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

a recently discovered negative transcription factor with unusual effects on period, timeless, vrille, and par domain protein 1. To understand the actions of this protein, we introduced a new system of ordinary differential equations to model regulatory networks. The model is faithful in the sense that it replicates biological observations. CWO loop-actions elevate CLK-CYC; the transcription of direct targets responds by integrating opposing signals from CWO and CLK-CYC. CWO, a transcriptional repressor of direct targets in one-dimensional in vitro experiments, is actually a transcriptional activator in vivo. Loop regulation and integration of opposite transcriptional signals appear to be central mechanisms as they also explain paradoxical effects of period gain-of-function and null mutations.

3843-Pos

Cellular Dynamics of Embryomas within Adult Neoplasms

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During vertebrate life, a single cell will contain a single genome until cell death. This genome contains some genes for embryonic development, some genes for adult expression, and other genes which are used both in embryonic life and in adult life. Embryonic-exclusive genes are exquistively selected for complicated expression during organ formation before birth, and are under close supervision by embryonic regulator proteins and RNAs. After birth, the embryonic-exclusive genes are no longer needed, but they are retained in the inactive form within the cell until cell death. The embryonic regulator proteins and RNAs are then replaced by adult regulator proteins and RNAs. Thus, within the adult cell, the inactive embryonic-exclusive genes are left without embryonic regulator proteins and RNAs. In this open state, such embryonic-exclusive genes may again become active. The activation of as little as one embryo-exclusive gene within an adult cell is capable of initiating a new neoplasm within that cell. Such neoplasms are termed embryomas, and as they divide and recruit other embryonic genes to regain similar activity, the new neoplasm will progress to kill the host animal. The dynamics of such embryoma activation and progression is often as explosive, cumulative, and destructive as an avalanche in character. Recently it has been found that providing the missing embryonic regulatory micro RNAs, either *in vitro* or in *vivo*, will reduce the activity of such embryomas, without apparent toxic effects on the subject. Taulli R, Bersani F, Foglizzo V, Linari A, Vigna E, Ladanyi M, Tuschl T, and Ponzetto C, ''The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation'', J. Clin. Investigation. 119: (8), 2366-2378 (Aug. 2009).
http://www.embryomas.net

3844-Pos

Analytic Parameter Fitting in Stochastic Stem Cell Models

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Pluripotent stem cells produce all of the body's diverse cell types though lineages of cell division and differentiation. One goal of current stem cell research is to create predictive models of cell development hierarchies to validate our understanding of these hierarchies and to guide the design of artificial lineages to produce specific cell types for medical purposes. Stochastic models have been used to study stem cell lineages for nearly 50 years, but a continuing challenge is how to fit the parameters of complex models to noisy experimental cell population data. We have developed a technique that addresses this problem by creating algorithms to automatically generate exact analytical expressions for the probability distributions of different cell types at each successive generation as a function of the model parameters. These expressions can be used with conventional optimizers to find the best-fit model parameters. Although the analytic expressions grow exponentially for complex differentiation hierarchies, the resulting equations are manageable out to the number of generations typically used in experiments. We have tested our parameter fitting strategy using, as ''experimental'' data, Monte Carlo simulations of a three-parameter stochastic model we have developed for T-cell formation from lymphocyte progenitor cells. We organized the Monte Carlo results to mimic two possible experimental protocols. One mimicked replicate measurements of cell differentiation starting with single stems cells, and the other mimicked measurements starting with pooled groups of cells. Our approach yielded good convergence to the stochastic model parameters, even for relatively small numbers of starting cells, showing that this should be practical for fitting models to experimental data. Additionally, our results show that data from replicate single cell experiments allow more reliable model fitting than pooled experiments using the same number of initial stem cells.

3845-Pos

The Oscillatory Rhythmic Activity of Retinal Ganglion Cell Spikes Might Be Induced by Slow Wave Component in *rd1* Mice Retina

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The abnormal rhythmic activities with ~10 Hz frequency were reported not only from field potential (slow wave component) but also from spontaneous spikes of retinal ganglion cells (RGCs) in *rd1* mice. However, there have been only a few studies on the electrically stimulated RGCs of *rd1* mice, and none of them have mentioned the effect of the abnormal rhythmic activity. Therefore, in this study we focused on the mechanism of oscillatory rhythm in RGC spikes to clarify if RGC responses are well evoked with the current stimulus even with this aberrant rhythm. Extracellular recording of *in vitro* retina was performed using 8x8 multi-electrode array (MEA).Biphasic current pulse trains were applied to one channel of MEA and neural activities of RGCs were recorded from the other channels of MEA. The raw waveforms were separated into field potentials and spike train waveforms using low- and high-pass filtering. Typical RGC responses to current stimulus showed multiple peaks with inter-peak intervals of ~100 ms in PSTH. When treated with CNQX + AP7 or strychnine or SCH23390, the frequency of oscillatory rhythm in RGC spikes decreased from ~10 Hz to ~5 Hz. While neither picrotoxin nor gap junction blockers affected the frequency of oscillatory rhythm. All the blockers showed exactly same effects on the oscillatory rhythm in field potential with that in RGC spikes. This strongly suggests that slow wave component might induce the oscillatory rhythm of RGC spikes. With current amplitude modulation from 2-60 uA, the numbers of evoked RGC spikes increased as a function of pulse amplitude, which means that RGC responses are well evoked with current stimulus even with aberrant oscillatory rhythm in *rd1* mice.

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3846-Pos

An Accelerated Algorithm for Stochastic Simulation of Reaction-Diffusion Systems using Gradient-Based Diffusion and Unified Tau-Leaping

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Stochastic simulation of reaction-diffusion systems enables the investigation of stochastic events arising from the small numbers and heterogeneous distribution of molecular species in biological cells. Stochastic variations in intracellular microdomains and in diffusional gradients that are especially prominent in neurites with elongated morphology play a significant part in the spatiotemporal activity and behavior of cells. Although an exact stochastic simulation that simulates every individual reaction and diffusion event occurring in the system gives a most accurate trajectory of the system's state over time, it can be too slow for many practical applications. We present an accelerated algorithm for discrete stochastic simulation of reaction-diffusion systems designed to improve the speed of simulation by reducing the number of time steps required to complete a simulation run. Our method is unique in that it employs two strategies that have not been incorporated in existing spatial stochastic simulation algorithms. First, we treat diffusive transfers between neighboring subvolumes based on concentration gradients. Our treatment necessitates sampling of only the net or observed diffusion events from higher to lower concentration gradients rather than sampling all diffusion events regardless of local concentration gradients. Second, we extend the non-negative Poisson tau-leaping method that was originally developed for speeding up non-spatial or homogeneous stochastic simulation algorithms. Our method calculates each leap time in a unified step for both reaction and diffusion processes while satisfying the leap condition that the propensities do not change appreciably during the leap and ensuring that leaping does not cause molecular populations to become negative. We also present numerical results that illustrate the improvement in simulation speed achieved by incorporating these two new strategies.