Unlike other voltage-gated ion channels, Hv1 is composed by two subunits each containing a voltage-sensing domain (VSD) and lacking the pore domain. On the other hand, Hv1 shares striking similarities with the M2 proton channel of the influenza A virus (A/M2): in both cases a bundle of four helices lines a proton conduction pathway and positively charged ''fragment-like'' molecules act as blockers.
Starting from the recently solved structure of Hv1, we combined homology modeling, molecular dynamics simulations and site finding methods to unveil potential binding pockets in the most relevant conformational states. We discovered that, similarly to the case of A/M2, the distribution of water molecules inside the channel lumen is state-dependent, offering a rationale to designing novel pore blockers.

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Allosteric Coupling between Open Subunits in the Hv1 Proton Channel Probed by Guanidino-Thiazoles
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The Hv1 channel extrudes protons from cells in response to membrane depolarization, and regulates the production of reactive oxygen species by NOX enzymes. Its excessive activity has been implicated in cancer development and in brain damage during ischemic stroke. The channel is a dimeric complex made of two voltage-sensing domains (VSDs), each containing a gated proton permeation pathway. We have previously identified 2-guanidinobenzymidazole (2GBI) derivatives that block proton permeation through the two VSDs (Hong et al. 2013, Neuron 77(2): 274-286). For these compounds, the binding occurs independently in the two open subunits, and resembles the binding to a monomeric version of the channel. Here we describe a separate class of guanidine derivatives - 2-guanidinothiazoles - that block Hv1 in a cooperative way. Comparison of the dose dependences of inhibition of dimeric and monomeric channels shows that blocker binding to one open subunit causes an increase in binding affinity of the neighboring open subunit. Using a site-directed mutagenesis approach, we identify the residues in the binding site responsible for cooperativity and then explore the residues at the interface between subunits that mediate allosteric coupling in the open state. Understanding the molecular features of the inhibitor that modulate cooperative binding can help develop better drugs targeting the Hv1 channel. This work is supported by NIH -National Institute of General Medical Sciences, grant R01GM098973.

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Characterization and Subcellular Localization of Hv1 in Lingulodinium Polyedrum Confirms its Role in Bioluminescence
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In 1972, J. Woodland Hastings and colleagues predicted the existence of a proton selective channel that opens in response to depolarizing voltage (H_V1) across the vacuole membrane of bioluminescent dinoflagellates and conducts protons into specialized luminescence compartments (scintillons), thus causing the pH drop that triggers the light flash. RNA-Seq data from several luminescent dinoflagellate species provided candidate H_V1 genes. When expressed in mammalian cells, the predicted H_V1 from *Lingulodinium polyedrum* displays the hallmark properties of bona fide proton channels, including time-dependent opening with depolarization, perfect proton selectivity, and characteristic ΔpH dependent gating. RT-PCR and Western blotting confirm expression of H_V1 in *L. polyedrum*. Fluorescence confocal microscopy of *L. polyedrum* cells stained with antibodies to luminescence proteins luciferase and luciferin binding protein and to H_V1 reveal structures consistent with H_V1's proposed function in bioluminescence.
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Engineered Voltage Sensing Phosphatases: What do they tell us about the Gating Mechanism?
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Voltage sensing phosphatases (VSPs) contain a phosphoinositide phosphatase domain (PD), which is under the control of a voltage sensor domain (VSD). Here, the underlying coupling mechanism is not fully understood. Recently, Liu et al. (NSMB 2012) and Hobiger et al. (Biophys J 2012, PLoS one 2013) proposed for Ciona intestinalis (Ci-) VSP an interaction of positively charged amino acid residues in the linker, which connects the PD to the VSD, with the negatively charged residue Asp^{400} in the 'TI' loop of the PD.
In light of these findings we revisited the engineered VSPs $PTEN_{CiV}$ (Lacroix et al., JBC 2011) and $hVSP1_{CiV}$ (Halaszovich et al., J Lipid Res 2012), chimeras consisting of Ci-VSP's VSD and the PD of PTEN or hVSP1, respectively. In those chimeras Asp^{400} (Ci-VSP numbering) is not conserved. Nonetheless, they show robust voltage dependent phosphatase activity. Mutations within the linker showed effects mirroring those previously reported for Ci-VSP, suggesting a gating mechanism similar to the one found in *wild-type* Ci-VSP. We mutated Asp^{400} in Ci-VSP to Asn or Arg and the corresponding Asn (hVSP1) or Arg (PTEN) in the chimeric enzymes to Asp and measured their voltage dependent enzymatic activity as well as sensing currents to elucidate the importance of Asp^{400} for VSD-PD-coupling.
We propose that the interactions involving Asp^{400} described by Liu et al. and Hobiger et al. are not a necessary prerequisite for the voltage dependent activation of VSPs, but allow for a more efficient VSD-to-PD coupling. Further work will be required to establish the essential interactions between VSP, linker, and PD.
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The Role of the C2 Domain of Voltage Sensing Phosphatase (VSP)
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The voltage-sensing phosphatase (VSP) is a voltage-activated enzyme that dephosphorylates phosphatidylinositol phosphates (PIPs). VSP is unique because it is the only example of an enzyme activated by voltage, providing the first direct link between lipid signaling pathways and membrane potential. Along with the voltage sensing domain, VSP also has a catalytic domain and a C2 domain. To date, the role of the VSP C2 domain is not understood. C2 domains are generally known as lipid binding domains, however, previous work has suggested the VSP C2 has a role in catalytic activity. Specifically, the crystal structures [1,2] show the 522-loop of the C2 forms a portion of the enzyme active site and the mutation Y522A altered enzyme activity [2]. We further probed the role of Y522 by introducing a phenylalanine instead of an alanine. We found the Y522F mutation also shifts the voltage dependence of catalytic activity, suggesting that hydrogen bonding is not a factor when this residue participates in VSP activity. A role in catalysis does not exclude the VSP C2 from also contributing to lipid binding. To investigate this possibility, we started by fully deleting the C2 domain from Ci-VSP and found that the presence of the full C2 domain is necessary for normal phosphatase function. We then combined more specific C2 mutations with the ability to manipulate PIP concentrations using a rapamycin-induced system in order to further address the possible roles of the C2 in VSP function. Our goal is to investigate how the C2 domain contributes to VSP function.
[1] Matsuda, M. et al. JBC, 286, 23368-77 (2011).
[2] Liu, L. et al. NSMB, 19, 633-641 (2012).

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Investigating the Function of a Novel Voltage-Sensing Protein
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We have identified a protein coded by the C15orf27 gene that we named NVS (Novel Voltage Sensor). NVS contains 531 residues, and contains an S1-S4 domain, a 90 residue N-terminus and a 307 residue C-terminus, both of which are predicted to be intracellular. The most critical residues found in S1-S4 domains of other voltage sensors are conserved in NVS, including 3 Arg and a Lys in the S4 helix, 4 conserved acidic residues in S1-S3 and the charge-transfer Phe in S2. In addition, the C-terminus is predicted to contain a coiled-coil domain, similar to voltage-activated proton (Hv1) channels. Our hypothesis is that NVS functions as a voltage sensor that couples to intracellular signaling pathways or interacts with Hv1 to form heteroligomers through the C-terminal coiled-coil domain. We used site-specific voltage-clamp fluorometry and identified several positions at the outer ends of S3 and S4 where labeled Cys residues produced changes in fluorescence as a function of membrane potential. Several positions give complex fluorescence responses, starting with a rapid increase in fluorescence followed by slower decrease in fluorescence. We also investigated whether NVS can oligomerize with Hv1, but observe no change