Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Delineating synchronized control of dynamic covalent and noncovalent interactions for polymer chain collapse towards cargo localization and delivery

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1. Materials & methods:

Solvents used in the syntheses were reagent grade. Ethanol, DCM, DMF, THF, *N,N*-dimethylacetamide were dried as per literature protocol.[S1] The reagents triethyl amine, thionyl chloride were purchased from Alfa-aeser and used without further purification. The chemicals 7-hydroxy coumarin, β-cyclodextrin, hexamethyl diisocyanate (HDI), Cu(I)Cl were purchased from Sigma. The reagents methyl α-bromo phenyl acetate (MeBrPhAc), tris[2-(dimethylamino)ethyl]amine (Me_6TREN) were purchased from TCI and were used without further purification. The monomers, 2-hydroxy ethyl acrylamide (HEAm) was purchased from TCI and were passed through basic alumina and *N*-isopropylacrylamide (NIPAM) was recrystallized from *n*-hexane to remove inhibitor MEHQ before using them for ATRP. A regenerated seamless cellulose dialysis tubing wherein the membrane is partially permeable, having molecular weight cut off between 12,000 to 14,000 Da was purchased from Himedia and used after activation.[S2]

NMR spectra were acquired on a 400 MHz Bruker machine. The chemical shifts were reported in ppm with downfield of tetramethylsilane using the resonance of the deuterated solvent as internal standard. Splitting patterns were designated as singlet (s), doublet (d), triplet (t) and multiplet (m). %Functionality was calculated using the formula, %Functionality = $\{(I_x)/(I_x+I_f)\}$x100 where, I_x is integral per proton of corresponding functional group, I_f is integral proton of end group (*e.g.* isopropyl methyl group of NIPAM chain).

Size exclusion chromatography (SEC) was performed on a Malvern Viscotek instrument having RI, right angle light scattering (RALS) detectors using D_{4000} column with DMF with 0.05 M LiBr as eluent at 25 ^oC with a flow rate of 0.7 mL/min. The results were analyzed by using Omnisec software. The sample peaks were analyzed for Absolute M_n, M_w, *PDI* by PMMA multi-detector calibration using PMMA-60k narrow standard and verified by PMMA-95 k broad standard.

Dynamic light scattering measurements were done on Malvern Zetasizer Nano ZS ZEN3600 equipped with a Helium−Neon laser (wavelength, λ= 633 nm with backscattering angle of 173°.

ITC Samples were prepared by filtering solutions through a 0.2 μm PTFE-filter in a glass cuvette. Isothermal titration calorimetric measurements were performed on a Malvern MicroCal PEAQ-ITC, which is composed of a reference cell and a sample cell of 300 μL. Degassing of the sample solutions were done prior to titration and kept constant temperature. In a typical run, a 40 μL syringe was full of host (1.00 mM) and the cell was loaded with guest **CP1-2** polymer (0.02 mM, 300 μL). The titration of the host with the guest was carried out at 25 ^oC, with a constant rate of 750 rpm, 19 injections of 2 μL, a time interval of 150 s. The enthalpy change per mole of each added host **bis-β-CD** in the sample cell was recorded continuously. The control titrations of **bis-β-CD** into water were also completed under similar conditions. The enthalpy changes of the titrations of the blank test were deducted from the sample titration data.

UV crosslinking was performed using a UV chamber equipped with 2* 8W UV_B lamp (λ_{max} = 320 nm, Intensity at 15 cm = 790 μW/cm^2) and 1*8W UV_C lamp (λmax = 254 nm, Intensity at 15 cm = 820 μW/cm^2) and the aqueous samples were kept at a distance of 15 cm for irradiation. Luminous intensity of irradiation at time *t* was determined in J/cm^2 by the formula $[(\mu W/cm^2)/10^6]*t$ (s). UV spectra including absorbance and transmittance studies was recorded using Shimadzu UV-6000 UV-vis spectrophotometer in a wavelength range of 800 to 200 nm.

The samples were drop-casted on silicon wafer and AFM height images were recorded using tapping mode on a Bruker multimode 8 scanning probe microscope with silicon cantilever.

Samples were dropcasted on a carbon coated Cu grid and TEM images were recorded using JEOL JEM 2100 with a Tungsten filament at an accelerating voltage of 120 kV.

2. Synthesis of the polymers and bis-β-CD crosslinker:

A. Random copolymerization of Hydroxy ethyl acrylamide (HEAm) with *N*-Isopropyl acrylamide (NIPAM) by ATRP method followed by grafting with coumarin moieties.

ATRP polymerization was performed with NIPAM and HEAm as co-monomers (Scheme 1). Coumarin carboxylic acid chloride was then tethered to the resulting copolymers using esterification. The detailed synthetic procedures of **CP1-3** are described as follows.

i. Random copolymerization by ATRP:

NIPAM, HEAm, Cu(I)Cl, Me_6TREN, and ethanol:water (1:1) were added in a schlenk tube under N_2 environment, capped with a rubber septum followed by degassing using N_2 bubbling for 15 minutes. Initiator (MeBrPhAc) was then added to the mixture and was degassed again for 15 minutes. The reaction mixture was stirred at room temperature for 24 hours followed by dilution with water. The solution was dialyzed against water for 3 days to remove the copper catalyst as well as unreacted monomers. NMR and GPC analysis confirmed the polymer with the percentage of comonomers and M_w, M_n, *PDI*.

P-1. Random copolymer of HEAm with NIPAM (DP = 200): NIPAM (16 mmol), HEAm (4mmol), Cu(I)Cl (0.1mmol), Me_6TREN (0.2 mmol), and Ethanol:Water (1:1) were added in a schlenk tube under N_2 environment, capped with a rubber septum followed by degassing using N_2 bubbling for 15 minutes . Initiator (MeBrPhAc, 0.1 mmol) was then added to the mixture and was degassed again for 15 minutes. The polymerization was carried out by stirring at room temperature for 24 hours. Then the reaction mixture was diluted with water and was dialyzed against water for 3 days to remove the copper catalyst as well as unreacted monomers. ^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.89– 3.71 (m, 17H, C***H***-$(CH3)_2$), 3.64-3.57 (m, 9H, ***CH***$_2CH_2OH$), 3.36–3.11 (m, 9H, CH_2C***H***$_2OH$), 2.12-1.8 (m, 22H, C***H***$_2$), 1.72-1.25 (m, 41H, C***H***$_2$), 1.10-0.9 (m, 103H, CH-$(C$***H***$_3)_2$ %HEAm = 19%. SEC analysis (DMF, PMMA triple detection standard): M_n = 26605 g/mol; M_w =32078 g/mol; M_w/M_n = 1.20.

P-2. Random copolymer of HEAm with NIPAM (DP = 400): NIPAM (32 mmol), HEAm (8 mmol), Cu(I)Cl (0.1 mmol), Me_6TREN (0.2 mmol), and ethanol:water (1:1) were added in a schlenk tube under N_2 environment, capped with a rubber septum followed by degassing using N_2 bubbling for 15 minutes. Initiator (MeBrPhAc, 0.1 mmol) was then added to the mixture and was degassed again for 15 minutes. The polymerization was carried out by stirring at room temperature for 24 hours. Then the reaction mixture was diluted with water and was dialyzed against water for 3 days to remove the copper catalyst as well as unreacted monomers. ^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.89–3.71 (m, 24H, C***H***-$(CH_3)_2$), 3.64-3.57 (m, 8H, C***H***$_2CH_2OH$), 3.36–3.11 (m, 8H, CH_2C***H***$_2OH$), 2.12-1.8 (m, 25H, C***H***$_2$), 1.72-1.25 (m, 42H, C***H***$_2$), 1.10-0.9 (m, 170H, CH-$(C$***H***$_3)_2$ %HEAm = 12 %. SEC analysis (DMF, PMMA triple detection standard): M_n = 41693 g/mol; M_w =48393 g/mol; M_w/M_n = 1.16.

ii. Grafting of courmain moieties on the polymers:

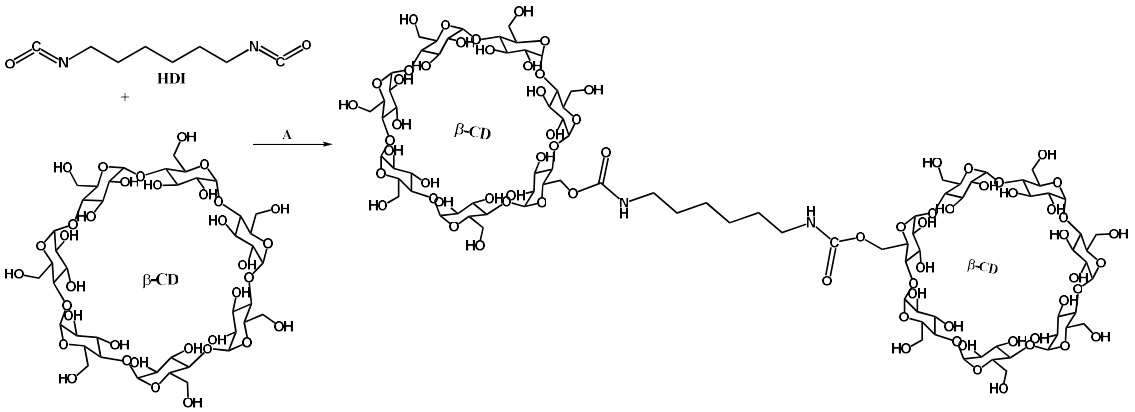
The synthesis of coumarin carboxylic acid chloride was performed according to literature procedure.[S3] The coumarin carboxylic acid chloride solution in THF was added to a stirred solution of precursor polymer **P1-2** with Et_3N in THF under N_2 environment. The solution was stirred under N_2 environment at 50 °C for 5 hours. Then cooled back to 25 °C under N_2 environment for 48 h. The solution was dialyzed against water for 3 days to remove the Et_3N as well as unreacted functionalities.

Polymer CP1: The solution of coumarin carboxylic acid chloride (1.01 mmol) in 20 mL THF was added to a stirred solution of polymer **P1** (0.02 mmol) with Et_3N (1.62 mmol) in 10 mL THF under N_2 environment. The solution was stirred under N_2 environment at 50 °C for 5 hours. Then cooled back to 25 °C under N_2 environment for 48 hours The solution was dialyzed against water for 3 days to remove the Et_3N as well as unreacted functionalities. ^{1}H NMR (400 MHz, D_2O): δ (ppm) 7.95-7.90 (m, 1H,

>C=***CH***-),7.59-7.52 (m, 1H, >C=***CH***-), 6.96-6.84 (m, 2H, , =CH-***CH***=C-***CH***=), 6.24-6.23 (m, 1H, =***CH***-CO-O-), 3.89– 3.71 (m, 22H, C***H***-$(CH3)_2$), 3.64-3.57 (m, 11H, $***CH_2***CH_2OH$), 3.36–3.11 (m, 10H, CH_2C***H_2***OH), 2.12-1.8 (m, 27H, C***H₂***), 1.72-1.25 (m, 57H, C***H₂***), 1.10-0.9 (m, 146H, CH-$(C***H_3***)_2$ %Coumarin = 4%. SEC analysis (DMF, PMMA triple detection standard): M_n = 27357 g/mol; M_w =37183 g/mol; M_w/M_n = 1.31.

Polymer CP2: The solution of coumarin carboxylic acid chloride (1.3 mmol) in 20 mL THF was added to a stirred solution of polymer **P2** (0.01 mmol) with Et_3N (2.02 mmol) in 10 mL THF under N_2 environment. The solution was stirred under N_2 environment at 50 °C for 5 hours. Then cooled back to 25 °C under N_2 environment for 48 hours The solution was dialyzed against water for 3 days to remove the ET_3N as well as unreacted functionalities. ^{1}H NMR (400 MHz, D_2O): δ (ppm) 7.95-7.90 (m, 1.3H, >C=***CH***-),7.59-7.52 (m, 1H, >C=***CH***-), 6.96-6.84 (m, 2H, , =CH-***CH***=C-***CH***=), 6.24-6.23 (m, 1H, =***CH***-CO-O-), 3.89– 3.71 (m, 23H, C***H***-$(CH3)_2$), 3.64-3.57 (m, 6H, $***CH_2***CH_2OH$), 3.36–3.11 (m, 6H, CH_2C***H_2***OH), 2.12-1.8 (m, 25H, C***H₂***), 1.72-1.25 (m, 43H, C***H₂***), 1.10-0.9 (m, 168H, CH-$(C***H_3***)_2$. % Coumarin = 4%. SEC analysis (DMF, PMMA triple detection standard): $***M_n***$ = 45197 g/mol; $***M_w***$ =53897 g/mol; $***M_w/M_n***$ = 1.19.

