Supplementary Information (SI) for Soft Matter.

Protein-Polyelectrolyte Complexation – Effects of Sterically Repulsive Groups, Macromolecular Architecture and Hierarchical Assembly

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Supplementary Information

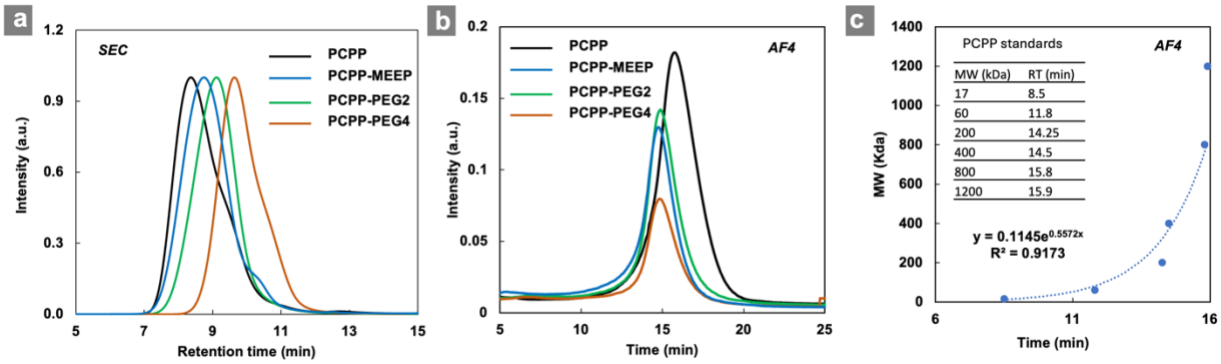

Fig. S1. (a) DLS profiles, (b) AF4 fractograms of PCPP, PCPP-MEEP, PCPP-PEG2 and PCPP-PEG4 and (c) AF4 calibration curves using PCPP standards (0.5 mg/mL polymer, 50 mmol phosphate buffer, pH 7.4).

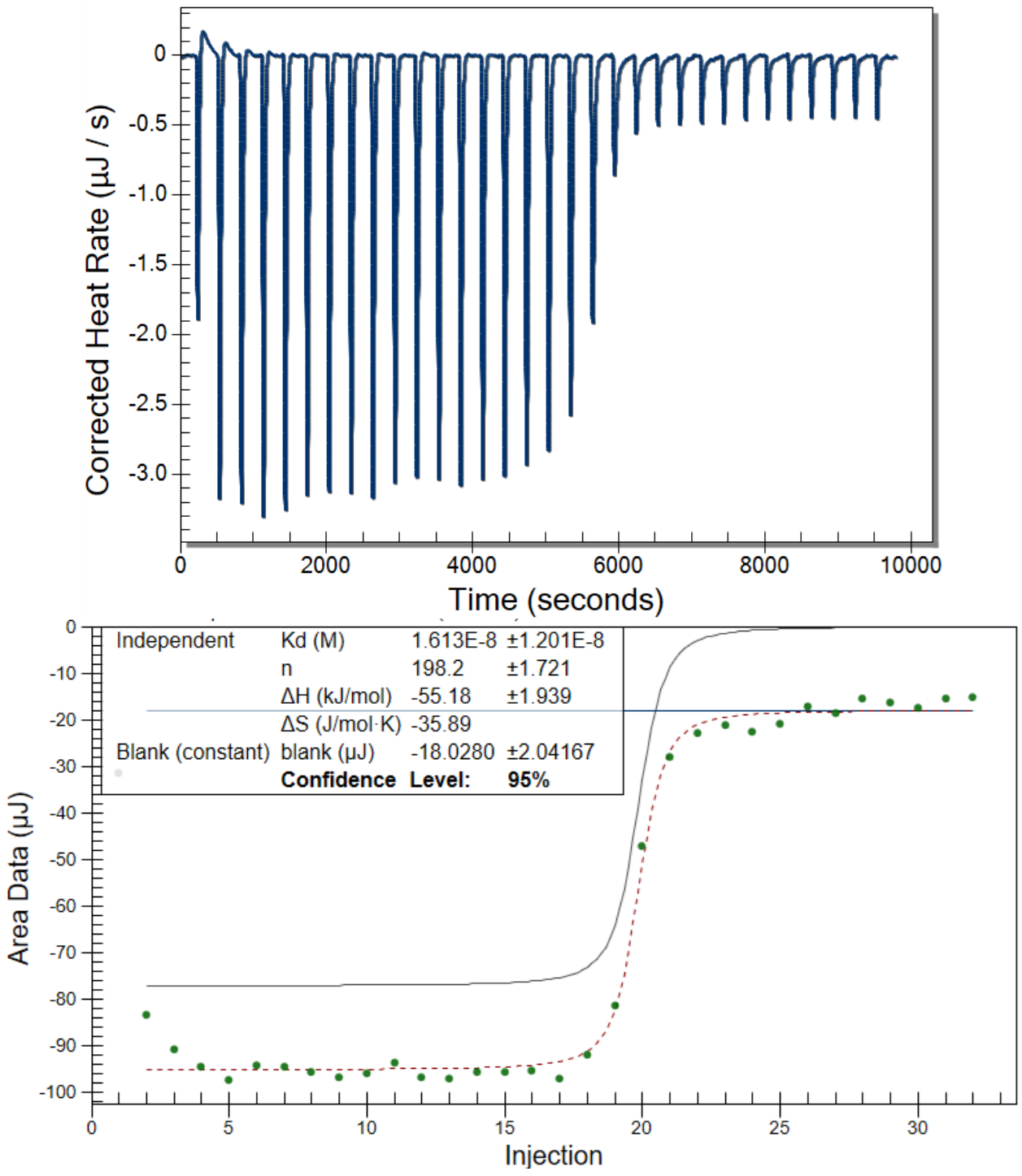

Fig. S2. ITC titration of PCPP with lysozyme (0.125 mg/mL polymer, 2.5 mg/mL protein, 50 mM phosphate buffer, pH 7.5)

