Supporting Information

Formation of Protamine and Zn-Insulin Assembly: Exploring Biophysical Consequences

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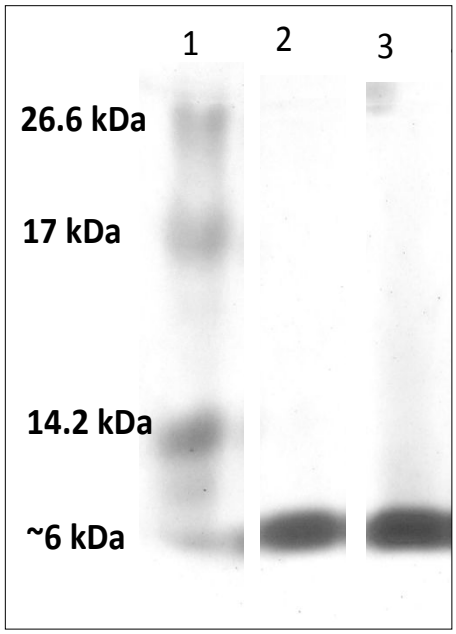

Figure S1: Insulin characterization using Native PAGE. Lane 1: Ultralow molecular weight marker, Lane 2: Insulin, Lane 3: Zn-Insulin.

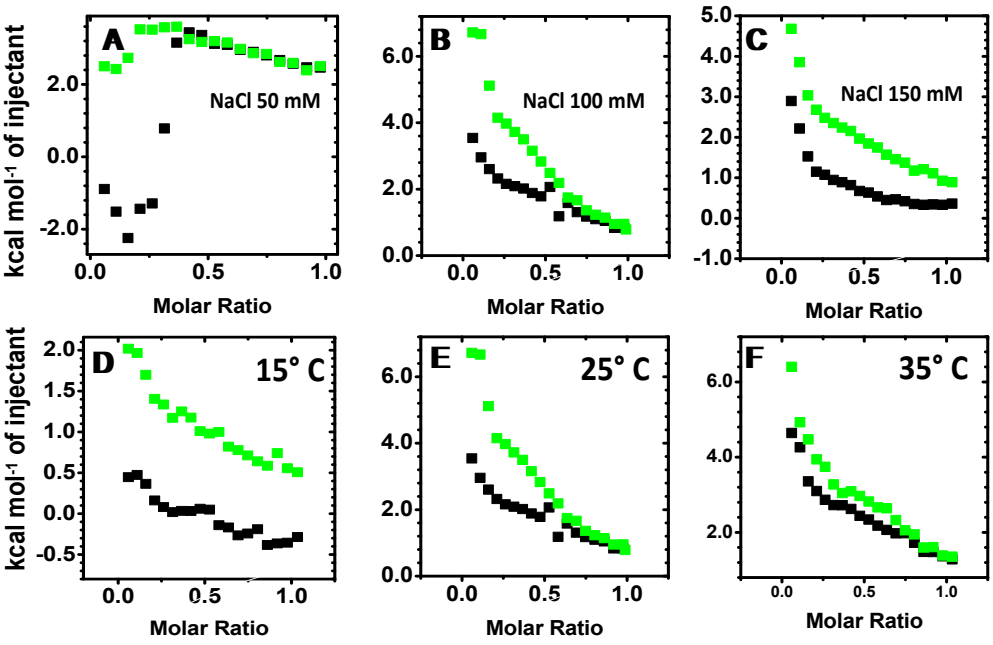

Figure S2: ITC thermogram showing titration of protamine into insulin (A-C) at different salt concentrations, and (D-F) at different temperature. Black points represent raw data and green points represent control experiment. All the experiments were performed in 10 mM phosphate buffer at pH 8.0 on a MicroCal iTC200 (Malvern Instruments Ltd. UK).

