Supporting Information

Glucose and H_2O_2 Dual-responsive Polymeric Micelles for Self-regulated Release of Insulin

Xiaoyu Liu[†,‖] Chang Li,[‡,‖] Juan Lv,[†] Fan Huang,[§,*] Yingli An,[†] Linqi Shi[†,#,*] and Rujiang Ma[†,*]

[†]Key Laboratory of Functional Polymer Materials of Ministry of Education, State Key Laboratory of Medicinal Chemical Biology, College of Chemistry, Nankai University, Tianjin 300071, China.

[‡]Department of Polymeric Materials, School of Materials Science and Engineering, Key Laboratory of Advanced Civil Engineering Materials of Ministry of Education, Tongji University, 4800 Caoan Road, Shanghai 201804, China.

[§]Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300192, China.

[#]Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, China.

[‖]X.L. and C.L. contributed equally to this work.

*Corresponding Authors

Fan Huang − Email: huangfanforever@126.com;

Linqi Shi − Email: shilinqi@nankai.edu.cn;

Rujiang Ma − Email: marujiang@nankai.edu.cn.

1. Synthesis and characterization of the block copolymer PEG-*b*-PAPBE

In this study, the polymer poly(ethylene glycol)-*block*-poly(amino phenylboronic ester) (PEG-*b*-PAPBE) was synthesized by a Michael addition polymerization of mono-functional mPEGA, and bi-functional hydroxymethyl phenylboronic ester diacrylate (HPBEDA) and 1,3-bis(4-piperidyl)propane (BPP) as indicated in Figure S1, where mPEGA with mono acrylate group and HPBEDA with double acrylate groups acted as the Michael addition acceptor and BPP with double imino groups acted as the Michael addition donor. Details about the synthesis of HPBEDA can be found in our recent work.[1] During the reaction, BPP and HPBEDA would undergo a linear step growth Michael addition polymerization, forming poly(amino phenylboronic ester) (PAPBE) chains composed of alternatively distributed BPP and HPBEDA, while mPEGA played a role of termination due to its mono-functionality. Because the feeding molar amounts of BPP and HPBEDA were ten times more than that of mPEGA, once the short PAPBE chains were terminated by mPEGA at one end, the other end would most likely propagate with BPP and HPBEDA. As a result, block copolymer PEG-*b*-PAPBE was obtained. The similar Michael addition polymerization was also used in our previous works, where block polymers were obtained and well characterized.[2,3]

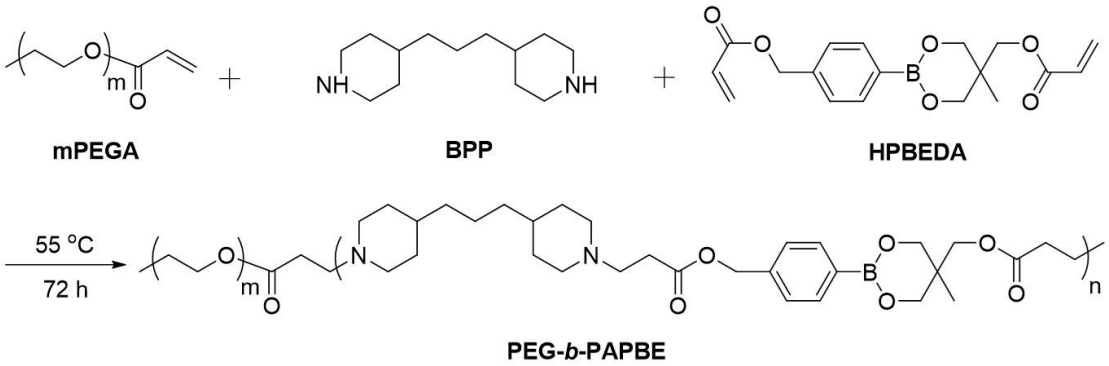

Figure S1. Synthesis of the block copolymer PEG-*b*-PAPBE.

