In vivo carrier-driven crystallization for novel structural determinations

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It is hard to do crystallography without a crystal, and unfortunately obtaining a crystal is often the most iterative or precipitous step within macromolecular crystallography. There is however a family of proteins that have evolved with strong crystal packing interactions, and crystallize in vivo. These Cry (as in CRY-stal) proteins are extremely well researched as they are important to the agricultural industry, but interestingly to date no one has tried to use these proteins to crystallize anything else.

Recently the full length structure of Cry1Ac (the most well researched member of this family) was determined (1), including the previously undetermined C-terminal extension which was thought to be responsible for crystallization. The current work will attempt to repurpose this system to crystallize other proteins by creating fusion proteins of Cry1Ac and a protein of interest.

To test this system, GFP-Cry1Ac fusions were made to reproduce literature precedent (2, 3) that were overexpressed in E. coli. Though near identical constructs were created, structural characterization was never attempted previously, so the crystallinity of these inclusions is unknown. Currently, they are being tested using X-ray powder diffraction and electron diffraction.

This work is part of a larger process to generate a tool for crystallographers that will eliminate the need for chromatographic purification and crystallization for proteins that are compatible with this system. The exact constraints of this system is unknown, but represents a unique way to overcome many of the greatest hurdles in crystallography.

References:

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