

Supporting Information for

Vesicular assembly and thermo-responsive vesicle-to-micelle transition from an amphiphilic random copolymer

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Materials and methods: All the reagents and solvents were purchased from commercial sources and purified by standard protocol. ^[1] ¹H NMR spectra were obtained on a Bruker DPX-300 MHz NMR spectrometer and spectra were calibrated using TMS as the internal standard. TEM measurements were carried out in a JEOL-2010EX machine operating at an accelerating voltage of 200KV. Dynamic Light Scattering (DLS) measurements were carried out in Malvern instrument. Fluorescence emission spectra were recorded in a FluoroMax-3 spectrophotometer from Horiba Jobin Yvon. Molecular weight of a polymer was estimated in a Waters GPC machine with respect to polystyrene standard using RI detector and THF as eluent.

Physical Studies:

Determination of Critical Aggregation Concentration (CAC) of Polymer P2: CAC of **P2** was estimated by fluorescence method using pyrene as a probe. Measured amount (20 μ L) of stock solution of pyrene (0.1mM) in CH₂Cl₂ was taken in different glass vials and the solvent was removed by blowing air. To each of these pyrene containing vials, varying amount of aqueous **P2** solution (1mM) was added and then the final volume was adjusted with distilled H₂O to get a series of solutions with constant pyrene concentration (1×10^{-6} M) and varying polymer concentration (0.3 mM to 0.002 mM). Each vial was sonicated for approximately 4-5 minutes and then allowed stand for 3 h before the emission spectra were recorded ($\lambda_{\text{ex}}= 337\text{nm}$, excitation band width 2.5 nm, emission band width 2.5 nm). The ratio of the emission intensities of the first (373 nm) and the

third (384 nm) peaks (**Fig S3**) were plotted against the concentration of **P2** and CAC was determined from the inflection point observed in this plot.

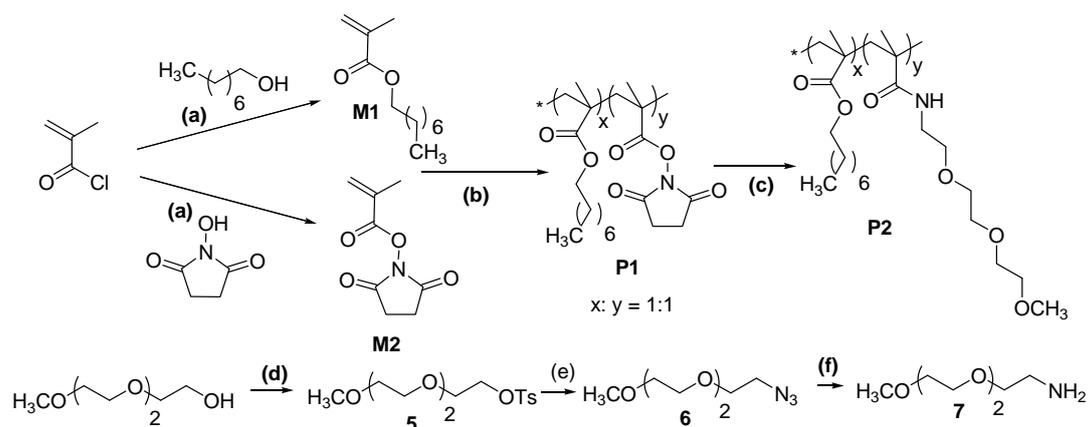
Dynamic Light Scattering (DLS) studies: DLS experiment was carried out with aqueous polymer solution of 0.1 mM concentration at a scattering angle of 173°. For temperature variable studies, the solution was heated from rt to higher temperature at a rate of 5 °C/min and each time after the desired temperature is reached the sample was allowed to stand at that temperature for 15 min before the measurement.

Transmission Electron Microscopy (TEM): For TEM studies a drop of the appropriate aqueous polymer solution (10^{-4} M) was put on a copper grid coated with carbon. After 10 minute, the surface solvent on the grid was removed by tapping on a filter paper and then the grid was kept for 12 h for air drying before the experiment was performed.

