

116f Fluorescent Approaches to High Throughput Crystallography

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X-ray crystallography remains the primary method for determining the structure of macromolecules. The first requirement is to have crystals, and obtaining them is often the rate-limiting step. The numbers of crystallization trials that are set up for any one protein for structural genomics, and the rate at which they are being set up, now overwhelm the ability for strictly human analysis of the results. Automated analysis methods are now being implemented with varying degrees of success, but these typically cannot reliably extract intermediate results. By covalently modifying a subpopulation, ~ 0.5 to 1%, of a macromolecule solution with a fluorescent probe, the labeled material will add to a growing crystal as a microheterogeneous growth unit. Labeling procedures can be readily incorporated into the final stages of purification. The covalently attached probe will concentrate in the crystal relative to the solution, and under fluorescent illumination the crystals show up as bright objects against a dark background. As crystalline packing is more dense than amorphous precipitate, the fluorescence intensity can be used as a guide in distinguishing different types of precipitated phases, even in the absence of obvious crystalline features, widening the available potential lead conditions in the absence of clear "hits." Non-protein structures, such as salt crystals, will not incorporate the probe and will not show up under fluorescent illumination. Also, brightly fluorescent crystals are readily found against less fluorescent precipitated phases, which under white light illumination may serve to obscure the crystals. Intensity is a much simpler analysis parameter than linear features, and automated image analysis to find crystals should be greatly facilitated, without having to first define crystallization drop boundaries and by having the protein or protein structures all that show up. The trace fluorescently labeled crystals will also emit with sufficient intensity to aid in the automation of crystal alignment using relatively low cost optics, further increasing throughput at synchrotrons. X-ray diffraction experiments show that the presence of the fluorescent probe does not affect the nucleation process or the quality of the X-ray data obtained.