Method for Enhancing Contrast in Protein Crystal Imaging

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Technology description

Background

Recently, due to the steep annual growth rate of approximately 25 percent of new protein crystal structures entered into the protein data bank, a need for high throughput technologies for protein crystal analysis throughout the entire structure determination pipeline has been realized. Analysis of proteins with high intrinsic fluorescence can be made using current methods. For proteins that do not exhibit intrinsic fluorescence, such as insulin, UV laser-stimulated fluorescence is used for imaging. This method has been shown to damage disulfide bonds within the protein due to exposing the protein crystals to a 266nm excitation laser.

Technology Summary

Researchers at Purdue University have developed a method for intercalating dye into the protein crystals to extend the detection range for second harmonic generation (SHG) microscopy and enhance signal-to-noise (S/N) images. This method uses SHG active dyes to enhance the SHG response of protein crystals. By intercalating SHG active dyes into the formed protein crystal, the dyes adopt a well-ordered orientation within the hydrophobic regions of the protein crystal, thus yielding a strong SHG response. By intercalating SHG active dyes into the protein crystal, a higher protein crystal coverage by

SHG can be realized along with lower integration times for high throughput analysis of protein nanocrystals. Furthermore, the dye is added to the crystal post-crystallization, so it does not have the opportunity to influence the folding structures of the protein.

Related Publications

Justin A. Newman, et al. Intercalating dyes for enhanced contrast in second harmonic generation imaging of protein crystals. Acta Crystallographica, 2015, D71, pp 1471-1477. https://doi.org/10.1107/S1399004715008287. Supporting Information for article: https://doi.org/10.1107/S1399004715008287/rr5104sup1.pdf

Web Links

Application area

SHG Microscopy

Advantages

Extend the detection range for SHG microscopy
Ability to see several orders of magnitude increase in SHG activity of dark protein crystals
Dye does not influence the folding structures of the protein

Institution

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