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Electronic Supplementary Information

Temperature-Responsive Nanobiocatalysts with an Upper Critical Solution Temperature for High Performance Biotransformation and Easy Catalyst Recycling: Efficient Hydrolysis of Cellulose to Glucose

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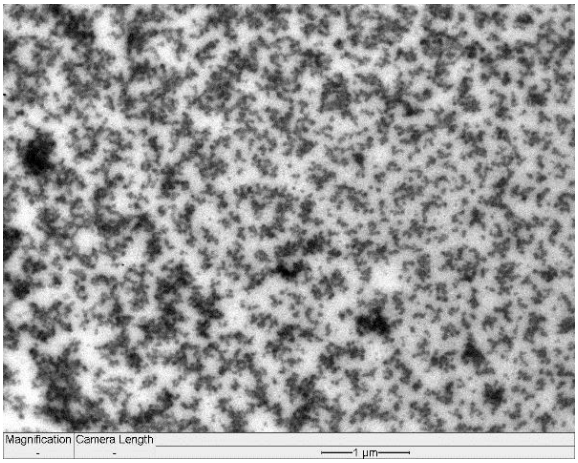

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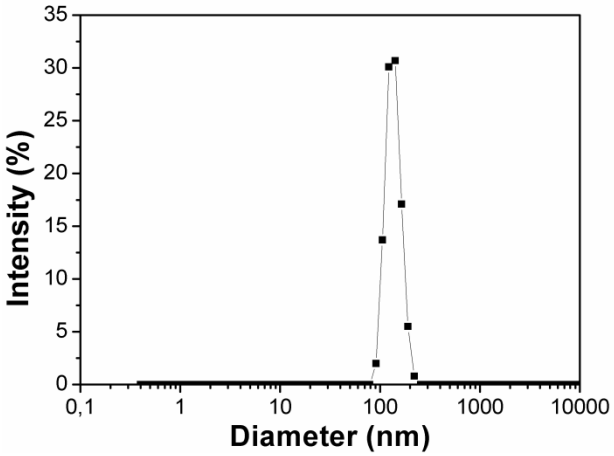
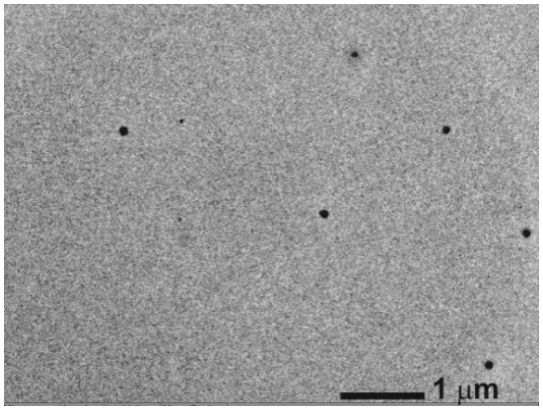


Figure S2. a) TEM of IPN PGA; b) Hydrodynamic size distribution of IPN PGA; c) Photos of IPN PGA (25 mg IPN PGA/mL) in water. (1) At 25°C and (2) at 4°C for 5 min

a)



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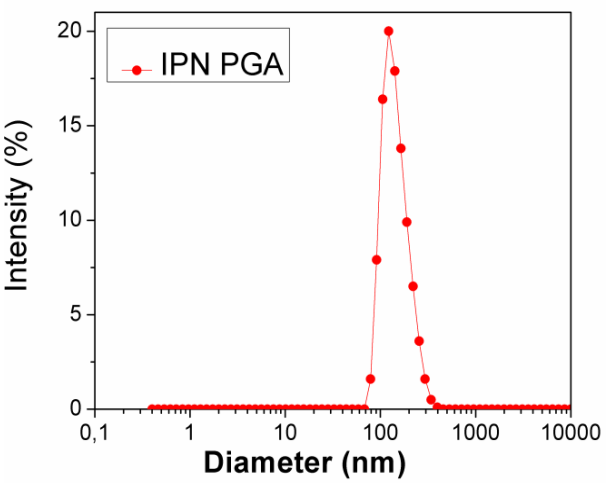


c)

