

Supporting information:

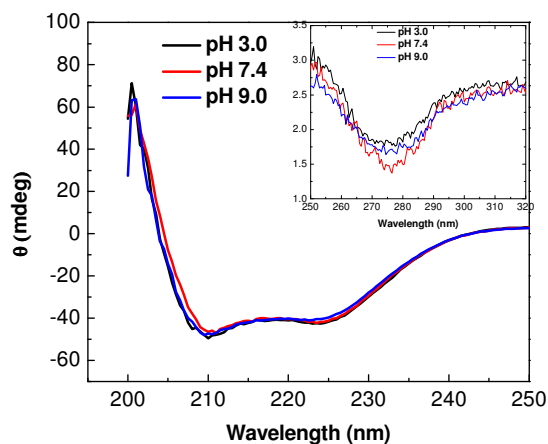


Figure S1. CD spectra of 0.6 g/L Zn-insulin in 10 mM phosphate at different pH conditions, 25 °C. Inset shows peaks at 275 nm corresponding to tertiary structure of protein.

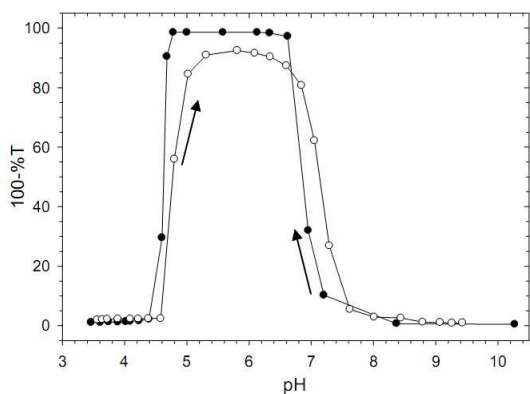


Figure S2. Forward and reverse titration curves for 1 g/L insulin solution titrated from high to low pH with HCl (closed symbols) and from low to high pH with 0.1N NaOH (open symbols). Data obtained from Giger et. al¹

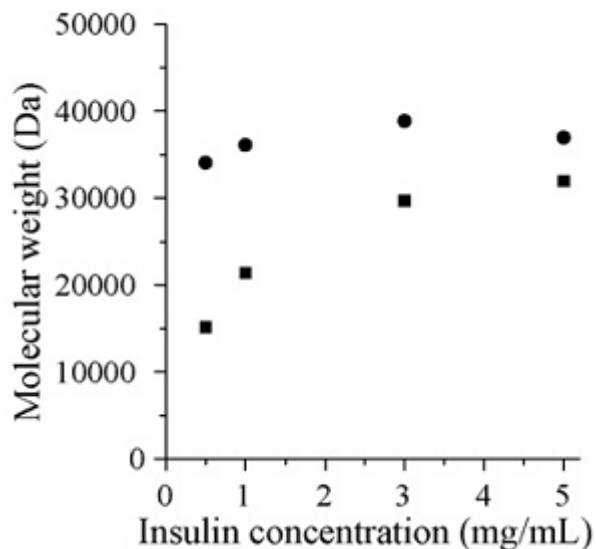


Figure S3. Apparent molecular weight of insulin as a function of protein concentration for elution from SEC (column) in mobile phase with (●) and without (■) 60 μM Zn^{2+} . Courtesy of Dr. Wim Jiskoot²