R6G encapsulation: Measured amount of aqueous R6g solution was mixed with measured amount of aqueous polymer solution so that in the mixture the polymer and R6G concentration were 1 mM and 0.5 mM, respectively. This solution was sonicated for 5 min and dialysed against water through 3000Da MWCO membrane for 24 hr. During this period the outside water was replaced with fresh water in every 2 h interval. After that UV and fluorescence spectra of the dialyzed solution was measured and analyzed. To estimate the concentration of encapsulated R6G dye, UV-vis absorption spectrum of the dye-encapsulated vesicular solution was recorded and the absorbance at 535 nm was matched with an aqueous R6G solution of known concentration without any polymer (see **Fig S6**). Emission spectra of these two absorbance matched solutions were compared to demonstrate self-quenching among the vesicle encapsulated dye molecules² (**Fig 1d**).

Measurement of lower critical solution temperature: An aqueous polymer solution of desired concentration was placed in a quartz cuvette of 1 cm path-length and it was placed in a UV-vis spectrometer. The solution was heated from 25 °C to 70 °C at 10 °C/min and % transmittance at 520 nm was recorded at various temperature intervals. Each time after the desired temperature was reached 10 min equilibration time was provided before spectral measurements. Then the % transmittance @ 520 nm was plotted against temperature to estimate the LCST.

Synthesis: Synthesis of monomers, random co-polymer **P1** and the substituted polymer **P2** are depicted in Scheme S1.



Reagents and conditions: (a) Et₃N, CH₂Cl₂, 0 °C to rt, 12 h; (b) AIBN, CIDB, Anisole, 95 °C, 1h; (c) Amine **7**, DMF Et₃N, 80 °C; (d) TsCl, NaOH, THF/ H₂O, 3h, 0-5 °C; (e) NaN₃, DMF, 100 °C, 8h; (f) H₂ (50 psi), 10 % Pd-C, EtOH

Scheme S1: Synthesis of monomers and polymer **P1** and **P2**

Octyl methacrylate (**M1**): 1-octanol (4.67 g, 0.035mol) and 9.95 mL (0.071mol) triethylamine were added to a round bottom flask containing 10 mL dry dichloromethane and the solution was cooled to ~ 0 °C in an ice-bath. To this cold solution, a solution of freshly distilled methacryloyl chloride (5.2 mL, 0.053mol) in anhydrous dichloromethane (10mL) was added drop wise over an hour under N₂ atmosphere. After the addition was over the temperature was allowed to come to rt and the reaction mixture was stirred for 12h. Stirring was stopped and the reaction mixture was washed with H₂O (3 x 30 mL) and then the organic part was dried over Na₂SO₄ and excess CH₂Cl₂ was removed under reduced pressure to get the crude product as yellowish liquid. It was purified by column chromatography using silica gel as stationary phase and 20% dichloromethane in petroleum ether (v/v) as eluent to get the desired monomer **M1**. Yield=70%. ¹H NMR (300MHz, CDCl₃, TMS): δ (ppm) = 6.02 (s, 1H), 5.47 (s, 1H), 4.06 (t, 2H), 1.87 (s, 1H), 1.57 (m, 2H), 1.26 (m, 10H), 0.83 (t, 3H).

N-Hydroxysuccinimide methacrylate (**M2**): N-Hydroxy succinimide (2.346g, 0.024mol) and triethylamine (3.3 mL, 0.024mol) was added to 10 mL anhydrous dichloromethane and the solution was cooled to 0°C in an ice-bath. To this cold solution, a solution of methacryloyl chloride (2 mL, 0.022mol) in 10mL anhydrous CH₂Cl₂ was added dropwise under N₂ atmosphere. After the addition was over temperature was allowed to come to rt and the reaction mixture was stirred for another 12h. Then it was washed with distilled

H₂O (3 x 30 mL) and the organic part was dried over anhydrous Na₂SO₄ and CH₂Cl₂ was evaporated under reduced pressure to get the crude product as light yellowish solid. The crude product was dissolved in 6 mL CH₂Cl₂ and dropwise added to 50 mL petroleum ether to get the product as white solid. Yield=67%. ¹H NMR (300MHz, CDCl₃, TMS): δ (ppm) = 6.34 (s, 1H), 5.81 (s, 1H), 2.78 (s, 4H), 1.99 (s, 3H).

