Support Information

Bio-Inspired Synthetic Nanovesicles for Glucose-Responsive Release of Insulin

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1. Materials

All chemicals were obtained from commercial sources and used without further purification. O-Acetyl-L-serine hydrochloride, triphosgene, lithium hydroxide, pyridinium *p*-toluenesulfonate (PPTS), Pluronic® F-127, glucose oxidase (GOx) and bovine catalase (CAT) were purchased from Sigma Aldrich. Poly(ethylene glycol) amine (PEG2000-NH_2) was ordered from Laysan Bio, Inc. (USA). 2-ethoxy-1-propene was obtained from Synthonix Inc.. Recombinant human insulin (Zn salt, 27.5 IU/mg) was purchased from Life Technologies (USA). All the organic solvents for synthesis and analysis were ordered from Fisher Scientific Inc. and used as received.

2. Chemical labeling of insulin with Oregon Green® 488

Thirty milligrams of Insulin were dissolved in 5 mL of dry DMSO. After insulin was completely dissolved, Oregon Green® 488 (1 mg, Life tech, Inc) was added into the solution. The reaction was initiated by adding 20 μL of triethylamine. After 24 hours, the reaction was poured into the distilled water (10 mL). The yellow solution was then dialysis against DD water and lyophilized. The molecular weight of chemical labeled insulin was measured by MALDI-TOF (Figure S10).

3. Preparation and analysis of nanovesicle by Confocal Laser Scanning Microscopy

The Oregon Green® 488 labeled insulin was encapsulated into the nanovesicle as described above. The diluted vesicle solution was transferred into the confocal microscopy dish (MatTek Corp.). The confocal image was taken using Confocal Laser Scanning Microscopy (Zeiss, LSM710). The excitation and emission wavelength were set at 488 nm and 518 nm, respectively. The image was analyzed and overlayed using Image J software.

4. GOx stability study

To confirm the stability of GOx during the polymersome-preparation process, the catalytic bioactivity of the treated GOx was compared with the native enzyme by measuring their pH-decreasing ability in glucose saline. After the THF evaporation during polymersome preparation, the supernatant of the polymersome suspension, which represents the un-encapsulated GOx/CAT solution, was collected and considered as the treated GOx/CAT enzymes. The native GOx/CAT and the treated enzymes were diluted to 0.2 mg/ml GOx with 400 mg/dL glucose saline, separately. The enzyme glucose solutions were incubated at 37 ^{o}C and their pH value was closely monitored as the function of time using pH meter (Fisher Scientific, AB15).

5. The stability of polymersome in PF127 thermogel

The stability of polymersome in PF127 thermogel was evaluated by measuring the insulin leakage during the gelation. Briefly, the insulin encapsulated polymersome was suspended into the ice-cold 30% PF127 solution, which was then warmed to 37 ^{o}C for gelation. Twenty-four hours after incubation at 37 ^{o}C, the hydrogel was cooled to 4 ^{o}C and liquefied. The polymersome was spin down and the supernatant was collected to quantify the released insulin.

6. Supplementary figures

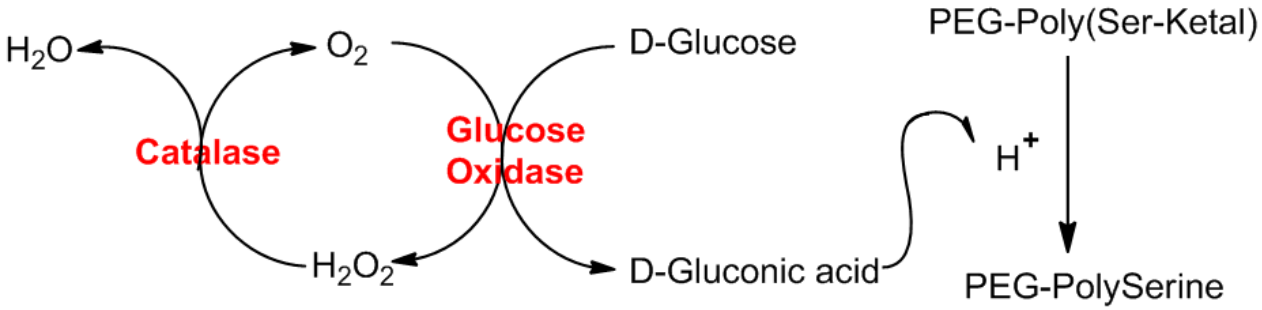

Figure S1. Schematic of enzymatic reaction with glucose oxidase (GOx) and catalase (CAT), together with its effect on ketal hydrolysis. GOx converts glucose into gluconic acid and hydrogen peroxide (H_2O_2) by consuming oxyogen (O_2), while CAT. breaks down harmful H_2O_2 into water and O_2. Gluconic acid decreases the local pH value and hydrolyzes the ketal containing polymer, leading to nanovesicle dissociation.

