Supporting information for

Alternating vs Random Amphiphilic Polydisulfides: Aggregation, Enzyme Activity Inhibition and Redox-responsive Guest Release

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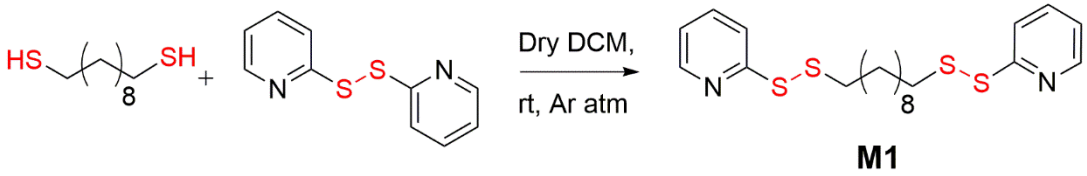
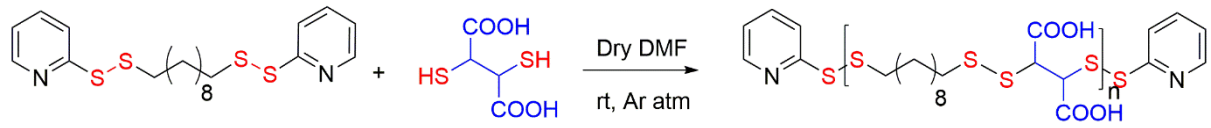
Materials and methods: 1,10-decane dithiol and 2,2′-dithiodipyridine were obtained from Tokyo Chemical Industry (TCI) Co. 2,3-meso-dimercapto succinic acid was purchased from Sigma Aldrich Chemical Co. UV/Vis spectra were recorded in a Jasco V-750 spectrophotometer and for experiments spectroscopic grade solvents used. Fluorescence spectra were recorded in a FluoroMax-3 spectrophotometer by Horiba Jobin Yvon. Size exclusion chromatography (SEC) analysis was performed in a Waters APC instrument at 45 °C using DMF as the eluent and maintaining a constant flow rate of 0.5 mL/ min. AFM images were captured in a Bruker Innova instrument. Powder X-ray Diffraction (XRD) experiment was performed on a Seifert XED 3000P diffractometer using Cu Kα radiation. ^{1}H NMR spectra analysis was conducted using Bruker DPX-400 MHz or DPX-500 MHz NMR spectrometer and calibration was done against TMS as the internal standard.



M1

Synthesis of monomer M1:

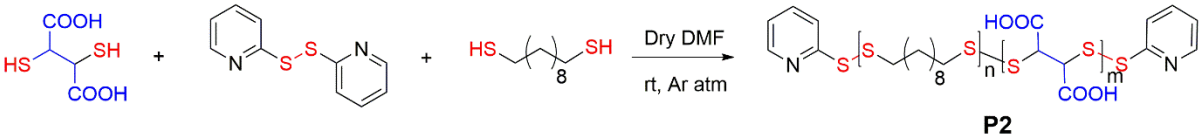
1,10-decane dithiol (0.5 gm, 2.42 mmol) was added dropwise to a dry dichloromethane (5 mL) solution of aldrithiol-2 (3.2 gm, 14.5 mmol), followed by addition of few drops of acetic acid. The reaction mixture was stirred at rt under inert atmosphere for 12h. After that, solvent was evaporated and the crude mixture was subjected to purification by column chromatography using combination of ethyl acetate and pet-ether as eluent. Desired product was isolated as a pale-yellow oil in 58 % yield. ^{1}H NMR ($CDCl_3$ 400 MHz) δ (ppm): 2.79-2.79 (t, 4H), 1.71-1.63 (m, 4H), 1.37-1.34 (br, 4H), 1.25-1.23 (br, 8H), 7.08-7.05 (q, 2H), 7.65-7.60 (t, 2H), 7.73-7.71 (d, 2H), 8.45-8.4 (d, 2H).



