3025-Pos  Board B795
Studies of the Shape of Growing Tumors
Shiva Rudraraju1, Kristen L. Mills2, Ralf Kemkemer3, Krishna Garikipati1.
1University of Michigan, Ann Arbor, MI, USA, 2Max Planck Institute for Intelligent Systems, Stuttgart, Germany.

We consider the biological, chemical and mechanical factors that dictate the shapes of growing tumor spheroids. In our published experiments MB-MDA-231 tumors growing in agarose gel cultures demonstrate a very strong tendency to break symmetry and grow into ellipsoidal shapes. We investigate this symmetry-breaking for the clues that it may yield on the interaction of biological, chemical and mechanical conditions that control tumor growth. We use confocal microscopy to monitor the tumor shapes, and histology to record the internal distribution of live and dead cells. Additionally, we impose mechanical interventions by controlling the agarose gel stiffness and imposing external tractions on the growing tumors. These studies suggest the following alternatives for tumor shape symmetry breaking: (a) The rise of a population of cancer stem cells, distinct from the non-stem cells, which proliferate faster and out-compete the latter for glucose and oxygen. (b) Lower rates of stress-induced cell death along microscopic planes of greater mechanical compliance in the agarose gel. (c) Stress-driven cell migration to positions of lower stress along the tumor surface. We explore these scenarios with a multi-physics continuum model that treats cell proliferation, death and migration, glucose and oxygen transport and consumption, and nonlinear mechanics of the growing tumor. On the basis of our own experiments as well as others in the literature, we include interaction source and sink terms that introduce complex, nonlinear coupling between these phenomena. We find that the interfacial tension of the tumor-gel interface plays an important role in all the above scenarios.

The numerical issues that arise in carrying out large scale computations include interaction source and sink terms that introduce complex, nonlinear coupling between these phenomena. We find that the interfacial tension of the tumor-gel interface plays an important role in all the above scenarios. The numerical issues that arise in carrying out large scale computations are discussed. This work is set in the context of our published work on a broader effort to delineate the free energy changes that accomplish the growth of a cancerous tumor.

3026-Pos  Board B796
A Likelihood Based Calculation for Elements of a Single Particle Tracking Cost Matrix
Peter K. Relich1, Patrick J. Cutler1, Fang Huang2, Keith A. Lidke1.
1University of New Mexico, Albuquerque, NM, USA; 2Yale, New Haven, CT, USA.

Single Particle Tracking (SPT) over extended time periods is often performed using quantum dots (QDs); however, the fluorescence intermittency of QDs means that particles may not be observed for some period of time during the acquisition. This blinking complicates the construction of trajectories from the found positions. One elegant method for constructing trajectories from coordinates is to implement a ‘Cost Matrix’, whose elements contain costs for ‘birth’, ‘death’, and ‘linking’ of trajectories [1]. The optimization is framed as a linear assignment problem, which is solved using existing numerical methods. We advance this approach by making a statistically rigorous calculation of the cost matrix such that each element in the cost matrix is the negative log-probability of the observation. We show how to include the effect of boundaries and how to include any additional information that can identify particles (such as spectral features). We also show how to include various blinking models such as for fluorescent probes that can be photo-activated, that blink and that bleach. We show results from simulations as well as experimental data from tracking QDs, dyes bound to fluorogen activating peptides, and photo-activated probes.

3027-Pos  Board B797
Energy Landscape Evolution of Single-Molecule Protein Guided by Conformational Fluctuation
Chien Y. Lin1, June Y. Huang1, Leu-Wei Lo2.
1Department of Photonics and Institute of Electro-Optical Engineering, National Chiao Tung University, Hsinchu, Taiwan, 2Division of Medical Engineering Research, National Health Research Institutes, Zhunan, Miaoli, Taiwan.

Recent molecular dynamics simulations and experimental observations have shown that the dynamics of single-molecule protein can be described with hopping among energy basins caused by backbone motions and side-chain related processes. We formalize the dynamic evolution by combining a stochastic approach with a Lindblad equation. In our model, the open quantum system is comprised of the active center and an environment relating to the protein matrix. The effects of the protein matrix are separated into two categories: fast fluctuations with frequencies higher than the dynamics of the active center, and slow modulations that allow the active center to respond adiabatically. We describe the response of the active center to the fast environmental fluctuations with a memory kernel. The active center responds to the slow modulations with a dynamical evolution on an energy landscape, which can be properly described by a series of stochastic transition matrices. We applied this method to analyze the photon emission from photoactivated fluorescent protein KFP1 in viewing that the light emission behavior of KFP1 is highly sensitive to the hydrogen bonding between its chromophores and the neighboring amino acid groups. We found the conformation fluctuation of KFP1 can vary the hydrogen bonding, thus encodes the energy-landscape evolution of the chromophore into the photon emission traces. By dynamically adjusting the trapping strength and the number of traps locating between two basins on the energy landscape, the conformation fluctuation can vary the photon emission behavior of KFP1. The distribution of the trapping strength exhibits two peaks with one near the thermal energy and the other higher than the thermal energy by a factor of 1.8. Conformational changes of AK1 undergo mutual adjustment process responded to thermal-driven modulation.

