MS08-2-6 Living cells: crystallization chambers for serial synchrotron crystallography
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Abstract
Despite the advent of AlphaFold and the resolution revolution in cryo-electron microscopy, the X-ray crystallography remains a powerful tool for structural biology. While the focus of crystallographers is shifting from conventional crystallography methods towards more advanced techniques elucidating mechanisms of enzymatic reactions in time-resolved manner. In cellulo crystallization is one such emerging alternate technique that directly utilizes living cells for generation of protein crystals [1-3]. In cellulo crystallography circumvents the conventional and laborious steps of heterologous expression, purification, and in vitro crystallization. Moreover, it allows to study the protein structure in the environment of living cells and thus provides a platform for drug discovery in cellular conditions. However, the underlying mechanisms of the intracellular protein crystallization are still poorly understood, and the identification of novel in cellulo crystallization targets is not at all straightforward. In recent years several techniques were developed that allow for fast screening of suitable novel in cellulo targets and improve hit rates of low efficacy in cellulo crystals [4-5]. Here we present our approach to the in cellulo crystallization method pipeline that is applied at EMBL – Hamburg at the PETRA III beamlines to identify novel in cellulo crystallization targets, to improve the crystal containing cells concentration and to prepare cryo-samples for serial synchrotron diffraction data collection in robust and reliable manner.

References
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