Title

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Competing financial interests
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CHANNELS

Sticking to nooks and crannies

Drug design for voltage-gated ion channels has long been hampered by the absence of crystal structures and the challenge of achieving subtype selectivity. A combination of mutagenesis, electrophysiology and molecular modeling has led to the identification of a new side pocket binding site for the small molecule Psora-4 between the pore and the voltage-sensor domain of Kv1.5, offering opportunities to design allosteric ion channel modulators.

Heike Wulff & Vladimir Yarov-Yarovoy

The human genome contains 143 voltage-gated-like ion channels[1] that are involved in generating cardiac and neuronal action potentials; regulating hormone, neurotransmitter and fluid secretion; and driving cellular proliferation and migration. Modulators of voltage-gated ion channels are used in the clinic as antihypertensives, anticonvulsants and local anesthetics and offer tremendous opportunities for the discovery of new drugs for neurological, cardiovascular and metabolic disorders as well as for immunosuppression and cancer. However, in contrast to some other therapeutic targets such as protein kinases, voltage-gated ion channels have not been popular with medicinal chemists in the pharmaceutical industry during the last decade. The reasons for this include the difficulties associated with automating electrophysiology and running true high-throughput screens for ion channel modulators as well as the absence of co-crystals of medically relevant channels with bound drug molecules, a prerequisite for structure-based drug design. To address the second challenge, molecular modeling, which recently made substantial advances owing to improved computational methods, expanded databases of high-resolution ion channel structures and growing computational power, is increasingly being used to interpret the results of mutational, electrophysiological and ligand binding experiments in structural terms. The work by Marzian *et al.*[2] is a particularly good example of this trend in that it identifies a previously unrecognized binding site in a 'side pocket' between the pore and the voltage-sensor domain of Kv1.5 (**Fig. 1a**).

Alkoxypsoralens such as Psora-4 (ref. 3), the compound used in the Marzian *et al.*[2] study, and PAP-1, a Kv1.3 blocker[4] starting phase 1 clinical trials for psoriasis, are high-affinity inhibitors of Kv1 family channels and have steep concentration-response curves with Hill coefficients of 2–3, indicating that more than one inhibitor molecule binds in a cooperative way to one tetrameric Kv1 channel. Using a combination of alanine-scanning mutagenesis, electrophysiology,