3028-Pos  Board B798
Dynamic Clamp Conductance Injection Software Based on the QNX Real Time Operating System with Error Control and an Implicit Integration Method
Hugo Zebedge1, Abraham Wolk1, Hugh Robinson2, Peter Arhen1.
1Dept of Neuroscience, Karolinska Institutet, Stockholm, Sweden, 2Dept of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom.

Dynamic clamp or conductance injection is an electrophysiological method that uses a real-time interface between living cells and a computer to simulate conductance changes in the membrane. To date, the numerical integration methods used for this purpose have been explicit. These methods have an upper bound for the length of the step size when the method becomes numerically unstable. This becomes a practical problem particularly when injecting fast voltage-dependent conductances. Here, we improve the numerical integration of the equations underlying such conductances by implementing an implicit method (Isersles & Norsett,IMA J. Num. Anal. 10:463, 1990), suited for stiff equations. Specifically, this is an A- and L-stable diagonal implicit Runge-Kutta method, which can exploit information about the membrane potential sampled at subintervals of the current update period, which we demonstrate can greatly improve the accuracy and stability of the injected conductance.

Furthermore, the integration method let us implement real-time error monitoring.

We have built our implementation of this on the QNX microkernel real-time operating system, widely used for high-speed mission-critical real-time applications in industry. Its flexibility and reliability in timing, interrupt control and processor scheduling in multiprocessor systems, and its microkernel architecture (which frees up more CPU time than other comparable systems for running the model), make it an ideal platform for more complex integration methods such as the one we describe. The here offered prospect of an improved accuracy and stability of the conductance injection technique, opens up for analyzing membranes with faster channels than was previously possible.

3029-Pos  Board B799
Kristofer Gryte1, Alistair Wardrope2, Geraint Evans1, Stephan Uphoff7, Ludovic Le Reste1, Achillef N. Kapanidis1.
1University of Oxford, Oxford, United Kingdom, 2The University of Sheffield, Sheffield, United Kingdom.

Single-molecule Förster Resonance Energy Transfer (smFRET) experiments provide time-series data offering insight into biomolecular structure and dynamics. Extracting insight and dissecting mechanism, however, are not trivial, particularly when smFRET is extended to complex systems involving multiple molecular components or FRET pairs. Hidden Markov models (HMMs) provide a powerful and extendable framework to address molecular function and structure. Here, we demonstrate an extension of HMM-based inference called ‘Regime-Changing HMM’ exploiting alternating laser excitation (ALEX) spectroscopy, and we test its limitations using simulated data and previously reported switchable FRET data involving complex stoichiometries. We further compare regime-changing HMM to previously described statistical
analysis methods and demonstrate the method’s superiority in inferring correct HMM topology and kinetic parameters. We then apply the method to DNA polymerase binding and replication to identify patterns of multiple polymerases to a DNA overhang construct and to extract binding, dissociation, and polymerization kinetics. The presented statistical algorithm provides objective quantification of single-molecule trajectories and successfully identifies, segments, and analyses photophysical, dynamical, and stoichiometric ‘regimes’ within these trajectories. Our work illuminates important mechanisms in DNA replication and paves the way for experimental extension to studies of large complexes and molecular machines and to the field of single-molecule enzymology.

3030-Pos Board B800
Specification, Construction, and Exact Reduction of Finite State Transition System Models of Biochemical Processes
Scott M. Bugenhagen, Daniel A. Beard.
Medical College of Wisconsin, Milwaukee, WI, USA.

Biochemical reactions may be viewed as discrete event processes characterized by a finite number of states and transitions. These processes may be modeled as finite state transition systems where state transitions represent individual reaction events. The time-evolution of the state occupancy probabilities of such systems is described by the master equation. Since these systems often involve a large number of interactions, it can be difficult to construct the master equation for a model describing a system, and since the resulting models can involve huge numbers of states, solving the associated master equation can be difficult or impossible. Here, we describe a method for the specification, construction, and reduction of finite state transition system models of biochemical processes using the symmetry and invariant manifold reduction techniques. The method allows a user to specify transition rules using an intuitive graphical representation, and to automatically construct the transition matrix of a differential equation characterizing exactly the dynamics of a model, with a potentially significant reduction in dimension when compared to the master equation of the model. The application of the method to a biological process is illustrated by models describing a hypothetical ion-channel at several levels of complexity.

