dehydration product of rhizoferrin, named imido-rhizoferrin. A particular focus is the effect of deprotonation of hydroxyl and carboxyl groups on binding energy of ferric-ion complexes of rhizoferrin-analogs has been investigated. In addition, we have calculated Nuclear Magnetic Resonance properties, such as chemical shifts for ^{13}C and ^{1}H, and indirect spin-spin couplings for methylene protons, and compared our results with available experimental data. The nuclear quadrupole interaction parameters will be discussed within the context of charge distributions and electric field gradients. Our study provides a structural basis for ferric-ion chelation by rhizoferrin analogs for these biomolecules, for which there exist no experimentally determined structures. Argonne National Laboratory is a US DOE Science Laboratory operated under contract no. DE-AC02-06CH11357 by UChicago Argonne, LLC. The research was performed, in part, at Argonne National Laboratory as a research participant in the Visiting Faculty Program. The program is administered by Argonne's Division of Communication, Education, and Public Affairs (CEPA) with funding provided by the U.S. Department of Energy.

4076-Pos Board B804

A Quantitative Method to Track Protein Translocation between Intracellular Compartments in Real-Time in Live Cells using Weighted Local Variance Image Analysis

Guillaume Calmettes, James N. Weiss.
Medicine, University of California Los Angeles, Los Angeles, CA, USA.

The genetic expression of cloned fluorescent proteins coupled to time-lapse fluorescence microscopy has opened the door to the direct visualization of a wide range of molecular interactions in living cells. In particular, the dynamic translocation of proteins can now be explored in real time at the single-cell level. Here we propose a reliable, easy-to-implement, quantitative image processing method to assess protein translocation in living cells based on the computation of spatial variance maps of time-lapse images. The method is first illustrated and validated on simulated images of a fluorescently-labeled protein translocating from mitochondria to cytoplasm, and then applied to experimental data obtained with fluorescently-labeled hexokinase 2 in different cell types imaged by regular or confocal microscopy. The method was found to be robust with respect to cell morphology changes and mitochondrial dynamics (fusion, fission, movement) during the time-lapse imaging. Its ease of implementation should facilitate its application to a broad spectrum of time-lapse imaging studies.

4077-Pos Board B805

Extracting Functional Information from Single Particle Trajectories

Benjamin Regner[1,2], Daniel Tartakovsky[3], Terrence Sejnowski[1].
[1]Salk Institute for Biological Studies, La Jolla, CA, USA, [2]Mechanical and Aerospace Engineering, University of California - San Diego, San Diego, CA, USA, [3]Mechanical and Aerospace Engineering, University of California - San Diego, La Jolla, CA, USA.

Single particle tracking (SPT) experiments offer a powerful means of probing the diffusive characteristics of biomolecules. A common result from SPT is a globally averaged anomalous diffusion exponent. However, methods of calculating the average exponent are typically unable to determine the local, time-dependent characteristics. We have developed a simple classification algorithm to calculate the time-dependent anomalous diffusion exponent of single stochastic trajectories. Classification is based upon the behavior of a renormalization group operator rather than the commonly used mean-square displacement. We present both simulated and experimental examples of the proposed method. Furthermore, we hypothesize that time-dependent fluctuations of the anomalous diffusion exponent will correlate with functional activity in certain cases. We present preliminary results supporting this hypothesis.

4078-Pos Board B806

Tracking Inhomogeneously Distributed Particles

Javier Mazzaferri, Stephane Lefrancois, Santiago Costantino.
Hôpital Maisonneuve Rosemont, Montréal, QC, Canada.

