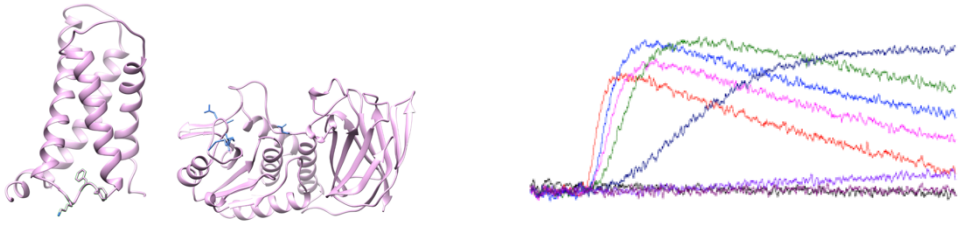
Isacoff Lab-UC Berkeley: Conformational dynamics and allostery in Voltage Sensing Phosphatases



Voltage Sensing Phosphatases (VSPs) dephosphorylate PIP signaling lipids in response to membrane depolarization to couple the electrical activity of the cell to the regulation of PIP_2-sensitive ion channels and transporters. We are studying the molecular mechanism of function of VSPs using our method of voltage clamp fluorimetry (VCF) (Mannuzzu, *Science* 1996; Glauner, *Nature* 1998) and single molecule fluorescence (Ulbrich, *Nature Methods* 2007; Vafabakhsh, *Nature* 2015) to monitor real-time conformational rearrangements in the functioning protein in living cells as well as in purified protein. We complement this with FRET reporters that reveal the dynamics of consumption and production of PIPs (Grimm, *Nature Chem. Bio* 2016) and cryo-EM to define structural pathways.

VSPs contain a membrane-spanning Voltage Sensing Domain (VSD), similar to that of voltage-gated ion channels, and a cytosolic lipid phosphatase domain (PD). We have shown that voltage turns VSP "on" by a gating mechanism, analogous to that of ion channel pores, where a linker that connects S4 to the PD, plays a role in coupling voltage sensing movements in S4 to motion of a loop in the PD that opens and closes access to the catalytic site (Kohout, *Nature Chem. Bio*. 2010; Liu, *Nature Struct. Molec. Bio* 2012).

But the system is more complicated than just "on" and "off" and coupling involves more than just the inter-domain linker. First, VSP has two "on" modes: one that converts PIP_3 to PIP_2 (modest depolarization) and the other that converts PIP_2 to PIP (strong depolarization) (Grimm, *Nature Chem. Bio,* 2016). Current studies reveal distinct conformations that belong to these two activity states. Second, we find new molecular components that control occupancy of the "states, indicating that VSD-PD coupling involves (indeed may be dominated by) other molecular interactions. The goal of this project is to reveal the mechanisms of allosteric control and substrate-switching. Understanding of this system will provide unique insight into how voltage-driven motions in the charged S4 helix can gate a remote functional site, such as an ion pore or, in this case, a catalytic site. It will guide our engineering of novel probes for remote control of PIPs and reporters of membrane voltage, which we will use to study synaptic transmission and homeostasis.

We seek a motivated postdoc to continue this work with experience in electrophysiology. Knowledge of fluorescence spectroscopy and/or biochemistry will be a plus. The candidate will work alongside molecular biophysicists studying metabotropic and ionotropic glutamate receptors (using single molecule optical methods and molecular dynamics simulation), neuroscientists performing super-resolution quantal imaging of synaptic transmission in Drosophila and mammalian neurons, and chemical biologists developing methods for optical control of glutamate and dopamine receptor signaling.

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