Mono-tosylate of tri-ethyleneglycol monomethyl-ether (5): A solution of tri-ethyleneglycol monomethyl ether (3.0 g, 18.2 mmol) in 40 mL THF was added with an aqueous NaOH solution (1.0 g in 5 mL) and the mixture was cooled down to 5°C. To this cold reaction mixture, a solution of *p*-toluene sulphonyl chloride (3.82g, 20 mmol) in 5 mL THF was added dropwise maintaining the temperature at 4-5°C. After the addition was over the reaction mixture was stirred at same temperature for another 3h and then poured to 100 mL cold water. The product was extracted with CH₂Cl₂ (3 x 25 mL) and the combined organic layer was washed with H₂O (2 x 30 mL) followed by brine (1 x 30 mL) and dried over anhydrous Na₂SO₄. Volatile solvents were removed under reduced pressure to get crude product as colorless oil which was purified by column chromatography using silica gel as stationary phase and 20% EtOAc in petroleum ether as eluent. Yield=74%. ¹H NMR (300MHz, CDCl₃, TMS): δ (ppm) = 7.77 (d, 2H), 7.31 (d, 2H), 4.12 (t, 2H), 3.66-3.50 (m, 10H), 3.34 (s, 3H), 2.42 (s, 3H).

Azido tri-ethyleneglycol monomethyl ether (6): 5.0 g tosylate ester of triethylene glycol monomethyl ether (**5**) and 20.4g of sodium azide were taken in 30mL dry DMF and the reaction mixture was stirred at 100 °C under N₂ atmosphere for 8 h. The heating was stopped, solution was cooled to rt and poured into 100 mL ice-cold water and the product was extracted with (3 x 30 mL) diethyl ether. The combined organic layer was dried over Na₂SO₄ and concentrated to get the crude product as light brown oil (2.8 g). Yield: 94 %. ¹H NMR (300MHz, CDCl₃, TMS): δ (ppm) = 3.68-3.63 (m, 10H), 3.55 (t, 2H), 3.37 (s, 3H). As it was pure from NMR and TLC the product was taken to the next step as such without further purification.

Amino tri-ethyleneglycol monomethyl-ether (7): 2.8 g of compound **6** was dissolved in 25 mL ethanol and taken in a high pressure hydrogenation vessel along with 0.120 g 10% Pd-C. The reaction mixture was stirred for 5h under 50-psi H₂ pressure and then the solid catalyst was filtered and the filtrate was concentrated to get the crude product as light

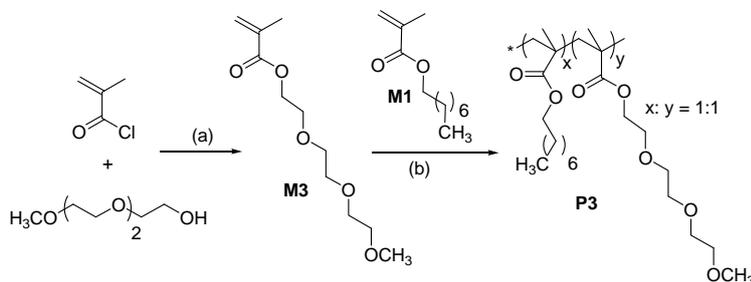
yellow oil. Yield=60%. ^1H NMR (300MHz, CDCl_3 , TMS): δ (ppm) = 3.73 (d, 2H), 3.65(m, 6H), 3.56 (d, 2H), 3.38 (s, 3H), 3.10 (d, 2H), 2.70 (broad peak, 2H).