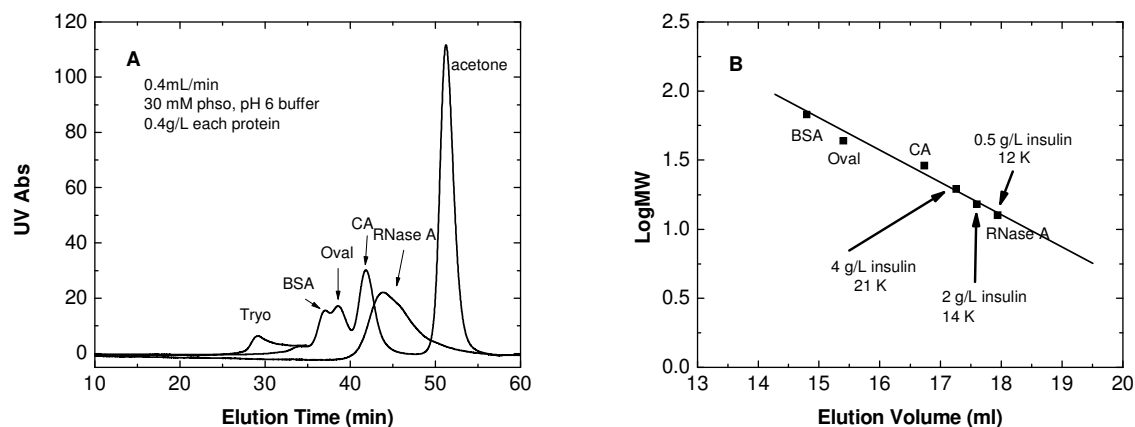


Figure S4. Elution profile (A) and calibration curve (B) of protein standards (0.4 g/L). On xx column, flow rate: 0.4 mL/min, buffer: 30 mM pH 5.9 phosphate buffer. The error in determination of apparent MW of eluted insulin peak is no greater than 5% based on Figure B.

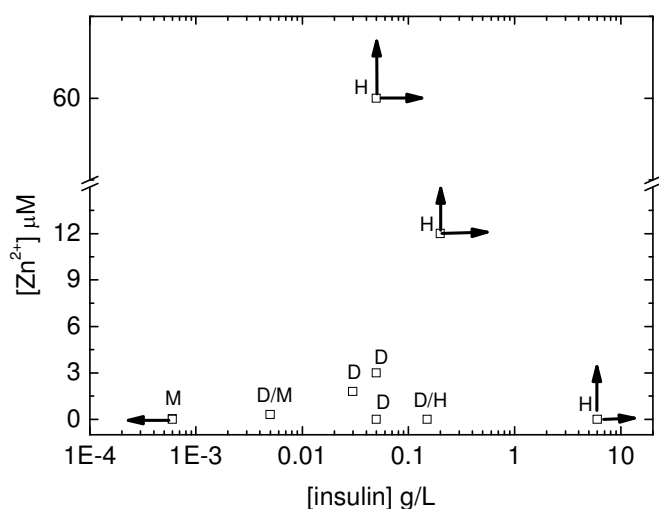


Figure S5. The major forms of insulin at different protein and Zn^{2+} concentrations. The data points were summarized from our data and from the literature.²⁻⁶ In the absence of Zn^{2+} , hexamer is reported to form at $[insulin] > 0.15$ g/L. Note: M, D, H, D/M and D/H represent monomer, dimer, hexamer, dimer/monomer mixture and dimer/hexamer mixture respectively. The arrows represent a value above (or below) which that species is preserved.

References:

- (1) Giger, K.; Vanam, R. P.; Seyrek, E.; Dubin, P. L. *Biomacromolecules* **2008**, *9*, 2338.
- (2) Tantipolphan, R.; Romeijn, S.; Engelsman, J. d.; Torosantucci, R.; Rasmussen, T.; Jiskoot, W. *J. Pharm. Biomed.* **2010**, *52*, 195.
- (3) Bocian, W.; Sitkowski, J.; Tarnowska, A.; Bednarek, E.; Kaw□cki, R.; Koźmiński, W.; Kozerski, L. *Proteins: Structure, Function, and Bioinformatics* **2008**, *71*, 1057.
- (4) Brange, J.; Langkjoer, L. *Pharm. Biotechnol.* **1993**, *5*, 315.
- (5) Andra, N.; Julia, G.; Julia, S.; Vello, T.; Peep, P. *Biochem. J.* **2010**, *430*, 511.
- (6) Attri, A. K.; Fernández, C.; Minton, A. P. *Biophys. Chem.* **2010**, *148*, 23.