Supplementary Information

Effect of Weakly Interacting Cosolutes on Lysozyme Conformations

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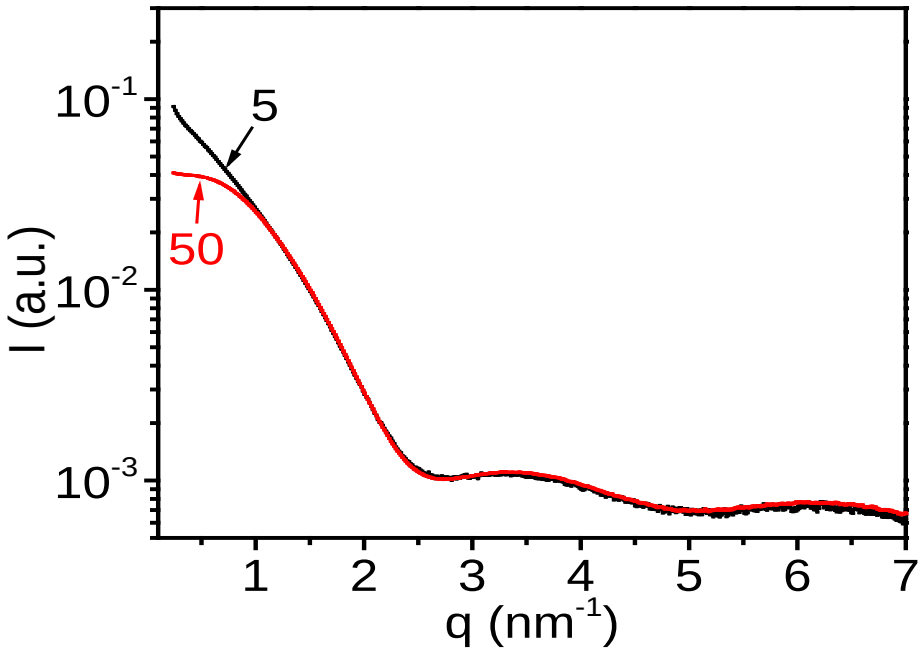


Figure S1: Solution X-ray scattering intensity, *I*, as a function of the magnitude of scattering vector, *q*, from 5 and 50 mg/ml lysozyme solutions in native buffer (50 mM sodium acetate). Scattering intensities were normalized by protein concentrations.

