Poster Presentation

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Protein Crystal Staining for Second Harmonic Generation Imaging

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Recently, second harmonic generation (SHG) microscopy has become a useful tool in the field of structural biology for the detection of protein crystals. SHG, or the frequency doubling of light, is a process specific to crystalline media lacking inversion centers. Through theoretical models and experimental data, it is estimated that ~84% of the known protein crystal structures give detectable SHG signal using current SHG microscopy instrumentation. Extending this coverage could be extremely useful to structural biologists who routinely screen entire 96 well plates, with hundreds of crystallization conditions, in search of diffraction-quality protein crystals. A series of SHG active dyes, including Malachite Green (MG) and trans-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (DMI) were investigated to assess their ability to enhance the nonlinear optical (NLO) response across a broad range of protein crystals with varying degrees of inherent SHG activity. MG and DMI were shown to enhance the SHG activity of tetragonal (P43212) lysozyme crystals, a protein that typically generates little to no SHG signal. SHG enhancements for lysozyme of approximately 16000x and 20x were achieved by intercalation of MG and DMI, respectively. These results are consistent with predictions based on the differences in symmetry and structure for the two dyes. The kinetics of the dye intercalation and uptake were investigated with SHG time-lapse images taken of a lysozyme crystal after the addition of MG dye into the crystallization well. Kinetic results indicate that an increase in SHG activity becomes easily noticeable within minutes of exposure to the dyes. These results show a significant progress towards increasing the coverage of SHG microscopy for protein crystal detection.

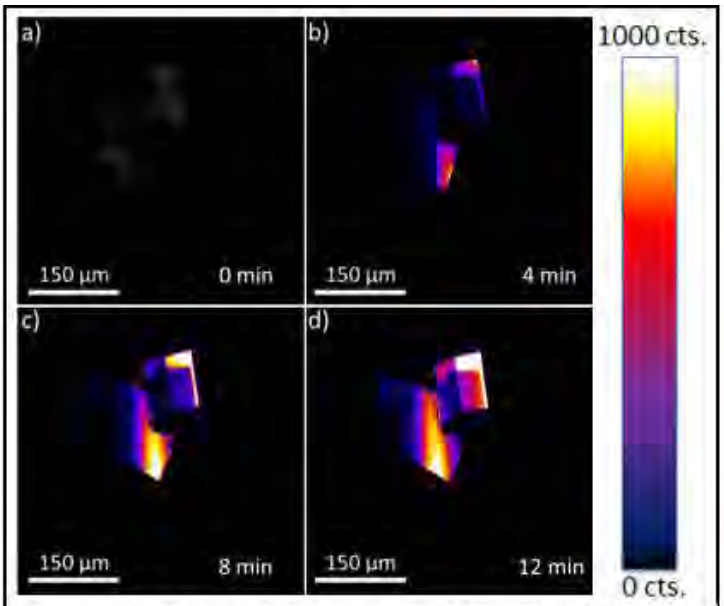

Figure 1. Intercalation of a 500 µM solution of malachite green into lysozyme crystals. At time zero **(a)** there is no detectable SHG. Within minutes detectable signal is observed **(b-d)** with an overall SHG enhancement of ~1000 after 12 minutes of dye intercalation. The scale of **(a)** was adjusted to show low counts.

Keywords: Nonlinear optical microscopy, Crystal screening, High throughput