fluoroproteins of interest for imaging suggests that this coupling is a quite general mechanism for photoswitchable proteins. These insights will also guide the rational design and optimization of photoswitchable proteins.

Computational Methods, Cellular - IV

2454-Pos Modeling Diffusion In 3-dimensions In Brain Extracellular Space: Comparison With Microfiberoptic Photobleaching Measurements

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Board B568

Diffusion through the extracellular space (ECS) in brain is important in drug delivery, intercellular communication, and extracellular ionic buffering. The ECS comprises ~20% of brain parenchymal volume, and contains cell-cell gaps down to ~50 nm. We developed a random-walk model of macromolecule diffusion in brain ECS in 3-dimensions using realistic ECS dimensions. Model inputs included ECS volume fraction (α), cell size, cell-cell gap geometry, intercellular 'lake' dimensions, and the molecular size of the diffusing solute. The model output was relative solute diffusion in water vs. brain ECS (D_o/D). Experimental D_o/D was measured using a microfiberoptic fluorescence photobleaching method involving stereotaxic insertion of a micron-size fiber into mouse brain. Measured D_o/D for the small solute calcein was 3.3 ± 0.1 in brain cortex and 4.1 ± 0.4 in cerebellum. D_o/D was size-dependent, with 5.6 ± 1.1 fold slowing in deep brain nuclei for 20 kDa FITC dextran. Moderate water intoxication (3% body weight) increased D_o/D from 3.4 ± 0.3 to 4.0 ± 0.1 at 10 min and 4.8 ± 0.3 at 20 min. Simulation of measured D_o/D using realistic α, cell size and cell-cell gap required the presence of intercellular 'lakes' at multi-cell contact points and cell-cell contact length of at least 50-fold smaller than cell size. With ECS geometric parameters fixed, the model predicted D_o/D for different solute sizes and cell-cell gap heterogeneity. The effect of cell swelling on ECS geometry was accurately simulated by altering the input parameters, revealing the importance of cell-cell gaps for diffusion through brain ECS. Our model establishes the geometric constraints to account quantitatively for the relatively modest slowing of solute and macromolecule diffusion in brain ECS.

2455-Pos Towards Realistic Modeling Of Dynamic Processes In Cell Signaling: Quantification Of Macromolecular Crowding Effects In Several Model Systems

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Board B569

One of the major factors distinguishing molecular processes in vivo from biochemical experiments in vitro is the effect of the environment produced by macromolecular crowding in the cell. To achieve a realistic modeling of processes in the living cell based on biochemical data it becomes necessary, therefore, to consider such effects. We describe a protocol based on Brownian dynamics (BD) simulation to characterize and quantify the effect of various forms of crowding on diffusion and bimolecular association in a simple model of interacting hard spheres. We show that by combining the elastic collision method for hard spheres and the mean field approach for hydrodynamic interaction (HI), our simulations capture the correct dynamics of a monodisperse system. The contributions from excluded volume effect and HI to the crowding effect are thus quantified. The dependence of the results on size distribution of each component in the system is illustrated, and the approach is applied as well to the crowding effect on electrostatic-driven association in both neutral and charged environment; values for effective diffusion constants and association rates are obtained for the specific conditions. The effects of crowding on both diffusion-limited and chemistry-limited reactions can be quantified through this protocol based on BD simulations. To investigate the contributions of other features of proteins, beyond the hard sphere, to the macromolecular crowding, a coarse-grained model has been incorporated into the simulation protocol. The quantitative results from these simulation approaches can be incorporated directly in the modeling of cell signaling to improve them without significant computational burdens.

2456-Pos Super-Diffusion in a Model for Diffusion in a Molecularly Crowded Environment

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Board B570

We present a model for diffusion in a molecularly crowded environment. The model consists of random barriers in percolation network. Random walks in the presence of slowly moving barriers show normal diffusion for long times, but anomalous diffusion at intermediate times. The effective exponents for square distance versus time usually are below one at these intermediate times, but can be also larger than one for high barrier concentrations. Thus we observe sub- as well as super-diffusion in a crowded environment.

