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Supplementary Information

Structure and function of proteins in hydrated choline dihydrogen phosphate ionic liquid†

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Experimental

Small angle x-ray scattering

Guinier analysis

SAXS from globular proteins will exhibit a linear relationship at low Q (where $Q \times R_g < 1.30$), and according to the following relationship (Equation S1), the Guinier approximation method was used for estimating values for R_g and I_0.

$$I(Q) = I_0 \times e^{\left(\frac{-R_g^2 Q^2}{3}\right)} \qquad \text{S1}$$

P(r) analysis

A method for evaluating the I_0 and R_g values that uses the entire measured scattering profile is to calculate them from the P(r) function as the 0^{th} and 2^{nd} moment, respectively. The P(r) function for a given set of scattering data was determined by an indirect inverse Fourier Transform method using the program package GNOM.[1] The P(r) reflects the probability of finding scattering centers within the protein separated by a distance of r. Because the scattering data is measured over a finite q-range, P(r) calculations are done via indirect methods and are based on the assumption that the P(r) will approach zero at r = 0 and at the maximum linear dimension,

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D_{max}. Thus, D_{max} is a 'soft' or model parameter in interpretation of scattering data. The uncertainty in D_{max} is highly dependent on the quality of the data and is generally not reported.

Table S1: Lysozyme-CDHP formulations used for solubility assay[a]

Lys, mg/mL	Absorbance at 520 nm									
	Percent (w/w) CDHP									
	0	5	10	20	30	40	50	60	70	80
0.8	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NT
1.0	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.006
2.0	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.000	NT
4.1	0.001	0.000	0.001	0.002	0.001	0.000	0.001	0.001	0.000	NT
6.2	0.001	0.002	0.001	0.003	0.001	0.001	0.001	0.001	0.000	NT
8.2	0.002	0.002	0.002	0.003	0.001	0.001	0.002	0.001	0.001	NT
10.0	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.024 P
20.5	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.000	NT	NT
25.0	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.042 P
41.0	0.002	0.002	0.003	NT	0.003	NT	NT	NT	NT	NT
82.1	0.003	NT	NT	NT	NT	NT	NT	NT	NT	NT

[a] NT: not tested; P: particulates observed by macroscopic examination. The level of aggregation was monitored by assessing the absorbance at 450-750 nm. A concentrated stock lysozyme solution of 85.9 mg/mL was prepared in NaOAc buffer (0.1 M, pH 4.00). A stock solution of hydrated CDHP 80% (w/w) was prepared by dissolving anhydrous CDHP salt with 0.1 M NaOAc solution at pH 4.00, followed by pH readjustment (H_3PO_4) so that the final solution was pH 4.00. All test sample solutions were prepared and maintained at room

