the CASP8 competition. This work suggests that physics-based simulations provide an important complement to bioinformatics structure prediction methods.

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3375-Pos Board B422

Accuracy of Ion Channels Homology Models is Significantly Improved by Symmetry-Restrained Molecular Dynamics Simulations

Adina L. Milac[1], Andriy Anishkin[2], H. Robert Guy[1].
[1]Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, [2]Department of Biology, University of Maryland, College Park, MD, USA.

Ion channels are an important target for drug development, however 3-D structures of biomedically relevant targets are usually unknown. Structural information required for structure-based drug design is often filled by homology models. Making models sufficiently accurate is challenging because few templates are available and these often have substantial structural differences. Most crystallized homo-oligomeric ion channels are highly symmetric, which dramatically decreases conformational space. In molecular dynamics (MD) simulations, channels deviate from the ideal symmetry and accumulate thermal defects. We have tested whether incorporating symmetry restraints in the MD simulations stage improves the accuracy of homology models. Our testing set consisted of three crystal structures of distantly related channels in closed conformation (KcsA, NaK and KirBac3.1), from which six homology models, two for each channel, were built using the remaining two structures as templates. These were embedded in POPC bilayer, solvated, and subjected to unrestrained MD simulations for 8 ns. Two approaches were then used to restore symmetry: 1) symmetry annealing gradually imposed symmetry through soft harmonic restraints during short 1ns simulations. 2) instantaneous symmetrization involved averaging the structures at a given timestep. Both techniques were followed by 8ns unrestrained simulation. This process was repeated three times. Our results show that the symmetry-annealing method improved the accuracy of homology models in 4 out of 6 cases, decreasing the RMSD against the x-ray structure by ~30%. The symmetricized models are also more stable during subsequent unrestrained simulations. The pore of the channel, which is the drug binding region, is improved most, making the modeled structures suitable for drug design. Instantaneous symmetrization produced effects similar to the gradual annealing, but was not as effective in mimicking the target protein's crystal structure and lowered structure stability.

3376-Pos Board B423

The combination of Small-Angle X-ray Scattering fitting and protein structure modeling in Integrative Modeling Platform

Seung Joong Kim, Benjamin Webb, Friedrich Förster, Andrej Sali.
University of California at San Francisco (UCSF), San Francisco, CA, USA.

We have shown that Small Angle X-ray Scattering (SAXS) data can be combined with protein structure modeling to determine the quaternary structures of multi-domain proteins and multi-subunit assemblies (Förster et al., J.Mol.-Biol., 2008, 382(4):p. 1089-1106). To maximize the utility of this approach, we further improved the scoring and sampling algorithms, and implemented them in our Integrative Modeling Platform (IMP) software (http://salilab.org/imp). This implementation will facilitate further integration of different kinds of data for determining the structures of proteins and their assemblies.

3377-Pos Board B424

Cryo-EM Guided de novo Protein Fold Elucidation

Steffen Lindert, Rene Staritzbichler, Nils Woetzel, Mert Karakas, Phoebe Stewart, Jens Meiler.
Vanderbilt University, Nashville, TN, USA.

Using cryo-electron microscopy (cryoEM) numerous sub-nanometer resolution density maps of large macromolecular assemblies have been reported recently. Although generally no atomic detail is resolved in these density maps, at 7 Å resolution α-helices are observed as density rods. Here we present the development of a computational protein structure prediction algorithm that incorporates the experimental cryoEM data as restraints. The placement of helices is restricted to regions where density rods are observed in the cryoEM density map. The Monte Carlo based protein folding algorithm is further driven by knowledge based energy functions.

The method has been benchmarked with ten highly α-helical proteins of known structure. The chosen proteins range in size from 250 to 350 residues. Starting with knowledge of the true secondary structure for these ten proteins, the method can identify the correct topology within the top scoring 10 models. With more realistic secondary structure prediction information, the correct topology is found within the top scoring 5 models for seven of the ten proteins. The algorithm has been applied to human adenovirus protein IIIa. This protein, for which there is no high resolution structure, is predicted to be highly α-helical. It is resolved in a 6.9Å resolution cryoEM adenovirus structure as a bundle of ~13 α-helical density rods.

3378-Pos Board B425

Membrane Protein Structure Determination by Coupling Sparse Experimental Data with Protein Structure Prediction Techniques

Nathan Alexander, Hassane Mchaourab, Jens Meiler.
Vanderbilt University, Nashville, TN, USA.