2457-Pos The Effect of Crowding on Rod Ordering

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Board B571

Macromolecules occupy 20–40% of a cell's total volume, making a cell's interior densely packed. Crowding shifts the equilibrium state of many macromolecules. An important driving force behind this effect is the minimization of steric interactions, as determined by the size, shape, and density of the molecules. Macromolecules can be separated into two shape classes: rods (ex. cytoskeletal filaments) and spheres (ex. globular proteins). Here, we investigate crowding and confinement in mixtures of rods and spheres in a model experimental granular system and with Monte Carlo simulations. We find that crowding promotes rod patterning. In a pure rod system, crowding induces a transition from a disordered to an orientationally ordered (nematic) state. The inclusion of spheres destabilizes this nematic state, and small independent clusters of rods form, instead. As both rod and sphere densities increase, these aggregates coalesce to form specific structures that resemble polymers.

2458-Pos Time evolution of multicellular structures studied by the Kinetic Monte Carlo method

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Board B572

Metropolis Monte Carlo (MMC) simulations of tissue morphogenesis proved successful in predicting long-lived structures (of lowest adhesion energy) of complex multicellular systems. A serious drawback of the MMC method is that it cannot provide information on how these long-lived structures are arrived at in time. To address this problem, we propose a kinetic Monte Carlo (KMC) approach, in which a transition rate is associated with each possible rearrangement of the system. We represent the system on a three-dimensional hexagonal close-packed lattice with sites occupied either by cells or by volume elements of cell culture medium. Rates are associated to swapping cells with nearest neighbors of different types (cells or medium). The time evolution is expressed in terms of these rates. We first qualitatively tested the new approach against experiments and MMC simulations of cell aggregate fusion and cell sorting within an aggregate composed of two cell types with differential adhesion. For quantitative studies we determined the time evolution of the interfacial area between two fusing spherical cell aggregates experimentally, analytically and by KMC simulations. Experiments involved spheroids of smooth muscle cells. In the analytic study we applied continuum hydrodynamics to describe the coalescence of two identical, highly viscous liquid droplets, which gave good agreement with experiments on cell aggregates. Apart from the early stages of fusion, the KMC method predicted a fusion pattern similar to the continuum hydrodynamics predictions and the experiments. Comparison with experiments allowed relating KMC transition rates to measurable time scales. We conclude that the KMC method may account for the time evolution of complex cellular structures, and thus be used for tissue engineering applications.

Work supported by NSF-056854.

2459-Pos Modeling Some Biological Processes Through Cellular Automata

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Board B573

Cellular automata are discrete or continuous dynamical systems studied in computability theory, mathematics, physic, chemical, artificial life and biology.

It consists of an infinite, regular grid of cells, each one cell take an element from a finite set of states. Cellular automata can evolve in one, two, three or any dimension over time. The local function have a relation between a central cell and their neighbors, then each one of them represent a neighborhood that determine a relation with an element of finite set of states. All possible relations produce the specific local function. Thus, we can calculate the history of the evolution, each time or generation represent a global state of our system. Where we can see the dynamic of the function. In this way, all cells are updated to the local function in parallel each time.

We study two-dimensional binary-state cellular automata case, where every cell take two states: 0 (dead) or 1 (alive), and updates its state depending on sum of states of its 8 closest neighbors as follows. In individual we studied a special complex cellular automaton called "Diffusion Rule". The local function working in the following way: cell in state 0 takes state 1 if there are exactly two neighbors in state 1, in otherwise the cell remains in state 0. Cell in state 1 remains in state 1 if there are exactly seven neighbors in state 1, in otherwise the cell switches to state 0. Complex cellular automaton governed by such local transition rule exhibits reaction-diffusion like patterns, stationary or mobiles particles, particle-guns and chaotic behavior.

Using Diffusion Rule we can generate a dynamical patterns or cellular reactions, like turn on/off light with alive o dead cells that shows a luminescence. Examples include fluorescence, bioluminescence and phosphorescence.

2460-Pos Simulation And Modeling Of Reaction Kinetics In Crowded Media

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Board B574

Reaction kinetics in intracellular environments is characterized by the effects of molecular crowding. These effects can be modeled within the framework of standard time-dependent kinetic equations when the law of mass action is appropriately modified. Two models have been proposed: the model ST [1] based on time-dependent association rate coefficient previously introduced by Kopelman and collaborators [2], and the model S based on the power law approxi-

mation [3]. To evaluate these models we have simulated reaction progress curves for A+B ↔ C reaction. Software was developed to run 2D lattice based simulations as previously described in [4] and 2D off-lattice simulations as described in [5].