M1 **P1**

Synthesis of P1:

Monomer M1 (0.364 gm, 0.856 mmol) and 2,3-meso-dimercapto succinic acid (0.15 gm, 0.823 mmol) were taken together in a Schlenk tube along with dry and degassed DMF (1.5 mL) and was added with 4-5 drops of acetic acid. The reaction mixture was stirred at rt under inert atmosphere for 12h. Then the polymer was purified by precipitation from excess cold diethyl-ether + dichloromethane mixture and dried under vacuum to obtain the desired product as a white solid in 40 % yield. ^{1}H NMR (DMSO-d6, 400 MHz), δ (ppm): 3.69 (s, 2H), 2.78-2.68 (m, 4H), 1.60-1.56 (br, 8H), 1.25 (br, 8H), 13.14 (acid group), 8.475 (2H), 7.95-7.78 (4H), 7.24 (2H). M_w = 8450, Đ = 1.25.



P2

Synthesis of P2:

1,10-decane dithiol (0.1 gm, 0.548 mM) and 2,3-mercapto succinic acid (0.113 gm, 0.548 mM) were introduced into a degassed Schlenk tube and dissolved in anhydrous degassed N,N-dimethyl formamide solvent (1.4 mL). After that, aldrithiol-2 (0.251 gm, 2.281 mM) was added to the reaction mixture followed by addition of few drops of acetic acid and the mixture was stirred at rt for 12h under inert atmosphere. The polymer was purified by precipitation from excess cold diethyl-ether + dichloromethane mixture and dried to get the desired product as a white solid in 42 % yield. ^{1}H NMR (DMSO-d6, 400 MHz), δ (ppm): 3.69 (s, 2H), 2.75-2.66 (br,4H), 1.59 (br, 8H), 1.26 (br, 8H). M_w = 8700 (Đ = 1.21).

Estimation of molecular weight by end group analysis (UV-Vis and NMR studies): UV-Vis spectra of both polymers (*c* = 0.1 mg/ mL) were recorded in DMF, which showed presence of characteristic band in the region of 270-300 nm (Fig. S1). Form the band intensity (285 nm) and extinction coefficient of 2-(pyridin-2-yldisulfaneyl) ethan-1-ol (available previously in the laboratory) at the same wavelength, concentration of terminal pyridine-disulfide groups was estimated to be 8560 and 8330 gm/ mole, respectively.

Likewise, from the ^{1}H NMR spectra (Fig. 1, S3) by comparing the relative intensity of the end-group proton (He) and backbone proto (Ha), molecular weights of P1 and P2 were estimated to be 6780 and 7220 gm/ mole, respectively.

Solution preparation and self-assembly studies: Aqueous polymer solution was prepared by directly dissolving polymer (~ 1.0 mg) in milli-Q water (1 mL) in presence of 1.0 mole equivalent (with respect to the COOH group) of NaOH. This was used as the stock solution and for different experiments, quantitative dilution was made with water as per the concentration requirement.

Atomic Force Microscopy (AFM): Freshly-prepared aqueous polymeric solution (c = 100 μg/ mL) was spin- coated on freshly-cut mica and then was dried overnight at rt. AFM images were captured using Innova instrument from Bruker. All AFM data were processed using WSxM 5.0 develop 10.2 software.

Wide-angle X-ray diffraction: Aqueous solutions of the polymer (P1 or P2) was lyophilized to make dry powder, which was used for the XRD studies. Data were recorded from 0^o - 40^o of 2θ on a Seifert XRD3000P diffractometer with Cu Kα radiation (α=0.154 nm).

Determination of Critical Aggregation Concentration (CAC): Critical Aggregation Concentration (CAC) of polymeric solutions were calculated using emission intensity vs concentration plots from fluorescence spectra of Nile Red (NR) encapsulated polymeric samples. For encapsulation, NR stock in tetrahydrofuran (THF) (4μL) was taken in different eppendorf and THF was evaporated out. Then aqueous solution of polymer was added to this, so that the final concentration of Nile red was 10^{-5} M and polymer concentrations varied from 0.125 μg/mL to 120 μg/mL. After that these solutions were sonicated for 20 min and allowed to equilibrate for 2h. Fluorescence spectra of NR-encapsulated polymer samples were recorded (λ_{ex} - 530 nm, excitation bandwidth 3.0 nm and emission bandwidth 3.0 nm) at rt. CAC was estimated plotting emission intensity at 626 nm with respect to polymer concentrations (μg/mL).