B. <u>Synthesis of bis-β-CD crosslinker:</u>



Scheme S1: Scheme for synthesis of coumarin functionalized polymers: (A) DMAc, 70 °C, 3 h, 70%.

β-CD (2 g, 2.6 mmol) was dissolved in 30 mL of dried *N,N*-dimethylacetamide. After adding hexadimethyl isocyanante (0.16 g, 1 mmol), the solution was reacted at 70 °C for 3 hours. The solution was then cooled to room temperature and then precipitated from methanol/water 9:1 mixture. The precipitate was collected after centrifugation at 1000 rpm for 10 min and dried under vacuum with P_2O_5 at room temperature. The product (**bis-β-CD**) was obtained with a yield of 70%. ^{1}H NMR (400 MHz, D_2O,): δ (ppm) 1.40, 1.63, and 3.32 (hexyl group), 3.49, 3.84, and 4.95 (β-CD)

3. Monitoring chain collapse mediated by photodimerization:

A. Monitoring photodimerization by UV spectroscopy:

The aqueous solution of polymers **CP1-2** (0.01 mM) were exposed to UV irradiation (2x8W UV_B lamp, λ_{max} = 320 nm) and monitored for decrease in absorbance in the range of 280-350 nm with time. Retrieval of absorbance on irradiation of UV_c light (λ_{max} = 254 nm) for dimerized polymers were monitored.

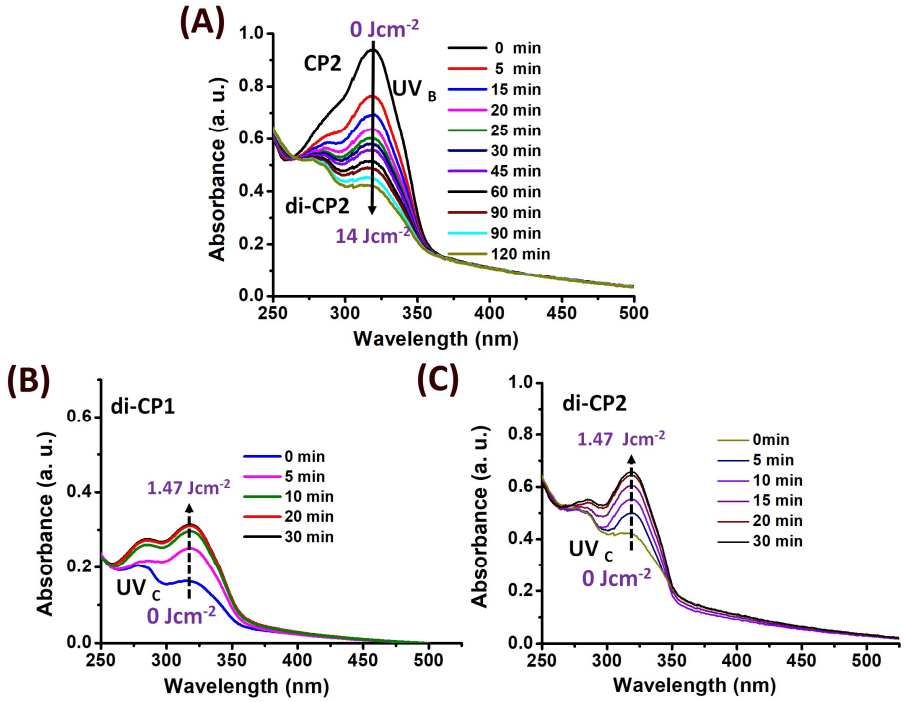


Figure S1: UV spectra showing photodimerization of the polymers upon irradiation with UV light. (A) Decrease in absorbance in the range of 280-350 for **CP2** polymer upon UV_B irradiation. Retrieval of absorbance on irradiation of dimerized polymers (B) **di- CP1** and (B) **di- CP2** with UV_c irradiation, λ_{max} = 254 nm).

B. Monitoring photodimerization by ^{1}H NMR:

1 mM solution of the **CP1** polymer was prepared in D_2O and exposed to UV irradiation in NMR tube. ^{1}H NMR spectra were recorded at different time intervals (0, 3, 6, 9 h). %Photodimerization degree ***(%PD***) was calculated according to the formula $\boldsymbol{\%PD= [(I_o-I_t)/I_o]*100}$, $\boldsymbol{I_t= I_{(a+e)}/I_{(b+d)}}$, where $\boldsymbol{I_{a+e}}$ and $\boldsymbol{I_{b+d}}$ are the integral area of *a, e, b, d* protons, $\boldsymbol{I_t}$ is the ratio of integral area of *a*+*e* with *b*+*d* protons at a specific time of irradiation (*t*). $\boldsymbol{I_o}$ is the ratio of integral area of *a*+*e* with *b*+*d* protons for the polymer.

Table S1: Monioring photodimrization of **CP1** by ^{1}H NMR			
Irradiation time (h)	**Irradiation dosage (Jcm^{-1})**	**I_t^a= I $_{(a+e)}$/I $_{(b+d)}$**	**%PD^b**
0	0	0.5	0
3	17	0.44	12
6	34	0.38	24
9	68	0.26	48

[a] integral ratio $I_t = I_{(a+e)}/I_{(b+d)}$ ratio of *a,e* (protons at photodimerization site) to *b, d* protons, [b] ***%PD={(I$_o$-I$_t$)/I$_o$}x100***, where I_o is initial integral ratio and I_t is integral ratio at time *t*.

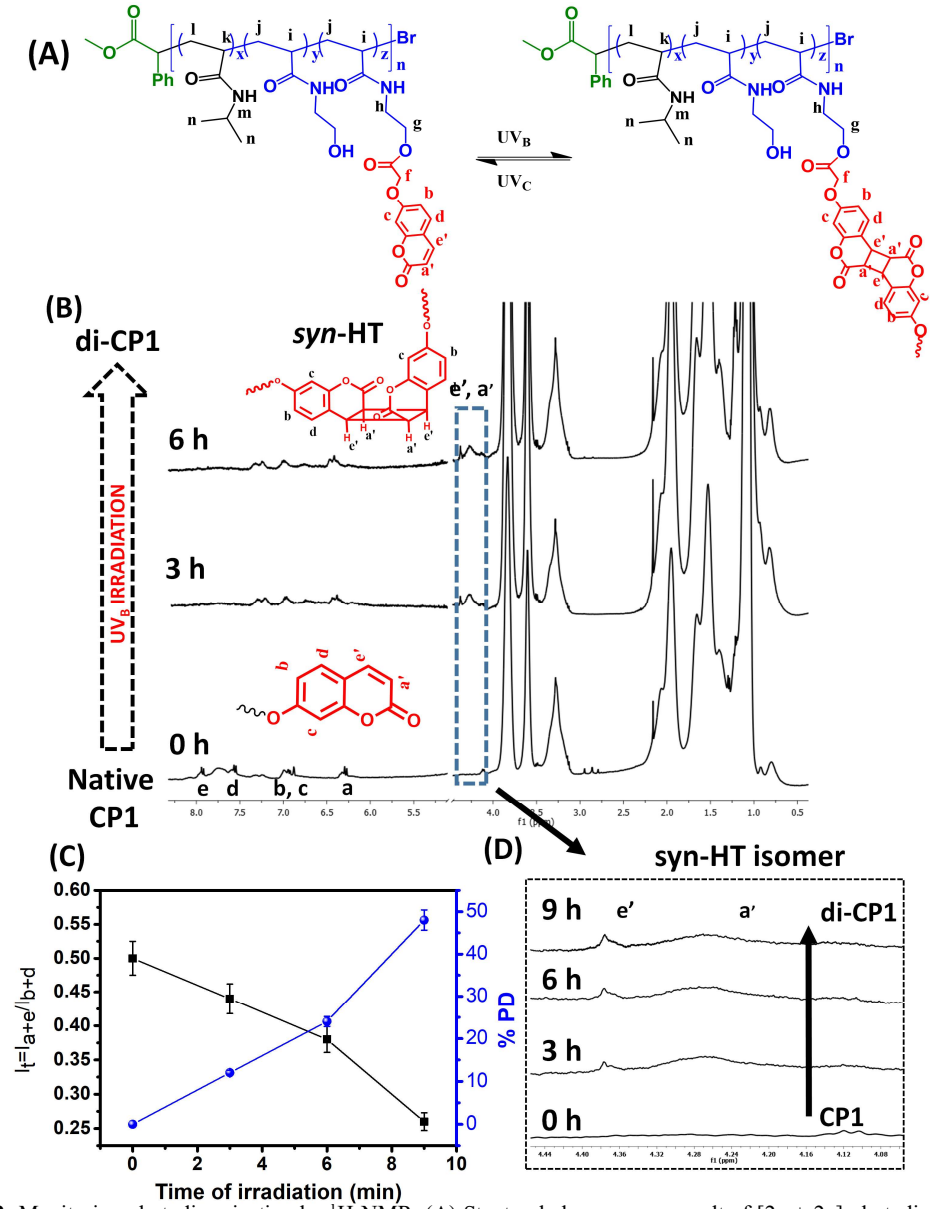


Figure S2: Monitoring photodimerization by ^{1}H-NMR. (A) Stuctural changes as a result of [2π + 2π] photodimerization of coumarin moiety in **CP1** polymer to form cyclobutane adduct. (B) Partial ^{1}H NMR spectra depicting the changes in peak intensity ascribed for coumarin along with appearance of new peak at δ (ppm) 4.2-4.4 which denotes presence of ***syn*-HT** cyclodimer as the major photodimerization product. (C) Change in integral ratio $I_t = I_{(a+e)}/I_{(b+d)}$ and ***%PD*** during photodimerization of **CP1** polymer solution (1 mM) in D_2O with UV_B Light. (D) Zoomed area of ^{1}H-NMR spectra showing appearance of new peaks at δ (ppm) 4.2-4.4 that denotes presence of *syn*-HT cyclodimer as the major photodimerization product.

C. Monitoring chain collapse by DLS:

Dynamic light scattering measurements were done on Malvern Zetasizer Nano ZS ZEN3600 equipped with a Helium-Neon laser (wavelength, λ= 633 nm with backscattering angle of 173°. Aqueous solution of **CP1-2** (0.01 mM) was filtered through a 0.2 μm PTFE-filter in a glass cuvette with a path length of 1 cm. The samples were irradiated with UV_B light (2x8W) for chain collapse *via* photodimerization to form **di-CP1-2** and were analyzed by DLS.