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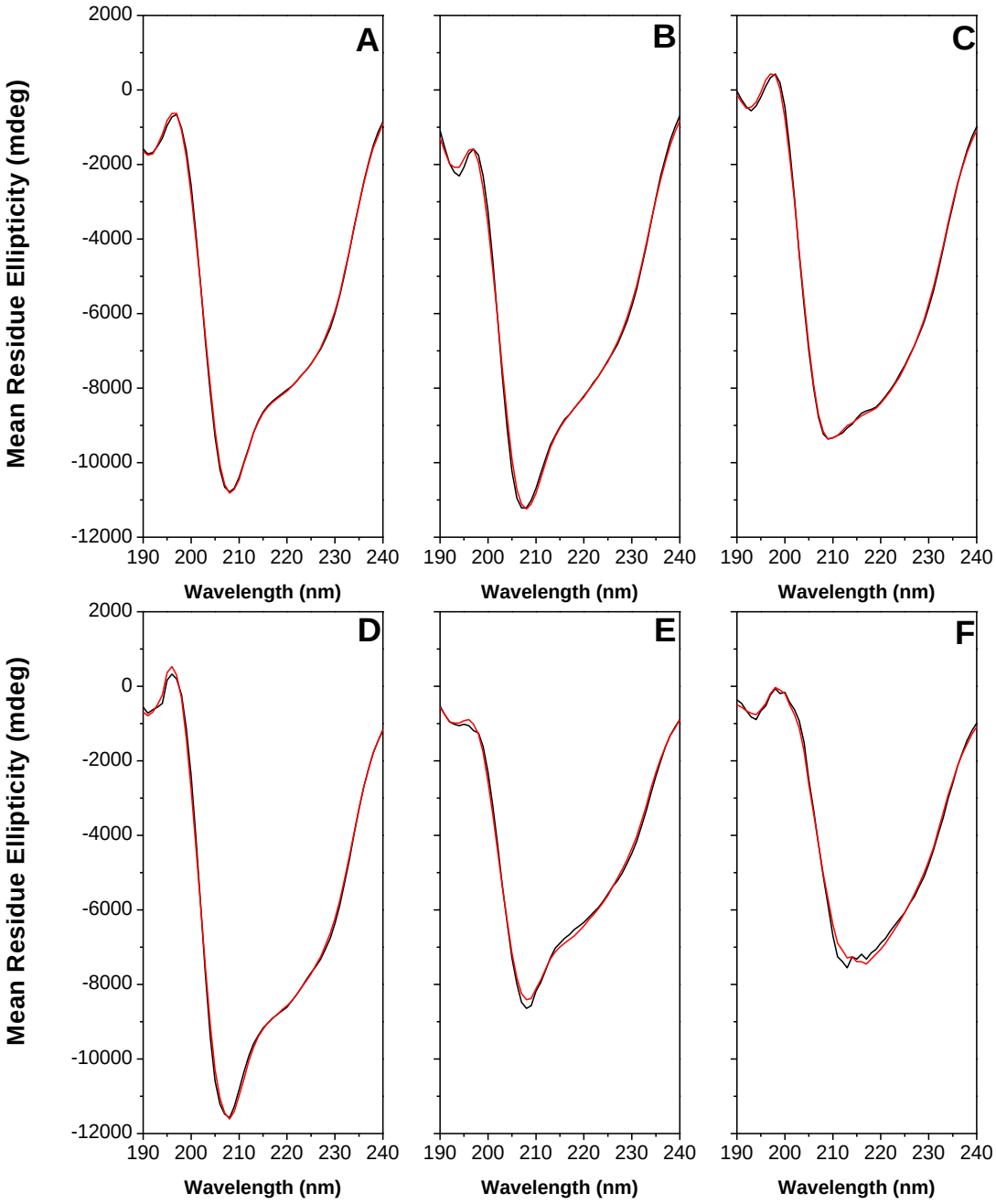
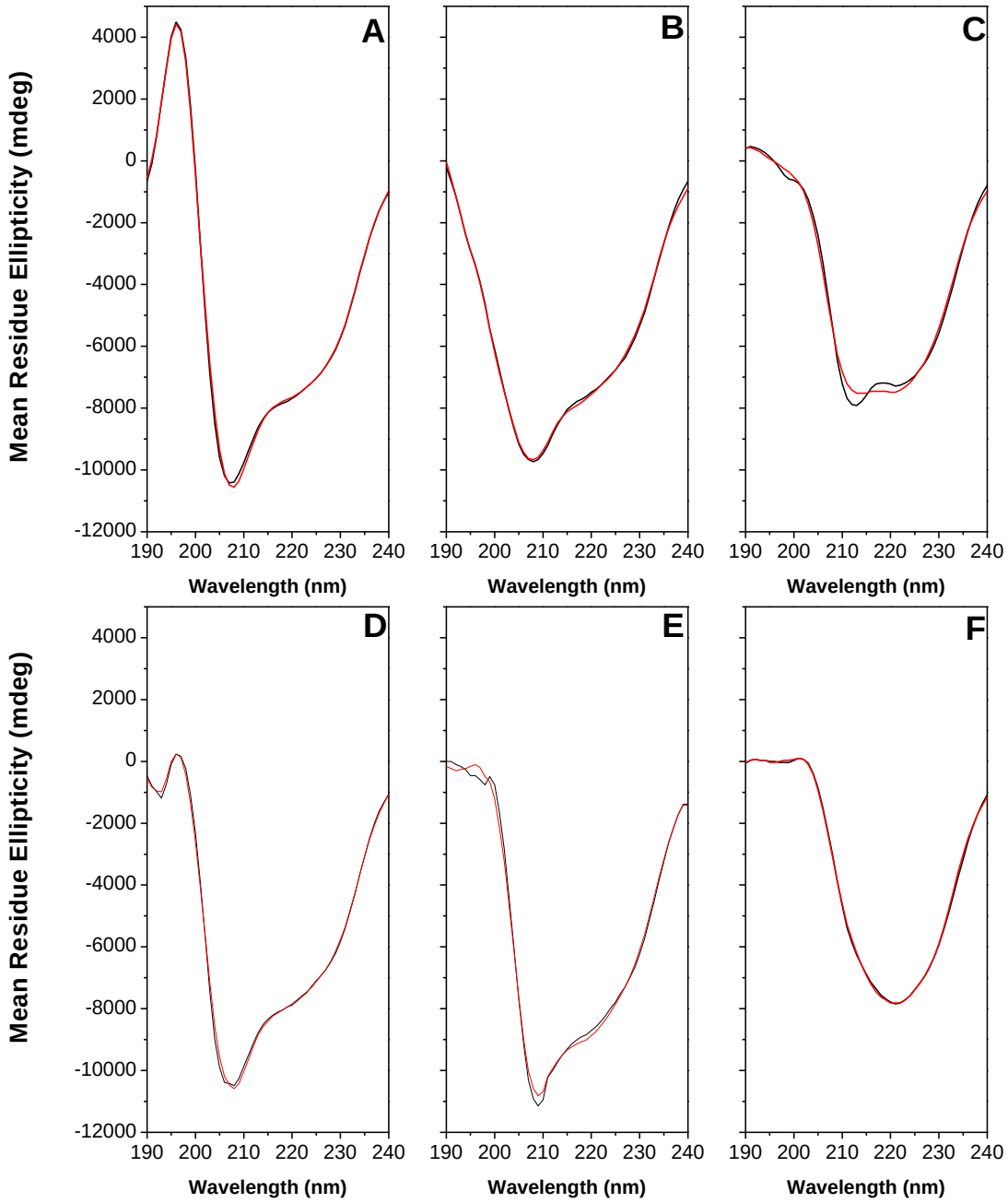
temperature in silanized glass vials. For each assessment of turbidity, an aliquot of stock protein solution was diluted with an appropriate volume of CDHP, and stock NaOAc solutions to a final volume of 0.5 mL, and mixed gently. For lysozyme solutions in 80% (w/w) CDHP a solvent composed of 80% (w/w) CDHP, 0.1 M NaOAc, pH 4.00 was added directly to lyophilized-protein. Observations were consistent over the 13 week time period of study, and the final absorbance readings at week 13 are listed here.

