## Electronic Supplementary information

Design of self-assembled glycopolymeric zwitterionic micelles as removable protein stabilizing agents

R. Rajan\*, and K. Matsumura\*

**Scheme S1.** Synthesis of trehalose methacrylate. Methacrylic anhydride was added to dried trehalose to substitute hydroxyl groups for a polymerizable methacrylate group.

Scheme S2. Synthesis of poly-caprolactone (PCL). Ring opening polymerization of caprolactone using hydroxyl-terminated reversible addition—fragmentation chain-transfer (RAFT) agent in the presence of stannous(II) octoate.

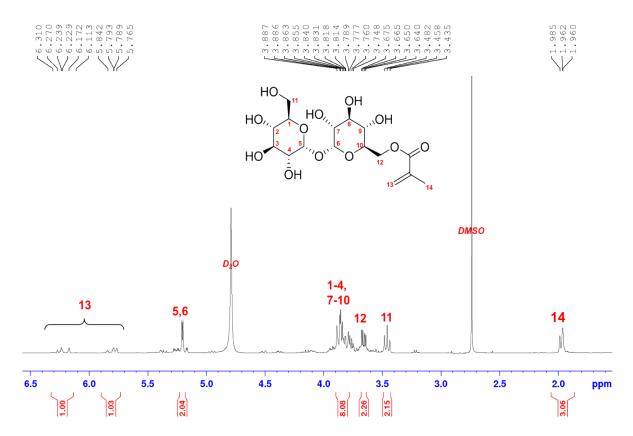


Fig. S1. <sup>1</sup>H NMR spectrum of TrMA in D<sub>2</sub>O.

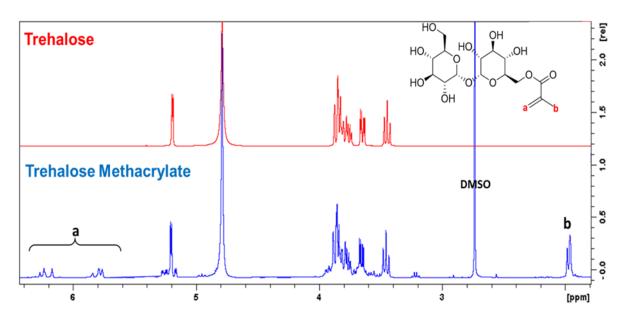
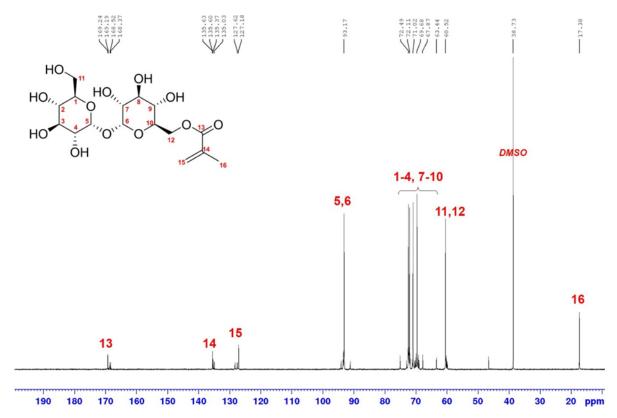


Fig. S2. Comparison of the <sup>1</sup>H NMR spectra (in D<sub>2</sub>O) of trehalose and TrMA.



**Fig. S3.**  $^{13}$ C NMR spectrum of TrMA in  $D_2$ O.

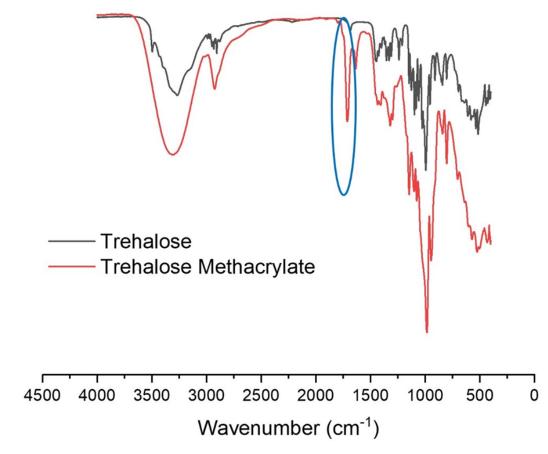


Fig. S4. Comparison of the FTIR spectra of trehalose and TrMA.