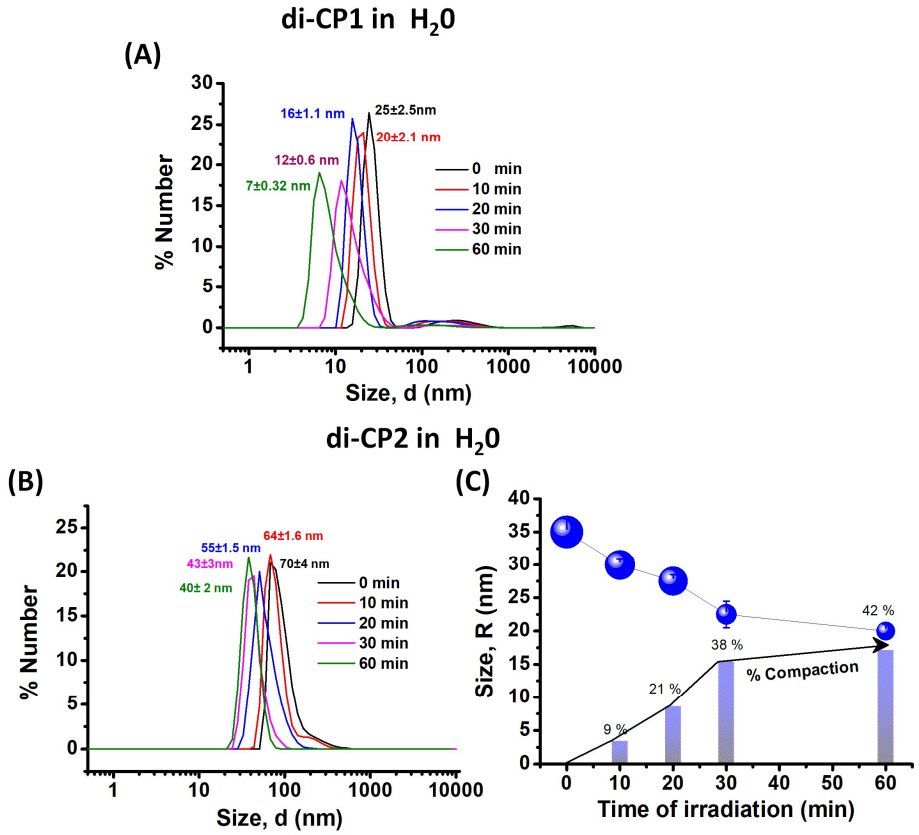


Figure S3: Dynamic Light scattering studies showing variation in size on chain collapse *via* photodimerization in **CP1-2** polymer solution (0.01 mM). % Number *vs* size (Diameter) plot for (A) **di-CP1** (B) **di-CP2**. (C) Hydrodyanamic radii with corresponding % compaction with time of irradiation for **di-CP2.**

D. Monitoring chain collapse by SANS:

Small-angle neutron scattering experiments were performed at the SANS diffractometer at Guide Tube Laboratory, Dhruva Reactor, Bhabha Atomic Research Centre, Mumbai, India.[S4] The instrument covers a Q-range of 0.017– 0.3 $Å^{-1}$ ($Q=4\pi \sin\theta/\lambda$, where 2θ is the scattering angle and λ is the incident neutron wavelength). The mean wavelength of the monochromatized beam from neutron velocity selector is 5.2 Å with a spread of $\Delta\lambda/\lambda \sim 15\%$. The angular distribution of neutrons scattered by the sample (in quartz cuvette) is recorded using a 1 m long one-dimensional He^3 position sensitive detector. Measured SANS data were corrected for background, empty-cuvette contribution and sample transmission, and normalized to absolute cross-section units. The differential scattering cross-section per unit volume ($d\Sigma/d\Omega$) as measured for a system of monodisperse particles in a medium can be expressed as[S5-6]

$$\frac{d\Sigma}{dW}(Q) = nV^2(\rho_p - \rho_s)^2 P(Q)S(Q) + B \text{ ---------(1)}$$

where n denotes the number density of particles, ρ_p and ρ_s are, respectively, the scattering length densities of particle and solvent and V is the volume of the particle. P(Q) is the intra-particle structure factor and S(Q) is the inter-particle structure factor. B is a constant term representing incoherent

background, which is mainly due to the hydrogen present in the sample. Intra-particle structure factor P(Q) is decided by the shape and size of the particle and is the square of single-particle form factor F(Q) as determined by

$$P(Q) = \langle |F(Q)|^2 \rangle \text{-------(2)}$$

For a spherical particle of radius R, F(Q) is given by

$$F(Q)=3\left[\frac{\sin(QR)-QR\cos(QR)}{(QR)^3}\right] \text{-------(3)}$$

The polymer chains are modeled using the form factor of Gaussian chains. The form factor of Gaussian chain coils with the radius of gyration R_g is given by

$$P(Q)=2\frac{\exp(-Q^2R_g^2)+Q^2R_g^2-1}{(Q^2R_g^2)^2} \text{----------(4)}$$

The excluded volume parameter from the Flory mean field theory (scaling exponent, ν) is an important parameter to measure the macromolecular conformation in solution. The scaling exponent can be derived from the double logarithmic SANS scattering curve as follows

$$\upsilon = \frac{-1}{Slope_{scattering\ curve}} \text{----------(5)}$$

The polydispersity in size distribution of particle is incorporated using the following integration [S7]

$$\frac{d\Sigma}{d\Omega}(Q) = \int \frac{d\Sigma}{d\Omega}(Q, R) f(R)\, dR + B \text{---------(6)}$$

where $f(R)$ is the particle size distribution and usually accounted by a log-normal distribution as given by

$$f(R)=\frac{1}{\sqrt{2\pi}R\sigma}\exp\left[-\frac{1}{2\sigma^2}\left(\ln\frac{R}{R_{med}}\right)^2\right] \text{----------(7)}$$

where R_{med} is the median value and σ is the standard deviation (polydispersity) of the distribution. The mean radius (R_m) is given by $R_m = R_{med}\ exp(\sigma^2/2)$. The data have been analyzed by comparing the scattering from different models to the experimental data. Throughout the data analysis, corrections were made for instrumental smearing, where the calculated scattering profiles smeared by the appropriate resolution function to compare with the measured data.[S8] The fitted parameters in the analysis were optimized using nonlinear least square fitting program to the model scattering.[S9]

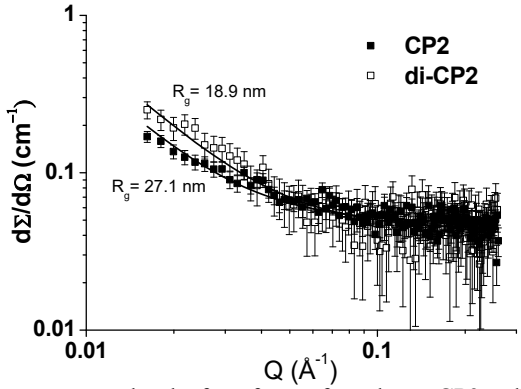


Figure S4: SANS data show macromolecular form factors for polymer **CP2** and the corresponding **di-CP2** nanoparticle after UV_B irradiation for 3h (1 mg/mL in D_2O). Lines are fitted to generalized Gaussian form factors. UV_B light (2*8 W, λ_{max} = 320 nm)

4. Monitoring chain collapse mediated by host-guest complexation:

A. Monitoring host-guest complexation by UV spectroscopy:

The polymer **CP1** (0.01 mM) and **bis-β-CD** (1 mM) were taken in milli-Q water. Job's plot was constructed by varying the Molar ratio of **CP1** to **bis-β-CD** from 0 to 1 keeping the total concentration of **CP1** constant at 0.01 mM. Binding constant was determined monitoring the increase in absorbance at 300 to 350 nm on titration with gradual addition of 2 mM **bis-β-CD** at 10μL per injection. The reciprocal plot of change in absorbance (ΔA) of **CP1** against concentration of **bis-β-CD** was fitted by non-linear least square equation to obtain binding constant value.

$$\Delta \boldsymbol{A} = \frac{1}{2}\{\boldsymbol{\varepsilon}([\boldsymbol{H}]_{\boldsymbol{O}} + [\boldsymbol{G}]_{\boldsymbol{O}} + \frac{1}{\boldsymbol{K_a}}) \pm \sqrt{\boldsymbol{\varepsilon}^2([\boldsymbol{H}]_{\boldsymbol{O}} + [\boldsymbol{G}]_{\boldsymbol{O}} + \frac{1}{\boldsymbol{K_a}})^2 - \boldsymbol{4\varepsilon}^2[\boldsymbol{H}]_{\boldsymbol{O}}[\boldsymbol{G}]_{\boldsymbol{O}}} \quad \text{..... (8)}$$

Where ΔA is the chemical shifts change of **CP1** upon addition of **bis-β-CD** as $\Delta A = A_{(\text{with bis-β-CD})} - A_{(\text{without bis-β-CD})}$, ε is the sensitivity factor, and $[H]_0$ and $[G]_0$ are the initial concentrations of **bis-β–CD** and **CP1**, respectively.

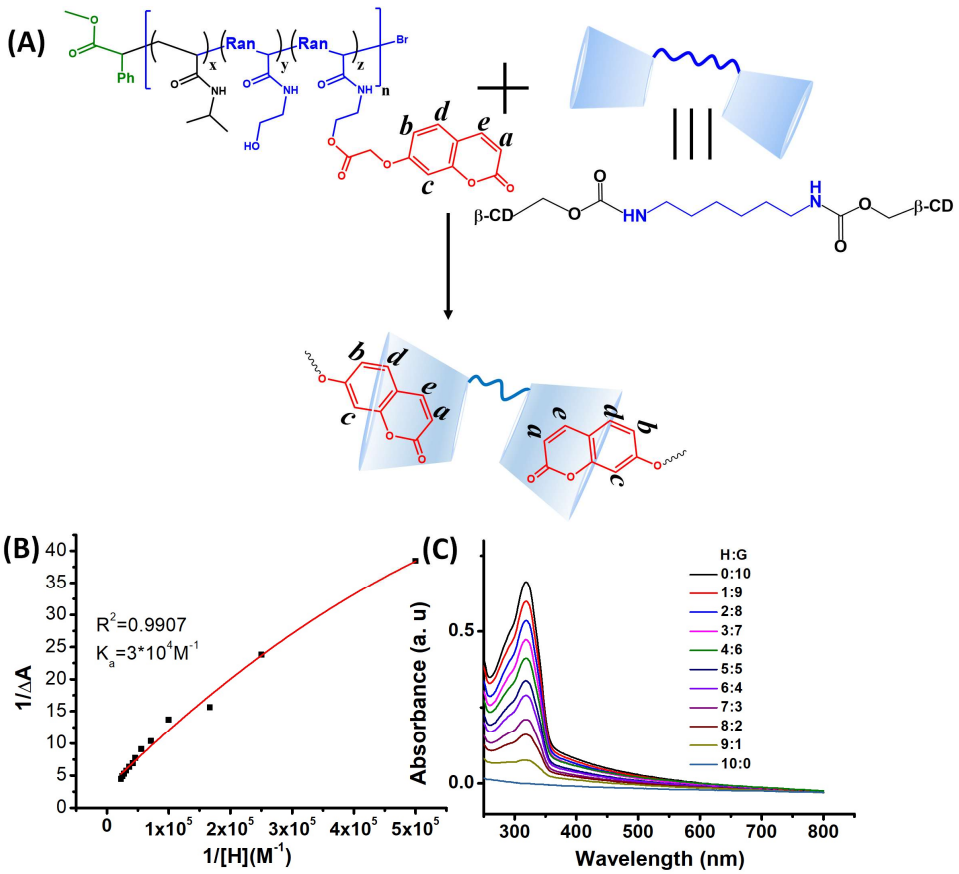


Figure S5: Monitoring host-guest complexations by UV studies. (A) Stuctural details depicting host-guest interaction of coumarin moiety inside **bis-β-CD** crosslinker to form inclusion complex. (B) Determination of the binding constant from increase in absorbance on titration of 0.01 mM **CP1** with **bis-β-CD** upto 44 μM, (C) Change in absorbance on varying the concentration of host and guest keeping total concentration at 0.01 mM to derive complexations stoichiometry from Job's plot of **CP1** against **bis-β-CD**.

B. Monitoring host-guest complexation by ^{1}H-NMR :

The **CP1** polymer solution in D_2O (1 mM) was mixed with 4 mM **bis-β-CD** and was monitored for the characteristic shift in proton peaks which are responsible for the complexations reaction.

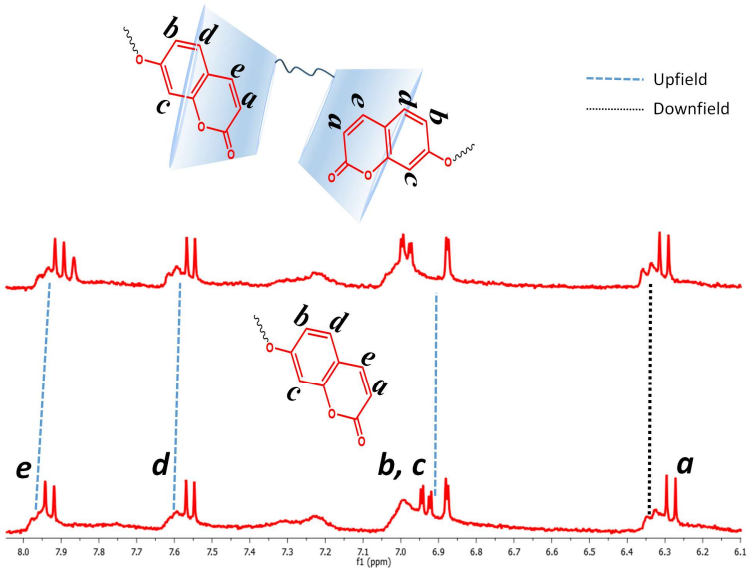


Figure S6: ^{1}H NMR spectra showing upfield shift of proton signals of coumarin moiety in **CP1** polymer, e, c, b and d except a on the addition of 4 equvalent of **bis-β-CD** due to the shielding effects on coumarin inside cyclodextrin cavity environment.

