

## Fab-mediated Crystallization of a Potassium Channel

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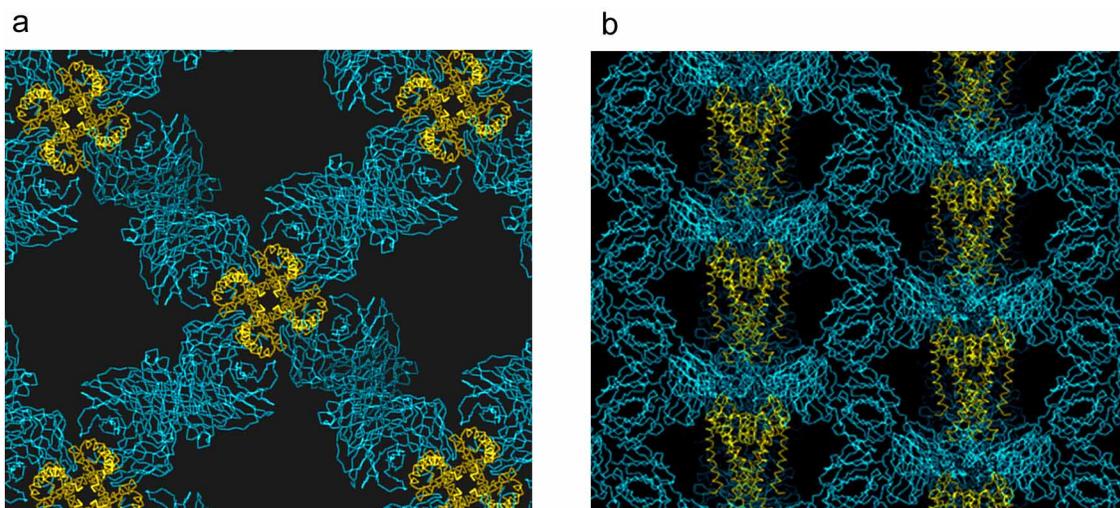
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In order to study structures of integral membrane proteins by x-ray crystallography, one has to overcome multiple obstacles including low expression levels and poor protein stability in detergents. After achieving sufficient quantities of membrane protein, there comes the most difficult part of the journey: crystallization. If integral membrane proteins crystallize at all, they rarely form high quality crystals, presumably because the interference of detergent micelles hinders the specific protein-protein contacts that are required to produce well-ordered crystals. A few novel approaches have been developed to overcome these difficulties<sup>1,2</sup>, each of which has yielded only a handful of high quality structures. One of these approaches, developed by Michel, H. et. al.<sup>1</sup>, is to co-crystallize membrane proteins with antibody Fv fragments. After inspecting the small database of existing membrane protein structures, they observed that most crystal contacts are formed between the polar groups of proteins, whereas hydrophobic interactions appear to be exceptional. Based on this observation, they produced the Fv fragment of a monoclonal antibody to the bacterial cytochrome c oxidase, and co-crystallized the Fv fragment bound to its protein epitope. (This specific cytochrome c oxidase can be crystallized alone but no structure determination has been reported.) The crystal structure of the complex was solved at 2.8Å resolution. The result showed that the Fv fragment indeed mediated the important crystal lattice contacts through polar interactions: each Fv fragment binds to one oxidase molecule through antibody-antigen recognition; at the same time it contacts another oxidase molecule as well as another Fv fragment through crystal packing. Years after the first publication, however, cytochrome c oxidase remains the lone example of a membrane protein co-crystallized with an antibody fragment. Recently, we employed a similar method to obtain a high-resolution structure of the potassium channel KcsA, implying that this method can

serve as a general approach to aid membrane protein crystallization.

