Meeting-Abstract

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Instrinsically Disordered Proteins Subgroup

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3-Subg Protein Intrinsic Disorder and Oligomericity in Immune Signaling

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Recent structural and genomic data have clearly shown that many proteins contain long regions termed intrinsically disordered (ID) or unstructured that do not adopt any globular structures under native conditions. Intriguingly, a highly flexible, random coil-like conformation constitutes the native and functional state for many proteins known to be involved in cell signaling. Reports in recent years revealed that these long ID domains preferentially occur on the cytoplasmic side. Examples include key components of immune signaling: the cytoplasmic regions of the multichain immune recognition receptor (MIRR; i.e., T and B cell receptors, Fc receptors, etc.) signaling subunits. Surprisingly, these unstructured domains exhibit specific dimerization distinct from non-specific aggregation behavior seen in many systems. Circular dichroic analysis and diffusion and chemical shift mapping NMR data show that the dimerization of these molecules is not accompanied by a structural transition to a folded form. This finding opposes the generally accepted view on the behavior of ID proteins and provides evidence for the existence of specific dimerization interactions for these protein species, thus opening a new line of research in this new and quickly developing field. The unusual homotypic interactions between ID cytoplasmic domains of MIRR signaling proteins have been used to develop a novel model of immune signaling that has been successfully applied in different fields of immunology and pharmacology. Application of this model to platelet signaling has already led to the development of a novel concept of platelet inhibition and the invention of new platelet inhibitors.

4-Subg Intrinsically Disordered Proteins in Human Diseases

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Intrinsically disordered proteins (IDPs) lack stable tertiary and/or secondary structure under physiological conditions *in vitro*. They are highly abundant in nature and often they are involved in regulation, signaling and control pathways, where binding to multiple partners and high-specificity/low-affinity interactions play a crucial role. Functions of IDPs may arise from the specific disorder form, from inter-conversion of disordered forms, or from transitions between disordered and ordered conformations. The choice between these conformations is determined by the peculiarities of the protein environment, and many IDPs possess an exceptional ability to fold in a template-dependent manner. IDPs are key players in protein-protein interaction networks being highly abundant among hubs. Numerous IDPs are associated with such human diseases as cancer, cardiovascular disease, amyloidoses, neurodegenerative diseases, diabetes and others. Overall, there is an intriguing interconnection between intrinsic disorder, cell signaling and human diseases, which suggests that protein conformational diseases may result not only from protein misfolding, but also from misidentification and missignaling. IDPs, such as α-synuclein, tau protein, p53, BRCA1 and many other disease-associated hub proteins represent attractive targets for drugs modulating protein-protein interactions. Therefore, novel strategies for drug discovery are based on intrinsically disordered proteins.

Permeation Transport Subgroup

5-Subg Structure of the Na,K-pump with Occluded Rb Ions

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The Na,K-ATPase belonging to the P-type ATPase family uses energy derived from ATP to pump Na ions out of the cell and K ions into the cell across the plasma membrane. It is composed of alpha- and beta-subunits and interacts with regulatory FXYD proteins, such as the gamma-subunit in kidney. This lecture will present and discuss the recently determined X-ray crystal structure of the pig renal Na+,K+-ATPase at 3.5 Å resolution with bound potassium or rubidium ions. It provides the first view of the architecture of multi-subunit P-type ATPases and the first sight of occluded countertransported ions. The C-terminus shows unique structural features, whose functional consequences have been probed by mutagenesis studies. Other aspects of the structure also point to new regulatory principles.

Motility Subgroup

6-Subg Three-dimensional Reconstruction of Cardiac Muscle Myosin Filaments from a Mouse MyBP-C Knockout Model for Human Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is an inherited disease caused mainly by mutations in myosin and myosin binding protein-C (MyBP-C), proteins that together with titin form the thick filaments. The mechanisms by which these mutations cause HCM are unknown. Most MyBP-C mutations result in failure of MyBP-C to bind to the thick filament. To determine the structural effects of the absence of MyBP-C, we have analyzed thick filaments from a mouse MyBP-C knockout model for HCM (Harris et al., 2002. Circ Res. *90*:594). Filaments isolated from wild type and knockout hearts