Particle tracking has been extensively applied to biological problems. It is mostly used to describe the dynamics of single molecules embedded within the cell membrane, to analyze cell migration in very different contexts, or to study intracellular trafficking. Several methods have been proposed during the last two decades, for both sparse and high density systems of particles. These methods mostly rely on minimizing the overall displacement of all objects in the field, but some include using spot intensity and historical features of movement to improve the reliability of particle identification. Adjusting tracking parameters of most algorithms is a relatively long and subtle procedure determined by the trade-off between several performance indicators.
In particular, systems where the spatial distribution of particles is inhomogeneous are especially ill-conditioned. Their overall performance is severely impaired because a single set of parameters cannot be optimally adjusted across the field of view. In this work we solve this shortcoming by developing an approach that locally optimizes the particle tracking parameters. A local particle tracking is first performed followed by a global reevaluation of all the trajectories found. To this end, we compute locally the optimal tracking parameters based on the features of the specific time series, which makes the process consistent and less prone to bias. We demonstrate a significant reduction of missing connections (false negatives) thereby improving the length of tracked trajectories. We thoroughly describe the algorithm and systematically compare its performance with state of the art alternative procedures using both, numerical simulations and biological image time series. As measures of tracking efficiency, we compute false positive and false negative errors, and the length of the tracked segments by comparing tracked and true simulated trajectories.

4079-Pos Board B807

Dynamic Quantification of Antigen Molecules on Cells with Flow Cytometry

Darya Yu. Orlova[1,2], Aaron B. Kantor[1], Andrei V. Chernyshev[2,3], David R. Parks[1], Wayne A. Moore[1], Leonore A. Herzenberg[1].
[1]Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA, [2]Institute of Chemical Kinetics and Combustion, Novosibirsk, Russian Federation, [3]Novosibirsk State University, Novosibirsk, Russian Federation.

Flow cytometry is a powerful tool for the quantification of target molecules on cells. Traditional methods for estimating the number of expressed molecules, based on the detection of target antigens bound with fluorescently labeled antibodies, assume that the antigen-antibody reaction reaches equilibrium. A calibration procedure, with carefully prepared reagents is needed to convert the intensity of the fluorescence signal to the number of target antigens. The new method presented here may be more robust and has application to lower affinity antibodies and non-equilibrium labeling conditions.
We have developed a novel kinetic approach to the antigen quantification problem. Instead of using a static calibration system, we analyzed fluorescence profile dynamics measured by flow cytometry in the general case of reversible antibody-antigen binding. The model considers the full fluorescence profile on a distribution of cells. Experimental data obtained with the LSRII cytometer were fitted by the diffusion-reaction mathematical model using the Levenberg-Marquardt nonlinear least squares curve-fitting algorithm in order to obtain the reaction rate constants and the number of target antigens per cell. Results were compared with the QuantiBRITE calibration system, which uses calibration beads with known amounts of covalently bound PE and unimolar (1:1) anti-Ag-PE reagents bound to capture beads or cells under saturating staining conditions.
Our approach is independent of specially prepared calibration beads and antibody reagents and can be applied to both low and high affinity antibodies, under both saturating and non-saturating binding conditions.

4080-Pos Board B808

Bridging the Gap Between PALM and Qdots Single Particle Tracking using Bayesian Inference and the Gillespie Scheme

jean-baptiste masson[1,2], Mohamed el beheiry[3], Charlotte Salvatico[4,5], Marianne Renner[4,5], Christian G. Specht[4,5], Antoine Triller[4,5], Maxime Dahan[3].
[1]insitut pasteur, paris, France, [2]CNRS UMR 3525, Paris, France, [3]Institut Curie, CNRS UMR 168, paris, France, [4]Institut de Biologie de l'Ecole Normale Superieure (IBENS),, paris, France, [5]Institut National de la Sante et de la Recherche Medicale U1024,, Paris, France.

There are many differences between what can be extracted from biomolecules motion using small numbers of long trajectories (Qdots, Nanoparticles, fluorophores) or large numbers of short, dense trajectories (PALM, uPaint, Storm). Long time recordings allow various estimators to converge towards mostly accurate values, but don't allow access to the statistical significance of these trajectories furthermore with this approach large portions of the membrane surface areas remain unexplored. Conversely, large number of short trajectories allows mapping large cellular surfaces but prevents good convergence of most estimators. Here, we show how to bridge the gap between these two types of recordings by using Bayesian Inference and the Gillespie scheme. To this aim, large numbers of short trajectories are recorded. Biomolecules are modeled as random walkers following the Langevin Equation with space varying diffusivities and interaction potentials [1,2]. The diffusive and interaction landscapes over the complete surface of the cells are inferred using a Bayesian inference scheme [3]. The inference allows combining local information within a map capturing most of the statistical properties of the trajectories. Then, trajectories generated from these maps using the Gillespie scheme [4] display all the statistical properties of the experimental ones. With these

generated trajectories, various estimators can be accurately evaluated such as the propagator, the first time of passage, the mean square displacement etc. Furthermore, these estimators can be ensemble averaged which is much more selective than time average estimators. We applied this method to the GlyR receptor diffusion dynamics in the neuronal plasma-membrane.

