study we used a selection of peptide neurotoxins against the cardiac Na\textsubscript{1.5}
channel to demonstrate the feasibility of screening slow-binding molecules by
applying sophisticated voltage protocols, and acquiring stable, high quality
recording for more than 30mins. The toxins used were: 1) Jing/Zhaoxin-II
which is a potentiator or positive modulator of Nav1.5 channels, 2) Pro-
Tx-II, which is an inhibitor of Na\textsubscript{1.5} channels, and 3) u-Conotoxin PIIIA a
Na\textsubscript{1.4} inhibitor was used as a negative control. The IonWorks Barracuda
Plus platform was used for these studies. Results are presented which are in
good agreement with peer-reviewed publications. Taken together, these results
demonstrate the high-throughput capabilities of this platform for measuring
peptide toxins targeted against ion channels.

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Searching for the Interaction Sites of the Betal Subunit with the Voltage-
Sensing Domains of Sodium Channels Using LRET
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Mammalian voltage-gated sodium channels (Nav) are composed of two sub-
units: a monomeric pore-forming subunit (a-subunit), that contains four
domains (DI-DIV), each composed of a voltage-sensing domain (VSD, S1-S4) and
a pore domain (S5-S6) and auxiliary subunits (b-subunits) that are transmembrane proteins with type I topology: containing an extracellular
amino-terminus, a single transmembrane segment and an intracellular
carboxyl terminus. A large body of literature has shown that interaction between
\(\alpha\)- and \(\beta\)-subunits results in Nav with altered gating kinetics suggest-
ing that \(\beta\)-subunits may directly interact with VSD. However, aspects of the
stoichiometry, arrangement and molecular interaction between \(\alpha\) and \(\beta\) sub-
units remain unclear. In this study, we explored the location of \(\beta\) in relation to
the rat skeletal muscle sodium channel \(\alpha\)-subunit (Nav1.4) using
lanthanide-based resonance energy transfer (LRET) via two strategies. 1)
Four Nav1.4 constructs were designed to encode a Tb\textsuperscript{3+} binding-tag
(Nav1.4-LBT) on top of the S4 of each domain (DI-LBT, DII-LBT, DIH-
LBT and DIV-LBT) as energy donor. A hexa-histidine-tag was inserted in
\(\beta\) (6His-\(\beta\)), which binds a Cu\textsuperscript{2+} ion to act as acceptor. 2) \(\beta\) constructs
were designed to encode at an extracellular site an LBT that binds Tb\textsuperscript{3+}
as energy donor while the acceptor was Alexa488 conjugated to Ts1, a
scorpion toxin which binds to DII-VSD in Nav1.4. For both experiments, we
used Xenopus laevis oocytes co-expressing Nav1.4 and \(\beta\) constructs
injected with the cRNA at 1:1 molar ratio. In preliminary results, DIV-
LBT + 6His-\(\beta\) oocytes displayed robust energy transfer between Tb\textsuperscript{3+}
and Cu\textsuperscript{2+}, indicating that at least one \(\beta\)-subunit is located at -28 Å of S4-
DIV. Support: 13POST14800031 (AHA), MOP-10053 (CIHR), GM68044-
07, U54GM087519 and GM030376.

