

Generally Physiological

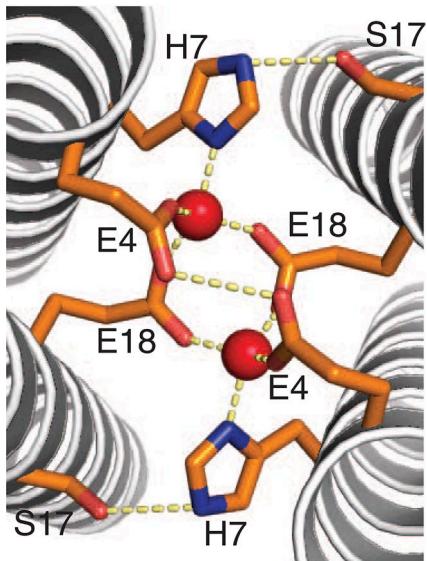
Of transporter design, screening for gating modifiers, and how TRAAK gates



This month's installment of *Generally Physiological* considers how to design a transporter, an approach to screening for drugs that target the Na_v voltage-sensing domain IV paddle motif, and how the mechanosensitive TRAAK channel is gated by membrane tension.

Designing a Zn^{2+} transporter

The ability to design an artificial protein with functional activity has exciting implications for biomedical engineering, and can also substantiate



Rocker's di- Zn^{2+} -binding site. (From Joh et al. 2014. *Science*. <http://dx.doi.org/10.1126/science.1261172>. Reprinted with permission from AAAS.)

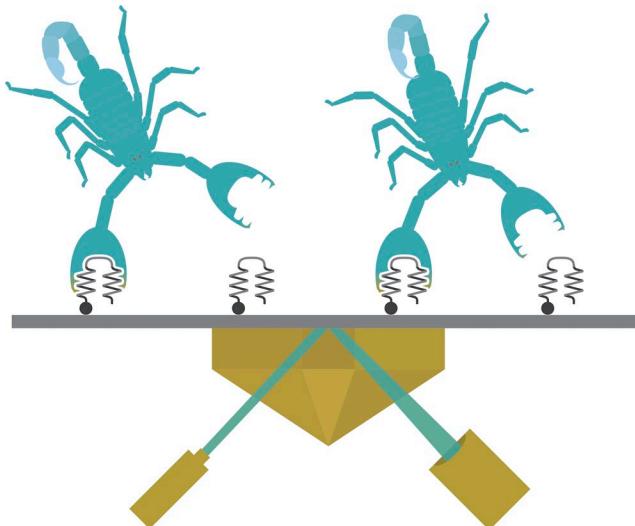
our understanding of the mechanisms through which natural proteins act and of the relationship between amino acid sequence and protein structure. Whereas substantial progress has been made in the design of water-soluble and catalytically active proteins, membrane proteins have presented more of a challenge (see

Lupas, 2014). Noting that membrane transporters are hypothesized to undergo a conformational change that allows alternating access of a substrate-binding site to either side of the membrane, Joh et al. (2014) designed a minimal protein based on a four-helix bundle fold that recapitulated this process, transporting Zn^{2+} and H^+ in opposite directions. The engineered protein, called "Rocker" because it was designed to rock between two alternating states, contained two di-metal binding sites. Rocker selectively transported Zn^{2+} and Co^{2+} , but not Ca^{2+} , and was capable of using the Zn^{2+} concentration gradient to transport H^+ against a pH gradient in a remarkable demonstration of the ability to design a membrane protein with defined structural and functional properties.

instance, α -scorpion toxins that target the Na_v voltage-sensing domain IV (VSD IV) paddle motif inhibit fast inactivation to prolong action potential duration, whereas toxins targeting the VSD I-III paddle motif typically disrupt channel activation. Conversely, agents that target specific sites on Na_v channels, such as the VSD IV paddle motif, could potentially be beneficial under pathophysiological conditions that involve aberrant channel activity. However, identifying such compounds is a nontrivial task. In this issue, Martin-Euclaire et al. used surface plasmon resonance to investigate the pharmacological sensitivity of the isolated VSD IV paddle motif. They found that the isolated motif, immobilized on sensor chips, remained sensitive to α -scorpion toxins, providing an approach that does