To characterize the block copolymer PEG-*b*-PAPBE more clearly, Size exclusion chromatography (SEC) measurements were carried out and the traces of mPEGA and PEG-*b*-PAPBE are shown in Figure S2. The reduced elution time of the copolymer compared to that of mPEGA and the unimodal molecular weight distribution indicate successful synthesis of the block copolymer PEG-*b*-PAPBE. The number-average molecular weights (M_n) of mPEGA and PEG-*b*-PAPBE are 5300 and 19500 Da, and the poly dispersity indexes (PDI) are 1.06 and 1.18 respectively based on the SEC results. In fact, the M_n of mPEGA is 5000 Da according to the supplier and the M_n of PEG-*b*-PAPBE is more accurately calculated to be 16400 Da by comparing the peak integration area of PEG and PAPBE in the ^{1}H NMR spectrum shown in Figure S3 and the degree of polymerization (DP) of the PAPBE block is determined to be 21.

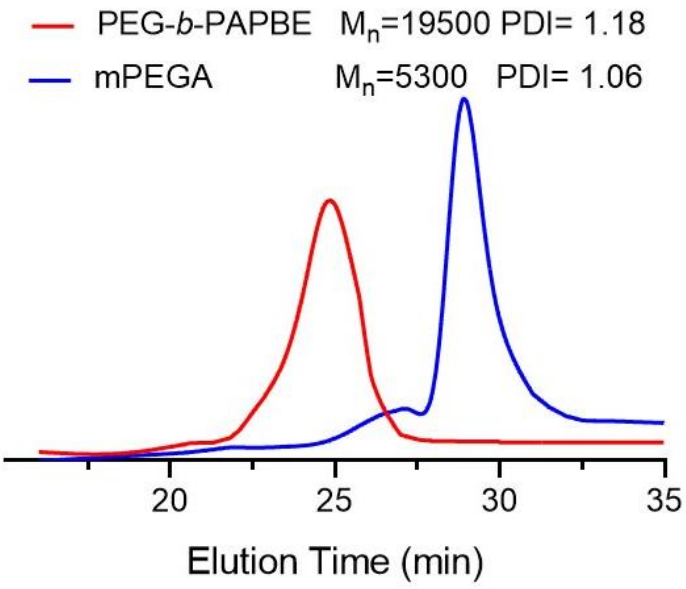

Figure S2. SEC traces of mPEGA and PEG-*b*-PAPBE in DMF.