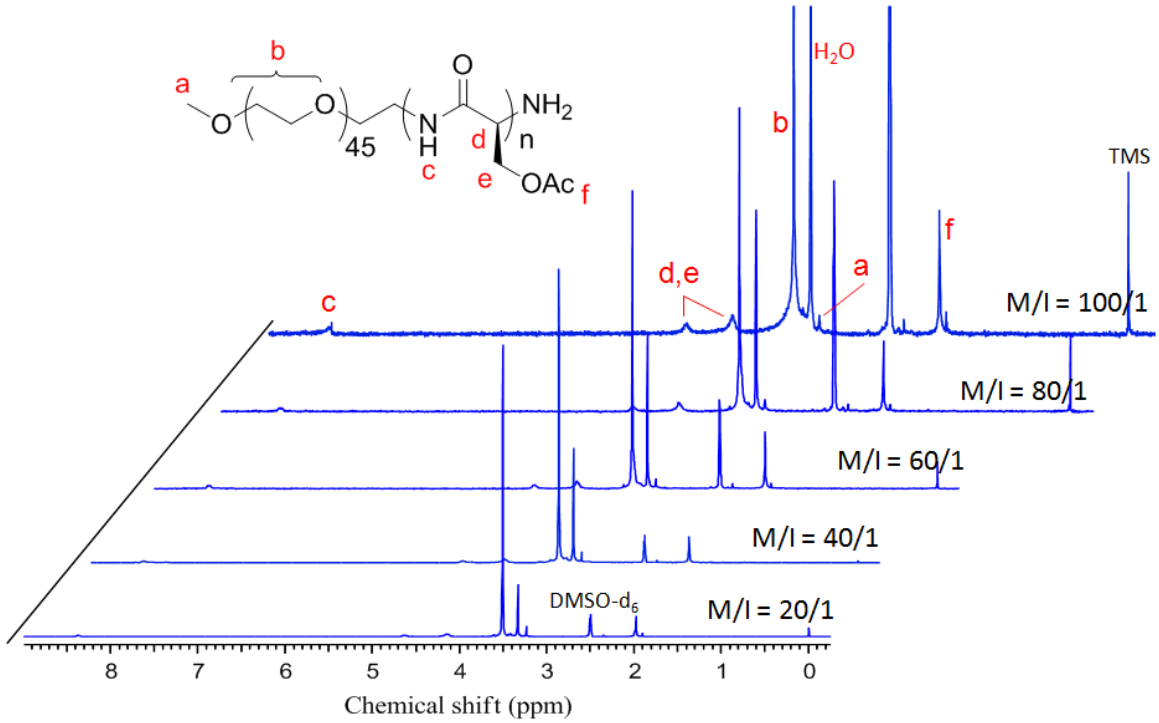

Figure S2. ^{1}H NMR spectra of polymer **3** synthesized from different M/I ratios.

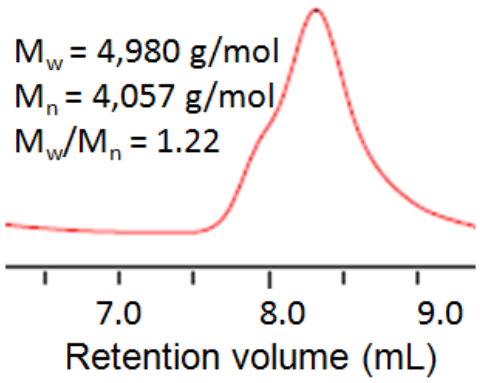

Figure S3. GPC chromatogram of polymer PEG-PolySerine$_{34}$ which was used for ketal grafting and polymersome assembly.

