Characterizing Proteins Using XRPD and SAXS Techniques on a Laboratory Diffractometer

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Proteins often form 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. Protein crystals, when exposed to controlled humidity environments can show a large change in unit-cell parameters when the humidity is decreased. The effect of relative humidity (rH) on the crystal structures of protein polycrystalline precipitates can be monitored via in-situ laboratory X-ray Powder Diffraction (XRPD) measurements.

On the other hand, Small-Angle X-ray Scattering (SAXS) applied to protein solutions has become an accepted and rapidly growing structural biology technique. Measurements can be done under native conditions, while varying concentration, pH, ionic strength or temperature. Such data provides information about molecular weight, size, shape and stability of the biomolecules and ultimately allow for a (low-resolution) molecular shape envelope reconstruction. The information is complementary to that obtained from XRPD, NMR or cryo-EM. Although the setup for SAXS is easy in theory, it is in practice demanding with respect to the instrumentation and until recently it required dedicated, costly laboratory instruments or the usage of synchrotron beam lines.

Here we present how a multipurpose Empyrean diffractometer can be configured for XRPD and SAXS measurements at ambient conditions and for in-situ rH and temperature (T) experiments. The performance is demonstrated on a number of protein examples.