923-Symp Intracellular calcium (Ca^{2+}) signals have distinct spatial and temporal components

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Intracellular calcium (Ca^{2+}) signals have distinct spatial and temporal components. Spatially, cytosolic Ca^{2+} changes have been linked to cell events such as secretion whereas nuclear Ca^{2+} changes are necessary for transcription, regenerative processes and cell death. Temporally, the frequency of Ca^{2+} oscillations and the rate of Ca^{2+} wave propagation have each been linked to cell-specific responses. To investigate the basis of these differences we have taken several approaches: structural, immunocytochemical, electrophysiological, and imaging. The structure of purified InsP3R was obtained from single particle reconstruction of cryo-electron microscopic images. This reconstruction shows the InsP3R as an uneven cylinder with lateral arms extending into the cytoplasmic domain. These arms appear to move when ligand and cofactors bind to the receptor. Using immunocytochemical methods, we found that the distribution of InsP3R isoforms was non-uniform both in tissue (hippocampal slices) and individual cells (epithelial and neuronal cells). Single channel measurements were used to study the functional implications of ligand and cofactor binding. Channel activity could be modulated by addition of cofactors to either the lumenal or the cytoplasmic side of the channel. Many of these modulators also are non-uniformly distributed in tissues and cells, adding another layer of complexity. Ca^{2+} imaging revealed a regional specificity in signal initiation and propagation. In many cell types the rate of InsP3 production and surface-to-volume effects play minor roles in determining temporal and spatial Ca^{2+} signaling patterns. Conversely, the combination of a non-uniform distribution of InsP3R isoforms and unique isoform-specific single channel properties do play a role in establishing patterns. Similarly, the reagents found to modulate single channel activity were also important in altering subcellular Ca^{2+} signals. These functional interactions provide the basis for understanding the consequences of channel modulation for intracellular Ca^{2+} signaling.

Minisymposium 2: Structural Refinement & Modeling Guided by Low-Resolution Experimental Poster

923.01-Minisymp Merging Data from Different Resolutions to Reveal Biomolecular Function

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A key to understanding how biological systems function is to look at their structures captured at work. Experimental techniques reveal different levels of macromolecular structure: high-resolution techniques such as X-ray chrystallography yield atomistic details, but the structures captured are often in undefined functional states; alternatively, techniques such as cryo-electron microscopy (cryo-EM) capture the system in a biologically functional state, albeit at lower resolution. Computational techniques can help bridge the resolution gap by adapting high-resolution crystallographic structures to electron microscope density maps, providing the details of the molecules in different functional states, and thus revealing astonishing views of cellular processes.

We developed a novel method to fit atomic structures into EM maps using molecular dynamics simulations. EM data are incorporated into the simulation as an external grid potential added to the molecular dynamics force field, allowing all internal features present in the EM map to be used in the fitting process, while the model remains fully flexible and stereochemically correct. The novel method has been applied to several macromolecular systems. As an example application, a high-resolution structure of the bacterial ribosome bound to the ternary complex derived from a 6.7Å EM map will be presented.

924-Minisymp Improving Structures of Supramolecular Complexes and Membrane Proteins at Moderate Resolutions of X-ray Diffraction

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We report a novel X-ray crystallographic refinement protocol for modeling anisotropic thermal parameters of supramolecular complexes and membrane proteins. Based on that, a very small set of low-frequency normal modes (e.g., 25 $\sim$ 50 modes) was used to reconstruct the thermal motions in X-ray diffraction. The method was applied on a series of supramolecular complexes and membrane proteins, all of which structures were solved at moderate resolutions. The results universally showed that the Rfree values of the normal-mode-refined models were lower than the original isotropically refined models. Most importantly, the refinement resulted in improvement in electron density maps that allowed for building of a substantial amount of missing atoms including those from functionally important residues. The distribution of anisotropic thermal ellipsoids also revealed structure flexibility that is functionally important. We believe that the new protocol will help to significantly improve the structures of many highly-flexible supramolecular complexes and membrane proteins, for which further refinement is beyond any currently available methods.

