CRYOPROTECTION AND LIGAND SOLUBILIZATION: EVALUATION OF MULTICOMPONENT MIXTURES

Lidia Ciccone1,2, Laura Vera1, Livia Tepshi1,2 and Enrico A. Stura1

1CEA, ibiTeC-S, Service d’Ingenierie Moléculaire des Protéines, Laboratoire de
2Dipartimento di Farmacia, Università Pisa, Via Bonanno 6, 56126 Pisa, Italy.

Crystallographic structure determination of protein-ligand complexes is an important step in the progress from a low affinity lead compound to an active molecule. The combination of low affinity and low solubility is a hurdle for structural studies of ligand-protein complexes. Complete solubilization of hydrophobic ligands is required for their use in co-crystallization or crystal soaking experiments to obtain interpretable electron density maps for the ligand. High-throughput screening is used to select among a library of millions of compounds those that bind to a target protein, inhibit a particular enzymatic reaction or block a cellular transport mechanism. Typically, the chemical compounds are dissolved in dimethyl sulfoxide (DMSO)[3] to produce an aqueous solution. This solution is diluted during the screening to ensure that the concentration of DMSO does not exceed 10%, as higher concentrations can be damaging to the target protein.[2]

The new cryosolutions were developed using Transthyretin as reference protein (Figure 2). About 200 samples were tested at synchrotron facilities, namely at the ESRF (beamlines ID31-1 and ID32-2) in Grenoble and on beamline Proxima-2 at the Soleil storage ring in Saclay.

![Figure 2](image)

**Figure 2** CRYOMIXES TO PREPARE A CRYOSOLUTION FOR WET-GRID EXPERIMENT

**SOLUTION**

The strategy of using a mixture of solvents to solubilize ligands is well known. Two or more solvents are tested in order to optimize the solubility of insoluble compounds.[4] The approach used to create cryoprotectant solutions by mixing together compounds that inhibit ice formation can be extended to solutions that are also effective in solubilizing ligands. For inhibitors poorly soluble or insoluble in DMSO, the mixed solutions with DMSO/dioxane/ethylene glycol mixtures of different ratios should improve ligand solubilization. DMSO, ethylene glycol and dioxane belong to different selectivity classes: III, IV and V, respectively (Figure 4)[5] and combination of these compounds should cover a relatively wide range of selectivity values to render water soluble a large variety of organic compounds.

![Figure 4](image)

**Figure 4** Classification of the Solvent Properties of Common Liquids

**Co-crystallization experiment**

The SM1-6 mixes were tested in co-crystallization experiments with TTR in the presence of curcumin and 16α-bromo-estradiol.[7] In these two cases, these hydrophobic compounds were solubilized in a DMSO/dioxane mix and co-crystallized with a precipitant consisting of high/low molecular weight PEG which helps to keep the inhibitor soluble in drop during crystal growth (Figure 6).

**Precaution!!!** An appropriate amount of cryozone was added to the reservoir to avoid disequilibrium between the drop and the reservoir. This is an important correction that must be applied to the reservoir solutions after the protein-ligand drop has been mixed with the reservoir solution, because the cryozone contain glycerol and other hygroscopic compounds that affect vapour diffusion equilibration.[8]

**RESULTS**

16α-bromo-estradiol is completely soluble in dioxane and not in DMSO, while curcumin is completely soluble in DMSO and not in dioxane. The combination of the two solvents has allowed the preparation of protein-ligand solutions suitable for crystallization (Figure 7). Dioxane acts as a precipitant in TTR crystallization, its effect is counterbalanced by glycerol and other diols that tend to increase protein solubility. The addition of 2,3-dimethylsulfoxide and glycerol in the cryomixes was a virtual guarantee that the crystals are protein-ligand drop and the reservoir. This is an important correction that must be applied to the reservoir solutions after the protein-ligand drop has been mixed with the reservoir solution, because the cryozone contain glycerol and other hygroscopic compounds that affect vapour diffusion equilibration.[8]

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