

In vivo Crystallography: A New Facet of Structural Biology

“In vivo crystallography opens a new door for protein crystallography of cytosolic and membrane proteins from diverse organisms at a native-functional state”

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Most of the proteins are under negative selection pressure for crystallizing inside the cells of organisms. However, the knowledge of several proteins crystallizing *in vivo* is also known. These proteins are proposed to be under positive selection pressure for crystallizing inside the cells with functional importance. One of the most interesting examples, amongst many others, includes the *in vivo* crystallization of δ -endotoxins or crystal (Cry) toxin proteins formed during sporulation of *Bacillus thuringiensis*. The crystallization of these proteins enables the toxin to be stored within a small volume in high concentration. Additionally, sf9-baculovirus systems have been engineered that induce *in vivo* crystallization of the over-expressed proteins.

The conventional *in vitro* X-ray crystallography involves recombinant protein purification and crystallization using various modifications to make the protein homogenous and monodisperse. This usually drives the protein away from its native state in which it naturally occurs. Further, proteins with post-translation modifications have been observed to be heterogeneous and mostly polydispersity in physiological conditions. It is argued (in spite of significant evidence otherwise) that purification and crystallization of proteins *in vitro* result in crystal structures that might not represent the true native structure of a protein or an assembly of interacting proteins or the catalytic enzyme-substrate complex as observed in cells.

One of the major challenges of *in vivo* crystallography has been the sizes of the *in vivo*-grown crystals that are limited by the volume of the cells and are usually of the micro-nanometer range. However, recent advancements in X-ray free electron lasers (XFEL) and serial femtosecond crystallography (SFX) have resulted in the increase of structures from *in vivo* crystals. The crystal structure of *BtCry3A* toxin was determined directly from the bacterial cells containing the *in vivo* crystals using SFX. As the scope of *in vivo* crystallography is broadening, protein structures from diverse *in vivo* crystals grown naturally and artificially are being determined. Since the need for structures of multiple pathogenic targets is increasing continually, *in vivo* crystallography can provide a new field for structure determination at native and natural state.

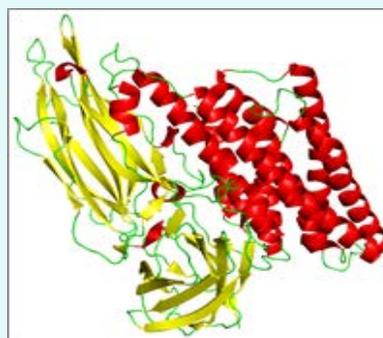


Figure: An example of X-ray crystal structure of *in vivo* grown crystals of Cry3A toxin (adapted from Sawaya., et al. 2014).

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