Dye release studies: Firstly, a stock solution (0.1 mM) of Nile Red (NR) was prepared in tetrahydrofuran (THF). Stock NR-solution was taken in an Eppendorf, and solution of the polymer in THF was added to it. THF was removed and the mixture was added with aqueous NaOH solution so that the final concentration of NR and polymer were adjusted to 10^{-5} M and 0.5 mg/ mL, respectively. To this solid GSH was added (*c* = 10 mM) and emission intensity was recorded as a function of time. After that, dye release percentage was calculated

using the equation [($I_0 - I_t$) / I_0 X 100] where I_t and I_0 are emission intensity (622 nm) at a given time *t* and just after the GSH addition, respectively.

α-Chymotrypsin (Cht) enzymatic activity assays: Stock solution of Cht (*c* = 3.2 μM) was prepared in 5.0 mM sodium phosphate buffer (pH 9). Stock solution of SPNA (*c* = 2.0 mM) substrate was prepared in ethanol/ DMSO mixture (9:1). Cht and polymer solution (*c* = 0.1 mM) were mixed together and 1h after mixing, the solution was added to the SPNA substrate solution and absorption intensity (405 nm) was monitored as a function of time for 120 min. Then absorption intensity was plotted against time (min) and the data were fitted to a straight-line equation. Slope of the lines (first 20 min) for P1, P2 and the control were taken as k1, k2 and k. Relative activity of Cht in presence of P1 or P2 was calculated using the formula k1/ k X 100 % or k2/k X 100 %.

Chain-exchange dynamics by FRET studies: DiO (3,3′-di-octadecyloxacarbocyanine perchlorate) and DiI (1,10-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) stock solutions were prepared in THF (1.0 mM). 5μL of each stock solution was taken in two different eppendorfs and to this polymer solution in THF was added separately. THF was evaporated and the mixture was added with aqueous NaOH solution, sonicated and the final solutions were dialysed against water (molecular weight cut off= 35 kD). Final concentration of polymer was adjusted to 20 μM. These DiI and DiO encapsulated polymer solutions were mixed together and fluorescence spectra were recorded as a function of time. FRET ratio was calculated using the formula $I_a/(I_a + I_d)$, were I_a and I_d are the emission intensities at 575 nm and 510 nm, respectively.

Cell Viability assay: To test cellular viability of polymers (P1 and P2), MTT assay was done in HeLa cell line. Cells of density ~ 10^4 were seeded in each well of 96 well plate. After overnight incubation at 37 ^oC and 5% CO_2, different concentrations (50 μL, 100 μL, 200 μL, 300 μL, 400 μL) of solutions of P1 or P2 solutions were added (100 μL) in each well and incubated for 24h and 48h. After that, 50 μL solution of MTT (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, concentration = 5.0 mg/mL) was added along with 100 μL fresh DMEM media after removing polymer solutions. It was allowed to incubate for 4h in 37 ^oC and 5% CO_2. After that, 100 μL DMSO was added to each well after carefully removing the MTT solutions, and it was then kept in incubator for 15 min. After putting in shaker for 5 min to homogenize, absorbance was recorded at 570 nm in microplate reader (VARIOSKAN, Thermo Fisher). Then percentage of cell viability was measured by the percentage of the ratio

of absolute absorbance of the cells treated with polymer solution to that of without polymer solution.

