

gate in the pore. Alanine replacement of H434 abolished the delay in channel deactivation and the generation of I<sub>ins</sub> by the photodynamic modification. This study provides insights into the instantaneous current conducted by HCN channel and establishes a well-defined model for studying IO<sub>2</sub> modification of ion channel proteins.

### 3831-Pos Board B559

#### Two Separate Sites Compete for Singlet Oxygen in the Photodynamic Modification of HCN Channels

Weihua Gao, Zhuocheng Su, Qinglian Liu, Lei Zhou.

Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA, USA.

Molecule oxygen has three electronic configurations, the triplet ground state, the first and the second singlet excited states. Singlet oxygen (IO<sub>2</sub>) is highly reactive and oxidizes a wide range of biomolecules including DNA and protein. Because of the short life time in microseconds and the short working distance in nanometers, IO<sub>2</sub> eliminates the function of specific protein molecules at high temporal and spatial precisions and thus holds the potential as an effective photonics tool to probe protein dynamics. Recently we discovered that HCN channels are sensitive to the modification by the singlet oxygen (IO<sub>2</sub>) generated through photosensitization processes. IO<sub>2</sub> modification slows down the channel deactivation and enhances the expression of the voltage-insensitive instantaneous current (I<sub>ins</sub>). A histidine residue located in the pore of the HCN channel, H434, was found to be critical. The alanine replacement mutation, H434A, abolished the above effects so that IO<sub>2</sub> modification does not prolong the channel deactivation nor enhance the I<sub>ins</sub>. However, the reduction in I<sub>h</sub> amplitude in H434A mutant channel becomes more significant than that in WT channel. After five short laser pulses, the amplitude of the I<sub>h</sub> current was reduced to 76.9 ± 1.7 % of the control level in the WT channel but to 43.4 ± 2.4 % in the H434A mutant channel. This result suggests that H434 is the major target of IO<sub>2</sub> modification and consumes the majority of the IO<sub>2</sub> generated through the photosensitization process. In the H434A mutant channel, other parts of the channel reacts with the IO<sub>2</sub> that becomes transiently more abundant and the modification leads to the reduction in I<sub>h</sub> amplitude. Possibly, IO<sub>2</sub> modification of protein molecules is not a process of random modifications but distinctly targets certain conformational and functional states.

### 3832-Pos Board B560

#### Different Effects of Alkaline Phosphatase on HCN4 Channels in CHO Versus HEK Cells

Julie Juchno, Joshua R. St Clair, Cathy Proenza.

University of Colorado, Aurora, CO, USA.

Hyperpolarization-activated, cyclic nucleotide-sensitive type 4 (HCN4) channels are regulated by cAMP, which can bind directly to a conserved cyclic nucleotide binding domain (CNBD) in the C-terminus. We previously found that the response of HCN4 channels to cAMP depends on the cellular context: cAMP binding to the CNBD shifts the midpoint activation voltage (V<sub>1/2</sub>) to more depolarized potentials when HCN4 is expressed in HEK cells, whereas cAMP has no effect on the V<sub>1/2</sub> when HCN4 is expressed in CHO cells because the channels are already "pre-activated," with a depolarized V<sub>1/2</sub> in the absence of cAMP (Liao et al., 2012 J Gen Physiol 140(5):557). Here we have tested the hypothesis that differential phosphorylation may underlie the differences in HCN4 behavior in CHO versus HEK cells. We found that while alkaline phosphatase (AlkPhos) restored the hyperpolarized basal V<sub>1/2</sub> of HCN4 in CHO cells, it did not restore the cAMP sensitivity of the channels. Quite different results were obtained in HEK cells, in which AlkPhos had little or no effect on the V<sub>1/2</sub> of HCN4 in the absence of cAMP but, remarkably, was found to significantly reduce the shift in V<sub>1/2</sub> in response to cAMP. These results indicate that dual regulation of HCN4 channels by cAMP and phosphorylation is complex and is sensitive to cellular context. And, phosphorylation alone is insufficient to account for the different behaviors of HCN4 channels in CHO versus HEK cells.

### 3833-Pos Board B561

#### The Auxiliary Subunit TRIP8B Inhibits the Binding of cAMP to HCN2 Channels Through an Allosteric Mechanism

Andrea C. Saponaro<sup>1</sup>, Manolis Matzapetakis<sup>2</sup>, Bina Santoro<sup>3</sup>, Sofia R. Pauleta<sup>4</sup>, Anna Moroni<sup>1</sup>.

<sup>1</sup>University of Milan, Milan, Italy, <sup>2</sup>ITQB, New University of Lisbon, Lisbon, Portugal, <sup>3</sup>Columbia University, New York, NY, USA, <sup>4</sup>TCF, New University of Lisbon, Lisbon, Portugal.

