Designed Ankyrin Repeat Proteins as Tools for the Crystallization of Proteins

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Ankyrin repeat proteins (ARPs) are ubiquitous protein-protein interaction molecules fundamental to many biological processes. By consensus sequence and structure analyses of ARPs, we derived a repeat module of 33 amino acids with fixed framework residues and randomized surface residues suitable for target binding. The random assembly of such modules yields combinatorial libraries of naive ARP's of varying length and diversities larger than 10^{10}. Unselected library members are well expressed and stable and show the correct fold [1]. Using ribosome display we selected specific binders against different protein targets with affinities in the low nanomolar range [2]. This opens the possibility to crystallize a target protein in complex with ARPs and enhances the chances of obtaining structures of target proteins difficult to crystallize. We have applied this technology to a variety of different proteins such as proteases, kinases and membrane proteins. The methodology and structures of unselected ARP's alone as well as of an ARP-maltose binding protein complex and an ARP-kinase complex will be presented proving the usefulness of selected ARP's in structural biology. The technology opens a new avenue in macromolecular crystallization and is an attractive alternative to antibodies in the crystallization of membrane proteins.

[1] Kohl A., Binz H.K., Forrer P., Stumpp M.T., Plückthun A., Grütter M.G., *PNAS*, 2003, **100**, 1700-05. [2] Binz H. K., Amstutz P., Kohl A., Stumpp M.T., Briand C., Forrer P., Grütter M.G., Plückthun A., *Nature Biotech*, 2004, **22**, 575-82.

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