Figure S1 – Panel 1



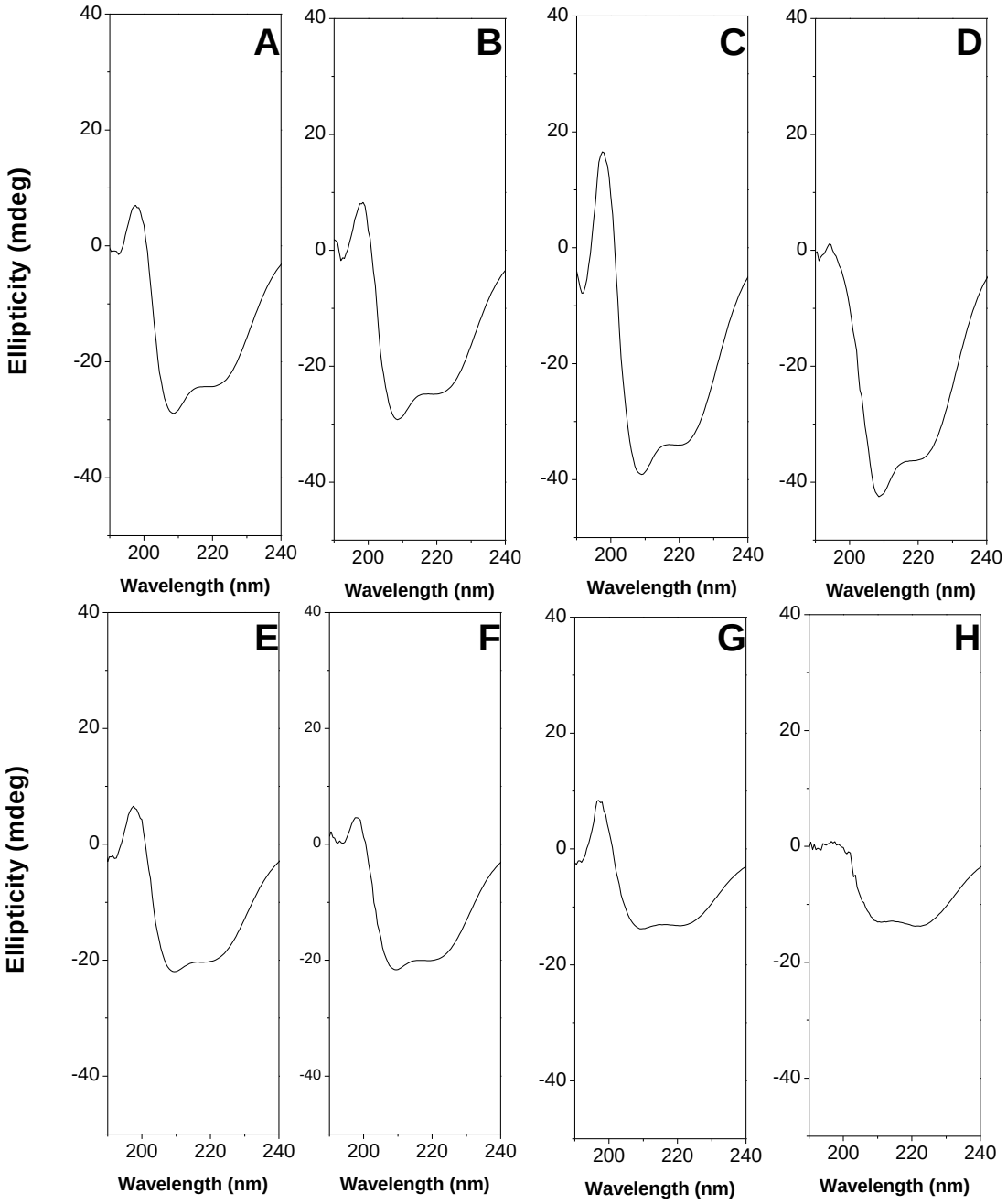
Representative Dichroweb circular dichroism spectral output [experimental (—), reconstructed (—)]: 0.50 mg/ml lysozyme in (A) 0.1 M NaOAc, 0% CDHP, pH 4.00, (B) 0.1 M NaOAc, 20% CDHP, pH 4.00, (C) 0.1 M NaOAc, 40% CDHP, pH 4.00, and 0.50 mg/ml lysozyme after incubation at 37 °C for 12 weeks in (D) 0.1 M NaOAc, 0% CDHP, pH 4.00, (E) 0.1 M NaOAc, 20% CDHP, pH 4.00, (F) 0.1 M NaOAc, 40% CDHP, pH 4.00.

Figure S1 – Panel 2



Representative Dichroweb circular dichroism spectral output [experimental (—), reconstructed (—)]: 0.50 mg/ml lysozyme in (A) 0.1 M NaH_2PO_4, 0% CDHP, pH 7.20, (B) 0.1 M NaH_2PO_4, 20% CDHP, pH 7.20, (C) 0.1 M NaH_2PO_4, 40% CDHP, pH 7.20, and 0.50 mg/ml lysozyme after incubation at 37 °C for 12 weeks in (D) 0.1 M NaH_2PO_4, 0% CDHP, pH 7.20, (E) 0.1 M NaH_2PO_4, 20% CDHP, pH 7.20, (F) 0.1 M NaH_2PO_4, 40% CDHP, pH 7.20.

