# Supporting Information

## A Dual Stimuli Responsive Fluorescent Probe carrier from Double Hydrophilic Block Copolymer Capped with *β*-cyclodextrin

*Guang Yang[a], Zhen Yang[b], Chengguang Mu[b], Xiaodong Fan[b], Wei Tian*[b], Qing Wang*[a]*

[a] Department of Materials Science and Engineering, The Pennsylvania State University, University Park, Pennsylvania 16802, USA. E-mail: wang@matse.psu.edu
[b] The key lab of Space Applied Physics and Chemistry, Ministry of Education and Shaanxi Key Lab of Macromolecular Science and Technology, School of Science, Northwestern Polytechnical University, Xi'an 710072, Shaanxi, China

**Table S1**. Polymerization conditions and results

| Polymer Samples | Polymerization Condition | Polymer Result | | | | |
|---|---|---|---|---|---|---|
| | [M]：[CTA]：[I][a)] | Conversion(%)[b)] | $M_{n,\ Theo}$ [c)] | $M_{n,\ SEC}$ [d)] | $M_w/M_n$ [d)] | D.P.[e)] |
| PDEA | 30:1:0.1 | 70.1 | 4167 | 4560 | 1.17 | 23 |
| PDEA-b-PNIPAM-b-PDEA | 65:1:0.1 | 83 | 10647 | 11100 | 1.14 | 23,58 |

[a)][M]：[CTA]：[I]=[monomer]:[chain transfer agent]:[initiator], molar ratio.

[b)]Evaluated by $(W_{(product)}-W_{(CTA)})/W_{(monomer)}\times 100\%$, $W_{(product)}$, $W_{(CTA)}$ and $W_{(monomer)}$ are weight of product, CTA and monomer.

[c)]Calculated by ([monomer]/[CTA]) ×Conversion×$M_{n\ (monomer)}$ +$M_{n\ (CTA)}$, $M_{n\ (monomer)}$ and $M_{n\ (CTA)}$ are molecular weight of monomer and CTA.

[d)]Determined by SEC/MALLS

[e)] Degree of polymerization is calculated from SEC/MALLS results. 23 is for PDEA, 58 is for PNIPAM.

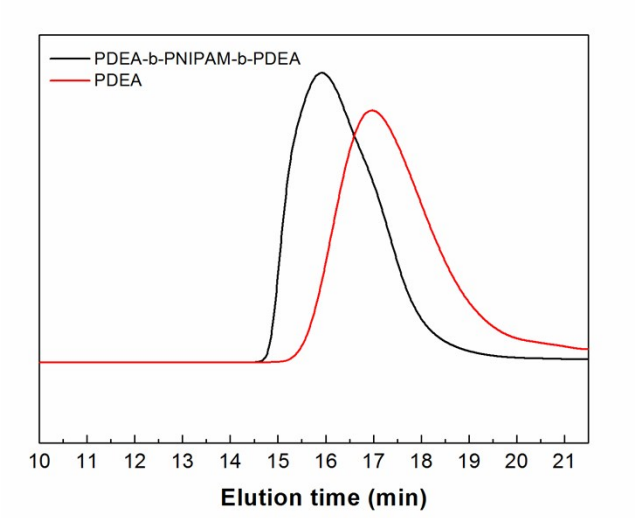


**Figure S1**. DRI traces of SEC/MALLS chromatograms of PDEA (red) and PDEA-b-PNIPAM-b-PDEA (black)

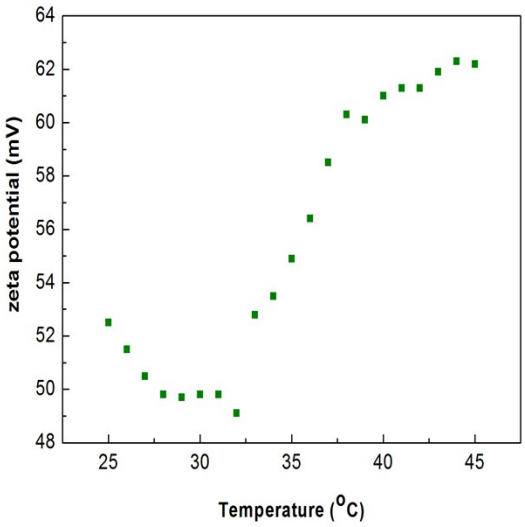

**Figure S2**. Zeta potential change of CD-PDEA-b-PNIPAM-b-PDEA-CD solution (0.1 mg $mL^{-1}$) as a function of temperature (pH=4)