3031-Pos Board B801
Reported Cellphone Effects on Brain Energetically Consistent with Electrostriction
William J. Bruno1,2.
1Los Alamos National Laboratory, Los Alamos, NM, USA, 2New Mexico Consortium, Los Alamos, NM, USA.

Cellphone exposure reportedly alters brain EEG [1], blood flow [2] metabolism [3], and blood-brain barrier [4]. Others claim slight heating is the only plausible effect. We find deformation of soft tissue by electrostriction forces energetically possible. The pressure pushing the hemispheres together could be transduced by pres- 

3032-Pos Board B802
Global Sensitivity Analysis of Arrhythmic Risk Biomarkers in Cardiac Single Cell Models
Anna A. Sher1, Paul Carter2, Blair Bethwaite3, Denis Noble2, David Abramson3, David J. Gavaghan1.
1Oxford University, Oxford, United Kingdom, 2Monash University, Melbourne, Australia.

Global sensitivity analysis is critical in understanding the role of ionic currents variability in modulating cellular electrophysiological properties of ventricular myocytes as well as in assessing the cardiac single cell arrhythmic risk biomarkers. This work involves a systematic investigation into the sensitivity of various preclinical cellular biomarkers of arrhythmic risk to changes in ionic current conductances and kinetics. We compare local and global sensitivity analysis approaches. The nonlinearity of the system is confirmed to play an important role when considering the effect of parameters on markers that involve information from different frequencies. In the case of such biomarkers as action potential duration (APD), maxima Ca2+ and Na+ concentrations, we find no significant changes in the overall order of the parameters’ significance between local and global sensitivity studies. However, even for these single frequency markers we are able to give a more precise relative contribution of each parameter. Importantly, in the case of maximum SI-S2 slope marker, which integrates the information across a spectrum of frequencies of electrical stim- ulation, we find significant changes in the order of parameters as compared to the previously used methods for studying the sensitivity of cellular biomarkers.

In this study we have focused on conformational structure of the β-alanine molecule isolated in low-temperature argon matrices. The main purpose was to determine the set of the β-alanine conformers, which can occur in the matrices. Matrix isolation FTIR-spectroscopy was combined with quantum chemical calculations performed by the MP2 and DFT(B3LYP) methods using the aug-cc-pVDZ and aug-cc-pVTZ basis sets. Gibbs free energies of the β-alanine conformers also were calculated at the CCSD(T)/CBS level of theory.

Totally 21 β-alanine conformers were found at the MP2/aug-cc-pVDZ level of theory. But 10 of them are separated from lower energy conformers by low energy barriers and they cannot be present in the matrices. High-resolution FTIR spectra were registered for the samples immediately after deposition as well as for the samples which were UV irradiated or annealed to 25K. Both the UV irradiation and the matrix annealing result in redistribution of the band intensities in the FTIR spectra. It allows us to distinguish spectral bands of different β-alanine conformers. Assignment of the spectral bands was performed based on calculated vibrational spectra of the β-alanine conformers. As a result we detected presence of 4 β-alanine conformers in the Ar matrices. Further data about β-alanine conformational structure can be used in molecular dynamics simulations and also they can be useful for searching of β-alanine in the interstellar space.

Key words: β-alanine, matrix isolation, quantum chemical calculations.

3033-Pos Board B803
Low Temperature Matrix Isolation IR-Spectroscopy and Quantum Chemical Study of β-Alanine Structure
Daryna Smyrnova1, Stepan Stepanian2.
1The Katholieke Universiteit Leuven, Leuven, Belgium, 2B.Verkin Institute for Low Temperature Physics and Engineering of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine.

In this study we have focused on conformational structure of the β-alanine molecule isolated in low-temperature argon matrices. The main purpose was to determine the set of the β-alanine conformers, which can occur in the matrices. Matrix isolation FTIR-spectroscopy was combined with quantum chemical calculations performed by the MP2 and DFT(B3LYP) methods using the aug-cc-pVDZ and aug-cc-pVTZ basis sets. Gibbs free energies of the β-alanine conformers also were calculated at the CCSD(T)/CBS level of theory.

Totally 21 β-alanine conformers were found at the MP2/aug-cc-pVDZ level of theory. But 10 of them are separated from lower energy conformers by low energy barriers and they cannot be present in the matrices. High-resolution FTIR spectra were registered for the samples immediately after deposition as well as for the samples which were UV irradiated or annealed to 25K. Both the UV irradiation and the matrix annealing result in redistribution of the band intensities in the FTIR spectra. It allows us to distinguish spectral bands of different β-alanine conformers. Assignment of the spectral bands was performed based on calculated vibrational spectra of the β-alanine conformers. As a result we detected presence of 4 β-alanine conformers in the Ar matrices. Further data about β-alanine conformational structure can be used in molecular dynamics simulations and also they can be useful for searching of β-alanine in the interstellar space.

Key words: β-alanine, matrix isolation, quantum chemical calculations.