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P2X subunits also efficiently form heterotrimeric P2X receptors, heterotrimer formation involving the P2X7 subunit is suggested only for the P2X4 subunit. Here, we performed co-purification experiments with the P2X7 subunit co-expressed with P2X1 or P2X4 subunits in X. laevis oocytes. A structural peculiarity of the P2X7 subunit is its C terminal intracellular domain, which is 120-200 resides longer than that of the other P2X family members. Genetic deletion of the long C-terminal tail of the P2X7 subunit after residues 408, 436 or 505 does not interfere with homotrimerization, because assembly is determined by the P2X ectodomain. We expressed the truncated P2X7 subunit as either non-Strep3-tagged prey protein or Strep3-tagged bait protein in reciprocal co-purification experiments. For comparison, we also co-expressed hP2X71-408-GFP-His or hP2X71-436-GFP-His as prey with His-hP2X1-Strep3 and His-hP2X4-Strep3 as bait. We found that hP2X71-408-GFP-His and hP2X71-436-GFP-His assemble with hP2X4 to stable heterotrimers consisting of two and one copies of P2X7 and P2X1, and vice versa, as indicated by their migration positions in native polyacrylamide gels. In contrast to hP2X4, we could not co-isolate hP2X1 with the truncated hP2X7-GFP subunit, indicating that the hP2X1 subunits do not form heterotrimers with hP2X7-GFP. We also observed heterotrimers formed from full-length GFP-tagged P2X7 subunits and P2X4 subunits, and vice versa, from GFP-tagged P2X4 subunits and non-tagged P2X7 subunits. Altogether, our results provide strong evidence for the existence of P2X4-P2X7 heterotrimers and against P2X1-P2X7 heterotrimers. Electrophysiological experiments underway to elucidate the functional phenotype of the P2X4-P2X7 heterotrimers by the concatamer approach.

1606-Pos

AAA+ regulator MuB distorts DNA to drive forward transposition

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Transposons are mobile genetic elements that drive genomic rearrangements and evolution. Many transposons, such as Tn7, CRISPR-Tn7-like, and Mu, depend on AAA+ proteins to enable target-site selection. MuB, a component of the transposition system from bacteriophage Mu, is an AAA+ protein that regulates the activity of the MuA transposase. Here, we solved a cryo-EM structure of the MuB helical filament that reveals the mechanism of its association with DNA and gives context to existing data on how MuB interacts with MuA. We show that each MuB monomer interacts with DNA through a positively charged loop, and that the DNA is distorted to match the helical parameters of the MuB filament and may engender additional topological deformations in DNA directly outside of the MuB filament. Additionally, comparison of this structure to the AAA+ regulator, TnsC, from the Type V-K CRISPR-Transposase family reveals a startling level of structural similarity. Nevertheless, we also observe crucial structural distinctions that could hint at the adaptations that are needed to allow physical interactions with a DNA-binding target-site recognition module. Our observations that MuB distorts DNA and, from prior research, that mismatched DNA are favored by MuA for transposition suggest that DNA deformation is a key prerequisite of transpososome assembly. All together, these results support a conserved manner of higher-order assembly for AAA+ regulators of transposition and a mechanistic model where DNA distortion by the AAA+ regulator primes the target DNA for subsequent transposition.

1607-Pos

The role of the osteocalcin-osteopontin protein complex in bone biomechanics: a key contributor to energy dissipation at mineral-mineral interfaces