C. Monitoring chain collapse by DLS:

Aqueous solution of **CP1** (0.01 mM) was filtered through a 0.2 μm PTFE-filter in a glass cuvette with a path length of 1 cm. Further the polymer **CP2** guest solution was added with gradient concentration of **bis-β-CD** keeping guest concentration constant to achieve chain collapse *via* host-guest interaction to form **CP1⊂bis-β-CD**.

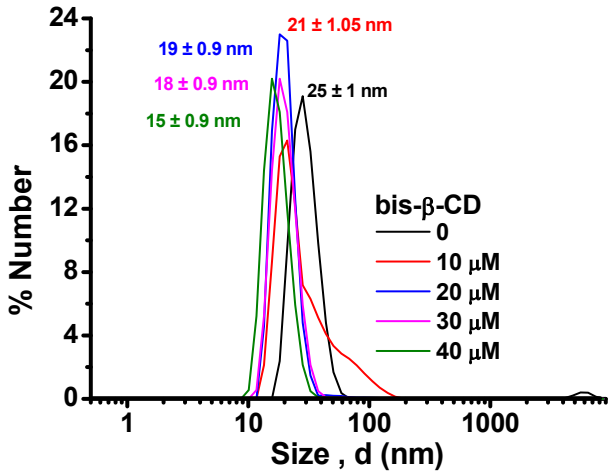


Figure S7: Dynamic Light scattering data showing change in hydrodynamic radii of **CP1** polymer upon chain collapse mediated by the addition of host molecule **bis-β-CD** to form **CP1⊂bis-β-CD complex.**

D. Monitoring host-guest interaction by ITC:

The samples **CP1, di-CP1, CP2** was titrated in ITC against **bis-β-CD** under same conditions and compared for the association constant and thermodynamic parameters

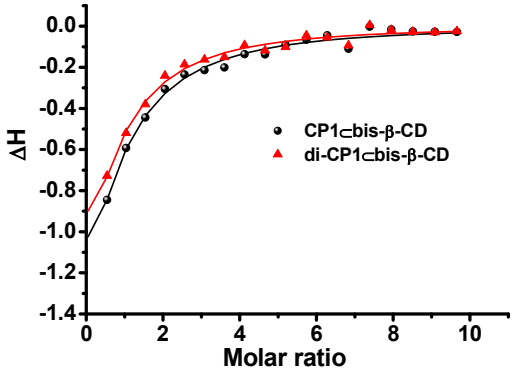


Figure S8: ITC data of host-guest interaction of native nad folded polymers with (A) ITC binding isotherm representing integrated heat of interactions on titration of **CP1, di-CP1** against **bis-β-CD** as a function of molar ratio and the solid line represent the line of best fit.

Table S2: Binding constant and other thermodynamic parameters for different polymer samples on host guest interactions (ITC titration for 1 mM **bis-β-CD** solution in 0.02 mM **CP1, di-CP1, CP2** solution at 298 K.

	$K_a(M^{-1})$	ΔH(KJ/mol)	ΔG(KJ/mol)	-TΔS (KJ/mol)
CP1⊂bis-β-CD	**$2.8x10^4$**	**-21.38**	**-25.43**	**-4.05**
di-CP1⊂bis-β-CD	**$3.1x10^4$**	**-18.48**	**--26**	**-7.32**
CP2⊂bis-β-CD	**$1.9x10^4$**	**-17**	**-24**	**-7.48**

5. Visualisation of nanostructures by microscopy

A. AFM Microscopy:

20 µL of polymer sample solution (0.01 mM) were drop-casted on silicon wafer followed by washing with water and dried overnight by keeping in desiccator. AFM height images were recorded by tapping mode on a Bruker Multimode 8 scanning probe microscope with silicon cantilever (Bruker) and analyzed using the software NanoScope Analysis 1.5.

B. Transmission Electron Microscopy (TEM): 6 µL of sample solution was drop-casted on a 300 mesh carbon-coated copper grid. After ~ 5 min, excess solution was blotted using Whatmann filter paper. Extra solution was the wicked off from all edges of the grid carefully followed by drying in the desiccator under vacuum for 1 day. TEM images were recorded using JEOL JEM 2100 with a Tungsten filament at an accelerating voltage of 120 kV.

C. Histogram analyses

The size distributions of the nanoparticles were analyzed using image-J software, from the U.S. National Institutes of Health. 50 random nanoparticles were selected from different areas of the images and a histogram was generated by choosing bin and frequency in Microsoft Excel. The number average radii (R_n), weight average diameter (R_w), and the ratio R_w/R_n were estimated using eqn. 8-10, where N_i

is the number of nanoparticles of radius R_i and n is the number of nanoparticles examined in each sample.

$$R_w = \frac{\sum_{i=1}^{n} N_i R_i^2}{\sum_{i=1}^{n} N_i R_i} \text{ ------ (9)} \qquad R_n = \frac{\sum_{i=1}^{n} N_i R_i}{\sum_{i=1}^{n} N_i} \text{ ----------(10)} \qquad PDI = \frac{R_w}{R_n} \text{---------- (11)}$$

D. Microscopical investigation of chain collapse:

The polymer samples **CP1-2** (0.01 mM) was irradiated with UV_B light (2*8W) for 1 h and thus resulted in formation of **di-CP1-2**. The aqueous polymer sample **CP1** solution (800 µL, 0.0125 mM) was mixed with aqueous **bis-β-CD** solution (200 µL, 0.3 mM) to form **CP1⊂bis-β-CD**.

Microscopical investigation showed that polymer samples after chain collapse either by photodimerization or by host-guest interaction showed a clear differences in structural signatures. The from highly diverse nanostructures of considerably variable long height to nearly uniform nanoellipsoidal structures of short height. Precise investigation into some of the zoomed area of **diCP1-2** as well as **CP1⊂bis-β-CD** showed clearly well-separated nanoparticles that are nearly spherical in shape.

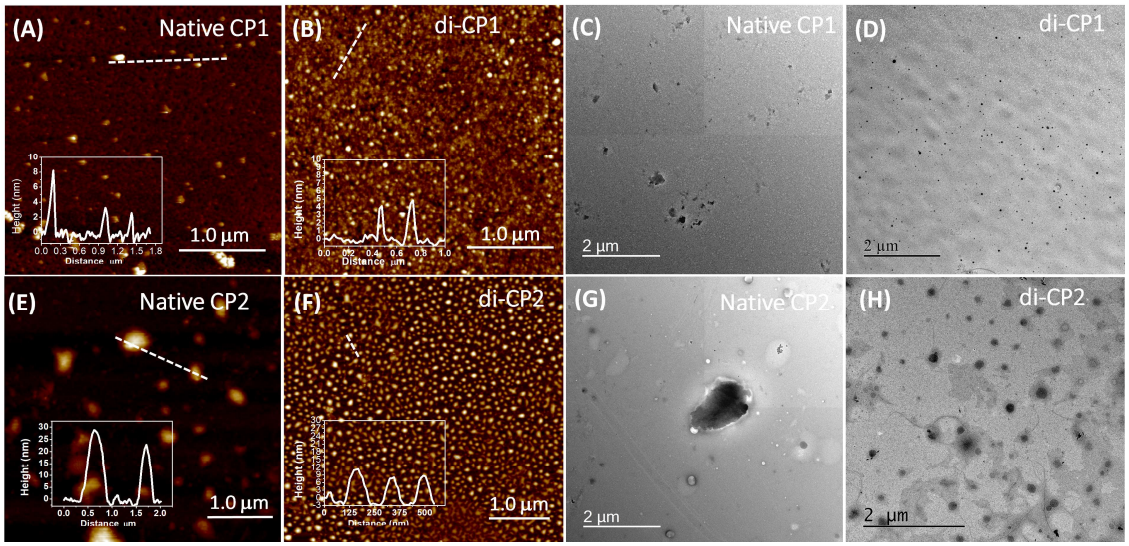


Figure S9: Morphological transformation polymers **CP1-2** to nanoparticles as a result of chain collapse *via* photodimerization. AFM and TEM images for native polymers showing nonspecific unconrtrolled nanostructures in case of (A,C) **CP1,** (E,G) **CP2** that transform in to discrete uniform nanoparticles in (B, D) **di-CP1**, (F, H) **di-CP2** (Inset showing corresponding AFM height profiles)

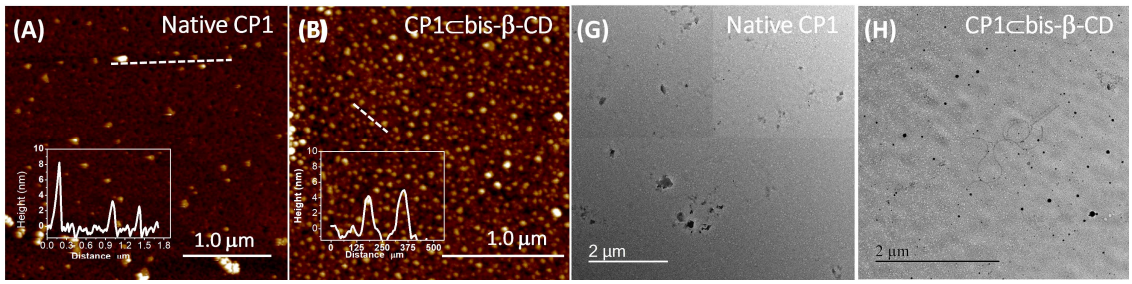


Figure S10: Morphological transformation of the polymers to discrete nanoparticles as a result of chain collapse *via* host-guest complexation. AFM & TEM images for (A, C) native **CP1** polymer showing nonspecific unconrtrolled nanostructures that formed nanoparticles mediated by (B, D) inclusion complex **CP1⊂bis-β-CD** showing spherical nanoparticles. (Inset showing AFM height profiles)

E. Spherical diameter from the hemi-ellipsoidal particle adsorbed on silicon or TEM grid surface:

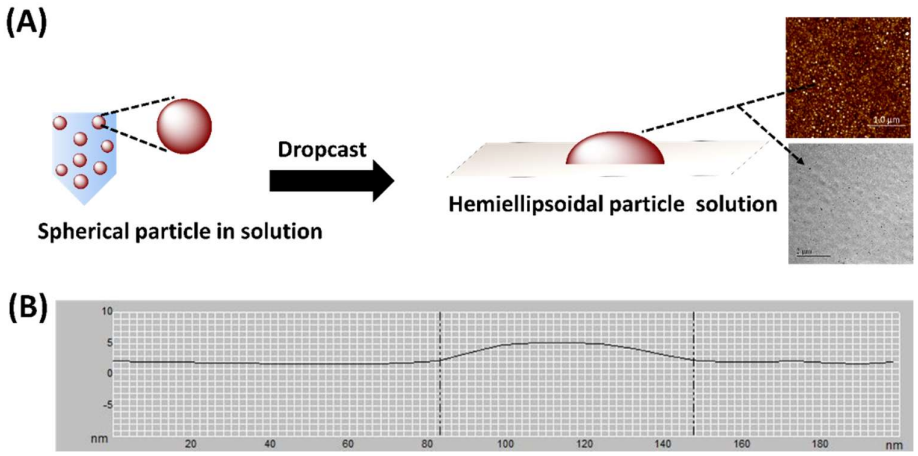


Figure S11: Determination of spherical radii from half-ellipsoid particle adsorbed on silicon wafer surface. (A) Schematic representation of spherical nanoparticles drop-casted on silicon wafer to form half-ellipsoid shape due to surface adsorption. Representative AFM and TEM image showing nanoparticles from **CP1** polymer. (B) AFM height profile of a selected nanoparticle from the same AFM image.

