Both substances displayed concentration dependent down-regulation of UcP2 expression induced by THs in neonatal rat cardiomyocytes. Because DHSB uncoupled the respiration of isolated rat heart mitochondria while limiting reactive oxygen species (ROS) formation, it may be affecting UcP2 expression in a feedback control fashion. However, SB does neither. Therefore we explored the possibility of both substances limiting TH uptake into cardiomyocytes. TH presence in cardiomyocytes was evaluated by mass-spectrometry and we did not observe any limitation to the uptake of hormones. Because TH actions are primarily mediated by nuclear thyroid receptors and their transcriptional activation, we used reporter plasmid system to assess the SB and DHSB effect on thyroid hormone receptor transcriptional activity in cardiomyocytes. Our data suggest that SB and DHSB modulation of TH-mediated UcP2 expression is not related to their antioxidant ability (DHSB) or lack thereof (SB). Rather both substances influence TH-related processes by affecting the TH-dependent signaling pathway with possible beneficial effects in hyperthyroid patients.
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3832-Pos

Uncoupling and Inward Migration of Subsarcolemmal Mitochondria in Rat Heart during Early Diabetes

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Myocyte loss is an established feature in hearts of both individuals with diabetes mellitus and in several animal models of the disease. Studies attribute this to an increase in apoptosis resulting from elevation in cytoplasmic cytochrome *c* and activation of caspase-3. To date, spatial, structural and functional changes subsarcolemmal mitochondria (SSM), which protect myocytes from circulating insults, undergo during early diabetes remains poorly characterized. Using the streptozotocin-induced diabetic rat model we show that after 5-6 weeks of diabetes, SSM disaggregate and migrate inwards. Diabetic SSM (dSSM) also exhibited increased biogenesis, were smaller with more compact cristae, possessed higher citrate synthase activity, produced more reactive oxygen species (ROS), increased interaction with sarcoplasmic reticulum (SR), and took up more Ca^{2+}. Atomic force microscopy also revealed that forty percent of dSSM also possessed a circumferential ''ribbon-like'' structure and 12% of these were leaky. dSSM also contained 65% less superoxide dismutase-I and 66% less connexin 43, a protein that regulates the activity of mitochondria K_{ATP} channels and opening of the mitochondrial permeability transition pore. Insulin-treatment blunted these changes. The inward migration of SSM during diabetes is likely to leave myocytes vulnerable to plasmalemmal Ca^{2+} spikes resulting from the barrage of circulating agonists. Persistent increases in ROS production and lower connexin 43 content are also likely to trigger leaking of dSSM and elevate cytoplasmic levels of cytochrome c and apoptosis-inducing factors. Thus, we propose that compensatory changes to ensure adequate ATP production and maintenance of ionic homeostasis during diabetes switches SSM from protecting myocytes to inducing their demise. (This work was funded in part by grants from NIH to WGM and KRB and AHA to MCZ).

3833-Pos

FGF21 and Pancreatic Islet Fatty Acid Metabolism

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Pancreatic islet β-cells maintain blood glucose through the regulated secretion of insulin. A rise in blood glucose stimulates β-cell production of NAD(P)H and increases the ATP/ADP ratio resulting in a cascade of events including closure of ATP-sensitive potassium channels, membrane depolarization, Ca^{2+}-influx, and insulin secretion. During the course of Type II diabetes, the glucose stimulated insulin response is dampened by glucose and lipid toxicity. It has recently been shown that the novel endocrine factor, FGF21, protects metabolically active tissues by regulating fatty acid metabolism. To test this effect in islet β-cells, we measured the levels of Acetyl-CoA carboxylase (ACC) in response to FGF21. ACC is an enzyme involved in the synthesis of malonyl-CoA, the substrate used in fatty acid synthesis and a regulator of fatty acid oxidation. We show that FGF21 causes an increase in ACC levels in βTC3 cells, a pancreatic islet β-cell line. We propose that this increase in ACC acts as a protective mechanism for maintaining β-cell sensitivity to glucose by lowering β-oxidation of fats for energy. To measure fatty acid metabolism in the islet, we will extend our biochemical studies of ACC to mouse islet tissue. Furthermore, we will examine mitochondrial metabolism of β-cells in the presence of fatty acid using two photon microscopy of NAD(P)H. More specifically, we will examine glucose-stimulated mitochondrial NAD(P)H response of β-cells under normal and high fat environments. Overall, these studies will determine whether metabolic changes in the β-cells occur under varying nutritional states and understand the effects of FGF21 regulation of ACC levels in controlling β-cell metabolism.