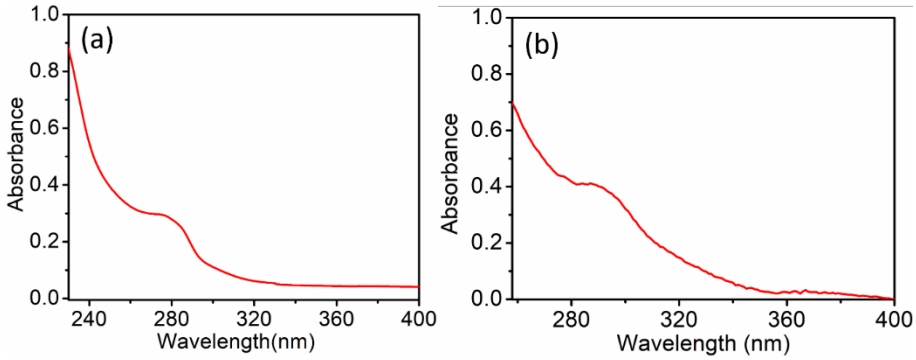


Figure S1: UV-Vis spectra of (a) P1 and (b) P2 (concentration = 0.1 mg/ mL in DMF, pathlength = 1 cm).

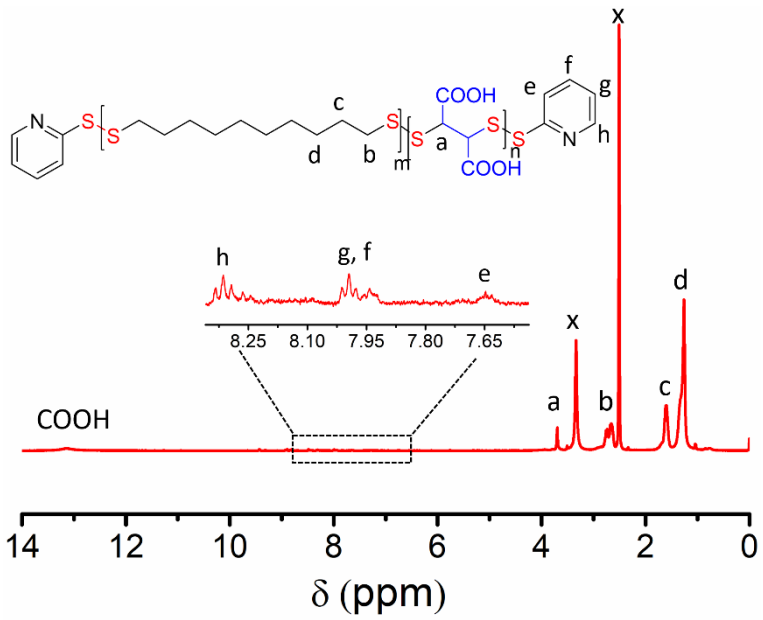


Figure S2: SEC trace of (a) P1 and (b) P2 in DMF (concentration = 5 mg/ mL).

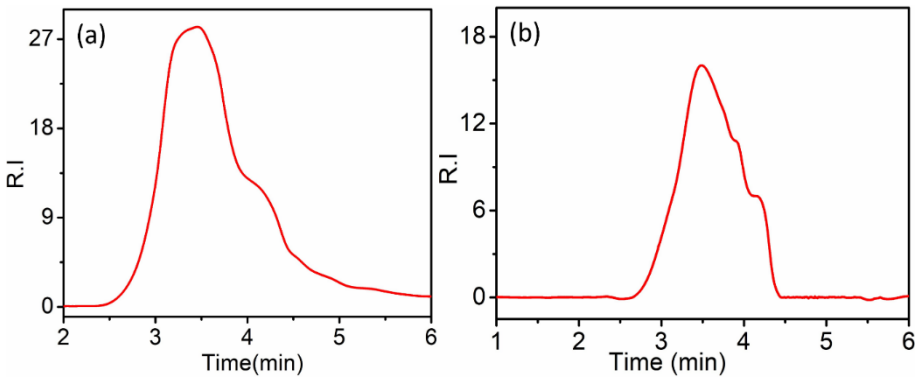


Figure S3: [1]H NMR of P2 (solvent DMSO-d6, 400 MHz). X indicates residual solvent peak.

