MS08-1-7 A metal-binding GFP-derivative reused as novel standard sample for serial crystallography approaches at FELs
#MS08-1-7

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Abstract
Since (time-resolved) serial crystallography experiments at an FEL source differ vastly from conventional cryo-crystallography measurements, sample requirements do as well. Hence, standard samples for FELs are needed for several reasons. There is an ongoing need for improving sample delivery methods as well as for data analysis of diffraction images for serial crystallography and time-resolved experiments.
One potential standard sample is iq-mEmerald, a GFP-derivative, with an engineered metal binding-site close to its chromophore, which was initially designed by Yu et al. (2014) as a metal sensor for live cell imaging [1]. Upon metal binding, fluorescence quenching and small reversible conformational changes can be induced, making it a candidate for mixing experiments, as well as for phase determination approaches.
A simple, one day, three-step purification procedure results in up to 300 mg of iq-mEmerald per liter *Escherichia coli* BL21 (DE3) cell culture. Microcrystals of various sizes can be obtained by batch crystallization by varying the pH and ammonium sulfate concentration of the precipitant solution. Crystals can be soaked, among others, with a $ZnCl_2$ solution, resulting in a change of the space group. The anomalous signal of the zinc atoms was exploited for single-wavelength anomalous dispersion (SAD) phasing and a structural model could be built using automatic model-building tools.
Thus, iq-mEmerald is a possible standard sample for serial mixing experiments and phasing approaches.

References
Yu, Xiaozhen, Marie-Paule Strub, Travis J. Barnard, Nicholas Noinaj, Grzegorz Piszczek, Susan K. Buchanan, und Justin W. Taraska. „An Engineered Palette of Metal Ion Quenchable Fluorescent Proteins“. PLoS ONE 9, Nr. 4 (21. April 2014): e95808. https://doi.org/10.1371/journal.pone.0095808.