Example: Calculation of spherical diameter ($D_{spherical}$) from half volume of ellipsoidal particle adsorbed on silicone surface[S10]

$$V = 1/2 \times 4/3 \times \pi \times R_{AFM}^2 \times h$$

Where R_{TEM} = Radii of the hemiellipsoid from the TEM, h = height from AFM (in nm)

$$R_{he} = 11 \text{ nm}, h = 4.1 \text{ nm}$$

$$V = 0.5 \times 4/3 \times \pi \times (11^2) \times 4.1$$

$$V = 1035 \text{ nm}^3$$

Volume of a sphere = $4/3 \times \pi \times R_{spherical}^3$

$$4/3 \times \pi \times R_{spherical}^3 = 1035 \text{ nm}^3$$

$$R_{spherical} = 6.27 \text{ nm}$$

6. Monitoring thermo-responsive property

UV–vis Transmission measurements: CP1, **di-CP1**, **CP1⊂ bis-β-CD** (0.02 mM) was monitored for decrease in Transmittance (*%T*) with increase in temperature from 20 to 70 °C at a rate of 2.5 °C /min at a wavelength of 563 nm. The temperature accouting for 50% transmittance is determined as the cloud point temperature (T_{cp}) signifying lower critical solution temperature (LCST).

DLS temperature trend analyses: The polymer **CP1, di-CP1** (**CP1** polymer solution irradiated with UV_B light for 1 h**)**, **CP1⊂ bis-β-CD** (0.02 mM) were monitored in a temperature trend analysis experiment in DLS instrument for the increase in size due to aggregation on heating above LCST. The polymer sample was heated from 20-60 °C and the size was measured at each interval of 5 °C.

SANS Temperature analyses: The sample **CP1** in water at a concentration of 5 mg/mL was analyzed at different temperatures *i. e*. 25, 35 and 45 °C and observed for the change in the scattering intensity.

Microscopical analyses: CP1 polymer sample (0.01 mM) heated to 50 °C was drop-casted on Silicon wafer for AFM. After 2 minutes, the silicon wafer was left to be air-dried in a desiccator. 6 µL of polymer sample solution (0.01 mM) at 50 °C was drop-casted on a 300 mesh carbon-coated copper grid. After ~ 2 min, excess solution was blotted using Whatmann filter paper was left to be dried in a desiccator under vacuum for one day.

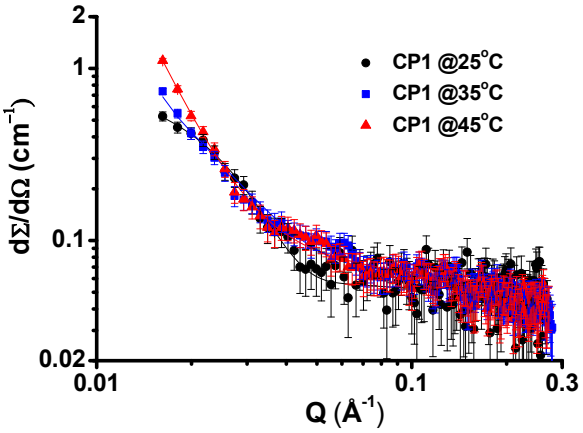


Figure S12: Monitoring thermoresponsiveness via SANS Macromolecular form factors obtained by SANS for CP1 polymer solution (5 mg/mL) at 25 °C, 35 °C and 45 °C. Lines are fits to generalized Gaussian form factors.

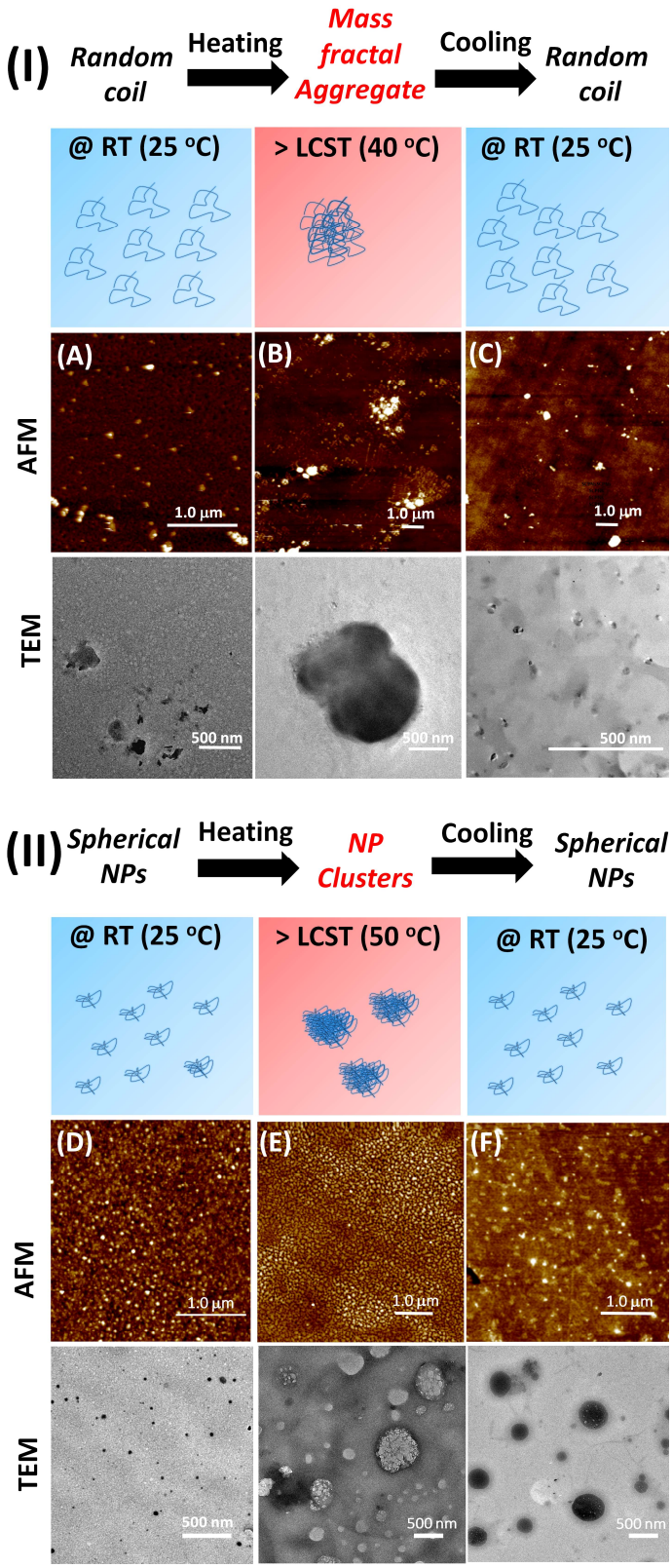


Figure S13: : Microscopical investigation of the thermoreponsive character of **CP1** solution (0.02 mM). I. (A-C) Extended random coils and micellar aggegare of **CP1** as observed in AFM and TEM images upon heating and cooling temperature beyond LCST temperature. II (D-E) Discrete nanoparticles and nanoparticle aggegare of **di-CP1** as observed in AFM and TEM images upon heating and cooling temperature beyond LCST temperature.

7. Sequential folding-defolding studies

The host **CP1** (0.02 mM) and guest **bis-β-CD** were mixed in 1:1 ratio to form partially complexed **CP1⊂ bis-β–CD** nanoparticle (**2**). The resulting solution was irradiated with UV_B light (2x8W) for 30 min to form **di-CP1⊂bis-β-CD** NPs (**3**). Further, the solution was irradiated with UV_C light (1x8W) for 15 min to form de-crosslinked **di-CP1⊂ bis-β-CD** *via* decycloaddition (**4**). Finally, solution was heated above LCST to observe the hydrophilic to hydrophobic transition and aggregation to **CP1⊂ bis-β-CD** clusters (**5**)**.**

UV studies were conducted for **CP1** (0.02 mM) to monitor the change absorbance upon addition of **bis-β-CD** host in a gradient manner upto 20 μM to form partially complexed **CP1⊂ bis-β–CD**. Further, the solution was monitored for decrease in absorbance on irradiation with UV_B light (2x8W) for 30 min. and increase in absorbance on irradiation with UV_C light (1x8W).

Size change at various stages of folding and defoding was monitored by DLS data. Aggregation of de-crosslinked **di-CP1⊂ bis-β-CD** to form clusters on heating above LCST was monitored by DLS temperature trend analysis. Samples at different stages of folding and defolding process were diluted to 0.01mM was dropcasted onto silicon wafer or Cu grid surface for AFM and TEM analysis respectively.

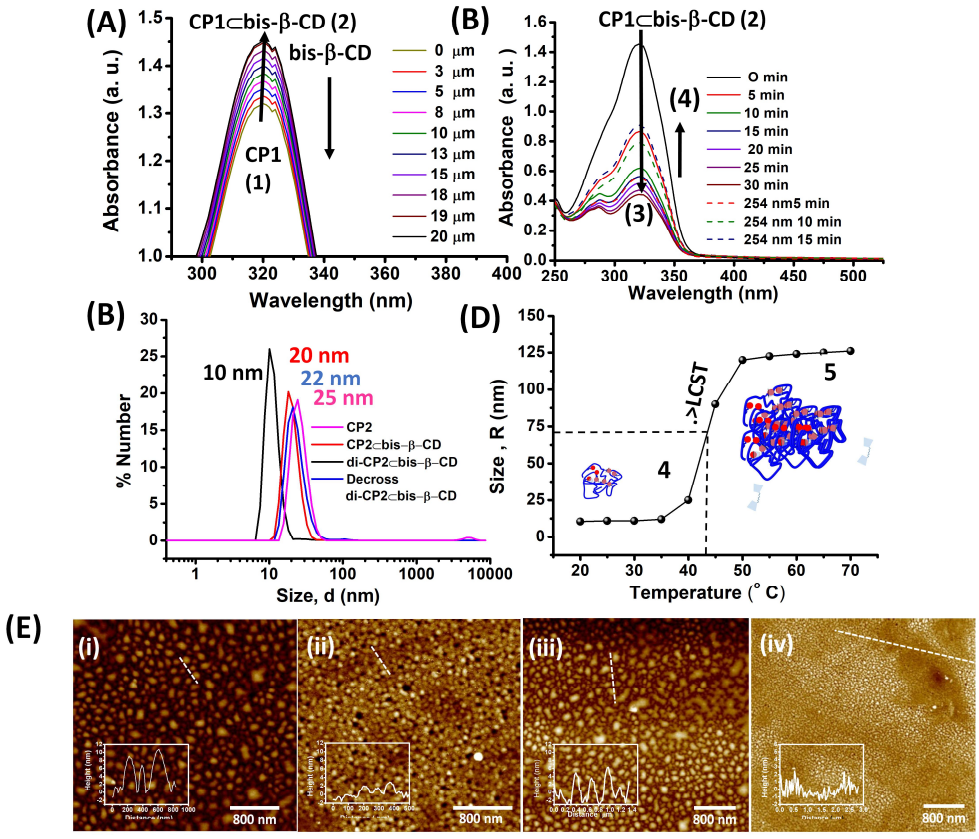


Figure S14: Sequential folding and defolding studies on **CP1** *via* cooperative interaction of host-guest ineraction and photodimerization. (A) UV spectra showing increase in aborbance on addition of 1 equivalent of **bis-β-CD** molecule to 0.02 mM native **CP1** polymer to form **CP1⊂bis-β-CD** inclusion complex. (B) Graph showing decrease in absorbance on photodimerization to form **di-CP1⊂bis-β-CD** *via* irradiation with UV_B light (2 x8W) for 30 min(C) DLS data showing variation of hydrodynamic diameter at various stages of stepwise folding. (C) DLS temperature ramp-up study showing the aggregation of NPs. (E) AFM images with corresponding profiles showing nanoparticles formed from (i) **CP1⊂bis-β-CD (2),** (ii) **di-CP1⊂bis-β-CD (3), (iii)** decrosslinked **di-CP1⊂bis-β-CD (4)** and (iv) nanoparticle cluster (**5**) (formed by heating decrosslinked **di-CP1⊂bis-β-CD** solution above LCST)

Table S3: Table showing size and dimensional details at various stages of sequential folding and defolding studies.