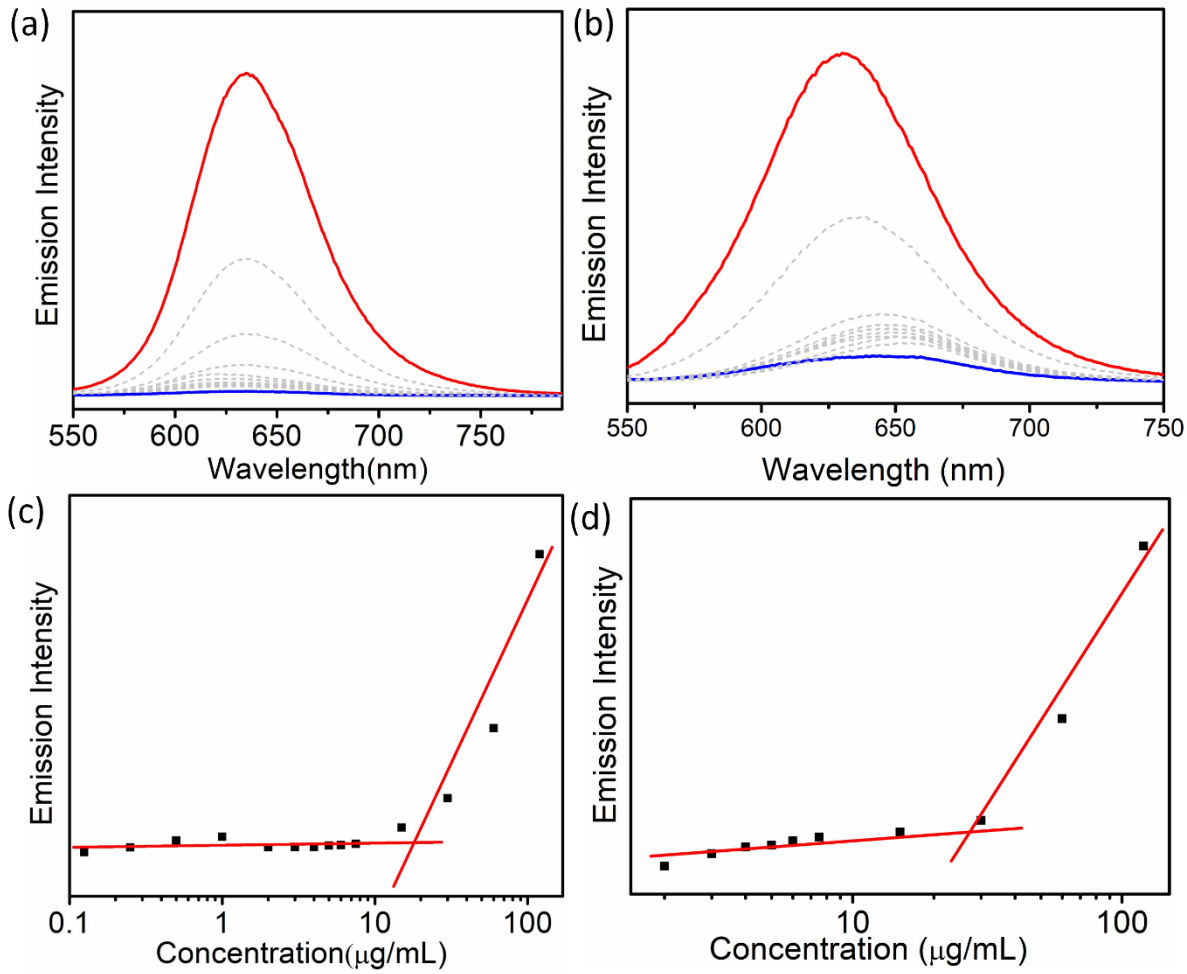


Figure S4: Concentration dependent emission spectra of Nile Red (NR) (c = 10^{-5} M) encapsulated in (a) P1 (b) P2 (λ_{ex} = 530 nm) and plot of emission intensity (626 nm) as a function of polymer concentration.

