] = 2.5 mg / ml, λ_{ex} = 450 nm

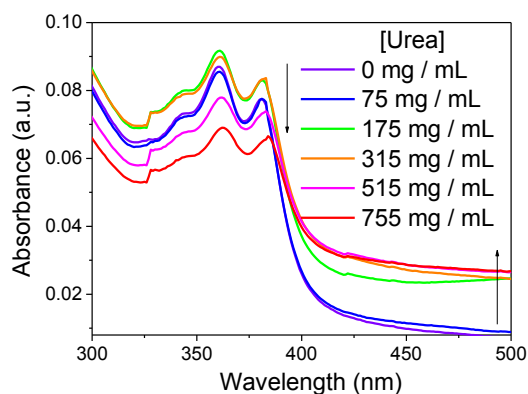


Figure S14: Variable temperature UV/vis spectra of **P2** in H₂O (2.5 mg / ml); Path length of cuvette = 0.1 cm, T = 25 °C. No change in spectra was noticed even at 85 °C

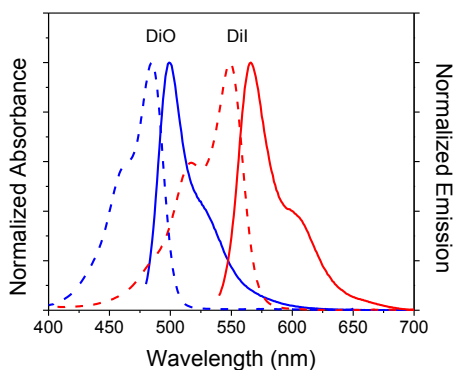
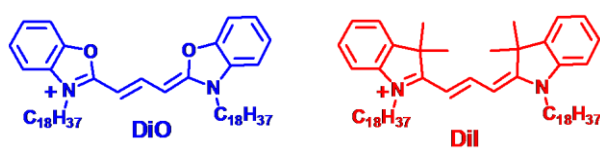


Figure S15: Structure of DiO and DiI (Top); Intensity normalized absorption (dashed line) and emission (solid line) spectra of DiO and DiI in MeOH (bottom). Dye concentration = 10^{-4} M, T = 25 °C; Path length of cuvette = 1.0 cm

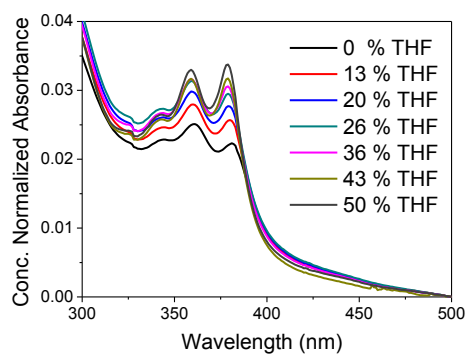


Figure S16: Absorption spectra of an aqueous solution of **P2** (0.5 mg / ml) as a function of increasing % of THF (v/v). $T = 25\text{ }^{\circ}\text{C}$; Path length of cuvette = 0.1 cm. Intensity reversal of S_0 - S_1 and S_0 - S_2 bands takes place above 26 % THF suggesting disassembly