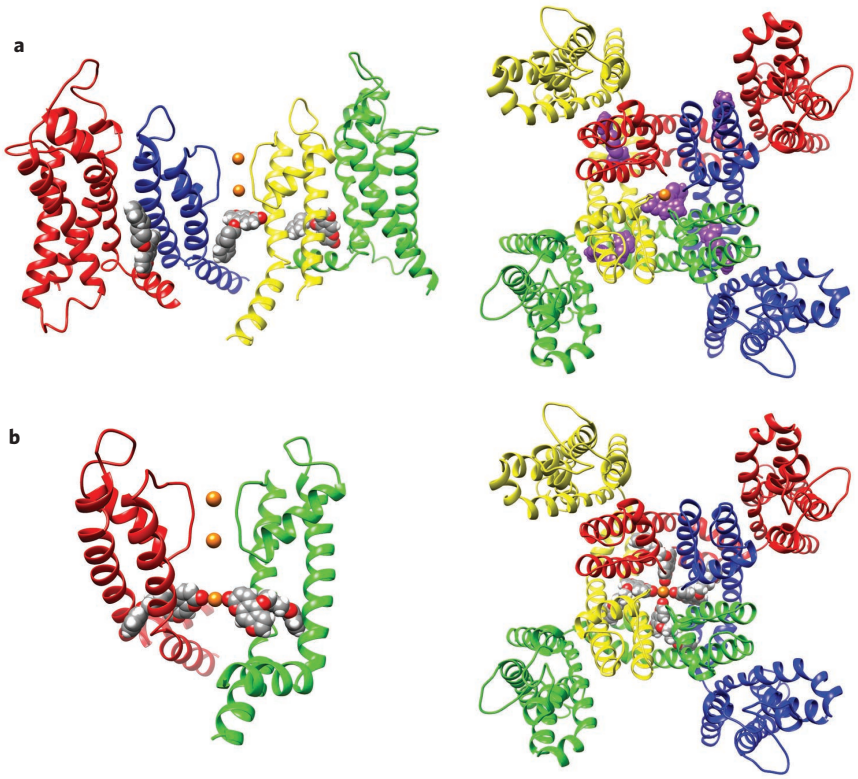

Figure 1 | Structural models of Psora-4 binding to Kv1.5 and PAP-1 binding to Kv1.3. (**a**) Transmembrane (left) and extracellular (right) view of the Kv1.5–Psora-4 open-state model. Each subunit is colored individually. Psora-4 and the K^+ ion are shown as space-filling models. (**b**) Transmembrane (left) and extracellular (right) view of the Kv1.3–PAP-1 open-state model. Each subunit is colored individually. PAP-1 and the K^+ ion are shown as space-filling models.

computational ligand docking and molecular dynamics simulations, Marzian *et al.*[2] constructed a Kv1.5 homology model based on the Kv1.2–Kv2.1 chimera structure[5] that can bind up to five Psora-4 molecules, one in the central pore cavity and four in side pockets formed by the backsides of the pore-forming S5 and S6 segments and the voltage-sensor domain (**Fig. 1a**). The authors confirm the side pocket binding site by introducing the residues involved in central cavity and side pocket binding into Kv2.1, a channel normally insensitive to alkoxypsoralens, and show that the cooperativity observed in Kv1 channels is recapitulated. Interestingly, our own group previously mapped the binding site of PAP-1 in Kv1.3 (ref. 6) and explained the Hill coefficient of 2 with a model that has two or four PAP-1 molecules coordinating a K^+ ion in the pore with their coumarin moieties and extending the lipophilic phenoxyalkoxy side chain into the intersubunit interfaces between helices S5 and S6 (**Fig. 1b**). This older Zimin *et al.*[6] model offers an explanation for why the coumarin moiety is essential for high-affinity block: it has the carbonyl groups of PAP-1 chelating a K^+ ion, and water molecules in the K^+ hydration shell form hydrogen bonds with the ether oxygens in the coumarin ring. The central pore Psora-4 molecule in the Marzian *et al.*[2] model is postulated to primarily make hydrophobic interactions, which would not necessarily require the coumarin moiety. However, the ability to transfer binding to Kv2.1 is of course a strong argument in favor of the Marzian model, and it should be pointed out that the Zimin *et al.*[6] Kv1.3 model did not include the voltage sensor domains and was therefore bound to miss the side pockets. Another intriguing feature of the Marzian study[2] is that the Psora-4 molecule in the side pocket seems to be able to adopt two different orientations (**Fig. 1a**), one of which has the phenyl ring in the side chain facing upwards and reaching the pore helix from the back, a binding mode that explains how Psora-4 can induce an inactivation-like process at the selectivity filter, leading to a very stable nonconducting drug–channel complex.

But, stepping back from the details, what is striking when looking at the two models (**Fig. 1**) is how well small molecules such as Psora-4 and PAP-1 seem to be able to snuggle into every available nook and cranny of voltage-gated ion channels: the central cavity, the side pockets between the pore domain and the voltage-sensor and the S5-S6 interface. Overall, the Marzian *et al.*[2] study illustrates to the field what had been suspected on the basis of the lateral fenestration visible in the crystal structure of the NavAb sodium channel[7]: that voltage-gated ion channels are highly accessible to small-molecule drugs and that channel inhibition does not simply always consist of physically 'plugging' the pore with a molecule that enters the central cavity through the open channel gate but can be achieved through more subtle allosteric mechanisms. Because sequence homology among channels is lower in the side pockets than in the pore, the side pockets provide new opportunities for the design of channel subtype–specific drugs, a major challenge remaining in the ion channel field.

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