Application Note

Protein crystallization in water only in presence of crystallophore

Polyvalan / Laboratoire de Chimie, ENS de Lyon / IBS, Grenoble - https://crystallophore.fr/

Protein structure crystallography is driven by the ability to obtain exploitable crystals. In order to get them, hundreds of different crystallization conditions have been developed since few decades. These crystallization mixtures are composed by many different components like salts, buffers, cations, PEGs…

The presence of these components is supposed to favour the nucleation and crystal growth. This empirical approach led to the commercialization of various crystallisation kits with a large variety of precipitant formulations. Our group has developed a family of nucleating and phasing agents, called 'crystallophore' (ie 'Xo4'), which improves the nucleation process in a broad scope of conditions and the quality of the obtained crystals from one side. On the other side, the presence of a lanthanide cation (Tb^{3+} or Lu^{3+}) allows the phasing of the structure without producing the Se-Met mutant.

To illustrate the nucleating efficiency of the Xo4 additive, crystallisation of several model proteins was achieved without any other additives into the crystallization drop, simply in pure water. (Figure 1).

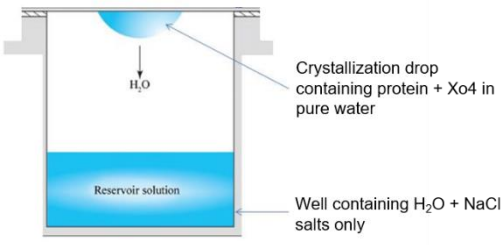

Figure 1: Crystallization process in pure water

To force the vapour diffusion from the drop to the well, we have added a low concentration of sodium chloride salt only into the well (300 and 350 mM salt concentrations were the best to obtain nice crystals in only few days).

Using these conditions, we were able to obtain protein crystals of both HEWL and thaumatine proteins solubilised in pure water in presence of a small concentration of crystallophore (2 mM or 5 mM of crystallophore for a 20 or 10mg/mL concentration of protein) (Figures 2 and 3).

Figure 2: Crystal of Thaumatine. Drop: 20mg/mL protein in water + TbXo4OH 2 mM (observation: + 7 days).

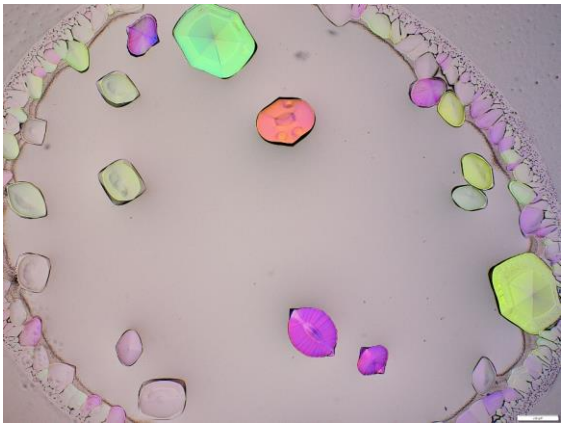

Figure 3: Crystals of HEWL. Drop: 20mg/mL protein in water + TbXo4 2mM (observation + 4 days).

Thanks to the action of the crystallophore, we think that a minimal set of components, as the ones found in protein stock buffers, should be sufficient to get crystals. This procedure should become a routine test to evaluate the direct crystallization of a protein in its stock buffer in the presence of Xo4.