**Experimental Section:**

*Materials*

Propargylamine (Bo Rui Chemical Co. Ltd. (Shang hai)). N,N'-Dicyclohexylcarbodiimide (DCC, 95%, Sinopharm Chemical Reagent Co. Ltd). 4-Dimethylaminopyridine (DMAP, 95%, Sinopharm Chemical Reagent Co. Ltd.). Sodium Azide (Alfa Aesar). Pentamethyldiethylenetriamine (PMDETA, redistilled, Yu Tian Chemical Reagent Co. Ltd. (Li Yang)). Cuprous bromide (CuBr, AR, Xi'an Chemical reagent Factory). Dialysis bag (molecular weight cutoff 3500, Hua Mei Biotech Co. Ltd.). Dimethylformamide (DMF), diethyl ether, 1, 4-dioxane (AR, Bo Di Chemical Co.Ltd. (Tian Jin)). Azobisisobutylonitrile (AIBN, recrystallized before use, CP, Shan Pu Chemical Reagent Co. Ltd. (Shang Hai)). Adamantanamine Hydrocholoride (Ada-NH3·Cl, M.W.=187.7) (Alfa aesar). Pyrene (Alfa aesar, 99%), CD azide (CD-N3) was synthesized according to literature.[1] N-isopropylacrylamide (NIPAM, ACROS, 99%). 2-(diethylamino) ethyl methacrylate (DEA, Alfa Aesar). S, S-bis(α, α'-dimethyl-α"-acetic acid)-trithiocarbonate (BDATTC) was synthesized according to reported method.[2] Mono-6-tosylated CD was synthesized according to literature. [3]

*Synthesis of PDEA macro chain transfer agent (CTA):*

BDATTC (84.6 mg, 0.3 mmol), DEA (1.67 g, 9 mmol), AIBN (4.9 mg, 0.03 mmol) were dissolved in 4.5 mL 1, 4-dioxane. The system was bubbled with $N_2$ for 30 min and degassed 15 min twice before sealed and placed in an oil bath at 75 ℃. The reaction flask was cooled down to room temperature after reaction for 6 h and most of the solvent was removed rotatory evaporation. 1 N HCl was added to the reaction flask and the system was dialyzed against water for 4 d (molecular weight cutoff=3500). Finally, lightly yellow solid was obtained by lyophilization to constant weight. Conversion: 70.1%. FT-IR (KBr): 1730 $cm^{-1}$ (*v*, C=O); 1254 $cm^{-1}$ (*v*, C-N). $^1H$ NMR (DMF-$d_6$, TMS): =4.55(2H, -O-C***H***$_2$-$CH_2$-); 3.56(2H, -O-$CH_2$-C***H***$_2$); 3.35(4H, -N-(C***H***$_2$-$CH_3$)$_2$); 2.18(2H, -C***H***$_2$-); 1.48(6H, -N-($CH_2$-C***H***$_3$)$_2$); 1.03(3H, -CC***H***$_3$). $M_{n.SEC}$=4560, $M_w/M_n$=1.17.

*Synthesis of PDEA-b-PNIPAM-b-PDEA:*

The synthesis of PDEA-b-PNIPAM-b-PDEA was accomplished by using PDEA as macro-CTA. PDEA (0.69 g, 0.15 mmol), NIPAM (1.1 g, 9.8 mmol), AIBN (2.46 mg, 0.015 mmol) were dissolved in 6 mL DMF. The system was bubbled with N2 for 30 min and degassed 15 min twice before it was sealed and placed in an oil bath at 75 ℃. The reaction flask was cooled down to room temperature and 1 N HCl was added to the reaction flask after reaction for 6 h. The system was dialyzed against water for 4 days (molecular weight cutoff=3500). Finally, light yellow solid was obtained by lyophilization to constant weight. Conversion(%): 83.0 %. FT-IR (KBr): 1730 $cm^{-1}$ (*v*, C=O); 1254 $cm^{-1}$ (*v*, C-N). $^1H$ NMR (DMF-$d_6$, TMS): =4.55(2H, -O-C***H***$_2$-$CH_2$-); 3.56(2H, -O-$CH_2$-C***H***$_2$); 3.35(4H, -N-(C***H***$_2$-$CH_3$)$_2$); 2.18(2H, -C***H***$_2$-); 1.48(6H, -N-($CH_2$-C***H***$_3$)$_2$); 1.03(3H, -CC***H***$_3$). $M_{n.SEC}$=4560, $M_w/M_n$=1.17. FT-IR (KBr): PDEA block, 1730 $cm^{-1}$ (*v*,