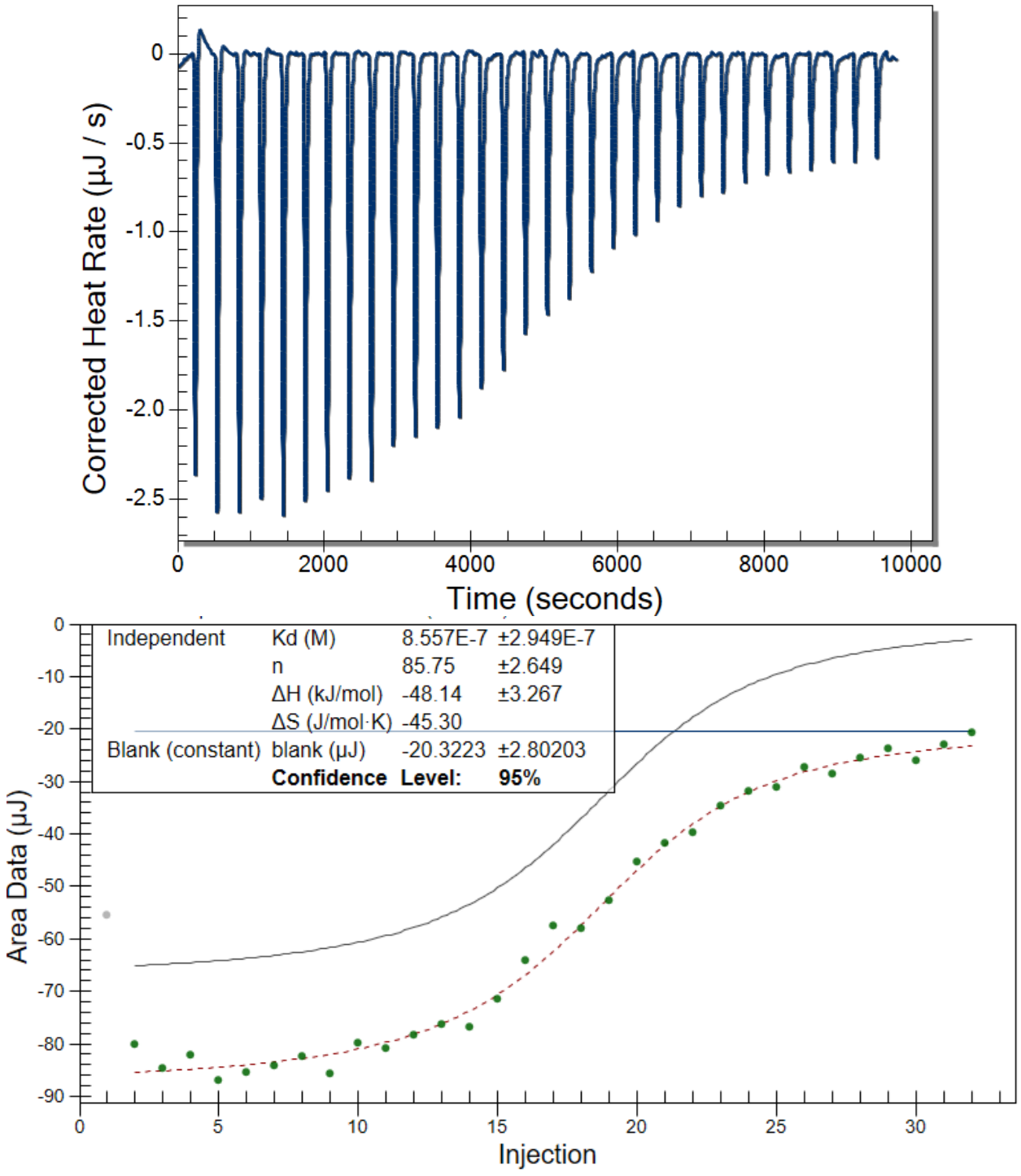

Fig. S3. ITC titration of PCPP-PEG2 with lysozyme (0.125 mg/mL polymer, 2.5 mg/mL protein, 50 mM phosphate buffer, pH 7.5)