Polymer P1: Monomer **M1** (0.270g, 0.0013mol), **M2** (0.250g, 0.0013mol), 2-cyano isopropyl di-thiobenzoate (3 mg, 0.015 mmol) and AIBN (0.8 mg, 0.0048 mmol) were dissolved in dry degassed anisole (500 μL) in a schlenk flask and the solution was degassed by bubbling Ar for 15 min and sealed and was transferred to a pre-heated oil bath at 95 $^\circ\text{C}$ in which it was stirred for 1 h while the reaction mixture solidified. Heating was stopped and the contents were dissolved in 1 mL THF and precipitated from excess di-ethyl ether. The precipitate obtained was centrifuged and dried in vacuum to get the desired polymer as white solid (0.320g, 62%). $M_n=26000$, PDI=1.16, ^1H NMR (300MHz, CDCl_3 , TMS): δ (ppm) = 3.96 (broad peak, 2H), 2.78 (broad peak, 4H), 1.59 (broad peak, 3H), 1.27 (broad peak, 3H), 0.88 (broad peak, 3H).

Polymer P2: Polymer **P1** (0.2g, 0.5 mmol), compound **7** (0.25g, 1.53mmol) and Et_3N (0.105 mL, 0.75 mmol) were dissolved in 0.4 mL dry DMF and the solution was stirred at 80 $^\circ\text{C}$ for 12h under N_2 atmosphere. Heating was stopped and the solution was cooled to rt and then dissolved in 6 mL distilled H_2O and purified by centrifugation using excess water in a Amicon ultra-10K (MWCO: 10 KD) centrifugation tube to remove all low molecular weight water soluble compounds. The centrifuged solution was freeze dried to get the pure polymer as an off white sticky compound (0.120g, 60%). ^1H NMR (300MHz, CDCl_3 , TMS): δ (ppm) = 3.92 (broad peak, 2H), 3.64 (broad peak, 12H), 3.38 (broad peak, 3H), 1.29 (broad peak, 3H), 0.89 (broad peak, 3H).

Tri-ethylene glycol methacrylate (M3): To an ice-cold solution of tri-ethylene glycol monomethyl ether (12.55g, 0.076mol) and triethylamine (7.94 mL, 0.057mol) in 10mL dry dichloromethane, a solution of methacryloyl chloride (3.7mL, 0.038mol) in anhydrous dichloromethane (20mL) was added dropwise under N_2 atmosphere and after the addition was over the reaction mixture was stirred at rt for 12h. Then reaction mixture was washed with H_2O (3 x 30 mL), dried over Na_2SO_4 and excess CH_2Cl_2 was removed under reduced pressure to get the crude product as yellowish liquid. It was purified by column chromatography using silica gel as stationary phase and dichloromethane as eluent to get the desired monomer as colorless oil. Yield=80%. ^1H NMR (300MHz,

CDCl_3 , TMS): δ (ppm) = 6.12 (s, 1H), 5.56 (s, 1H), 4.29 (t, 2H), 3.66 (m, 10H), 3.37 (s, 3H), 1.94 (s, 3H).



Reagents and conditions: (a) Et_3N , CH_2Cl_2 , 0°C to rt, 12 h; (b) AIBN, CIDB, Anisole, 95°C , 24h

Scheme S2: Synthesis of control polymer P3

Polymer P3: Monomer **M1** (0.100g, 0.5mmol), **M3** (0.082g, 0.5mmol), 2-cyano isopropyl di-thiobenzoate (0.6 mg, 0.003 mmol) and AIBN (0.15 mg, 0.001 mmol) were dissolved in dry degassed anisole (100 μL) in a schlenk flask and the solution was degassed by bubbling Ar for 15 min and sealed and stirred at 95°C for 24 hour. The resulting solidified product was dissolved in THF and precipitated from di-ethyl ether to get the desired polymer as colorless sticky material. $M_n=33000$, $\text{PDI}=1.17$, ^1H NMR (300MHz, CDCl_3 , TMS): δ (ppm) = 4.10 (m, 2H), 3.92 (m, 2H), 3.65 (m, 10H), 3.39 (m, 3H), 1.30 (m, 12H), 0.88 (m, 3H).