Figure S3. TEM of UCST-nanobiocatalyst IPN-Cellu

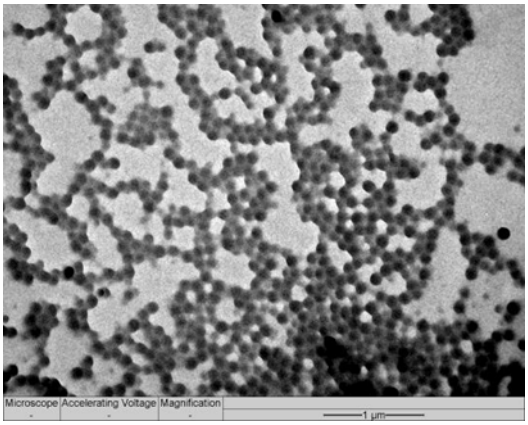


Figure S4. Time courses for the hydrolysis of filter paper (2.5 mg/mL) with UCST-nanobiocatalyst IPN-Cellu at different cellulase loading and 50 ºC

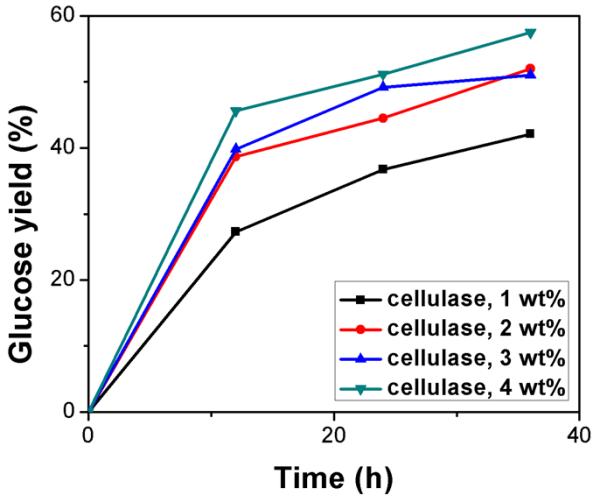
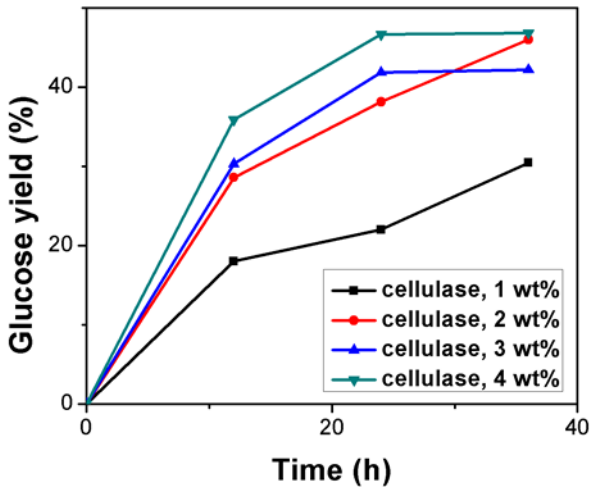


Figure S5. Time courses for hydrolysis of pre-treated EFB (2.5 mg/mL) with UCST-nanobiocatalyst IPN-Cellu at different cellulase loading and 50 ºC



2) Supplementary Tables

Table S1. Hydrolysis of carboxymethylcellulose (CMC) with reported recyclable immobilized cellulase.

	Carrier	Immobilization method	Enzyme loading (mg protein/g carrier)	Reaction temperature (°C)	Reaction time (min)	Retained enzyme activity (%)[a]	Recyclability[b]		Ref.
							Retained productivity (%)	No. of cycles	
1	Polyaniline coated PS-DVB-g-PS microspheres[c]	Adsorption	3.6-7.2	35	30	67-78	69	15	S1
2	Clay-poly(glycidyl methacrylate) composite	Covalent bonding	39	45	60	73	79	12	S2
3	Modified PVA coated chitosan beads[d]	Covalent bonding	0.14	50	5	87	52	8	S3
4	Chitosan (with glutaraldehyde as cross-linker)	Covalent bonding & cross-linking	20	25	3	66	60	6	S4
5	Chitosan-L-glutamic acid (with glutaraldehyde as cross-linker)	Covalent bonding & cross-linking	26	25	3	85	70	25	S4

[a] The activity of the immobilized enzyme related to the activity of the free enzyme. [b] Same reaction conditions (time, temperature, enzyme loading, etc) were used in each cycle of the recycling experiment. [c] PS-DVB-g-PS: Polystyrene-Divinylbenzene-*graft*-polystyrene. [d] PVA: polyvinyl alcohol.

