Supplementary Information

A glucose-responsive complex polymeric micelle enabling repeated on-off release and insulin protection

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1-1 Synthesis of t-Boc-aminoethyl 2-bromoisobutyrate (BEBIB)

BEBIB was synthesized similar to ref.[1] A solution of *t*-Boc-aminoethyl alcohol (1.61 g, 10 mmol) and triethylamine (1.11 g, 11 mmol) in methylene chloride (50 mL) was cooled in an ice bath. Into the solution was added 2-bromoisobutyryl bromide (2.29 g, 10 mmol) in methylene chloride (20 mL). The reaction was slowly warmed to room temperature and stirred for 4 h. The salt was filtered off and the reaction mixture was extracted sequentially with water and saturated sodium bicarbonate solution. The organic phase was dried over magnesium sulfate. Solvent was evaporated and the crude product was purified by column chromatography using heptane/diethyl ether (80/20, w/w) as an eluent to get a pale-yellow liquid. Then it was dissolved and crystallized in a little bit of n-hexane at 4 °C to give 2.6 g of BEBIB as a white acicular crystal (yield 84%). ^{1}H-NMR (CDCl3) δ (ppm): 1.45 (e, 9 H), 1.95 (a, 6 H), 3.44-3.46 (c, 2 H), 4.23-4.25 (b, 2 H), 4.78 (d, H) .

1-2 Synthesis of Monoamino-terminated poly(N-isopropylacrylamide) ($PNIPAM_{130}$-NH_2)

Firstly N-tBoc-PNIPAM was synthesized by ATRP of *N*-isopropylacrylamide using BEBIB as initiator and CuCl/Me_6TREN as catalyst. The detailed process is introduced as follows. CuCl (110 mg, 1.1 mmol) and Me_6TREN (276 mg, 1.2 mmol) were introduced into the reaction flask and then DMF (4 mL) was added. The sample was first stirred with ultrasonic followed by the addition of *N*-isopropylacrylamide (11.3 g, 0.1 mol) dissolved in H_2O (8 mL) and DMF (6 mL), and then degassed by two freeze-thaw cycles. Subsequently, BEBIB (217 mg, 0.7 mmol) dissolved in DMF (2 mL) was introduced into the flask and the mixed solution was degassed by freeze-thaw again. Polymerization was performed at 35 ºC for 3 h and monomer conversion is about 75%. The N-tBoc-PNIPAM was purified by passing through a Al_2O_3 Column to remove the copper catalyst and then the sample was deposited in 10-fold excess of cold diethyl ether. The precipitate of N-tBoc-PNIPAM was then filtered under vacuum and dried in vacuum at room temperature.

To obtain PNIPAM-NH_2, N-tBoc-PNIPAM was firstly dissolved in CH_3OH followed by adding excess K_2CO_3 solid to totally substitute the Br- to CH_3O- group. After stirring at room temperature for 12 h, CH_2Cl_2 was added following the evaporation of

CH_3OH to dissolve the polymer. Then the polymer was treated with excess trifluoroacetic acid under vigorous stirring to remove the *t*-Boc protection group. Finally, after dialyzed against NaOH aq at pH 10 for three days and deionized water for another 4 days in a dialysis bag with a molecular cutoff of 3500 D, 7 g of PNIPAM-NH_2 was obtained by lyophilizing the solution. The DP was calculated as 130 from GPC (THF as the eluent).

