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Supplementary Information

Preparation of Functionalized Star Polymer Nanoparticles by RAFT Polymerization and its Application in Positionally Assembling Enzymes for Cascade Reactions

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Synthesis of 3-(Benzylsulfanylthiocarbonylsulfanyl)-propionic acid (BSPA). BSPA was synthesized using the method reported by Cyrille Boyer.[1] First, 3-mercaptopropionic acid (7.75 g, 73 mmol) was added to a round bottom flask containing 400 mL of acetone; then, sodium bicarbonate (10.7 g, 80 mmol) was introduced to the solution. After stirring for 10 min, benzyl bromide (10.26 g, 73 mmol) was slowly added to the round bottom flask in a dropwise manner with stirring for another 10 min at room temperature. Afterwards, the solution was filtered and the filtrate was placed under reduced pressure to remove the large quantities of solvents. The crude product was added to a saturated solution (700 mL) of brine and extracted with dichloromethane (2 x 200 mL). The organic phase washed with a saturated brine solution (3 x 200 mL) and dried over anhydrous magnesium sulfate. After the solvents were removed under reduced pressure, the crude product was purified on a silica gel column (chloroform) to yield a brilliant yellow crystalline solid (yield: 67%). ^{1}H NMR (600MHz, $CDCl_3$, ppm): δ 2.85(t, 2H, -C**H**$_2$COOH), 3.62(t, 2H, -CH_2C**H**$_2$S-), 4.61(s,

2H, Ph-C**H**$_2$-), 7.32(m, 5H, Ph). ^{13}C NMR (600MHz, $CDCl_3$, ppm): δ 176.73, 134.81, 129.28, 128.75, 127.86, 41.56, 32.83, 30.88. (Fig. SI-1)

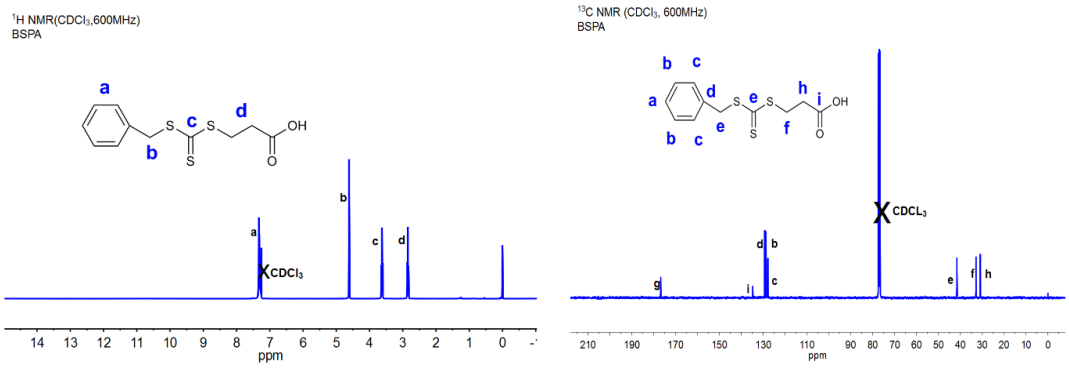

Figure S1. ^{1}H NMR and ^{13}C NMR spectrum of BSPA in $CDCl_3$.

Synthesis of *N*-Adamantylacrylamide. Based on procedures previously reported in the literature.[2] *N*-adamantylacrylamide was synthesized as described below. 1-Adamantylamine (3.03 g, 20 mmol) and trimethylamine (3.47 mL, 25 mmol) were added to a round bottom flask containing 75 mL of tetrahydrofuran and stirred in ice-water bath to dissolve the compounds. Then, acryloyl chloride (1.79 mL, 20 mmol) in 25 mL of tetrahydrofuran was slowly added to the solution in a dropwise manner within 30 min. After stirring for 2 h in the ice-water bath, the mixture was stirred for another 12 h at room temperature. The precipitate was filtered and the filtrate was placed under reduced pressure to remove the solvents. The crude product was purified on a silica gel column (chloroform) to yield a white solid and dried under a vacuum (yield: 83%). ^{1}H NMR (600MHz, $CDCl_3$, ppm): δ 1.69(m, 6H, ADH), 2.05(m, 6H, ADH), 2.09(m, 3H, ADH), 5.43(s, 1H, -CON**H**-), 5.57(dd, 1H), 6.04(dd, 1H), 6.24(dd, 1H). ^{13}C NMR (600MHz, $CDCl_3$, ppm): δ 164.56, 132.24, 125.43, 52.05, 41.57, 36.34, 29.42. (Fig. SI-2)

