showed that insertion of Hsp70 into the bilayer was proportional to the PS concentration. In contrast, Hsp90 did not incorporate into PS liposomes. Hsp70 was found integrated into the lipid bilayer as demonstrated by lack of extraction by sodium carbonate or sonication treatment. Hsp70 inserted into PS liposomes could only be solubilized by non-ionic detergents. In cells, the presence of Hsp70 on the plasma membrane correlates with the flipping of PS to the outside of the membrane. These results demonstrate that Hsp70, which does not contain a predictable hydrophobic trans-membrane region, can spontaneously get inserted into a lipid environment by a process that may require the traslocation of PS across the lipid bilayer.

180-Pos

Single-Molecule Imaging of 1:2 Groel-Groes Complexes in Zero-Mode Waveguides

Tomoya Sameshima[1], Taro Ueno[1,2], Junichi Wada[3], Ryo Iizuka[1], Mutsuko Aoki[3], Naonobu Shimamoto[3], Iwao Ohdomari[3], Takashi Tanii[3], Takashi Funatsu[1,4].
[1]The University of Tokyo, Tokyo, Japan, [2]Stanford University, Stanford, CA, USA, [3]Waseda University, Tokyo, Japan, [4]Core Research for Evolutional Science and Technology, Tokyo, Japan.

GroEL is an *Escherichia coli* chaperonin which is composed of two heptameric rings. GroEL interacts with its cofactor GroES and assists protein folding in an ATP dependent manner. Because of negative cooperativity between two rings of GroEL in the binding of ATP, it has been generally believed that an asymmetrical 1:1 complex is only a functional form for over a decade. Contrary to the belief, we revealed that a symmetrical 1:2 GroEL-GroES complex can be formed in the presence of denatured protein using fluorescence resonance energy transfer (FRET) and fluorescence correlation spectroscopy (FCS). However, the dynamics of GroEL-GroES interaction including a 1:2 GroEL-GroES complex is still unclear. To clarify this issue, 1:2 GroEL-GroES complexes were observed using zero-mode waveguides (ZMWs) at a single molecule level. ZMWs are nanoholes array fabricated in a thin metal film. They can reduce excitation volume compared to total internal reflection illumination; therefore, they make it possible to observe individual 1:2 GroEL-GroES complexes at sub-μM concentrations that are required to form the complexes. Cy3-GroES and Cy5-GroEL binding to and dissociating from Alexa488-GroES immobilized on the bottoms of ZMWs were visualized. Cy3-GroES and Cy5-GroEL were co-localized in GroES-immobilized ZMWs for ~ 3 s. The duration time in ZMWs without immobilizing Alexa488-GroES, which reflected non-specific adsorption of GroEL-GroES complex to the bottoms of ZMWs, was ~ 1 s. These results showed that 1:2 GroEL-GroES complexes were successfully observed at a single-molecule level and their duration time was estimated to be ~3 s. Furthermore, the same experiment was carried out in the absence of denatured protein. The duration time of Cy3-GroES and Cy5-GroEL in Alexa488-GroES-immobilized ZMWs was ~1 s. This result indicated that 1:2 GroEL-GroES complexes disappeared in the absence of denatured protein, being consistent with FRET and FCS experiments.

181-Pos

Crystal Structure of *Drosophila* Unc-45, a Putative Myosin Chaperone

Chi F. Lee[1], Arthur V. Hauenstein[1], William C. Gasper[1], Banumathi Sankaran[2], Sanford I. Bernstein[1], Tom Huxford[1].
[1]San Diego State University, San Diego, CA, USA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

UNC-45 is a chaperone that may aid in folding myosin's motor domain. Mutations in UNC-45 cause muscle defects and dysfunction, due to the importance of myosin in muscle structure and contraction. UNC-45 is composed of an N-terminal tetra-tricopeptide (TPR) domain, a C-terminal UCS (UNC-45/Cro1/She4 homology) domain, and a central region that links these. Here we present the crystal structure of *Drosophila* UNC-45 (dUNC-45), which should serve as a basis for attaining a detailed understanding of its mechanism of action. Bacterially expressed recombinant His-tagged dUNC-45 was purified sequentially using immobilized metal affinity chromatography and size exclusion chromatography. The protein eluted as a single peak, indicating a homogeneous population of protein suitable for crystallization. Crystals were prepared by hanging drop vapor diffusion and x-ray diffraction data were collected to a limit of 3.0 Å resolution. For phase determination, a seleno-methionine derivative dUNC-45 crystal was prepared for single wavelength anomalous dispersion (SAD) experiments. Synchrotron data were collected at the Berkeley National Laboratory Advanced Light Source. The diffraction data were processed in HKL2000. Selenium positions were determined and refined in Phenix. The resulting structure was refined against native data using Phaser and maximum-likelihood refinement with Refmac5. Model building was performed in COOT. Our current model has *R*-cryst and *R*-free values of 0.24 and 0.28 respectively. The TPR domain is not visible in the model, likely due to flexibility of the domain within the crystal. Overall, the UCS domain is composed of α-helices that form armadillo repeats which stack together to form a supercoiled structure. Based on our structure, it appears that the central and UCS domains make up a single structural unit. Currently, we are pursuing structure-based biochemical assays to pinpoint the protein surfaces that are involved in the binding and chaperone activities of dUNC-45.