Figure S1 – Panel 3



Representative experimental circular dichroism spectral output: 0.38 mg/ml IL-2 in (A) 30 mM NaH_2PO_4, 0% CDHP, pH 7.40, (B) ~0.5% (30 mM) CDHP, pH 7.40, (C) ~3.3% (185 mM) CDHP, pH 7.40, (D) ~12% (680 mM) CDHP, pH 7.40. Solutions were rescanned after cooling following thermal denaturation: (E) 30 mM NaH_2PO_4, 0% CDHP, pH 7.40, (F) ~0.5% (30 mM) CDHP, pH 7.40, (G) ~3.3% (185 mM) CDHP, pH 7.40, (H) ~12% (680 mM) CDHP, pH 7.40.

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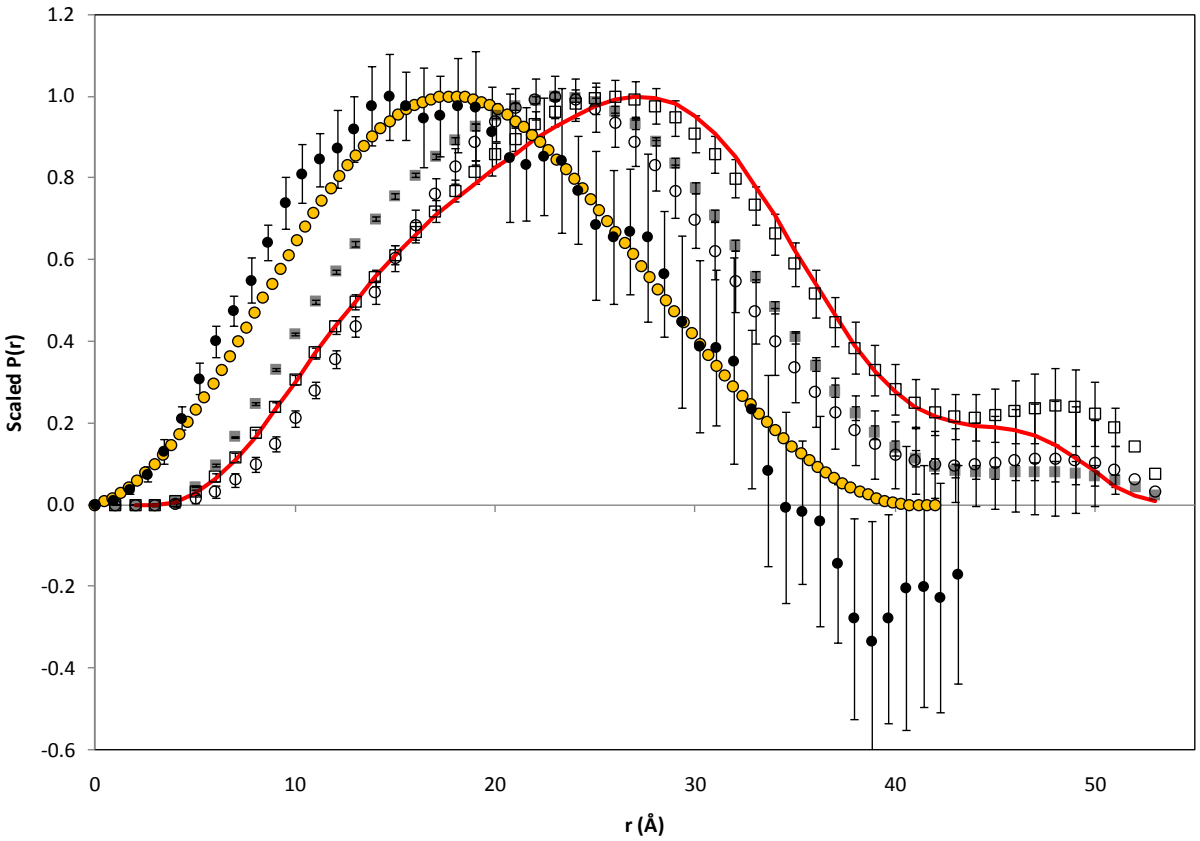
Several methods were explored using the DICHROWEB server for the deconvolution of the lysozyme and IL-2 CD data. The CONTIN, SELCON, and K2d algorithms were rejected because the standard goodness-of-fit parameter [the normalized root mean square (NRMSD)] values were not less than 0.1 using the CONTIN and SELCON algorithms, and the reported error was high for the Kd2 algorithm for the highest concentration of CDHP used.[2] In addition, the correlation between experimental and calculated spectra was poorer when using CONTIN, SELCON, or K2d algorithms.

