

Ion channels and protein-protein interactions: novel approaches to mining validated targets

by D. S. Hogg, D. Ryan and R. Z. Kozlowski

Ion channels represent a well-validated class of drug targets for a diverse range of disorders. The development of new screening technologies to identify small molecule inhibitors of the interaction between ion channels and their unique accessory proteins affords a novel approach to further exploiting this target class.

Ion channels as validated and tractable drug targets

Ion channels have proved to be valuable therapeutic targets in the search for drugs for the treatment of patient populations across a range of indications, including hypertension, epilepsy, postherpetic neuralgia, type II diabetes, insomnia, severe chronic pain and chronic idiopathic constipation. Indeed, the value of inhibitors of ion channels can be further exemplified by calcium channels blockers (e.g. amlodipine and nifedipine) for the treatment of hypertension, which alone generate in excess of \$6 billion in annual worldwide sales.

Ion channels are composed of a number of protein sub-units that consist of a principal multimeric transmembrane-spanning protein complex that forms the basis of the channel 'pore'. Ion flux through these channel pores in response to stimuli (e.g. voltage, ligands, pH etc.), serves to control and regulate an array of important cellular functions including, for example, control of neuronal excitability, regulation of arterial tone and control of hormone secretion. Given the diversity of physiological functions that are regulated by certain ion channels, these targets have been implicated in the prophylaxis, and/or treatment of disorders of high unmet medical need, such as overactive bladder (OAB).

Historically ion channel targets have proved tractable, as demonstrated by the first generation of approved ion channel therapeutics: those which bind to the pore-forming protein sub units of ion channels. Given this success and the

potential to exploit new therapeutic markets, it is perhaps not surprising that this target class has received considerable interest over recent years from both biotechnology and pharmaceutical companies. In order to identify new ion channel drugs, significant resources have been utilised to develop improved screening methodologies for identifying new chemical entities targeted at the pores of specific ion channels [1,2]. However, compounds identified by utilising these methodologies are anticipated to possess a mode of action analogous to that of a number of first generation compounds.

Recent advances in the understanding of the structure and function of ion channels have identified diverse classes of 'accessory' proteins that form part of the channel complex and serve to regulate the function (including biophysical properties and cell surface expression) of the channel pore. Thus, pharmacological manipulation of the function conferred by accessory proteins to ion channels, may represent a significant advance towards 'selectively' modulating ion channels for therapeutic benefit. Compounds that exert their effects through novel mechanisms of action, such as ion channel accessory proteins, may therefore represent the 'next-generation' of ion channel therapeutics.

Protein-protein interactions as drug targets

Protein-protein interactions are pivotal in most biological processes, including regulation of ion channels, and therefore appear to represent an attractive target for novel therapeutics. However, one of the challenges faced by targeting the interaction between two proteins is the identification of the small molecule starting points for subsequent development. Until recently, targeting protein-protein interactions with 'drug-like' small molecules has been perceived as difficult since protein-protein interactions have typically not been shown to possess small deep cavities that have the appearance of traditional binding pockets [3]. However, if such a cavity or cleft is present upon the target protein, including potential allosteric sites, then the interaction is clearly likely to be 'drug-gable'. This is evidenced by the recent success in discovering a potent small molecule, as a potential cancer therapy, that

binds MDM2 in the p53-binding pocket to modulate the MDM2-P53 protein-protein interaction [4].

Thus, the success of finding hits that target the protein-protein interactions between ion channels and their accessory proteins relies upon the capability of the proteins involved to bind small molecules. With regard to ion channels and their accessory proteins, this is supported by the high resolution crystal structures of known ion channel accessory proteins, which reveal the presence of potential 'drug-like' binding sites.

Targeting accessory proteins of ion channels

To exploit the interaction between ion channels and their accessory proteins, several strategies to identify small molecule inhibitors have been employed including the widely used yeast-two hybrid system. Recently, a counter-selection yeast-two hybrid assay was developed to study the interaction between the Cav β 3 accessory proteins of the Cav2.2 calcium channel. The assay was used to screen a library of 156,000 compounds from which WAY141520 was identified. This protein specifically inhibits the interaction between the intracellular linker of domains I and II of the pore forming sub unit of Cav2.2 and the Cav β 3 accessory protein sub unit [5]. Subsequently, WAY141520 was shown to inhibit Cav2.2 calcium channels in neuronal cells in functional electrophysiological assays, and also demonstrated selectivity over other ion channels such as sodium and potassium channels.

More recently, a novel cell-free assay technology, LEPTICS (Leveraged Enabling Proteomics Technology for Ion Channel Screening), has been developed for the identification of small molecule inhibitors of ion channel protein-protein interactions. LEPTICS utilises recombinant fusion proteins immobilised to a solid substrate.