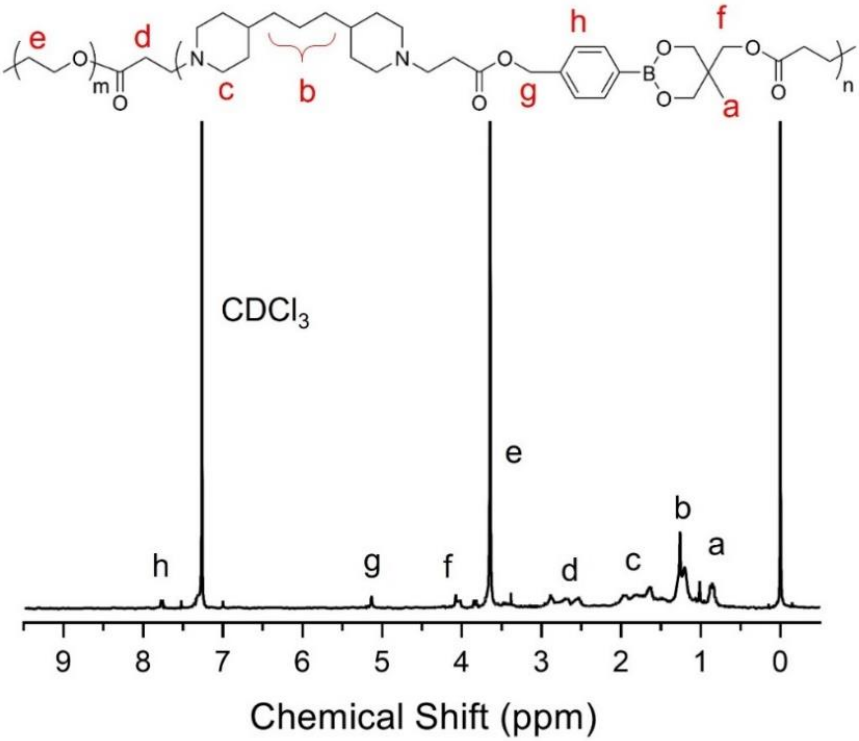

Figure S3. ^{1}H NMR spectrum of PEG-*b*-PAPBE in $CDCl_3$.

2. Time-dependent size variation of the PMs under glucose.

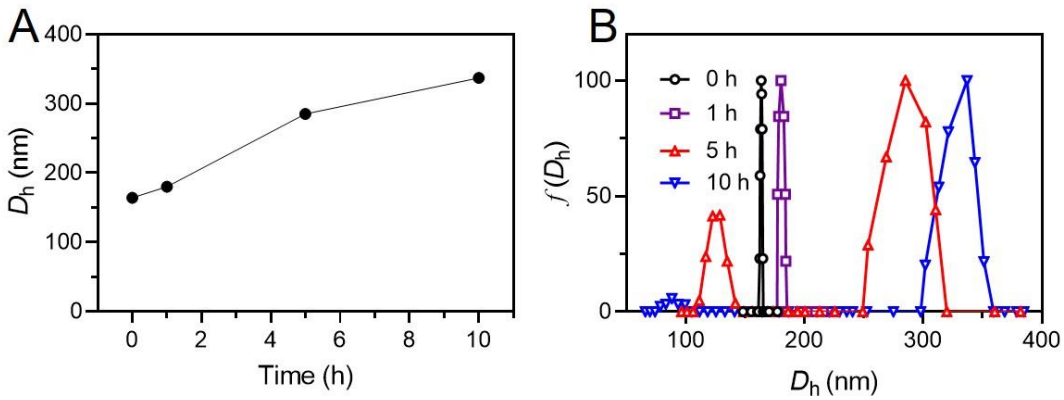

Figure S4. Variations of (A) average size and (B) size distribution of the blank micelles in the presence of 5 g/L glucose as a function of time at 37°C.

3. Circular dichroism (CD) characterization of insulin.

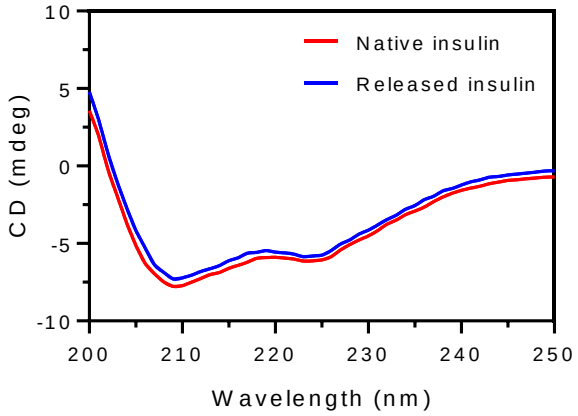

Figure S5. CD spectra of the released insulin and the native insulin at 37 °C.

