

Supporting information for

Recyclable Thermoresponsive Polymer- β -Glucosidase Conjugate with Intact Hydrolysis Activity

Ishita Mukherjee,^{a,#} Sushant K. Sinha,^{b,#} Supratim Datta^{b,c,*} and Priyadarsi De^{a,c,*}

^a*Polymer Research Centre, Department of Chemical Sciences,* ^b*Protein Engineering Laboratory, Department of Biological Sciences,* ^c*Centre for Advanced Functional Materials, Indian Institute of Science Education and Research Kolkata, Mohanpur - 741246, Nadia, West Bengal, India*

*Corresponding Authors. E-mails: p_de@iiserkol.ac.in (PD), supratim@iiserkol.ac.in (SD)

[#]These authors contributed equally to this work.

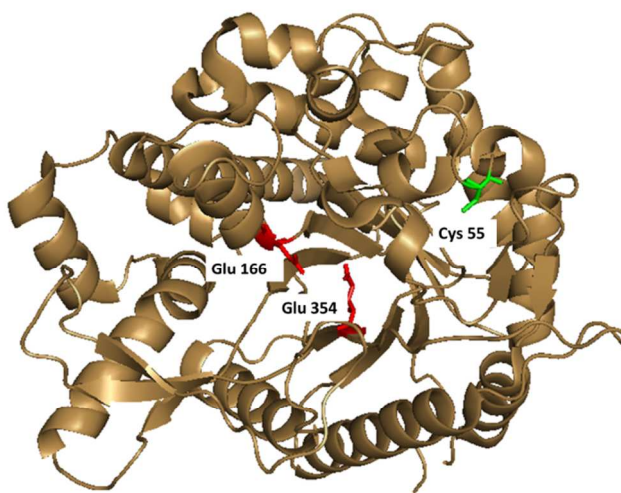


Figure S1. Schematic representation of B8CYA8 showing polymer attachment site (Cys 55). The catalytic residues (Glu 166 and Glu 354) are shown in red.

Table S1. Results from the Synthesis of B8CYA8-PNIPAM Conjugate Using B8CYA8-macroCTA with Two Different [VA-044] : [B8CYA8-macroCTA] Ratios

Compound	Conv. ^a (%)	$M_{n,theo}$ ^b (g/mol)	$M_{n,APC}$ ^c (g/mol)	\bar{D} ^c
B8CYA8	-	-	50000	1.13
B8CYA8-PNIPAM conjugate prepared at [NIPAM]/[B8CYA8-macroCTA]/[VA-044] = 1000:1:1 ^d	43	99500	110000	1.21
B8CYA8-PNIPAM conjugate prepared at [NIPAM]/[B8CYA8-macroCTA]/[VA-044] = 1000:1:2 ^e	84	145000	115000	1.24

^a Conversions were calculated by ¹H NMR study. ^b $M_{n,theo} = ([NIPAM]/[B8CYA8-macroCTA] \times \text{molecular weight (MW) of NIPAM} \times \text{conversion}) + (\text{MW of B8CYA8-macroCTA})$. ^c Measured by APC. ^d Polymerization time = 24 h. ^e Polymerization time = 4 h.

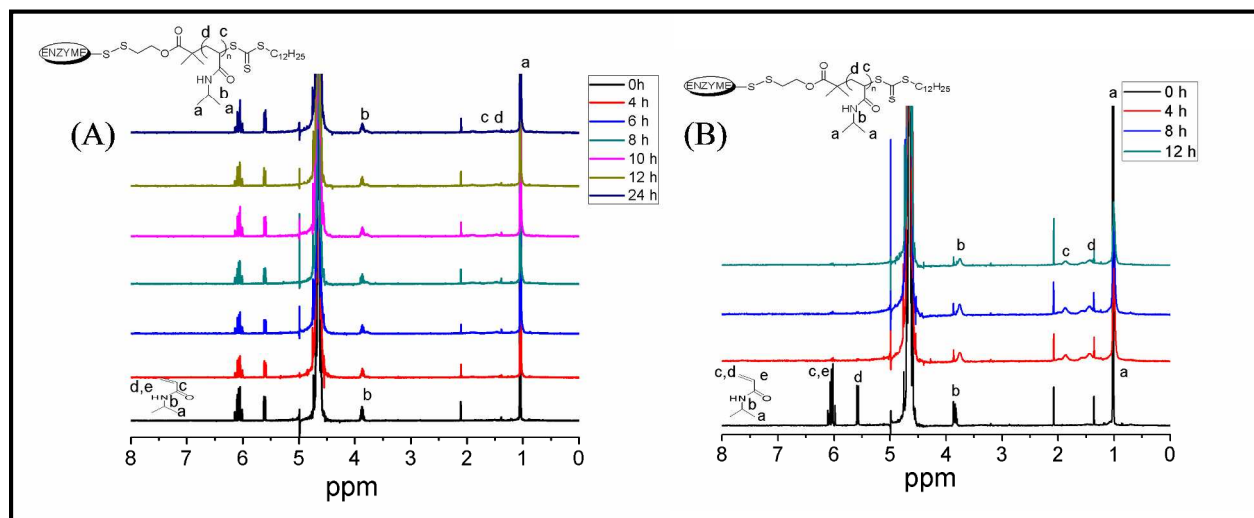


Figure S2. ¹H NMR spectra obtained after the polymerization of NIPAM in the presence of B8CYA8-macroCTA as a function of time at [NIPAM]/[B8CYA8-macroCTA]/[VA-044] = 1000/1/1 (A) and 1000/1/2 (B).