Computational Methods III

3834-Pos

A New Semi-Explicit Solvation Model: Fast Physics for Better Results

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Computational physicists, chemists, and biologists have a critical need for better models of water and aqueous solutions. We present an exciting new solvation model called Semi-Explicit Assembly, which combines the speed of the fastest continuum models available with the strong physical basis and discrete water treatment afforded by explicit solvent simulations. We base our model on several simple physical properties of water as a solvent, collected directly from explicit solvent simulations for individual atomic solutes. As a first test and application of our method, we compute solvation free energies based on dispersion and electrostatics. Our approach, which is purely physical and involves no fitting of parameters to data sets, executes as fast as the popular Generalized Born solvation model, but with substantially improved accuracy in prediction of experimental solvation free energies. Also, the structure of our model means that improvements in simulation forcefields will improve our results as well. All of this comes without any artificial parameter adjustments; our model's properties are the same as those used directly in molecular dynamics. Our model's energetic accuracy and detailed structural information have wide-ranging implications for molecular modeling research.

3835-Pos

Non-Linear Analysis of Voltage Clamp Data in the Investigation of Mechanisms of Inherited Arrhythmias

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Several mutations in genes encoding cardiac ion channels render the heart at high risk of incidence of life-threatening arrhythmias. Although the genes, loci, and phenotypes have been identified, the mechanism by which these mutations lead to fatal arrhythmias is still poorly understood. Progress on this problem requires a thorough quantification of the phenomena involved over multiple scales. An aspect of this is the precise quantification of membrane current kinetics. Here, we present a methodology that addresses this problem and apply it to the testing of a hypothesis on the initiation of abnormal beats in LQT2 and LQT3 syndromes.

We show that the traditional estimation of the functions of voltage composing the Hodgkin-Huxley model through non-linear least square fitting (NLLSF), has numerous limitations and present a novel non-linear method that overcomes these limitations.

An important result is the demonstration that we can determine a-priori whether the voltage clamp data fully constrains the model in a given voltage range. Then, based on voltage clamp data gathered in two complementary protocols, we can evaluate the voltage dependence of the steady state through a sequence of non-linear transformations, i.e. an inversion. The voltage dependence of the time constants is obtained by inverting the model at each data point and applying constraints as well as continuity criteria on the inverted solution. Importantly, we show how the methodology allows us to derive experimental protocols constraining the model; thus allowing us to thoroughly test our hypothesis.

In conclusion, we have presented a theory to perform a high quality non-linear analysis of voltage clamp data and applied it to provide credence to a plausible mechanism for the initiation of arrhythmias in LQT2 and LQT3 syndromes.

3836-Pos

Misty Mountain Clustering: Application to Fast Unsupervised Flow Cytometry Gating

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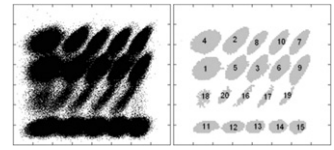
Flow cytometry experiments record, in individual cells, the fluorescent intensity of different fluorophores that correspond to features such as the levels of specific proteins. An assay typically generates a large number (order 10^6) of data points in a two or



higher dimensional space. The grouping of cells (data points) having similar features, which is referred to as gating, is usually done manually by an expert. We developed software that performs efficient unsupervised gating determining the number of clusters, and the points belonging to each cluster. The program analyses the cross-sections of the histogram created from the data points. The method is particularly efficient in the case of large number of data points such as 10^4-10^6. The overall run time for the composite steps of the algorithm increases linearly by the number of data points. In our example 1 million data points, shown in the left part of the figure, were analyzed within 6 seconds on a standard laptop PC. The analysis resulted in 20 clusters, shown in the right side of the figure. The code number of the largest cluster is 1, the second largest is 2, etc.

3837-Pos

An Investigation of Glutamic Acid 242 as a Proton Pump Valve in Bovine Cytochrome C Oxidase using QM/MM Monte Carlo Simulations

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Cytochrome c Oxidase (CcO) is a mitochondrial inner membrane protein which catalyzes the reduction of oxygen to water and utilizes the free energy of this reaction to pump protons across the membrane from a lower concentration of protons (N-side) to a higher concentration of protons (P-side). This generates an electrochemical proton gradient which is ultimately used by ATP synthase to convert ADP to ATP. A key question is how CcO is able to maintain unidirectional translocation of protons across the membrane in the presence of this gradient. Glutamic acid 242 (bovine numbering) is a conserved residue in CcO which is found in the X-ray crystal structure to be a physical connection for protons from the N-side to the P-side of the membrane. It is hypothesized that Glu242 acts as a proton pump valve by delivering protons in one direction and preventing the backflow of these protons through protonation state dependent changes in its conformation. A model of CcO has been developed and the conformation space of Glu242 has been sampled using Monte Carlo simulations with energies calculated using the ONIOM QM/MM method. These calculations suggest a mechanism by which Glu242 facilitates unidirectional pumping and the prevention of proton leakage.

3838-Pos

Ionic Effect on MD-SAXS Profile

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The combination of small-angle X-ray solution scattering (SAXS) experiment and molecular dynamics (MD) simulation is now becoming a powerful tool for studying protein structures in solution at an atomic resolution. Several studies have developed the calculation methods of SAXS profile from protein atomic structures, in which scattering from hydration structure around the protein was calculated using uniform density layer or explicit water molecules in the MD simulations. Although general SAXS experiments of protein solutions are carried out at certain ionic concentrations, in these calculations the effects of ionic strength on SAXS profile has not been considered explicitly. In this study, we investigate the effect of ionic strength on the SAXS profile by using the MD simulations of hen egg white lysozyme at various NaCl concentrations.