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4081-Pos Board B809

High Density Single Particle Tracking with Various Probes

Peter K. Relich, Keith A. Lidke.
Physics, UNM, Albuquerque, NM, USA.

Single Particle Tracking (SPT) is a set of single molecule techniques that identify and characterize the motion of individual particles in a medium. High Density Single Particle Tracking (HDSPT) is a subset of advanced SPT techniques designed to overcome some of the limitations of current SPT methods, primarily to compensate for poor statistical information due to low trajectory counts per observed area as seen in conventional SPT. Quantum Dots (QDs), Fluorogen Activating Peptides (FAPs), and Organic Dyes are recognized probes that have been used in SPT experiments. QDs are very bright, photo stable probes with blinking dictated by power law behaviors. A FAP will bind to a receptor and activate, emitting photons at a constant rate and intensity until it bleaches. ODs have emission behaviors characterized by Jablonski diagrams; depending on the particular OD and buffer combination, they may blink or bleach. Variable emission dynamics provide a challenge in SPT analysis because it requires localization techniques that can properly account for varying probe behaviors. We have modified our multi-emitter fitting algorithm [1] with a Bayesian approach in order to localize overlapping probes with variable intensity profiles. This advancement in localization capturing allows us to overcome one of the more difficult challenges present in high density SPT- reliable probe identification in high density regions. We have tested our modified SPT software on simulated data as well as on live Rat Basophil Leukemia (RBL) cells with various probes to demonstrate our approach to high density SPT.

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4082-Pos Board B810

Cell Adhesion Sensitivity to Cell Size and Surface Receptor Densities

Srikanth Raghavan[1], Shripad Joshi[1], Alexander Dawson-Eli[2], Aravind R. Rammohan[1], Matthew E. McKenzie[1], Ramakrishnan Natesan[3], Ravi Radhakrishnan[3].
[1]Corning Incorporated, Corning, NY, USA, [2]Rochester Insititute of Technology, Rochester, NY, USA, [3]University of Pennsylvania, Philadelphia, PA, USA.

Using a Monte Carlo based code in conjunction with a Weighted Histogram Analysis Method (WHAM) we explore the impact of the size of Cells/Nanocarriers on their binding to functionalized surfaces. In our study we vary the size of Cells/Nanocarriers between 50 nm up-to 1 micron and also vary the number of receptors on these Cells/Nanocarriers such that the surface coverage of receptors ranges between 45%-95%. We adopt a two level framework: (i) where we explicitly model all surface receptors and their interactions with the surfaces for the smaller Cell/Nanocarrier systems (< = 200 nm) and (ii) for larger Cells/Nanocarriers we perform coarse grained simulations where we develop receptor ligand interaction potentials for smaller regions of the Cells and incorporate them to then derive a potential of mean force (PMF) for the full Cell surface interactions. We compare the results from the two approaches to establish the validity of the coarse graining methodology. We then evaluate the sensitivity of predicted equilibrium dissociation constants to cell size and surface coverage by receptors.

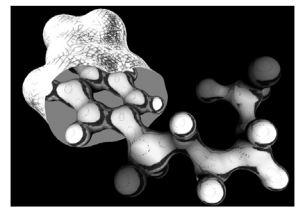
4083-Pos Board B811

Game on, Science - How Video Game Technology may Help Biophysicists Tackle Visualization Challenges

Alexandre Kouyoumdjian[1], Erwan Ortie[1], Alex Tek[2], Aurélien Pluot[3], Eric Henon[3], Matthieu Chavent[4], **Marc Baaden**[1].
[1]IBPC, CNRS UPR9080, Paris, France, [2]Uppsala University, Uppsala, Sweden, [3]University of Reims, Reims, France, [4]University of Oxford, Oxford, United Kingdom.