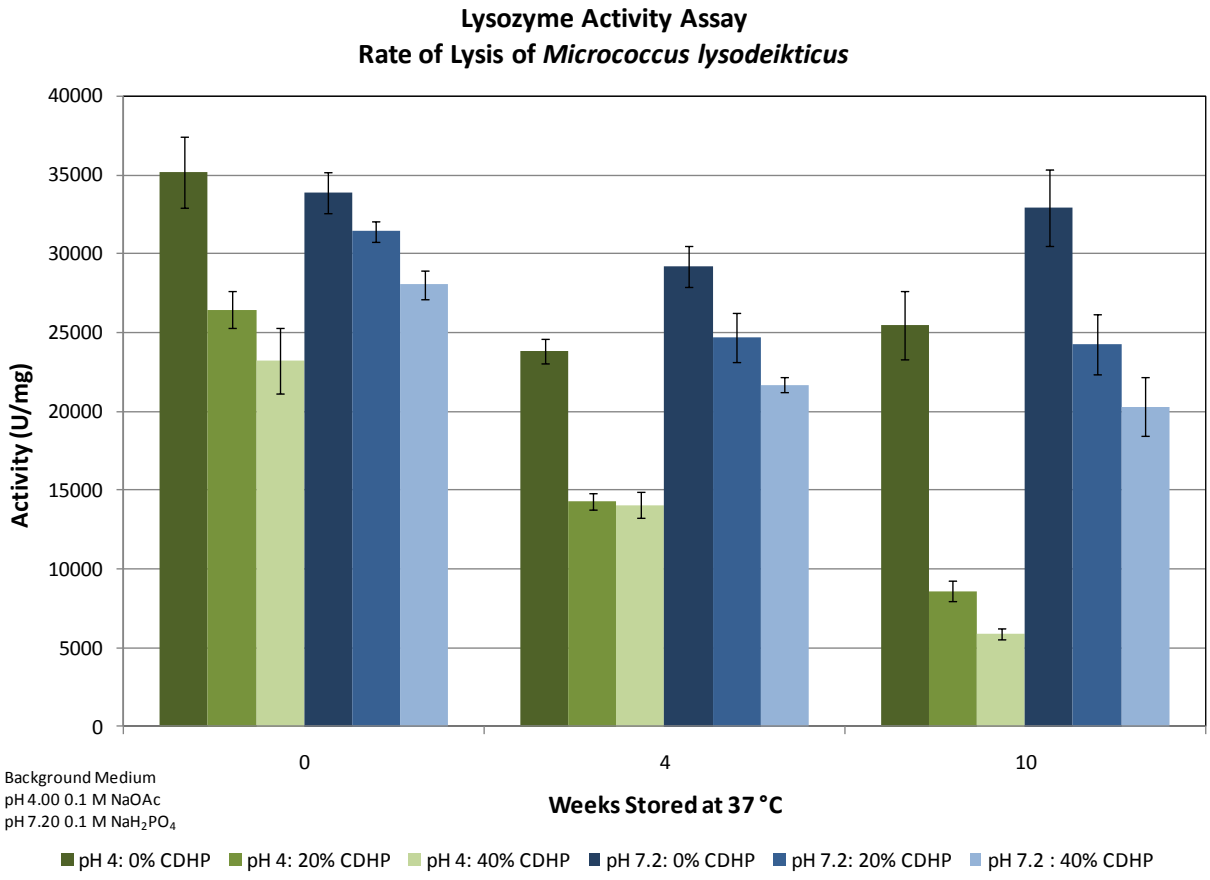
With NRMSD values all below 0.1, and excellent correspondence of the calculated and experimental secondary structure, the CDSSTR algorithm using reference database sets 4, 7, and SP175 proved to be the best method for assessing secondary structure for IL-2 and lysozyme. Out of the three databases used, reference set 7 yielded the lowest NRMSD values, and the closest spectral fits, for IL-2 (see manuscript, Table 2) where as reference set SP175 proved best for lysozyme (see manuscript, Table 1). The reported secondary assignments from CDSSTR are split into six classes: regular α-helix, distorted α-helix, regular β-strand, distorted β-sstran, turns, and unordered.[3] The regular and distorted α-helix, and β-sheet content have been combined for this report (see manuscript, Tables 1, 2).

