Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic supporting information for:

Protein conformation in pure and hydrated deep eutectic solvents

A. Sanchez-Fernandez,[a,b]† K. J. Edler,[a] T. Arnold,[c] D. Alba Venero,[d] and A. J. Jackson[b,e]

[a]*Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK.*

[b]*European Spallation Source, Lund, Sweden.*

[c]*Diamond Light Source, Harwell Campus, Didcot, OX11 0DE, UK.*

[d]*ISIS Spallation Neutron Source, Harwell Campus, Didcot OX11 0QX, UK.*

[e]*Department of Physical Chemistry, Lund University, SE-221 00, Lund, Sweden.*

†Corresponding author: a.sanchez.fernandez@bath.ac.uk

Experimental

Materials

Choline chloride:urea and choline chloride:glycerol were prepared by mixing and heating at 80 ˚C one mole equivalent of choline chloride (>98 %, Sigma) and two mole equivalents of hydrogen bond donor (urea, >99.5 %, Sigma or glycerol, >99 %, Sigma) until an homogeneous and transparent liquid was obtained. After the synthesis the liquid was equilibrated for at least for 24 h in an oven at 40 ˚C. The deuterated versions of the deep eutectic solvent (DES) were prepared following the same procedure. d_9-choline chloride (N,N,N-trimethyl-d_9, 99 % atom D, 99 % purity), d_4-urea (98 % atom D, 99 % purity) and d_8-glycerol (99 % atom D, 98.5 % purity) were supplied by QMX Laboratories and manufactured by CDN Isotopes. Samples containing water were prepared using ultrapure water (18.2 Ω, Elga) and D_2O (99.9 atom % D, Sigma).

Bovine serum albumin (BSA, >98 %) and lysozyme from chicken egg white (Lysozyme, ~70,000 U/mg) were supplied by Sigma and used without further purification. Samples were prepared through freeze-drying, following the procedure explained by Guiterrez *et al.*[1, 2] A small volume of DES was mixed with water in order to reduce the viscosity and facilitate the solvation of the proteins. Afterwards DES was added to adjust the concentration of protein, DES and water. For the protein samples in pure DES, the same procedure was followed but the resulting solutions were freeze-dried to remove water from the sample. Samples were stored at 4 ˚C and measured within 5 days after preparation.

An aliquot of pure DES was included in the procedure of addition and removal of water in order to determine the water content in the pure DES samples. Water content was determined through Karl-Fischer titration (Mettler Toledo DL32 Karl-Fischer Coulometer Aquiline electrolyte A (Fisher Scientific), Aqualine Catholyte CG A)). The water content was maintained below 0.6 wt% for choline chloride:glycerol and 0.4 wt% for choline chloride:urea during the experimental procedure explained here.

Samples were prepared in different concentrations and isotopic mixtures in order to fulfil the different requirements of each experiment. Circular dichroism required two different concentrations. A low concentration (~18 μM) was measured in a 1 mm path length, 1 cm wide, quartz cell for both proteins on the far-UV region. Near-UV region measurements required a higher concentration of protein (~82 μM) for all solvents. These samples were measured in a 1 cm path length, 1 cm wide, quartz cell.

Small-angle neutron scattering required isotopically substituted solvents in order to improve the contrast between solvent and proteins. Samples were prepared in one isotopic mixture combining deuterated solvent (d_9-choline chloride:d_8-glycerol or d_9-choline chloride:d_4-urea) and protonated proteins (750 μM for lysozyme and 400 μM for BSA).

Methods

Circular dichroism

The determination of the CD spectra was done using a Jasco CD J-815 spectrometer (Jasco Coorp., Tokyo, Japan) with a Peltier cell holder for temperature control. Two different ranges were investigated in terms of wavelength. The far-UV region (200-250 nm) provided information regarding the secondary structure of the proteins, and the near-UV region (250-310 nm), showed information about the conformation of the protein. Spectra at different temperatures were collected with a scan rate of 50 nm min^{-1}, a bandwidth of 1 nm and a response time of 2 s. Measurements were repeated 7 times and averaged in order to improve the statistics and check repeatability.