C=O); 1254 $cm^{-1}$ (*v*, C-N). PNIPAM block, 1554 $cm^{-1}$ (*v*, N-H), 1644 $cm^{-1}$ (*v*, C=O). $^1H$ NMR (DMF-$d_6$, TMS): PDEA block, =4.55(2H, -O-C***H***$_2$-$CH_2$-); 3.56(2H, -O-$CH_2$-C***H***$_2$); 3.35(4H, -N-(C***H***$_2$-$CH_3$)$_2$); 2.18(2H, -C***H***$_2$-); 1.48(6H, -N-($CH_2$-C***H***$_3$)$_2$); 1.03(3H, -CC***H***$_3$). PNIPAM block, =7.71-7.30(1H, -N***H***-CH-); 3.95(1H, -NH-C***H***-); 2.18(1H, -C***H***-$CH_2$); 1.55(2H, -C***H***$_2$-); 1.11(6H, -CH-(C***H***$_3$)$_2$). $M_{n.SEC}$=11100, $M_w/M_n$=1.14.

Synthesis of pg-PDEA-b-PNIPAM-b-PDEA-pg:

PDEA-b-PNIPAM-b-PDEA (0.605 g, 0.05 mmol), DMAP (18.3 mg, 0.15 mmol), DCC (30.9 mg, 0.15 mmol) were dissolved in 5 mL DMF. Propargylamine (11 mg, 2 mmol) was added to the reaction flask in an ice bath. The reaction took place for 72 h before precipitation in ethyl ether. Yellow solid was obtained after filtration and was vacuum dried at 30 ºC for 2 d. Conversion: 88.1%. $^1H$ NMR (DMF-$d_6$, TMS): =1.92 (-C≡C***H***). PDEA block, =4.55(2H, -O-C***H***$_2$-$CH_2$-); 3.56(2H, -O-$CH_2$-C***H***$_2$); 3.36(4H, -N-(C***H***$_2$-$CH_3$)$_2$); 2.17(2H, -C***H***$_2$-); 1.48(6H, -N-($CH_2$-C***H***$_3$)$_2$); 1.02(3H, -CC***H***$_3$). PNIPAM block, =7.71-7.30(1H, -N***H***-CH-); 3.89(1H, -NH-C***H***-); 2.18(1H, -C***H***-$CH_2$); 1.55(2H, -C***H***$_2$-); 1.11(6H, -CH-(C***H***$_3$)$_2$).

*Synthesis of CD-PDEA-b-PNIPAM-b-PDEA-CD:*

pg-PDEA-b-PNIPAM-b-PDEA-pg (0.5 g, 0.04 mmol), CD-N3 (0.186 g, 0.1 mmol), PMDETA (13.84 mg, 0.08 mmol), and CuBr (11.52 mg, 0.08 mmol) were dissolved in 5 mL DMF. The system was bubbled with N2 for 30 min and degassed 15 min twice before it was sealed and placed in an oil bath at 75 ℃. The reaction took place for 24 h before precipitation in 250 mL ethyl ether. The solid was vacuum dried at 30 ºC for 2 d

after filtration. The coarse product was dissolved in 1 N HCl and dialyzed against water to eliminate unreacted CD-N3 and copper residues. Finally, the product was obtained by lyophilization to constant weight. Conversion: 86.5%. FT-IR (KBr): PDEA block, 1730 $cm^{-1}$ (*v*, C=O); 1254 $cm^{-1}$ (*v*, C-N). PNIPAM block, 1554 $cm^{-1}$ (v, N-H), 1644 $cm^{-1}$ (*v*, C=O). *β*-CD on polymer chain ends, 1080 $cm^{-1}$ (*v*, C-O), 1031 $cm^{-1}$(*v*, C-O-C). $^1$H NMR (DMSO-$d_6$, TMS): PDEA block, =4.18(2H, -O-C***H***$_2$-$CH_2$-); 3.16(2H, -O-$CH_2$-C***H***$_2$); 2.98(2H, -N-(C***H***$_2$-$CH_3$)$_2$); 1.97(2H, -C***H***$_2$-); 1.26(6H, -N-($CH_2$-C***H***$_3$)$_2$); 0.98(3H, -CC***H***$_3$). PNIPAM block, =7.45-7.00(1H, -N***H***-CH-); 3.86(1H, -NH-C***H***-); 1.97(1H, -C***H***-$CH_2$); 1.41(2H, -C***H***$_2$-); 1.00(6H, -CH-(C***H***$_3$)$_2$). 5.15-5.25(1-H from *β*-CD )

