Detection of Protein Crystals Formed in the Crystal Former using the UVEX UV-Fluorescence Microscope.

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ABSTRACT:

PURPOSE: Effective in situ discrimination between protein and salt crystals without the requirement for X-ray diffraction greatly improves the workflow of protein crystallographers. One such technique is based on the UV-fluorescence of the amino acid tryptophan, which fluoresces at 360 nm when excited with UV-light at 280 nm. In this study we evaluate the suitability of the JANSi UVEX (JAN Scientific, USA) microscope to detect protein crystals grown in the microcapillaries of the Crystal Former (Microlytic ApS, DK).

METHODOLOGY: Crystals of the protein chicken egg white Lysozyme were formed in the microchannels of the Crystal Former under standard crystallization conditions known to promote protein crystals. The Crystal Former channels were subsequently imaged using the JANSi UVEX microscope.

RESULTS: Protein crystals formed within the channels of the Crystal Former could be identified clearly based on their UV fluorescence using the JANSi UVEX system. It was found that even protein micro-crystals (~10 µm) could be detected in situ with the higher-power (15X) objective.

CONCLUSIONS: Protein crystals formed in the Crystal Former can be identified based on their UV fluorescence. When the JANSi UVEX system is used clear images are obtained in UV-fluorescence mode allowing researchers to evaluate whether crystals are of protein or salt in minutes without extracting the crystals from the Crystal Former.

INTRODUCTION: Atomic resolution structural models of biological macromolecules have enabled a detailed understanding of biological processes at the molecular and mechanistic level. Outside of furthering our basic scientific understanding of biology these models enable rational design of drug compounds and have been used with great success to develop various pharmaceutical drugs.

A major technique for experimentally determining atomic resolution structures is X-ray crystallography in which a well ordered crystal of the target molecule is subjected to an X-ray beam resulting in a diffraction pattern from which the molecular structure of the target molecule can be deduced. Currently a major limitation of X-ray crystallography is the challenges associated with creating well ordered protein crystals with good diffraction properties. This challenge is further complicated by the fact that certain precipitants used to promote crystallization of the target molecule can themselves crystallize over the course of the experiment leading to false positive crystal hits.

The ultimate verification of whether a crystal is a target molecule crystal or just a precipitant crystal requires subjecting the crystal to X-ray diffraction studies. However, this necessitates a significant pre-processing effort including the extraction, cryo-protection and flash cooling of the crystal followed by mounting the crystal in the X-ray beam. An alternative approach is based on the fact that certain amino acid residues have detectable fluorescence. The amino acid tryptophan emits light at ~360 nm when excited with light at ~280 nm; hence if a target protein contains the amino acid tryptophan it should be possible to distinguish target molecule crystals from precipitant crystals based on crystal fluorescence. The UVEX microscope (JAN Scientific, US) is designed to image tryptophan-containing protein crystals in crystallization trays. An impediment to in situ imaging of protein crystals is that certain plastic containers of protein crystals themselves can attenuate the UV excitation light and/or generate large background fluorescence, degrading image brightness and contrast. Given the improved crystallization hit rates obtained in the Crystal Former (Microlytic ApS, DK) we wanted to evaluate whether it also is UV compatible so that protein crystals formed in it could be detected in situ.

**METHODS:** Lysozyme kit from Hampton Research (HR7-108) was used to make a stock solution of 20 mg/ml.

Solutions of Wizard I crystal screen from Emerald Biosystems were used to induce crystallization by loading 400 nl of the screen solution at one end of the capillary and 400 nl of protein solution at the other end. The microfluidic chip was allowed to incubate at room temperature (20 °C) and crystals ranging in size from ~10 µm to ~200 µm formed within 2 days.

**RESULTS:** Crystals formed consistently in the crystallization channels of the Crystal Former and the channels were imaged by placing the microfluidic device in a slide holder mounted on the XY stage of the UVEX microscope. Fluorescence from the larger crystals (>100 µm) could be visualized with the 5x objective, whereas the small crystals (~10 µm) could be seen only with the 15x objective by virtue of the higher fluorescence excitation/collection efficiency and higher spatial resolution of the higher-power objective.

The background signal from the material of the Crystal Former is sufficiently low that fluorescence from even the smallest crystals is detected reliably (Figure 1).

**CONCLUSIONS:** The sensitivity and the resolution of the UVEX UV fluorescence microscope, and the materials and geometry of the Crystal Former are well matched so that protein crystals formed in the microcapillaries of the Crystal Former are readily identified *in situ.*

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![Figure 1](image-url) | Brightfield (A, C and E) and UV fluorescence (B, D and F) images of lysozyme crystals in the microcapillaries of Crystal Former using the UVEX microscope. Channel width is 150 µm. Exposure was 0.5 s for brightfield and 1 s for fluorescence images.