925-Minisymp Modeling Protein Complexes by Combining High-Resolution Structure with Small-Angle X-ray and Neutron Contrast Variation Data

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Small-angle solution scattering yields low-resolution structural information that complements high-resolution techniques such as crystallography and NMR. The ever increasing desire to understand

more complex biological systems, has brought about a recent surge in interest in the technique, greatly facilitated by developments in sources, instrumentation, and computing power that enables 3D modeling. Modeling 3D structures from solution scattering data is an appealing prospect, however, the results may not be uniquely determined by a single scattering profile. This ambiguity can be resolved, in part, through the inclusion of neutron contrast variation data in the modelling process. We have been using this approach to study biomolecular signalling and regulation, specifically looking regulation the regulatory mechanisms controlling bacterial sporulation (1). More recently we have used these methods to study a receptor-ligand complex in nerve synapses whose alteration via mutations can cause autism (2). This presentation with describe the strengths and limitations of these approaches in the context of understanding bio-molecular function and also compare and contrast what can be done with small-angle X-ray scattering for which contrast variation is not generally feasible using examples drawn from our studies of IP3 signalling (3) and other signalling molecules.

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926-Minisymp Structural Analysis of Cellulolytic Multi-enzymatic Complexes by combining X-ray diffraction, Small Angle X-ray Scattering and Molecular Dynamics

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Cellulose, the main structural component of plant cell walls, is the most abundant carbohydrate polymer in nature. To break down plant cell walls, anaerobic microorganisms have evolved a large extracellular enzyme complex termed cellulosome. This megadalton catalytic machinery organizes an enzymatic assembly, tenaciously bound to a scaffolding protein via specialized intermodular "cohesin-dockerin" interactions that serve to enhance synergistic activity among the different catalytic sub-units. All these different domains are separated by intrinsically disordered linker peptides. Because of the intrinsic flexibility of these complexes, the atomic structure of only the isolated domains (enzymes, cohesin, dockerin) could be solved. We therefore analyzed the solution structure properties of cellulosome-like assemblies from Clostridium cellulolyticum and C. thermocellum by combining small angle X-ray scattering, molecular dynamics and X-ray crystallography. We thus investigated the conformational events occurring upon complexation of the enzymes onto the scaffolding protein, and showed that the linker peptide separating the enzymes from their dockerin undergoes a dramatic structural rearrangement upon complexation. With this strategy, we could also generate atomic models of mini-cellulosomes of increasing size, made of one, two, and three enzymes, which reveal the existence of various conformational states existing in solutions. These results provide the first clues on the mechanisms by which these protein assemblies attain their remarkable synergy. The quality of our SAXS data also allowed us to model the atomic structure of the Clostridium cellulolyticum dockerin-cohesin interface, using homology modeling, crystallographic data and molecular dynamics. This modeled structure highlights the local differences between both organisms responsible for the species specificity, and was very recently confirmed by collaborators who solved the crystal structure.

927-Minisymp Applications of a Continuous Representation of Proteins in Diffraction

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We have developed a continuous representation of proteins in terms of space curves which is an alternative to the more conventional atomic coordinate models. Differential geometry allows the construction of arbitrary curves and in particular can be employed to construct curves which follow the path of a protein backbone in three dimensions. Such curve models can be specified with significantly less information than coordinate models, and so may be especially useful for systems where only low-resolution structural techniques are applicable. Here we describe a method for simulating diffraction patterns from our continuous representation and investigate the accuracy and efficiency of this approach.

928-Minisymp Multiple Models And Simulation, Allied With AFM And IMS, Show Quantized E4 Nano-lipo-protein Particle Sizes

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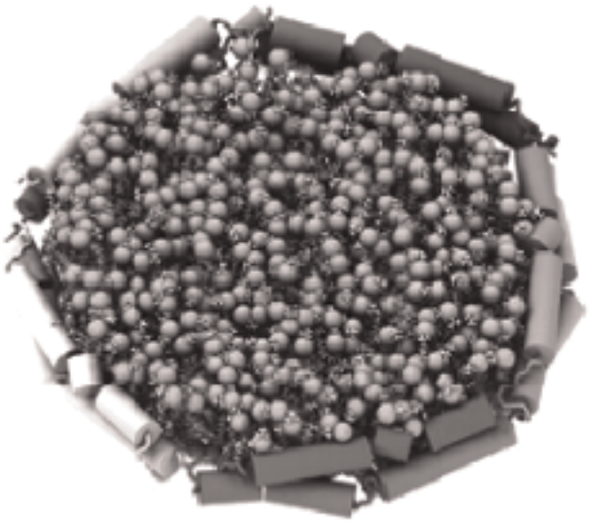
NLPs are important for their ability to solubilize amphipathic molecules such as phospholipids, cholesterol and membrane proteins. Traditionally, SEC is used to separate heterogeneous populations of particles into several purified populations. However SEC purification does not always perfectly filter particles by size. Atomic-force microscopy (AFM) and Ion Mobility Spectroscopy (IMS) following fine exclusion chromatogaphy indicate a quantization of NLP disc sizes. Multiple initial models were constructed using a wide range of diameters and E4 folds. These large systems were analyzed for their stability and dynamics in over 1 microsecond of all-atom and multiple microsecond coarse-grained MD simulations.