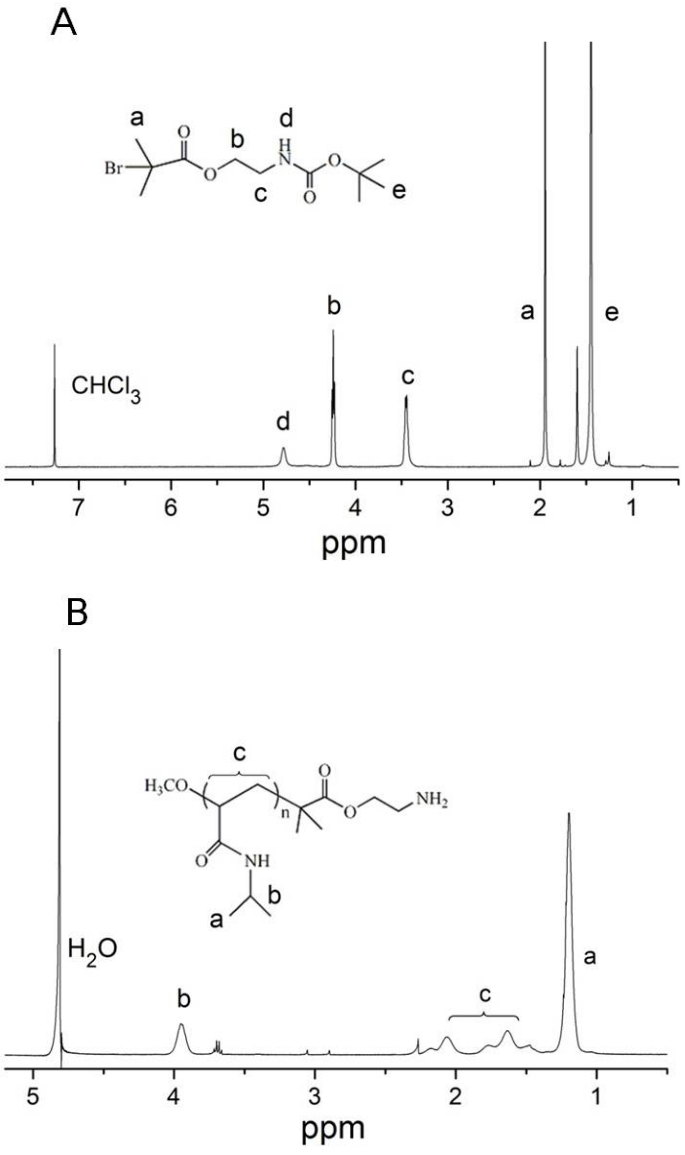


Figure S1. ^{1}H-NMR spectra of BEBIB in $CDCl_3$ (A) and $PNIPAM_{130}$-NH_2 in D_2O (B).