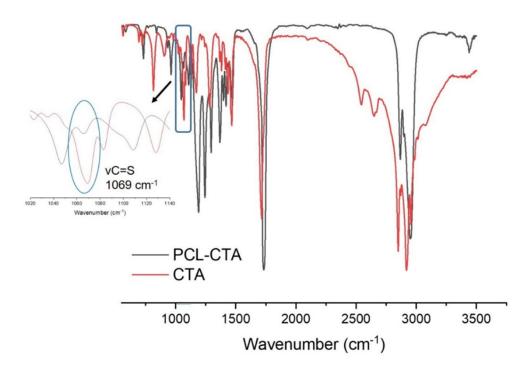


Fig. S5. Comparison of the FTIR spectra of PCL-CTA and CTA.

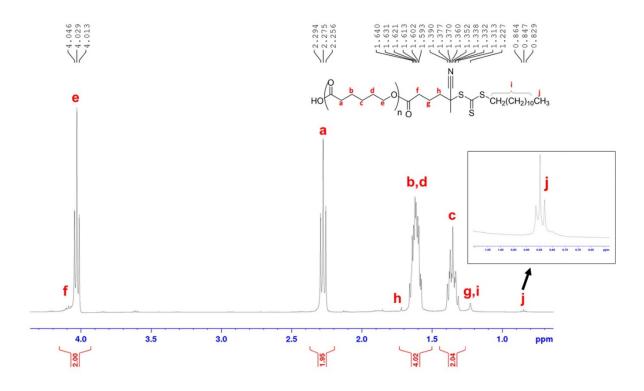


Fig. S6. <sup>1</sup>H NMR of PCL-CTA in CDCl<sub>3</sub>.

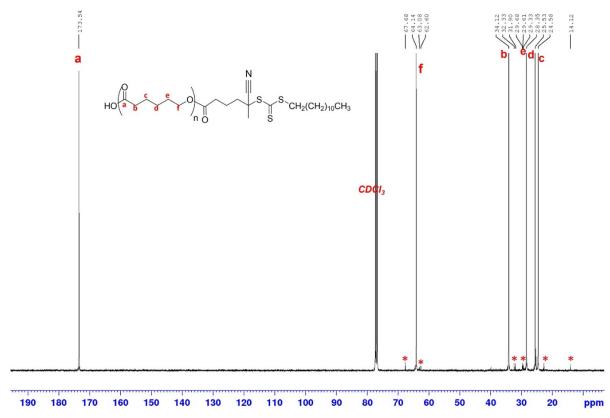
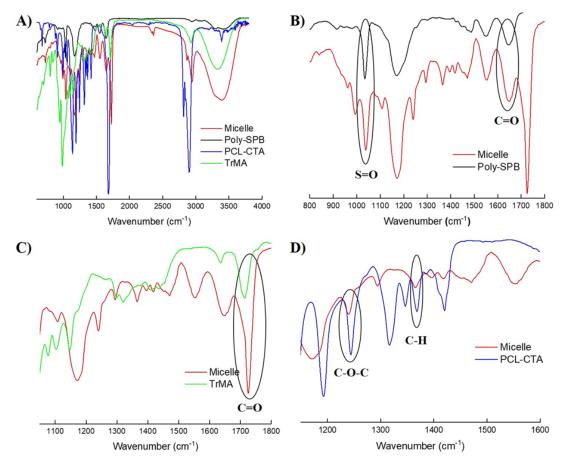
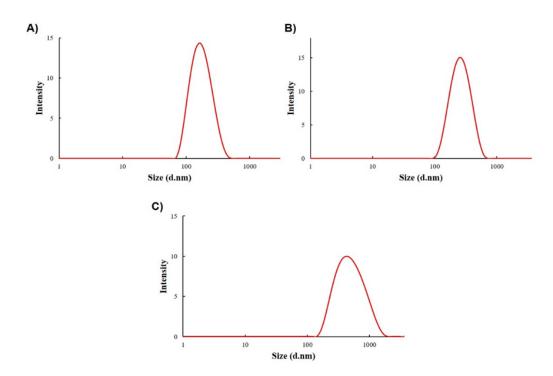


