Poster

Efficient enrichment of in cellulo grown protein crystals

Jan Blaha

EMBL Hamburg, DE
jan.blaha@embl-hamburg.de

Among the well-established methods for protein crystallization, in cellulo crystallography has a special place, as it allows for investigation of protein structure in the cellular milieu. However, due to current limits in current understanding why some proteins undergo spontaneous ordered assembly, in cellulo crystallization has remained a niche technique to date. Many protein targets that can be identified in cellulo crystallization are inaccessible for X-ray diffraction experiments due to insufficient yields of these crystals in cell culture. Here, we introduce a novel approach for enrichment of cells housing in cellulo crystals. We employ linked co-expression of a marker protein and in cellulo crystallizable protein target to obtain a direct correlation between the marker protein's signal and the crystallization probability of the target. We demonstrate this approach on established in cellulo crystallization targets HEX-1 from N.crassa and cathepsin B from T.brucei, by sorting subpopulations of crystal containing cells from the cell culture. This technique is part of the in cellulo crystallization pipeline that is utilized by EMBL at the PETRA III beamlines to identify novel in cellulo crystallization targets, to improve the concentration of crystal containing cells and to prepare cryo-samples for serial synchrotron diffraction data collection in a robust and reliable manner. This study presents necessary innovations with the potential to make the in cellulo protein crystallography more available for structural biology studies of proteins without known experimental structures in the intricate cellular environment.