Membrane protein structure determination by classic experimental methods such as X-ray crystallography and nuclear magnetic resonance (NMR) continues to be extremely challenging, as demonstrated by the extremely low proportion of such structures in the Protein Data Bank. However, more than 50% of pharmaceuticals target membrane proteins, and it is estimated that membrane proteins make up 30-40% of all proteins. Therefore, a novel method was developed for membrane protein structure determination, taking advantage of alternative experimental techniques which are not hindered by the size or environment of membrane proteins. These alternative experimental techniques, such as electron paramagnetic resonance (EPR) and cryo-electron microscopy, provide sparse or low resolution structural data but cannot alone uniquely define a protein's structure. In order to obtain atomic detail models, the method incorporates one or more types of sparse or low resolution experimental data into a protein structure prediction algorithm. The method was benchmarked on a set of membrane proteins with known structure using sparse or low resolution data. This demonstrated the feasibility of obtaining membrane protein models of biologically relevant quality. The method was then applied to the multidrug resistance membrane transporter protein EmrE, for which extensive EPR and electron density data exist, giving a model with a high confidence of being a valid structure for EmrE.

Voltage-gated K Channels-Gating III

3379-Pos Board B426

Voltage-Clamp Fluorimetry Of Kv1.2 Channels Show Two Unique Phases Of Quenching Associated With Channel Activation

Andrew J. Horne[1], Tom W. Claydon[2], David Fedida[1].
[1]University of British Columbia, Vancouver, BC, Canada, [2]Simon Fraser University, Vancouver, BC, Canada.

Voltage-clamp fluorimetry can be used to visualize real-time changes in protein structure relative to a locally introduced fluorophore, and thus enhance our understanding of ion channel gating. Since the crystal structure is available for Kv1.2, it seems important to compare the voltage-dependent fluorescence reports from this channel with those already recorded from Shaker potassium channels. In the absence of introduced cysteine residues, we were unable to record voltage-dependent fluorescence signals from wild-type channels incubated with tetramethylrhodamine maleimide (TMRM). However, signals were obtained from an introduced cysteine at A291 in the Kv1.2 S3-S4 linker. Depolarization resulted in two separate components of quenching, which may underlie two different conformational changes in the protein. A slow quenching phase was observed upon depolarization from a holding potential of −120 mV and was essentially complete by −50 mV. The rate of this quenching was not significantly voltage-dependent, with time constants between 35 ms at −70 mV and 29 ms at +80 mV. The voltage-dependence of the slow component suggests that it reports on conformational changes preceding opening. A more rapid quenching component was observed upon depolarizations positive to −40 mV, with time constants from 2-10 ms. It had a similar voltage-dependence to the conductance-voltage relationship for potassium currents through Kv1.2, suggesting that it may report on channel rearrangements associated with opening. Dissociation of channel gating charge movement from pore opening by the incorporation of the ILT triple mutation in the S4 domain abolished the fast phase of fluorescence quenching at potentials up to +80 mV. This suggests that the fast quenching reports on conformational changes associated with channel opening, and that slow fluorescence quenching reflects protein rearrangements occurring earlier in the gating process.

3380-Pos Board B427

Kinetics Of Open- And Closed-state Inactivation Of Kv1.5 At Low pH Or With Ni2+ Ions

Steven J. Kehl, David Fedida, May Cheng.
University of British Columbia, Vancouver, BC, Canada.

Previous studies have shown that Ni^{2+}_o and H^+_o inhibit Kv1.5 current by enhancing open state inactivation (OSI) and promoting closed state inactivation

(CSI). By combining fast changes of $[H^+]$ or $[Ni^{2+}]$ with test depolarizations, the kinetics of OSI and CSI were studied to assess the possible kinship of closed-inactivated and open-inactivated states. At −80 mV, the mean onset time constant of CSI (τ_{CSI}) in nominally K^+ -free medium was 3.1, 1.2 and 0.18 s at pH 6.9, 6.4 and 5.4, respectively; τ_{OSI}, measured at 50 mV, and at the same pHs, was 1.2, 0.6 and 0.16 s. With 0.1 mM Ni^{2+}, τ_{CSI} and τ_{OSI} were 7.5 s and 1.8 s; in 10 mM Ni^{2+} these values decreased to 0.6 and 0.4 s, respectively. Following CSI or OSI, either at pH 5.4 or in 0.1 mM Ni^{2+}, recovery in control solution (pH 7.4, Ni^{2+}-free) was biphasic, and the recovery time constants were comparable to those fitted to recovery following OSI in control solution. However, the relative weight of the fast and slow components depended on the ligand used. Following OSI and CSI induced at low pH_o, recovery was dominated by the faster process (τ approx. 1s); with Ni^{2+}, recovery occurred mainly by the slower process (τ approx.10 s). Thus, Ni^{2+} and H^+ cause a concentration-dependent increase of CSI and OSI, with τ_{CSI} approaching τ_{OSI} at high concentrations. Inactivation at low pH_o, either from the open- or the closed state, is to a state that is also visited at pH 7.4 and from which recovery is relatively rapid. The latter conclusion also applies for Ni^{2+}, except that the inactivated state is predominantly that from which recovery is slow.