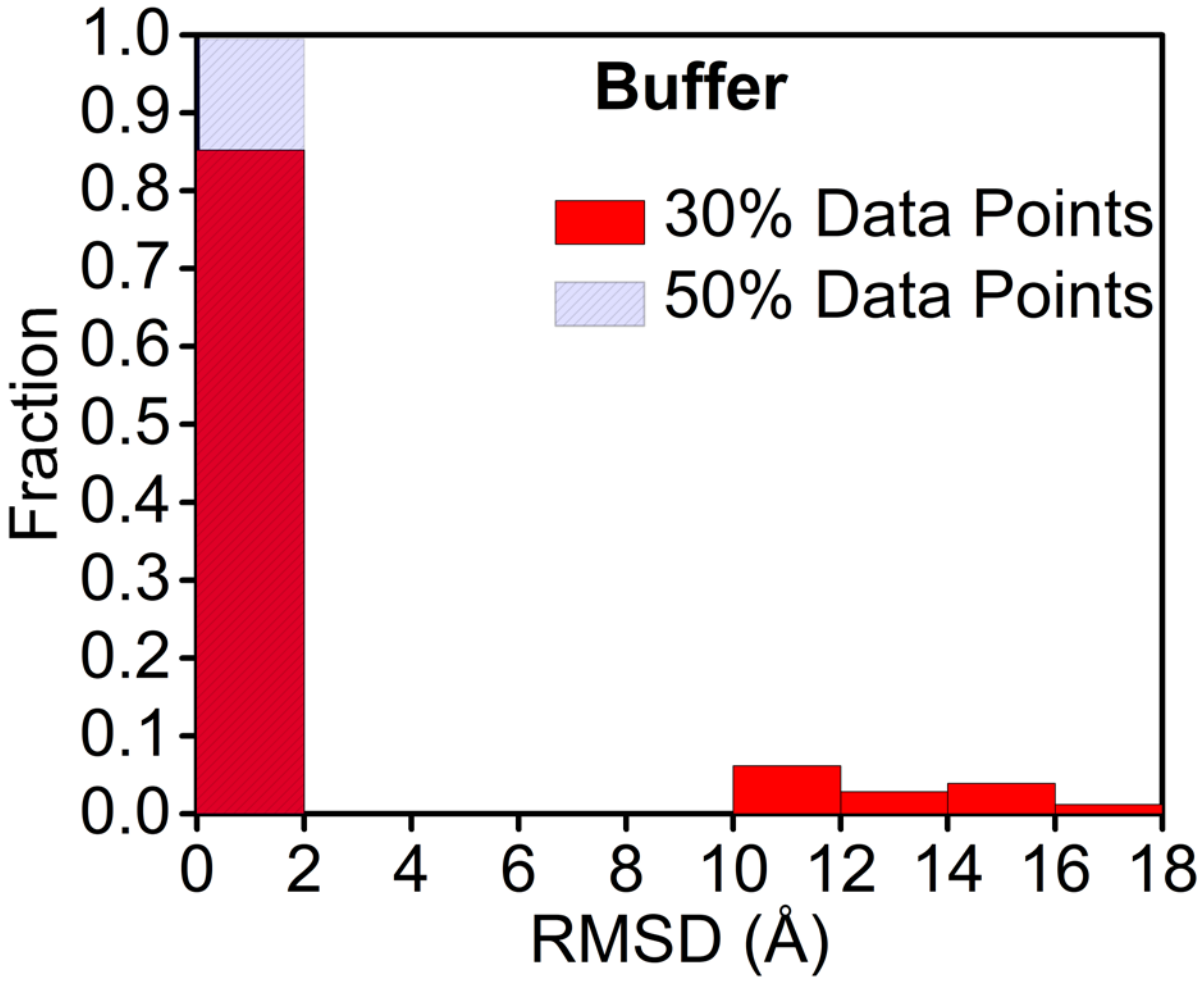


Figure S2: Distribution of the conformations of 2.5 mg/mL lysozyme samples in 50 mM sodium acetate buffer (pH = 4.5) as a function of their root mean squared displacement (RMSD) values (with respect to the crystal structure). The patterned blue columns represent the RMSD distribution of the conformational ensemble using 50% of randomly selected data points. The solid red columns represent the RMSD distribution of the conformational ensemble using 30% of randomly selected data points. Similar results were obtained when the procedure was repeated a few more times. In some of the cases, however, 30% of randomly selected points gave similar results to the 50% data set.

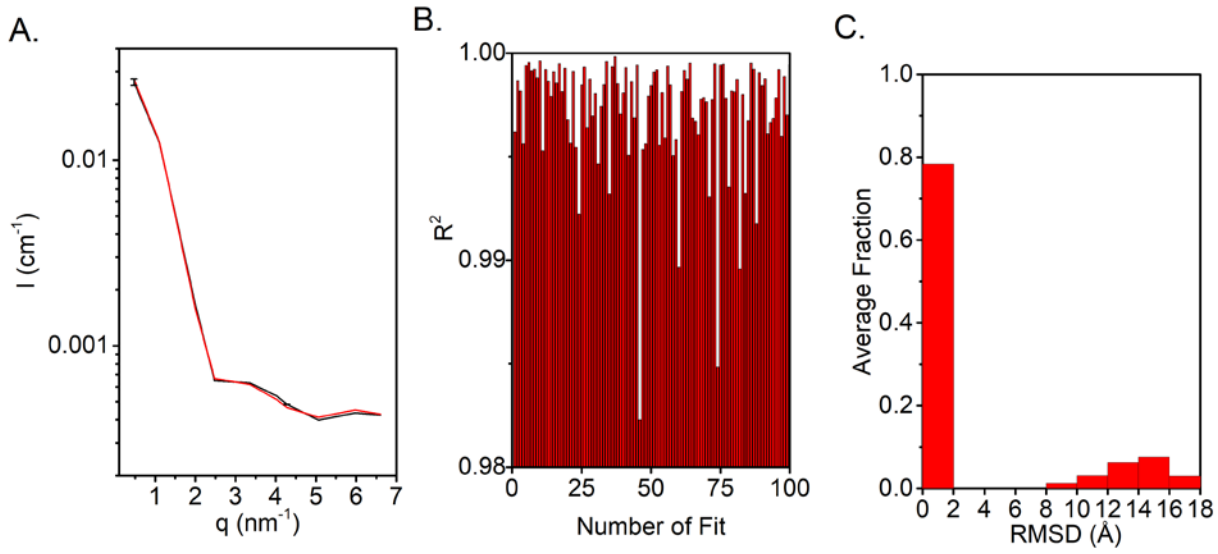


Figure S3: Fitting using randomly selected 10 data points. A. 10 randomly selected data points (black line) from the scattering curve under native buffer conditions (shown in Figure 3) were fitted to our set of models. The fit was repeated 100 times, each time 10 points were randomly selected. B. Distribution of R^2 values of the all fits. C. Average fraction of RMSD values based on all the fits.

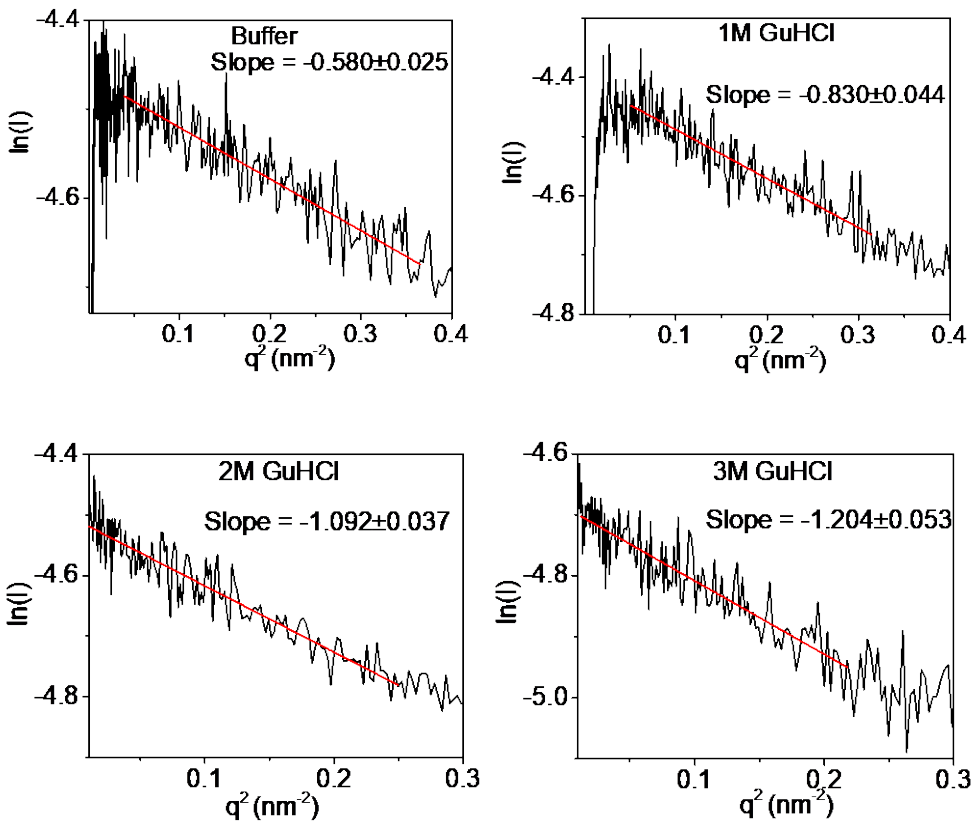


Figure S4: Guinier plots of 2.5 mg/mL lysozyme in 50 mM sodium acetate buffer (pH = 4.5), and in the same buffer with 1M, 2M, and 3M GuHCl, as indicated in the figures. The slope of the linear fit (red line) was used to calculate the gyration radius according to Eq. 1.