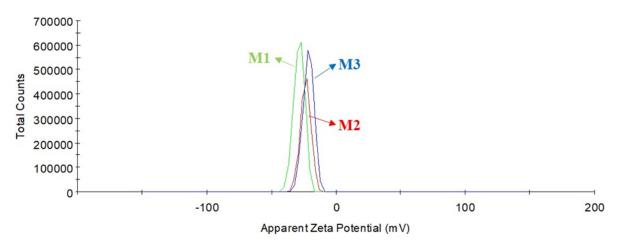
Fig. S7.  $^{13}$ C NMR spectrum of PCL-CTA in CDCl<sub>3</sub> (\* indicates RAFT agent peaks).



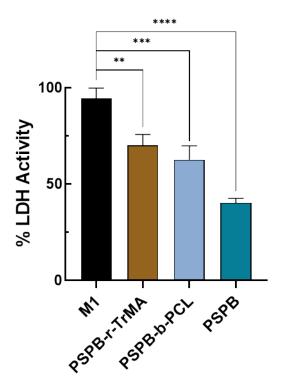
**Fig. S8.** Investigating the presence of all three components in the micelles. FTIR spectra of micelle and A) its corresponding components, B) poly-SPB, C) trehalose methacrylate, and D) PCL-CTA.



**Fig. S9.** Size of micelles. Size distribution by number obtained for the micelles by dynamic light scattering (DLS) for A) M1, B) M2, and C) M3.



**Fig. S10.** Surface charge of micelles. Zeta potential distributions of the micelles were observed at 4 mg/mL in deionized water.



**Fig. S11.** Residual enzymatic activity. Enzymatic activity of LDH after incubation in presence of different additives (2 mg/mL) after incubation at 37 °C for 1 h.

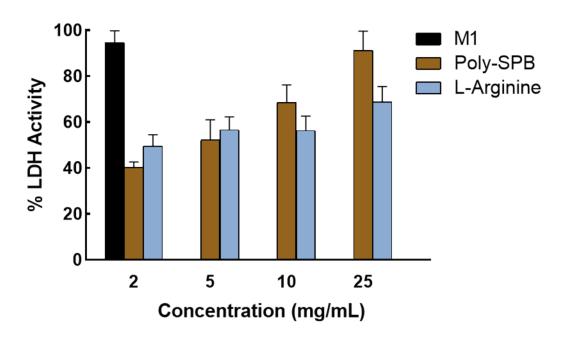
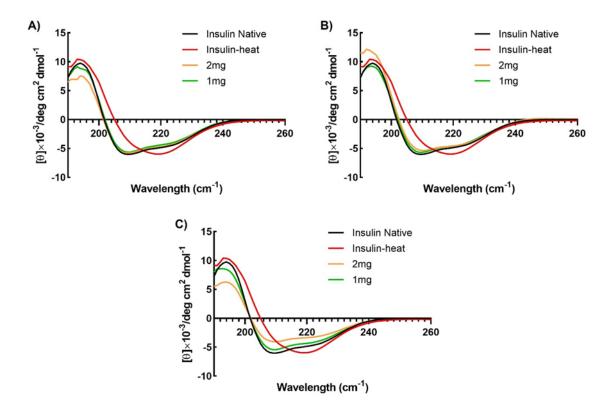
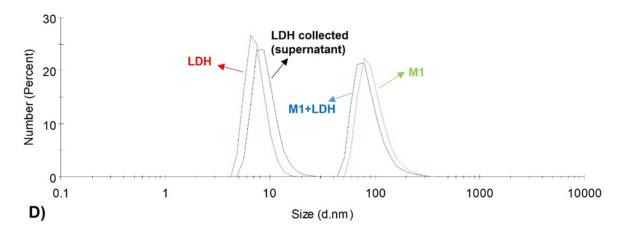


Fig. S12. Residual enzymatic activity. Enzymatic activity of LDH after incubation in the presence of different additives at different concentrations after incubation at 37 °C for 1 h.