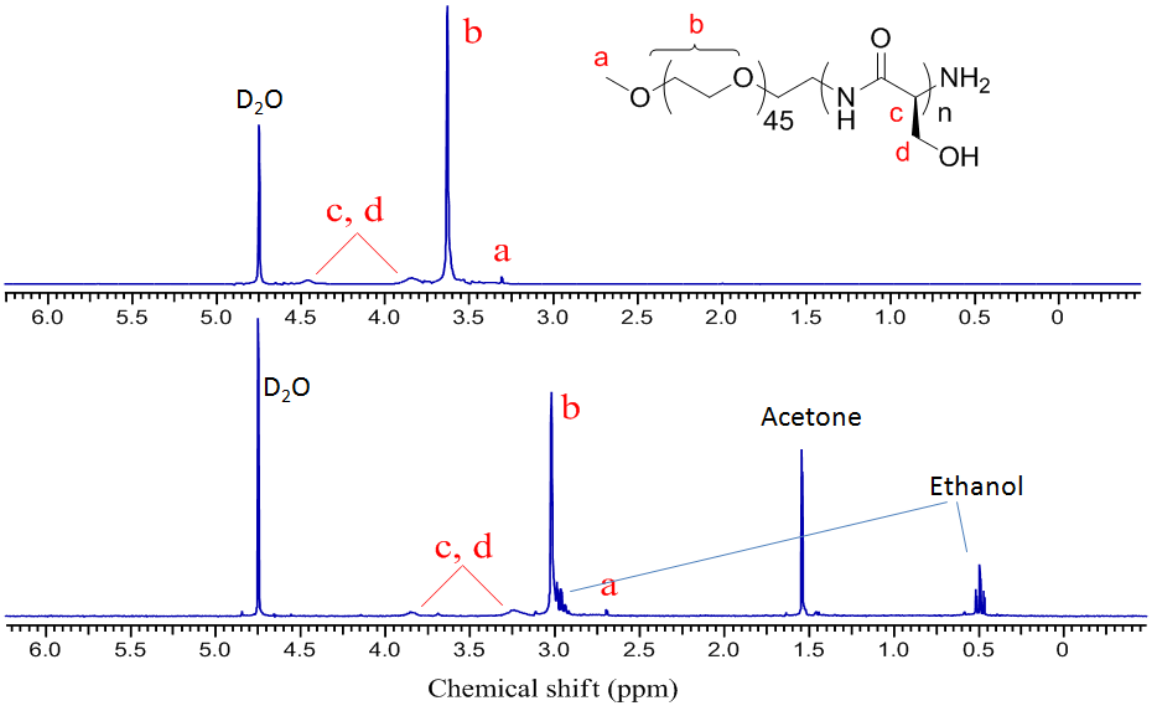

Figure S4. ^{1}H NMR analysis of PEG-PolySerine (upper), and PEG-Poly(Ser-Ketal) after DCl induced hydrolysis in D_2O (lower).

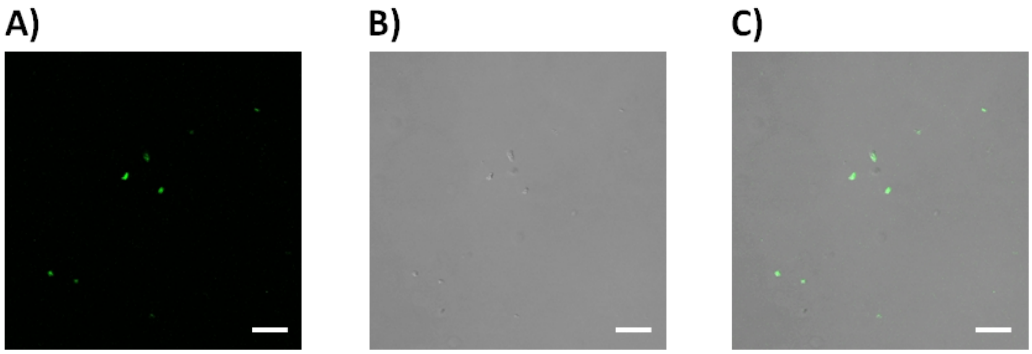

Figure S5. CLSM images of nanovesicle encapsulating Oregon Green® 488-labeled human insulin. **A)** Oregon Green® 488 channel; **B)** Bright field; **C)** Merged. Scale bar: 10 µm.

