MS12-P11 A method for high-throughput, low volume soaking of protein crystals in rapid screening fragment libraries

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Co-crystallisation is a common method for producing protein-ligand complex structures. It is especially useful when drug-like compounds trigger conformational changes in proteins. This can result in variations in the growing conditions or crystal form and may necessitate wider screening strategies for co-crystallisation in general. Alternatively, soaking protein crystals with ligands is the fastest route to high-throughput structure if the starting crystal form is easy to grow reproducibly, able to accommodate the desired ligand and is robust to physical and chemical changes. This poster will describe a miniaturised, high-throughput soaking method developed by Dr. David Hargreaves, AstraZeneca, UK for screening a fragment-based lead generation (FBLG) library. This automated screening method uses TTP Labtech's mosquito® Crystal enabling small volumes of fragment libraries (10-25 nL) to be prepared systematically. The mosquito® liquid handler was used to make up a variety of soaking solutions containing fragment library cocktails at high concentrations, high affinity ligand soaks at lower concentrations and gradients of compound concentrations. Having already grown crystals in a standard crystallisation plate the solutions were prepared and transferred quickly and automatically reducing potential errors and improving reproducibility. The success of this soaking method relied on the accuracy and reproducibility of mosquito Crystal's low volume pipetting which is in the range of 25 nL to 1,200 nL. This reduced material cost and, together with its ease-of-use makes this method a highly attractive initial high-throughput screen for all structure–based compound screening.

Keywords: protein crystallography, soaking, fragment based drug design

MS12-P12 Humidity Induced Phase Transitions of HEW Lysozyme Investigated by Microcrystalline Powder Diffraction

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Proteins often crystallize in microcrystalline precipitates. The protein molecules are then surrounded by solvent and their packing arrangement is retained by limited intermolecular contacts. A change in the crystal environment first affects the bulk solvent that fills the intermolecular space, with resulting changes in the crystal structure. In literature it is reported that protein crystals in controlled humidity environments show a large change in unit-cell parameters when the humidity is decreased [1-2]. When a protein crystal is carefully dehydrated, it is in a metastable state in which the crystal initially still retains the original packing structure [2]. Further dehydration may cause the collapse of the crystal lattice: the crystal no longer maintains its packing structure because of the loss of a large amount of bulk solvent. However in some crystals, the dehydration induces a molecular arrangement change resulting in a new crystal structure. This has been already reported for hen egg-white (HEW) lysozyme [3]. While dehydration can induce structural changes, this is also likely to happen upon hydration of the same crystals.

Here, we present our results of microcrystalline tetragonal and monoclinic HEWL samples on a laboratory X-ray powder diffractometer including in situ measurements under variable relative humidity conditions. The observed gradual structural changes as well as phase transitions upon dehydration and hydration of HEWL are analyzed in the relative humidity range 50% - 95%. D

ehydration and hydration processes are reversible in humidity cycles in the range of 95% rH to 75% rH. Without stabilizing PEG the lower limit for dehydration of tetragonal HEWL is around 75% rH. With PEG the tetragonal HEWL samples remain crystalline below 75% rH, but show phase transitions and larger variations of the cell parameters. Below 75% rH a new tetragonal polymorph was discovered.

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