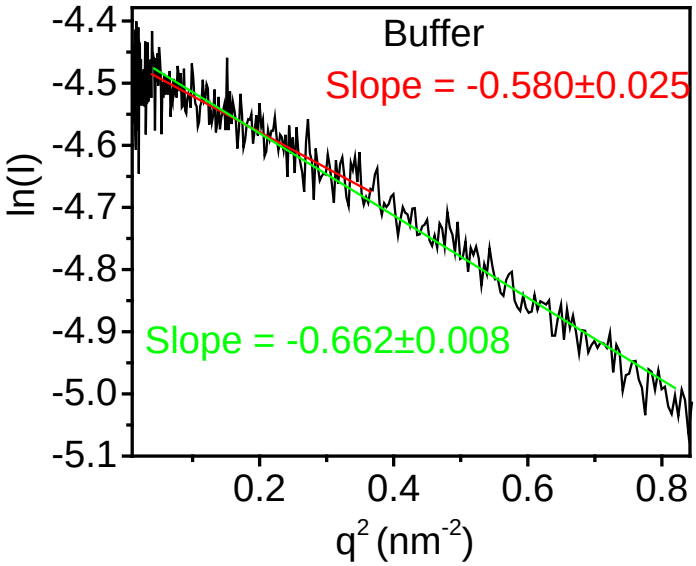


Figure S5: Guinier plots of 2.5 mg/mL lysozyme in 50 mM sodium acetate buffer (pH = 4.5). The slope of the linear fit (green line) was used to calculate the gyration radius according to Eq. 1, using $\boldsymbol{q_{max} \leq \frac{1.3}{R_g}}$ the resulting gyration radius was 1.41±0.1 nm, which is larger than in the value obtained in Figure S4, yet within the error.

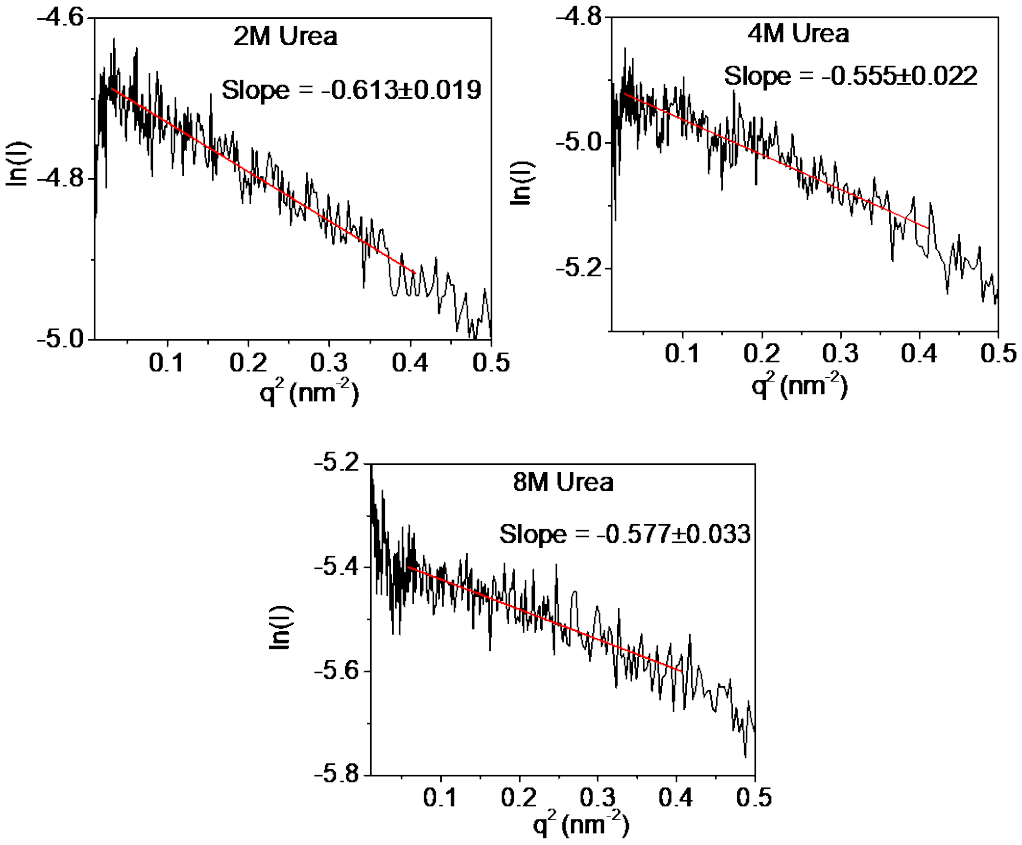


Figure S6: Guinier plots of 2.5 mg/mL lysozyme in 50 mM sodium acetate buffer (pH = 4.5) with 2, 4, and 8M urea as indicated in the figures.

