Symposium 14: Mechanisms of Exo- and Endocytosis

1733-Symp Dynamin Structure

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The dynamin family of proteins are large GTPases involved in membrane remodeling events throughout eukaryotic cells. For example, dynamin is involved in the final stages of fission during endocytosis, and the dynamin-related protein (Drp1) is necessary for mitochondrial division. During endocytosis, dynamin is believed to wrap around the necks of coated pits and facilitate vesiculation. In support of this model, purified dynamin self-assembles into spirals (50 nm diameter) and readily form dynamin-lipid tubes, which constrict and fragment upon addition of GTP. Structural changes induced by GTP addition were characterized by solving the three-dimensional structure of dynamin in the constricted and non-constricted states using cryo-electron microscopy. Using a rigid-body Monte Carlo algorithm, the crystal structures of the GTPase and pleckstrin homology domains were fit to the cryo-EM densities. The GTPase domain is placed at the periphery of the helical array while the PH domain is in a position that allows for interactions with lipid headgroups. The placement of the crystal structures into the cryo-EM densities revealed a twisting motion of the GTPase, middle and GTPase-effector domains, which suggests a corkscrew model for dynamin constriction.

To determine if a common mechanism of action exists among the dynamin family members, we examined the structure and function of the Drp1 homologue in yeast, Dnm1. In collaboration with Dr. Jodi Nunnari (UC Davis) we have shown that Dnm1 assembles into large spirals with a diameter of 100 nm. Remarkably, the Dnm1 spirals have the same diameter as the observed mitochondrial constriction sites seen in vivo. Dnm1 also assembles onto liposomes and form Dnm1 decorated tubes that constrict significantly upon GTP addition. These results suggest that although dynamin family members share common characteristics, their structural properties are uniquely tailored to fit their function.

1734-Symp Pka Activation Bypasses The Requirement For Unc-31 In The Docking Of Dense Core Vesicles From *C.elegans* Neurons

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The nematode *C. elegans* provides a powerful model system for exploring the molecular basis of synaptogenesis and neurotransmission. However, the lack of direct functional assays of release processes has largely prevented an in depth understanding of the mechanism of vesicular exocytosis and endocytosis in *C. elegans*. We address this technical limitation by developing direct electrophysiological assays, including membrane capacitance and amperometry measurements, in primary cultured *C. elegans* neurons. In addition, we have succeeded in monitoring the docking and fusion of singledense core vesicles (DCVs) employing total internal reflection fluorescence microscopy. With these approaches and mutant perturbation analysis, we provide direct evidence that UNC-31 is required for the docking of DCVs at the plasma membrane. Interestingly, the defect in DCV docking caused by-UNC-31 mutation can be fully rescued by PKA activation. We alsodemonstrate that UNC-31 is required for UNC-13-mediated augmentation of DCV exocytosis.

1735-Symp Intermediates in and Regulations of SNARE-mediated membrane fusion

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SNAREs are the central components of the intracellular membrane fusion machinery. Association of v- and t-SNAREs bridges two membranes, which facilitates the fusion. We use spin-labeling EPR to determine the structural transitions that SNAREs undergo during SNARE complex formation and membrane fusion. Additionally, we also use the newly developed single fusion assay based on wide field TIRF microscopy to dissect the individual steps along the fusion pathway. The SNARE assembly intermediates that are found with EPR and the fusion intermediates that are discovered with the single fusion assay will be discussed. We will also discuss the regulation of the SNARE-mediated membrane fusion by Ca2+, complexin, and synaptotagmin.

Platform AG: Protein Conformation

1736-Plat Probing the Cross-β Core Structure of Amyloid Fibrils by Hydrogen-Deuterium Exchange Deep UV Resonance Raman Spectroscopy

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Studying the structure of amyloid fibrils is important for the detailed understanding of fibrillogenesis at a molecular level. Amyloid fibrils are non-crystalline and insoluble, and thus are not amenable to conventional X-ray crystallography and solution NMR. Several specialized techniques with less general capabilities have been developed and utilized for probing fibrillar structure. Transmission electron microscopy and scanning probe microscopy provide general information on fibril topology. The application of fiber X-ray diffraction and scattering has been limited to short peptides mimicking the core structure of the fibrils formed from amyloidogenic protein. Solid state NMR probes inter-atomic distances and torsion angles, which define local secondary structure and side-chain conformations. Deep UV resonance Raman (DUVRR) spectroscopy have been found to be a powerful tool for protein structural characterization at all stages of fibrillation (1, 2).

