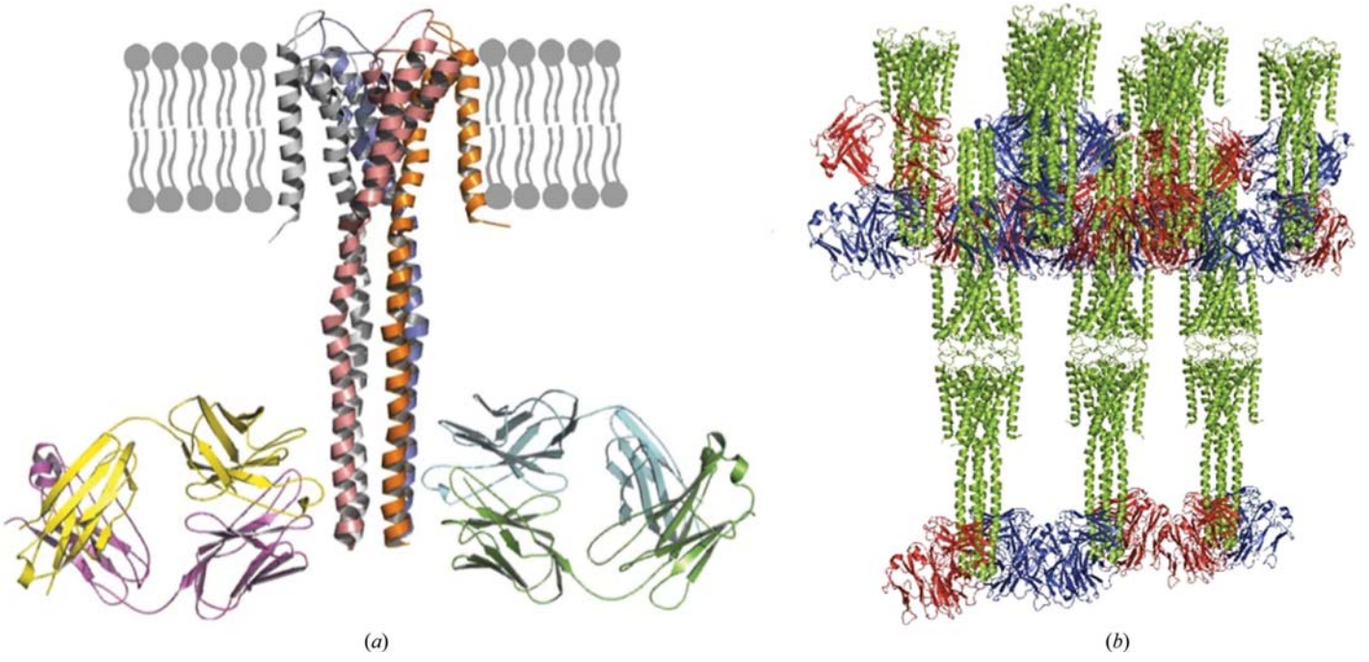

Figure 4.3.6.1
Phage-display-generated Fab fragments as crystallization chaperones: the structure of the KcsA channel in the closed conformation in complex with a synthetic Fab (PDB code 3eff; Uysal *et al.*, 2009). (*a*) A diagram showing how the Fab binds to the cytosolic portion in reference to the transmembrane domain. (*b*) The crystal structure of the complex showing how the synthetic Fab molecules mediate the major crystal contacts. (Figure courtesy of Dr Anthony Kossiakoff, University of Chicago.)

and expensive. In principle, a more efficient approach is to carry out *in vitro* selection of Fab fragments using phage display (Lee *et al.*, 2004) or ribosome display (Lipovsek & Pluckthun, 2004). However, since a typical antibody–antigen interface involves ∼30 amino acids, the total number of possible sequences of a given template Fab significantly exceeds the available combinatorial libraries. Consequently, traditional phage-display libraries greatly diminish diversity at the mutated sites, which explains why synthetic antibodies were initially weaker binders than natural ones (Hawkins *et al.*, 1992; Koide, 2009). This problem was successfully overcome using a different type of phage-display library based on a 'reduced genetic code' and comprised of only a few amino acids, *e.g.* four, which produces high-affinity binders based on a single Fab scaffold (Fellouse *et al.*, 2004; Lee *et al.*, 2004). In contrast to natural antibodies, such synthetic Fab fragments can be generated against unique conformations, complexes or weak antigens such as RNA. Among recent examples are the crystallization and structure determination of the closed form of the full-length KcsA potassium channel with its cognate synthetic Fab (Uysal *et al.*, 2009; Fig. 4.3.6.1) and the crystallographic study of the ΔC209 P4-P6 domain of the *Tetrahymena* group I intron, a structured RNA molecule (Ye *et al.*, 2008).

The *in vitro* display methods also allow the engineering of non-antibody scaffolds as alternative protein binders and crystallization chaperones (Koide, 2009). For example, a fibronectin type III domain (FN3) scaffold was successfully used to generate binders with a reduced genetic code phage-display library (Koide, Gilbreth *et al.*, 2007; Gilbreth *et al.*, 2008). A similar approach was used for DARPins, *i.e.* designed ankyrin-repeat proteins (Sennhauser & Grütter, 2008), based on ribosome-display selection (Lipovsek & Pluckthun, 2004; Sennhauser & Grütter, 2008). Several new protein structures have been solved as complexes with DARPin chaperones, including polo-like kinase 1 (Bandeiras *et al.*, 2008), the trimeric integral membrane multidrug transporter AcrB (Sennhauser *et al.*, 2007) and the receptor-binding protein (RBP, the BppL trimer) of the baseplate complex of the lactococcal phage TP901-1 (Veesler *et al.*, 2009).

4.3.7. Removal of post-translational modifications

A number of proteins undergo post-translational modifications which can adversely affect crystallization. By far the most ubiquitous is N- and O-glycosylation, primarily of membrane-associated, secreted and lysosomal proteins. In a number of cases successful crystallization of glycoproteins purified from natural sources has been reported and carbohydrate groups have often been found to be ordered and occasionally sequestered between the protein molecules, thus even contributing in a positive way to crystallization (Mark *et al.*, 2003; Aleshin *et al.*, 1994). In general terms, however, the flexible and heterogeneous carbohydrate moieties, particularly the oligosaccharides linked by N-glycosylation, can account for a significant fraction of the surface area of the protein and can therefore be detrimental to crystallization. The preparation of recombinant proteins in *E. coli* eliminates these post-translational modifications and may sometimes solve the problem (Mohanty *et al.*, 2009), but N-glycosylation is often required for appropriate folding and solubility, so this approach is not always possible. However, if a eukaryotic expression system is a necessity, the problem can often be resolved by mutating the asparagines within the relevant glycosylation motifs (Asn-*X*-Thr/Ser), *e.g.* to aspartates, as was performed in the case of the extracellular domain of the metabotropic glutamate receptor expressed in insect cells (Muto *et al.*, 2009), or to glutamines, as was performed for the human testis angiotensin-converting enzyme (Gordon *et al.*, 2003). Alternatively, glycosylation at these sites can be eliminated by mutation of the Thr/Ser residues in the glycosylation motif to alanine or other amino acids, as described for rat cathepsin B (Lee *et al.*, 1990), or valine, as was the case with the Ebola virus glycoprotein (Lee *et al.*, 2008, 2009). Similarly, potentially glycosylated threonines or serines in O-