*Characterization Instrument*

FTIR measurements were performed on Nicolet iS10 infrared spectrometer. $^1$H-NMR was measured using Bruker AV-500 Nuclear Magnetic Resonance Spectrometer using DMF-$d_6$ or DMSO-$d_6$ as solvent. The number average molecular weight ($M_n$) and polydispersity ($M_w/M_n$) of the polymers were estimated by DAWN EOS size exclusion chromatography/multi angle light scattering (SEC /MALLS). HPLC grade DMF was used as the mobile phase (containing LiCl, 0.01 mol $L^{-1}$) at the flow rate of 0.5 mL/min (40 ºC). A Waters 515 pump and a differential refractometer (Optilab rEX) were used. Low critical solution temperature (LCST) was recorded by Ultraviolet spectroscopy (Shimadzu 2550). Polymer samples were lyophilized using Freeze Drier (FREEZONE4.5, LABCONCO Co.). Fluorescence spectrophotometer (Hitachi F-4600) was used to study pyrene emission spectra. The Dz and PDI of CD-PDEA-b-PNIPAM-b-PDEA-CD

aggregate at various solution pH and temperature were determined by dynamic laser scattering (DLS) using a Malvern Zetasizer Nano ZS instrument.

*Aggregation behaviors of CD-PDEA-b-PNIPAM-b-PDEA-CD*

The LCST of this triblock copolymer was determined by UV-vis spectrometer. The polymer was dissolved in acidified water (1 mg $mL^{-1}$, PH=4) and placed in the chamber of UV-vis spectrometer. Transmittance at 550 nm was recorded as a function of temperature. The temperature was elevated at the rate of 0.2 ºC $min^{-1}$ from 25 ºC～50 ºC. The LCST was defined as the temperature where the transmittance is 90% of the original value at 25 ºC. The size and distribution of CD-PDEA-b-PNIPAM-b-PDEA-CD aggregates in aqueous solution were studied by DLS: The polymer was dissolved in water (0.1 mg $mL^{-1}$) and passed through a microfilter. The scattered light of a vertically polarized He-Ne laser (633 nm) was measured at an angle of 173° and collected on an autocorrelator. As the external condition (temperature and pH) was changed, the diameter distribution was recorded. Temperature and pH was varied from 25 to 45 ºC and from 2 to 11, respectively. Before data collection at certain temperature or pH, the solution was stabilized for 3 min.

*Temperature Responsiveness and Encapsulation of small molecules*

The temperature responsiveness and encapsulation of CD-PDEA-b-PNIPAM-b-PDEA-CD were characterized by fluorescence spectroscopy. Polymer was dissolved in $6\times10^{-6}$ M pyrene solution to form 0.1 mg $mL^{-1}$ mixed solution and stabilized for 12 h. The emission spectra were measured by fluorescence spectrophotometer using the

following parameters: excitation wavelength 335 nm, emission wavelength range 355-550 nm, excitation slit width 5 nm, emission slit width 2.5 nm. The emission intensity ratio of the first vibronic band to the third of the spectra ($I_1/I_3$ ratio) was recorded and plotted as a function of temperature. To study the functionality of *β*-CD units at chain ends of CD-PDEA-b-PNIPAM-b-PDEA-CD in $6\times10^{-6}$ M pyrene solution, Ada-$NH_3$·Cl was added to CD-PDEA-b-PNIPAM-b-PDEA-CD. At 45 ºC, Ada-$NH_3$·Cl was gradually added to 0.1 mg $mL^{-1}$ polymer solution at predetermined intervals (5 h). Data was collected immediately before adding Ada-$NH_3$·Cl each time. The value of $I_1/I_3$ was recorded and plotted as a function of amount of Ada-$NH_3$·Cl added.

*Critical Micelle Concentration (cmc) of* CD-PDEA-b-PNIPAM-b-PDEA-CD

The CMC of CD-PDEA-b-PNIPAM-b-PDEA-CD was investigated by fluorescence spectroscopy. Polymer was dissolved in $6\times10^{-6}$ M pyrene solution with concentration ranging from 10 to $1\times10^{-4}$ mg $mL^{-1}$ at 25 ºC. The excitation spectra were measured; the ratio of excitation intensity at 337 nm to that of 335 nm was calculated and plotted as a function of polymer concentration. The CMC was estimated according to the intersection-point after extrapolating the $I_{337}/I_{335}$ ratio at low and high concentration regions.

References:


1. H. Liu, Y. Zhang, J. Hu, C. Li and S. Liu, *Macromol Chem Physic*, 2009, 210, 2125-2137.
2. J. T. Lai, D. Filla and R. Shea, *Macromolecules*, 2002, 35, 6754-6756.
3. R. C. Petter, J. S. Salek, C. T. Sikorski, G. Kumaravel and F. T. Lin, *J Am Chem Soc*, 1990, 112, 3860-3868.