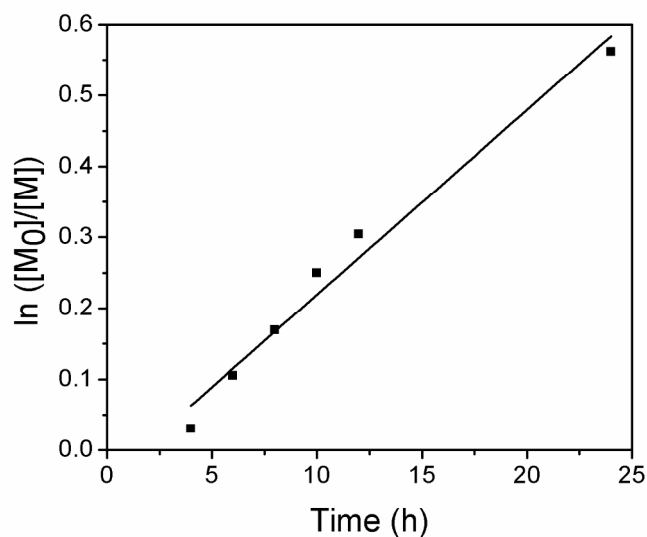


Figure S3. Pseudo-first-order kinetics plot for the RAFT polymerization of NIPAM at $[\text{NIPAM}]/[\text{B8CYA8-macroCTA}]/[\text{VA-044}] = 1000/1/1$.

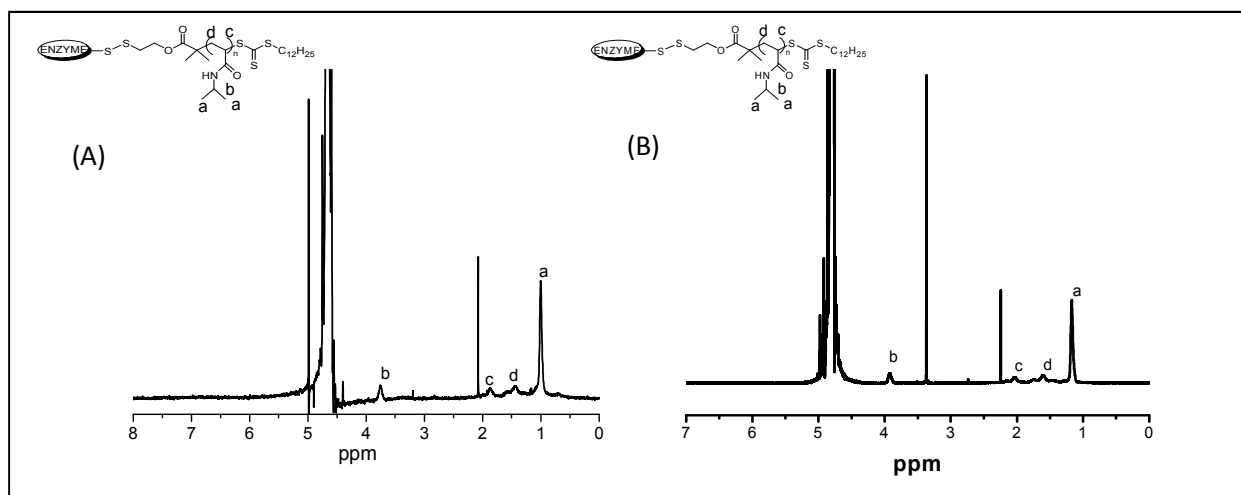


Figure S4. ^1H NMR spectra obtained after the polymerization of NIPAM in the presence of B8CYA8-macroCTA after purification through dialysis at $[\text{NIPAM}]/[\text{B8CYA8-macroCTA}]/[\text{VA-044}] = 1000/1/1$ (A) and $1000/1/2$ (B).

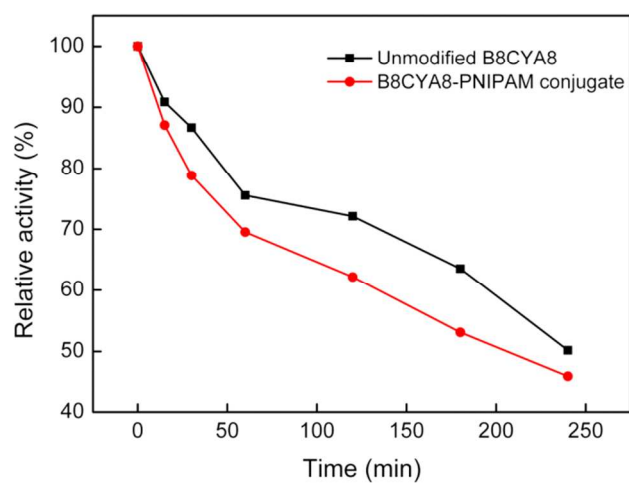


Figure S5. Thermostability of the native B8CYA8 and B8CYA8-PNIPAM conjugate. The enzyme was incubated at 70 °C for different time intervals, cooled down and assayed for the residual activity at 30 °C. The activity without any incubation at 70 °C was taken as 100 %.

Table S2. Concentration and Specific Activity of the Conjugate after Each Cycle of Thermal Precipitation.

Cycle	Conjugate (mg)	Specific activity (%) ($\mu\text{mol}/\text{min}/\text{mg}$)
1	0.0427	100.0 \pm 2.4
2	0.0306	95.6 \pm 3.4
3	0.0159	100.6 \pm 4.9
4	0.0109	99.8 \pm 3.4