Platform V: Protein-Ligand Interactions

929-Plat Reduced complexity of T cell recognition: Gamma Delta T cells recognize the MHC ligand T22 using a single CDR loop

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Reduced complexity of T cell recognition: Gamma Delta T cells recognize the MHC ligand T22 using a single CDR loop. Antigen recognition by the adaptive arm of the immune system is mediated by receptors expressed on T cells or antibody producing B cells that are generated through a process of genomic rearrangement. The diversity inherent in the rearrangement process, at the protein level, is concentrated in the receptor's six Complementary Determining Region (CDR) loops and endows these receptors with the ability to recognize a diverse array of ligands. We show that a unique subpopulation of T cells that express rearranged T cell receptors (TCR) composed of a gamma and delta chain as opposed to the more ubiquitous alpha and beta chains, recognize their ligand, the non-classical MHC class I molecule T22, through only one CDR loop, CDR3delta. Fusion of this loop from two T22 reactive gamma delta TCRs onto a naïve TCR transfers reactivity, with similar affinities to those of the wildtype receptors. The reactivity of these fusion constructs to ligand confirms that the recognition "glue" is solely through their CDR3delta loop. However, amino acid and length variation exists between the two loops that were examined; alanine scanning confirms that ligand binding is accomplished through use of different registers of amino acids in these loops. The reduced recognition complexity of T22 reactive gamma delta TCRs therefore contrasts with alpha beta TCR or antibody recognition of antigen, which involves a combination of the six CDR loops inherent in these receptors structure. However, the use of different amino acid residues within these loops for ligand binding suggests within each loop there exists complexity that is a result of a convergent recognition strategy.

930-Plat Aberrations in the Mechanism of Protein Kinase A Mediated Phosphorylation Caused by Phospholamban Mutants

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Protein phosphorylation is fundamental in the modulation of myocardial contractility. One mechanism which controls this modulation occurs through alterations of Ca^{2+} flux formed across the sarcoplasmicreticulum (SR) membrane, which has profound dependence on the interactions of three proteins: protein kinase A (PKA), sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA), and a single pass transmembrane protein, phospholamban (PLN). Phosphorylation of PLN by PKA is associated with an augmented rate of SR Ca^{2+} uptake and relaxation of the myocardium. Mutants of PLN (PLN-R9C and PLN-R14-Delete) have been shown to be linked with forms of the fatal hereditary disease, dilated cardiomyopathy. We have proposed that this form of the disease is largely attributed to abnormalities in protein phosphorylation of these mutants of PLN. Here, we examine the structural perturbations, thermodynamics of substrate binding, and kinetics of phosphorylation for the interactions between the full catalytic subunit of PKA and peptides corresponding to the cytoplasmic region of PLN ($PLN_{1–19}$) and its mutants ($R9C_{1–19}$ and $R14Del_{1–19}$) using NMR spectroscopy and coupled enzyme kinetic assays. We found that the binding thermodynamics for $PLN_{1–19}$ or $R9C_{1–19}$ are nearly identical ($K_d \sim 14\ \mu M$), but the kinetics of phosphorylation for $R9C_{1–19}$ are drastically reduced (k_{cat} is decreased 5-fold for $R9C_{1–19}$). By using TROSY-based NMR techniques, we also mapped the residue specific differences in the amide fingerprint of C-subunit PKA (PKA-C) during substrate binding. Our results suggest that changes in the perturbation of key allosteric residues in the enzyme may implicate a decrease in the ability of $R9C_{1–19}$ to become phosphorylated. Finally, we present a model of the protein-protein complexes formed, using a novel technique which measures residual dipolar couplings of the backbone amides for PKA-C in alignment media containing phospholamban embedded in the matrix.

931-Plat Live Cell Single Molecule Imaging and Micropatterning of CD4 reveals Novel Binding Mechanisms to Lck

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