3381-Pos Board B428

Low External pH_o Induces Closed-state And Enhances Open-state Inactivation Of *Shaker*IR Channels Expressed In A Mammalian Cell Line

Yen May Cheng, Steven J. Kehl.
University of British Columbia, Vancouver, BC, Canada.

In fast-inactivation removed *Shaker* (*Shaker*IR) channels, external acidification reduces peak current. Previous studies have suggested that this is due to the low pH_o-induced acceleration of P/C-type inactivation from the open state (OSI). However, recent fluorimetric studies of *Shaker*IR channels suggested that reduced channel availability due to a closed-state inactivation (CSI) process may also be involved, although the time-dependence of this process is unclear. To determine the relationship, if any, between OSI and CSI, we examined the pH_o- and time-dependence of both OSI and CSI of *Shaker*IR channels expressed in an HEK cell line. Consistent with previous findings in *Xenopus* oocytes, both peak current and the time constant of OSI (τ_{OSI}) at +50 mV decreased with reductions in pH_o; the pK_as for these relationships were 4.5 and 5.2, respectively. Thus, at pH_o 4.5, peak current was ~41% of that seen at control pH_o 7.4, while τ_{OSI} decreased from ~1.3 s to ~140 ms. The time constant of CSI (τ_{CSI}) at pH_o 4.5, estimated by monitoring the decrease in the peak current evoked by a pulse to +50 mV after a variable exposure time to pH 4.5 solution at −80 mV, was ~200 ms. Measurements of τ_{CSI} for comparison with τ_{OSI} at other pH_o values are currently in progress.

3382-Pos Board B429

Addition of a Charged Residue at Position 363 Focuses the Electric Field Sensed by S4 During Activation of *Shaker* K-channels

Vivian M. Gonzalez Perez, Katherine Stack, Katica Boric, Tania Estevez, David Naranjo.
Centro de Neurociencia de Valparaiso, Valparaiso, Chile.

Voltage-gated potassium channels contain a voltage sensor domain in each of its four subunits that confers exquisite sensibility to the trasmembrane electric field. The fourth transmembrane segment (S4) of a *Shaker* K-channel subunit has seven highly conserved basic amino acids, periodically spaced every two hydrophobic residues. The four outermost arginine residues (R1-R4) in S4 move through most of the electric field upon depolarization. This charge movement (12-13 e0 per channel) is tightly coupled to the conformational change leading the opening of the potassium conduction pathway. Any residue located within the R1-R4 limits would be dragged through the electric field, and if charged would contribute to the charge movement. Surprisingly, when we replaced V363 (located between R1 and R2) by either an arginine or an aspartate, both charge adding mutations decreased the effective valence of opening by 50%. This reduction in effective valence was not attributable to an uncoupling between charge movement and channel opening because the Q-V curve remained preceding the G-V curve. To test if the added charges promote a remodeling of the electric field sensed by S4, we assessed the state-dependency of external accessibility of R1 or R2 in the presence of the additional charge. Then we tested the accessibility to methanethiosulfonate derivates in R1C/V363R, R1C/V363D, R2C/V363R or R2C/V363D double-mutants. We measured the rates of cysteine modification within a ten-fold change in the overall open probability for each mutant. Unlike the R1C control, the modification rates were not state-dependent for R1C/V363X double-mutants, while they remained state-dependent for R2C/V363X double-mutants as the R2C control. These results suggest that in channels carrying a charged residue at position 363, R362 remains outside the field during voltage dependent activation.

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3383-Pos Board B430

Gating Differs Between an Antarctic and a Tropical K_v1 Channel Because of RNA Editing

Sandra C. Garrett-Rodrigues, Joshua J.C. Rosenthal.
University of Puerto Rico, San Juan, PR, USA.