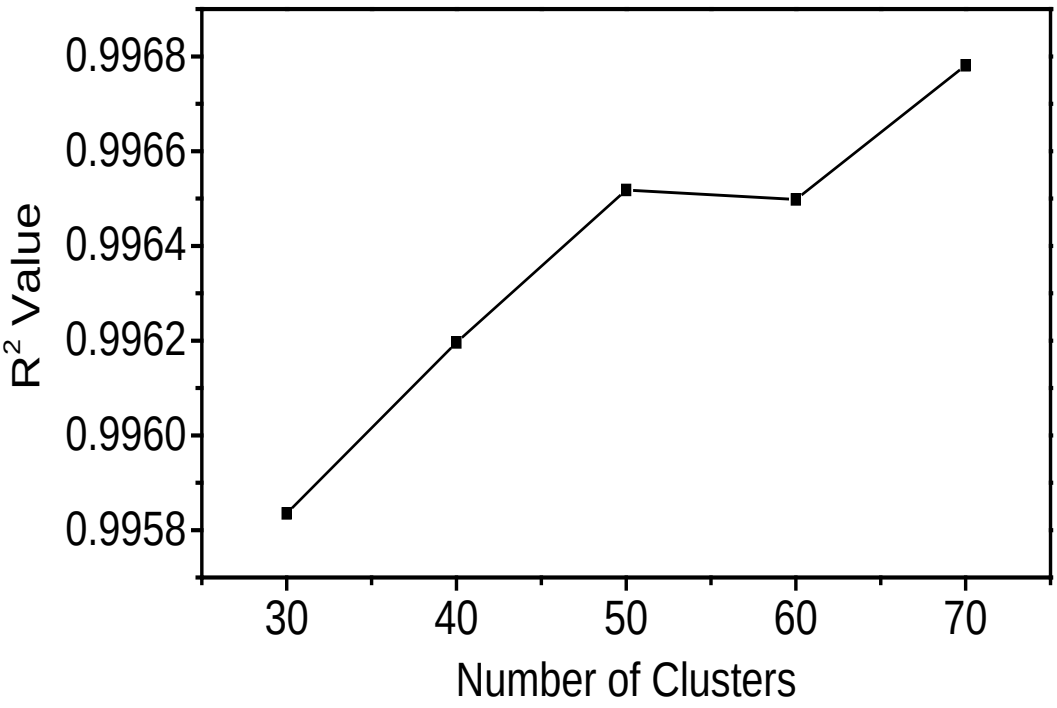


Figure S7: R^2 values of the fitting to the solution X-ray scattering curve of 2.5 mg/mL lysozyme in the native buffer solution, as a function of the number of clusters in the K-means algorithm used for the fit.

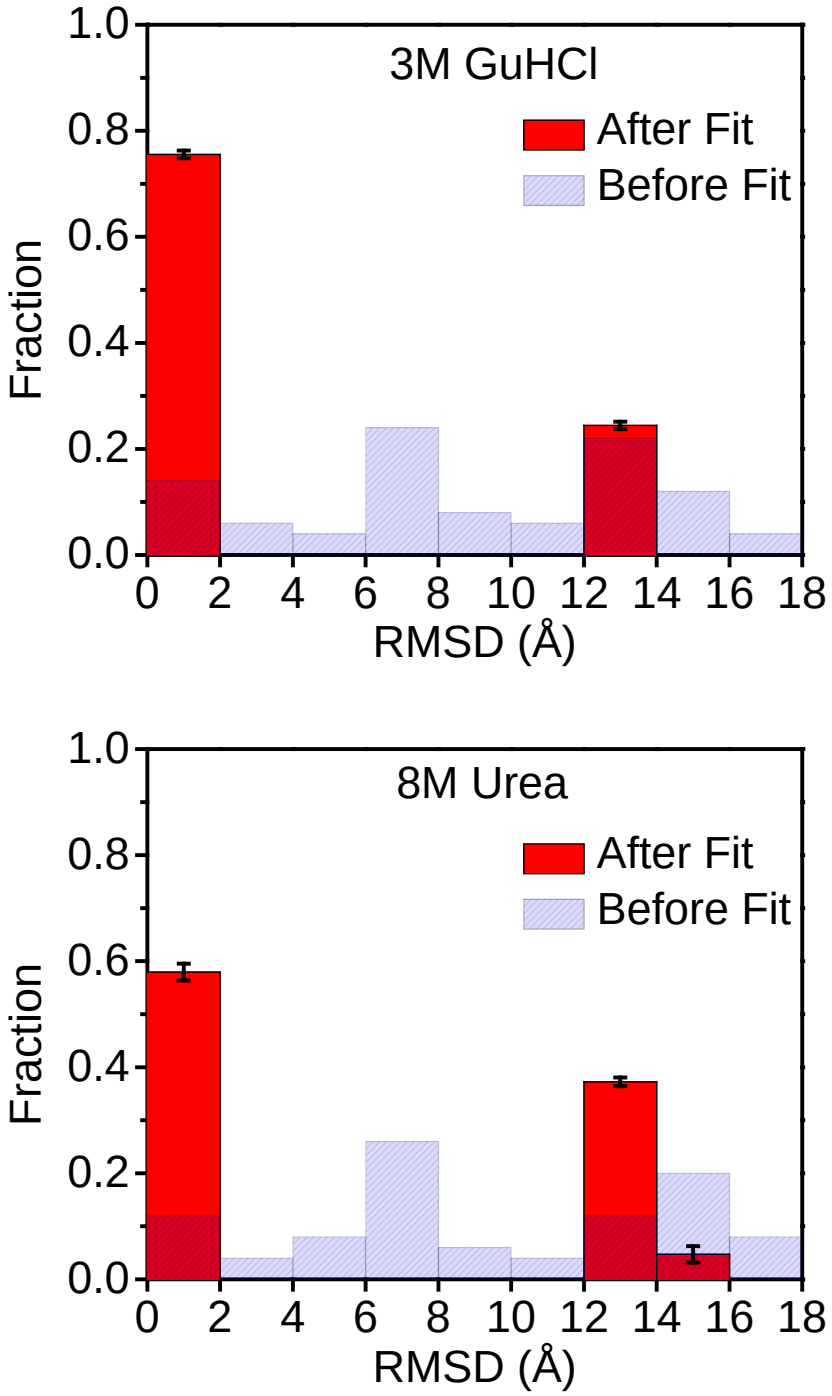


Figure S8: Distribution of the conformations of 2.5 mg/mL lysozyme samples in 50 mM sodium acetate buffer (pH = 4.5) with 3M GuHCl and 8M urea as a function of their RMSD values (with respect to the crystal structure). The patterned blue columns represent the RMSD distribution of the conformational ensemble before the fitting procedure; the solid red columns represent the RMSD distribution of the conformational ensemble according to the fit.