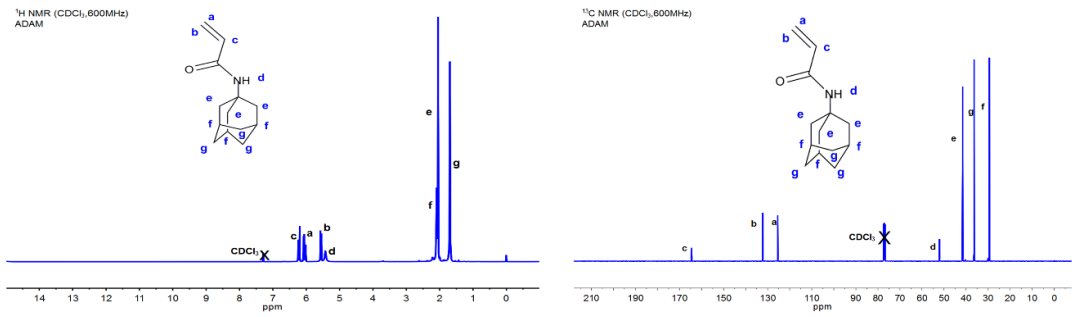

Figure S2. ^{1}H NMR and ^{13}H NMR spectrum of ADAM in $CDCl_3$.

Synthesis of Mono-6-thio-β-cyclodextrin (CD-SH). Mono-6-thio-β-cyclodextrin (CD-SH) was synthesized as described in a previous report, with slight modifications.[3] β-cyclodextrin (17.22 g, 15 mmol) was added to a round bottom flask containing 200 mL of an NaOH solution (0.25 mol/L). Paratoluensulfonyl chloride (2.90 g, 15 mmol) was dissolved in 7 mL of acetonitrile, and the mixture was added dropwise to the round bottom flask. Then, the mixture was stirred for 6 h at room temperature and filtered to remove the white precipitates. The pH of the filtrate was adjusted to 2. Next, the filtrate was refrigerated overnight at 4°C to induce crystallization. The resulting precipitate was recrystallized with hot water three times. The product was dried under a vacuum at 50°C to obtain mono-6-deoxy-6-(p-tolylsulfonyl)-β-CD (yield: 84%). ^{1}H NMR (600MHz, DMSO-d_6, ppm, Figure S3): δ 7.77(d, 2H, Ph), 7.40(d, 2H,Ph), 5.71(m, 14H, OH-2,3), 4.85(d,4H, H-1), 4.77(d, 3H, H-1), 4.35-4.18(d, 6H, OH-6), 3.74-3.56(m, 28H, H-3, 5, 6), 3.48-3.16(m, overlaps with HOD), 2.44(s, 3H, -CH_3).