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Bone is a naturally occurring composite mainly made of HydroxyApatite (HAp mineral phase and an organic matrix composed of collagenous and Non-Collagenous Proteins (NCPs). It has become apparent that NCPs role in intrinsic toughening, one of the primary contributors towards healthy tissue's excellent fracture resistance, is considerable. Osteocalcin (OC) and Osteopontin (OPN) are the most abundant NCPs and, together, these are known to form protein complexes that mediate bonding at nanoscale interfaces in the extrafibrillar space. It has been established that these NCP complexes facilitate plastic sacrificial sliding, which is a major contributor to the tissue's excellent fracture toughness. In this study, we use a combination of molecular docking and Steered Molecular Dynamics (SMD) to elucidate the mechanism by which the energy dissipation in the OC-OPN protein complex occurs. The molecular docking results illustrate that the complex formation is mediated by both the random-coil and α-helix structures on the OC surface. Two different structures of straight and compact are identified for the OPN through homology modelling. The central residues of the straight structure contributed to the protein-protein interface, while the residues at both ends of the amino-acid sequence formed the interface for the compact case. SMD simulations are done on two complex structures chosen among tens of structures identified for the complex to study its ability to dissipate energy. In summary, this study for the first time obtained the structure for the OC-OPN complex and identified that it can dissipate large amounts of energy at mineral-mineral interfaces and play a major role in bone toughening. Implementing the SMD results in a CGMD framework, it is deduced that the presence of NCPs in bone extrafibrillar space increases the bone fracture resistance.

1608-Pos

Characterization of ESCRTs proteins encoded by Asgard archaea highlights evolutionary conserved features of the ESCRT-III complex

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Conserved from archaea to mammals, the Endosomal Sorting Complex Required for Transport (ESCRT) constitutes one of the most versatile cellular machinery for membrane remodeling across evolution. Recently, genes encoding for ESCRT proteins have been found in Asgard archaea: a newly discovered superphylum that, according to current evolutionary theories, is the closest archaeal relative to eukaryotes. Interestingly, Asgard-ESCRT homologs were recently found to partly compensate for the loss of the equivalent proteins in yeast raising the possibility that these ancient Asgard-ESCRT proteins can interact with their eukaryotic counterparts. To test this notion, we have investigated the biochemical and cellular characteristics of ESCRTs derived from different Asgard archaea species in mammalian cells. Our data point to potential interactions and functional similarities between specific mammalian and Asgard ESCRTs and highlight new, evolutionary conserved, functions for the ESCRT. This study provides the first characterization of ancient ESCRT proteins in the cellular milieu and facilitates new models for the role of this versatile membrane remodeling machine across evolution.

1609-Pos

Developing lectins as inhibitors of SARS-CoV2

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The Spike protein of SARS-CoV2, is a highly glycosylated trimer that interacts with the ACE2 receptor on host cells via the receptor binding domain (RBD) to facilitate viral entry. As such, the Spike trimer has become the target for vaccines and therapeutics used to prevent or treat SARS-CoV2 infection. As variants of concern emerge and mutations associated with immune escape accumulate on the RBD the effectiveness of these antibodies decreases. Thus developing Spike inhibitors that target other features of Spike is highly attractive. Glycans are the ligands for lectins, thus placing lectins in a unique position for exploitation as antivirals. BOA is an antiviral lectin that binds viral glycoproteins via N-linked high mannose glycans. Here we show that BOA interacts with the spike protein and is a potent inhibitor of SARS-CoV2 viral entry at nanomolar concentrations. Using a variety of biophysical tools we demonstrate that the interaction is avidity driven and that BOA crosslinks spike into soluble aggregates. Furthermore, we demonstrate BOA remains effective for all current variants of concern.

1610-Pos

Monitoring the hepatitis B viral (HBV) capsid assembly by charge transfer spectroscopy

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HBV virions are double-shelled particles, 40–42 nm in diameter containing a viral nucleocapsid, or the HBV core which is an ordered assembly of stable homo-dimeric HBV Core protein (Cp). Both the full-length Cp containing 183 residues as well as the first-149 residues fragment, Cp149 can assemble spontaneously *in vitro* into capsids triggered by high ionic strength (0.2-0.5M NaCl) into 120 Cp dimers in an icosahedral symmetry (T=4) or into 90 Cp dimers with (T=3) icosahedral symmetry. Both the Cp149/Cp183 capsids and their assembly-disassembly is generally studied by Size Exclusion Chromatography, Light Scattering, Electron microscopy, Atomic Force Microscopy and other high-end labour-intensive techniques. Here, we report a simple, robust and a label free technique using Protein Charge Transfer Spectra (ProCharTS) to characterize the capsid assembly formation of both