and selective cPKC activation by cell-permeable cPKC activator peptide mimicked this effect. A mutation in a putative PKC phosphorylation site in the auxiliary subunit KCNE1 (S102A) specifically abolished the voltage shift by α_1-AR stimulation or cPKC activation, suggesting cPKC phosphorylation of KCNE1(S102) increases IKs current through facilitation in voltage dependence of activation. LQT1 associated mutations at the voltage activation domain (S1-S5) of KCNQ1 subunit, also impaired cPKC regulation of the IKs channel independently of β-AR regulation, suggesting that the interactions between the KCNE1 and the voltage-gating domain of KCNQ1 is crucial for cPKC regulation. In conclusion, our study indicates that the voltage activation of IKs by cPKC isoform is important in the IKs channel regulation under adrenergic stimulation and that impairment of this regulation may be linked to LQT1 clinical phenotype.

PLATFORM AF: Protein Assemblies

1082-Plat
Conical Tomography: A Simple Method to Study Proteins in Cells at High Resolution
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Despite advances in molecular biology and genetics, the location of thousand of proteins in cells remains undetermined. The principal problems are their small dimensions and their capacity to form large assemblies by associating either with themselves or other proteins. We took advantage of the tendency to form aggregates and developed a simple method that describe the three-dimensional structure of these assemblies in cells at high resolution (2-3nm) and in three-dimensions. As a proof of principle, we studied the alphaA-crystalline, a small chaperone that plays an important role in lens transparency and cataract formation. To identify the assemblies containing the chaperone, lens tissues labeled with primary anti-alphaA-crystalline and probed with both 2nm and 5nm diameter gold particle conjugates were reconstructed by conical tomography. First, we determined the location of all gold particles contained within the reconstructed volume. From maps of their 3D-distribution, we determined that gold particles formed files that repeated at 6-7nm center-to-center apart and bent at angles measuring ~90° or ~120°. Second, we identified the tethers that linked each gold particle to the assemblies containing the chaperone. Independent of the diameter of the gold particle, tethers formed by the association of primary and secondary antibodies measured ~14nm in length. Finally, by applying the constraints represented by the repeat period, the angles and the structure of the assemblies, we identified the chaperone in unlabeled tissues as small globules spaced 6-7nm apart decorating thin filaments of the cytoskeleton. In conclusion, the high resolution in three-dimensions, the reliance on geometric constraints instead of exogenous probes and the technical simplicity are unrivaled properties of our method for studying the contribution that proteins made to normal cell homeostasis and pathological conditions.
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1083-Plat
Bind'NGO: Flexible Docking Model for Multiprotein Complexes with Intrinsically Disordered Segments
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Protein-protein interactions play an essential role in many biological processes, such asincluding DNA transcriptional regulation, signal transduction, membrane-protein trafficking and, immune response, etc. Many proteins contain flexible loops or intrinsically disordered segments, limiting the ability of atomic simulationsrigid-body docking models to sample all possible configurations and interaction modes between proteins. Here we develop a residue-level coarse-grained model for simulating multi-protein complexes with intrinsic flexibility. The intermolecular interactions are described by the transferable energy function, which was developed and applied to many weakly binding protein complexes. For the intramolecular interactions, G[[Unable to Display Character: ō]]-type potentials are derived from experimental structures. To controlinvestigate the effectdegree of flexibility of each protein, umbrella potentials along a reaction coordinate of native contacts or distance-root-mean-square deviation (dRMS) are applied with varying spring constants to control the extent of fluctuations. The model is applied to the complexes of ubiquitin and various ubiquitin binding domains. We show that the flexibility generally weakens the binding free energy, owing to the entropic penalty upon binding. However, for some cases, a weak flexible motion in the binding regions increases the binding free energies modestly. The model can be an effective tool for simulating multi-protein complexes with flexible loops or linkers, and be applied to studies of theparticularly where conformational changes upon binding are important.

1084-Plat
Adhesive Water Networks Facilitate Binding of Hydrophilic Protein Interfaces
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It is well known that bulk water plays a crucial role in the biological assembly of hydrophilic surfaces. In this work, we emphasize the special molecular nature of bridging water networks in the formation of biomolecular contacts driven by electrostatic interactions. We have studied the assembly of two hydrophilic protein interfaces. Extensive atomistic molecular dynamics simulations reproducably recovered the native bound state of the Barnase:Barstar complex as seen in the crystal structure of the complex and thus give atomistic insight into the mechanism of binding. The simulations showed the structured water in the interfacial gap to play an adhesive role between the interfaces by forming a strong hydrogen bond network between the interfaces with a reduced dielectric constant compared to bulk. The role of this network is relevant already during the diffusive phase and stabilizes the early intermediate states before native contacts are formed. The convergence to the stereo-specific complex was accompanied by maximizing the interfacial water-mediation and formation of the highly hydrated stereo-specific complex. We introduce a new graph-based methodology to quantify the connectness of water networks.

1085-Plat
Crystal Structure of a Ten-Subunit Human Spliceosomal U1 snRNP at 5.5 Å Resolution
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Most eukaryotic protein-coding genes contain non-coding regions (introns) that separate those coding for protein (exons). The introns must be excised and exons spliced together from the precursor-mRNA transcript of such genes. This process (RNA splicing) is catalyzed by the spliceosome, integral to which are five RNA-protein complexes (U1, U2, U4, U5 and U6 snRNPs). A first step in RNA splicing, functioning to initiate spliceosome assembly, involves recognition of the junction between the 5′-exon and intron (5′-splice site) by U1 snRNP. Human U1 snRNP (~250 kDa) is composed of one RNA (U1 snRNA) and ten polypeptides (seven Sm proteins, U1-A, U1-70K, and U1-C). An experimental electron density map at 5.5 Å resolution enabled us to build U1 snRNA and, in conjunction with site-specific labeling of individual proteins, to place the seven Sm proteins, U1-C and U1-70K into the map. The structure reveals a hierarchical network of interactions between subunits. The seven Sm proteins interact to form a heptameric ring with a single-stranded segment of U1 snRNA leafing through its center. Sm proteins form multiple and varied interactions, with other regions of U1 snRNA as well as other protein subunits, to stabilize the structure of the particle overall. A striking feature is the amino terminus of one subunit (U1-70K), which extends over a distance of ~180 Å, wrapping around the Sm protein heptameric ring, to contact the protein U1-C. The U1-C protein is crucial for 5′-splice-site recognition. In the crystal, the zinc-finger of U1-C interacts with an RNA duplex formed between the single-stranded 5′-end of U1 snRNA and its counterpart from an adjacent complex. This unexpected interaction provides important insight into the critical role of U1-C in recognizing the pre-cursor-mRNA transcript 5′-splice site.

1086-Plat
Nanomechanics of Clathrin Protein Shells
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The nanomechanical properties of protein shells, ranging from virus capsids to clathrin-coated vesicles, are functionally important and of notable recent interest. Using atomic force microscopy (AFM) and, more recently, quartz crystal