182-Pos

Clpxp Degradation of Proteins Probed By Single-Molecule Fluorescence

Yongdae Shin, Joseph H. Davis, Ricardo R. Brau, Andreas Martin, Tania A. Baker, Robert T. Sauer, Matthew J. Lang.
MIT, Cambridge, MA, USA.

ClpXP is an AAA+ protease that unfolds and degrades target proteins. ClpX, a hexameric ring-shaped ATPase, recognizes specific proteins and then powers their mechanical denaturation and translocation into the degradation chamber of ClpP where polypeptide bond cleavage occurs. Although ClpXP degradation activities have been widely studied at the bulk solution level, the operating principles and detailed mechanisms of this complex macromolecular machinery remain unanswered. Here, we probe the kinetics of substrate unfolding and degradation by ClpXP using a single-molecule fluorescence assay. These assays employ a covalently crosslinked ClpX hexamer, immobilized on PEG coated surface illuminated by total internal reflection fluorescence. A series of substrates are engineered to contain fusion of an N-terminal Cy3 and a C-terminal GFP-titin-ssrA module. In the presence of ATPγS, ClpX stalls at GFP after degradation of titin-ssrA domains. These stalled pre-engaged substrates are stably bound to ClpXP even in the absence of Mg^{++}, but are released quickly upon the introduction of nucleotide-free solution. Exchange into ATP for pre-engaged substrate-ClpXP complexes allows synchronous resumption of unfolding and degradation of GFP and any following domains. The time required for complete degradation is measured by loss of the N-terminal Cy3 from the protease complex. GFP unfolding can also be monitored directly with quenching of intrinsic fluorescence by denaturation. Global fitting of single-molecule data for a set of related substrates yields time constants for ClpX unfolding, translocation, and a terminal step which may involve product release, and shows strong agreement with bulk solution measurements. It should be possible to extend these methods to allow single-molecule studies such as FRET for real-time assays of ATP-fueled conformational changes that drive the mechanical operations of the ClpXP protease. Support from the NSF Career Award (0643745) is gratefully acknowledged.

Protein-Ligand Interactions I

183-Pos

Efficacy As An Intrinsic Property of the M2 Muscarinic Receptor in Its Oligomeric State

Dar'ya S. Redka, Heiko Heerklotz, James W. Wells.
University of Toronto, Toronto, ON, Canada.

G protein-coupled receptors exhibit a characteristic dispersion of affinities for agonists, and the breadth of that dispersion is correlated with efficacy. The implied heterogeneity is commonly attributed to a ligand-regulated transient complex between the receptor and the G protein; that is, agonists bind with higher affinity to the complex than to the receptor alone. Such an arrangement is at odds with observations that the receptor-G protein complex is less transient than required by such models, and that GPCRs exist as oligomers. The present investigation has been directed toward the alternative notion that heterogeneity emerges as a property of the oligomer and is independent of G proteins. M_2 muscarinic receptors were purified as monomers from *Sf*9 cells and reconstituted as tetramers devoid of G proteins in phospholipid vesicles (POPC/POPS/cholesterol). The antagonists *N*-methylscopolamine and quinuclidinylbenzilate recognized a single class of sites in assays with *N*-[^{3}H]methylscopolamine; in contrast, seven agonists recognized at least two classes (log K_H and log K_L). The magnitude of that dispersion was quantified empirically as the product of the difference in affinity for the two classes ($\Delta\log K$) and the fraction exhibiting higher affinity (F_H). The value of $F_H\Delta\log K$ equals the area between the observed curve and a curve of equal amplitude and a single affinity corresponding to log K_L. It therefore prefigures the shift that could be effected by GTP acting via a G protein, were it present. The values of $F_H\Delta\log K$ generally were higher for full agonists than partial agonists. This distinction among agonists and antagonists recalls the propensity of those ligands to elicit a response. It follows that the heterogeneity revealed by agonists, which is predictive of efficacy, is a property intrinsic to the receptor in its oligomeric state.