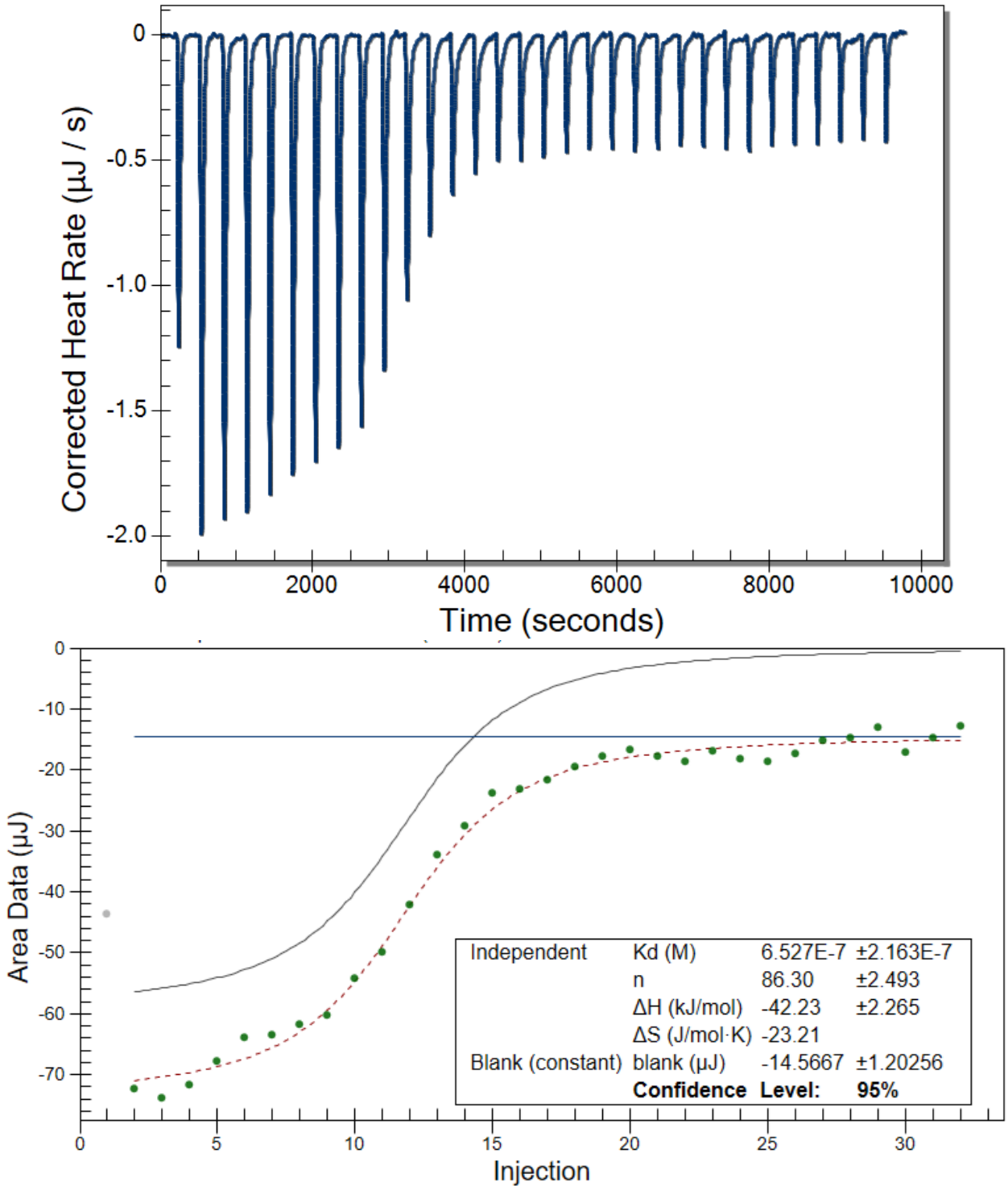

Fig. S4. ITC titration of PCPP-MEEP with lysozyme (0.125 mg/mL polymer, 2.5 mg/mL protein, 50 mM phosphate buffer, pH 7.5)

Table S1. Assessment of apparent distances between adjacent charges in PCPP-PEG2 and PCPP-MEEP copolymers

Polymer	MW[a] (kDa)	mw_r[b]	N[c]	e[d]	L[e] (Å)	b[f] (Å)
PCPP-PEG2	360	521	691	1353	2182	1.6
PCPP-MEEP	600	305	1967	1967	6216	3.2

[a] Molecular weight of the polymer; [b] molecular weight of the polymer repeat unit; [c] degree of polymerization; [d] number of elementary charges (carboxylate ions) per polymer chain; [e] polymer counter chain length - calculated assuming 1.58 as the average length of polyphosphazene skeleton bond (H. R. Allcock, *Chemistry and Applications of Polyphosphazenes*, Wiley, Hoboken, NJ, 2002, page 405); [f] apparent distance between adjacent charges in the polymer chain (b=L/e).