Table S2. Hydrolysis of CMC (2.5 mg/mL) at 50 ºC with IPN-Cellu and free cellulase (enzyme concentration: 25 μg/mL), respectively.

Time (h)	Free cellulase			IPN-Cellu		
	Sugar (mg/mL)	Glucose (mg/mL)	Glucose yield (%)	Sugar (mg/mL)	Glucose (mg/mL)	Glucose yield (%)
0	0	0	0	0	0	0
6	1.29	0.78	32.4	1.13	0.68	28.2
12	1.63	0.99	40.9	1.46	0.89	36.7
24	2.00	1.21	50.0	2.04	1.24	51.1
30	2.19	1.32	54.7	2.22	1.34	55.6
36	2.22	1.34	55.6	2.22	1.34	55.6

Table S3. Hydrolysis of filter paper (2.5 mg/mL) and pre-treated EFB (2.5 mg/mL) at 50 ºC with IPN-Cellu (enzyme concentration: 25 and 50 µg/mL; enzyme to substrate: 1 and 2 wt%), respectively.

Time (h)	Cellulase loading (%)	Filter paper		Pre-treated EFB	
		Glucose (mg/mL)	Glucose yield (%)	Glucose (mg/mL)	Glucose yield (%)
0	1	0	0	0	0
12	1	0.71	27.3	0.20	18.0
24	1	0.96	36.7	0.24	22.0
36	1	1.10	42.1	0.34	30.5
0	2	0	0	0	0
12	2	1.01	38.7	0.32	28.6
24	2	1.16	44.5	0.42	38.1
36	2	1.36	52.0	0.51	46.0

Table S4. Hydrolysis of filter paper (10 mg/mL) to glucose (50 ºC) with the mixture of IPN-Cellu & IPN-Cello at different enzyme loading and reaction time.

IPN-Cellu (mg cellulase/mL)	IPN-Cello (mg cellobiase/mL)	Time (h)	Glucose yield (%)
0.4	0.8	24	57
0.2	0.4	24	54
0.1	0.2	24	43
0.2	0.4	48	94
0.2	0.1	48	73

Table S5. Hydrolysis of filter paper (10 mg/mL) to glucose at 50 ºC with the mixture of IPN-Cellu & IPN-Cello and the mixture of free cellulase & cellobiase (enzyme concentration: 0.20 mg cellulase/mL & 0.40 mg cellobiase/mL), respectively.

Time (h)	Free cellulase & free cellobiase		IPN-Cellu & IPN-Cello	
	Glucose (mg/mL)	Glucose yield (%)	Glucose (mg/mL)	Glucose yield (%)
0	0	0	0	0
12	4.18	40.0	2.98	28.5
24	6.95	66.6	5.68	54.4
36	8.62	82.5	8.42	80.6
48	9.74	93.2	9.87	94.2
72	10.1	96.2	10.1	97.1

Table S6. Hydrolysis of pre-treated EFB (10 mg/mL) to glucose at 50 ºC with the mixture of IPN-Cellu & IPN-Cello and the mixture of free cellulase & cellobiase (enzyme concentration: 0.20 mg cellulase/mL & 0.40 mg cellobiase/mL), respectively.

Time (h)	Free cellulase & free cellobiase		IPN-Cellu & IPN-Cello	
	Glucose (mg/mL)	Glucose yield (%)	Glucose (mg/mL)	Glucose yield (%)
0	0	0	0	0
12	1.68	37.8	1.08	24.2
24	2.59	58.2	1.80	40.4
36	3.24	73.0	2.96	66.5
48	3.70	83.1	3.83	86.2
72	4.04	90.8	4.13	92.8

Table S7. Retained activity of IPN-Cellu and free cellulase after storage.