Potassium channels are integral membrane proteins that control the electric potential across cell membranes by catalyzing the rapid, selective diffusion of potassium ions<sup>3</sup>. The basic ion permeation pathway within this class of proteins was outlined in the 3.2Å resolution structure of a bacterial potassium channel KcsA<sup>4</sup>. The ion conduction pore has a water-filled cavity roughly in the middle of the membrane and a narrow selectivity filter near the extracellular side. The cavity is apparently designed to overcome the dielectric barrier by keeping a K<sup>+</sup> ion in a fully hydrated state, and the selectivity filter is designed to catalyze the dehydration, transfer and re-hydration of K<sup>+</sup> ions. Due to the limited resolution, K<sup>+</sup> ions within the selectivity filter were not clearly resolved, nor could the hydration shell of the K<sup>+</sup> ion in the cavity be directly observed. To elucidate the mechanism of ion translocation in potassium channels, it was necessary to solve the K<sup>+</sup> channel structure at a resolution that would reveal detailed protein chemistry and ordered water molecules with high accuracy.

In order to obtain a high-resolution structure of KcsA, we raised monoclonal antibodies against the channel and selected clones that recognized the tetrameric but not the denatured, monomeric form of the channel. This selection step ensured us that we would obtain an antibody directed against the native structure. The Fab fragment of the antibody was easily produced by standard papain proteolysis followed by ion exchange chromatography. A KcsA-Fab complex with stoichiometry of one Fab fragment per channel subunit was produced and crystallized in space group I4. Frozen crystals diffracted up to 2.0Å at the National Synchrotron Light Source, beamline X25, and the phases were solved by molecular replacement using a published Fab structure<sup>5</sup>. All Fab fragments contain a well-

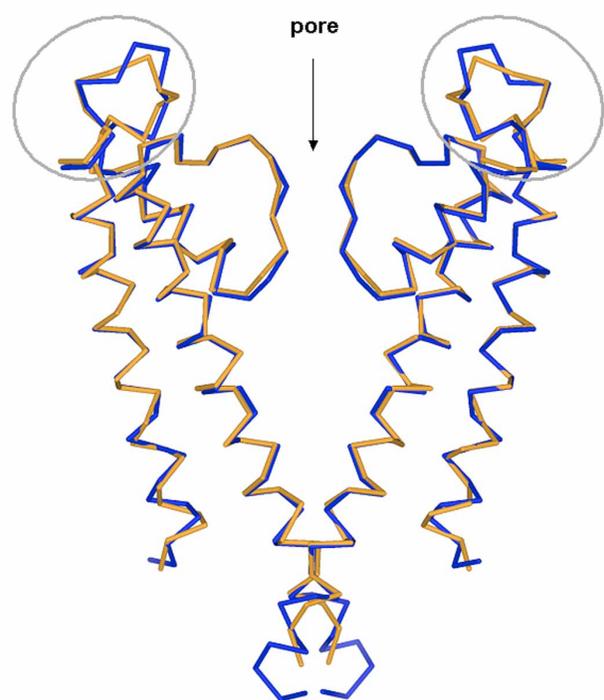


**Figure 1.** Crystal packing. KcsA (yellow) was crystallized as a complex with an antibody Fab fragment (blue). **a**, View down the four-fold crystallographic axis of the  $I4$  cell, which corresponds to the molecular four-fold axis of the  $K^+$  channel. **b**, View perpendicular to the four-fold axis.

conserved constant domain and an antigen binding variable domain, and the loop region connecting them is in general flexible, therefore it was necessary to use the constant domain and variable domain as separate search models for molecular replacement.

Our structure shows that nearly all of the crystal contacts are formed between neighboring Fab fragments, which constructed a cage-like frame into which the  $K^+$  channel is conveniently anchored (Fig. 1). This packing effectively avoided detergent micelle in crystal contacts and undoubtedly accounts for the high resolution. Compared with the original KcsA structure<sup>4</sup>, the presence of Fab has little effect on the conformation of the channel, except for a small change in the extracellular loop (the turret) region where the Fab binds (Fig. 2). Binding of Fab to the turret also leaves the ion pathway wide open; therefore, ion binding should not be affected by the presence of Fab fragments. The structure revealed detailed chemistry of ion coordination and hydration in the channel<sup>6</sup>. It shows how the  $K^+$  channel displaces water molecules around an ion at its extracellular entryway, how it holds a  $K^+$  ion in a shell of 8 water molecules in its central cavity, and how the selectivity filter mimics the hydration shell around each  $K^+$  binding site (Fig. 3). This unprecedented view of a hydrated potassium ion is made possible by the high-resolution data obtained through co-crystallizing KcsA with an Fab.