4. MTT assay of polymeric micelles and monomers.

The cytotoxicity of the polymeric micelles and synthetic monomers were evaluated by MTT assay according to our recent works.[4, 5] NIH 3T3 mouse fibroblast cells were seeded into a 96-well plate with a density of 10^4 cells per well in 100 μL of RPMI1640 complete media containing 10% FBS and incubated at 37 °C under a humidified 5% CO_2 atmosphere for

24 h. The culture medium of each well was replaced with 100 μL of fresh medium containing polymeric micelles or the monomers with the final concentrations range from 0.001 to 0.5 g/L. After incubation for 24 h, 25 μL of MTT solution with a final concentration of 1 g/L was used to replace the mixture in each well. The cells were incubated for another 4 h. Then, the solution was replaced with 150 μL of DMSO and the plates were slightly shaken for 10 min. The optical absorbance was measured at 492 nm using a microplate reader (Labsystem, Multiskan, Ascent, Finland). The NIH 3T3 mouse fibroblast cells without any treatment were used as the control.

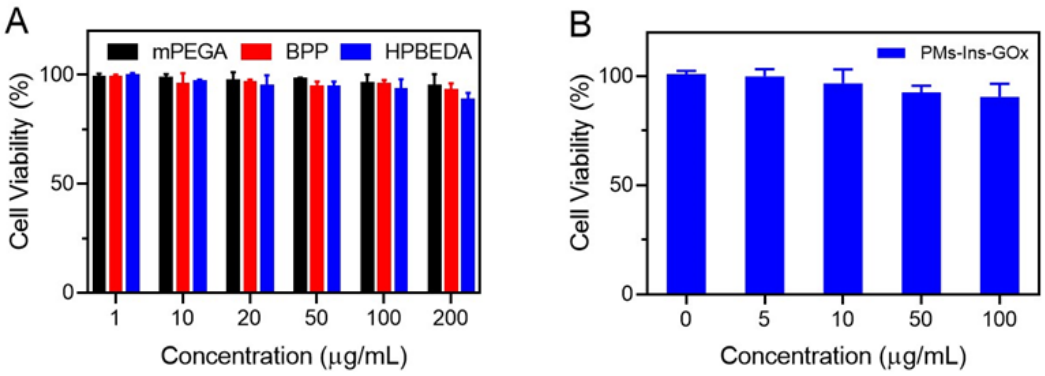

Figure S6. MTT assay with NIH 3T3 mouse fibroblast cells (A) under varying concentrations of the synthetic monomers mPEGA, BPP, and HPBEDA and (B) under varying concentrations of the GOx/insulin co-loaded polymeric micelles (PMs-Ins-GOx) in the presence of 1 g/L glucose. The measurements were carried out in triplicate (n = 3) and the data were shown as mean ±SD (standard deviation).

5. Hematology analysis.

Hematological examination was performed by an automatic blood analyzer (Celltace, Japan). Five healthy BABL/c mice were injected with

the blank polymeric micelles via tail vein at a dose of 20 mg/kg every other day and another five healthy mice injected with PBS 7.4 were used as the control.[6] Hematological analysis was performed at 1 day after the last administration (day 1) and 1 day after seven successive administrations (day 7). The blood samples (100 μL) were collected from the eyes of mice at the designated time points and were added into a heparinized tube for hematological analysis.

6. In vivo studies with diabetic mice.

In this study, the streptozotocin (STZ)-induced diabetic mice (male BABL/c, 17-22 g) were provided by Chinese Academy of Medical Sciences & Peking Union Medical College. The diabetic mice were randomly divided into four groups (n = 5) to evaluate the therapeutic efficacy. The detailed process is as follows: free insulin dissolved in PBS 7.4 (PBS-Ins), insulin-loaded polymeric micelles (PMs-Ins) and GOx/insulin-coloaded polymeric micelles (PMs-Ins-GOx) were subcutaneously injected into each diabetic mouse with a dose of insulin (20 IU/kg), respectively. The blood samples (3 μL) were collected from the tail vein of the mice, and the BGLs were measured immediately by the blood glucose meter (Sinocare Inc., Changsha, China). Besides, an intraperitoneal glucose tolerance test (IPGTT) was carried out to confirm the in vivo glucose responsiveness of the PMs-Ins-GOx. Briefly, the diabetic mice were administrated with PBS-Ins and PMs-Ins-GOx (the

insulin dose is 20 IU/kg for each mouse), healthy mice without any treatment were used as the normal control group. When the BGLs of diabetic mice decreased to 1 g/L after administration of therapeutics, a glucose solution in PBS 7.4 was intraperitoneally injected into all the mice with the glucose dose of 1.5 g/kg, and the BGLs were monitored over time.