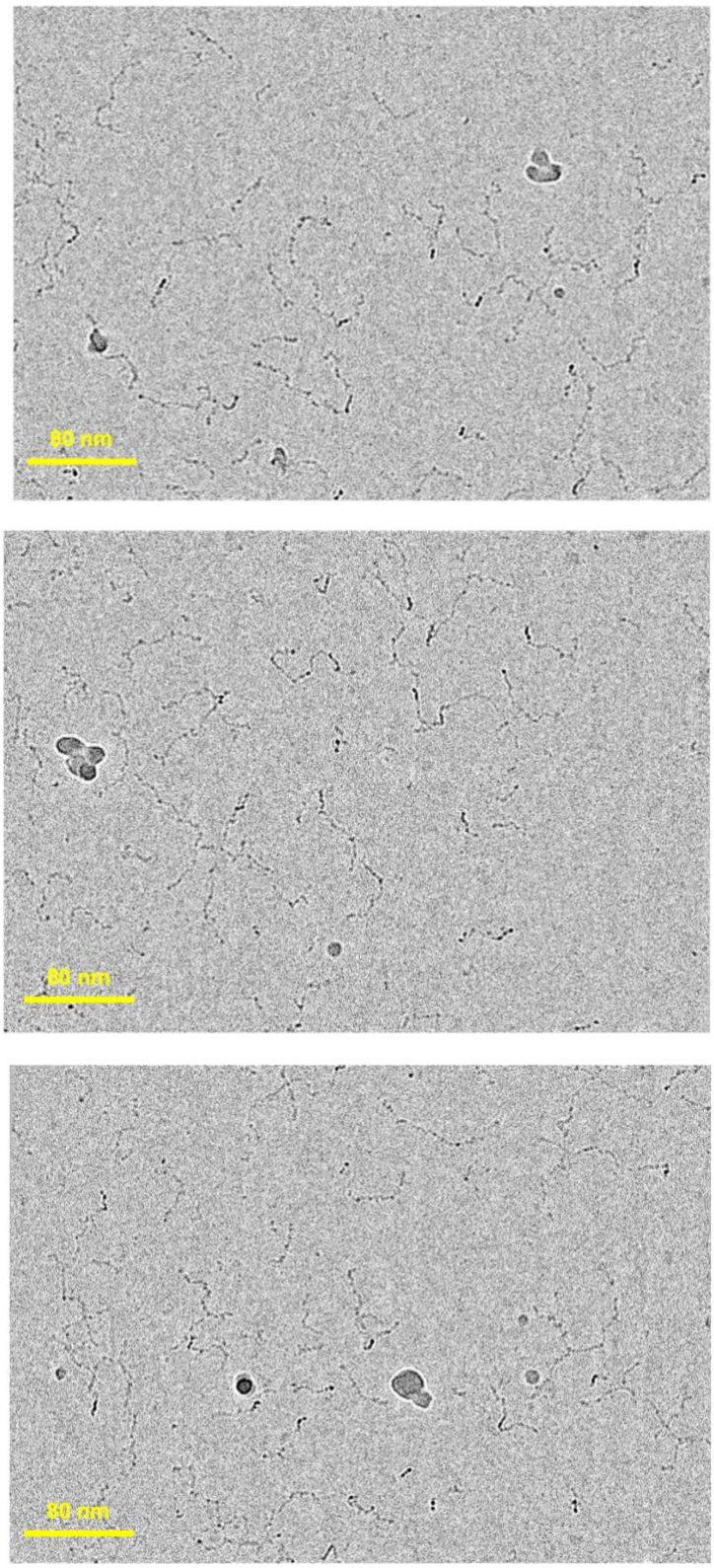

Fig. S5. cryoEM images of PCPP-PEG2 (0.25 mg/mL polymer, 50 mM phosphate buffer).

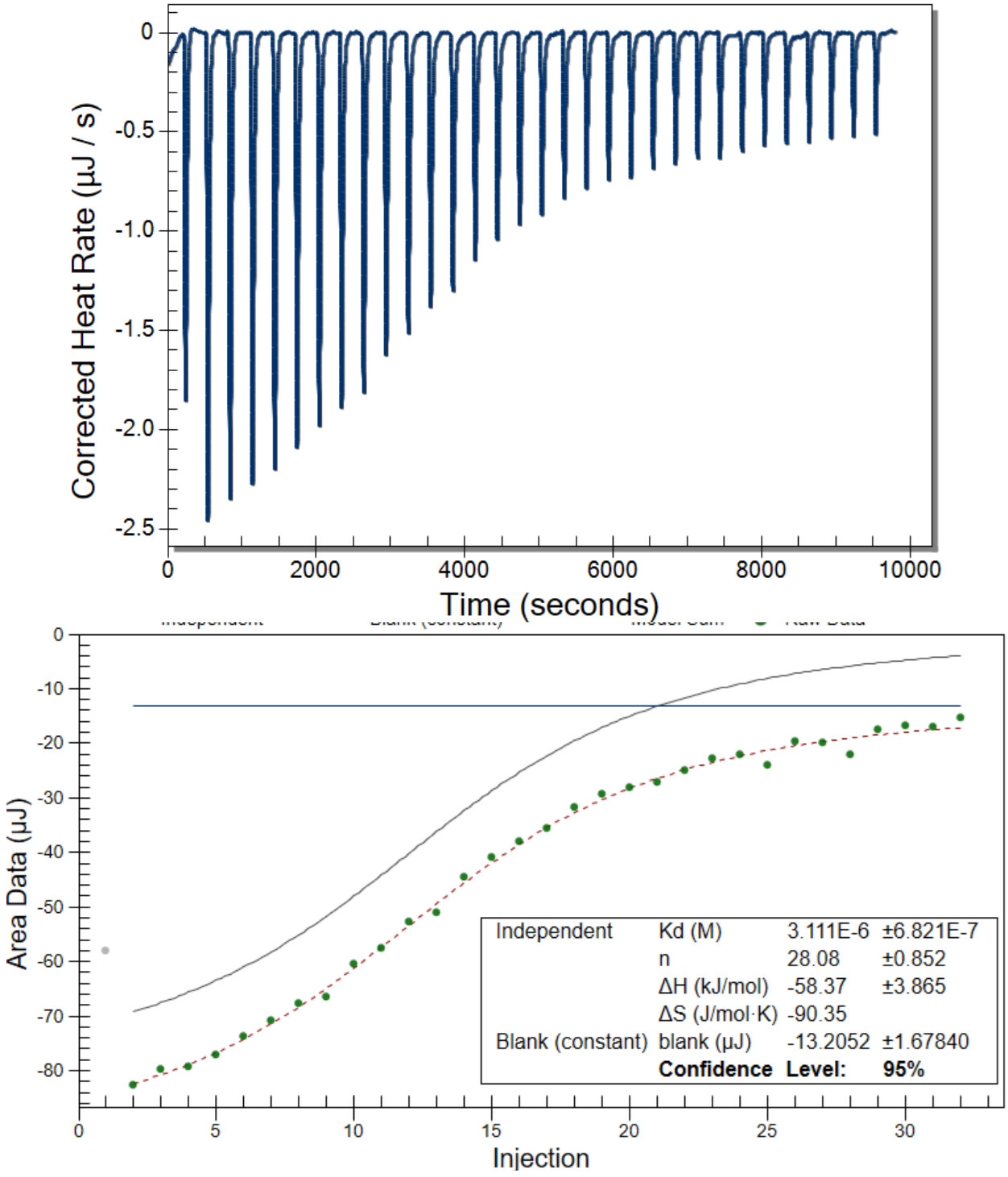

Fig. S6. ITC titration of PCPP-PEG4 with lysozyme (0.125 mg/mL polymer, 2.5 mg/mL protein, 50 mM phosphate buffer, pH 7.5)

