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protein active site is observed without biased sampling methods. During the course of MD simulation, an average of 6.4 water molecules is observed in the camphor-binding region of the apo form and zero water molecules in the binding site of the substrate-bound form, in agreement with the number of water molecules observed in their crystal structures. However, as many as 12 water molecules can be present at a given time in the camphor-binding region of the active site in apo-P450cam, revealing a highly dynamic process for hydration of the protein active site with water molecules exchanging rapidly with the bulk solvent. Water molecules are also found to frequently exchange locations inside the active site, preferentially clustering in regions surrounding the water molecules observed in the crystal structure. Potential of mean force calculations identify thermodynamically favored trans-protein pathways for the diffusion of water molecules between protein active site and the bulk solvent. Binding of camphor in the active site modifies the free energy landscape of P450cam channels toward favoring the diffusion of water molecules out of protein active site.

680-Pos Board B466

Computational Systems Analysis of Glucose Sensitivity, Electrical Activity and Glucagon Secretion in Pancreatic Alpha-Cells

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Glucagon, a 29-amino acid hormone secreted from the alpha-cells of the islets of the endocrine pancreas, is critical for blood glucose homeostasis by promoting liver production of glucose in hypoglycemia. A clearly defined, testable model for alpha cell glucagon secretion is lacking. Alpha-cells respond to rising blood glucose by increasing oxidative metabolism, closing ATP-sensitive K+ channels and the resulting depolarization of the plasma membrane leads to decreased glucagon secretion. This mechanism of glucose sensing involves the coupling of electrophysiological, cytoplasmic and mitochondrial processes. Here we describe a computational systems analysis of pancreatic alpha-cells including metabolic processes , electrical activity and Ca2+ dynamics in the regulation of glucagon secretion. This mathematical model of alpha-cell sensitivity to glucose is based on a previous model for pancreatic beta-cells. We also formulated a Hodgkin-Huxley-type ionic model for action potentials in alpha-cells that incorporates voltage-gated Ca2+, K+, Na+ and Cl- currents. The metabolic and ionic models are coupled to the equations describing intracellular Ca2+ homeostasis and glucagon secretion that depends on an activation of specific voltage-gated Ca2+ channels. This model simulates the behavior of alpha-cell action potentials under a wide range of experimental conditions, including block of action potential firing at increased glucose levels or due to channel blockade. Paracrine regulation of glucagon secretion can be modeled following the effects of gamma aminobutyric acid or somatostatin secreted by other islet cells on corresponding alpha-cell ion channels and metabolism. This computational systems analysis aids in providing a more complete understanding of the complex process of alpha-cell glucose sensing and the pathways of treatment of diabetes.

681-Pos Board B467

Molding the Business End of Neurotoxins by Diversifying Evolution

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A diverse range of organisms utilize neurotoxins that target specific ion channels and modulate their activity. Typically, toxins are clustered into several multigene families, providing an organism with the upper hand in the never-ending predator-prey arms race. Several gene families, including those encoding certain neurotoxins, have been subject to diversifying selection forces, resulting in rapid gene evolution. Here, we sought a spatial pattern in the distribution of both diversifying and purifying selection forces common to neurotoxin gene families. Utilizing the Mechanistic Empirical Combination model, we analyzed various toxin families from different phyla affecting various receptors and relying on diverse modes of action. By this approach, we were able to detect clear correlations between the pharmacological surface of a toxin and rapidly evolving domains, rich in positively selected residues. On the other hand, patches of negatively selected residues were restricted to the non-toxic face of the molecule and most likely help in stabilizing the tertiary structure of the toxin. We thus propose a mutual evolutionary strategy of venomous animals in which adaptive molecular evolution is directed towards the toxin active surface. Furthermore, we propose that the binding domains of unstudied toxins could be readily predicted using evolutionary considerations.