Figure S2



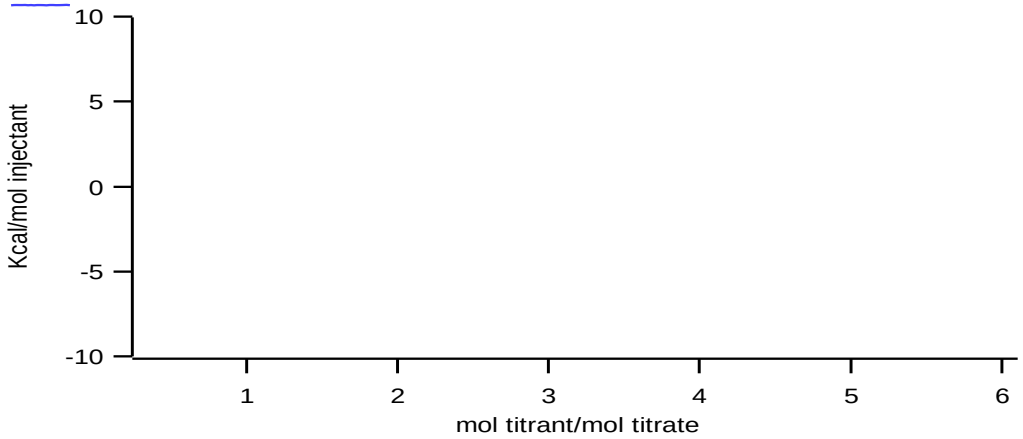
Representative P(r) profiles calculated from the SAXS data (Q-range 0.015 – 0.30 $Å^{-1}$) for approximately 5 mg/mL lysozyme in: 0.1 M NaOAc at pH 3.80 without CDHP (●) and with 20% (w/w) CDHP (○); 0.1 M NaH_2PO_4 at pH 7.20 without CDHP (■) and with 20% (w/w) CDHP (□) versus that calculated for the X-Ray crystal structure form the protein data bank, 1BHZ (red). The yellow circles represent the scattering profile extrapolated from a concentration series of lysozyme in 0.1 M NaOAc at pH 3.80. The amount of variation indicated by the error bars is related to the beam intensity at the time of sampling and counting statistics.

Figure S3



Time course of lysozyme activity: Lysozyme dissolved in either 0.1 M NaOAc at pH 4.00 or 0.1 M NaH_2PO_4 at pH 7.20 with 0, 20 and 40 % (w/w) CDHP. Two sets of samples were incubated at 37 °C in sealed silanized vials for the time indicated. Aliquots were removed for activity and other testing at specified times.

Figure S4



Representative integrated peak plot from raw ITC data: Lysozyme: 14.87 μM, CDHP: 300 μM. Buffer-lysozyme titration was used as a blank subtraction from plotted peak data. Small heats are representative of nonspecific effects, such as CDHP dilution, and solvation as well as thermal energy from mechanical mixing. These small exotherms cannot be fit to known binding models, and indicates no direct binding between lysozyme and CDHP.

References

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