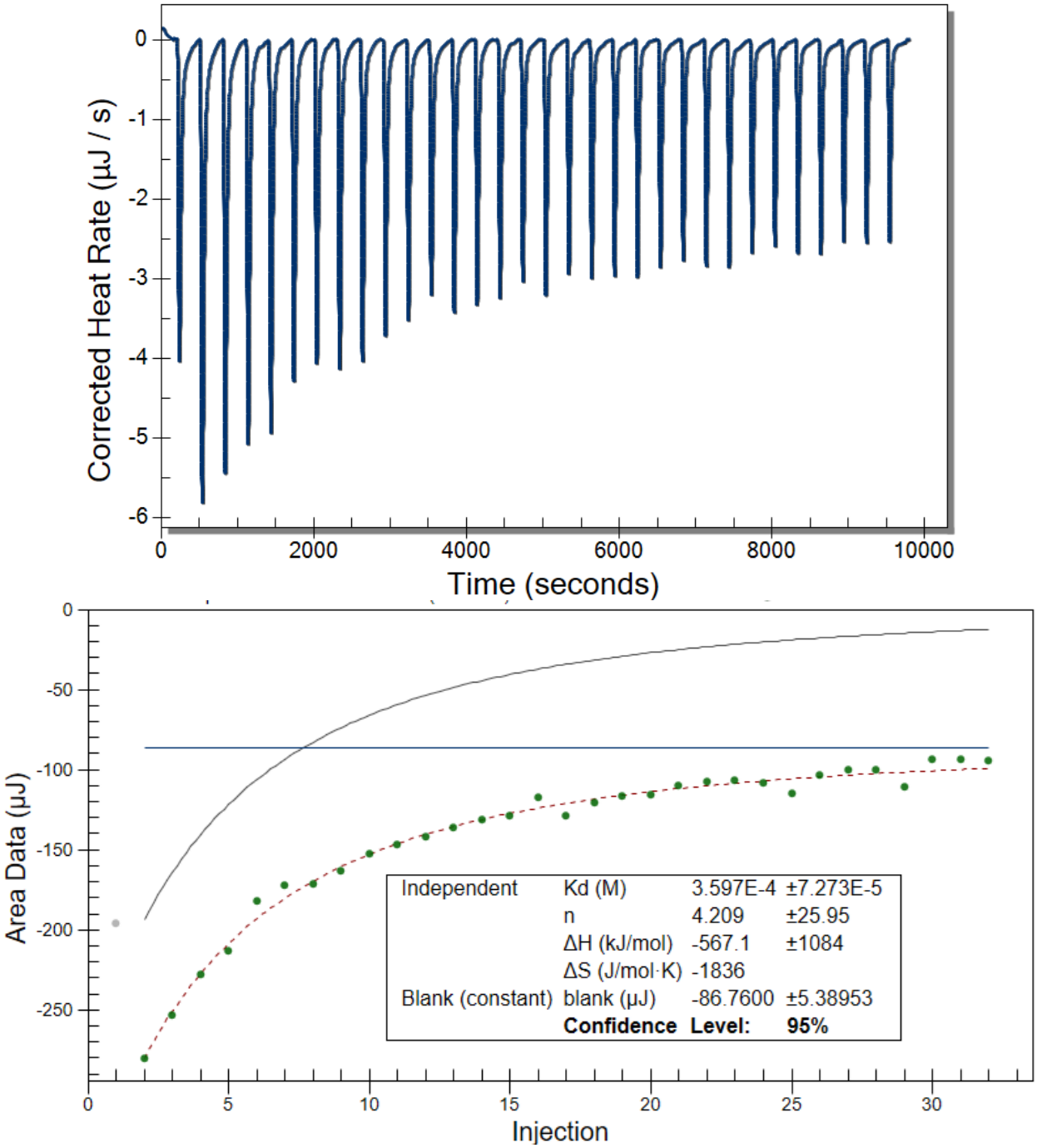

Fig. S7. ITC titration of PCPP with PEG (1 mg/mL PCPP, 20 mg/mL PEG (5 kDa), 50 mM phosphate buffer, pH 7.5)