The comparison of fits to simulated progress curves by S and ST models showed that in some cases the S model was preferred, while in the other cases the ST model was preferred. As previously observed [6], the ST model predicts a maximum in [C](t) at some finite time and an asymptotic steady-state approaching [C]=0. Such a behavior was not observed in simulated progress curves. We proposed a modification of the ST model in which both the association rate and dissociation rate coefficients share the same time-dependent factor. This model has an analytical solution for the considered reaction and predicts the classical shape for [C](t). It provides the best fits to most of the simulated progress curves.

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2461-Pos Self-assembly of Artificial Peptides

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Board B575

The self-assembly of β-peptides molecules (in which the backbone has an additional carbon atom compared to natural peptides) into nano-structures is studied using computer simulations. Each peptide forms a stable helix in solution and the self-assembly into larger scale objects is very sensitive to the nature and sequence of the side chains. Experiments [Pomerantz, W. C et. al., JACS, *128*, 8730 (2006)] have shown that some sequences self-assemble into long hollow cylinders, which further assemble into a liquid-crystalline mesophase. The self-assembly into cylinders is sensitive not only to the side chains but also to their arrangement. We employ a minimal model where each helix is represented as a short "shish-kabob" rod with interaction sites at the positions of the side chains. We conduct Monte Carlo (MC) simulations with a cleaving algorithm and cluster moves that allow us to simulate the self-assembly on large length-scales. The simulations show a sequence dependence of self-assembly consistent with experiments. Interestingly, it is found that not only the sequence of the interaction type (hydrophilic or hydrophobic) but also the range of interaction sites plays a significant role in determining the nature of the self-assembled structure. The model is used to predict the behavior of the peptides at solid surfaces.

2462-Pos Kinetic model and Monte Carlo simulations of in vitro platelet aggregation

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Board B576

Platelets are anuclear, disk shaped cells that circulate in the blood. They are deployed to sites of blood vessel injury, where they elicit blood clot formation by adhesion to the site of injury and subsequent aggregation. Low platelet concentration favors bleeding, whereas high platelet concentration, such as that observed in the myeloproliferative disorder, favors thrombosis. A method for diagnosing platelet function defects is platelet aggregometry. It is based on monitoring the evolution of the extinction of platelet-rich plasma during the progress of platelet aggregation triggered by agonists, such as ADP, epinephrine, collagen, arachidonic acid or ristocetin. We propose a novel kinetic model of in vitro platelet aggregation in platelet-rich plasma, and use it as a basis for Kinetic Monte Carlo (KMC) simulations. The kinetic model is nonlinear, since it takes into account that only cells on aggregate surfaces have a chance for deaggregation, but it is simple enough to allow for parameter estimation by fitting experimental data. We solved the system of differential equations numerically, and used the nonlinear least squares method to fit the results of optical density measurements. The time evolution of the system was also simulated by the KMC method implemented in a three-dimensional, hexagonal, close-packed lattice model of platelets in suspension. Lattice sites were attributed either to individual platelets or to volume elements of plasma. Movements were described by associating rates to possible movements of platelets. The simulations yielded the time course of the concentration of single platelets in the plasma along with the aggregate size distribution. Our study suggests that kinetic and KMC model parameters may be used to quantify platelet function in various hematological conditions.

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2463-Pos Torsional Moments and Flexural Vibration Effects on Glass Microneedles during Myofibril Kinetic Force Measurements (a finite element assessment)

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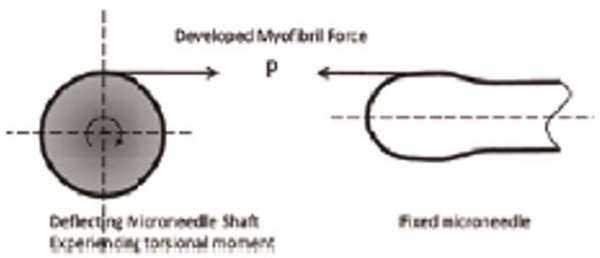
Board B577