682-Pos Board B468

Cooperative Regulation of Slack Channel by Na^+, Cl^- and PIP2

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Slack (or Slo2.2) channel activity is regulated by Na+ and Cl- ions. The Na+ coordination site of Slack channels bears similarity to the analogous site in Kir3 channels. Here, we show that the activity of Slack channels, like BK (or Slo1) and Slo3 channels, is also dependent on PIP2. We next ask: How are these multiple positive cooperative factors interact with each other to regulate Slack channel activity? We first examined the relationship of Na+ and PIP2 regulation. Unlike Kir3 channels, the D818N mutation, which decreases Na+ sensitivity greatly, showed no effect on PIP¬2 sensitivity. Manipulations that weaken channel-PIP2 interactions in Kir2.1 channels induce residence into subconductance states (Xie et al., 2008, J. Physiol. 586:1833). Unitary Slack channel activity rarely visited sub-conductance levels in the absence of Cl-. Yet, in the presence of Cl-, the Slack single channel conductance was shifted from 145 pS to 80~100 pS, while the open probability of Slack channels was increased by 2-2.5 fold due to the cooperative regulation of Cl- with Na+. We are in the process of screening for potential Cl- binding site(s) in the cytoplasmic domain of Slack channels, utilizing a strategy similar to the one that enabled us to identify the D818 Na+ coordination site. We hypothesize a close cooperative relationship between PIP2 and Cl- regulation of Slack activity. We are working on investigating this cooperativity and developing a suitable model to explain this complex regulatory mechanism.

683-Pos Board B469

Structure and Elasticity of Genistein and Daidzein in Lipid Membranes using X-Ray Scattering and MD Simulations

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This work reports the effects of bioflavinoids genistein and daidzein on lipid bilayers as determined by volume measurements, X-ray scattering and molecular dynamics simulations. Upon adding 20 mole% genistein or daidzein to DOPC membranes, experimental and simulated volumes are found to be in outstanding agreement. Both bioflavinoids insert into the hydrocarbon region near the carbonyls of DOPC and also DiphytanoylPC, ~12 Å from the bilayer center. In DOPC, both experiments and MD simulations show the area/unit cell equals 83 $Å^2$ for 20 mole% genistein and 80 $Å^2$ for 14 mole% daidzein (its maximum solubility in oriented samples). Both bioflavinoids thin DOPC and DPhyPC membranes proportional to their solubility. The long axes of both bioflavinoids are oriented nearly parallel to the plane of the bilayer with their carbonyl groups preferentially pointed towards the proximal surface. X-ray diffuse scattering reveals that both bioflavinoids modestly reduce K_C, the bending modulus, daidzein slightly more than genistein. MD simulations determine that both bioflavinoids reduce K_A, the area compressibility modulus, daidzein slightly more than genistein. These elasticity results are in general agreement with the hypothesis that bioflavinoids increase membrane flexibility, thereby overcoming hydrophobic mismatch, and increase gramicidin (gA) lifetimes[1]. However, the small structural and elasticity differences between genistein and daidzein cannot account for genistein's having twice the effect of daidzein in increasing gA channel lifetimes; that is apparently due to the greater solubility of genistein in membranes when equal amounts are added in gA experiments.

[1]Hwang, Koeppe & Andersen, Biochemistry 42:13464(2003).

684-Pos Board B470

Electrophysiological and Gene Expression Profiling at Single Cell Level through an Improved Whole Cell Patch Clamp Quantitative Real-Time PCR Technique

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Cellular excitability andaction potential generation originates from a concerted action of different ion channels. The molecular diversity of channels (over 100 different genes) underlies the vast diversity in the CNS and even a specific type of neurons may display large differences in channel expression. Patch-clamp is a powerful technique to study the electrophysiology of excitability at the single cell level allowing to explore cell-to-cell variability. At present only few attempts have been made to link electrophysiological profiling to mRNA transcript levels and most suffered from experimental noise making correlations virtually impossible. Here we describe a significant refinement to the technique that combines patch-clamp analysis with quantitative real-time (qRT) PCR at the single cell level. Hereto the process was optimized such that the expression of a housekeeping gene could be used to normalize for cell-to-cell variability in mRNA isolation as this step relied on capturing most of the cytosol into the patch-pipette. However, the total amount of mRNA obtained from a single neuron remains the limiting factor to have enough cDNA yield for a valid qRT-PCR. This was resolved by designing an RNA amplification step and the technique was validated on a stable Ltk- cell line expressing the Kv2.1 channel. Current density and Kv2.1 transcript quantity displayed a nice correlation (R^2 = 0.9) when the qRT-PCR essay was done in twofold and the data