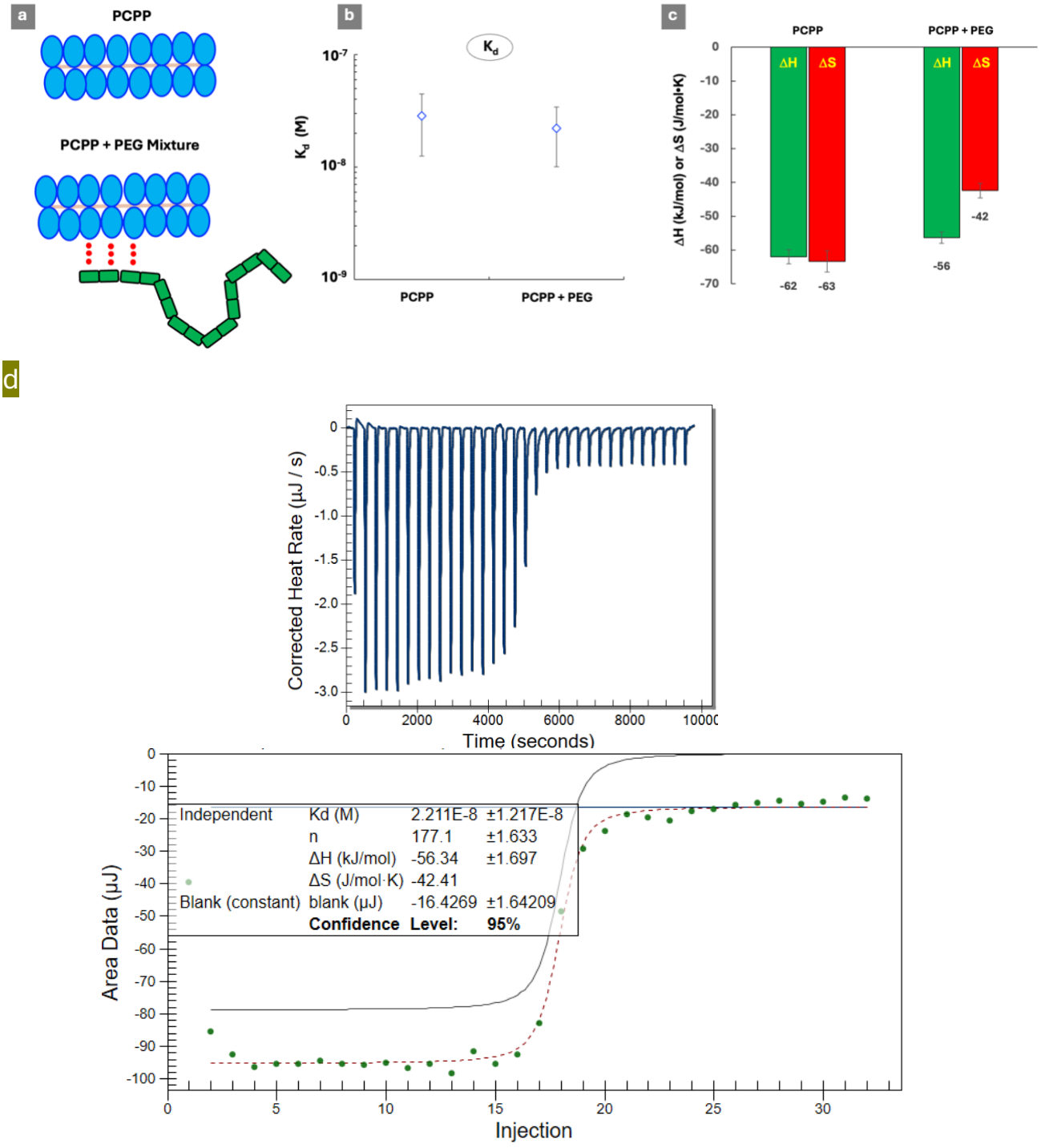

Fig. S8. ITC titration of PCPP and PEG mixture with lysozyme: (a) schematic of PCPP-PEG interactions via formation of hydrogen bonds, comparison of (b) dissociation constants of PCPP – lysozyme and (c) thermodynamic patterns of polymer-protein interactions in the absence and presence of PEG and (d) ITC raw data (0.125 mg/mL polymer, 0.1 mg/mL PEG (5kDa), 2.5 mg/mL protein, 50 mM phosphate buffer, pH 7.5)

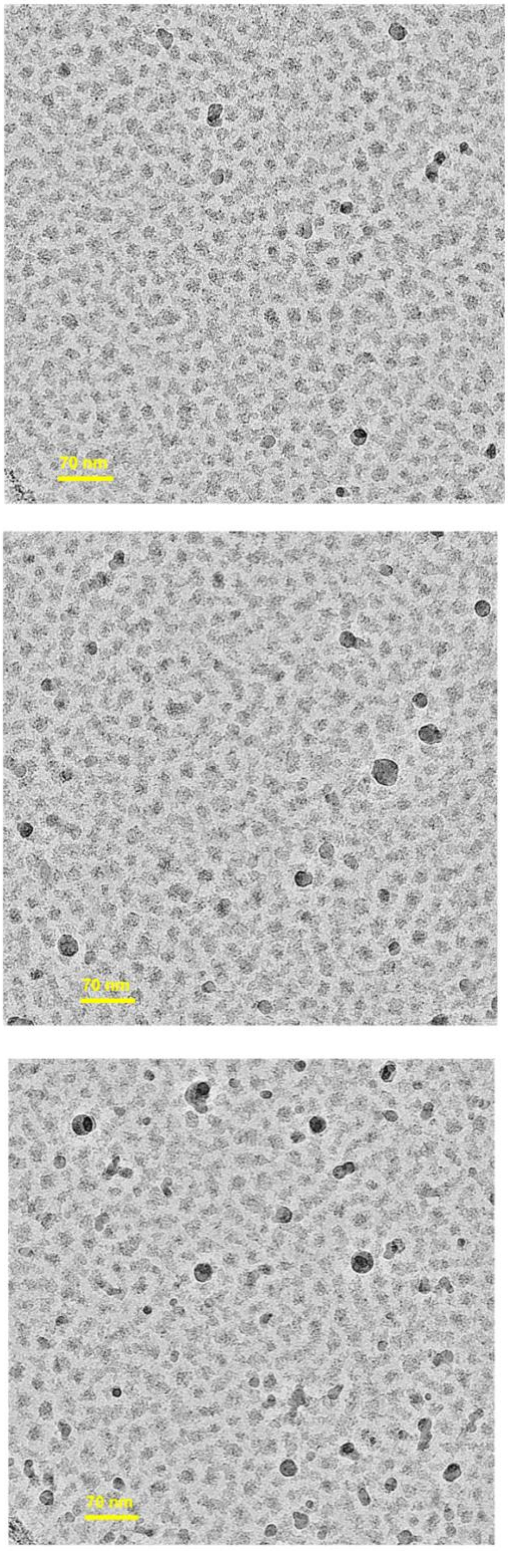

Fig. S9. CryoEM images of PCPP-PEG2 cross-linked with spermine (1 mg/mL PCPP-PEG2, 1mg/mL spermine, 50 mM phosphate buffer, pH 7.4)