Polymers	R_H^{DLS} [a] (nm)	% Compaction [DLS]	Radii$_{TEM}$ [b] ($R_{TEM,}$ nm)
Native **CP1 (1)**	13 ± 0.7	0	Nonspecific structures
CP-1⊂bis-β-CD (2)	10 ± 0.5	20	21 ± 2
di-**CP-1⊂bis-β-CD (3)**	5 ± 0.2	60	11± 1.5
Decrosslinked di-**CP-1⊂bis-β-CD (4)**	11 ± 0.6	12	23 ± 2.7
CP-1⊂bis-β-CD cluster (5)	12 ± 3	**Aggregation**	200-400

[a] Hydrodynamic radii determined by DLS studies [b] Diameter from analysis of TEM images

8. Cargo incorporation and loadng studies

Rhodamine B dye or Ibuprufen drug (1 mg/mL) was added to **CP1, di-CP1, CP1⊂bis-β-CD** (2 mg/mL) polymeric system on stirring at 11 ° C for 4h to result in polymeric drug delivery system containing 1 mg/mL polymer with 50 % w/w cargo. After that it was kept for release through dialysis in PBS buffer (10 mM, pH7, 200 mL) at 4 ºC for 24 h to release free drug *via* cellulose membrane dialysis (3000-5000 Da) bag. Amount of drug loaded was determined by UV spectroscopy monitoring absorbance at 272 nm (Ibu) or 549 nm (RhB), then lyophilized to obtain the total weight of cargo-loaded polymeric solution.

$$\% \text{ Cargo loading} = \frac{W_{Cargo}}{W_{Total}} * 100 \text{ -----} \quad (12)$$

Table S4: Table showing percentage loading of RhB or Ibu incorporated in native and folded polymers.

Loaded Cargo	Polymeric system	W $_{cargo}$ (mg)[a]	W $_{total}$ (mg)[b]	% Loading[c]	Average Cargo molecule per chain[d]
RhB	Nil	0.06	2.9	2	-
RhB	Native CP1	3.6	8.9	40	34
RhB	di-CP1	1.2	8.4	14	9
RhB	CP1⊂bis-β-CD	1.6	8.1	20	12
Ibu	Nil	0.11	2.7	4	
Ibu	Native CP1	3.1	8.7	35	66
Ibu	di-CP1	1.82	9.1	20	32
Ibu	CP1⊂bis-β-CD	1.48	8.2	18	26

[a] Amount of loaded drug in polymeric solution determined by UV spectra by monitoring the absorbance at 272 nm (Ibu) or 459 nm (RhB) [b] Weight determined by lyophilisation of the RhB or Ibu loaded polymer solution after dialysis with release of free drug and lyophilisation [c] % loading of drug was determined by equation (10) [d] as determined from the amount of cargo in one molecule of polymer in mmol taking Avogadro number ($N_A = 6.022*10^{23}$) into account.

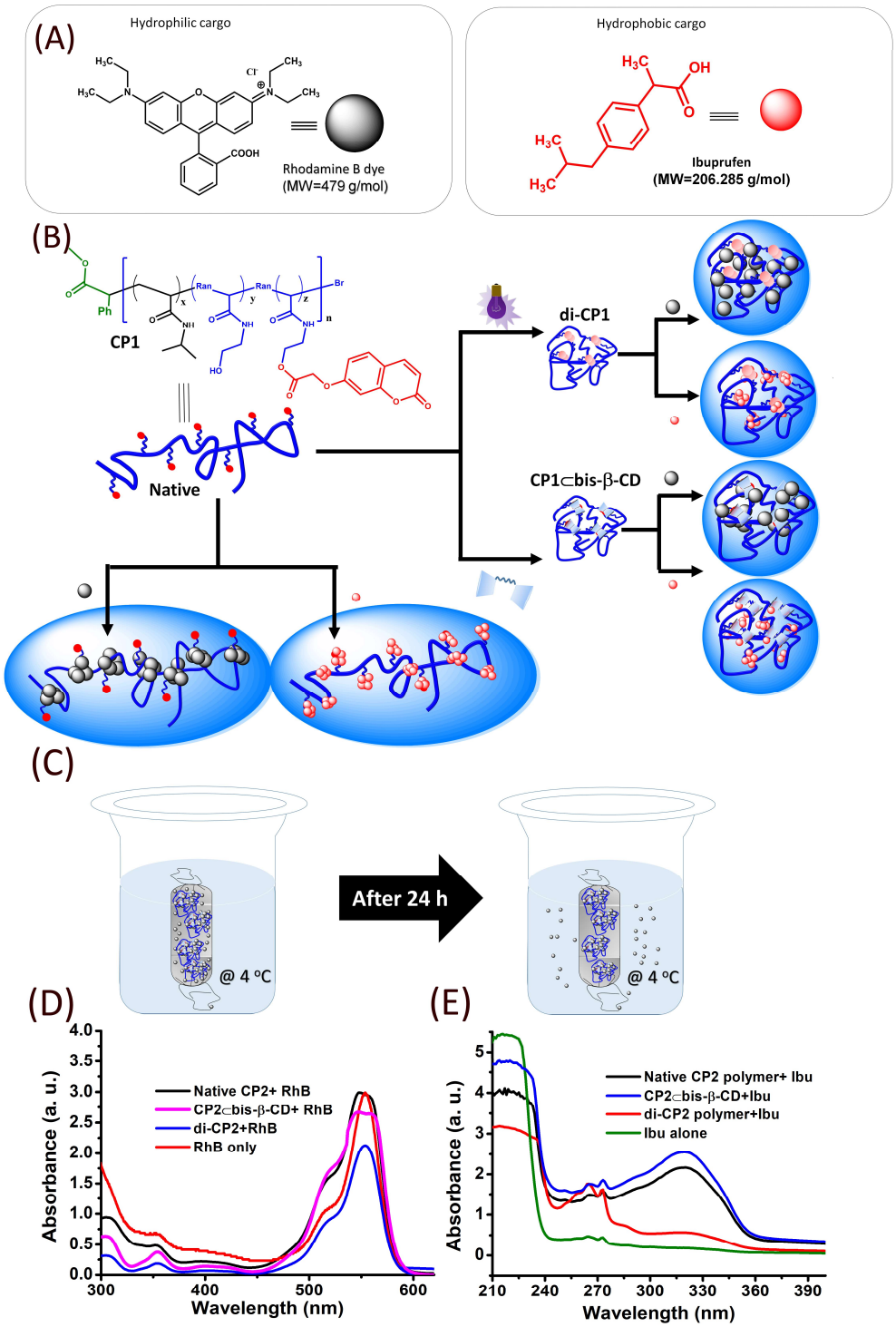


Figure S15: Cargo incorporation and loading studies. (A) Molecular structures of hydrophilic Rhodamine B, hydrophobic ibuprofen drug. (B) Scheme of RhB and Ibu loading into **CP1, di-CP1, CP1⊂bis-β-CD** polymers. (C) Schematic represention of the protocol for determination of loading percentage using dialysis of drug loaded polymers against PBS buffer (pH 7, 10 mM) at 4 °C. (D) The interaction of RhB (0.2 mM) loaded in **CP1, di-CP1, CP1⊂bis-β-CD** polymers (0.04 mM) by UV spectra by monitoring absorbance at 549 nm. (E) The interaction of Ibu (2.6 mM) loaded in **CP1**, **di-CP1, CP1⊂bis-β-CD** (0.04 mM) polymers by monitoring absorbance at 272 nm.

9. Release studies

RhB dye or Ibu drug was added to the 1 mg/mL aqueous polymers (CP1, di-CP1 or CP1⊂bis-β-CD) at corresponding loading percentage to result in polymeric drug delivery system (1 mg/mL) and kept for release in PBS buffer (10 mM, pH = 7, 200mL) at 37 ºC for 24 h to release drug *via* cellulose membrane bag dialysis (3-5 KDa). After 24 h, the temperature was increased to 50 ºC to monitor the drug release for 8h. Amount of drug released was determined by UV absorbance at 272 nm (Ibu) or 549 nm (RhB)

$$\% \text{ Cargo release} = \frac{W_{cumulative}}{W_{cargo}} * 100 \text{ ----- (13)}$$

Where $W_{cumulative}$ is cumulative cargo weight in mg released in buffer, W_{cargo} is amount of loaded cargo in mg.

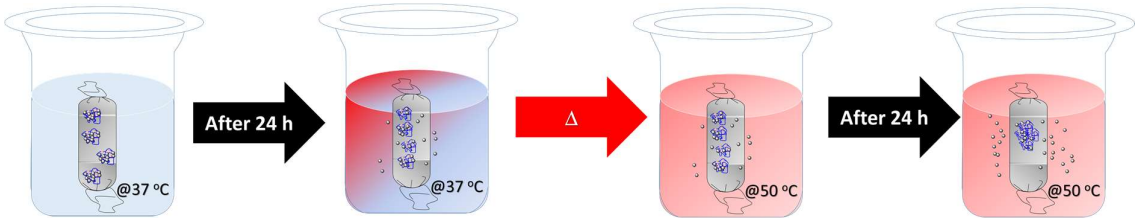


Figure S16: Schematic representation of release study of RhB loaded **di-CP1** nanoparticle by dialysis against PBS buffer (pH = 7, 10 mM) at 37 ºC for 24 h. Thereafter, the temperature was increased to 50 ºC and monitored for the release for additional 8 h.

10. Monitoring host-guest interaction through classical MD simulation

Syn di-coumarin molecule along with β-CD

To investigate the host-guest interactions **syn-di-HT** isomer with **β-CD**, the guest molecule was placed in the neighbourhood of the **β-CD** at initial stage and performed the MD upon suitably placing it a periodic bounding box filled with water as solvent.

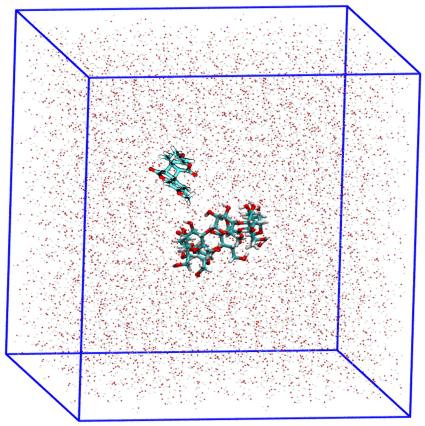


Figure S17. The simulation celle with the initial system of **β-CD** with di coumarin molecule solvated in water.

During the thermal equilibration at 300K, **syn-di-HT** enters inside the cyclic cage of **β-CD** and remains inside quite steadily. We did not observe any instance of deviation of this through the complete 200 ns long trajectory considered here. The close look into the dynamical structural fluctuations indicates quite strong host-guest interactions.

The RMSD fluctuations of the complete trajectories are plotted in Figure S18. It contains regular dynamical fluctuations mostly, however from 148 ns to 175 ns a slight increase in the RMSD values are observed due to flipping the side of one of the sugar units in **β-CD**. The maxima in the RMSD probability distribution appears in 0.53 Å for **β-CD** only, 1.14 Å and **β-CD** with **syn-di-HT** is 2.19 Å.

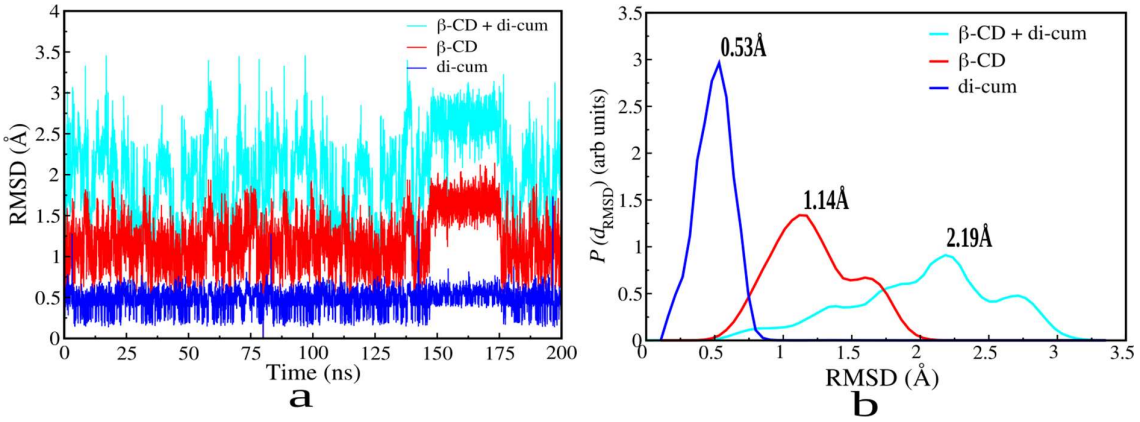


Figure S18. (A) A plot of the RMSD of the non-hydrogen atoms of **β-CD** with **syn-di-HT** (Cyan) and β-CD only(red) and **syn-di-HT** molecule only (Blue) along with simulation time. (B) Probability distribution functions of the β-CD and di-com system**.**

The distance between the centre of the mass (COM) of syn-di-HT with β-CD

Based on our visual observations of the trajectory and RMSD, it is quite clear that the cyclobutane of **syn-di-HT** is favorably placed in the hydrophobic site of **β-CD**. This could be further understood from the fluctuation of the COM as shown in Figure S19. The synergic hydrophilic and hydrophobic interaction between host and guest molecules provides expectational stability to the inter-locked systems.