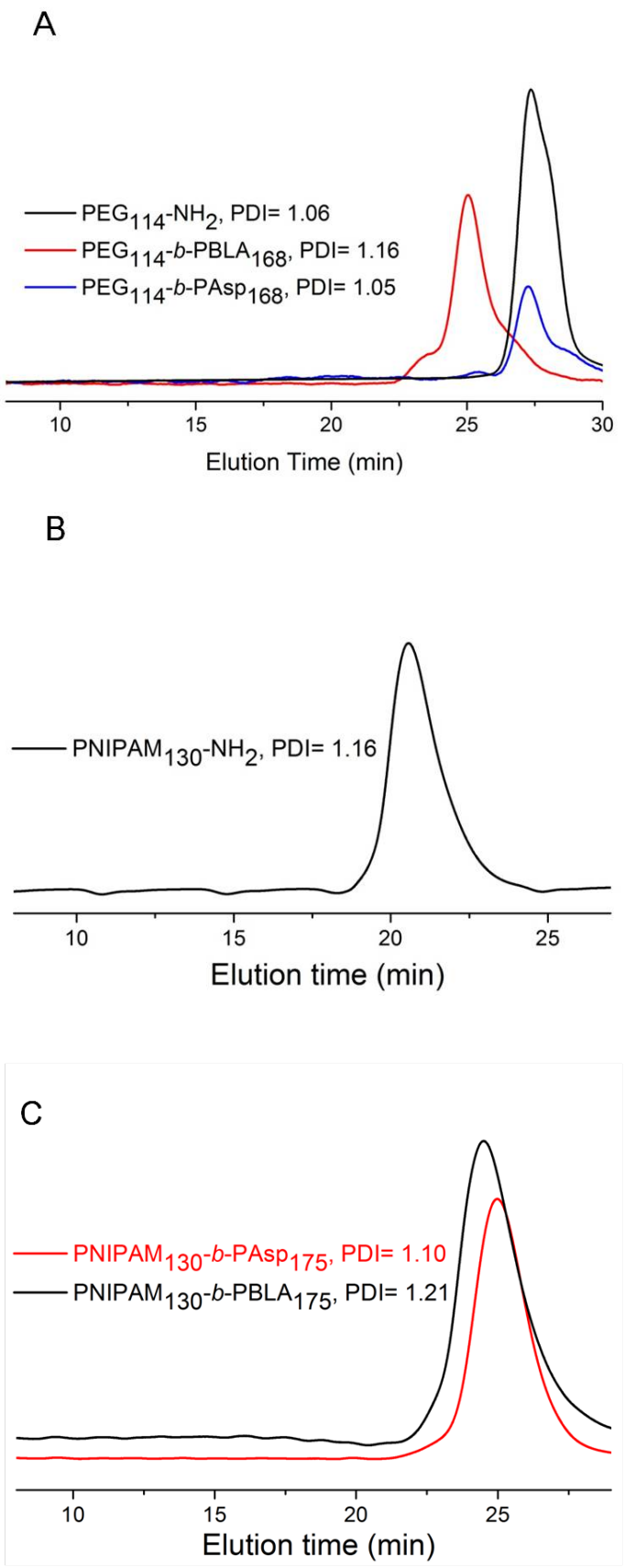


Figure S2. (A) GPC profiles of PEG_{114}-NH_2, PEG_{114}-*b*-$PBLA_{168}$ and PEG_{114}-*b*-$PAsp_{168}$ in DMF. (B) GPC profile of $PNIPAM_{130}$-NH_2 in THF. (C) GPC