In muscle kinetic measurements, the spring constant of the glass microneedle force probe, which relates the applied normal force to the normal deflection, is required. However in single myofibril applications a resultant lateral force is applied to the microneedle

shaft which has a tapered [1.6–8.0°], bent [90°], cylindrical geometry, making it difficult to describe by a simple set of mathematical equations. We use finite element methods to model the microneedles, validate their normal tension to applied force, and investigate their torsional spring constants which relate the applied torque to the angle of twist. Our simulations reveal a significant percentage of the force [from 0 – 37%] transferred into torsional moments, for our range of microneedles used, which is not reflected in the normal deflection observed. We also investigate the flexural vibration of these microneedles in different viscous fluids to quantify any related torsional vibration during kinetic measurements. We conclude that our model more reliably account for such dissipation, and propose a correctional utility for the torsional moments and flexural vibration that reflect a more saccurate measurement of kinetic force during single myofibril studies.



2463.01-Pos Computational Model for Cell Pattern Development and Tissue Growth

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Board B577.01

We develop a model for simulating dynamic pattern formation of cell populations. Unlike cellular automata which provide only caricatures of cell pattern formation, we model the shape and size of cells more realistically. Based on physical adhesive and tension forces experienced by cell walls, our model can also include other forces (e.g., concentration gradient, isotropic/anisotropic cell-cell adhesion). Our model parameters are determined by pathway-, cell-type, and/or tissue- specific biology. Cell population can be either homogeneous or heterogeneous. The absence/presence of arbitrary boundary conditions and initial conditions such as different cell distributions can also be enforced. At discrete time step, cells can grow, divide, differentiate, die, or experience changes in mechanical properties or environment. Cell growth from single or multiple locations, either in synchronized or asynchronized modes, can also be modeled. Our model is driven by a geometric engine, which accurately models primitive topological changes and cell rearrangements occurring when cells migrate, divide, insert, or die out. We give examples of studies on the role of directional cell-cell inhibition in cellular pattern transition. Our method can be used for studying embryo morphogenesis, cell differentiation, cell migration, tissue deformation, wound healing, as well as patterning of in silico tissue assembly.

2463.02-Pos Cellular Particle Dynamics Simulation of the Uniaxial Compression of Cellular Aggregates

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Board B577.02

Embryonic tissues and cellular aggregates are complex visco-elastic materials. While on a time scale shorter than their characteristic relaxation time \tau they behave as visco-elastic solids, on a time time scale longer than \tau they flow like highly viscous fluids. The relaxation time is determined by the viscosity (\eta) and the shear stress modulus (G) of the tissue/cell aggregate, i.e., \tau = = \eta/G. In this study we employ the recently proposed cellular particle dynamics (CPD) computer simulation method to investigate the visco-elastic response of a spheroidal tissue/cellular aggregate compressed between to parallel pressure plates. In CPD living cells are represented as ensembles of cellular particles (CPs), which interact via short ranged contact forces. Results of the CPD simulations are compared with the available experimental data. A detailed and systematic evaluation of the compression force relaxation obtained in the CPD simulations and experiments is provided in terms of a continuum theoretical model. This approach allows us to (1) estimate \eta, G and the surface tension \gamma of the compressed tissue/cell aggregate from the experimental data, and (2) relate these tissue level properties to the cellular level model parameters used in the CPD simulation. Once the CPD parameters have been determined, the dynamics of more complicated multicellular systems can be simulated and predicted for experimental validation.

This work was supported by the National Science Foundation [FIBR-0526854]. Computer time was provided by the Univ. of Missouri Bioinformatics Consortium.

2463.03-Pos Coupled Composition-Deformation Phase-Field Method for Biological Membranes