were observed by negative staining EM, and their 3D reconstructions (computed by single particle methods) compared. The wild type reconstruction showed, at 4 nm resolution, the near-helical organization of the myosin heads, intramolecular head-head interactions, and immunoglobulin/fibronectin domains of titin and MyBP-C (Zoghbi et al., 2007, Biophys. J. *92*. 373a.). MyBP-C knockout filaments looked similar to the wild type in negative stain (Kensler 2007, Biophys. J. *92*. 297a.). However, the MyBP-C knockout reconstruction had a lower resolution (7 nm), suggesting that MyBP-C helps to stabilize the relaxed array of myosin heads in wild-type filaments. Titin domains were not distinguished at this resolution. Some of the intramolecular head-head interactions observed in wild-type filaments were absent in the knockout. Since such head interactions are important for the relaxed conformation of the filament, our results suggest that MyBP-C knockout filaments might not relax as fully as the wild-type. Poorer relaxation in the absence of MyBP-C may help explain compromised cardiac relaxation in MyBP-C knockout mice, and may also be related to the abnormal diastolic function in humans with HCM.

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7-Subg Computational Dissection of Intramolecular Interface Binding Energies in Various Myosin States

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Several crystal structures of the head domain of myosin are thought to represent various states in the crossbridge cycle. Here we offer an objective way to examine the various aspects of the energies involved in the myosin molecule and test hypotheses of force production and regulation. For instance, the 50k/25k cleft, the HW helix[1] and the 7 strand beta sheet are currently implicated as substructures capable of storing stress (tension) and productively releasing it as work at later points in the crossbridge cycle. We examined these energies and the implication of intramolecular interface (intraface) formation, dissolution and motion by computing ΔΔG of specific amino acids and their binding to neighboring substructures by virtual alanine scanning[2] intrafacial energies. We then compared these values in identical amino acids at various states in the crossbridge cycle. This produced a ΔΔΔG value describing how energy shifts inside the head at various states in the crossbridge cycle. These values represent the shifts in stress within myosin and within the crossbridge cycle. These intrafacial energy values and stress and strain estimates, combined with the empirical values of free energy of ATP hydrolysis, enthalpy and work output set limits for values of the energy necessary (and perhaps mechanism) to produce regulation as well as the interfacial energies associated with the actin myosin interface.

References

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Biological Fluorescence Subgroup

8-Subg In Vivo and In Vitro Studies of Endophilin Oligomerization

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Endophilin, which is involved in membrane vesiculation in receptor mediated endocytosis and vesicle trafficking, is a 40-kDa SH3 domain-containing protein that binds to the PRD domain of dynamin and to synaptojanin, a phosphoinositide phosphatase implicated in endocytosis. The N-terminus of endophilin contains a so-called BAR domain and the recent solution of its structure has suggested a mechanism for the ability of endophilin to induce membrane curvature. It has been suggested that dimerization of BAR domains results in a concave, positively-charged surface that can interact with, and thereby deform, membranes containing negatively charged lipids. It has been also been suggested that endophilin may be a monomer in the cytoplasm which can then dimerize upon binding to membranes or perhaps upon binding, via its SH3 domain, to dynamin's PRD domain. To clarify these issues we have studied the oligomeric state of endophilin, both in vitro (using AUC and fluorescence polarization) and in vivo. EGFP-endophilin, expressed in CV-1 cells, were studied using two-photon fluorescence correlation spectroscopy (FCS). The FCS data were analyzed using the Q-analysis method which allowed for determination of the intrinsic "brightness" of the labeled protein complexes and hence its aggregation state in the cytoplasmic regions of the cell. Despite a relatively high Kd (~5 micromolar) observed in vitro, the in vivo measurements indicate that endophilin is dimeric in the cytoplasm even at submicromolar concentrations.

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Membrane Structure and Assembly Subgroup

9-Subg Hydration of POPC Bilayers Studied by ^{1}H-PFG-MAS-NOESY and Neutron Diffraction

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Location of water molecules in POPC bilayers and the lifetime of water-lipid associations was studied by nuclear Overhauser en-