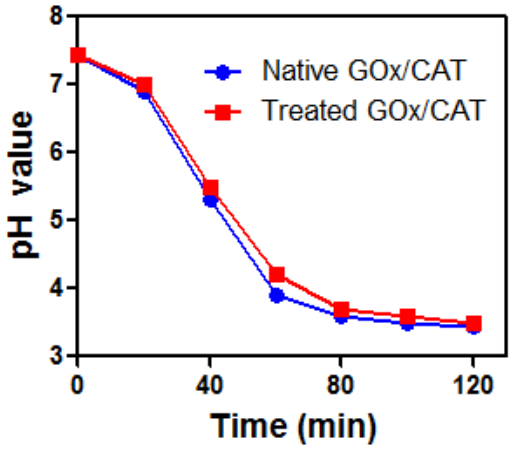

Figure S6. The catalytic activity of enzyme GOx/CAT after polymersome preparation treatment (treated GOX/CAT) was compared with the native GOX/CAT by measuring their pH-decreasing ability in 400 mg/dL saline solution. Similar trend indicated that the bioactivity of GOx/CAT was preserved during polymersome preparation.

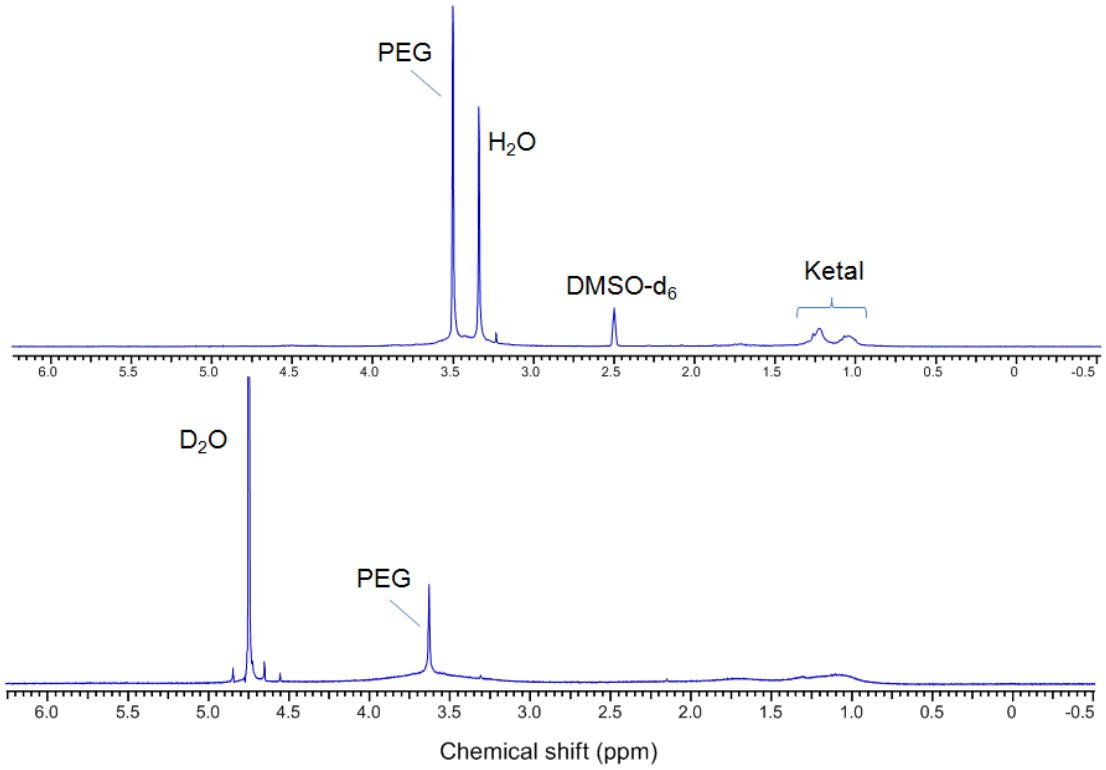

Figure S7. ^{1}H NMR spectra of nanovesicle in DMSO-d_6 (upper) and D_2O (lower). ^{1}H NMR spectra of nanovesicle in D_2O only shows PEG peaks, indicating that PEG is located on the surface of nanovesicle. Other peaks are shield off by its nanoparticle structures.