Additional Figures:

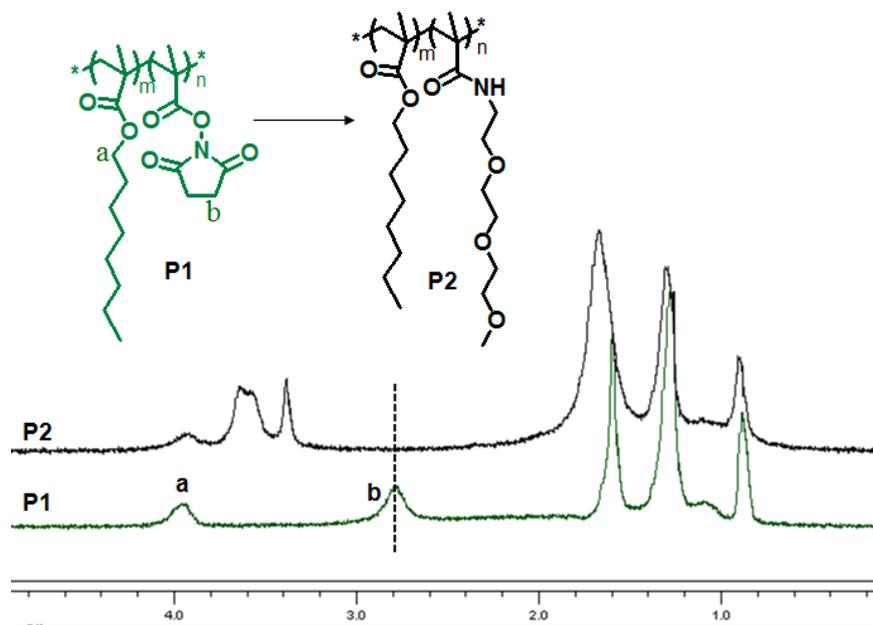


Fig S1: ¹H NMR spectrum of **P1** (green) and **P2** (black) in CDCl₃. NHS-proton peak (b) was completely absent in **P2** spectrum clearly suggesting complete substitution. Further new peaks in the region of 3.15-3.4 ppm suggest incorporation of oligooxyethylene moiety. Integration ratio of “a” and “b” protons was used to estimate the incorporation ratio (~ 1:1) of the two monomers in the parent random copolymer **P1**.

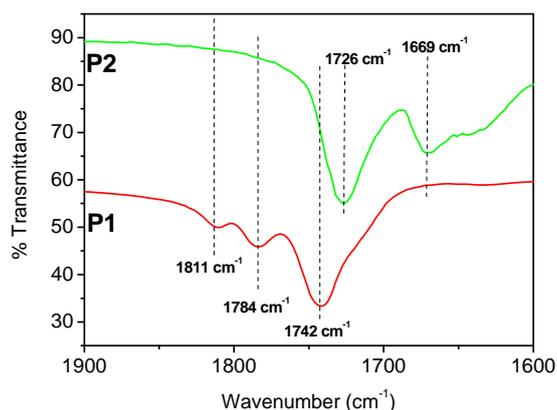


Fig S2: FT-IR spectra of **P1** (red) and **P2** (green). Peaks at 1811, 1784 and 1742 cm⁻¹ are absent in **P2** suggesting complete removal of the NHS-ester group.³ In **P2** spectrum, peaks at 1726 and 1669 cm⁻¹ are due to the ester and amide functionalities respectively.