Data collected were corrected for the different solvents, and subsequently normalised to the concentration and converted to molecular ellipticity (mdeg cm^2 $dmol^{-1}$) in order to facilitate comparison between the different samples.

Small-angle neutron scattering

Small-angle neutron scattering (SANS) measurements were performed on Sans2d at ISIS Pulsed Neutron Source.[3] SANS2D is a time-of-flight instrument with two movable detectors. The rear detector was placed at 4 m distance from the sample giving, together with the front detector, a momentum transfer range of 0.004-1.40 $Å^{-1}$. Samples were loaded into a modified Linkam DSC stage using 1 mm path length, 1 cm wide, quartz Hellma cells. Lysozyme samples were measured at room temperature. BSA samples were cycled between -80 ˚C and 80 ˚C (Set point). A first measurement was taken at 25 ˚C and then gradually decreased at 2 ˚C/min rate and measured at -40 ˚C and -80 ˚C. After cooling, samples were heated at the same rate and measured at 25 ˚C and 80 ˚C. Finally samples were gradually cooled down and measured again at 25 ˚C to check the structure after the cycle.

Data were reduced using the routines within Mantid.[4] The data were normalised to the sample transmission, and corrected for detector efficiencies, then scattering from the empty cell was subtracted. The output data were the absolute scattered intensity, I(q) in cm^{-1}, versus the momentum transfer, q in $Å^{-1}$. The scattering of the pure solvents were afterwards subtracted accounting for the incoherent contribution to each sample and data were normalised to the concentration using SasView.[5] Instrument resolution was accounted for by smearing of the model functions using a Gaussian function at a constant 8% dq/q.

Data analysis

Small-angle neutron scattering

Indirect Fourier Transformation (IFT) was used to analyse the conformation of the proteins in the different solvents. IFT is a model-free numerical method to analyse SANS data.[6] This technique uses a real-space distance distribution function to describe the structure, the maximum dimension (D_{max}) and the radius of gyration of the scatterer (R_g). IFT is limited to non-periodic centrosymmetric structures, where the interparticle interaction, and therefore the structure factor, is negligible. The pair distance distribution function (p(r)) differs from 0 in a limited region of real space, between 0 and D_{max}, and changes in the shape of the curve can be directly related to the conformation of the particle in solution. The resulting curve can be used to calculate the R_g as the second moment of the p(r).

Results

Here we include the log-log plots of the scattering data with the fits obtained through IFT (Fig. S1 a and b).

The Guinier plot allows a rapid estimation of the extrapolated intensity at momentum transfer zero (I(0)) and the radius of gyration (R_g) of the scatterers (Fig. S1 c and d). These values were subsequently used to obtain the normalised-to-R_g Kratky plot.

