Using ionic liquids to determine the origin of specific ion-binding modulation of key protein properties

Qi Han, Calum J. Drummond, Tamar L. Greaves

School of Science, STEM College, RMIT University, 124 La Trobe Street, Melbourne, VIC 3000, Australia

Email of communicating qi.han@rmit.edu.au

Keywords: ion binding, ionic liquid, lysozyme

Proteins often experience various stresses which restrict industrial applications. The solvent is one of the most important external factors, and specific ion effects have significant impact on protein solubilization, crystallization, folding-unfolding transitions, aggregation and inactivation of enzymes. However, current understanding of specific ionic liquid (IL) effects on proteins is limited, therefore, there is a need for understanding the IL effect on proteins and developing ILs to control protein behavior [1,2]. This work employs activity assays, spectroscopic techniques and protein crystallography to explore the effect of protic ILs on crystallization, activity, conformational change, crystal packing and ion-binding in dilute and concentrated ILs with the model protein lysozyme. Results show that ILs of ethylammonium nitrate and ethanolammonium formate, particularly their anions, have specific binding sites on protein surfaces. The anions had direct electrostatic interactions with the charged side chains and hydrogen bonding with hydrophilic residues (especially Asn) and aromatic residue Trp (for nitrate), whereas few of the cations were identified in the hydration layer. Importantly, a decrease in protein solubility and activity, induced aggregation, and changes in crystal packing are linked to an increase in the number of binding sites. The ion binding to the protein seems likely to have broad importance when designing solvents for protein stabilization and crystallization, controlling phase behavior, biochemical processes, and studies of biological function and disease.

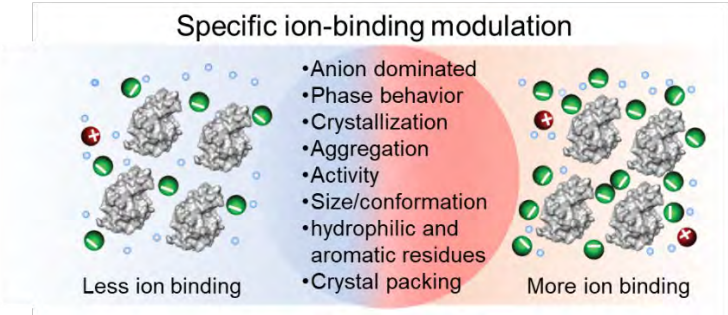

Figure 1. The specific ion-binding modulation of key protein properties.

[1] Han, Q., Brown, S. J., Drummond, C. J., & Greaves, T. L. *J. Colloid Interface Sci.* **2022**, *608*, 1173-1190.

[2] Han, Q.; Binns, J.; Zhai, J.; Guo, X.; Ryan, T. M.; Drummond, C. J.; Greaves, T. L. *J. Mol. Liquids*. **2022**, *345*, 117816.