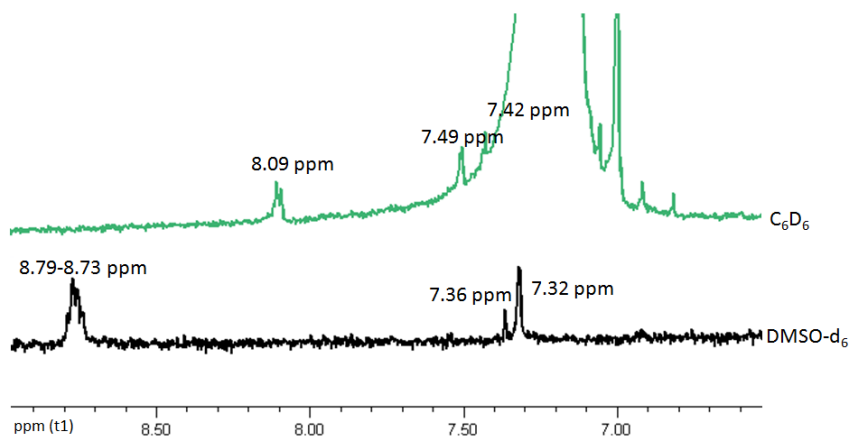


Figure S17: Solvent dependent ^1H NMR (selected region is shown) studies

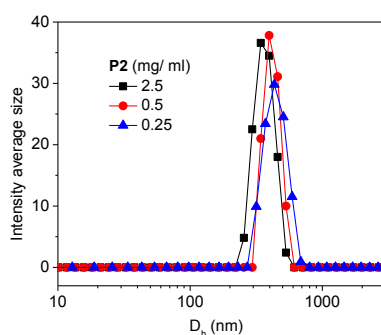


Figure S18: Intensity averaged size distribution from DLS measurements **P2** in benzene at three different concentrations. $T = 25\text{ }^{\circ}\text{C}$

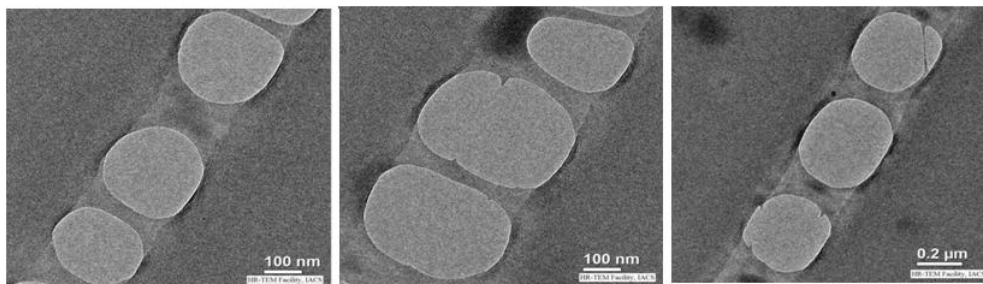


Figure S19: TEM images (collected from various sections of the grid) of the reverse vesicle formed by **P2** in benzene. $[P2] = 2.5 \text{ mg / ml}$

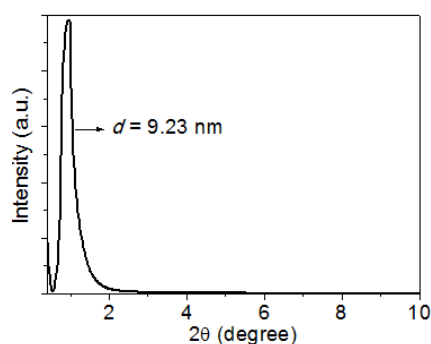


Figure S20: XRD pattern of air dried **P2** solution in benzene. It is interesting to note that the d -value in reverse vesicle is significantly less compared to that in vesicle (14.4 nm) (Figure S9) because in benzene due to interdigitation of longer hydrophilic segment (Scheme 1) extent of deviation from twice the length of the polymer chain was more pronounced.

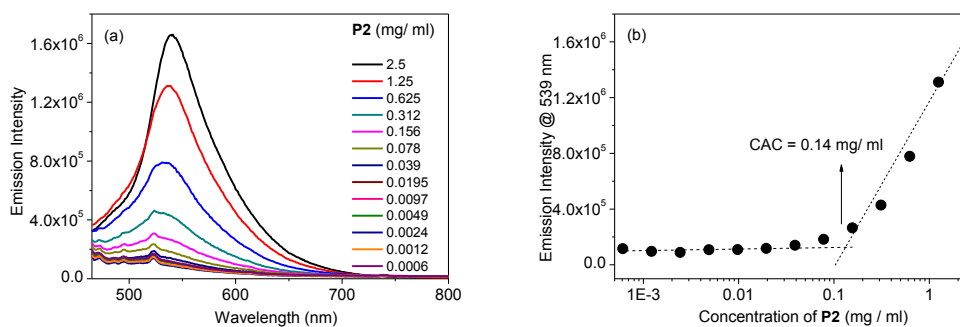


Figure S21: Emission spectra of Calcein ($\lambda_{ex} = 450 \text{ nm}$) in presence of various amount of **P2** in benzene. (b) Plot of emission intensity at 539 nm as a function of concentration of **P2**.