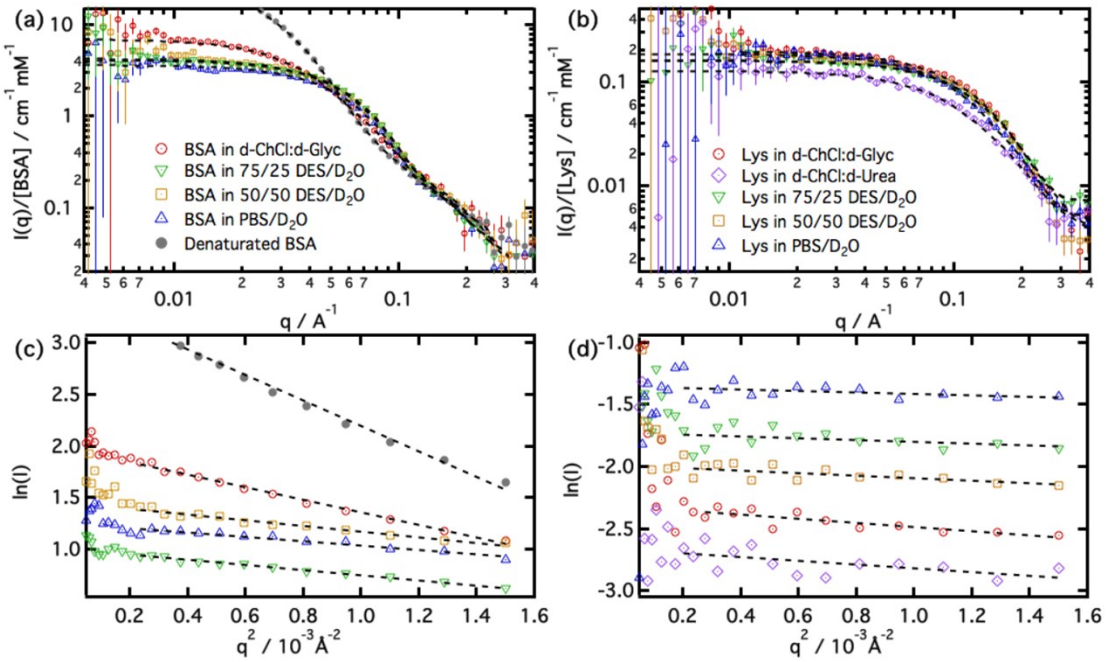

Fig. S1 Log-log plot of the SANS data of (a) BSA and (b) lysozyme in different solvents. The black-dashed lines correspond to the best fits obtained through the IFT analysis. Guinier plots of SANS data used to calculate the radius of gyration and the intensity at q = 0: (c) BSA and (d) lysozyme. The black-dashed lines correspond to the linear fits of the data.

The evolution of the protein conformation with temperature was also investigated. Fig. S2 includes the results from the temperature scan of the BSA in choline chloride:glycerol system. Samples were first equilibrated at 25 ˚C and cooled gradually down to -80 ˚C (2 ˚C/min), allowing equilibration at each temperature of interest for 20 minutes. Samples were heated up to 80 ˚C and measured upon denaturation. Finally, samples were re-equilibrated at 25 ˚C and measured at the end of the cycle to check reversibility in the system.

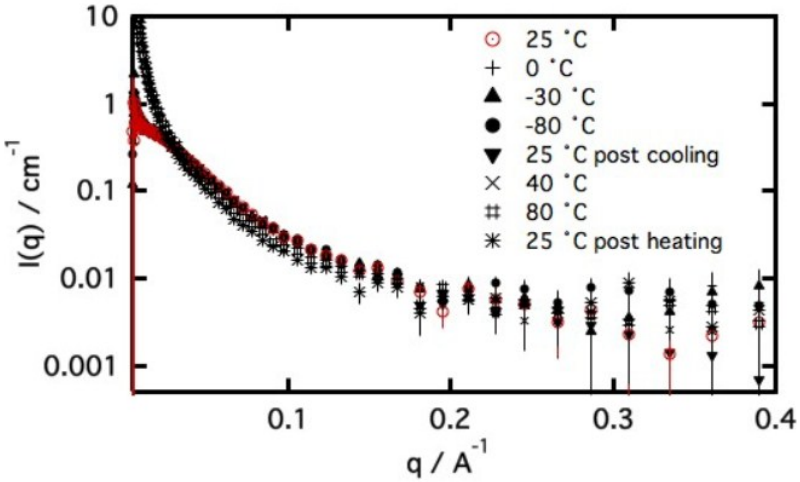

Fig. S2 SANS data from the temperature scan of BSA in d_9-choline chloride:d_8-glycerol at different temperatures: 25 ˚C, 0 ˚C, -30 ˚C, -80 ˚C, 25 ˚C (after cooling), 40 ˚C, 80 ˚C and 25 ˚C (after heating).

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