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Board B577.03

The plasma membrane, a lipid bilayer membrane surrounding all mammalian cells, is not homogeneous, but rather contains domains termed ‘rafts’, defined as regions enriched with cholesterol and saturated lipids. Understanding how and why these rafts form is of great importance to cell biologists and immunologists, since they are involved in many important cell functions and processes including endocytosis, cell adhesion, signaling, protein organization, lipid regulation, and infection by pathogens. These raft structures also show great potential for technological applications, especially in connection with biosensors and drug delivery systems. We present a method for modeling phase transitions and morphological evolution

of binary lipid membranes with planar and spherical background geometries. The local composition and the shape of the membranes are coupled through composition-dependent spontaneous curvature and bending rigidity in a Helfrich free energy. The evolution of the composition field is described by a Cahn-Hilliard-type equation, while shape changes are described by relaxation dynamics. Our method treats explicitly the full nonlinear form of the geometrical scalars, tensors, and differential operators associated with the curved shape of the membrane. The method is applied to investigate the dynamics of systems that have a lamellar structure as their lowest energy state. For the planar case, microstructures are characterized quantitatively using average domain size, shape anisotropy, and other statistical measurements. We find that evolution is very sensitive to initial conditions; only membranes with sufficiently large lamellar-type composition or shape perturbations in their initial configuration can deterministically evolve into a lamellar equilibrium morphology.

2463.04-Pos Ionic Wave Propagation and Processing Over Arbitrary Topologies of Multicellular Networks

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Board B577.04

Standing and traveling wave patterns are common occurances in biology over a wide range of scales [1]. Calcium waves, in particular, have been extensively studied *in vivo* and *in vitro* due to the richness in variety of patterns that they exhibit [2]. We developed a unified method based on Turing's *reaction-diffusion* mechanism to analytically track the spatiotemporal variations in a cell's biochemical constituents across a multicellular network of arbitrary shape [3]. As the network develops from an initial infinite length chain of cells into an intricate branching topology, the eigenvalues and eigenvectors of the resulting cellular connectivity matrix are computed using the Interface Response Theory (IRT). Assuming a fixed subcellular template of linear *activation-inhibition* interaction between two hypothetical biochemicals, traveling plane waves can form over the infinite backbone. Adding a side chain of finite length *L* leads to standing waves upstream from the contact site and traveling waves downstream with *L* distinct spatial frequencies filtered out. The time response of a finite length pulse, *i.e.* elevated concentration levels over a finite segment of the backbone, can be tracked by first approximating the pulse with a finite sum of fundamental planar waves (*Fourier decomposition*), computing the time progress due to each by using the presented method and finally summing the individual responses (*superposition*). Our method is directly applicable in modeling intercellular calcium waves when the intracellular nonlinear interactions effecting calcium concentrations are approximated by piecewise linear functions [4].

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2463.05-Pos Computational modeling of mitochondrial Ca ion flux via the Ca uniporter: Modulation by isoflurane

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Board B577.05

Background: We applied hypothesis-driven computational models to analyze and quantify experimental data on mitochondrial (m) Ca^{2+} handling by a volatile anesthetic, isoflurane. We assumed that isoflurane alters mitochondrial respiratory complex and cation channel and exchanger function in a manner that can be modeled and used to predict changes. Our biophysical models, based on detailed and validated catalytic and transport mechanisms, rely on iterations between experimental measurements and model development.

Methods: We used indo 1 fluorescence to measure [mCa^{2+}] in guinea pig heart mitochondria suspended in Ca- free, 2.5 mM EGTA buffer and respiring on Na-pyruvate. Adding ADP transiently increased [mCa^{2+}]. Adding $CaCl_2$ (0.5–2 mM) rapidly increased [mCa^{2+}] by 25–500 nM; this was blocked by ruthenium red, indicating all uptake was by the Ca uniporter (CaU). 1.5–2 mM isoflurane further increased [mCa^{2+}] by 50–100 nM.

Results: Computational modeling of the ADP data suggested dynamic mCa^{2+} buffering due to Ca^{2+} binding with ATP, ADP, P_i, and other TCA intermediates. By accounting for a Ca^{2+} regulatory mechanism in which increased [mCa^{2+}] inhibits CaU activity (non-competitive); $X'_{CaU} = X_{CaU}{}^{\bullet}K_{mi}{}^2/(K_{mi}{}^2 + [mCa^{2+}]^2)$; $J_{CaU} = X'_{CaU}{}^{\bullet}f([mCa^{2+}], [eCa^{2+}], \Delta\Theta_m))$, the model fit the rise in [mCa^{2+}] by $CaCl_2$, and by activating the CaU, also well fit the increase in [mCa^{2+}] by isoflurane.

Conclusion: By including in our model a biophysical mechanism for Ca^{2+} transport into mitochondria, we can analyze and interpret data on direct effects of isoflurane on Ca-induced mCa^{2+} uptake.

