Distinguishing Protein Nanocrystals from Amorphous Precipitate by Depolarized Dynamic Light Scattering

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Growth and preparation of high quality micro-sized protein crystals, optimal for data collection experiments at modern micro-focus synchrotron (SR) beamlines and growth of nanocrystals required for data collection at Free-Electron-Laser (FEL) radiation sources is a new and challenging task. Latest methods will be presented to precisely monitor crystal growth and to optimize the preparation of crystalline particles, too small to be observed by light microscopy. The identification of the presence of a spatial repetitive orientation of macromolecules (crystal nuclei) in the early stages of the crystallization process is essential to detect nanocrystals. The optical properties of a crystal lattice offer the potential to detect the transition from disordered to higher ordered particles. A unique experimental setup was designed and constructed to detect nanocrystal formation by analyzing depolarized scattered laser light. The ability of a lattice to depolarize laser light depends on the different refractive indices along different crystal axes. The results obtained so far demonstrate that the successful detection of nano-sized protein crystals at early stages of crystal growth is possible, by analyzing the signal intensity of the depolarized component of the scattered light. The method and approach allows an effective differentiation between protein-dense liquid cluster formation and ordered nanocrystals[1]. The data and results obtained so far were verified by complementary methods like X-ray powder diffraction, second harmonic generation, ultraviolet two-photon excited fluorescence and scanning electron microscopy.

Further, this particular advanced laser light scattering technique can be combined with a state of the art protein crystallization robotic setup (Xtal-Controller[2]), allowing the controlled nanoliter increments addition of protein, precipitant and additive solution towards a crystallization solution sitting on a microbalance. By this combination, crystallization phenomena can be characterized in detail and methods can be optimized for the efficient production of nanocrystals. Details and examples will be presented.

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[1] Schubert *et al.*, Journal of Applied Crystallography, Issue 48, 1476-1484, (2015)

[2] Meyer *et al.* Acta Crystallographica Section F68, 994-998, (2012)