The video games industry develops ever more advanced technologies to improve rendering, image quality, ergonomics and user experience of their creations providing very simple to use tools to design new games. In biophysics, only a small number of experts with specialized know-how are able to design interactive visualization applications, typically static computer programs that cannot easily be modified. Are there lessons to be learned from video games? Could their technology help us explore new molecular graphics ideas and render graphics developments accessible to non-specialists?

This approach points to an extension of open computer programs, not only providing access to the source code, but also delivering an easily modifiable and extensible scientific research tool. In this work, we use the Unity3D game engine to develop a visualization application for research and education. Classical and novel representations such as molecular structures, HyperBalls, surfaces, animated chemical reactions, animated electrostatic field lines and biological networks can be decorated with powerful, artistic and illustrative rendering methods. Our prototype is easily modifiable and extensible and may serve others as starting point and platform for their developments.



4084-Pos Board B812

Loos: A Tool for Making New Tools for Analyzing Molecular Simulations

Tod D. Romo, Alan Grossfield.
University of Rochester Medical School, Rochester, NY, USA.

We have developed LOOS (Lightweight Object Oriented Structure-analysis) as a tool for making new tools for analyzing molecular simulations. LOOS is an object-oriented library written in C++ with a Python interface and was designed to facilitate the rapid development of new methods for structural analysis in either C++ or Python. In addition, LOOS includes over 130 pre-built tools for common structural analysis tasks including 3D histograms for visualization, hydrogen bonding patterns, and assessing simulation convergence. LOOS also includes a set of libraries and tools for performing elastic network model calculations. LOOS supports reading the native file formats of most common simulation packages, such as Amber (including NetCDF-formatted), CHARMM, Gromacs, NAMD, and Tinker. LOOS can also write NAMD formatted PDB and DCD files. A dynamic atom selection language, based on the C expression syntax, is included in the library and is easily accessible to the tool writer. Through modern C++ design, LOOS is both simple to use, requiring knowledge of only 4 core classes, and easy to extend. LOOS makes extensive use of Boost and the Standard Template Library for correctness and ease of use, and relies on ATLAS for high performance numerical routines.

4085-Pos Board B813

CHARMM-Gui Pace Cg Builder for Solution, Micelle, Bilayer and Vesicle Simulations

Yifei Qi[1], Xi Cheng[1], Wei Han[2], Sunhwan Jo[3], Benoit Roux[3], Klaus Schulten[2], Wonpil Im[1].
[1]Department of Molecular Biosciences and Center for Bioinformatics, The University of Kansas, Lawrence, KS, USA, [2]Beckman Institute and Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [3]Department of Biochemistry and Molecular Biology, Gordon Center for Integrative Science, University of Chicago, Chicago, IL, USA.

Coarse-grained and multi-scale simulations are widely used to study large biological systems nowadays. However, building the simulation system is not trivial in some occasions. We have developed the CHARMM-GUI PACE CG simulator for building solution, micelle, bilayer and vesicle systems using the PACE force field, a united-atom model for proteins, and Martini, a coarse-grained force field for water, ion and lipids. The qualities of the output systems are validated by simulation of various examples and comparison of the coarse-grained simulation to all-atom simulation. We expect this module to be a useful tool for modeling large, complicated systems.

4086-Pos Board B814

Calculator for Mutual Information Between a Discrete and a Continuous Data Set

Brian Ross.
University of Washington, Seattle, WA, USA.

Mutual information (MI) is in many ways an ideal statistic for detecting relationships between two data sets. MI is easy to calculate when both data sets are discrete, but not when one or both data sets are real-valued. An accurate method for calculating MI between two real-valued data sets was previously developed (Kraskov et al. 2004). We present an accurate method for calculating MI between one discrete data set and one real-valued data set. For example, this calculator can quantify the correlation between base methylation (a discrete variable) and gene expression level (real-valued), or the effect of a clinical procedure (boolean; discrete) on patient survival time (real-valued). We use our