We report here on the first application of hydrogen-deuterium exchange DUVRR spectroscopy to probe the secondary structure of the fibril cross-β core (3). This method allowed for structural

characterization of the highly ordered cross-β core of lysozyme fibrils. No inhomogeneous broadening of Raman bands due to various amino acid residues was found that might indicate a sequence-independent structure of the cross-β core. The resolved Raman signature indicated that the anti-parallel β-sheet is the dominant secondary structural conformation of the core.

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1737-Plat Kinetic Study of Beta-amyloid residue accessibility

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Beta-amyloid peptide (Aβ) is the main protein constituent found in senile plaques in Alzheimer's disease (AD). Aβ plays a role in neurodegeneration associated with AD and its toxicity is related to its structure or aggregation state. Comprehension the relationship between Aβ structure and its function is fundamental to better understanding the toxicity behavior of this protein. Chemical Modification was used to identify residues on Aβ peptide that were exposed or buried upon changes in peptide structure associated with aggregation. The change in mass, after chemical modification, analyzed by MALDI-TOF was used to indicate the solvent accessibility of specific peptide residues in different aggregation states. Results indicate that different relative abundance of fully accessible species and partially accessible species were observed in the different Aβ aggregation states: monomer and fibrils. Several residues were modified: Lysine, Tyrosine and Aspartic acid; limited proteolysis was performed to identify with accuracy which residue was accessible in the structure. Determination of the specificity of residue modification and the rate of reaction for a specific site was the ultimate goal of this work, which was a step towards understanding how the peptide folds. Elucidation of Aβ peptide structure at the residue level is a first step in designing novel therapies for prevention of beta amyloid structural transitions associated with Alzheimer's disease.

1738-Plat Generating Conformational Transition Paths In Myosin Motor And Chaperonin Groel By A Mixed Elastic Network Model

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We have developed a mixed elastic network model (MENM) to investigate large-scale conformational transitions of proteins between two known structures. Elastic network potentials for the beginning and end states of a transition are combined, in effect, by adding their respective partition functions. The resulting effective MENM energy function smoothly interpolates between the original potential energy surfaces, and retains the beginning and end structures as two local minima. Saddle points, transition paths, potentials of mean force, and partition functions can be computed efficiently by largely analytic methods. To characterize the protein motions during a conformational transition, we follow "transition paths" on the MENM surface that connect the beginning and end structures (via a saddle point). To illustrate this general method, we study large-scale conformation changes of myosin II motor and chaperonin GroEL. We generate possible transition paths for these two proteins that reveal details of their conformational motions, including the sequence of forming/breaking of residue contacts. The MENM formalism is computationally efficient and generally applicable, even for large protein systems that undergo highly collective structural changes.

References

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1739-Plat A New Approach To the Analysis of Protein Structure

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The eigendecomposition of a square distance matrix is a complete and simple description of a system of points. It has at most 5 nonzero, interpretable terms: a dominant eigenvector is proportional to r^2 - the square distance of points to the center of the mass, and the next three are principal components of the system of points. This new observation has many possible applications in protein science.

(i) We show that knowing r we can approximate a distance matrix of a protein chain with the expected values of drms about 4.5 Å.
(ii) We can also explain the role of hydrophobic interactions for the protein structure, because r is highly correlated with a hydrophobic profile of the sequence.
(iii) Moreover, r is highly correlated with several sequence profiles which are useful in protein structure prediction, such as a contact number, residue-wise contact order (RWCO) or mean square fluctuations. Hence we expect that estimation of r from the sequence could improve protein structure predictions.

1740-Plat Unfolding of Single Maltose Binding Protein

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The mechanical unfolding of individual proteins by single-molecule force spectroscopy (SMFS) complements more classical methods using either temperature or chemicals as denaturants. In contrast to much of the literature, which focuses on mechanical proteins such as those in adhesion, in the work reported here we decided to explore whether structural transitions of proteins without an obvious mechanical function could be uncovered by mechanically forced unfolding. We chose maltose binding protein (MBP) to see whether two state behavior would be preserved. If not two-state behavior, then what would be observed? Comparisons of the measured forces with those from numerical simulations will be made. These results additionally assist in studies where maltose binding protein is included in a fusion protein.