2463.06-Pos A Universal Approach for Global Fitting of Experiments of Different Types to a Computational Model of a System

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Board B577.06

Fitting experimental data into a computational model of a system allows to verify that the model is consistent with all experimental

observations, to estimate the parameters and their confidence intervals. Implementation of the fitting routine involves coordinating flows of data between model, experimental conditions, observations, optimization engine, model parameters, constraints, and other entities. The data logistics becomes complicated when many different experiments are fit to a multi-parameter model. Currently this appears to be the main obstacle for building more accurate models of biological systems. Since patterns of the data flow are largely independent of the system being modeled, we have developed *gfit* (http://gfit.sf.net), an open-source software for model-based regression analysis. Computational models used by *gfit* may implement any algorithm for simulating experiments performed with the system. By reading the meta-model (description associated with the model), *gfit* discovers how to execute the model and how to interpret the experimental data. The language of meta-model is simple, but flexible enough to accommodate complex experiments as well as rule-based models. Our approach has been used for creating models and for global analysis of data from many different sources including rapid stopped-flow and quench-flow, fluorescence correlation spectroscopy, and surface plasmon resonance. Here we present analysis of nuclear transport, a system composed of many proteins engaged in a complex network of interactions. Although nuclear transport has been extensively studied, the large number of unknown parameters precludes validation of the model by any single experiment or even by many experiments of any single type. We achieve greater confidence in the model and more accurate estimates of parameters by globally fitting many live-cell measurements of equilibrium distribution, uptake kinetics, and mobility of the nuclear transport proteins.

2463.07-Pos The Octagonal Shape of Nuclear Pore Complex

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Board B577.07

The basic structure of the nuclear pore complex (NPC), conserved across almost all organisms from yeast to human, persists in featuring an octagonal symmetry involving the nucleoporins that constitute the NPC ring. In this project, we seek to understand and evaluate the potential biomechanical reasons for this 8-fold symmetry. Our theoretical and computational analysis shows that the octagonal shape maximizes the bending stiffness of each of the eight NPC spokes; suggesting that the high flexural stiffness of the vertical spokes facilitates the normal functioning of the NPC and contributes to the symmetry of the NPC. While analytical methods show that the octagonal shape maximizes the bending stiffness, numerical methods compute the most likely modes of NPC deformation and kinetic energies of the NPC. These modes have energies close to other published findings using membrane analysis of the nuclear membrane pore opening and deformation states that agree with experimental observations.

2463.08-Pos Modeling Field Induced Transmembrane Potentials in Cellular Organelles

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Board B577.08

We have developed both analytical and finite element models of the potential differences induced across plasma and internal organelle membranes in suspended cells exposed to oscillatory electric fields. The analytical model uses an iterative application of the single shell solution with mobile charges. Complex cell geometries are modeled using the finite element method. Frequency-dependent transmembrane potentials are calculated for weakly conducting membrane shells enclosing a conductive cytoplasm surrounding internal organelles. This is motivated, in part, by recent results suggesting the ability to use ac fields to non-invasively monitor enzyme activity within internal membranes including mitochondrial and chloroplast electron transport chains.

2463.09-Pos Normal Mode Flexible Fitting of High-Resolution Structures of Biological Molecules Toward Fluorescence Resonance Energy Transfer (FRET) Data

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Board B577.09

Conformational dynamics is important for protein function. Biological systems can indeed adopt several conformations but rarely all of these conformations are known. However, using appropriate computational methods one can predict those conformations using low-resolution experimental data and a high-resolution structure. Normal mode flexible fitting (NMFF) was developed for that purpose [Tama, F., Miyashita, O. and Brooks III, C.L. J. Mol. Biol., *337*: 985–999, 2004]. In NMFF a known initial high-resolution structure is deformed to fit into target low-resolution data obtained from cryo-electron microscopy experiments. It uses a linear combination of low-frequency normal modes in an iterative manner to deform the structure and it utilizes gradient following techniques (steepest descent method) to locally optimize the correlation coefficient between computed and measured electron density. In this work we present an extension of NMFF toward using experimental data derived from FRET experiments. Such experiments yield distances between pairs of atoms that can be used as constraints for fitting. In this case a scoring function (SF) between target distance constraints (DCs) and simulated, intermediate distances is optimized (via steepest descent). This SF is evaluated at each iteration step. The form of this SF is presented and we show test results for Adenylate Kinase, LAO binding protein, Maltodextrin

binding protein and Elongation Factor 2. In these test cases simulated DCs are used for the target. Having successfully tested the methodology, application to the ribosome for which experimental FRET data are available [R. Hickerson, Z.K. Majumdar, A. Baucom, R.M. Clegg, and H.F. Noller. J. Mol. Biol., *354*: 459–472, 2005], is presented. We compare results obtained by our approach with those from experiment.