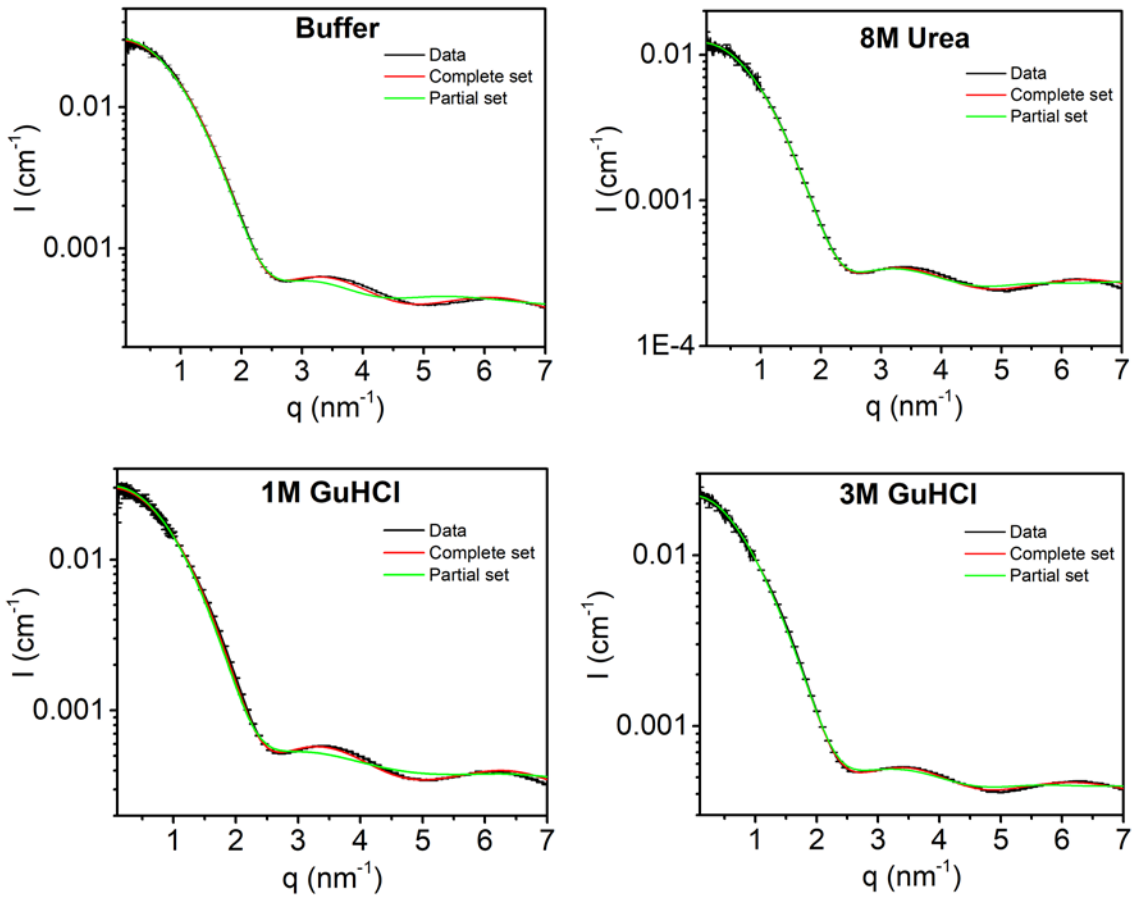


Figure S9: Background subtracted scattering intensity from 2.5 mg/mL lysozyme in 50 mM sodium acetate buffer (pH = 4.5), and buffer with 1 or 3 M GuHCl, and 8 M urea, as indicated (black curves). The red curves are the best fitted models based on the complete set of 50 computed ensemble of lysozyme conformations. The green curves are the best fitted models based on a partial set of computed ensemble of lysozyme conformations that includes only the unfolded states.