profiles of $PNIPAM_{130}$-*b*-$PBLA_{175}$ and $PNIPAM_{130}$-*b*-$PAsp_{175}$in DMF.

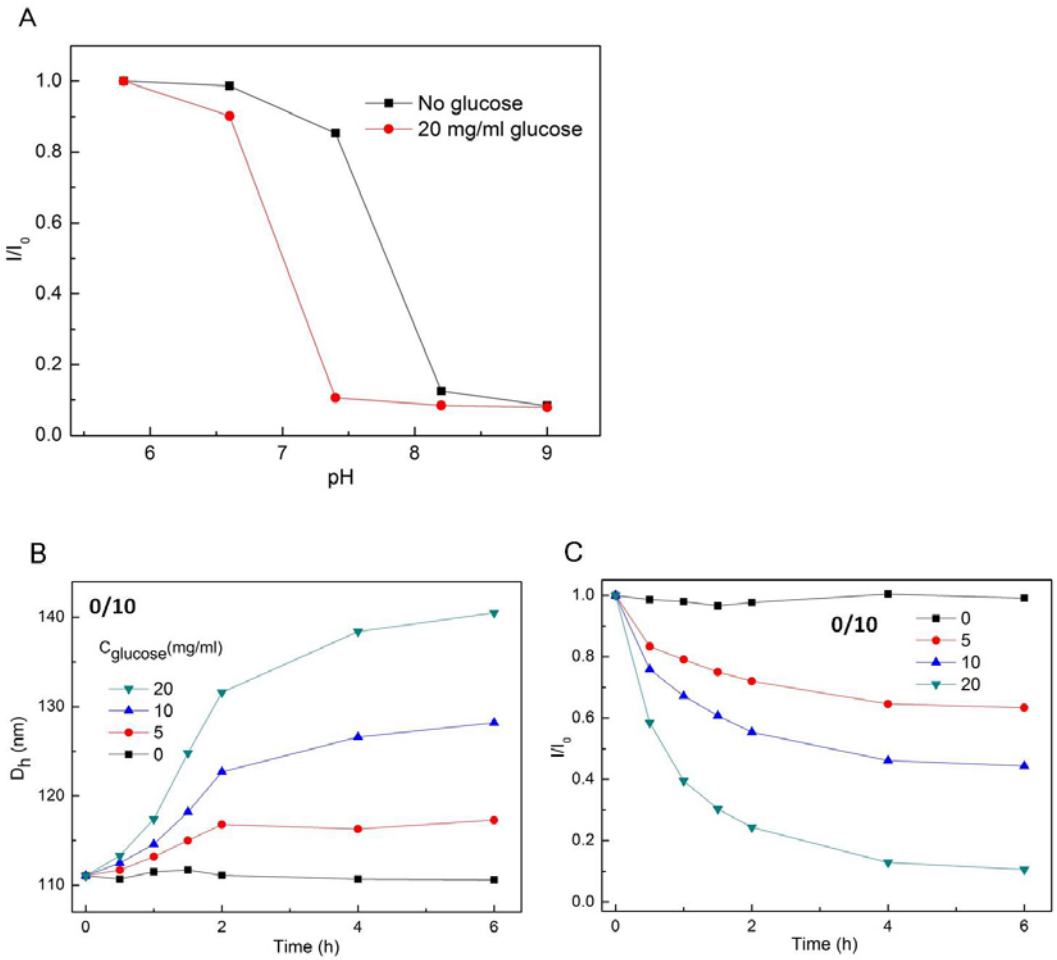
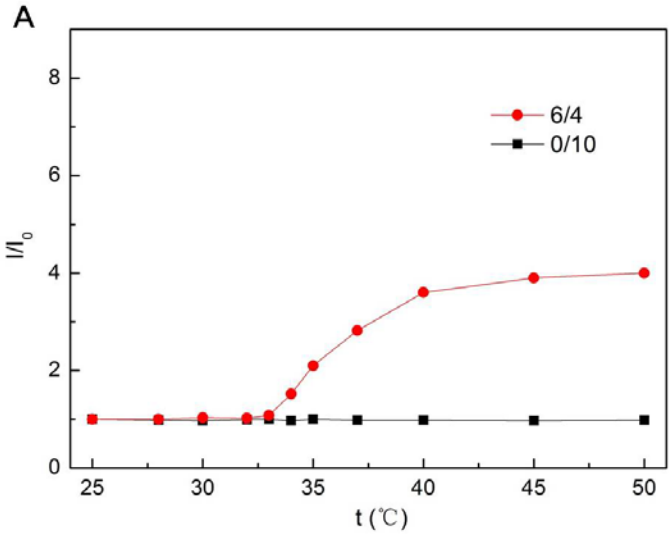


