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# Metamorphic Response of CLIC1 Chloride Intracellular Ion Channel upon Interaction with the Membrane

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Starting from the crystal structure of beta2-adrenergic receptor (B2AR), we have used LITiCon computational method to predict the ligand stabilized receptor state with full (epinephrine and norepinephrine), partial (salbutamol and dopamine), and inverse agonists (carazolol) bound. We have calculated the minimum energy pathway going from the inactive to the ligand stabilized state for each of the ligand/receptor complex. The activation pathways derived for all the agonists studied here are in agreement with fluorescence lifetime measurements<sup>1,2</sup>. We have also studied the norepinephrine activation pathway for the mutant m23 avian beta1-adrenergic receptor (B1AR) that has been crystallized, and the wild type B1AR. We show that the activation barrier for activation of the mutant m23B1AR by norepinephrine, is larger than for the wild-type B1AR, which is in agreement with the previous experimental finding that m23B1AR requires higher concentration of norepinephrine for activation<sup>3</sup>. Virtual ligand screening with the salbutamol-stabilized B2AR conformation shows enrichment of non-catechol agonists over norepinephrine-stabilized conformation. Our computational method provides an unprecedented opportunity to understand activation mechanisms in GPCRs.

1. Swaminath G, et al. (2004), *J. Biol. Chem.* 279:686-691.
2. Swaminath G, et al. (2005), *J. Biol. Chem.* 280:22165-22171.
3. Warne T., et. Al., (2008), *Nature*, 454, 486-491.

### 3365-Pos

#### Functional Analysis of a Conserved Histidine Residue of the Extracellular Loop of Acid-Sensing-Ion-Channel-1a

Benoite Bargeton, Stephan Kellenberger.

University of Lausanne, Dept. of Pharmacol. & Toxicol., Lausanne, Switzerland.

ASICs are non-voltage-gated neuronal sodium channels activated by protons. Activation of ASICs induces a membrane depolarization. ASICs are trimeric assemblies of homologous subunits. The structure of the chicken ASIC1 in its inactivated conformation is known. Each subunit has two transmembrane domains, a large extracellular loop (ECL) and two short intracellular termini. The ECL is the sensor of the extracellular acidity and the target of modulators. Zinc potentiates the acid activation of ASIC2a-containing channels. Two histidine residues are essential for this effect. Only the hASIC1a His163 is conserved in all ASICs and forms H-Bonds with the conserved Asn96 and Gln225 residues. To test their functional relevance, we mutated these and neighboring residues to cysteine. Most mutant channels showed poor expression in *Xenopus* oocytes suggesting that this part of the ECL is essential for the channel folding. The mutant channels were however functional and were unlike the wild type channel inhibited by Cd<sup>2+</sup>. The Cd<sup>2+</sup> IC<sub>50</sub> values (N96C: 71 ± 10 μM, Q225C: 73 ± 53 μM, H163C: 331 ± 36 μM) suggest together with the structural information that H163 participates in Cd<sup>2+</sup> chelation in the mutant channels. Covalent modification by the charged sulphydryl reagent MTSET was used to further investigate the structural and functional role of the His163 and its environment. After MTSET exposure the hASIC1a H163C channel current decreased to 4 ± 1% and the pH dependence of activation was shifted by -0.24 pH units, while the pH dependence of inactivation was not affected. The unitary conductance of the modified H163C mutant was not different from the conductance of unmodified H163C or wt ASIC1a. These observations suggest a role of this region in the gating, but likely not the pH-sensing of ASIC1a. Our study identifies a conserved region around His163 as critical determinant of ASIC1a expression and function.

### 3366-Pos

#### Structural Studies of the Integral Membrane Protein Human LTC4 Synthase by Electron Crystallography

Frederik Rudolph<sup>1</sup>, Stefania Mari<sup>2</sup>, Gengxiang Zhao<sup>1</sup>, Matthew C. Johnson<sup>1</sup>, Yoshihide Kanaoka<sup>3</sup>, Daniel Müller<sup>2</sup>, Ingeborg Schmidt-Krey<sup>1</sup>.

<sup>1</sup>Georgia Institute of Technology, School of Biology, School of Chemistry and Biochemistry, Atlanta, GA, USA, <sup>2</sup>Technical University Dresden, Biotechnology Center Dresden, Tatzberg 47/49, Dresden, Germany,

<sup>3</sup>Harvard Medical School and Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, MA, USA.

Human leukotriene C4 synthase (LTC4S), an 18kDa integral membrane protein and member of the MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) family, is critical to the biosynthesis of the cysteinyl leukotrienes. LTC4S catalyzes the conjugation of leukotriene A4 with glutathione to produce leukotriene C4. The cysteinyl leukotrienes, in particular leukotriene C4 and its metabolites, are important lipid mediators in inflammation and are central to acute and chronic inflammatory diseases of the respiratory system, in particular bronchial asthma. The structure of human LTC4S was studied by two-dimensional (2D) crystallization and electron crystallography.