1741-Plat Mapping alpha-helical Induced Folding Within The Intrinsically Disordered C-terminal Domain Of The Measles Virus Nucleoprotein By Site-Directed Spin-Labeling EPR Spectroscopy

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In this study, we used site-directed spin-labeling EPR spectroscopy to study the induced folding of the intrinsically disordered C-terminal domain of measles virus nucleoprotein (N_{TAIL}). 14 single-site NTAIL mutants were prepared and labeled with a nitroxide paramagnetic probe. EPR spectroscopy allowed us to monitor the gain of rigidity that N_{TAIL} undergoes in the presence of either the secondary structure stabilizer 2,2,2-trifluoroethanol (TFE) or one of its physiological partners, namely the C-terminal domain (XD) of the viral phosphoprotein. The mobility of the spin labels grafted within the region spanning residues 488–522 was significantly reduced upon addition of XD, with the 488–502 region being the most affected. Conversely, the mobility of the spin labels bound to positions 407 and 460 was unaffected. Furthermore, in the presence of 30% sucrose and of XD, the EPR spectra of N_{TAIL} variants bearing the spin label grafted within the 488–502 region, are indicative of the formation of an *a*-helix in the proximity of the paramagnetic probes. Such an *a*-helix is partly preformed also in the unbound state, as judged based on studies in the presence of urea. Using TFE we unveiled a previously undetected structural propensity within the N-terminal region of N_{TAIL}, and showed that its C-terminal region "resists" to gaining structure even at high TFE concentrations. Finally, we for the first time showed the reversibility of the induced folding process that N_{TAIL} undergoes in the presence of XD.

1742-Plat A Computational Approach to Predicting the Structures and Properties of Therapeutic Peptides

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Short peptides have become critical new sources of drugs in recent years. These molecules offer low toxicity, are extremely effective in inhibiting disease-implicated protein interactions, and treat a vast range of conditions. Computational methods potentially offer powerful rational peptide design tools for these systems. Unfortunately, current state-of-the-art bioinformatics-based protein structure algorithms often fail for peptide therapeutics because these molecules are small, hence often flexible, and because they frequently include non-canonical amino acids to modulate functionality or reduce degradation in vivo by serum proteases. Here, instead, we apply a novel physics-based approach, relying on proper thermal sampling and molecular potential energy functions, and a conformational search protocol based on a putative folding mechanism called the Zipping and Assembly Method (ZAM). ZAM works by breaking a polypeptide chain into small fragments, simulating each fragment independently, and then successively pairwise reassembling them along multiple folding pathways into larger segments with further sampling. It gives full conformational ensembles and folding pathways, learned by computing free energies as fragments fold. We use this approach to predict the conformational ensembles of a set of model therapeutic peptides. These structures are then coupled with coarse-grained theories of transport and association in order to model the interactions of peptides with membranes (for insertion processes), proteins (for binding processes), and other peptides (for aggregation processes). Importantly, this approach is able to capture highly sequence-specific effects due to the atomistic nature of the ZAM folding simulations, providing a broad-based predictive tool for targeted sequence mutations in peptide engineering.

1743-Plat NMR Investigations of Intrinsically Disordered Proteins: Examples of Conformational Selection and Membrane Environmental Induction of Structure

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The γ-subunit of cGMP phosphodiseterase (PDEγ) plays a central role in G-protein coupled visual signaling events through alternate interactions with the catalytic α, β-subunits of PDE6, the α-subunit of transducin (γ_t), and the G protein signaling regulator RGS9. In identifying transitory structure present in this intrinsically disordered protein we used a variety of NMR approaches: chemical shift analysis, residual dipolar coupling measurements, and paramagnetic relaxation enhancement NMR measurements from ten PDEγ variants, each with an introduced Cys residue reacted to introduce a paramagnetic spin-label. The solution structure revealed a loosely

compact fold with transient structural features resembling those seen previously in the X-ray structure of an active complex containing PDEγ46–87. NMR mapping of the interaction between PDEγ46–87 and the chimeric PDE5/6 catalytic domain confirmed that the C-terminal residues (74–87) of PDEγ are involved in the association and demonstrated that its W70 indole group, which is critical for subsequent binding to α_t, is left free. These results indicate that the interaction between PDEγ and α_t, which occurs during the phototransduction cascade, involves the selection of preexisting transient conformations. We compare these results with those from a published NMR study (1) of another intrinsically disordered protein, a plant-specific protein in the photosynthetic thylakoid membrane (TSP9). This study showed that non-phosphorylated TSP9 and a mimic of its tri-phosphorylated form both are disordered under aqueous conditions but adopt an ordered conformation in the presence of detergent micelles. The results provided a structural model for the role played by TSP9 in its biological function.