2463.10-Pos Visualization of Axon Conduction Failure at a Node of Ranvier in a Multi-scale Computational Model

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Board B577.10

The dysfunction of action potential (AP) conduction in myelinated axons is implicated in many human neuropathies and motor neuron diseases. Understanding the causes of conduction failure will require an understanding of axonal electrophysiology over a range of scales. Accordingly, we have developed a multi-scale simulation method for electrodynamic function. Our approach couples a one-dimensional cell-scale model based on cable equations using the NEURON simulator (Hines and Carnevale, 2001). to a three-dimensional (3D) electro-diffusion simulator (Lopreore et al., in press). This method utilizes finite volume techniques to solve the Nernst-Planck equations and evaluate ion fluxes within a Delaunay/Voronoi 3D mesh. The NEURON model included a soma, axon hillock and initial segment followed by 75 myelin-node segments. Single-node dimensions used in the model were derived from serial EM tomographic reconstruction of a peripheral sensory fiber node of Ranvier (Sosinsky et al., 2005). The placement and densities of specific voltage-gated sodium and potassium channels followed laboratory findings. Variables examined for contributions to conduction failure included the densities of ion channels in nodal compartments and demyelinated segments, internodal distance, changes in the equilibrium potential for K+, extra-nodal volume and rate of K+ clearance from the nodal region. Voltage wave-forms produced by the NEURON model under normal and compromised conditions were injected into the 3D electrodiffusion for evaluation of ion fluxes within subcellular micro- and nano-domains.

Supported by HHMI, NIH 1 P20 EB001432-01.

2463.11-Pos Universal Analysis Program for Multifrequency Phase and Modulation Fluorometers Facilitates Analysis with Fast ms-Kinetics for Mixing and Stop-Flow Applications

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HORIBA Jobin Yvon, Edison, NJ, USA.

Board B577.11

The recent advent of fast multifrequency phase and modulation fluorometers has enabled researchers to gather information on multi exponential pico- to nanosecond decay samples with millisecond per time point acquisition rates. Solving the resulting problem set requires a large scale analysis program that is able to handle data sets with hundreds to thousands of time-point files. This poster outlines our current 'universal' analysis program features and functionality. The current 'universal' program is a significant breakthrough because the state of the art 'global' analysis programs are normally software-limited to 50 data point files or less. Further, current global analysis programs are not designed to deal with concerted data sets but rather treat each multifrequency time point as a separate file requiring time-consuming manual loading, editing and parameterization of the data files prior to fitting. The current program is designed especially to accommodate large concerted data files with a conservative and convenient file loading and parameterization algorithm. A further breakthrough in the current program is the ability to link and solve the data sets using analytical functions that greatly reduce the number of free fitting parameters and solving time. Solving times in general are less than 1 minute for up to 500 time points. The program allows the user to test multiple models for the same data set using a convenient scenario function. The report generator yields a fully featured Excel workbook that can be opened and graphed with a specially designed presentation and analysis macro. In summary, the program facilitates picosecond to nanosecond time resolution of up to 5 exponential decay component fractions and lifetimes which allows, for the first time, convenient access to millisecond stop flow, mixing and even chromatographic analysis.