Screening for agents that modify Na_v gating

Voltage-gated sodium (Na_v) channels play a key role in action potential propagation and thus provide an enticing target for toxins from various venomous creatures. Indeed, different classes of toxins have evolved that bind to different sites on Na_v channels and, consequently, have different effects on channel function. For

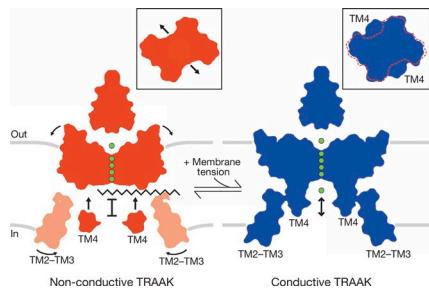


Stylized depiction of the interaction of a scorpion toxin (indicated by the unique shape of the claws) with the S3b-S4 paddle motif of a Na_v channel attached to an SPR chip. Polarized light to measure the refractive index near the sensor surface to which the paddle motif is attached is depicted below the chip. See Martin-Euclaire et al. (2015). Image provided by Kate Baldwin (<http://www.k8baldwin.com/>).

not require expression of the full-length channel that could potentially be used for the rapid identification of pharmacological agents that selectively modify Na_v activity.

Gated by tension

Like the bacterial MscL and MscS channels, the mechanosensitive eu-



Model for TRAAK gating. Left shows the closed conformation, with an acyl chain extending into the cavity to sterically block conduction; TM4 rotation (right) blocks lipid access to allow conduction. Insets show change in cross-sectional area, which together with reduced membrane bending in the open conformation, promotes channel opening with increased membrane tension (Reprinted by permission from Macmillan Publishers, Ltd. S.G. Brohawn et al. *Nature*. <http://dx.doi.org/10.1038/nature14013>, copyright 2014.)

Designing a transporter, screening for gating modifiers, and how TRAAK gates

karyotic channel TRAAK, a dimeric two-pore domain K^+ (K2P) channel, is gated by membrane tension. However, the underlying mechanism has been unclear. Brohawn et al. (2014) obtained TRAAK crystal structures and determined that a transmembrane helix (TM4) could rotate about a central hinge into either an “up” or a “down” conformation. In the “down” conformation, an $\sim 5\text{-}\text{\AA}$ wide intramembrane opening between subunits allowed a lipid acyl chain to extend into the channel cavity underneath the selectivity filter, whereas, in the “up” conformation, TM4 of one subunit packed against TM2 of the second to seal the cavity against membrane lipids. These down and up conformations corresponded to nonconductive and conductive states (assessed by

the absence or presence of the permeant ion Tl^+ in the cavity), a hypothesis substantiated by recording currents in the presence of branched or unbranched lipids and analyses of a mutant channel trapped in the up conformation. Channel opening was associated with an increase in TRAAK cross-sectional area and a change in channel shape predicted to favor the open conformation with increased membrane tension, thereby providing a mechanism for TRAAK gating and its mechanosensitivity.

Elizabeth M. Adler

Executive Editor, *JGP*

eadler@rockefeller.edu

REFERENCES

Brohawn, S.G., et al. 2014. *Nature*. 516:126–130. <http://dx.doi.org/10.1038/nature14013>
Joh, N.H., et al. 2014. *Science*. 346:1520–1524. <http://dx.doi.org/10.1126/science.1261172>
Lupas, A.N. 2014. *Science*. 346:1455–1456. <http://dx.doi.org/10.1126/science.aaa2721>
Martin-Euclaire, M.F., et al. 2015. *J. Gen. Physiol.* 145:155–162. <http://dx.doi.org/10.1085/jgp.201411268>