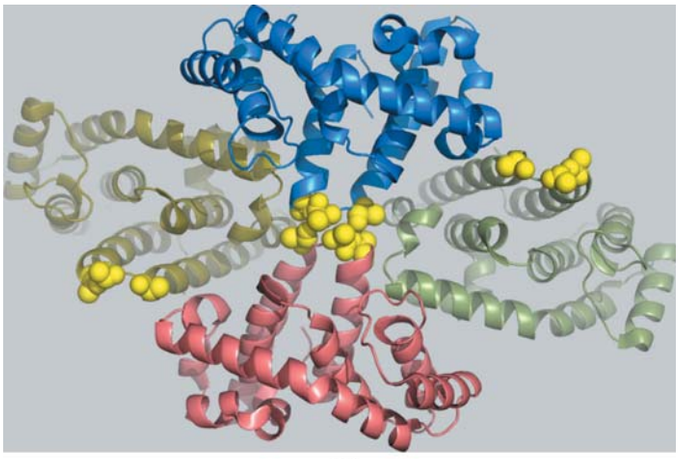
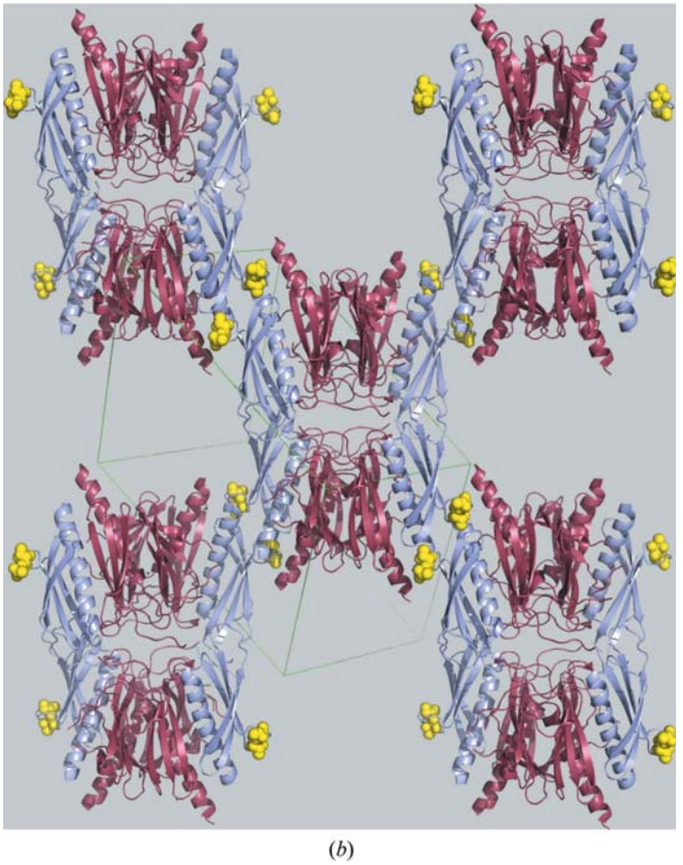


(*a*)



(*b*)

Figure 4.3.9.1
Two examples of proteins crystallized by the surface-entropy reduction (SER) method. (*a*) The RGSL domain of the PDZRhoGEF nucleotide-exchange factor (PDB code 1htj; Longenecker, Lewis *et al.*, 2001); the yellow spheres show the alanines introduced by mutagenesis, which mediate an isologous crystal contact across a crystallographic twofold axis. (*b*) The crystal structure of EpsI complexed with EpsJ (PDB code 2ret; Yanez *et al.*, 2008); the EpsI protein (pale blue) contains two surface mutations, shown by yellow spheres, which mediate heterologous crystal contacts.

protein only crystallized after an entropy-reducing mutation (E104Y) was introduced into the protein (Pornillos *et al.*, 2009). The protein–protein complexes solved to date underscore the utility of the method, which extends beyond individual proteins because high-entropy patches occur outside complex interfaces. For example, the complex of c-Src and its inactivator Csk was crystallized using a variant of Csk carrying K361A and K362A mutations (Levinson *et al.*, 2008). Similarly, it was possible to crystallize the complex of two pseudopilins EpsI and EpsJ from the type 2 secretion system of *Vibrio vulnificus* when a variant of EpsI carrying two mutations (E128T and K129T) was used (Yanez *et al.*, 2008; Fig. 4.3.9.1).

An interesting variation of the SER method was used in the investigation of the RACK1 protein, which was crystallized as an in-line fusion with an MBP variant carrying D82A, K83A and K239A mutations (Ullah *et al.*, 2008). This is the first example of the application of the surface-entropy reduction strategy to a carrier protein and not the crystallization target itself.

The SER strategy is attractive not only because of its efficacy but also because of its simplicity: once an expression construct for a target protein is available several rounds of mutagenesis can easily create variants with systematically enhanced crystallizability. To assist in the design of crystallizable variants, a server has been developed that uses the amino-acid sequence of the target to identify suitable mutation sites (Goldschmidt *et al.*, 2007).

4.3.10. Improvement of crystal quality

In most cases, protein engineering is used as a tool of last resort to obtain variants for proteins for which no crystals can be grown using the wild-type form. However, it may sometimes be necessary to obtain a new, different crystal form even when the wild-type protein does crystallize. Such a need may arise, for example, in drug-design investigations, where high-resolution structures are particularly critical for evaluation of the interactions between lead compounds and the target protein and may not always be possible using wild-type crystals. A novel crystal form may also be necessary if the wild-type crystals contain the target protein in an orientation in which the active site is obscured by crystal contacts, making it impossible to soak in drug lead compounds and screen small-molecule libraries by high-throughput crystallography (Blundell & Patel, 2004).

One possible strategy for obtaining a new crystal form is to modify the existing crystal contacts by replacing some of the participating amino acids. While this approach occasionally leads to improvement of the X-ray data resolution (Liu *et al.*, 2007; Mizutani *et al.*, 2008), modification of crystal contacts is typically counterproductive as it abolishes the propensity of the target to crystallize in one form but does not necessarily induce another (Charron *et al.*, 2002). A more successful strategy is to generate a novel crystal form by engineering new crystal contacts through SER. For example, a novel crystal form of the insulin-like growth factor 1 receptor kinase domain, a putative drug target, was obtained using a double mutant (E1067A and E1069A); the new form diffracted to 1.5 Å resolution, whereas the wild-type crystals only diffracted to 2.7 Å resolution (Munshi *et al.*, 2003). In the case of the catalytic domain of activated factor XI, a key enzyme in the blood coagulation cascade and another potential drug target, a single K437A mutation allowed the preparation of a crystal form that diffracted to 2.0 Å resolution (Jin *et al.*, 2005). Entropy-reducing mutations were also key in the preparation of a crystal form of HIV-1 reverse transcriptase for structure-based drug design that diffracted to 1.8 Å resolution, in contrast to the typical 2.5–3.0 Å range observed for the wild-type protein crystals (Bauman *et al.*, 2008).

4.3.11. Conclusions

Protein engineering has become a routine tool that is used to generate crystallizable macromolecules and their complexes. While some approaches may only apply to very specific targets, a