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Platform AH: Self-Assembled Session: KCNE Peptides (MiRPs): Essential Components of Voltage-gated Potassium Channels

1744-Plat KCNQ1 S6 Segment Determines Modulation by KCNE4

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The voltage-gated potassium (Kv) channel KCNQ1 is modulated by members of the KCNE family of single transmembrane proteins. The related Kv channel KCNQ4 can also be modulated by KCNE proteins but with very different effects. For example, KCNE4 completely suppresses current carried by KCNQ1 but not KCNQ4 channels. Using a KCNQ1/KCNQ4 chimera strategy, we have mapped structural determinants of the KCNE4 effect. Based upon previous work, we hypothesized that important structural determinants of KCNE4 interaction reside within the S6 segment. Using heterologous expression in CHO cells we first examined the functional properties of a KCNQ1 chimera having the S6 sequence of KCNQ4 in the absence or presence of KCNE4. Unlike the parent KCNQ1 channel, this chimera was resistant to the suppressive effects of KCNE4. By contrast, the converse chimera (KCNQ4 with the S6 of KCNQ1) was dramatically inhibited by KCNE4. Subsequent KCNQ1 chimeras incorporating subregions of S6 from KCNQ4 also exhibited resistance to KCNE4. However, a KCNQ1 chimera in which four residues in the N-terminal half of S6 were substituted with the corresponding residues of KCNQ4 (Q1–Q4 [324–328]) was resistant to KCNE4 inhibition. Interestingly, this chimera still generated ^{1}Ks when co-expressed with KCNE1. Our results indicated that the KCNQ1 S6 segment contains residues critical to the channel's response to KCNE4 but are independent of structures required for the modulation by KCNE1.

1745-Plat KCNQ1 and KCNE1 Make State-dependent Contacts in Their Extracellular Domains

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Previous studies on (KCN)Q1/(KCN)E1 interactions in the IKs channel complex largely focused on the transmembrane/cytoplasmic regions. However, intimate interactions between Q1/E1 in their extracellular domains are possible: short QT- or familial-atrial-fibrillation (fAF)-related Q1 mutations S140G & V141M (immediately outside S1) do not affect Q1 channel function when expressed alone, but markedly affect the IKs channel function when coexpressed with E1. Likewise, mutations in E1 extracellular domain have been linked to long QT or fAF, indicating perturbations of IKs channel function. We used the 'disulfide bond mapping' approach to explore extracellular contact points between Q1 and E1, focusing on Q1 positions 140–147 and E1 positions 36 – 43. Native Cys(teines) were removed. New Cys were engineered into positions listed above. We expressed different combinations of Cys-substituted Q1/E1 pairs in COS-7 cells and used Western blotting under non-reducing conditions to test which pairs could form disulfide-bonded Q1/E1 complexes (80-kDa, vs Q1 60-kDa and E1 20-kDa). WT-Q1/Cys-substituted E1 or Cys-substituted Q1/WT-E1 served as negative control, and we tested whether DTT could abolish the 80-kDa band. For positive Q1/E1 pairs, we used oocyte expression to check: (a) mutations per se did not disrupt Q1/E1 interactions, and (b) putative disulfide bond formation impacted on IKs channel function. We identified disulfide bond formation between Q1-144C and E1-40C or E1-41C, and between Q1-145C and the same E1 Cys residues. Oocyte experiments suggested that Q1-145C formed disulfide bond with E1-40C preferentially in the open state, and the disulfide-bonded conformation locked the IKs channel in a constitutively active state. In contrast, disulfide bond formation between Q1-I145C and E1-41C stabilized the IKs channel in the closed state. The state-dependent contacts between Q1 & E1 will be useful spatial constraints in building IKs structural models of different gating states.

1746-Plat S1 Constrains S4 Sensor In Kv7.1 K^+ Channels: Interaction With KCNE1 Auxiliary Subunit

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