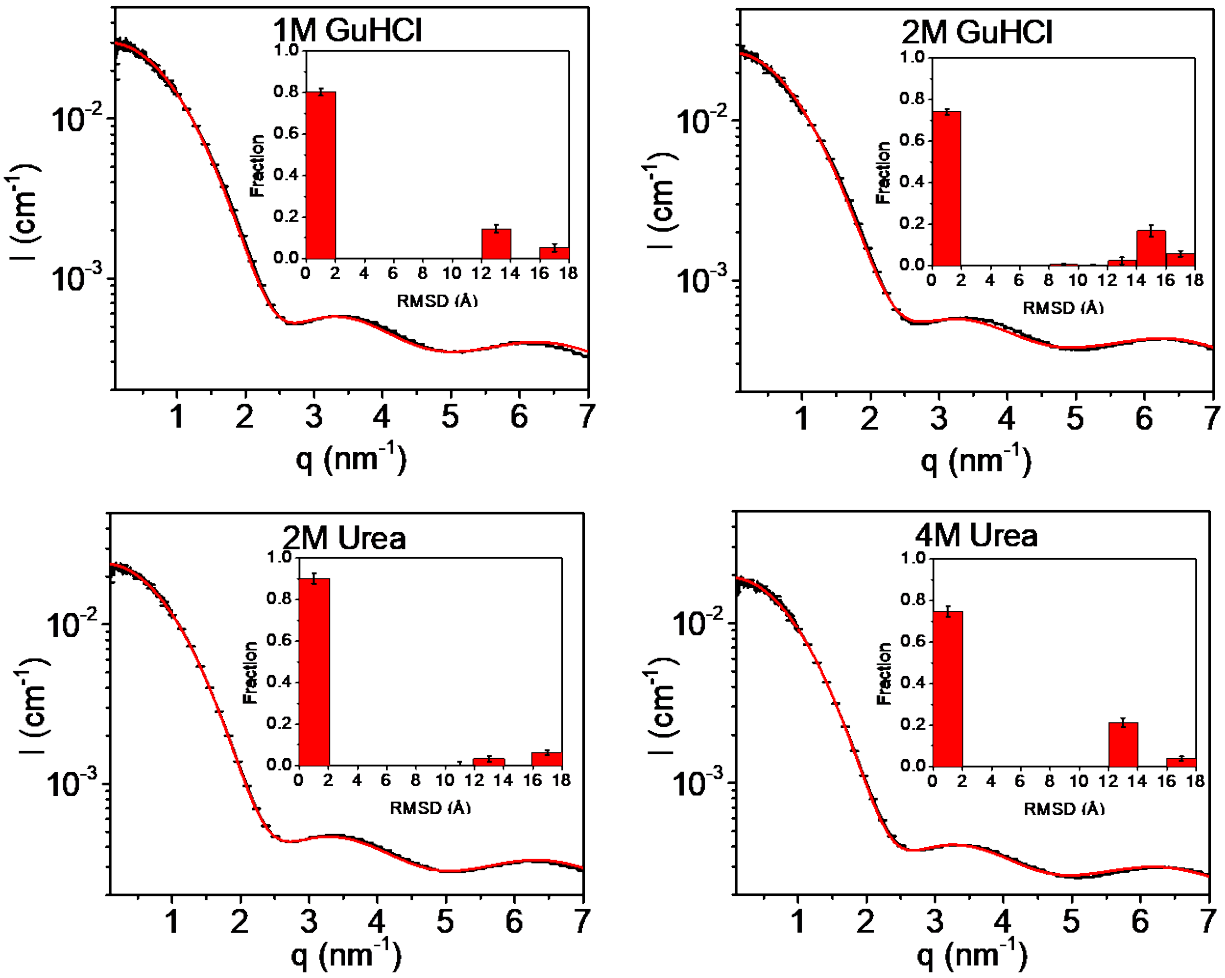
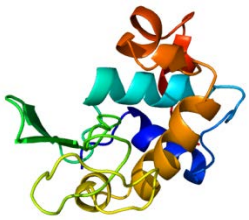
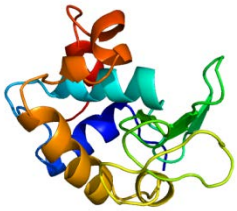


Figure S10: Background subtracted scattering intensity from 2.5 mg/mL lysozyme in 50 mM sodium acetate buffer (pH = 4.5), with 1 and 2 M GuHCl, 2 and 4 M urea, as indicated (black curves). The red curves are the best fitted models based on the computed ensemble of lysozyme conformations. The insets show the fraction of conformations as a function of their RMSD values (with respect to the crystal structure).

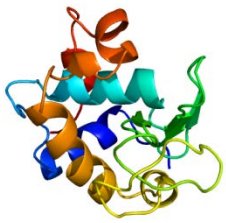


RMSD=1.1Å
Fraction=0.51±0.01
R_g=15.0Å

RMSD=1.6Å
Fraction=0.23±0.05
R_g=14.5Å

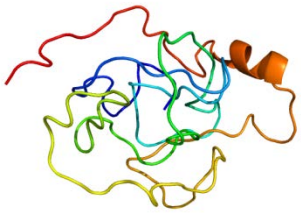


RMSD=1.7Å
Fraction=0.13±0.02
R_g=14.7Å



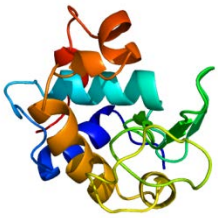
RMSD=0.88Å
Fraction=0.09±0.05
R_g=14.6Å

RMSD=1.6Å
Fraction=0.03±0.03
R_g=14.6Å

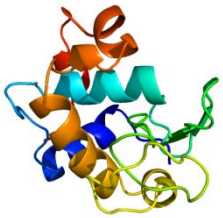


RMSD=14.4Å
Fraction=0.01±0.02
R_g=14.9Å

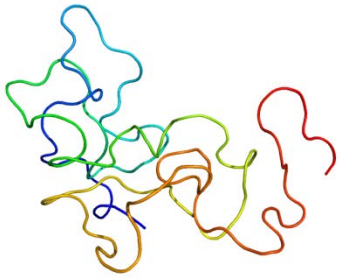
Figure S11: Ribbon diagram representation and parameters of the models of lysozyme that were selected to fit lysozyme in the buffer solution (50mM sodium acetate, pH 4.5).The gyration radii were determined using Guinier analysis of the WAXSiS calculated scattering curves.



