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De novo in-vivo protein crystal structure: is experimental phasing required?

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The combined use of modern microfocus synchrotron beamlines in conjunction with serial crystallography approaches allows the exploitation of a reasonably low number of micron-sized protein crystals for structure determination. Such micro-crystallography techniques open the window towards the investigation of the full potential of in-vivo crystallography.
In-vivo crystallography is a phenomenon in which protein crystals occur within the cell or living bodies, naturally or induced by heterologous expression, in the cytoplasm or in specific subcellular compartments [1]. Such crystals are limited in size but are produced in a native like environment. Anticipated benefits of such system are the possibility to obtain crystals of proteins for which classical in vitro crystal growth revealed unsuccessful and the opportunity to study functionally important post-translational modifications potentially providing a new tool in the protein crystallography area. Additionally, when properly understood, in vivo crystallography could minimize the amount of efforts provided in optimizing purification of samples prior to in vitro crystallization, currently presenting a very large bottleneck in the technique [2].
Whereas subsequent crystals purification can be performed for diffraction experiments, the more delicate ones are prone to dissolution upon cell lysis. Nevertheless in cellulo diffraction studies of such crystals have proved to be feasible and led to protein structure determination. Further characterization of properties of in cellulo diffraction is of great interest for several reasons.
Beside the fact of dealing with protein crystals grown in a native-like environment (presence of co-factors, ligands, proteolytic processing…), established advantages of in cellulo protein crystals diffraction represent a simplified and time-saving workflow for sample obtention and preparation, as the intracellular medium might act as a cryoprotectant for example, without trading for diffraction pattern quality due to the minimal crystal handling [3]. It gives also the possibility to deal with sensitive protein crystals which revealed not stable upon crystals purification, as well as it might help for increasing the hit rate during diffraction experiment via cell staining and simplified sample identification.
However, on the road to de novo crystal structure determination, little is known about the possibility to achieve the limiting step of phase determination. As in vivo crystallography stands, it may represent a clear challenge for phase determination without experimental phases. Further to the native nature of the crystals and proteins within, which eventually include post-translational modifications currently unseen in most of the in vitro prepared samples, the requirement to perform serial crystallography measurements adds to the complexity of the process. Our preliminary results tend to indicate that, although not documented, structure determination of in vivo grown crystals without experimental phasing may benefit from an iterative approach combining new methods to the latest advances in structure determination from native data sets and weak anomalous scatterers.
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