26S proteasome uses the same "Key-in-a-lock mechanism", but the C-termini of these six different Rpt ATPases' play distinct roles, some participate in complex formation while others cause gate-opening. This novel "key-in-a-lock" mechanism for gate opening contributes to the energy-dependence of intracellular protein degradation.

2596-Symp CryoEM Studies of Small Heat-Shock Proteins

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Small heat shock proteins (sHsps) are a family of chaperones that bind unfolded proteins and prevent irreversible aggregation. sHsp's share a conserved central alpha-crystallin domain, ~90 amino acid long, with variable N- and C-termini. The polydispersity of human alpha-crystallin and Hsp27 have precluded their high resolution structural determination. Hsp16.5 from *Methanococcus jannaschii* forms a symmetrical assembly and has been studied by x-ray crystallography, cryoelectron microscopy (cryoEM) and site-directed spin labeling EPR (SDSL-EPR). CryoEM and SDSL-EPR studies of several engineered Hsp16.5 variants have revealed how modifying the N-terminal region can modulate the oligomer size as well as its oligomeric state (monodisperse and symmetrical or polydisperse). CryoEM results of Hsp16.5 wild-type, as well as engineered variants, complexed with a model substrate will be presented. The Hsp16.5 variants include a truncated form (Hsp16.5TR) that lacks the N-terminal 33 residues of wt Hsp16.5 and a form (Hsp16.5-P1) that has a proline-rich 14aa peptide from Hsp27 inserted between the N-terminal region and the alpha-crystallin domain. We use a thermodynamically unstable mutant of T4 lysozyme (T4L) as the model substrate. Our results on the Hsp16.5 wt/T4L complex indicate that substrate binding can trigger significant changes in the quaternary structure of the oligomer. Our results from the Hsp16.5 variants indicate particular regions of Hsp16.5 that are likely to be involved in substrate binding. In addition a cryoEM structure of Hsp16.5-R107G will be presented, which offers a clue to the impaired functionality of the human disease related R120G mutation in alpha-crystallin. Our structural studies indicate that the adaptable quaternary structure of sHsps is related to their ability to bind substrate.

Symposium 19: Allostery and Dynamics in Protein Function

2597-Symp Defining Native Protein Ensembles Using X-ray Crystallography

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Although proteins populate large structural ensembles, protein X-ray diffraction data are traditionally interpreted using a single dominant model. We developed two methods – Ringer and tau – that use direct electron-density sampling to measure structural polymorphism. Ringer analysis around dihedral angles of high-resolution structures suggests that in addition to side chains already built in multiple conformations, 5–15% of residues populate "missing" unmodeled rotamers. The side-chain tau value affords a complementary, model-independent metric of rotamer ensembles. The tau value is correlated to the Shannon entropy, connecting for the first time a crystallographic measurement with a thermodynamic quantity, the residual entropy of each residue. These results show that crystalline proteins are more polymorphic than current crystallographic models. Examples of polymorphism in allosteric switching, ligand binding and enzymatic catalysis will be discussed. The structural and functional implications of this polymorphism depend critically on the degree of conformational coupling. Independent conformers would contribute residual entropy to the native state, while simultaneous structural changes can provide a mechanism for signaling. Overall, our analyses using Ringer and tau indicate that far from providing a static picture of proteins, X-ray crystallographic data reveal populations of alternate structures that reflect the dynamics essential for folding, binding, catalysis and regulation.

2598-Symp Conformational Waves in Receptors

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Acetylcholine receptor-channels (AChRs) are large (~300 kD, five subunits) allosteric membrane proteins that switch between C(losed) and O(pen) conformations. We use Φ, a parameter derived from the forward (f) and backward (b) rate constants of a reaction, to provide temporal information about the moving parts of the AChR in C↔O 'gating'. Φ is the slope of a linear fit to a plot of log f vs log Keq (=f/b) for a family of mutations of a single residue. First, we develop a Markov model of the transition region (TR) and show that Φ reflects the relative time in the TR that the perturbed residue switches, in an all-or-none fashion, from a C-like to an O-like structure. Second, we describe the map of Φ-values for >300 different AChR mutant constructs. There is, approximately, a coarse-grained and decreasing gradient in Φ between the allosteric (transmitter-binding) sites and the catalytic site (the 'gate'). Third, we use this map and theory to *calculate* the shape of the micro-barriers of the TR, which turns out to be nearly flat. Finally, we use the 'speed-limit' for channel-opening to estimate the rates of the microscopic transitions between the intermediates states that link stable C with stable O. AChR gating is, approximately, a brownian conformational 'wave' in which nm-sized domains move back-and-forth (on ~50 ns timescales) to connect the stable ground states of the reaction.