To get a structural change along the simulation time, we plotted corresponding selected snapshots from 0 ns to 200 ns for inclusion complexes formed by the **β-CD** and **syn-di-HT** (Figure S19). In 200 ns simulation time, **syn-di-HT** molecule is inserted, never losing the hydrophobic cavity in **β-CD**. Around 148 ns to 176 ns it was trying to leave the hydrophobic cavity at 150 ns inside view.

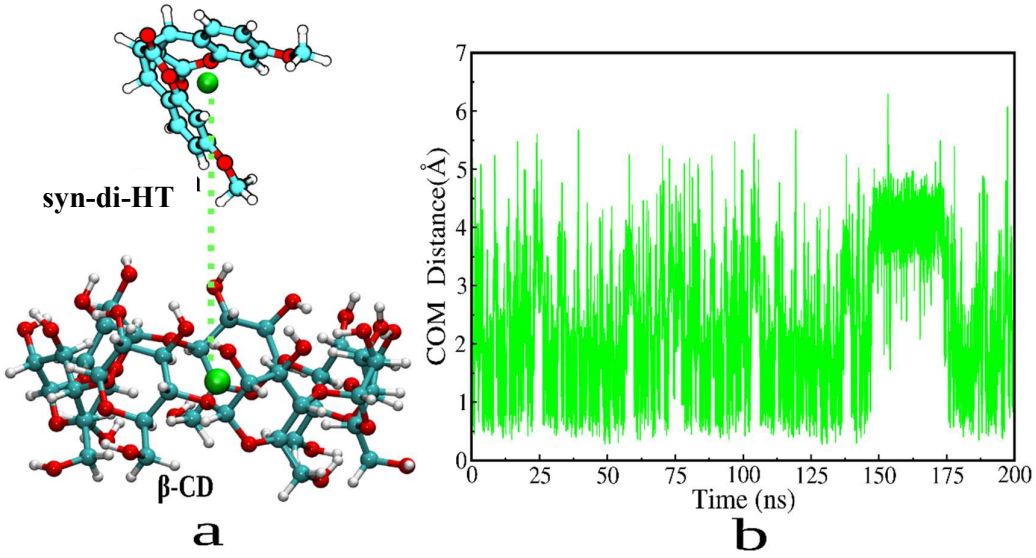


Figure S19. (A) Schematic representations of the Centre of the Mass (COM) distance between di-cum and β-CD cavity. (B) COM distance between the **syn-di-HT** and **β-CD** along the simulation time.

The time evolution of the host-guest interaction was captured along the 200 ns with 50 ns of interval. The snapshots are given in S20. At around 150 ns we observed the flipping of one of the sugar units that pushes the guest molecule out of its inter-locked stable position.

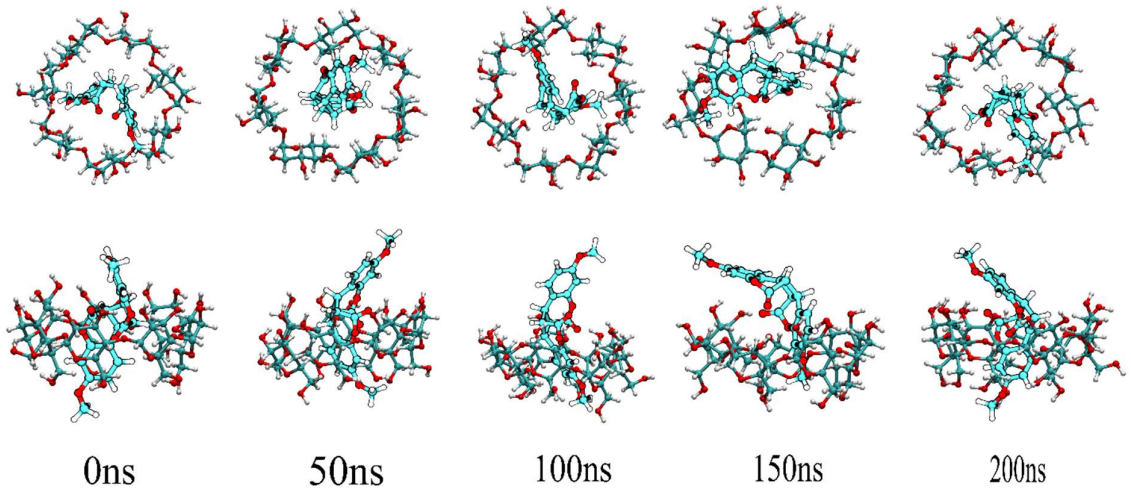


Figure S20. Selected time step snapshot of **syn-di-HT** and **β-CD** molecule. The upper panel contains top view from the second face and lower panel contains the side view of the complex.

11. References

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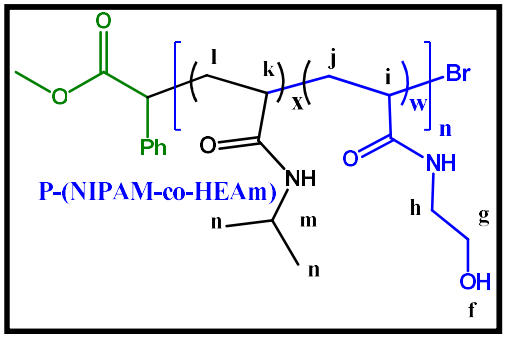
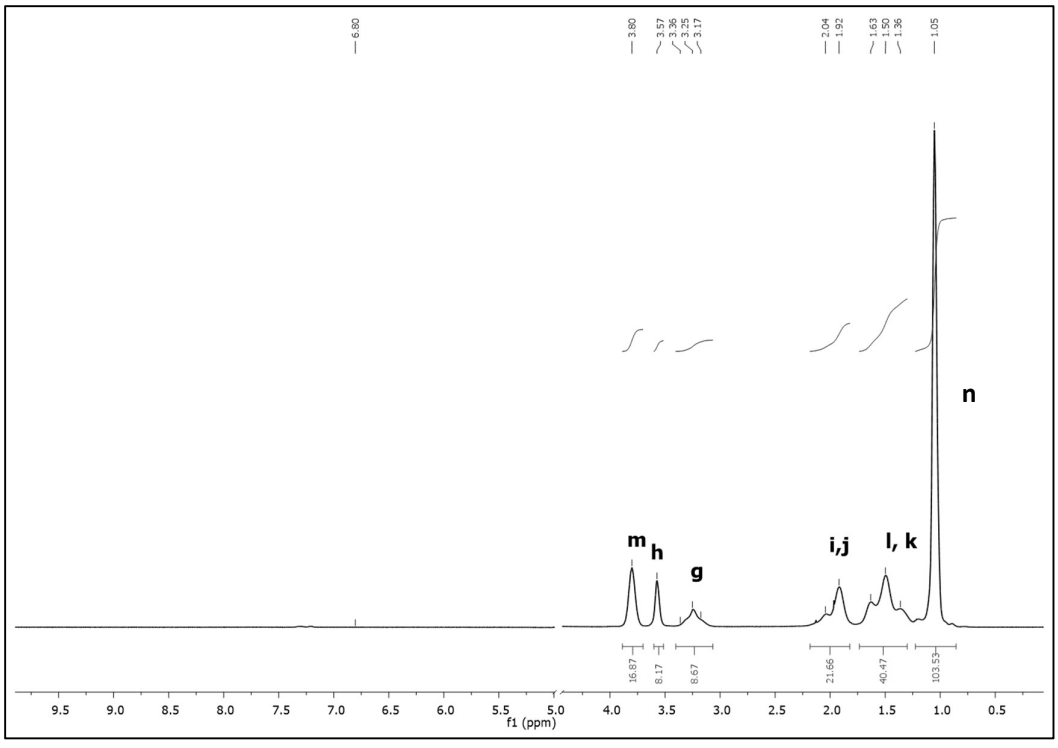
12. Appendix

A. ^{1}H NMR characterization of polymers (D_2O, 400MHz)

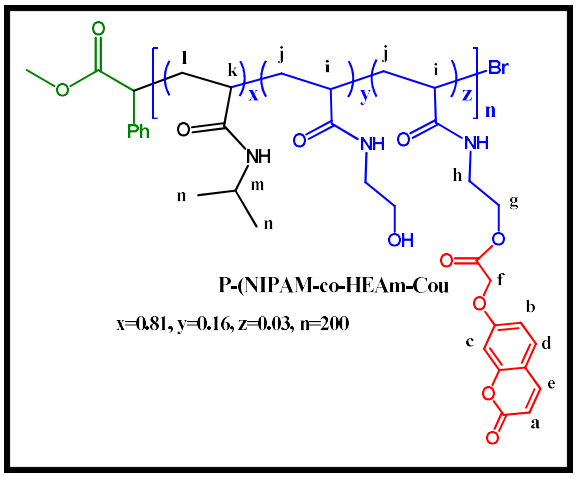
i. Representative Precursor polymer P1 (DP-200)



$$\%\ \boldsymbol{HEAm} = \frac{\frac{\boldsymbol{g}}{\boldsymbol{2}}}{\frac{\boldsymbol{g}}{\boldsymbol{2}} + \frac{\boldsymbol{n}}{\boldsymbol{6}}} * \mathbf{100} = \frac{{}^{\mathbf{8}}/_{\mathbf{2}}}{{}^{\mathbf{8}}/_{\mathbf{2}} + {}^{\mathbf{103}}/_{\mathbf{6}}} * \mathbf{100} = \mathbf{19}\ \%$$

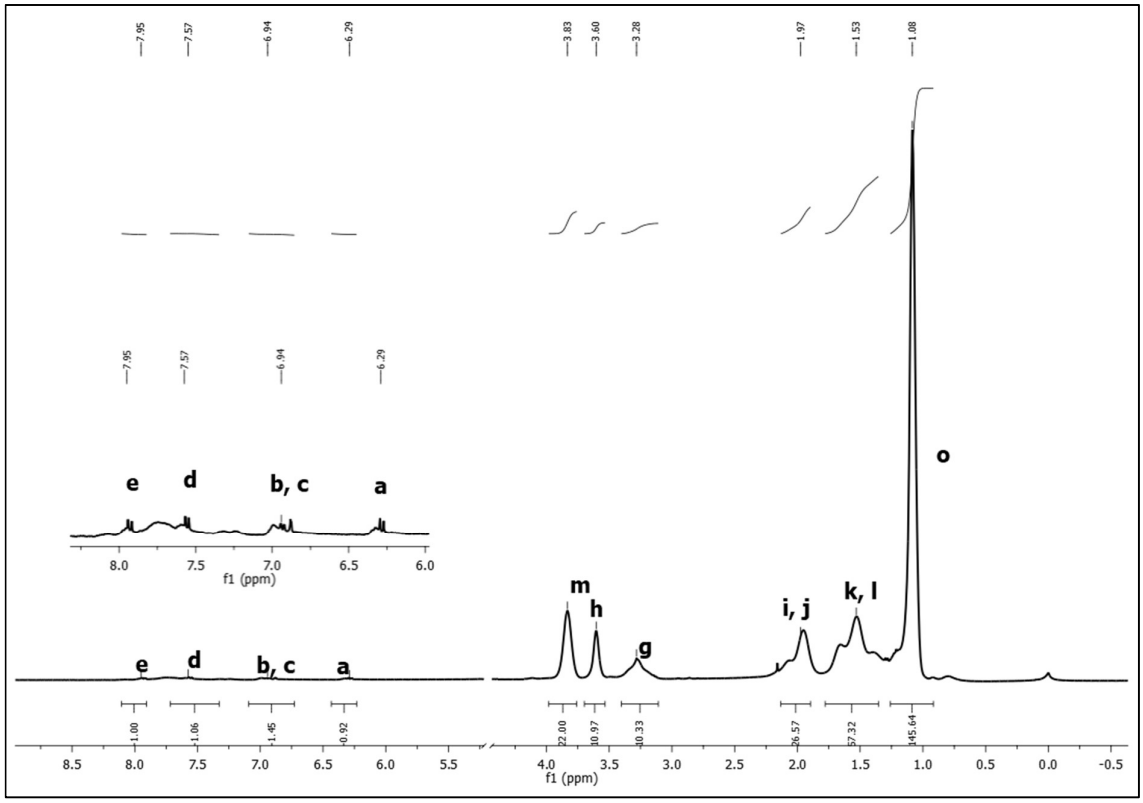


ii. Polymer CP1 (DP-200)