The high-resolution structure of the KcsA-Fab complex not only has revealed valuable information about the mechanism of  $K^+$  conduction, but it also offers important possibilities for membrane protein crystallography in general. First, although one additional example



**Figure 2.** The presence of Fab has little effect on the conformation of KcsA. KcsA structures in the absence (yellow) and presence (blue) of Fab are shown. Gray circles mark the Fab binding region. The arrow indicates the ion pathway. For clarity, only two diagonally opposed subunits of the channel are shown.

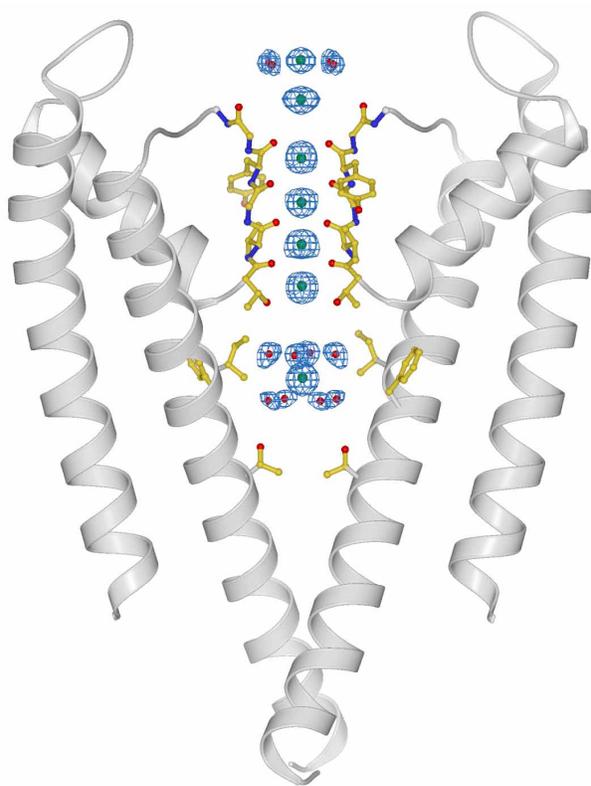
is hardly proof, it seems that using antibody fragments to assist crystallization should be applicable to many membrane proteins. Second, it has been argued that Fab fragments should be less suitable than Fv fragments in crystallization because of the flexible hinge region between the two domains<sup>1</sup>. This work shows that in the crystal packing a fixed relationship between the two domains of the Fab can occur, yielding very ordered crystals. Moreover, it is relatively easy to produce Fab fragments. Third, Fab co-crystallization might, as in the KcsA K<sup>+</sup> channel, offer a simple, straightforward solution to the phase problem through molecular replacement using the Fab structure.

This structure was recently published<sup>6</sup>, and the coordinates and structure factors are available under PDB code 1K4C. R.M. is an investigator in the Howard Hughes Medical Institute. We thank the staff at the National Synchrotron Light Source beamline X25 and Cornell High Energy Synchrotron Source A1, F1 for as-

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**Figure 3.** Structure of potassium channel KcsA at 2.0 Å resolution. Two diagonally opposed subunits of the tetrameric channel are shown in ribbon representation. Residues forming the selectivity filter and residues facing the central cavity are shown in ball-and-stick representation. The  $2F_o - F_c$  electron density map (contoured at  $2\sigma$ ) covers the potassium ions (green spheres) along the ion pathway and water molecules (red spheres) in the vicinity.