9. Catherine Vénien-Bryan

Electron microscope studies on signaling proteins

We are interested in allosteric regulation of biological molecules through protein/protein interactions and phosphorylation. We are using various approaches, 2D crystallisation of soluble and membrane protein and single particle electron microscopy.

KirBac3.1 potassium channel, domain motions during gating

C. Vénien-Bryan, A. Kuo, L. N. Johnson and D. A. Doyle

Ion channels are central to a wide range of biological processes including propagation of the neuronal action potential, cell volume regulation and muscle contraction (1). Ion channel proteins span the cell membrane, forming a conduction pathway, or pore, through which ions diffuse down their electrochemical gradient across the membrane. Inward-rectifier potassium (Kir) channels comprise a superfamily of potassium (K^+) channels that are expressed in most cells. The physiological role of Kir channels is to modulate the excitability and secretion of K^+ to maintain K^+ homeostasis. The gating process is modulated by ligands such as ATP, H^+, G-proteins, Na^+ and PIP_2, Mg^{2+} and spermine (2). All these ligands have different or overlapping binding sites but they all result in a change of diameter of the central ion conduction pathway either moving the channel into the open state or vice versa.

The atomic structure of the potassium channel KirBac1.1 elucidated in 2003 gave a clear description of a gated potassium channel in the closed state (3). The structure allowed identification of the main activation gate and the structural elements involved in its regulation. In order for the pore to open, movements of the pore helices coupled to other structural elements including the slide helix were proposed. In our work we are exploring this gating mechanisms by cryo-electron microscopy. Two-dimensional crystals of KirBac3.1 in 2 different states: closed and open state have been obtained by reconstitution of purified KirBac3.1 into lipid bilayers. Dramatic changes of conformation are observed between the 2 states (**Figure 1**) The unit cell of the closed state (99Å x 110Å) **(figure 1A)** is slightly smaller than this of the open state (99Å x 114Å) **(figure 1B)**. The size of the channel is about 15Å in the open state and hardly visible in the closed state, at a resolution of 9Å. This result shed light on the dramatic structural changes as the potassium channel moves between the closed and open states.

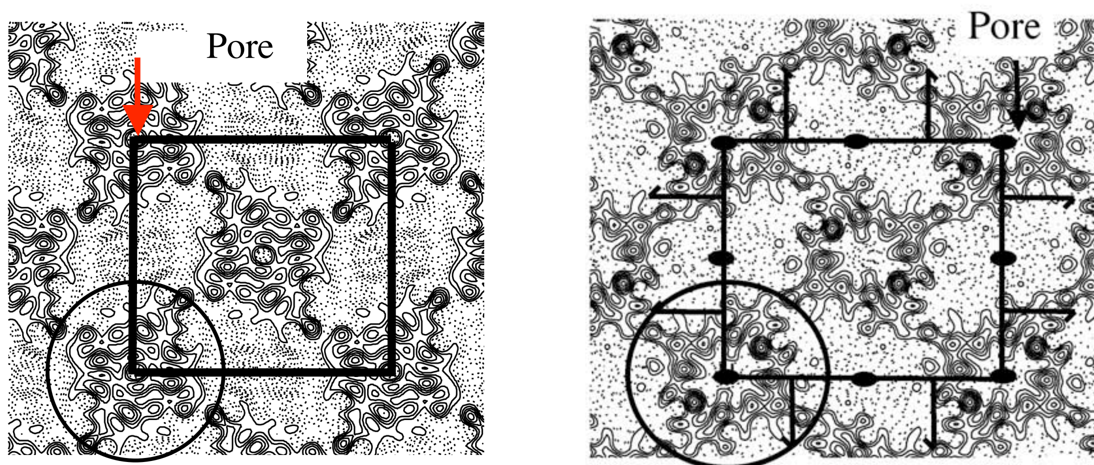

Figure 1. Projection density maps of KirBac3.1 from vitreous ice-embedded specimen. **A:** Kirbac3.1 in the closed state at 9 Å resolution calculated from eight cryo-images. A unit cell is displayed with the a-axis (99Å) vertical and b-axis (110Å) horizontal in two-sided plane group $p22_12_1$. In the bottom left KirBac is outlined. Negative and positive contours are shown as dotted and solid lines. **B**: KirBac3.1 in the open state at 9Å resolution calculated from six $p22_12_1$ two-dimensional crystals embedded in vitreous ice. The dimensions of a unit cell are: 99Å x 114Å. KirBac is outlined in the bottom left. The two-fold axes perpendicular to the membrane plane and the screw axes parallel to the membrane plane are indicated (with the central two-fold axis omitted for clarity).

3D structure of a transcription factor, HupR

Karen Davies and Louise Johnson

HupR protein (53Kda) is a transcriptional regulator from the photosynthetic bacterium *Rhodobacter capsulatus*. It is a response regulator of the NtrC subfamily; it activates the transcription of the structural genes hupSLC, of [NiFe]hydrogenase. A projection map of the full-length protein at 9Å resolution was obtained by electron cryo-microscopy and image analysis of frozen-hydrated two-dimensional crystals. The crystals have a p6 plane group with unit cell dimensions of a=b= 112Å, $\gamma = 120^{o}$ (4). We have calculated a 3D model of HupR at medium resolution using tilted images from 2D crystals. These results provide the first structure at medium resolution of a whole transcription factor, HupR from the NtrC family. The various domains of known structures have been fitted inside the enveloppe.

Anaphase Promoting Complex (APC)

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The anaphase-promoting complex (APC) promotes the selective destruction of key proteins during the mitotis in the cell cycle (5-7). The APC promotes the attachment of polyubiquitin chains to selected proteins that are then targeted to the proteasome for proteolytic degradation (8). The APC is composed of at least 13 subunits with a total molecular mass ~ 800 kDa. We have visualised the APC particles in the Philips CM120 electron microscope using negative stain (uranyl acetate) methods. Image pairs (tilted and untilted) were recorded under low-dose conditions. Image analysis was carried out with the WEB and SPIDER software package (9) using the random conical tilt method. The 3D model of APC is a complex asymmetric structure. The size of the reconstructed particle is approximately 180 Å x 145 Å x 160 Å. We have also identified the location of individual his-tagged subunits within the complex using gold cluster coupled to Ni^{2+}-NTA (nitrilotriacetic acid).

Phosphorylase kinase

Vicky Skamnaki, Ed Lowe, Dietbert Neumann and Louise Johnson in collaboration with Nicolas Bischler and Nikos Oikonomakos (Athens).

Phosphorylase kinase integrates signals from hormonal messengers and neuronal stimuli to produce rapid activation of glycogen phosphorylase and subsequent degradation of glycogen stores either to provide energy to sustain muscle contraction or, in the liver, to provide other tissues such as the brain with glucose. It is one of the most complex kinases comprising $(\alpha\beta\gamma\delta)_4$ assembly of subunits with a total molecular weight of 1.3×10^6. A 3D structure of the holoenzyme PhK has been produced at medium resolution by electron microscopy (10). We wish to extend the structure from the current 22Å resolution to higher resolution using cryo-electron microscopy in order to identify individual subunits and their arrangements with respect to the substract.

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