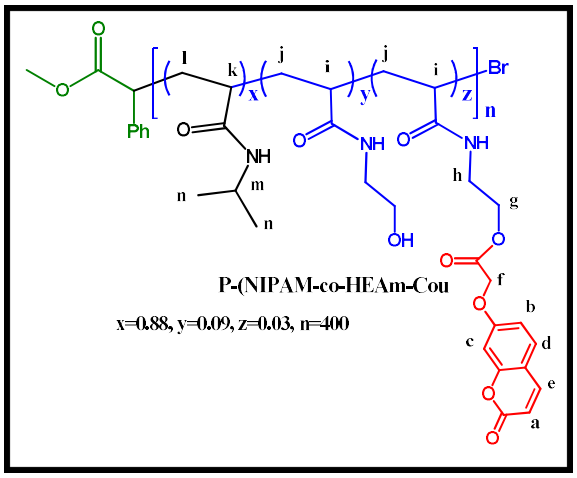


$$\%\,\boldsymbol{Cou} = \frac{\frac{\boldsymbol{x}}{\boldsymbol{5}}}{\frac{\boldsymbol{x}}{\boldsymbol{5}}+\frac{\boldsymbol{n}}{\boldsymbol{6}}} * \mathbf{100} = \frac{{}^{\mathbf{4.4}}/_{\mathbf{5}}}{{}^{\mathbf{4.4}}/_{\mathbf{5}}+{}^{\mathbf{144}}/_{\mathbf{6}}} * \mathbf{100} = \mathbf{4}\,\%$$

(x=a+b+c+d+e)

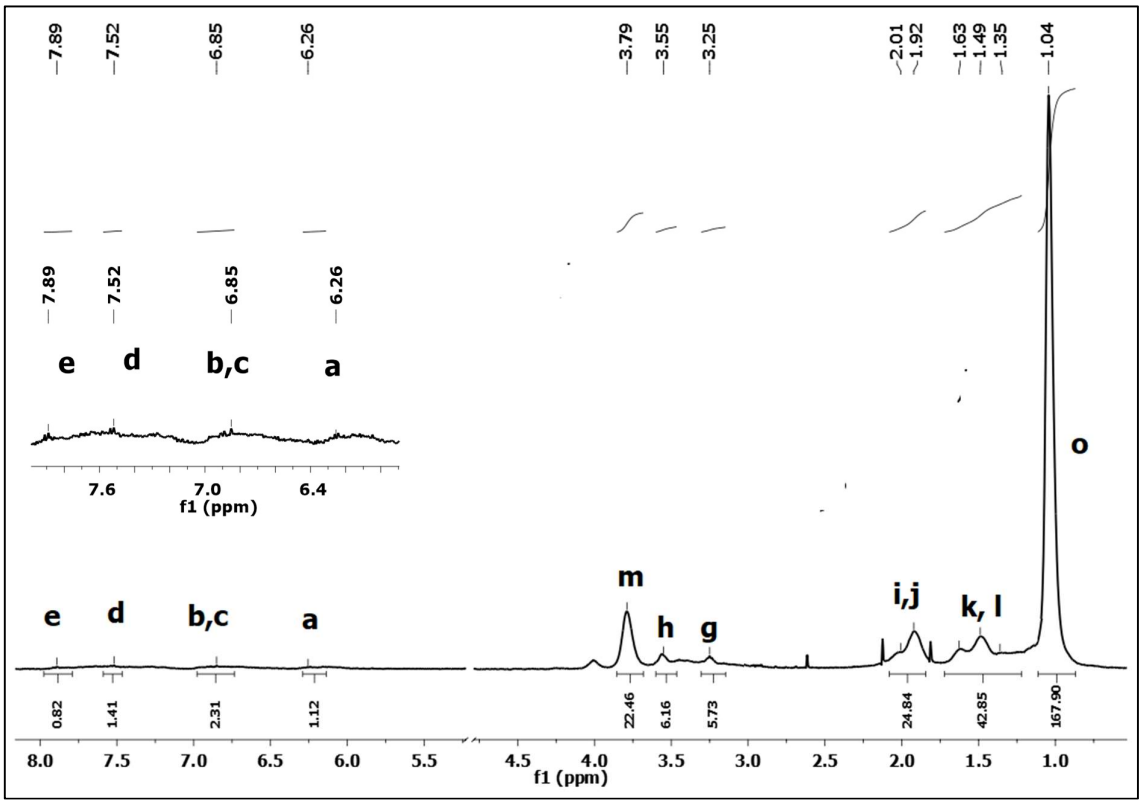


iii. Polymer CP2 (DP-400)

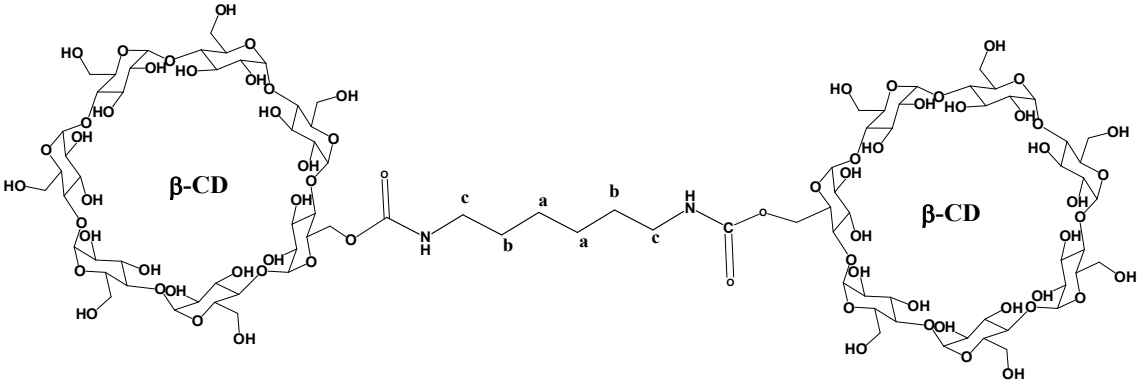


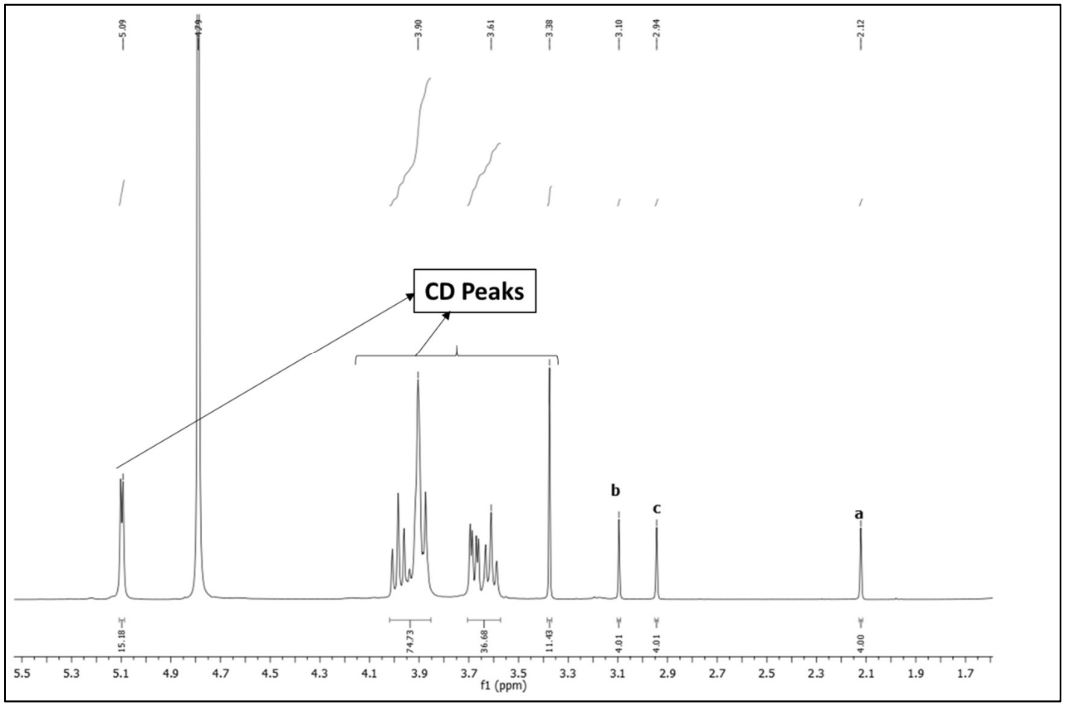
$$\%\,\boldsymbol{Cou} = \frac{\frac{x}{5}}{\frac{x}{5}+\frac{n}{6}} * 100 = \frac{5.4/5}{5.3/5 + 168/6} * 100 = 4\,\%$$

(x=a+b+c+d+e)



iv. bis-β-CD Crosslinker

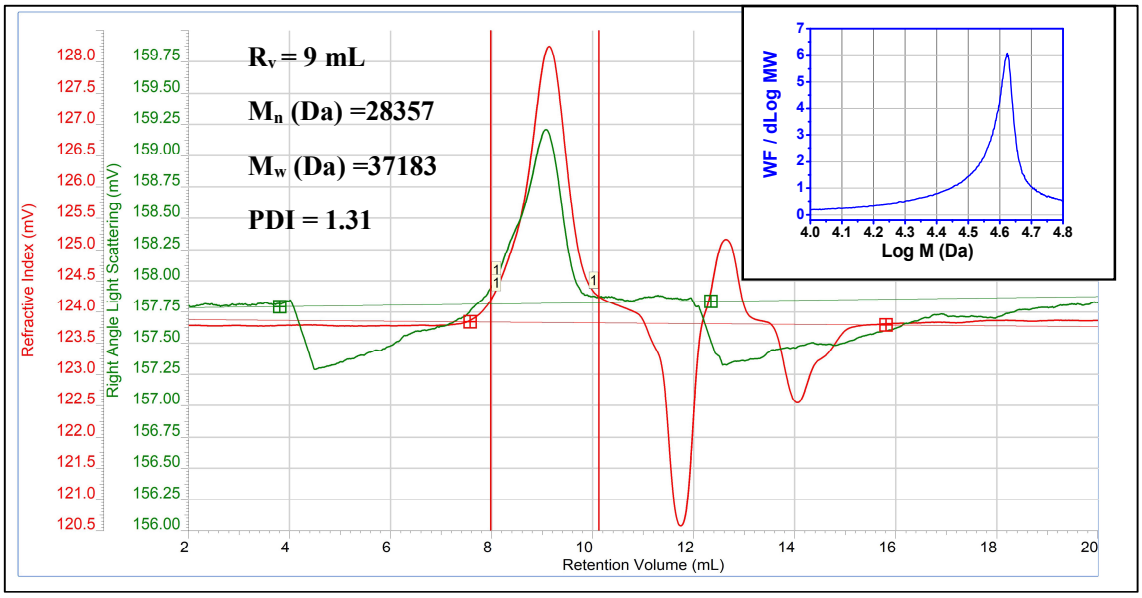




B. Characterization of polymers by Gel permeation chromatography

Size exclusion chromatography (SEC) was performed on a Malvern Viscotek instrument having RI, right angle light scattering (RALS) D_{4000} column using DMF with 0.05M LiBr as eluent at 25 ^{o}C with a flow rate of 0.7 mL/min. The results were analysed by using Omnisec software. The sample peaks were analysed for absolute M_n, M_w, *PDI* by means of multi-detector calibration method using PMMA 60 k narrow standard and verified by PMMA 95K broad standard. Concentration of polymer - 2 mgmL^{-1}

i. **Polymer CP1 (DP-200)**



ii. **Polymer CP2 (DP-400)**