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Solvent effects of protic ionic liquids on protein structures

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The design of solvents and buffers for use with many biomolecules is currently both very complex and limited in what is available. Currently buffered aqueous salt solutions are used as solvents for proteins, but these do not sufficiently control protein solubility and stability, which adversely affects protein activity, folding-unfolding transitions, aggregation and crystallisation. Therefore, there is a need for new solvents which can control protein and biomolecule solubility and stability.

Protic ionic liquids (PILs) are cost efficient "designer" solvents which can be tailored to have properties suitable for a broad range of applications.[1] These are liquid salts which are typically liquid at room temperature and miscible with water. Certain aqueous PIL solutions have beneficial properties, including stabilising biomolecules, suppressing aggregation and enhancing protein crystal growth. However, there is a lack of understanding about the interactions present, which prevents solvent design for specific protein applications.

Here, I will discuss our ongoing work into designing PIL solvents for proteins, including protein structural changes, activity and identifying specific ionic interactions of PILs, cations and anions, with the protein surface.[2] This also includes developing and adapting characterisation methods for use with proteins in PIL solutions.[3] Recently, we have combined results from Solution SAXS and Protein Crystallography, using the Australian Synchrotron SAXS/WAXS and MX2 beamlines, to obtain a deeper understanding of ionic liquid-protein interactions.[4] In these studies we have used model proteins, with a focus on hen egg white lysozyme.

Specifically, we have identified conformational changes of the protein in solution due to changes in the ionic liquid chemical structure and/or concentrations. We have also identified the ion-binding sites of the ionic liquid solvated cations and anions. From these results we have clearly shown that the anion has significantly more interactions with the protein, and preferentially binds to positively charged and aromatic side chains, whereas few of the cations were identified in the solvation layer. Crystallographic studies using the MX2 beamline on lysozyme crystals grown in the presence of PILs provided insight into which ions are present at the surface and the key amino acids ionically bonded to the PILs which is important for protein stability. These findings will contribute towards being able to produce designer solvents for specific biomolecule applications.

[1] Greaves, T. L., Drummond, C. J. (2008) *Chem. Rev.,* **108**, 206.
[2] Han, Q., Brown, S., Drummond, C. J. (2022) Greaves, T. L., *J. Colloid Interface Sci.,* **608**, 1173.
[3] Han, Q., Binns, J., Zhai, J., Guo, X., Ryan, T. M., Drummond, C. J., Greaves, T. L. (2022) *J. Mol. Liq.,* **15**, 2430.
[4] Han, Q., Smith, K. M., Darmanin, C., Ryan, T. M., Drummond, C. J., Greaves, T. L. (2021) *J. Colloid Interface Sci.,* **585**, 433.