Structural examination of biologically-relevant solutions using neutron diffraction

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Introduction

Over the past decade, significant advances have been made in the methods of neutron diffraction with isotopic substitution and in the development of more powerful computational tools. Neutron diffraction is an ideal probe for the structural study of liquids providing a full atomistic-level, structural examination of aqueous solutions.

Research theme 1: molecular mechanisms of cryoprotection

Glycerol–water liquid mixtures are intriguing hydrogen-bonded systems and essential in many fields, ranging from basic molecular research to widespread use in industrial and biomedical applications as cryo-protective solutions. Despite much research on these mixtures, the details of their microscopic structure are still not understood. One common notion is that glycerol acts to diminish the hydrogen bonding ability of water, a recurring hypothesis that remains untested by direct experimental approaches. We have characterised the structure of glycerol–water mixtures, across the concentration range, using a combination of neutron diffraction experiments and computational modelling. Contrary to previous expectations, we show that the hydrogen bonding ability of water is not diminished in the presence of glycerol. We show that glycerol–water hydrogen bonds effectively take the place of water–water hydrogen bonds, allowing water to maintain its full hydrogen bonding capacity regardless of the quantity of glycerol in the environment. We provide a quantitative measurement of all hydrogen bonding in the system and reveal a concentration range where a micro segregated, bi-percolating liquid mixture exists in coexistence with a considerable interface region (Figure 1). This work highlights the role of hydrogen bonding connectivity rather than water structuring/destructuring effects in these important cryo-protective systems.

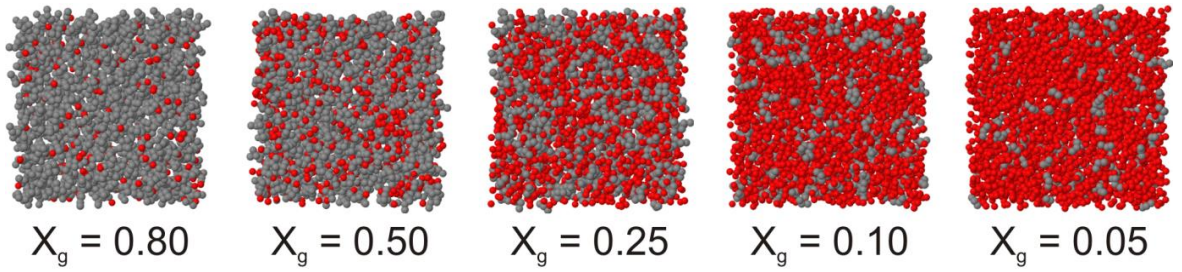

Figure 1: molecular scale segregation in glycerol-water solutions (x_g = mole fraction glycerol) is shown by clusters of glycerol (grey) and water (red) molecules. The snapshots show groups of water molecules and glycerol molecules hydrogen bonded together, forming clusters in the solution. To quantitatively assess this micro segregation, we completed a cluster analysis for glycerol and water across the concentration range.

Research theme 2: the role of hydrogen bonding in polyglutamine structure and association

Hydrogen bonding between glutamine residues has been identified as playing an important role in the intermolecular association and aggregation of proteins. To establish the molecular mechanisms of glutamine interactions, neutron diffraction coupled with hydrogen/deuterium isotopic substitution in combination with computational modelling has been used to investigate the structure and hydration of glutamine in aqueous solution. We find that the backbone of glutamine is able to coordinate more water molecules than the side chain, suggesting that charged groups on the glutamine molecule are more successful in attracting

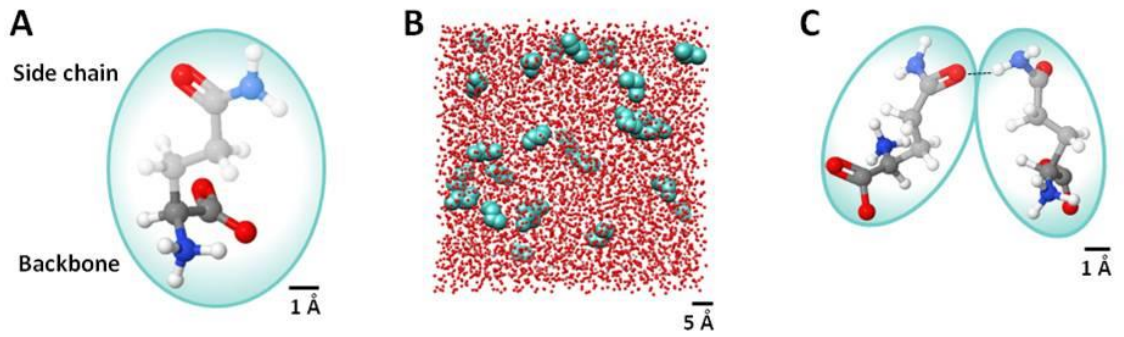

Figure 2: (a) the zwitterionic form of the glutamine molecule ($C_5H_{10}N_2O_3$). **(b)** Neutron diffraction experiments and computational modelling examine zwitterionic glutamine association in aqueous solution. The snapshot illustrates the association of glutamine molecules (blue) in a water solution (red) at 297 K. **(c)** Glutamine molecules associate into dimers through hydrogen bonds between the side chains.

water than the dipole in the side chain. In both the backbone and the side chain, we find that the carbonyl groups interact more readily with water molecules than the amine groups. We find that glutamine–glutamine interactions are present, despite their low concentration in this dilute solution. This is evidenced through the occurrence of dimers of glutamine molecules in the solution (Figure 2), demonstrating the effective propensity of this molecule to associate through backbone–backbone, backbone–side chain, and side chain–side chain hydrogen bond interactions. The formation of dimers of glutamine molecules in such a dilute solution may have implications in the aggregation of glutamine-rich proteins in neurological diseases where aggregation is prevalent.

Publications

Rhys, N., Soper, A. & Dougan, L. (2012) The hydrogen-bonding ability of the amino acid glutamine revealed by neutron diffraction experiments. *J. Phys. Chem. B* **116:** 13308-13319.

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Towey, J., Soper, A. & Dougan, L. (2012) Molecular insight into the hydrogen bonding and micro-segregation of a cryoprotectant molecule. *J. Phys. Chem. B* **116:** 13898-13904.

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Collaborators

External: A. Soper (Rutherford Appleton Laboratories, United Kingdom).