These fusion proteins incorporate both the ion channel accessory protein of interest and a tag moiety comprising a biotinylation domain (biotin carboxyl carrier protein) [Fig.1 see P.22] for increasing the solubility and/or determining the folded state of the fusion protein. The biotin carboxyl carrier protein is biotinylated post-translationally *in vivo*

during expression at a single lysine residue by biotin ligase in the host cell. This biotinylated tag can then be utilised to immobilise the fusion protein to a solid substrate. The immobilised accessory proteins can subsequently be interrogated with labelled interaction domains of the ion channel pore protein complex in the presence of test compounds.

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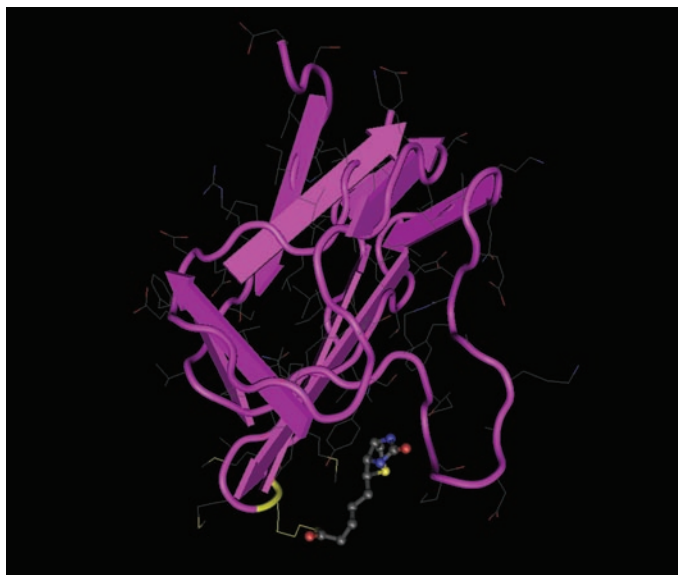


Figure 1. Structure of the biotinyl domain, biotin carboxyl carrier protein, of acetyl-coenzyme A carboxylase.

The LEPTICS assay technology has been utilised to construct an assay to study the protein-protein interaction between the voltage-gated potassium channel, Kv1.1 and its Kv β subunit accessory protein [Figure 2; 6]. Fusions proteins of Kv β subunits, incorporating the biotin carboxyl carrier protein tag, were immobilised to a screening substrate and interrogated with the interacting domain of the Kv1.1 channel (located on the Kv1.1 channel N-terminus). Following screening of a random library, inhibitors of the Kv1.1 channel - Kv β accessory protein interaction have been identified. The broader utility of the LEPTICS assay technology has also recently been demonstrated with the development of assays to exploit the interaction between other ion channel classes such as the interaction between Cav β 3 accessory proteins and Cav2.2 calcium channels [7].

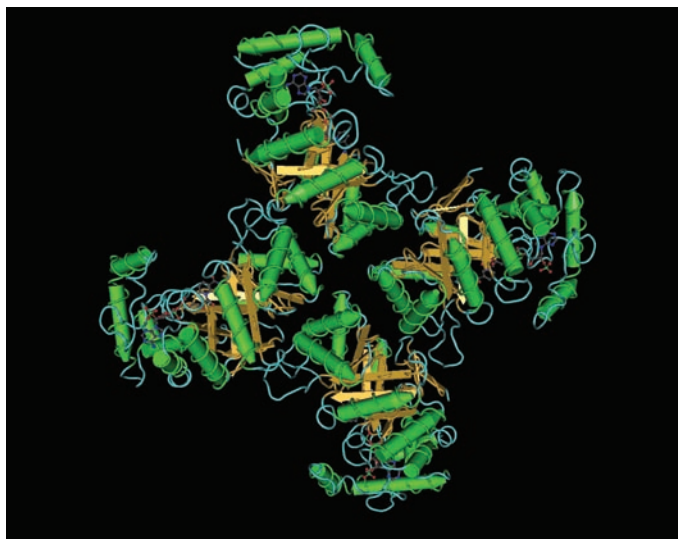


Figure 2. Structure of a voltage-dependent Kv channel β sub unit.

Future prospects

The recent emergence of hit compounds provides significant evidence to support the tractability of targeting the protein-protein interaction between well validated ion channel drug targets and their accessory proteins. However, considerable effort will be required to translate these small molecule hit compounds into high affinity compounds with therapeutic potential. Thus, ion channel protein-protein interaction technologies, such as the LEPTICS screening assay, will be a key tool for evaluating the affinity of potential therapeutic agents as they are progressed from discovery through to development. The existence of novel assay technologies to exploit interactions between ion channels and their accessory proteins provides the basis for identifying new classes of potential ion channel drugs across a number of important therapeutic areas. Such drugs are anticipated to exhibit a clearly different mode of action and selectivity profile from that of the first generation of ion channel drugs.

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The authors

Dayle S. Hogg, Diane Ryan
and Roland Z. Kozlowski,
Lectus Therapeutics Ltd,
Babraham Research Campus,
Cambridge CB22 3AT,
United Kingdom
Tel +44 (0)1223 496 182
info@lectusth.com

Hotline: www.drugplusinternational.com & tick 30853