number of strategies offer general applicability. Among these, gene-construct optimization or surface-entropy reduction are quickly gaining popularity as methods of choice. However, it should be stressed that none of the methods described here offer a guarantee that the target protein can be coerced to crystallize. To maximize the chances of success, one must frequently attack the problem on multiple fronts based on an understanding of the chemical and physical properties of a specific protein. This is particularly true of technically difficult targets such as membrane proteins. A classic example illustrating this principle is the study of the HIV gp120 envelope glycoprotein (Kwong *et al.*, 1998, 1999). The construct that was ultimately used in successful crystallization screens had deletions of 52 and 19 residues from the N- and C-termini and two flexible loops replaced by Gly-Ala-Gly linkages; additionally, the protein was 90% deglycosylated compared with the wild type. Moreover, this engineered gp120 was only crystallized in the form of a ternary complex with the CD4 receptor and an Fab fragment from a neutralizing antibody. In the recent case of the ATP-gated P2X4 ion channel, a crystallizable variant was obtained after a series of N- and C-terminal deletions were screened to identify the smallest functional unit and the introduction of three mutations (C51F/N78K/N187R) to eliminate both aggregation arising from oxidation and N-glycosylation (Kawate *et al.*, 2009).

The rapidly expanding database of macromolecular structures greatly enhances our understanding of the physical chemistry of proteins, ultimately enhancing our ability to predict the behaviour of a protein in solution from its sequence. It is therefore increasingly possible to rely on such theoretical predictions *in lieu* of tedious experimental screens. A number of online tools have been developed for this purpose. The propensity of a protein target to crystallize can be evaluated using the *XtalPred* server (http://ffas.burnham.org/XtalPred-cgi/xtal.pl), which offers insights into potential sources of problems arising from sequence features (Slabinski *et al.*, 2007). Automated design of optimally truncated constructs for structural analysis has been made possible by the *ProteinCCD* meta-server (http://xtal.nki.nl/ccd), which uses the cDNA sequence of the target (Mooij *et al.*, 2009). This server collects information about secondary structure, disorder, putative coiled coils, transmembrane segments, domains and domain linkers, and suggests constructs so that the user can interactively choose suitable options and obtain sequences of oligonucleotides needed for appropriate PCR amplification (Mooij *et al.*, 2009). For proteins recalcitrant to crystallization in their wild-type form, surface mutations enhancing crystallizability can be designed using the surface-entropy reduction server (http://nihserver.mbi.ucla.edu/SER/; Goldschmidt *et al.*, 2007).

As the focus of macromolecular crystallography shifts from the principles of protein architecture to increasingly complex biological questions, the approach to crystallization is also undergoing dramatic evolution. As we gain better understanding of the microscopic nature of protein crystallization, we will be able to develop rational protein-engineering strategies that systematically and significantly improve the success rate of crystallization.

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