2389-Pos Board B526
A Langevin Dynamics Algorithm for Coarse-Grain Modeling of Protein Clusters
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A variety of recent experimental results have highlighted the importance of spatial localization and clustering in cellular signaling pathways. We have called such non-stoichiometric dynamic clusters ''pleiomorphic ensembles'' (PE). The participation of PEs often significantly changes the dynamic and equilibrium properties of signaling networks, but the mechanisms behind such changes can be unclear, and computational modeling of these effects is hampered by the lack of efficient and appropriate methods to address such questions. Non-spatial models can reproduce some features of clustering, such as phase transitions, but cannot capture features such as steric hindrance and PE diffusion, which may have important biological consequences. Publicly available spatial stochastic solvers, such as Smoldyn or MCELL, treat all particles as points and thus cannot model spatial organization and excluded volume. In principle, clustering could be modeled with molecular dynamics simulations, but typical PE sizes and time scales preclude such models with current computational hardware.
To overcome these limitations we have designed a general purpose Langevin dynamics simulator that models proteins as a set of sites connected by stiff links. The sites are modeled as impenetrable spheres, which captures the effects of excluded volume and steric hindrance. The program allows for zero order creation reactions, a variety of first order reactions such as dissociation reactions and transitions between states of a given site (e.g., active or inactive), and second order binding reactions between sites.
We apply our program to study cluster formation in the nephrin-Nck-N-wasp system, which is important for kidney podocyte function. We compare our results to the previously published analysis of this system based on a non-spatial simulator (Falkenberg et al. Biophys. J. 2013), to explore the consequences of steric crowding and diffusion on cluster dynamics. (Supported by NIH grants TRO1DK087650 and P41GM103313)

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DFGmodel: Predicting Protein Kinase Structures in Inactive States for Structure-Based Discovery of Type-II Inhibitors
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Protein kinases exist in equilibrium of active and inactive states, in which the aspartate-phenylalanine-glycine motif in the catalytic domain undergoes conformational changes that are required for function. Drugs targeting protein kinases can bind the primary ATP-binding site of an active state (type-I inhibitors) or utilize an allosteric pocket adjacent to the ATP-binding site in the inactive state (type-II inhibitors). Limited crystallographic data of protein kinases in inactive state hampers the application of rational drug discovery methods for developing type-II inhibitors. Here, we present a computational approach to generate structural models of protein kinases in the inactive conformation. We first perform a comprehensive analysis of all protein kinase structures deposited in the Protein Data Bank. We then develop DFGmodel, a method that takes either a known structure of a kinase in active conformation or a sequence of a kinase without a structure, to generate kinase models in inactive conformation. Evaluation of DFGmodel's performance using various measures indicates that the inactive kinase models are accurate, exhibiting RMSD of 1.5 Å or lower. The kinase models also accurately distinguish type-II kinase inhibitors from likely non-binders (AUC > 0.70), suggesting that they are useful for the utility of virtual screening. Finally, we demonstrate the applicability of our approach with three case studies. For example, the models are able to capture inhibitors with unintended off-target activity. Our computational approach provides structural framework for chemical biologists to characterize kinases in the inactive state and explore new chemical spaces with structure-based drug design.

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Virtual Reality Environment for Patching and Imaging in Brain Slices
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We are developing a program that constructs a 3D representation of a virtual workspace that visualizes the brain slice and the perfusion chamber, the patch-clamp and other recording electrodes, and the imaging objectives. The user can control the operation and 3D positioning of the instruments relative to the sample, with real-time visual feedback. Specific cells can be bookmarked and linked to optical and electrical recordings, and the 3D workspace can be saved for later viewing and data analysis. We are using this software platform to explore ion channel properties and their contributions to cellular and network interactions in brain slices. This unified interface provides a more streamlined approach for combining electrophysiology with structural and functional imaging.

Optical Microscopy and Super-Resolution Imaging II

2392-Pos Board B529
3 Color - 3 Dimensional STED Nanoscopy
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The stimulated emission depletion microscopy (STED) is a super-resolution technique that enables to overcome the diffraction barrier, allowing to distinguish details of cellular as well as molecular structures, not visible with a conventional confocal microscope [1].
Here we present the development of a custom made versatile STED microscope, based on a super-continuum pulsed laser source endowed with 2 high power STED laser beams, at 715 and 745 nm, respectively [2]. In order to obtain three-dimensional super-resolved images, we split the STED beam into two parts. One of the beams passes through a vortex phase plate which creates a donut shape beam for lateral resolution enhancement. For the other beam we introduced a home-made phase plate which introduces a phase delay of π in the center, creating a z-donut to increase resolution in the axial direction [3]. We can tune the microscope PSF adjusting the ratio of the power in the two depletion pathways, to achieve, for example, an isotropic resolution. Additionally in order to perform three color imaging we paid particular attention to chromatic aberrations and we optimized the setup accordingly.
Therefore by using proper fluorophores, we are able to do 3 color super-resolved imaging.
Thanks to this completely custom made optical architecture, designed for fast scanning, 3D and three color acquisition, we are able to perform multimodal live cell imaging.
The achieved 3D resolution discloses morphometric properties at the nanoscale which are completely hidden to the confocal observation.
[1] S. W. Hell et al., Opt. Lett., 19, 780-782 (1994).
[2] S. Galiani et al., Opt. Express 20, 7362-7374 (2012).
[3] B. Harke et al., Nano Letters 8 (5), 1309-1313 (2008).

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Efficient Integrated 3D and Multi-Color Single Molecule Super-Resolution Imaging
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PALM, STORM, and related super-resolution localization microscopies were initially limited to a 2D resolution improvement in a single color. Since then a number of methods for both 3D localization and multi-color imaging have become well established. A particularly promising approach to multi-color is ratiometric imaging, which allows spectrally similar fluorophores to be distinguished and imaged simultaneously, minimizing the acquisition time as well as artifacts due to drift, sample motion, and chromatic aberration. By splitting each single molecule signal across 2 channels, however, ratiometric multi-color incurs a small penalty in signal to noise (SNR) and contrast. 3D methods either work by aberating the PSF or by splitting the image, also incurring an SNR penalty. Added to this are insertion losses from the imperfect optical systems used.
To date, the combination of 3D and multi-color has typically relied on 'chaining' a 3D method and a color method - e.g. using an image splitter for ratiometric color together with an astigmatic lens for 3D. This incurs an SNR 'double-hit', making it challenging to routinely obtain high quality multi-color 3D images. By devising a system from scratch to estimate both color and 3D position, much of the redundancy in the 'chaining' approach can be eliminated and the SNR hit minimized.
In this presentation we discuss our experience with two such integrated approaches - the use of a focal offset within a spectral splitting device to allow simultaneous biplane 3D and ratiometric color with only two images on the