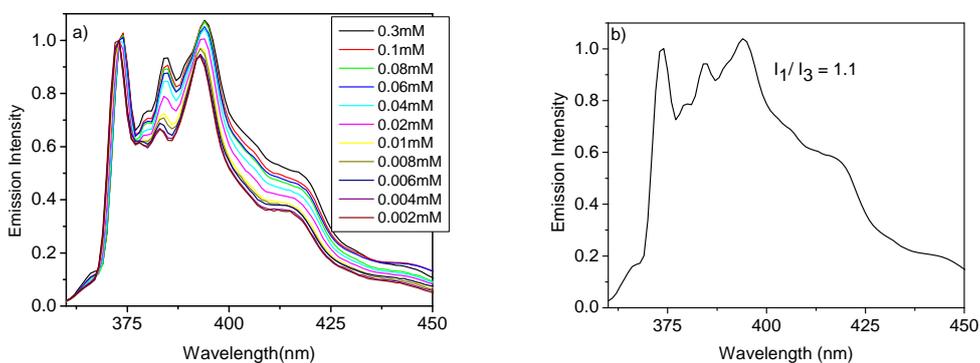


Fig S3: a) Normalized (intensity @ 373 nm is normalized to 1) emission spectra of pyrene encapsulated in aqueous **P2** solution with varying concentration; Concentrations of **P2** are shown in the figure legend; b) Normalized emission spectrum of pyrene encapsulated in aqueous **P2** solution of 1 mM concentration. Concentration of pyrene= 10^{-6} M. $\lambda_{ex} = 337$ nm in all the experiments.

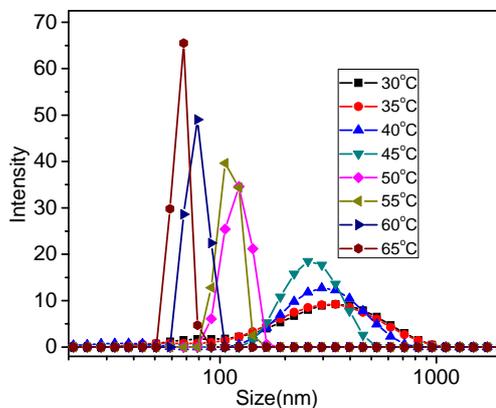


Fig S4: Intensity averaged size distribution from DLS measurements of aqueous **P2** solution (0.1 mM) as a function of temperature.

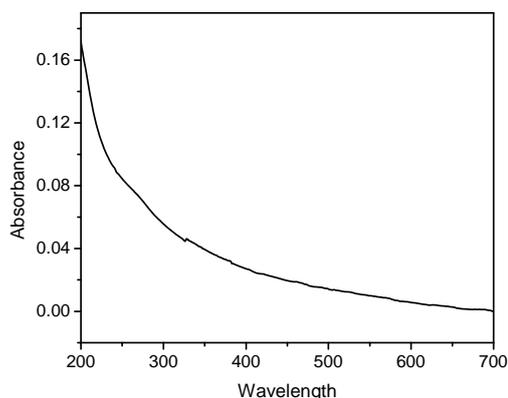


Fig S5: Absorption spectra of the aqueous solution of **P3** (0.1 mM) after it was treated with R6G for encapsulation and dialyzed as in case of **P2**. No absorption band can be visible here at 534 nm in sharp contrast with R6G encapsulated **P2** solution (**Fig 1d**).

References

1. D.D. Perrin, W. L. F. Armarego, D. R. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press: 1986.
- 2.(a) E. N. Savariar, S. V. Aathimanikandan, S. Thayumanavan, *J. Am. Chem. Soc.*, 2006, **128**, 16224; (b) P. Mahato, S. Saha, S. Choudhury, A. Das, *Chem. Commun.*, 2011, **47**, 11074-11076.
2. C. Wang, Q. Yan, H. B. Liu, X. H. Zhou, S-J. Xiao, *Langmuir*, 2011 (**DOI:** 10.1021/la202267p)