When reconstituted into proteoliposomes under rigorously controlled parameters, human LTC4S forms large and well-ordered two-dimensional crystals that

retain enzymatic activity. Despite two three-dimensional structures, many questions about the reaction mechanism of human LTC4S remain unanswered. To elucidate these structure/function questions by electron crystallography, we are crystallizing both the wild-type and mutant enzymes. Surprisingly, the number of subunits of human LTC4S is not limited to the trimers earlier observed by both electron and x-ray crystallography.

### 3367-Pos

#### Metamorphic Response of CLIC1 Chloride Intracellular Ion Channel upon Interaction with the Membrane

Sophia C. Goodchild<sup>1</sup>, Michael W. Howell<sup>1</sup>, Kenneth A. Sale<sup>2</sup>, Paul M.G. Curmi<sup>3</sup>, Louise J. Brown<sup>1</sup>.

<sup>1</sup>Macquarie University, Sydney, Australia, <sup>2</sup>Sandia National Laboratories, Livermore, CA, USA, <sup>3</sup>The University of New South Wales, Sydney, Australia.

The Chloride Intracellular Channel (CLIC) family can be characterized as 'metamorphic' proteins; namely, capable of reversibly shifting between two or more different-folded conformations. The CLICs are expressed as soluble proteins but can also auto-insert into the membrane to form active ion channels. Such a conformational transition must involve large-scale structural rearrangement to confer favourable interactions with the membrane. To date, little is known about the process and cellular triggers for CLIC membrane insertion; although a unique feature of mammalian CLIC1 is its ability to undergo a dramatic structural rearrangement from a monomer to a dimer upon oxidation in solution. Whether this oxidation-induced metamorphosis in solution facilitates CLIC1 membrane insertion is unclear. We have sought to characterize the structural response of CLIC1 upon interaction with the membrane. A novel labeling scheme for CLIC1 was devised to enable site-directed-labeling of single native cysteines with either fluorophores or spin labels. Fluorescence resonance energy transfer (FRET) and electron paramagnetic resonance (EPR) revealed that the interaction of CLIC1 with the membrane results in a large-scale inter-domain movement. The two phases for membrane interaction, i.e. association followed by insertion, were also monitored by probing the local environment of an N-terminal transmembrane tryptophan residue (Trp35) using fluorescent quenching. A sucrose-loaded-vesicle sedimentation assay was also used to quantify membrane binding. Our results suggest oxidation of CLIC1 monomer in the presence of the membrane promotes CLIC1 binding and insertion. Our current model for the structural transitions and environmental triggers of CLIC1 membrane-induced metamorphosis will be discussed.

### 3368-Pos

#### Structural Analysis of Heptameric Alpha-Hemolysin under Extreme Conditions that Facilitate Nucleic Acid Translocation

Marsiyana M. Henricus, Deapen Japrun, QiuHong Li, Giovanni Maglia, Hagan Bayley.

University of Oxford, Oxford, United Kingdom.

Protein nanopores are under intense investigation as sensors of various analytes, particularly for the rapid analysis of genomic material. In several important applications, notably ultrarapid sequencing, nucleic acids must be analyzed in unfolded single-stranded form. Therefore, conditions were examined that cause the denaturation of double stranded DNA (dsDNA), and single stranded DNA (ssDNA) and RNA with secondary structure. The behavior of the heptameric  $\alpha$ -hemolysin ( $\alpha$ HL) pore was investigated under alkaline pH conditions (8.0-13.0) and in varying concentrations of urea (0-8 M). The structural stability of the pore was examined by SDS-PAGE, intrinsic tryptophan emission fluorescence (ITFE) spectroscopy and circular dichroism (CD) spectroscopy. The pH studies revealed that the heptamer is resistant to structural change at up to pH 12.0. Electrical recordings revealed that DNA is capable of translocating through the pore at pH 11.7, a value at which its secondary structure is lost [1]. Studies conducted at various urea concentrations revealed that a substantial fraction of the heptamer remains stable in 8 M urea. At urea concentrations above 4.0 M, the secondary structure of single stranded DNA/RNA is denatured [2], permitting translocation.

[1] Zimmer C. Alkaline denaturation of DNA's from various sources. *Biochim Biophys Acta* 161 (2), 584-586 (1968).

[2] Levine, L., J. A. Gordon, and W. P. Jencks. The relationship of structure to the effectiveness of denaturing agents for deoxyribonucleic acid. *Biochemistry* 2, 168-175 (1963).

### 3369-Pos

#### Mtb Membrane Protein Expression, Purification and, Structure Determination : Solid State NMR Approach

Nabanita Das<sup>1,2</sup>, Timothy A. Cross<sup>1,2</sup>.

<sup>1</sup>Institute of Molecular Biophysics, Kasha Laboratory, Tallahassee, FL, USA,

<sup>2</sup>National High Magnetic Field Laboratory, Tallahassee, FL, USA.