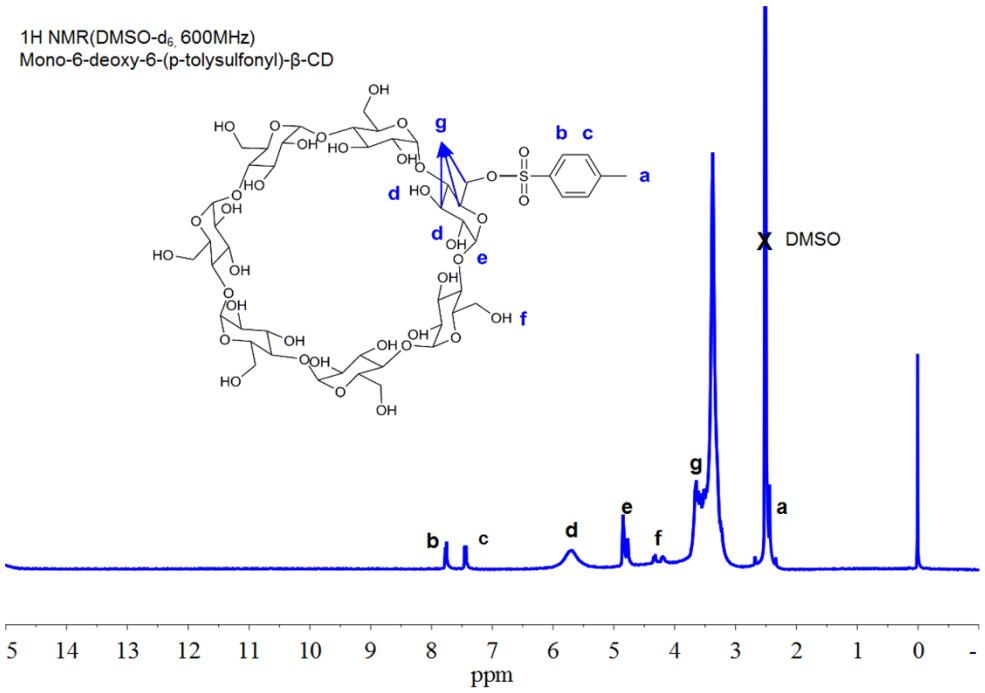

Figure S3. ^{1}H NMR spectrum of mono-6-deoxy-6-(p-tolylsulfonyl)-β-CD in DMSO-d_6.

Mono-6-deoxy-6-(p-tolylsulfonyl)-β-CD (1.0 g, 0.776 mmol) was added to a round bottom flask containing 20 mL of DMSO. Cystamine hydrochloride (1.6 g, 15.5 mmol) was dissolved in 5 mL deionized water, and NaOH (0.57 g, 1.55 mmol) was added to that solution to deprotonate the primary amines. Then, the resulting solution was slowly added to the flask in a dropwise manner. Afterwards, the flask was plunged into an oil bath and the mixture was stirred at 80°C for 4 h. The product was precipitated with acetone; this procedure was repeated three times. Mono-6-deoxy-6-cystamine-β-CD was dried under a vacuum overnight (yield: 65%). ^{1}H NMR (600MHz, DMSO-d_6, ppm, Figure S4): δ 5.74-5.68(m, 14H, OH-2,3), 4.84(d,7H, H-1), 4.46-3.93(d, 6H, OH-6), 3.65(m, 28H, H-3, 5, 6), 3.49-3.17(m, overlaps with HOD), 3.11(s, 1H, -N**H**-), 2.91(t, 4H, -C**H**$_2$-C**H**$_2$-NH$_2$), 2.68(s, 1H, -NH$_2$), 2.34(s, 1H,-NH$_2$), 2.10(t, 4H, -C**H**$_2$-C**H**$_2$-S-).

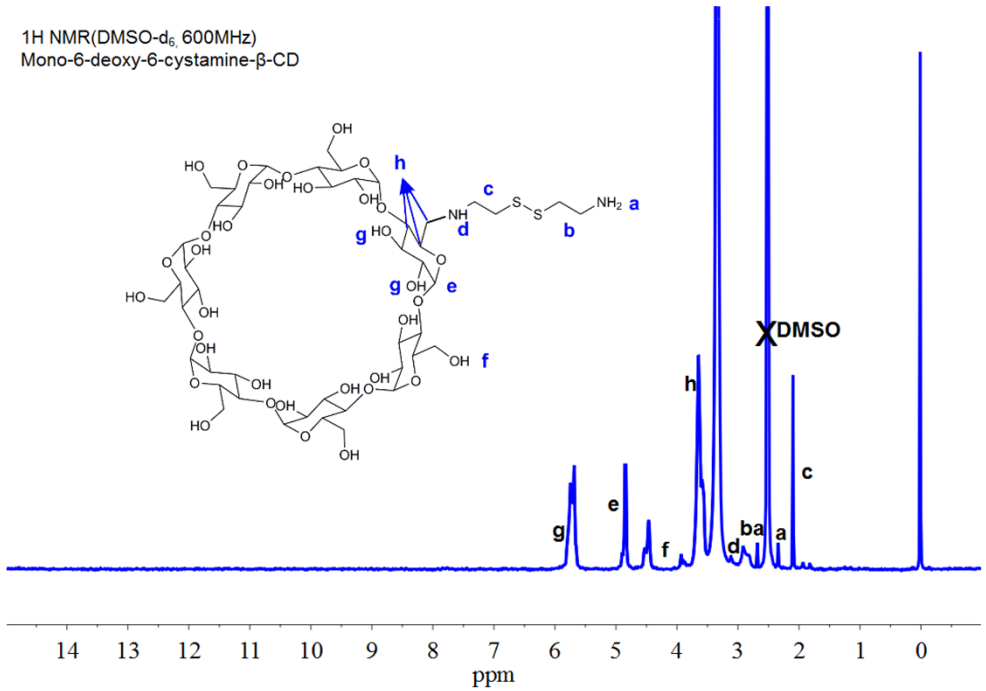

Figure S4. ^{1}H NMR spectrum of mono-6-deoxy-6-cystamine-β-CD in DMSO-d_6.