Figure S3. Glucose-responsiveness study of 0/10 PM. (A) pH-dependent Normalized light scattering intensity change with and without addition of glucose solution. Time-dependent hydrodynamic diameter (B) and normalized scattered intensity (C) changes at pH 7.4 and 37 ^{o}C under various glucose concentrations, where the scattering angle is 90^{o}.

Table S1. DLS and SLS measurements of PMs with different W_{PNIPAM}/W_{PEG} at 25 and 37 ^{o}C. *a* D_h represents the apparent average hydrodynamic diameter measured at the scattering angle of 90^{o}.

W_{PNIPAM}/W_{PEG}	t (^{o}C)	D_h[a] (nm)	R_h (nm)	R_g (nm)	R_g/R_h
0/10	25	111.5	65.9	58.0	0.88
	37	111.0	65.8	58.0	0.88
2/8	25	102.4	60.3	53.7	0.89
	37	97.6	58.2	49.5	0.85
4/6	25	109.8	63.1	56.2	0.89
	37	102.9	59.8	48.4	0.81
6/4	25	111.6	62.5	55.6	0.89
	37	99.4	57.6	44.9	0.78
8/2	25	105.4	61.4	55.3	0.90
	37	724.2	-	-	-



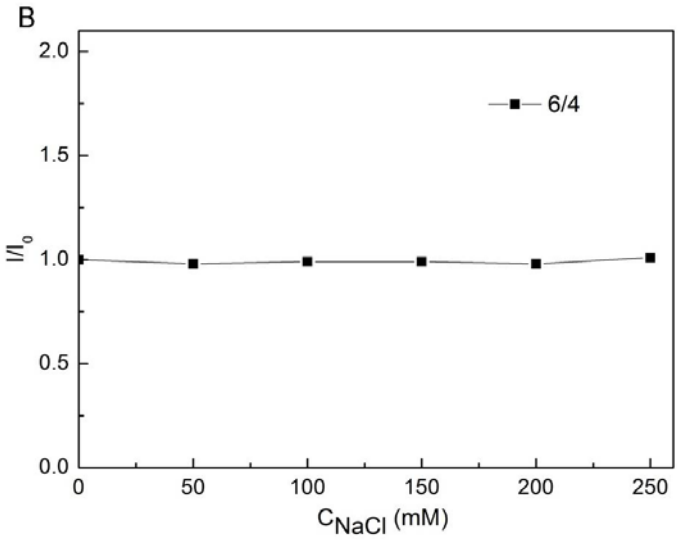
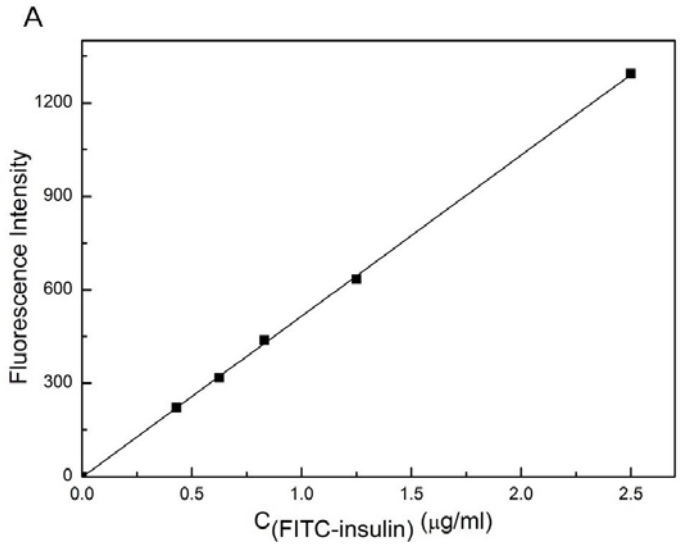


Figure S4. Structure characterization of PMs. (A) Temperature-dependent normalized scattered intensity of 0/10 and 6/4 PMs. (B) salt concentration-dependent normalized scattered intensity of 6/4 PMs.



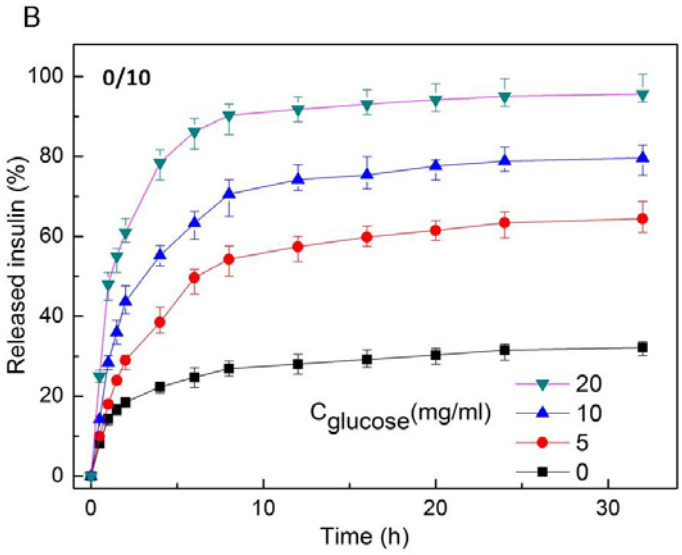


Figure S5. (A) Fluorescence intensity at various concentrations of FITC-insulin. (B) Release profiles of FITC-insulin from 0/10 PMs upon varying concentrations of glucose.

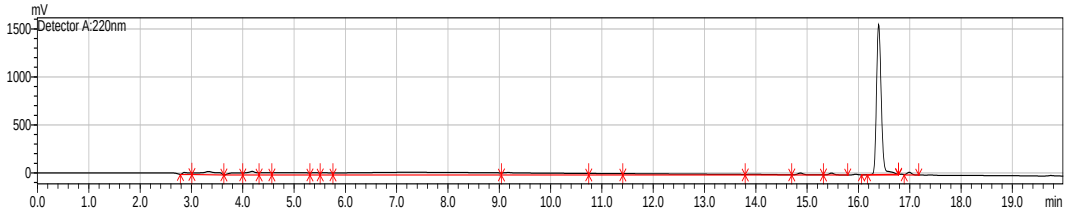


Figure S6. HPLC profile of insulin at 35 ^{o}C (mixed solvent: acetonitrile and 0.1% trifuoroacetic acid)

References:

1 Z. Yang, S. Y. Zheng, W. J. Harrison, J. Harder, X. X. Wen, J. G. Gelovani, A. Qiao and C. Li, *Biomacromolecules*, 2007, **8**, 3422-3428.