Insights into membrane protein function from molecular modelling

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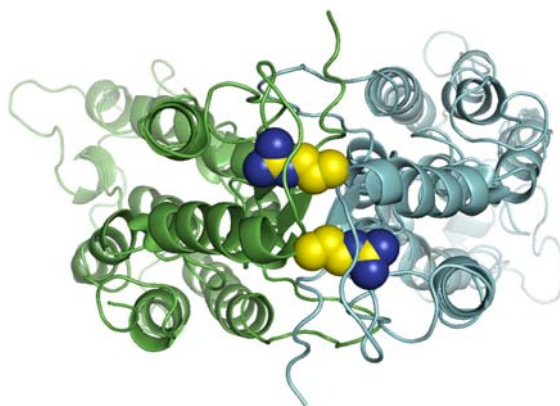
Introduction

In most organisms, up to 30% of the genome encodes membrane proteins, which perform diverse tasks ranging from the uptake of nutrients to communication between cells via chemical or electrical signals. These proteins represent more than half of current therapeutic drug targets in humans, and are involved in many serious diseases. Despite this importance, high resolution structures are available for fewer than 250 membrane proteins, reflecting the experimental difficulty of working with them. Our laboratory is currently engaged in research aimed at speeding up the rate at which novel membrane protein structures can be obtained, by developing improved, high-throughput technologies. However, in parallel we are exploiting the limited number of existing structures known, to gain insights into the molecular mechanisms of membrane proteins and the ways in which mutations can lead to disease. This typically requires specialised approaches to enable the modelling of the membrane protein targets of interest, because of their evolutionary distance from known structural templates. Three examples of the success of such modelling during the past year are described below.
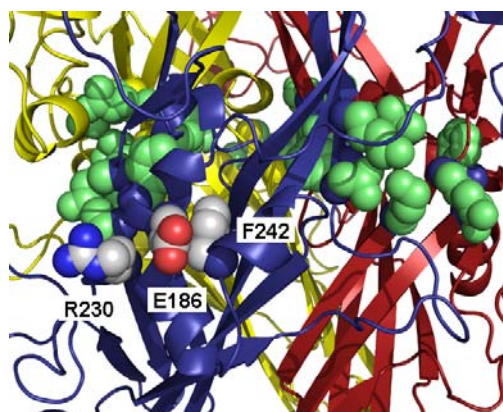
Results

Recent genome-wide studies have shown that a mutation in the human zinc transporter Znt8 gene, resulting in the replacement of a tryptophan residue with arginine, increases the risk of type 2 diabetes. This transporter is found in the insulin-containing secretory granules of pancreatic β-cells, consistent with a possible role of the mutation in decreasing insulin secretion and/or proinsulin processing. Working in collaboration with Prof. Guy Rutter's group at Imperial College, we sought to explore the underlying mechanism by modelling the human protein on the distantly-related bacterial zinc transporter YiiP. This revealed that the mutation is located at the interface between protomers in the dimeric transporter, where it may disturb structurally-important zinc-binding sites (Figure 1).

Figure 1: location of diabetes-associated mutations in the human zinc transporter Znt8. Subunits of the dimeric transporter, shown in blue and green cartoon form, are viewed from the cytoplasmic side of the membrane, with the arginine residues associated with diabetes risk shown in solid molecular representation.

Continuing the theme of using models of human membrane proteins to understand disease, we have also modelled the structure of the human P2X$_7$ receptor. This receptor is an ATP-activated cation channel, which plays critical roles in immune responses, inflammation and perception of pain. Moreover, single non-synonymous nucleotide polymorphisms (SNPs) in the corresponding gene have been linked to affective mood disorders such as major depression. In a collaboration between the laboratories of Lin-Hua Jiang and Steve Baldwin at Leeds, we have attempted to identify the mechanisms underlying such linkage by modelling the human protein on the recently-determined structure of the zebrafish P2X$_4$ receptor (Figure 2). Examination of the model revealed that several of the mutations, which were shown to affect channel function, were located close to the proposed ATP-binding site,

consistent with effects on ATP-binding and channel gating.

Figure 2: location of glutamate 186 in a model of the human P2X$_7$ receptor. Mutation of this residue to lysine causes complete loss of function, probably via altered interactions with adjacent residues R230 and F242. The three subunits of the trimeric receptor are shown in red, blue and yellow cartoon form, while residues predicted to comprise the ATP binding sites are shown, in solid molecular representation, in green.

In addition to our work on human proteins, we have also successfully applied our modelling strategies to gain an understanding of the link between mutation and phenotype in the peroxisomal ABC transporter "comatose" from the plant *Arabidopsis thaliana*. This protein is required for import of substrates for peroxisomal β-oxidation, and is a homologue of an ABC transporter, ALDP, involved in the human genetic disease adrenoleukodystrophy. In a collaboration between the laboratories of Alison Baker and Steve Baldwin at Leeds, we have built a model of comatose based on the structure of a bacterial multidrug transporter. This has allowed us to investigate the role of interactions between the transmembrane and nucleotide-binding domains of the protein in its transport function, and to predict likely substrate-binding residues for future mutagenesis studies.

Taken together, the results of the investigations described above indicate the utility of molecular modelling of membrane proteins, even in the majority of cases where structural templates are only distantly related to the proteins being modelled.

Publications

Dietrich, D., Schmuths, H., De Marcos, L., Baldwin, J., Baldwin, S., Baker, A., Theodoulou, F. and Holdsworth, M. (2009) Mutations in the *Arabidopsis* peroxisomal ABC transporter COMATOSE allow differentiation between multiple functions in planta: insights from an allelic series. *Mol Biol Cell* **20:**530-543.

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Roger, S., Mei, Z., Baldwin, J., Dong, L., Bradley, H., Baldwin, S., Surprenant, A. and Jiang, L. (2010) Single nucleotide polymorphisms that were identified in affective mood disorders affect ATP-activated P2X$_7$ receptor functions. *J Psychiatr Res* **44**:347-355.

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