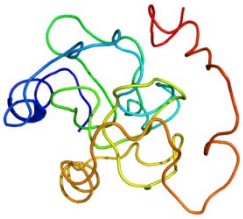
RMSD=0.9Å
Fraction=0.54±0.12
R_g=14.7Å



RMSD=0.8Å
Fraction=0.37±0.07
R_g=14.8Å

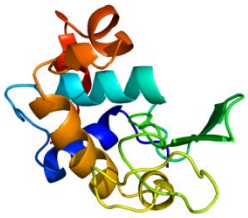


RMSD=17.3Å
Fraction=0.06±0.02
R_g=17.0Å

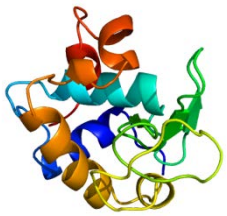


RMSD=13.1Å
Fraction=0.03±0.01
R_g=14.7Å

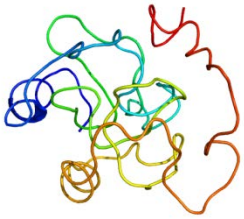
Figure S12: Ribbon diagram representation and parameters of the models of lysozyme that were selected to fit lysozyme in 2M urea solution. The gyration radii were determined using Guinier analysis of the WAXSiS calculated scattering curves.



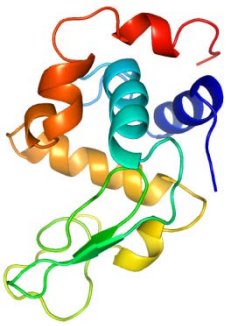
RMSD=1.1Å
Fraction=0.48±0.05
R_g=15.1Å



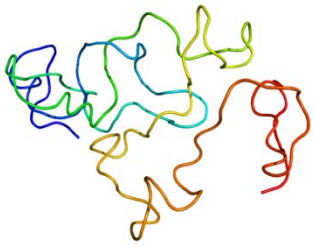
RMSD=1.6Å
Fraction=0.22±0.03
R_g=14.5Å



RMSD=13.1Å
Fraction=0.21±0.03
R_g=14.7Å

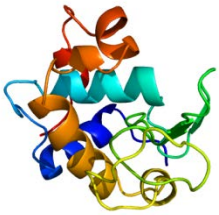


RMSD=1.6Å
Fraction=0.04±0.02
R_g=14.7Å

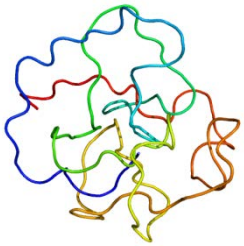


RMSD=16.7Å
Fraction=0.04±0.01
R_g=17.6Å

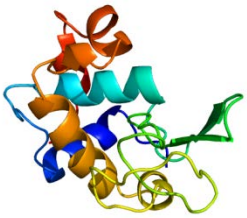
Figure S13: Ribbon diagram representation and parameters of the models of lysozyme that were selected to fit lysozyme in 4M urea solution. The gyration radii were determined using Guinier analysis of the WAXSiS calculated scattering curves.



RMSD=0.7Å
Fraction=0.42±0.02
R_g=14.7Å



RMSD=13.2Å
Fraction=0.20±0.02
R_g=14.6Å



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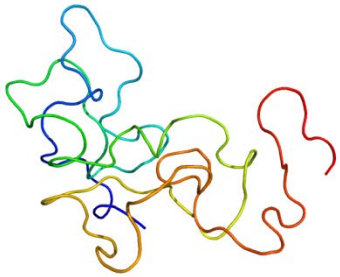
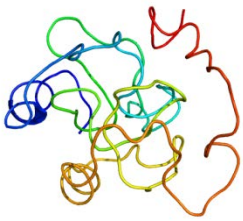
Fraction=0.16±0.05
R_g=15.3Å



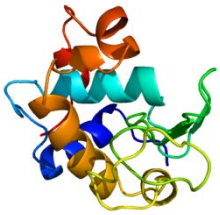
RMSD=17.1Å
Fraction=0.11±0.01
R_g=17.3Å

RMSD=13.2Å
Fraction=0.09±0.02
R_g=14.3Å

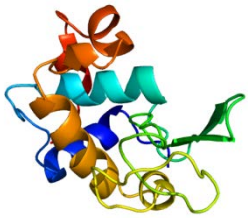


RMSD=13.1Å
Fraction=0.03±0.02
R_g=14.7Å

Figure S14: Ribbon diagram representation and parameters of the models of lysozyme that were selected to fit lysozyme in 8M urea solution. The gyration radii were determined using Guinier analysis of the WAXSiS calculated scattering curves.



RMSD=0.7Å
Fraction=0.42±0.04
R_g=14.5Å



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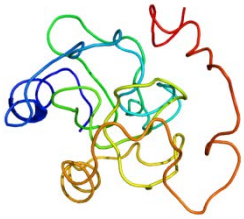
Fraction=0.38±0.05
R_g=15.0Å



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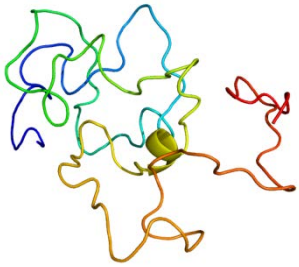
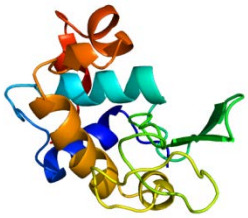
Fraction=0.14±0.03
R_g=14.7Å



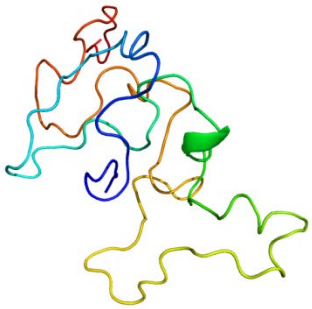
RMSD=16.5Å
Fraction=0.05±0.02
R_g=17.6Å

Figure S15: Ribbon diagram representation and parameters of the models of lysozyme that were selected to fit lysozyme in 1M GuHCl solution. The gyration radii were determined using Guinier analysis of the WAXSiS calculated scattering curves.