7. Characterizations.

^{1}H NMR spectra was recorded on a Varian UNITY-plus 400 M NMR spectrometer at room temperature. Dynamic light scattering (DLS) measurement was performed on a laser light scattering spectrometer at room correlator (BI-9000AT) at 636 nm. All samples were first prepared by filtering a certain volume of micelle solutions through a Millipore filter of 0.45 μm into a clean scintillation vial. Transmission electron microscopy (TEM) measurement was performed by using a FEI Talos F200C electron microscope at an acceleration voltage of 200 kV. Samples for TEM were obtained by depositing the diluted polymeric micelle solution (0.05 g/L) onto a carbon-coated copper EM grid and dried at a suitable temperature in a vacuum. Fluorescence emission spectra were measured on a Hitachi F-4600 fluorescence spectrophotometer under room temperature. Circular dichroism (CD) spectra were measured by MOS-500 spectropolarimeter equipped with a Peltier-type temperature

controller.

REFERENCES

(1) Li, C.; Liu, X. Y.; Liu, Y.; Huang, F.; Wu, G.; Liu, Y.; Zhang, Z. Z.; Ding, Y. X.; Lv, J.; Ma, R. J.; An, Y. L.; Shi, L. Q. Glucose and H2O2 dual-sensitive nanogels for enhanced glucose-responsive insulin delivery. *Nanoscale* **2019,** *11*, 9163−9175.

(2) Cheng, T. J.; Zhang, Y. M.; Liu, J. J.; Ding, Y. X.; Ou, H. L.; Huang, F.; An, Y. L.; Liu, Y.; Liu, J. F.; Shi, L. Q. Ligand-Switchable Micellar Nanocarriers for Prolonging Circulation Time and Enhancing Targeting Efficiency. *ACS Appl. Mater. Interfaces* **2018,** *10*, 5296−5304.

(3) Cheng, T. J.; Ma, R. J.; Zhang, Y. M.; Ding, Y. X.; Liu, J. J.; Ou, H. L.; An, Y. L.; Liu, J. F.; Shi, L. Q. A surface-adaptive nanocarrier to prolong circulation time and enhance cellular uptake. *Chem. Commun.* **2015,** *51*, 14985−14988.

(4) Li, C.; Wu, G.; Ma, R. J.; Liu, Y.; Liu, Y.; Lv, J.; An, Y. L.; Shi, L. Q. Nitrilotriacetic Acid (NTA) and Phenylboronic Acid (PBA) Functionalized Nanogels for Efficient Encapsulation and Controlled Release of Insulin. *ACS Biomater. Sci. Eng.* **2018,** *4*, 2007−2017.

(5) Li, C.; Huang, F.; Liu, Y.; Lv, J.; Wu, G.; Liu, Y.; Ma, R. J.; An, Y. L.; Shi, L. Q. Nitrilotriacetic Acid-Functionalized Glucose-Responsive Complex Micelles for the Efficient Encapsulation and Self-Regulated Release of Insulin. *Langmuir* **2018,** *34*, 12116−12125.

(6) Yang, C. H.; Chu, L. P.; Zhang, Y. M.; Shi, Y.; Liu, J. J.; Liu, Q.; Fan, S. J.; Yang, Z. M.; Ding, D.; Kong, D. L.; Liu, J. F. Dynamic biostability, biodistribution, and toxicity of L/D-peptide-based supramolecular nanofibers. *ACS Appl. Mater. Interfaces* **2015,** *7*, 2735−2744.