2463.12-Pos Pattern Recognition Informed Feedback for Channel Current Cheminformatics

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Pattern recognition-informed (PRI) feedback using channel current cheminformatics software and LabWindows control software has been reported previously. The accuracy of the PRI classification was shown to inherit the high accuracy of the off-line classifier. For the

molecular blockades studied here, the accuracy inherited is 99.9% to distinguish between terminal base pairs of two DNA hairpins. The pattern recognition software consists of Hidden Markov Model (HMM) feature extraction software, and Support Vector Machine (SVM) classification/clustering software, that is optimized for data acquired on a nanopore channel detection system. The PRI-feedback system is based on a server running a LabWindows protocol that is set up to control the amplifier settings that eject a molecule from the channel if the SVM determined confidence level is not high enough to accept the signal. The control server takes signal information and broadcasts it via TCP/IP to a collection of compute nodes to do the HMM and SVM computations, and decision-making, in a distributed fashion, to stay within the time-frame of the signal acquisition in-process – ending that process if identified as not in the desired subset of events, or once recognized as non-diagnostic in general.

Due to purification limitations and fragmentation of antibodies and some antigens, it is time consuming to acquire the required subset of signals during analysis and for biosensing with a passive, uninformed (or manual), sampling capability. The advantage of PRI data acquisition for an antibody-antigen system is due to the reduction in wasted observation time on eventually rejected "junk" (non-diagnostic) signals. The use of PRI feedback for nanopore channel data collection reduces the amount of time required to acquire data, using fixed objective criteria, and thus, considerably improves the quantity and quality of the data analysis.

2463.13-Pos Using a Small Number of Cryo-EM Particle Images to Build an Icosahedral Density Map at Subnanometer Resolution

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Several thousand cryo-EM particle images are now typically cited for generating a 3-D icosahedral reconstruction at subnanometer resolution. To achieve the same resolution, theoretically, only a few evenly sampled particles are needed. We developed Multi-Path Simulated Annealing, a Monte Carlo type of optimization algorithm, for globally and simultaneously searching the center and orientation parameters of each particle. This method avoids error propagation and model bias. A consistency criterion was derived to exclude particles in the iterative refinement process. Following this procedure with a new empirical double threshold particle selection method, we are able to pick small number of best quality particles to reconstruct a subnanometer resolution map without any model as a template. Using the best 62 particles of rice dwarf virus, the reconstruction reached 9.6Å resolution at which 4 helices of the inner capsid protein are resolved. Furthermore, with the 284 best particles, the reconstruction is improved to 7.9Å resolution, and 21 of 22 helices and 6 of 7 beta sheets are resolved. The relationship between numbers of particles and the best achievable resolution has also been studied.

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EPR Spectroscopy

2464-Pos Computational Studies Of A Nitroxide Spin Label At Solvent-exposed, Helix Surface Sites In Proteins

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Board B578

We study the conformational dynamics of the spin label R1 (1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl-methanethiosulfonate) at solvent-exposed, helix surface sites in proteins through molecular dynamics (MD) simulations. In addition, we develop a theoretical framework to compute electron spin resonance (ESR) spectra from the simulated trajectories. First, we characterize the behavior of the spin label on the surface of a solvated poly-alanine alpha-helix. This system serves as a model of R1 at an idealized, noninteracting, solvent-exposed site. It allows us to critically assess the chi4/chi5 model, commonly invoked to rationalize the observed ESR spectra in terms of the internal spin label dynamics at such sites. Then, we proceed with simulations of R1 at positions 131 and 72 in T4 Lysozyme. We observe that specific and non-specific interactions of the nitroxide with the neighboring amino acids, rather than putative hydrogen-bonding of the disulfide to the backbone, are responsible for the relative ordering and immobilization of the spin label. This raises questions regarding the extent to which the spectral line shape reports on fluctuations of the protein backbone. To evaluate the relevance of our observations, we calculate ESR spectra at 9, 95 and 170 GHz simulated MD trajectories. The results are in remarkably good agreement with experimental spectra. We conclude that the rigorous interpretation of ESR spectra has to take into consideration the molecular nature of the spin label and its environment.

2465-Pos α-Synuclein Fibril Structure Investigated by Continuous Wave and Pulsed EPR Spectroscopy

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The misfolding and aggregation of α-synuclein plays an important role in the pathogenesis of Parkinson and other neurodegenerative diseases. Misfolding of α-synuclein appears to involve specific structural conformation that populates certain steps of the misfolding. This includes the oligomeric state and the fibril state. We aim to decipher these structures in order to understand the mechanism of misfolding. Of particular interest have been fibrils inasmuch as they represent the endpoint product. α-Synuclein fibrils contain signifi-