Mono-6-deoxy-6-cystamine-β-CD (0.5 g, 0.4 mmol) was dissolved in 15 mL of DMF, and then the DTT solution (4 mmol DTT was dissolved in 5 mL of deionized water) was added to the mixture dropwise and stirred at room temperature. At the end of the reaction, a small amount of deionized water was added to dilute the reaction mixture, and the product was precipitated with acetone three times. Mono-6-thio-β-cyclodextrin was dried under a vacuum overnight (yield: 53%). ^{1}H NMR (600MHz, DMSO-d_6, ppm, Figure S5): δ 5.71(m, 14H, OH-2,3), 4.84(d,7H, H-1), 4.48(d, 6H, OH-6), 3.64(m, 28H, H-3, 5, 6), 3.52-3.16(m, overlaps with HOD), 2.80(s, 1H, -NH-), 2.09(t, 4H, -C**H**$_2$-C**H**$_2$-SH), 1.41(s,1H, -SH).

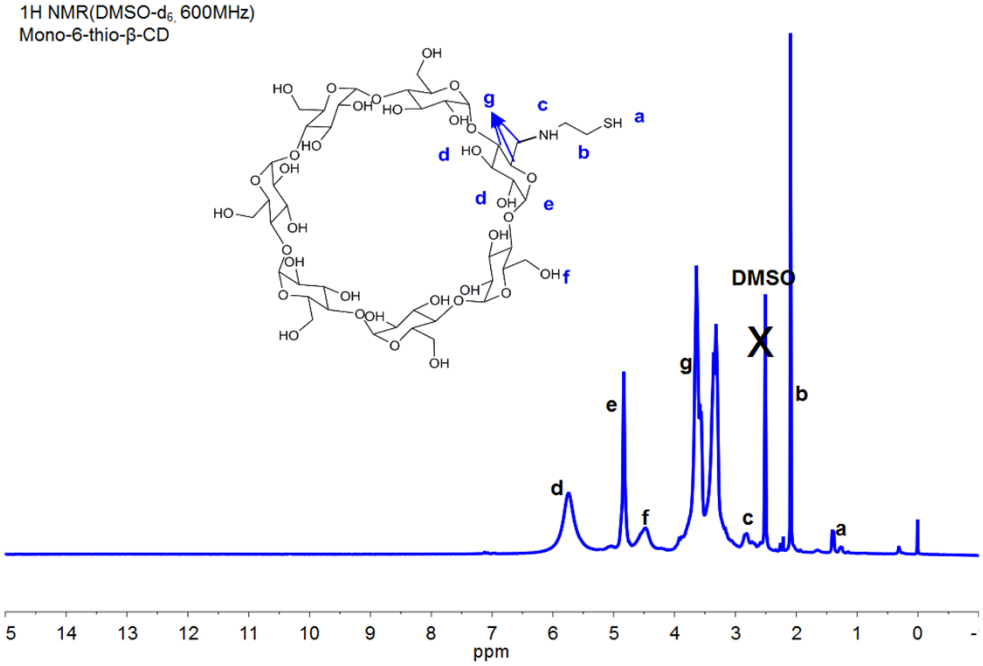

Figure S5. ^{1}H NMR spectrum of mono-6-thio-β-CD in DMSO-*d*$_6$.