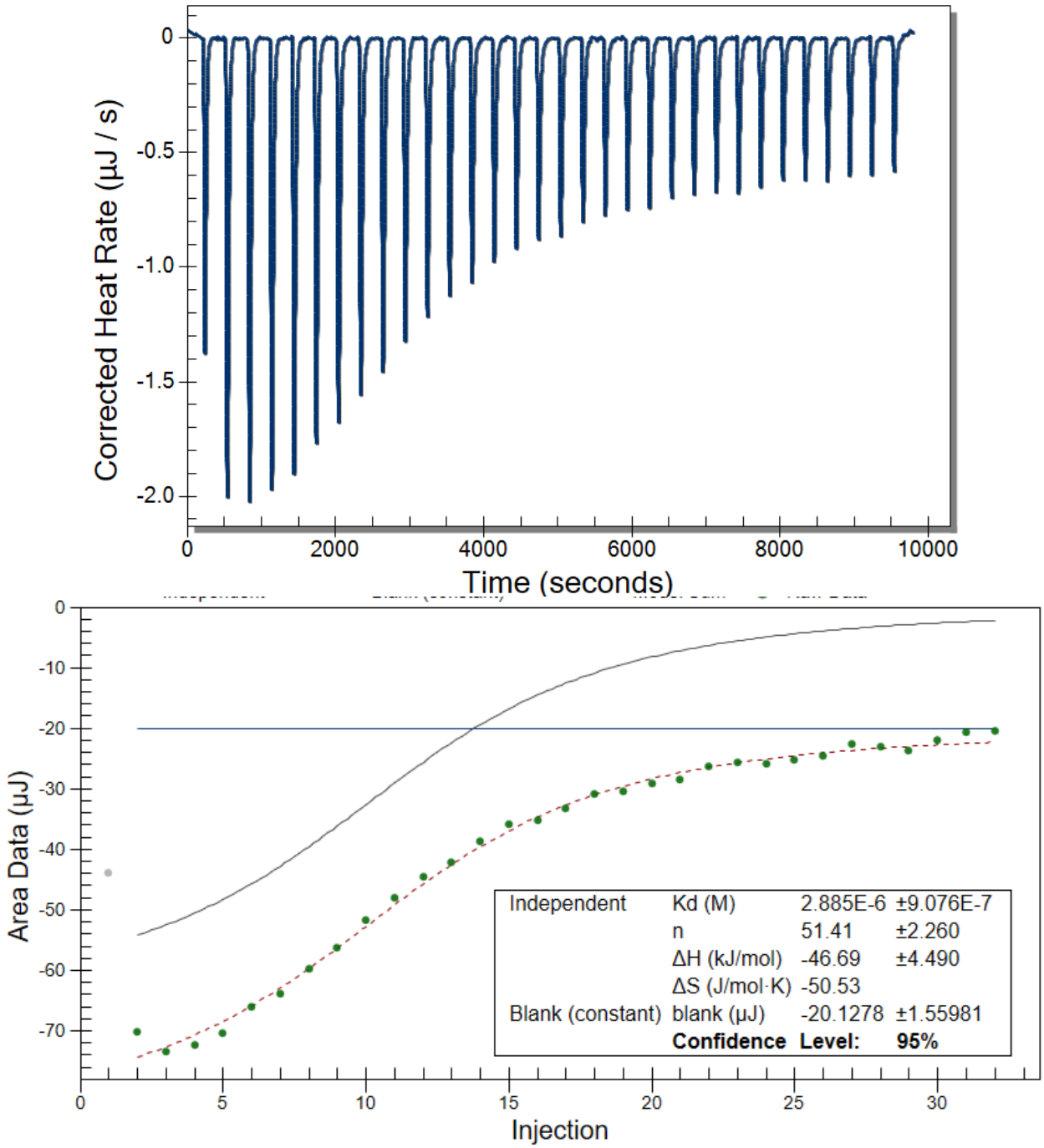

Fig. S10. ITC titration of spermine cross-linked PCPP-PEG2 (NG-01) with lysozyme (0.125 mg/mL nanogel, 2.5 mg/mL protein, 50 mM phosphate buffer, pH 7.5)