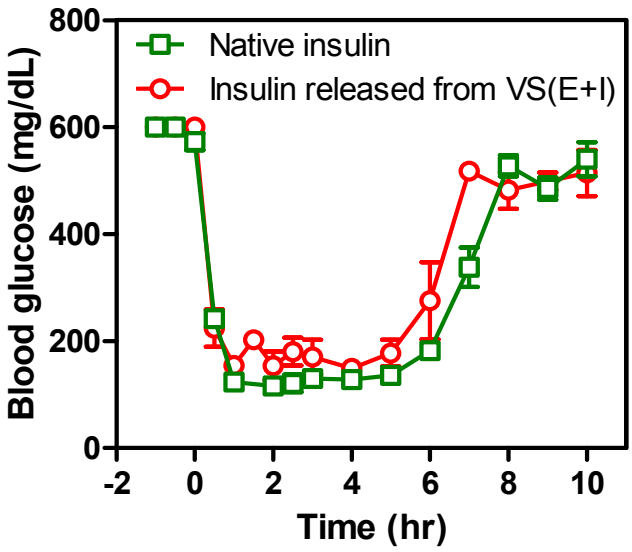

Figure S8. *In vivo* bioactivity comparison of the native human insulin and the insulin released from nanovesicle after incubation with 400 mg/dL glucose at 37°C for 6 hours.

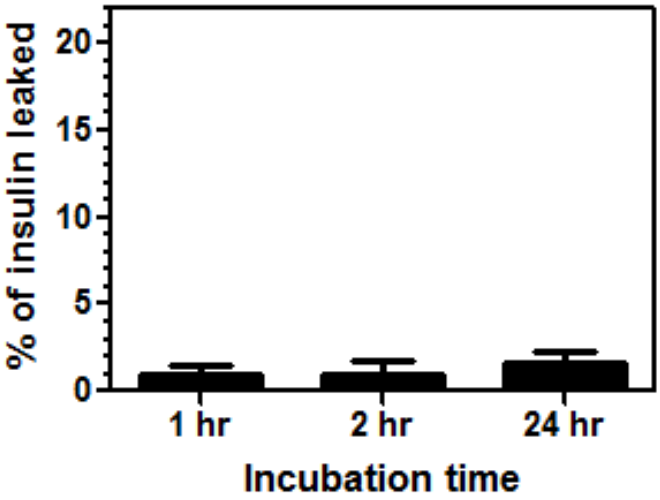

Figure S9. The stability of polymersome in PF127 thermogel was tested by monitoring the cargo leakage at different time points. Less than 5% leakage indicates that the polymersome is stable in the thermo-responsive hydrogel.

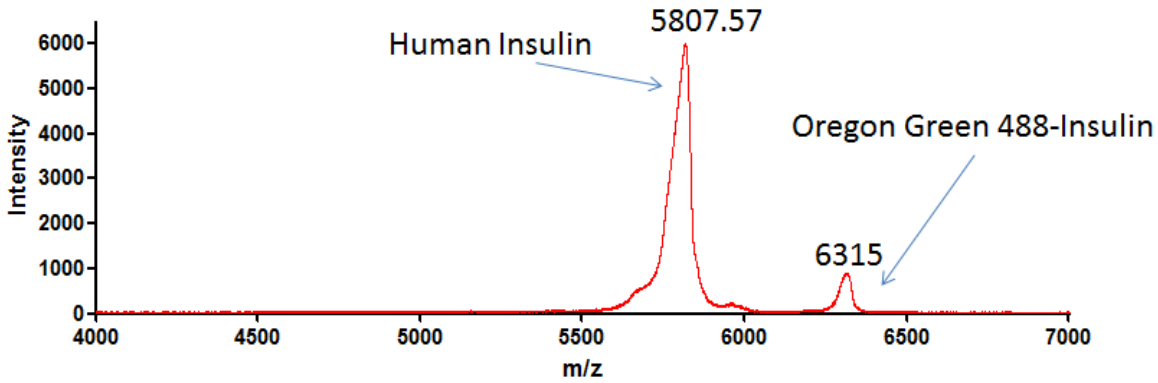

Figure S10. MALDI-TOF analysis of Oregon Green® 488-labeled human insulin.