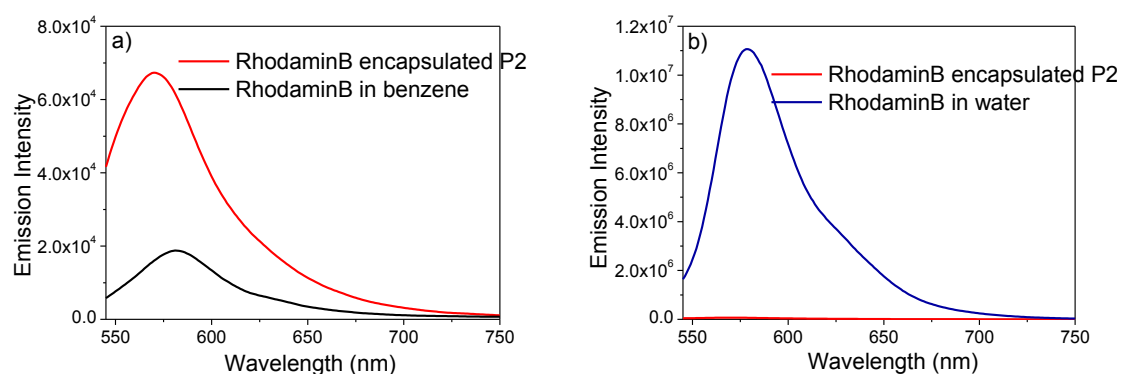


Figure S22: a) Emission spectra ($\lambda_{\text{ex}} = 530 \text{ nm}$) of Rhodamine B ($5 \times 10^{-5} \text{ M}$) in benzene with (red) and without (black) **P2**; b) Comparison of emission intensity of Rhodamine B solution in H₂O (blue) and encapsulated in **P2** reverse vesicle (red) in benzene; [**P2**] = 2.5 mg / ml) in each experiment. Dye concentration was $5 \times 10^{-5} \text{ M}$ in both cases

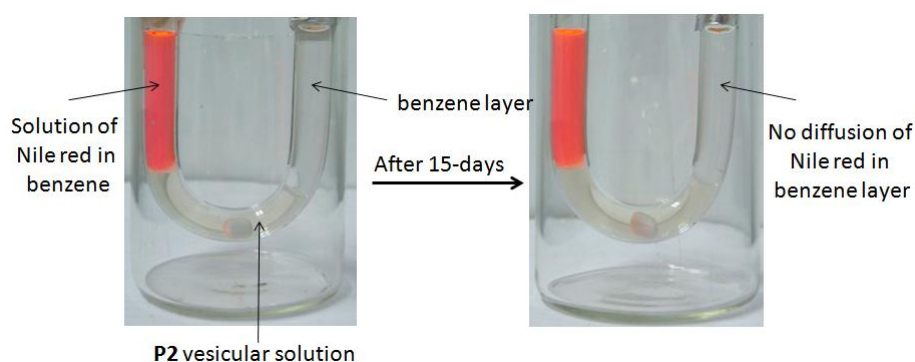


Figure S23: U-tube experiment to demonstrate diffusion controlled transport of Nile red from one arm to another through the vesicular solution in H₂O; [**P2**] = 2.5 mg / ml; Nile red concentration = $1 \times 10^{-4} \text{ M}$

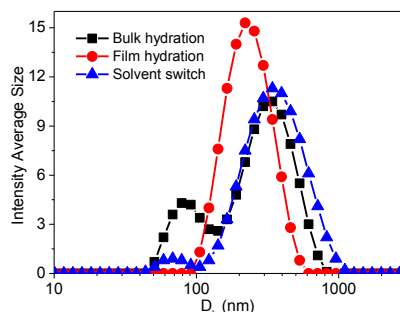


Figure S24: Particle size distribution from DLS for aqueous **P2** solutions (2.5 mg/ ml) prepared in various methods. Details of these sample preparation is elaborated in the experimental section of main text.

References:

- (1) Dan, K.; Bose, N.; Ghosh, S. *Chem. Commun.* **2011**, 12491.
- (2) Percec, V.; Peterca, M.; Tsuda, Y.; Rosen, B. M.; Uchida, S.; Imam, M. R.; Ungar, G.; Heiney, P. A. *Chem. Eur. J.* **2009**, *15*, 8994.
- (3) Wang, C.; Yan, Q.; Liu, H. B.; Zhou, X. H.; Xiao, S.-J. *Langmuir* **2011**, *27*, 12058.