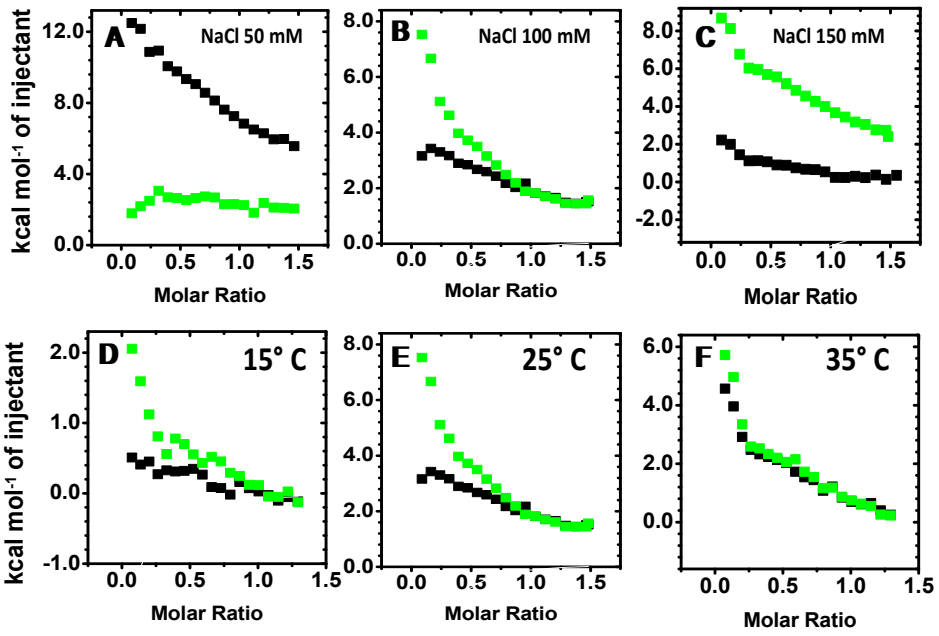

Figure S3: ITC thermogram showing titration of protamine into insulin-Zn-phenol (A-C) at different salt concentrations, and (D-F) at different temperature. Black points represent raw data and green points represent control experiment. All the experiments were performed in 10 mM phosphate buffer at pH 8.0 on a MicroCal iTC200 (Malvern Instruments Ltd. UK).

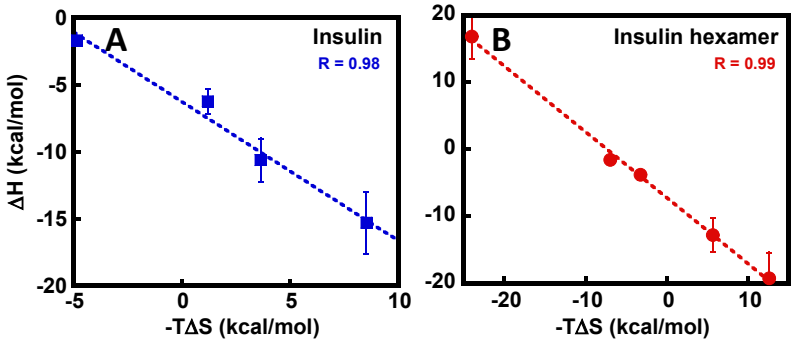

Figure S4: ΔH v/s -TΔS plot of protamine titration into (A) Insulin and (B)Zn- Insulin.

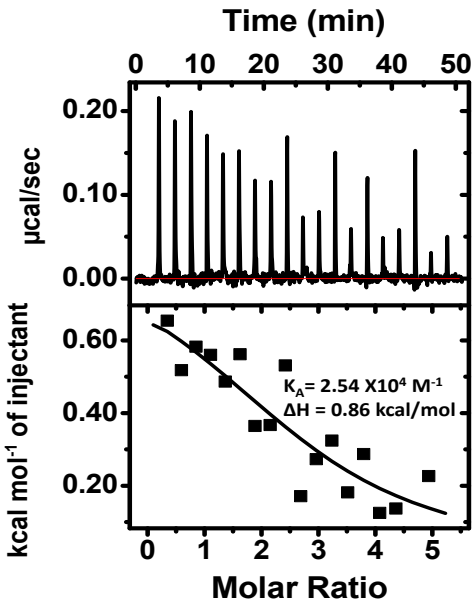

Figure S5: ITC thermogram showing titration of 1 mM Zn^{2+} into 40 µM insulin at room temperature in 10mM phosphate and 100mM NaCl.