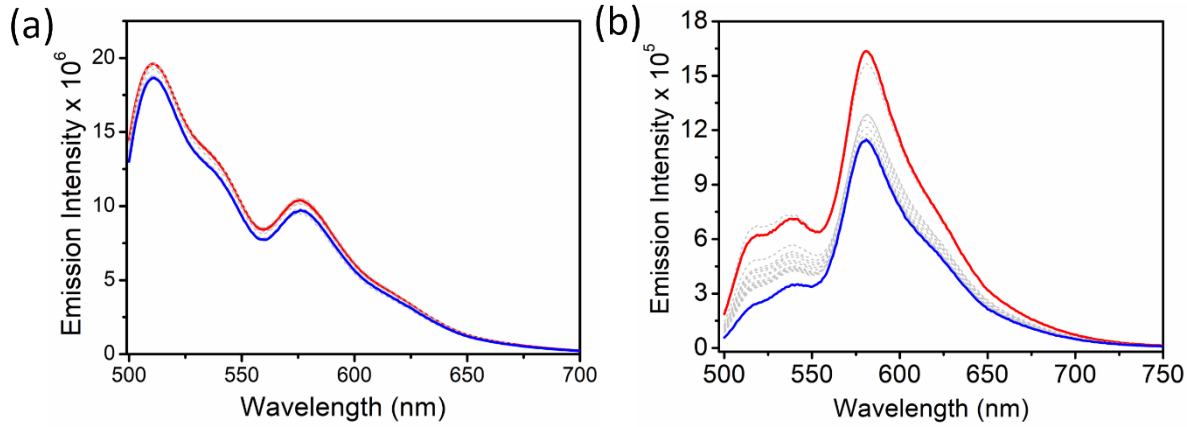


Figure S5: Time dependent emission spectra of a mixed solution of DiO (C = 10 μM) and DiI (C = 10 μM) encapsulated in (a) P1 and (b) P2 (c = 20 μM, λ_{ex} = 485 nm).

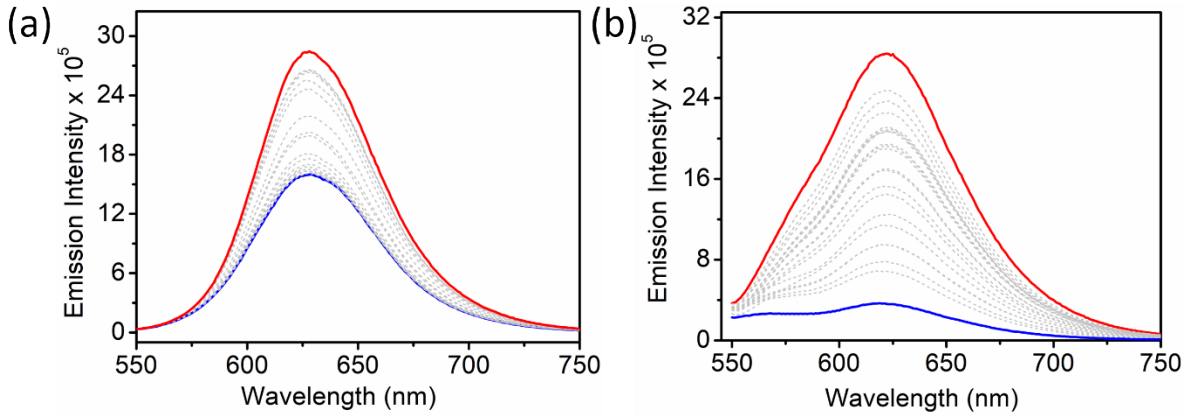


Figure S6: Time dependent emission spectra of Nile Red (NR) (c = 0.1 mM) encapsulated in (a) P1 (b) P2 (Concentration of polymer = 0.5 mg/ mL; λ_{ex} = 530 nm) in presence of 10 mM GSH.