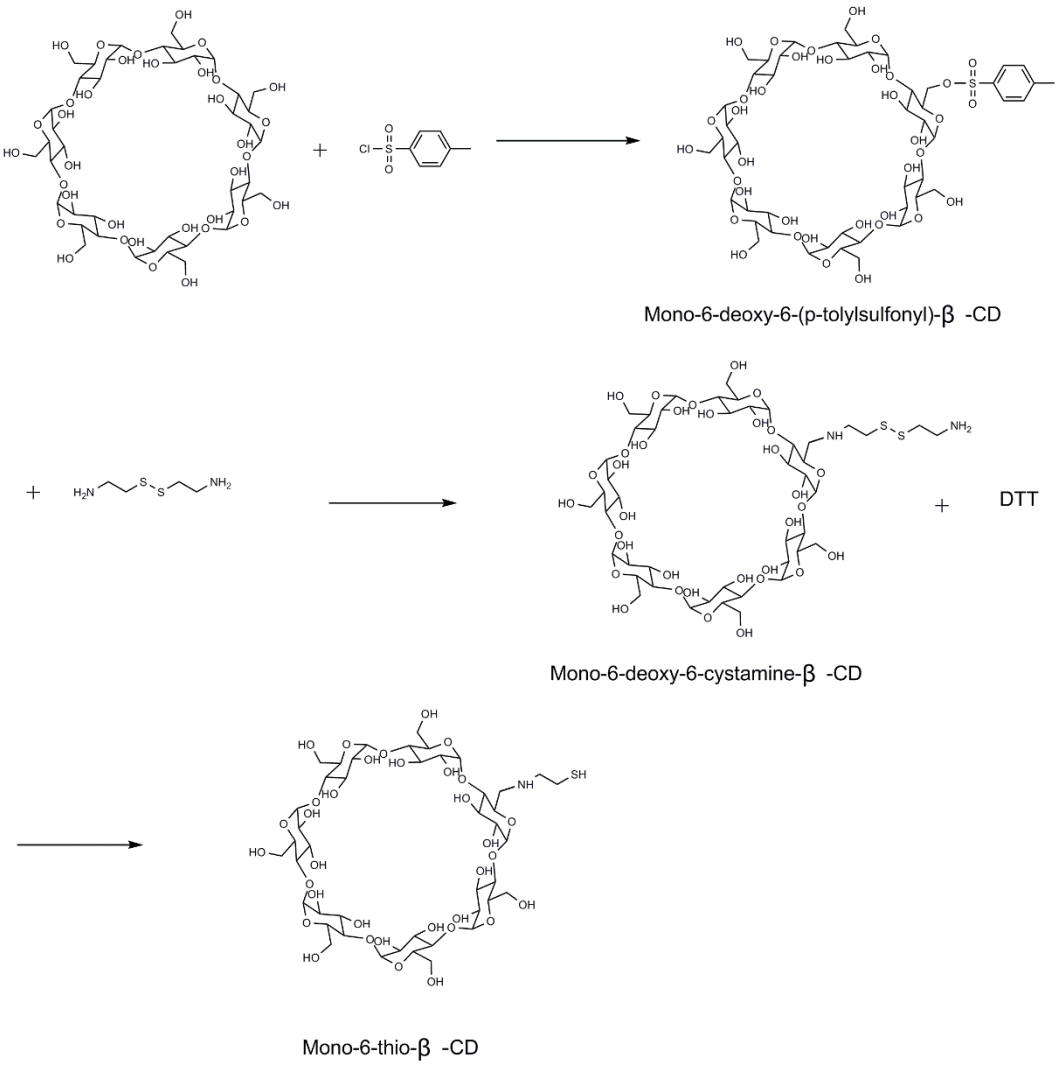

Figure S6. Schematic depicting the synthesis of mono-6-thio-β-cyclodextrin.

Labelling of Enzymes with FITC or Rhodamine B. Here, GOx-CD was labelled with 5(6)-isothiocyanate (FITC) and HRP was labelled with Rhodamine B.[4] One milligram of fluorescein FITC or Rhodamine B was dissolved in 200 μL of a DMSO solution. Four millilitres of the enzyme solution (2.5 mg/mL, carbonate buffer, pH 9.0) were added to the dye solution and the mixture was stirred at room temperature for 4 h in the dark. After the reaction was complete, the excess fluorescent dye was removed by centrifugal filtration using Amicon 10 KDa cutoff filters three times. The solution

was dialyzed (MW cutoff, 3 KDa) against ultrapure water for 48 h to further purify the enzyme. Then, the fluorescein-labelled enzyme was lyophilized prior to use.