Storage condition		Retained activity of nanobiocatalyst	Retained activity of free enzyme
Temperature	Time		
4°C	3 days	95%	85%
4°C	2 weeks	95%	84%
25°C	3 days	93%	82%

3) 10-Gram scale synthesis of UCST-nanoparticles IPN-PGA

4.779 g acrylamide (67.3 mmol), 9219.6 μL acrylic acid (134.6 mmol) and 0.267 g ammonium persulfate (389.5 μmol) were added in 450 mL water in a 3-neck round bottom flask under argon bubbling. The reaction was carried out at 60ºC and 500 rpm for 1 h, followed by centrifugation at 1000 g and 4ºC for 1 min. The precipitates (PAA NP) were washed by DI water for four times to remove remaining reactant. The obtained PAA NP obtained was dissolved in 450 mL water in a 3-neck round bottom flask, and 3.057 mL glycidyl methacrylate (22.4 mmol) and 0.267 g ammonium persulfate (389.5 μmol) were added to this PAA NP solution under argon bubbling. Reaction was carried out at 60°C and 500 rpm for 30 min. The product (IPN PGA) was separated by centrifugation at 1000 g and 4°C for 5 min, and then washed by DI water for three times to remove remaining reactant. The obtained IPN PGA was re-suspended in 75 mL water to a concentration of 136.5 mg/mL. This gave 10.2 g IPN PGA in 60% yield.

4) Larger scale immobilization of cellulase on IPN PGA as UCST-nanobiocatalyst IPN-Cellu

89 mL citric acid buffer (50 mM, pH 4.8) containing 1.113 g UCST-nanoparticles IPN PGA and 42.2 mg cellulase were stirred at 25°C and 250 rpm in a 100 mL round-bottom flask for 5 h. Following the procedure described for small scale immobilization of cellulase in the main text, 1115 mg IPN-Cellu were obtained with a cellulase loading of 37.9 mg/g support and 100% enzyme immobilization efficiency. The particles were re-suspended into 15 mL citric acid buffer to a concentration of 77 mg/mL for further use.

5) Larger scale immobilization of cellobiase on IPN PGA as UCST-nanobiocatalyst IPN-Cello

76 mL citric acid buffer (50 mM, pH 4.8) containing 950 mg UCST-nanoparticles IPN PGA and 41.8 mg cellobiase (from *A. niger*) in a 100 mL round-bottom flask were shaken at 25ºC and 250 rpm for 36 h. Following the procedure described for small scale immobilization of cellobiase in the main text, IPN-Cello was obtained in 983 mg, with a cellobiase loading of 35.1 mg/g support and 79% enzyme immobilization efficiency.

6) 25 mL-scale hydrolysis of filter paper with the mixture of UCST-nanobiocatalysts IPN-Cellu and IPN-Cello

25 mL citric acid buffer (50 mM, pH 4.8) containing 132 mg IPN-Cellu (5 mg cellulase), 285 mg IPN-Cello (10 mg cellobiase), and 250 mg filter paper in a 100 mL flask were shaken at 250 rpm and 50°C for 72 h. Mixtures of IPN-Cellu and IPN-Cello were separated from reaction mixture by centrifugation at 4°C and 6000 g for 5 min. The supernatant was used for the determination of glucose by using glucose assay kit. This batch of reaction gave 95% yield of glucose.

7) Pretreatment of EFB

A mixture of 1 g EFB in 20 mL 5% NaOH aqueous solution (1 gram NaOH per 1 gram EFB) in a round-bottom flask was stirred at 250 rpm and room temperature for 24 h. The solid fraction was separated from the liquid by filtration. The solid part was washed until the washing solution reached neutral pH. The compositions of the remaining solid (pre-treated EFB) were determined by using acid-hydrolysis following NREL LAP-002 protocol.[S5, S6] It contains 40% glucan and 23% xylan.

8) Comparison of storage stability of UCST-nanobiocatalyst IPN-Cellu and free cellulase

13 mg UCST-nanobiocatalyst IPN-Cellu (0.5 mg cellulase) and 0.5 mg free cellulase were stored in the buffer citric acid (50 mM, pH 4.8) at 4°C and 25°C for 3 days and 2 weeks, respectively. After storage, 2.6 mg IPN-Cellu (0.1 mg cellulase) or 0.1 mg free cellulase was used for the hydrolysis of 5 mg filter paper in 2 mL citric acid buffer (50 mM, pH 4.8). Reaction was carried out in a 10 mL flask at 50°C and 250 rpm for 3 h. After reaction, IPN-Cellu was separated from reaction mixture by centrifugation at 4°C and 6000 g for 5 min. In the case of using free enzymes, the mixture was centrifuged at 13500 g at room temperature for 5 min. The supernatant was used for the determination of glucose by using glucose assay kit. After storage, the retained enzyme activities for IPN-Cellu and cellulase are given in Table S7. UCST-nanobiocatalyst IPN-Cellu showed better storage stability than free cellulase.

9) References

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