MS082.O06

Design and application of crystallization aids comprising DARPin domains.

Peer Mittl[1], Yufan Wu[1], Patrick Ernst[1], Alexander Batyuk[1], Annemarie Honegger[1], Andreas Pluckthun[1]
[1]*University Zürich, Zürich, Switzerland*
E-mail: mittl@bioc.uzh.ch

Crystals of sufficient quality in terms of size and intrinsic order are still indispensable to determine the structures of biological macromolecules by X-ray diffraction techniques. Despite large progress in instrumentation and screening throughput, many proteins still resist yielding diffraction-quality crystals. To overcome this bottleneck, strategies like homology screening, fragmentation, surface entropy reduction, and synthetic symmetrization have been introduced, but they all require genetic manipulation of the target protein. Only the binding of crystallization aids, such as Fab fragments or nanobodies circumvent this limitation. We have developed a modular approach for crystallization aids that extends the initial concept in several ways, adding molecular dimension and surface composition as new screening variables. These crystallization aids comprise Designed Ankyrin Repeat Proteins (DARPins). Since DARPins are very stable, they can be expressed with high yields in E. coli and high-affinity binders can be selected by ribosome display from DARPin libraries. Among crystallization aids, DARPins are special because they have N- and C-terminal helices, allowing us to fuse two or three DARPins by means of a shared helix. The rigid helix linker fixes the DARPin domains in different relative orientations such that none of the paratopes obstructs the other. The DARPin recognizing the protein of interest is coupled either to a particularly well crystallizing DARPin or to a DARPin that binds yet another helper protein (e.g. MBP, GFP). Fourteen crystal structures of two- and three-DARPin fusions, encompassing eight of nine different connector geometries, without or with bound helper proteins, generally confirm the validity of the design. We also applied this concept to determine two crystal structures of human c-Jun N-terminal kinase 1. Particularly the modularity of this approach offers ample opportunity to overcome limitations in crystallogenesis.

Keywords: crystallization chaperone, protein engineering, scaffold protein