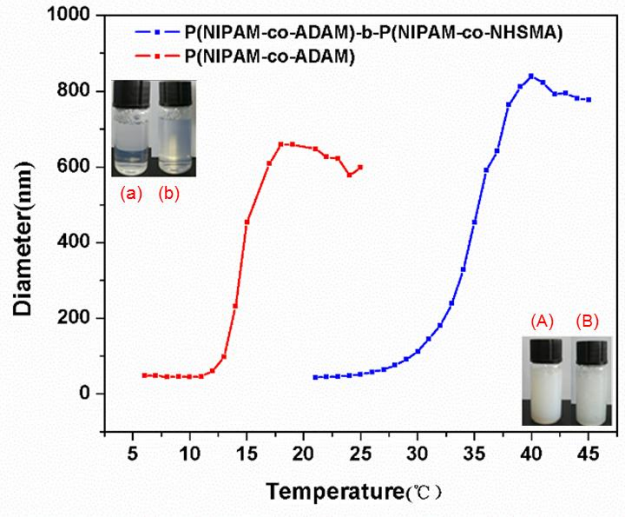

Figure S7. Variation of hydrodynamic diameter with temperature obtained for 1mg/mL aqueous solution of P(NIPAM-co-ADAM) and P(NIPAM-co- ADAM)-b-P(NIPAM-co-NHSMA).

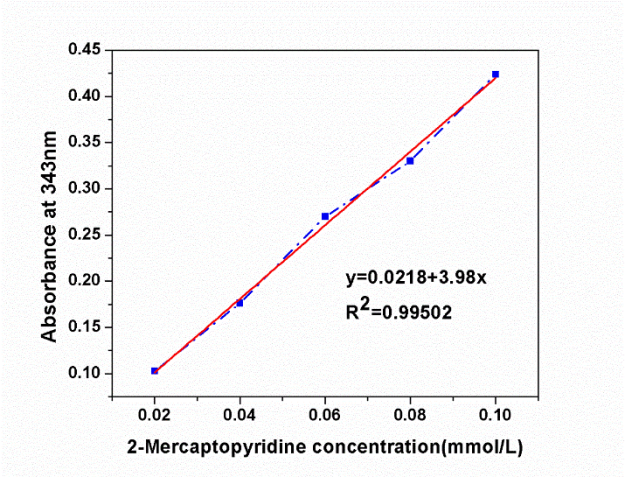

Figure S8. Calibration curve of pyridine-2-thione in PBS buffer (PH=8.5), extinction coefficient:7960 L $mol^{-1}cm^{-1}$.

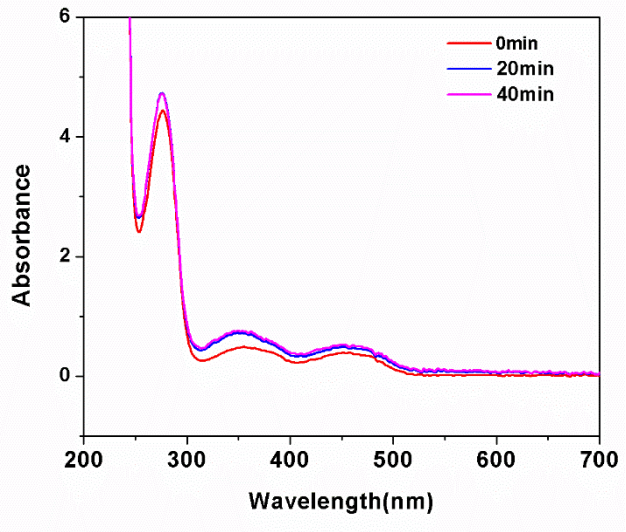

Figure S9. The reaction time of enzyme-SPDP with CD-SH was determined by monitoring changes in the absorbance during the reaction process at 343 nm.

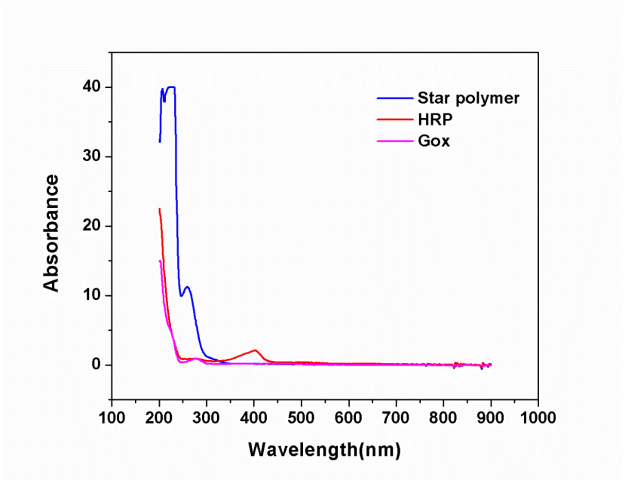

Figure S10. Full wavelength spectrum of star polymer, HRP and GOx; there is not absorbance at 415nm of the star polymer, so the loading capacity of HRP can determined by the absorbance of the redissolved star polymer@HRP solution at 415nm directly.