In squid, shaker-like potassium channel mRNA's are extensively edited by adenosine deamination. Because A-to-I RNA editing tends to recode for smaller amino acids, it could create more flexible proteins and be involved in cold adaptation. In this study we compare editing patterns and their functional consequences for a K_v1 K^+ channel mRNA from two species of octopus: *Pareledone sp*., from the extreme cold of Antarctica and *O. vulgaris* from tropical inshore waters of Puerto Rico. From each species, the same K_v1 gene was cloned and sequenced. An editing map was created by sequencing 50 cDNA clones and identifying sites with A/G variation. At the genomic level, the channels were nearly identical, differing by four amino acids. By contrast, their editing patterns differed substantially. In total there were 13 non-silent editing sites, with five sites unique to one species or the other. At four sites editing percentages differed by more than 50% between species: N40S and S54G are edited more in *O. vulgaris* while N105G and I321V are edited more in *Pareledone*. We tested the eletrophysiological effects of differences at both the genomic and RNA editing levels. Channels were expressed in *Xenopus* oocytes and their kinetics of activation, deactivation, and inactivation, along with their voltage-dependence, were compared. The two genomically encoded channels were nearly identical, although the activation kinetics were slightly faster for the Antarctic channel. Individual editing sites, however, changed multiple parameters. The ''tropical'' edits N40S and S54G, both in the tetramerization domain, slowed activation and accelerated inactivation. The ''Antarctic'' edit I321V shifted the voltage dependence of activation by +12 mV and more than doubled the rate of deactivation. I321V, which is in the S5 helix, appears to be a candidate for cold-adaptation.

3384-Pos Board B431

Closed-State Inactivation in Kv4.3 Splice Forms is Differentially Modulated by Protein Kinase C

Chang Xie[1], Vladimir Bondarenko[2], Michael J. Morales[1], **Harold C. Strauss[1]**.
[1]UB, SUNY, School of Medicine, Buffalo, NY, USA, [2]Georgia State University, Atlanta, GA, USA.

Kv4.3, with its complex open- and closed-state inactivation (CSI) characteristics, is a primary contributor to early cardiac repolarization. The two alternatively-spliced forms of Kv4.3 (L and S) differ by the presence of a 19 amino acid exon 81 amino acids downstream from the sixth transmembrane segment. The two isoforms are reported to be kinetically similar, however, the longer form has a unique PKC phosphorylation site. To test the possibility that inactivation is differentially regulated by phosphorylation, we stimulated PKC in *Xenopus* oocytes expressing Kv4.3 isoforms and examined their inactivation properties. There was no difference in open-state inactivation, there were profound differences in CSI; in Kv4.3-S, PMA (10nM) reduced the extent of CSI from 0.53 ± 0.06 to 0.69 ± 0.05 after 14.4 s at −50 mV. In contrast, CSI in Kv4.3-L increased from 0.71 ± 0.05 to 0.51 ± 0.03 under the same conditions. Mutation of the unique putative PKC phosphorylation site in Kv4.3-L eliminated its isoform-specific behavior. This effect was independent of the intervention used to increase PKC activity; identical results were obtained with either PMA or injected purified PKC. Our data demonstrate that Kv4.3 can be differentially regulated by PKC, and that the carboxy terminus of Kv4.3 plays an important role in regulation of CSI.

3385-Pos Board B432

Inverse Modes of Coupling in Leak and Voltage-activated K^+ Channel Pore Gates Underlie their Distinct Roles in Electrical Signaling

Yuval Ben-Abu[1], Yufeng Zhou[2], Noam Zilberberg[1], **Ofer Yifrach[1]**.
[1]Ben-Gurion University of the Negev, Beer-Sheva, Israel, [2]Yale University, New haven, CT, USA.

Voltage-activated (Kv) and leak (K_{2P}) potassium channels play key, yet distinct roles in electrical signaling in the nervous system. Here, we examined how differences in the operation of the activation and slow inactivation pore gates of Kv and K_{2P} channels underlie their unique roles in electrical signaling. We report that (1) leak potassium channels possess a lower activation gate, (2) the activation gate is an important determinant controlling the conformational stability of the K^+ channel pore, (3) the lower activation and upper slow inactivation gates of leak channels cross-talk and (4) in contrast to Kv channels, where the two pore gates are negatively-coupled these two gates are positively-coupled in K_{2P} channels. Our results thus demonstrate how basic thermodynamic properties of the K^+ channel pore, particularly conformational stability and coupling between the pore gates, underlie the specialized roles of Kv and K_{2P} channel families in electrical signaling.