At 0 mM NaCl, the calculation of the SAXS profile converged completely within ~ 200 ps MD simulation, but at concentrations larger than 100 mM NaCl, the convergence was not obtained even with 10-ns simulation due to large density fluctuations in the bulk region. We also observed certain dependencies of SAXS profile on NaCl concentrations. These results indicate that MD simulation at large NaCl concentrations is a disadvantage in obtaining accurate SAXS profile. To accommodate this problem, we investigated the dependency of solvation structure around the protein on NaCl concentration, and based on the obtained information, have develop the new calculation method that incorporates the effect of ionic strength in SAXS profile calculation derived from the MD simulation at 0 mM NaCl.

3839-Pos

Parameterization of CB1 Negative Allosteric Modulators for CHARMM Molecular Dynamics

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Recently, several allosteric modulators of the Class A G-protein coupled receptor CB1 were discovered. Among these modulators are PSNCBAM-1 and ORG27569 which act as CB1 negative allosteric modulators (M. R. Price, et al. Molec. Pharm. 68, 1484 (2005) and J. G. Horswill, et al. British J. of Pharm. 152, 805 (2007)). Molecular dynamics simulations would be useful in elucidating the interactions between these ligands and the CB1 receptor. In order to utilize molecular dynamics, CHARMM force field parameters for these ligands are necessary. The parameters that have been developed for molecular dynamics simulations using the CHARMM force field have mainly focused on proteins, lipids, and nucleic acids and therefore do not encompass many small molecules. Only recently have researchers begun to expand these parameters to small molecules that have compositions that differ from the more biological groups (K. Vanommeslaeghe, et al. J. Comp. Chem. Early View 2009). In order to prepare these CB1 allosteric modulators for use in molecular dynamics simulations, novel parameters were developed for PSNCBAM-1 and ORG27569 by calculating new atom charge, angle, and dihedral parameters that could not be found in the recently developed CGenFF database, which encompasses more small molecules than the previous CHARMM databases. The methods used to develop these parameters, developed by the MacKerrell group (http://dogmans.umaryland.edu/~kenno/tutorial/#charges_qm), will be reviewed, and the results of the parameterization will be presented.

Regulatory Networks & Systems Biology

3840-Pos

A Systems Biology Approach to Understanding Alzheimer's Disease

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A mathematical model for Alzheimer's disease (AD) has been developed using a systems biology approach. A cellular network of neurons, microglia and astrocytes has been created to model the levels of beta amyloid in the brain. The production and spatial distribution of beta amyloid, the key protein implicated in AD, has been modeled using the reaction-diffusion equation, where reaction rates have been modeled using stochastic functions. Neurons have been modeled using a previously developed McCulloch-Pitts neural network (Butz 2006) modified to account for neuronal cell death and loss of synaptic elements during high beta amyloid levels. Microglia are either in the ramified state (at rest) and modeled using a continuous random walk model, or in the activated state (actively moving towards a source of beta amyloid) and modeled using the Langevin equation of motion. Astrocytes are defined to set locations and contribute to removal of beta amyloid from the brain interstitial fluid. The roles that local cerebral blood flow, transport across the BBB, and local reactions play have also been modeled. Future work will look at the development of amyloid beta plaques in the cerebrovasculature and brain parenchyma, and their relationship to observed decreases in cerebral blood flow as the disease progresses.

3841-Pos

A Ratchet Mechanism for Low-Frequency Hearing in Mammals

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The sensitivity and frequency selectivity of hearing result from tuned amplification by an active process in the mechanoreceptive hair cells. The nature of the active process in the mammalian cochlea is intensely debated, for outer hair cells exhibit two forms of mechanical activity, active hair-bundle motility and membrane-based electromotility. Here we show theoretically that active hair-bundle motility and electromotility can together implement an efficient mechanism for amplification that functions like a ratchet: sound-evoked forces acting on the basilar membrane are transmitted to the hair bundles while electromotility decouples the active hair-bundle forces from the basilar membrane. Through a combination of analytical and computational techniques we demonstrate that the ratchet mechanism can naturally account for a variety of unexplained experimental observations from low-frequency hearing.

3842-Pos

Model of the Drosophila Circadian Clock: Loop Regulation and Transcriptional Integration

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Circadian clocks influence key features of daily life including timing of sleep, awakening, and feeding. Eukaryoticcircadian clocks include interconnected positive and negative feedback loops. The CLOCK-CYCLE dimer (CLK-CYC) and its homolog, CLK-BMAL1, are key transcriptional activators of central components of the Drosophila and mammalian circadian networks, respectively. In Drosophila, negative loops include period-timeless and vrille; positive loops include par domain protein 1. Clockwork Orange (CWO) is