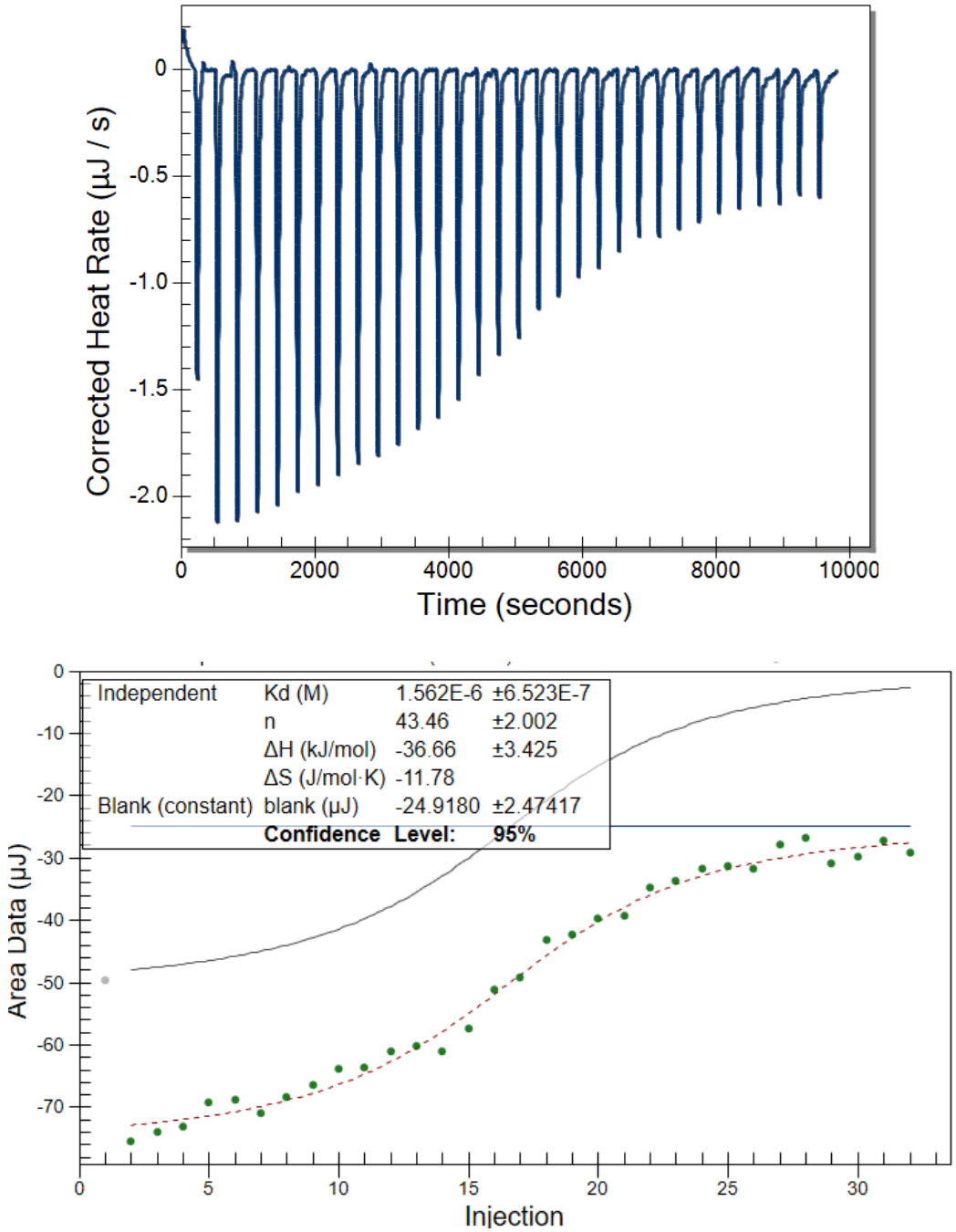

Fig. S11. ITC titration of PCPP-PEG2 with lysozyme in the presence of 50 mM of potassium chloride (0.125 mg/mL polymer, 2.5 mg/mL protein, 50 mM phosphate buffer, pH 7.5)

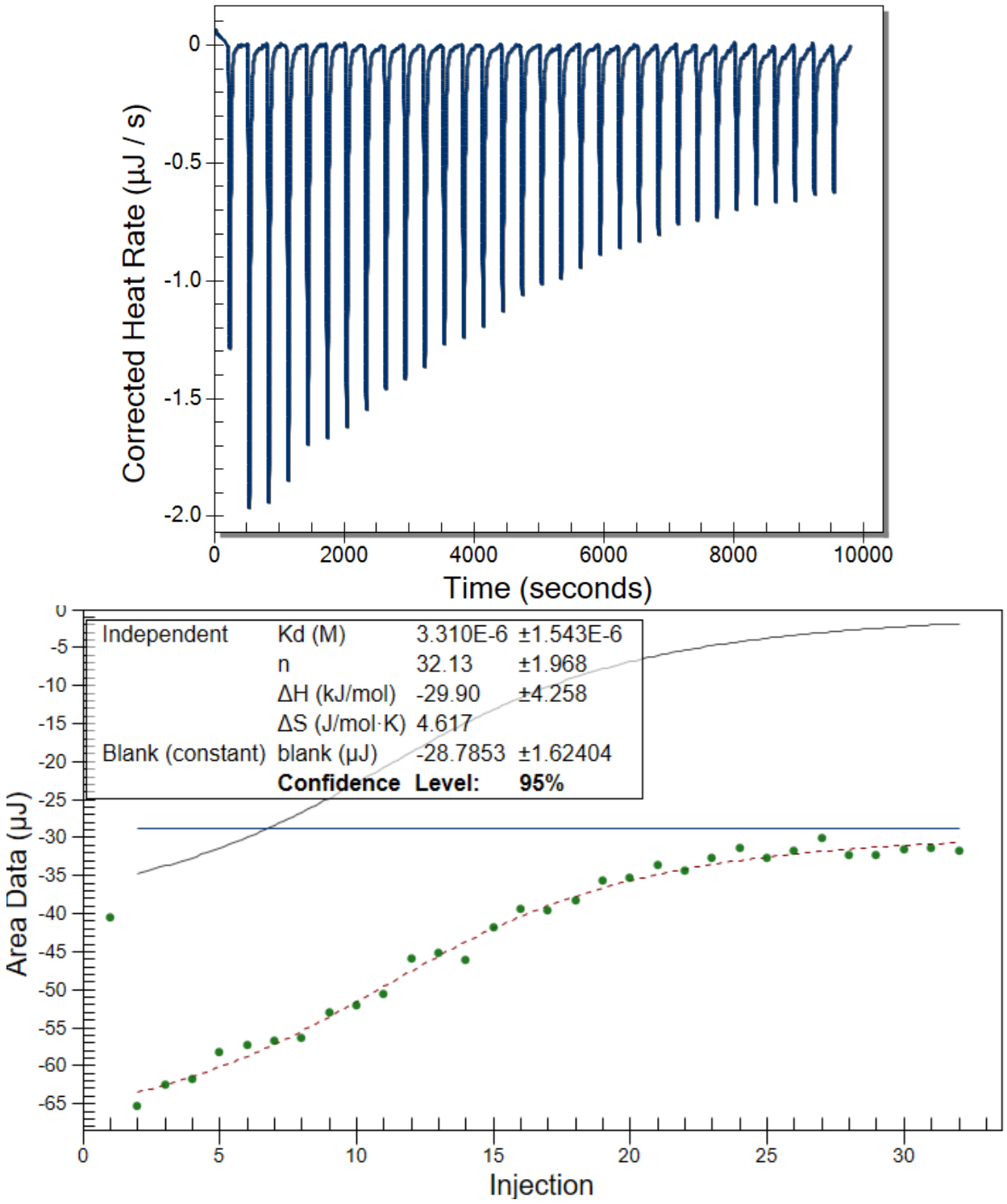

Fig. S12. ITC titration of PCPP-PEG2 with lysozyme in the presence of 100 mM of potassium chloride (0.125 mg/mL polymer, 2.5 mg/mL protein, 50 mM phosphate buffer, pH 7.5)