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Solving structures from in cellulo crystallized proteins: strategies and bottlenecks

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X-ray crystallography requires the growth of well-ordered, sufficiently sized protein crystals to obtain structural insights at atomic resolution. In addition to routinely performed parameter screening in vitro, protein crystallization in living cells, referred to as in cellulo crystallization, holds the possibility to grow a huge number of micron-sized protein crystals with comparable properties and of high quality in a short time [1,2]. The advantage of in cellulo crystallization is apparent: The cells grow the protein crystal without the need for purification or crystal screening steps. To systematically exploit the enormous potential of in cellulo crystallization for structural biology we streamlined this process by establishing a pipeline to elucidate the structural information of an in cellulo crystallized target protein in short time. After cloning of the target gene into baculovirus transfer vectors, the associated recombinant baculoviruses are generated to infect insect cells, and crystal formation is detected at day 4 to 6 after infection. If intracellular crystallization is successful, diffraction data of the isolated in cellulo crystals are collected using serial crystallography approaches at highly brilliant synchrotron sources [3] or XFELs [2], depending on the size of the obtained crystals. Although these efforts resulted in the successful crystallization of more than 25 different proteins so far, several bottlenecks currently restrict a more broad application of in cellulo crystallography: Depending on the recombinant protein, the number of crystal containing cells varies between more than 70 % and less than 1 %, and changes of environmental conditions during cell lysis and crystal purification result in a loss of crystal quality. Moreover, exploiting living cells as native crystallization bioreactors excludes solving the phase problem by experimental methods.

In this talk, strategies to overcome these limitations will be presented, including intracellular labelling of recombinant target proteins with heavy metal ions and serial diffraction data collection from in cellulo grown crystals directly within the living cell using synchrotron radiation. These innovative approaches avoid any transfer of the living, crystal-containing cells, allow direct screening of cell cultures for successful in cellulo protein crystallization using the X-ray beam, and will gain access to direct phasing methods, e.g. multiwavelength anomalous diffraction (MAD). Thus, the current in vivo crystallization pipeline will be further improved to elucidate structures of proteins without prior information, and limitations in data collection due to low intracellular crystallization efficiency will be overcome. Our results pave the way to more efficiently use crystal containing cells as suitable targets for serial diffraction data collection at synchrotrons and XFELs in the future.

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