PROTEIN BIOCHEMISTRY

Widening the protein crystallization bottleneck

Two recent reports describe simple methods that may help expand the range of proteins amenable to crystallization.

The Protein Data Bank (PDB) database of three-dimensional protein structures is growing by leaps and bounds thanks to large-scale structural proteomics efforts. Multi-institutional collaborations such as the Structural Genomics Consortium (SGC) have streamlined the process from target selection to data collection by optimizing methods for protein expression and purification as well as crystallization. But successfully getting proteins to crystallize still is the major rate-limiting step in the overall process.

Nevertheless, protein crystallographers have a few tricks up their sleeves to improve their chances of crystallizing a protein. Often the way to go is to stabilize the protein structure; rigid structures are easier to crystallize and in turn form better diffraction-quality crystals. Two groups use this idea to provide useful solutions to protein stabilization.

One solution to improve a protein's crystallization chances is to simply screen more conditions, a strategy that is widely used in both large-scale and individual efforts. Furthermore, the addition of a small-molecule ligand can often stabilize the protein structure. With this in mind, Aled Edwards of the University of Toronto and his colleagues in the SGC have for the first time systematically investigated the stabilizing power of ligands (Vedadi *et al.*, 2006).

"For the many proteins that undergo large conformational changes in the presence of a ligand, it is difficult to imagine that the use of small molecules would not be critical [for successful crystallization]," says Edwards. To screen for ligands that increase the stability, and by extension the 'crystallizability' of a protein, Edwards and his colleagues use the simple techniques of differential scanning fluorimetry and differential static light scattering to measure an increase in protein thermal stability. The fluorimetry technique measures the increase in fluorescence of a dye that binds as a protein unfolds, whereas the light scattering technique monitors the aggregation of proteins as they thermally denature. They used these two techniques to screen 221 proteins first against a series of 'generic' compounds such as common cofactors, metals and nucleotides, among others. They also screened a focused library of kinase inhibitors to identify ligands that stabilized serine-threonine protein kinases.

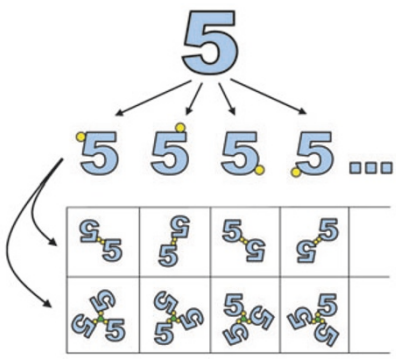

Figure 1 | A representation of protein symmetrization. The "5" represents the nonsymmetric protein. When cysteine point mutations are introduced (yellow dots) at various positions, disulfide bond formation results in protein dimerization and thus symmetrization. Protein trimerization (using a trivalent thiol-specific crosslinking reagent) is also theoretically possible. Reprinted with permission from the National Academy of Sciences.

For proteins of unknown structure and activity, the Edwards group and others are in the process of generating core metabolite libraries. To date, they have used both the generic and specific ligand screens to help crystallize more than 30 human therapeutic protein targets of the SGC. But Edwards stresses that these strategies are not just for large-scale implementation, as he says, "The methods are not only effective and robust, but are also cost-effective and able to be implemented in individual laboratories."

Alternatively, sometimes it is more efficient to endow the protein structure with 'crystallizable' properties rather than set up multitudes of screening experiments. Todd Yeates of the University of California, Los Angeles and his colleagues take this approach as reported in their recent paper (Banatao *et al.*, 2006). Yeates and his colleagues have understood for some time that it is easier to crystallize molecules that are relatively symmetric. Therefore, they hypothesized that by introducing cysteine residues at a solvent-exposed position on a protein surface and forming disulfide bonds between individual proteins, artificial dimers could be constructed, which are symmetric by default (**Fig. 1**). "You can actually create a whole series of different constructs depending on where you place your attachment point," explains Yeates.

They tested the method with phage T4 lysozyme and were able to successfully crystallize six novel crystal forms from protein dimers. This in itself was rather remarkable and a good sign for the future application of the strategy, as postdoc Rey Banatao explains: "Looking at the PDB and the number of occurrences of lysozyme in various crystal forms, it was pretty amazing that we could find six new crystal forms even though that protein is arguably the most crystallized protein in the database." Yeates is collaborating with the Joint Center for Structural Genomics, where the true potential of this method for aiding large-scale efforts will be put to the test. "We have selected a number of targets that haven't crystallized readily from their pipeline and settled on a series of constructs to be made from those to see if a handful of proteins that fell out of the pipeline can be rescued," says Yeates.

There is no one perfect method for achieving protein crystallization; it is still very much an empirical science. But these two reports demonstrate that the more tools in the arsenal, in particular simple tools to increase protein stability, the better the chances for crystallization.

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RESEARCH PAPERS

Banatao, D.R. *et al.* An approach to crystallizing proteins by synthetic symmetrization. *Proc. Natl. Acad. Sci. USA* **103**, 16230–16235 (2006).

Vedadi, M. *et al.* Chemical screening methods to identify ligands that promote protein stability, protein crystallization, and structure determination. *Proc. Natl. Acad. Sci. USA* **103**, 15835–15840 (2006).