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Tracking Voltage-Dependent Conformational Changes of the VSD in Nav with
LRET
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Voltage-gated sodium channels (Nav) are fundamental for the generation and
the propagation of action potentials. Mammalian Nav alpha subunits are sin-
gle macromolecules organized in four different domains (DI-DIV). Each is
composed of 6 transmembrane segments (S1-S6) from which S1-S4 consti-
tute the voltage sensing domain (VSD) and with S5 and S6 constituting the
pore. While Nav function has been studied extensively, the exact struc-
tural mechanisms of gating are not fully understood. Recently, the crystal
structure of the prokaryotic sodium channel, NavAb, has been solved, but
NavAb is a homotetrameric protein in contrast to the mammalian Navs. Thus
many questions were not answered by the prokaryotic channel struc-
tures. To resolve the voltage dependent conformational changes of Nav,
we tracked conformational changes of the VSD from each of the rat skeletal muscle sodium channel (Nav1.4) using Lanthanide-based Reso-
nance Energy Transfer (LRET), a FRET technique that allows for precise
measurement of intermolecular distances by taking advantage of the special
properties of lanthanide as an energy donor. We prepared Nav1.4 constructs
with a genetically encoded lanthanide binding tag (LBT), which holds a
lanthanide (Tb\textsuperscript{3+}) ion with high affinity, inserted at the top of the S4
segment in each domain. Also, we synthesized two toxins conjugated to
dyes to function as acceptors: the pore-blocking small molecule tetrodotoxin
conjugated with a HiLyte fluor488 (TTX-F), and the peptide \(\beta\) scorpion toxin
Ts1, from the Brazilian scorpion Tityus serrulatus, conjugated with Alexa488
(Ts1-Alexa488). Having several donor positions (Tb\textsuperscript{3+} ions in LBT’s) and
two different acceptor positions (TTX-F and Ts1-Alexa488), we calculated
multiple distances in voltage-clamped Xenopus laevis oocytes expressing
our Nav1.4 constructs that remained functionally active. The results provide
new insight to structure-function information in mammalian Nav channels.
Support: 13POST14800031 (AHA), MOP-10053 (CIHR), GM68044-07,
U54GM087519 and GM030376.

Voltage-gated Ca Channels I

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Observation of "Remote Knock-On", a New Permeation-Enhancement
Mechanism in Ion Channels
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We report observation of a novel "remote knock-on" mechanism for enhance-
ment of permeation in Brownian dynamics simulations of a simple model ion
channel. Unlike conventional knock-on, which requires a second ion of the
same species to enter the channel in order to knock forward and replace an
ion already in the channel, the new mechanism does not require the instig-
ating ion to enter the channel, nor that it be of the same species.

The figure plots the conditional probability distribution as a function of the position x
of the instigating ion at the instant of permeation when the ion initially trapped at
x=0 escapes to the right. The curves are plotted for different solute concentrations.
At the most probable position of the insti-
gating ion is clearly at the left mouth of the channel (x=-15Å). A 2nd small peak corre-
sponding to conventional knock-on appears at x=0 for high enough CL.

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A Mutational and Computational Study of Water and Ion Movement
through the S6 Bundle-Crossing of CaV1.2 Channel
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How exactly voltage-gated calcium and sodium channels open their physical
gate formed by the S6 TM segments is not clear yet. To study this, we modeled
the pore-forming region of CaV1.2 channel using a combination of homology-
modeling based and abinitio Rosetta algorithm. The obtained fold was stable
during 100-200 ns long molecular dynamics runs with implicit membrane
and solvent. In explicit membrane/water systems, it remained stable during
multiple 40-50 ns runs. When tested with the ZDOCK protein-docking algo-
rithm, the fold binds toxin Calcichedin at a site that incorporates residues,
which previously were shown to be involved in binding of the toxin. Molecular
dynamics with SW4-MDP water and polarizable ions reveals the presence of
novel water-filled cavities on the intracellular side of the channel. We mutated
the principal residues that form them and show their critical role in channel
gating.

According to molecular dynamics calculations, water polarizability appears to be
important for the filling of channel. We observe that water molecules form stable
structures (an enthalpy stabilized ice-like phase) inside the channel. We mutated
the principal residues that are predicted to alter water structures in the
pore and confirm their role in channel gating. Overall, our findings further elab-
orate previously proposed involvement of water in channel gating and uncover
a novel molecular view on the final steps of channel opening. Supported by NIH
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Poison-Fermi Model of a Calcium Channel: Correlations and Dielectric
Coefficient are Computed Outputs
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We derive a continuum model of biological calcium channels, called the
Poison-Fermi equation, designed to deal with crowded systems in which
ionic species and side chains nearly fill space. The model is evaluated in three
dimensions. It includes steric and correlation effects and is derived from

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