

Ionic Liquids as Protein Crystallization Additives

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Ionic liquids (ILs) are typically defined as salts composed of separate cations and anions having melting points below 100 °C. ILs typically have chaotropic anions and surfactant cations, have low vapour pressure, have a wide liquidus range, and have depressed melting points as a result of low-symmetry ions. The cationic and anionic components can be individually tailored for various applications, which characteristic has resulted in a rapid proliferation in their use for a wide range of applications. This ability to finely tune their properties also makes ILs attractive materials for use in biochemical applications. Several investigations have been carried out using IL's in protein crystallization¹⁻³, with no conclusions that could guide one in their use. We are currently working to remedy this, testing a panel of 23 commercially available ILs, with dH₂O as a control, as protein crystallization additives. The ILs are used as 1M solutions in dH₂O, at final concentrations of 0.1, 0.2, and 0.4 M in the precipitant solution. The proteins employed are not the usual models, but those being worked as part of other ongoing crystallization projects in our group. Optimization with ILs has resulted in 3D faceted crystals being obtained from all types of starting outcomes tested, including non-faceted crystals (dendrites, urchins, spheroids, etc.), bright spot leads⁴, and, most surprising, precipitate. The use of trace fluorescent labeling is critical to these experiments as the ILs often also crystallize from solution. Not every tested lead condition resulted in crystals, and some gave crystals with several ILs. In initial experiments the pattern of hits changes with the IL concentrations employed. Results from experiments currently under way, including diffraction analysis of crystals from IL+ and IL- conditions, will be reported.

1. Pusey et al., (2007), *Cryst. Growth & Des.* 7:787-793.
2. Judge et al., (2009), *Cryst. Growth & Des.* 9:3463-3469.
3. Kennedy et al., (2011), *Cryst. Growth & Des.* 11:1777-1785.
4. Pusey et al., (2015), *Acta Cryst.* F71, 806-814.