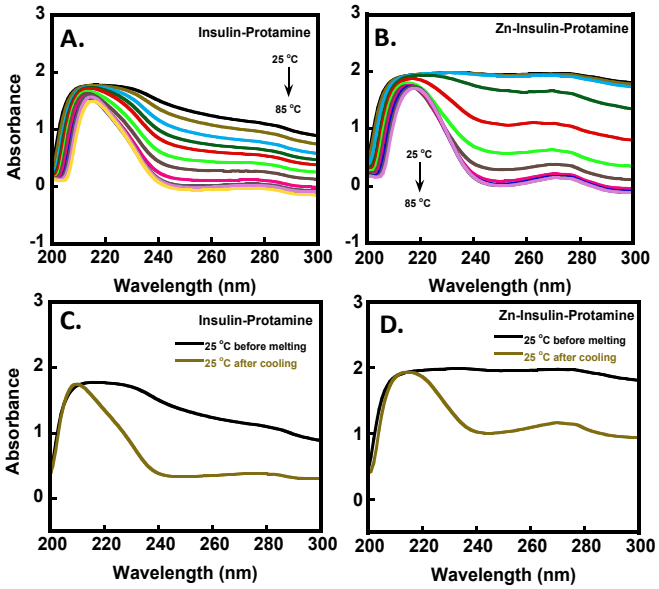

Figure S6: U.V absorbance spectra (A) Insulin-protamine, (B) Zn-Insulin-protamine at different temperature from 25 ^{0}C to 80 ^{0}C, (C) and (D) represent the spectra of Insulin-protamine and Zn-Insulin-protamine respectively at 25 ^{0}C before and after melting, and after cooling down.

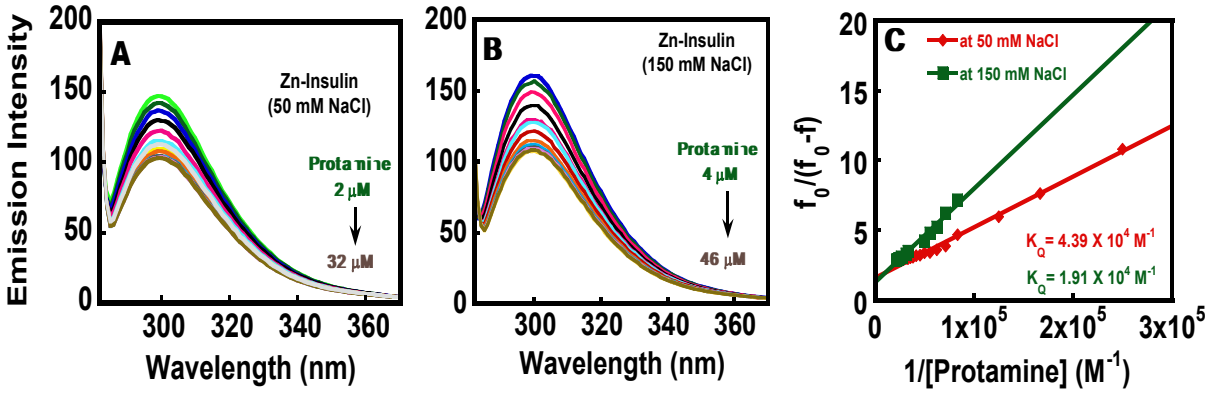

Figure S7: Salt based fluorescence titration of increasing concentration of protamine into Zn-insulin at (A) 50 mM NaCl concentration, and (B) 150 mM NaCl concentration; (C) plot of [(f_o/f -1)] vs 1/[Q] using modified Stern-Volmer equation to determine. Intensity was recorded at 304 nm. All the experiments were performed in 10 mM phosphate buffer at pH 8.0 and 25˚C. The insulin:Zn ratio was 1:0.5 and insulin:phenol ratio was 1:3

Table S1: Parameters obtained from the modified Stern-Volmer plot.