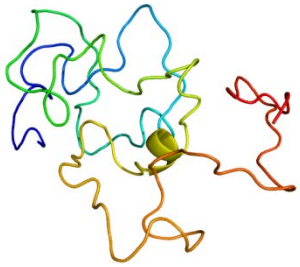


RMSD=1.1Å
Fraction=0.36±0.02
R_g=15.1Å

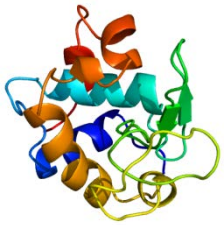
RMSD=16.1Å
Fraction=0.29±0.04
R_g=14.7Å



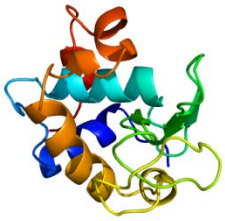
RMSD=15.0Å
Fraction=0.15±0.04
R_g=18.7



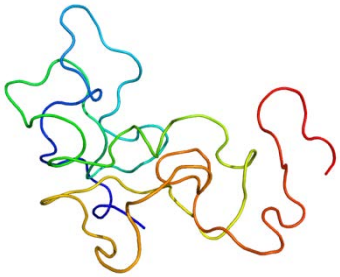
RMSD=16.5Å
Fraction=0.04±0.02
R_g=17.7Å



RMSD=1.7Å
Fraction=0.02±0.02
R_g=14.8Å

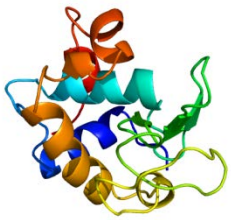


RMSD=1.6Å
Fraction=0.01±0.01
R_g=14.6Å

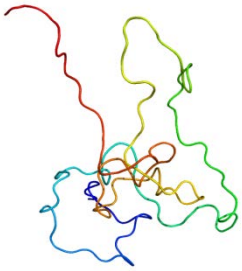


RMSD=17.3Å
Fraction=0.01±0.01
R_g=17.0Å

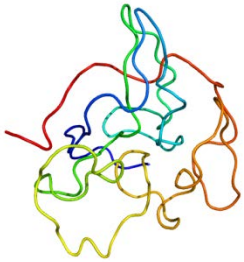
Figure S16: Ribbon diagram representation and parameters of the models of lysozyme that were selected to fit lysozyme in 2M GuHCl solution. The gyration radii were determined using Guinier analysis of the WAXSiS calculated scattering curves.



RMSD=1.1Å
Fraction=0.38±0.04
R_g=14.7Å

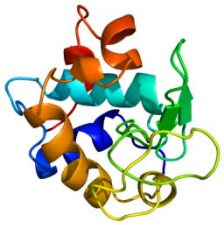


RMSD=18.1Å Fraction=0.25±0.01
R_g=19.9Å

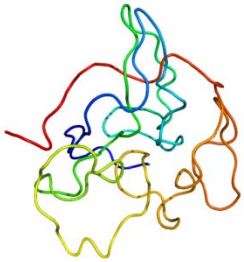


RMSD=11.6Å
Fraction=0.12±0.01
R_g=13.6Å

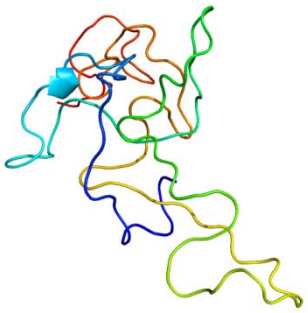
RMSD=1.4Å
Fraction=0.09±0.01
R_g=14.8Å



RMSD=1.8Å
Fraction=0.04±0.02
R_g=14.7



RMSD=13.9Å
Fraction=0.01±0.01
R_g=14.4



RMSD=14.5Å
Fraction=0.10±0.01
R_g=18.9

Figure S17: Ribbon diagram representation and parameters of the models of lysozyme that were selected to fit lysozyme in 3M GuHCl solution. The gyration radii were determined using Guinier analysis of the WAXSiS calculated scattering curves.

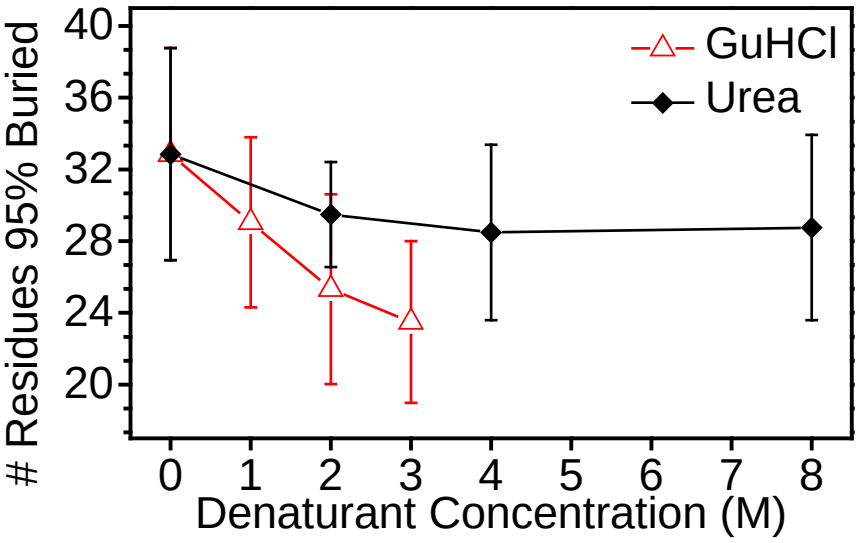


Figure S18: The weighted average of the number of residues that are 95% buried in the models that were selected to fit the data in Figures 3 and S10, as a function of denaturant concentration.

Table S1. The electron density of the solvents used in the experiments[42].

Solvent	Electron density $[e/\mathrm{nm}^3]$
Buffer	334 ± 1
2M urea	343 ± 1
4M urea	352 ± 1
8M urea	368 ± 1
1M GuHCl	341 ± 1
2M GuHCl	347 ± 1
3M GuHCl	354 ± 1