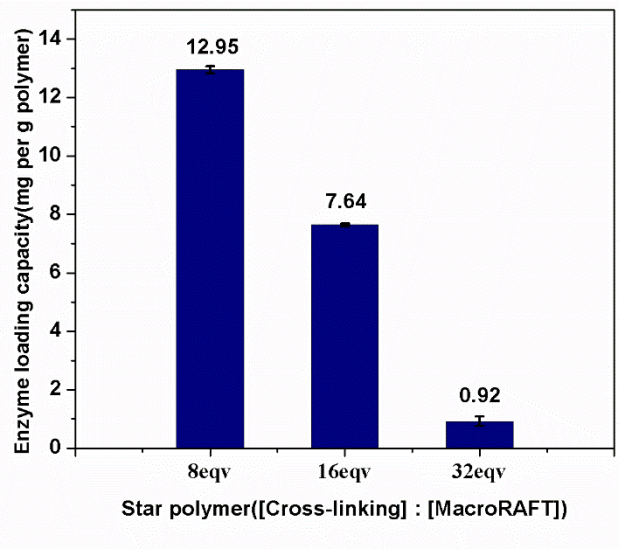

Figure 11S. HRP loading capacity of star polymers.

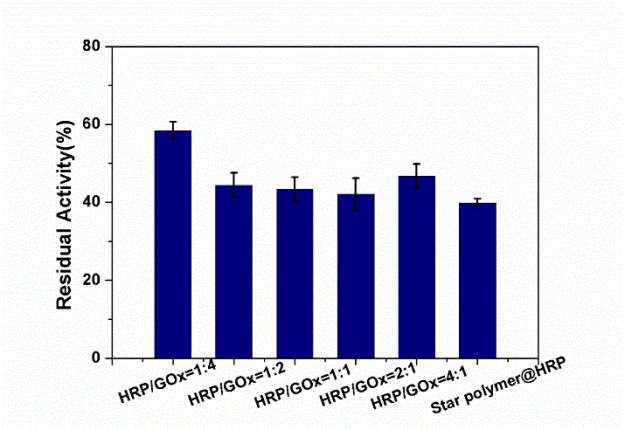

Figure S12. Residual activities of star polymer@HRP@GOx at different ratios of HRP to GOx.

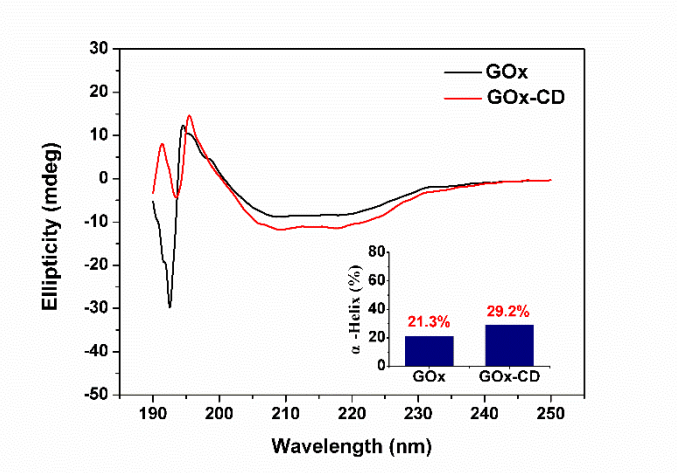

Figure S13. Far-UV CD spectra of GOx before and after modification; inset figure: α-helical contents of GOx and GOx-CD.

References

1 C. Boyer, V. Bulmus and T. P. Davis, *Rapid Commun.,* 2009, *30*, 493-497.

2 C. Koopmans and H. Ritter, *Macromolecules,* 2008, *41*, 7418-7422.

3 Y. Wang, H. Li, Q. Jin and J. Ji, *Chem. Commun.,* 2016, *52*, 582-585.

4 M. Buhl, B. Vonhoeren and B. J. Ravoo, *Bioconjugate Chem.,* 2015, *26*, 1017-1020.