System	K_{sv}	f_a	R^2
Insulin-protamine	22.34X 10^4	0.4	0.98786
(Zn-insulin)-protamine 50 mM NaCl	4.39 X 10^4	0.6	0.99707
(Zn-insulin)-protamine 150 mM NaCl	1.91 X 10^4	0.78	0.99786

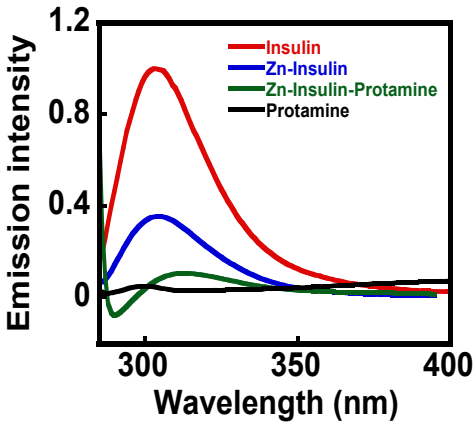

Figure S8: Fluorescence spectra for insulin, Zn-insulin and Zn-insulin-protamine and free protamine. The insulin:Zn ratio was 1:0.5, all the data is normalised w.r.t insulin 20 µM.

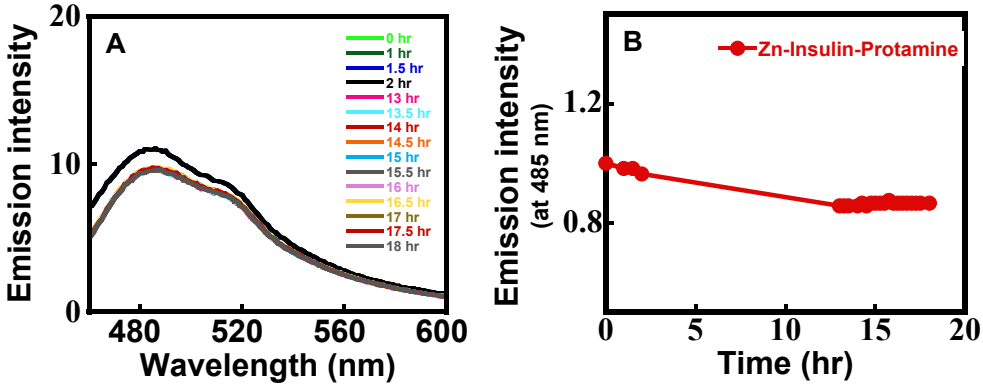

Figure S9: ThT fluorescence of ThT-Zn-Insulin-protamine (A)Emission spectra, excited at 440 nm and emission recorded for 460-600 nm and (B) Plot of emission intensity v/s time upto 18 hrs.

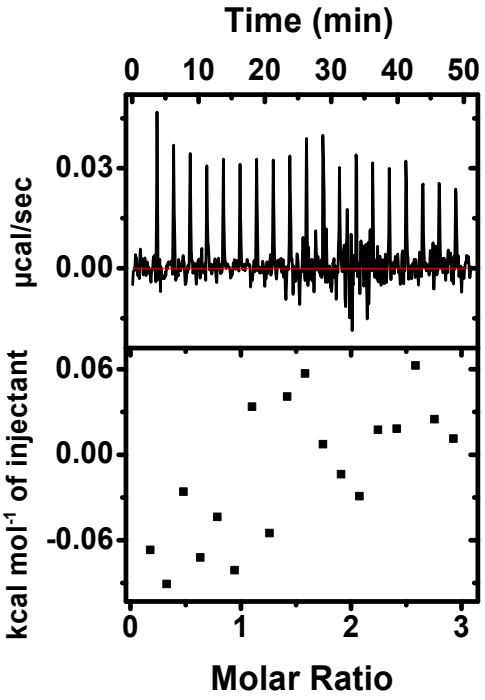

Figure S10 : ITC Binding study of protamine and Zn^{2+}.

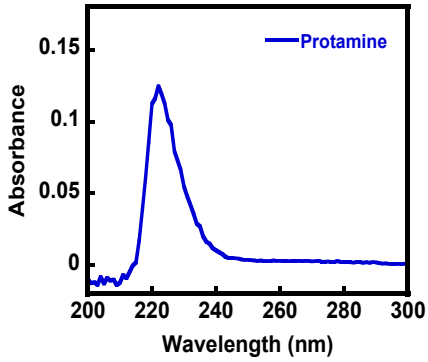

Figure S11: Absorbance spectra of freee protamine. While reporting melting transition of insulin and Zn-insulin in the presence of protamine, we have plotted the absorbance at 276 nm against temperature. As we can see here, protamine shows no absorbance at 276 nm. Thus it does not interfere with the insulin absorbance values.