2599-Symp Endopeptidases that cleave specific proteins

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Caspases are thiol endopeptidases that cleave specific proteins after aspartic acid residues and drive apoptosis or inflammation upon zymogen activation. Despite considerable interest in developing

active site inhibitors to these enzymes the highly charged nature of the substrate binding site has yielded relatively intractable chemotypes for drug discovery. We have discovered a novel allosteric site in both apoptotic capsases −3, and −7 and the inflammatory caspase−1. This involved the use of a site-directed fragment-based approach to drug discovery, called Tethering® or disulfide-trapping. In this case a native thiol at the dimer interface of caspases was allowed to react reversibly under thiol exchange conditions with a small library of disulfide-containing small molecules. The site-directed character of the approach can focus the discovery process on unactivated enzymes and allosteric sites which would otherwise be difficult to selectively target. We identified selective tethered compounds for these allosteric sites and will present their structures and mechanisms for inhibition (Hardy et al. PNAS *101*, 12461 (2004); Scheer et al., PNAS *103*,7579 (2006)). Mutational analysis reveals an "allosteric circuit" that connects these sites and we believe is naturally involved in their regulation. We have further developed chemical and immunologic tool that trap these transitions so they may be studied in vitro and in cells.

Platform AX: Membrane Proteins - III

2600-Plat Lateral Sorting of Influenza Virus Hemagglutinin in Membranes

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Hemagglutinin (HA) is a homotrimeric glycoprotein embedded in the envelope of Influenza virus. It mediates binding of the virus to the host cell as well as fusion between the viral envelope and the endosomal membrane. HA has been reported to play also an important role in budding of the new viral particles from the host cell. Rafts reflecting liquid-ordered lipid domains enriched of sphingomyelin and cholesterol have been suggested as sites for local recruitment of viral components. HA is supposed to entrap in those lipid domains. In order to elucidate the role of the HA transmembrane domain in lipid raft localization we expressed constructs harboring the transmembrane domain and the cytoplasmic tail but lacking the N-terminal ectodomain of HA in the plasma membrane of mammalian cells (MDCKII, CHO-K1). The N-terminus of the transmembrane domain was tagged with YFP (HA-YFP). We studied Foerster's energy transfer (FRET) between the artificial HA-YFP and a GPI anchored CFP as a raft marker by fluorescence lifetime imaging microscopy (FLIM). First results suggest that HA constructs are indeed sorted and enriched into cholesterol dependent lipid domains indicated by enhanced FRET efficiency. This is supported by the observation that cholesterol depletion of the plasma membrane caused a significant decrease of FRET. Likewise, deletion of the three highly conserved palmitoylation sites of HA is also accompanied by a reduction of FRET efficiency. Taken together, the results are in agreement with sorting of HA constructs into cholesterol-enriched lipid domains.

2601-Plat Solution Structure of Integral Membrane Protein from *Mycobacterium tuberculosis*: Insight into Rv0008c - an FtsZ Inhibitor and Its Complex

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Our goals are to determine the structure of Rv0008c, an FtsZ inhibitor, a critical component of the cell division machinery from *Mycobacterium tuberculosis* and to study the binding and interface of this protein and Rv0011c by solution NMR and biophysical methods. Rv0008c is a small, conserved integral membrane protein consisting of 145 residues. It is in a gene cluster that we have shown to form a multi-protein complex with Rv0010c and Rv0011c - a homologue of CrgA, an FtsZ inhibitor. Rv0008c is a potential tuberculosis drug target. A detailed three-dimensional structure of this protein will help to understand its critical role in FtsZ depolymerization and therefore this will help to suggest a possible cell division control mechanism in *M. tuberculosis*.

Backbone assignments of Rv0008c were obtained from 2-D and 3-D TROSY-based experiments on uniformly $^{13}C/^{15}N$-labeled Rv0008c protein in DPC micelles. Residual dipolar couplings were obtained for protein in compressed polyacrylamide gels by using gNtrosyS3 experiments. Paramagnetic relaxation enhancement (PRE) distances have been measured by using site-directed parallel spin-labeling method. These distances were derived by comparing the difference in peak volumes in the presence of paramagnetic or diamagnetic agents based on the HSQC experiments. We have completed 90% of the backbone assignments and achieved two different alignments for Rv0008c in uncharged and positive charged acrylamide gels.

We have obtained a good number of PRE distances for nine spin label sites and have calculated the initial structure for Rv0008c. We have also identified and mapped out the residues involving in the interaction of Rv0008c and Rv0011c. In addition, mutations have been made in the transmembrane helices of Rv0008c and Rv0011c to further study the interaction of Rv0008c and Rv0011c.

2602-Plat Controlling the Inhibition of the Sarcoplasmic CA^{2+}-ATPase by Tuning Phospholamban Structural Dynamics

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