In neurons, hyperpolarization-activated cyclic nucleotide-regulated (HCN1-4) channels are the molecular determinants of the I<sub>h</sub> current that controls

cell excitability and rhythmicity [1]. HCN channels are activated by membrane hyperpolarization and directly regulated by cAMP binding to the cyclic nucleotide binding domain (CNBD) [2]. HCN channels are further regulated by their association with an auxiliary protein, TRIP8b, which binds to the CNBD and opposes cAMP regulation [3]. We asked if the effect of TRIP8b could be attributed to a direct competition with cAMP. To this end, using NMR methodologies, we determined the 3D structure of the human HCN2 CNBD in the cAMP-unbound form and subsequently mapped onto it the TRIP8b binding site. Our results show that cAMP and TRIP8b do not directly compete for binding to the same residues, and support an allosteric inhibition model for the dual regulation of HCN channels by cAMP and TRIP8b.

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### 3834-Pos Board B562

#### Mechanism of Ionic Permeation in the Mimics of CNG Channels: A Structural, Functional and Computational Analysis

Luisa M.R. Napolitano<sup>1,2</sup>, Ina Bisha<sup>1</sup>, Manuel Arcangeletti<sup>1</sup>, Arin Marchesi<sup>1</sup>, Matteo De Marchi<sup>2</sup>, Silvia Onesti<sup>2</sup>, Alessandro Laio<sup>1</sup>, Vincent Torre<sup>1</sup>.

<sup>1</sup>SISSA, Trieste, Italy, <sup>2</sup>Elettra-Sincrotrone Trieste, Trieste, Italy.

Using an engineered cyclic nucleotide-gated (CNG) channel mimic based on the bacterial non-selective NaK channel, we examined how different ionic species interact with the pore by means of electrophysiology, all-atoms molecular dynamics simulations and X-ray crystallography.

Difference and anomalous difference electron-density maps of high-resolution crystal structures (up to 1.85Å) obtained after soaking with different ionic species (Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Rb<sup>+</sup>, Cs<sup>+</sup>, Methylammonium) clearly shows the presence of different peaks inside the pore. Depending on these ionic species Glu66 and Thr67, located at the extracellular entrance of the selectivity filter, adopt different rotameric states. In the presence of Methylammonium, Glu66 points towards Tyr55 of the same subunit, while in the presence of Na<sup>+</sup>, Glu66 forms H-bonds with Thr67 and Thr60 of a neighboring subunit (Derebe et al, 2011, PNAS). Moreover, large-scale molecular dynamics simulations in a hydrated lipid bilayer at 0 mV indicates that Glu66 and Thr67 side chains are capable of large structural fluctuations which are modulated by the nature of the ion residing in the pore. In particular, in presence of Rb<sup>+</sup> and Cs<sup>+</sup> Glu66 explores at least three different conformations, characterized by a significantly different distribution of the local charge. Single channel recordings from this chimeric ionic channel in symmetrical Rb<sup>+</sup> and Cs<sup>+</sup> conditions show a strong voltage dependency similar to what observed in the native CNGA1 channel (Marchesi et al, 2012, Nat. Communications). These results indicate that membrane voltage catalyzes a conformational change of these residues, especially of Glu66, perturbing the hydrogen bonding network behind the selectivity filter. Non-selective ionic permeation emerges as a robust feature of an intrinsic flexible pore and is a key microscopic factor controlling ionic selectivity in CNG and possibly in other non-selective channels.

### 3835-Pos Board B563

#### Architecture of the HCN Selectivity Filter and Control of Cation Permeation

Vincenzo Macri, Damiano Angoli, Eric Accili.

UBC, Vancouver, BC, Canada.

Hyperpolarization-activated Cyclic Nucleotide-modulated (HCN) channels are similar in structure and function to voltage-gated potassium channels. Sequence similarity and functional analyses suggest that the HCN pore is potassium channel-like, consisting of a selectivity filter and an activation gate at the outer and inner ends, respectively. In GYG-containing potassium channels, the selectivity filter sequence is 'T/S-V/I/L/T-GYG', forming a row of four binding sites through which potassium ions flow. In HCNs, the equivalent residues are 'C-I-GYG', but whether they also form four cation binding sites is not known. Here, we focus on the anomalous filter residue of HCNs, the cysteine located at the inner side of the selectivity filter. In potassium channels, this position is occupied by threonine or serine and forms the fourth and most internal ion binding site of the selectivity filter. We find that this cysteine in HCNs does not contribute to permeation or form a fourth binding site.