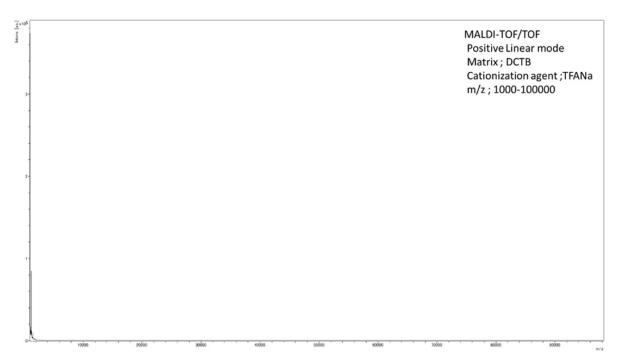


**Fig. S13.** Secondary structure of insulin. Representative far-UV CD spectra of human insulin after incubation at 45 °C for 72 h in the presence of A) M1, B) M2, and C) M3.

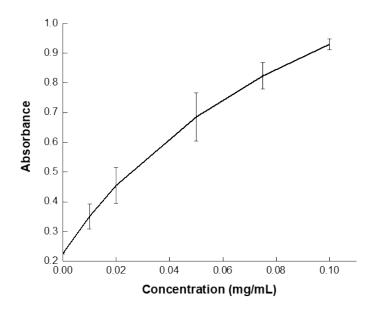
## Size Distribution by Number



**Fig. S14.** Removal of micelles from protein. DLS analysis of micelles before and after centrifugation.

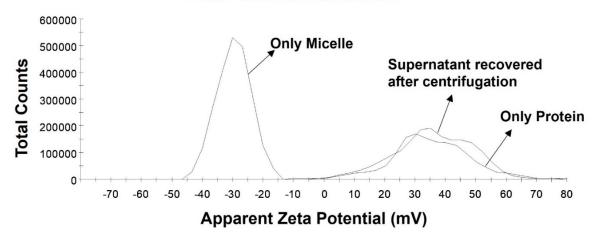


**Fig. S15.** Removal of micelles from protein. MALDI-TOF analysis of the supernatant recovered after centrifugation of micelles.



**Fig. S16.** LDH calibration curve. Bradford assay standard curve of LDH concentration versus absorbance (595 nm).

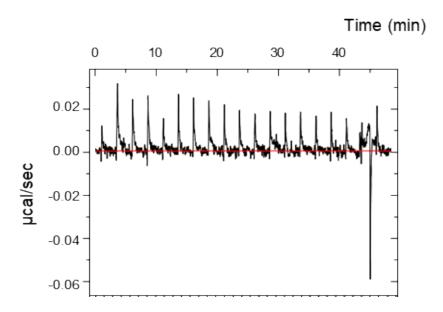
## **Zeta Potential Distribution**



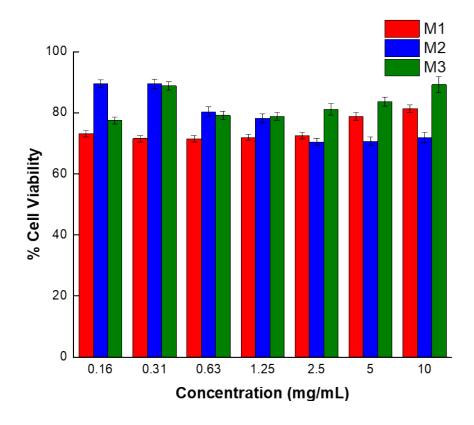
**Fig. S17.** Removal of micelles from protein. Zeta potential analysis of micelles before and after centrifugation.

**Table S1.** Summary of the recovery rates of LDH after ultracentrifugation.

	Protein recovered (µg)	Recovery rate (%)
M1	65.66	91.55 ± 2.83
M2	64.31	92.75 ± 1.87
М3	63.66	94.87 ± 1.29



**Fig. S18.** Isothermal titration calorimetry results of the interaction between poly-SPB and insulin at 25°C.



**Fig. S19.** Cytotoxicity of the micelles. The viabilities of L929 cells were tested after 24 h of treatment with different concentrations of micelles by MTT assay. Error bars indicate standard deviation of the mean.