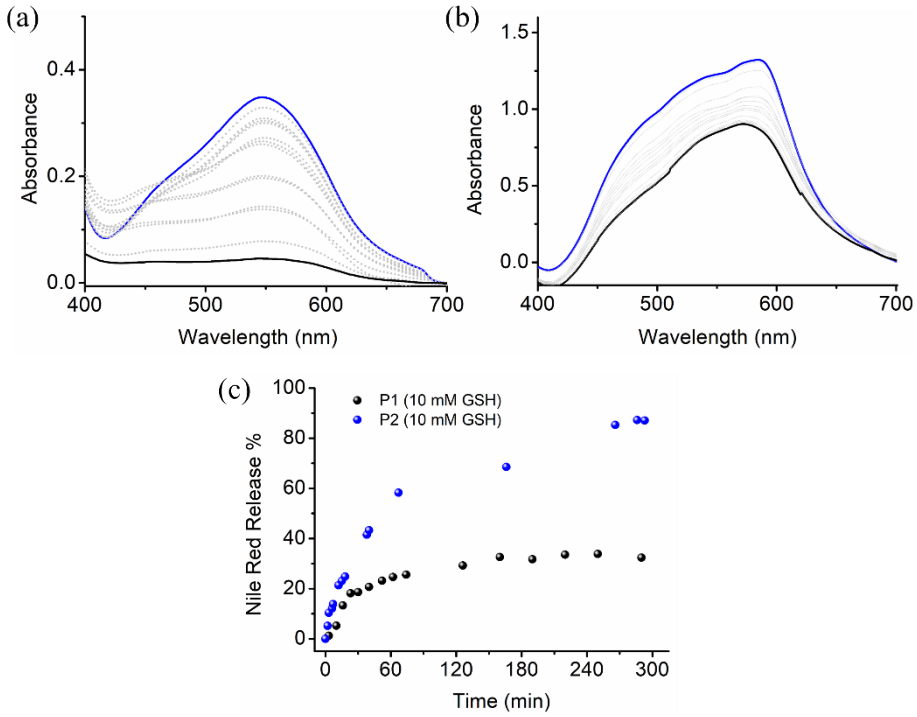


Figure S7: Time dependent UV/Vis spectra of Nile Red (NR) encapsulated (a) P1 and (b) P2 in presence of 10 mM GSH. Concentration of NR and polymer = 10^{-5} M and 0.5 mg/ mL, respectively; (c) Cumulative % release of Nile red as a function of time after GSH addition. Release % was calculated using absorbance at maxima.

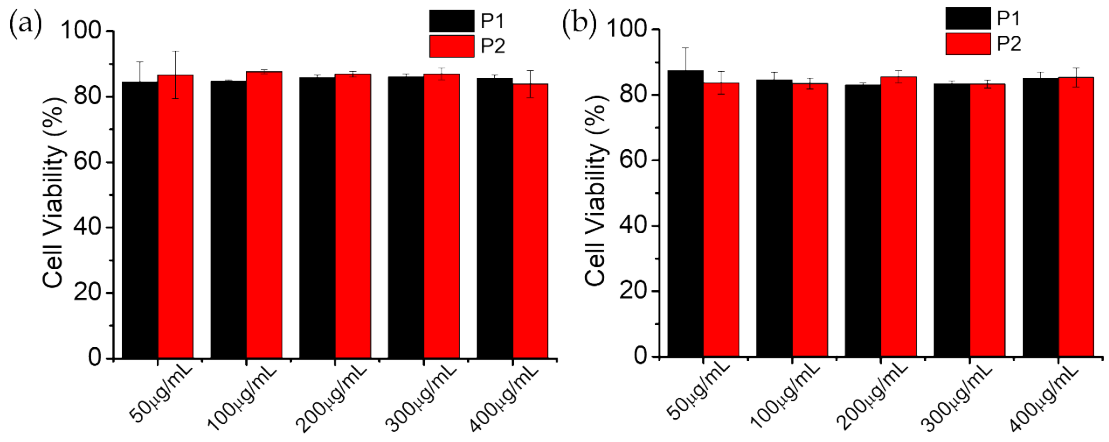


Figure S8: Cell viability (MTT) assay of P1 and P2 in HeLa cell lines for (a) 24 h and (b) 48 h (cell density = 10^4).