Our analysis revealed the micro-domain organization of heterochromatin structure with the mean domain size of 115 nm in the intact and hydrated state of cells. Upon addition of histone deacetylase inhibitor, that phenocopied aging, we observed a marked decrease in the heterochromatin density distribution, disruption of microdomains, and decrease in chromatin compaction length scales. To validate these observations, we also developed a bidisperse copolymer model in a confined volume using Brownian dynamics simulations. Collectively, our results provide direct evidence of mesoscale domains in heterochromatin packing and their alterations during cellular aging

Platform: Protein Structure and Conformation III

1495-Plat
Insights into the mechanism of the bacterial, periplasm-spanning transporter system 'Ton'
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The Ton system is a multi-protein transporter system embedded in the inner and outer membrane of Gram-negative bacteria spanning the periplasmic space. It utilizes the proton motive force (pmf) at the inner membrane to physically 'unplug' an outer membrane receptor - allowing for specific, active transport of scarce nutrients. Hereby, the electric potential at the inner membrane is translated into a kinetic potential at the outer membrane - traversing the periplasm - by means of the inner membrane proton channel forming ExbB-ExbD complex and the periplasm spanning protein TonB/HasB. However, the mechanism of kinetic potential transfer in between the membranes by means of TonB/HasB is controversial. Here, we present a novel structure of the periplasmic domain of ExbD and show that the protein exists in a closed and sparsely populated open state. This open state is conformationally selected upon binding to a partner within the Ton system. Also, we show interactions of the system with the periplasm dissecting peptidoglycan layer in a dynamic fashion. Based on those results and previous efforts in the field, we propose a model of action for the Ton system that highlights the bimodal nature of ExbD and the significance of peptidoglycan.

1496-Plat
Secondary structure and stability of a gel-forming tardigrade desiccation-tolerance protein
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Protein-based pharmaceuticals are increasingly important, but their inherent instability necessitates a 'cold chain' requiring costly refrigeration during production, shipment, and storage. Drying can overcome this problem, but most proteins need the addition of stabilizers, and some cannot be successfully formulated. Thus, there is a need for new, more effective protective molecules. Cytosolically abundant heat soluble proteins from tardigrades are both fundamentally interesting and a promising source of inspiration; these disordered, monodisperse polymers form hydrogels whose structure may protect client proteins during drying. We used Fourier transform infrared spectroscopy, differential scanning calorimetry, and small-amplitude oscillatory shear rheometry to characterize gelation. A 5% (w/v) gel has a strength comparable to human skin, and melts cooperatively and reversibly near body temperature with an enthalpy comparable to globular proteins. We suggest that the dilute protein forms α-helical coiled coils and increasing their concentration drives gelation via intermolecular β-sheet formation.

1497-Plat
Alternate conformational states of the HIV-1 capsid protein: Atomistic structures and dynamics of interconversion
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The HIV-1 capsid protein assembles into a conical shell that encases the viral RNA genome. The capsid protein is a two-domain protein, with the C-terminal domain (CTD) forming a dimer. Depending on being in a solution environment or the crystalline state, different dimer arrangements have been observed for the full capsid protein, as well as a truncated construct consisting of only the CTD. To characterize the structures and dynamics of these alternate quaternary arrangements, we carried out atomistic simulations using the weighted ensemble enhanced sampling strategy. Our simulations generated continuous pathways for interconversion between the two states with rate constants that agree with those measured by ^{19}F NMR exchange spectroscopy. Our results demonstrate the advantages of pairing atomistic simulations with ^{19}F NMR and have implications for the structural polymorphism of the HIV-1 viral capsid assembly process.

1498-Plat
Novel insights into the structure and function of dengue virus NS1 protein
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The dengue flavivirus is responsible for dengue fever. While generally mild, a proportion of cases develop into life-threatening dengue haemorrhagic fever. Flaviviruses produce a non-structural protein, NS1, that is essential to flavivirus genome replication, though it has no known catalytic function. Early in infection, dimeric NS1 localises on the endoplasmic reticulum membrane at the site of viral RNA replication and may be involved in the replication complexs formation. Later on NS1 is secreted as a hexameric lipoprotein that interacts with components of the complement-mediated immune system. In 2014, crystal structures of full-length, glycosylated, and truncated West Nile Virus and Dengue Type 2 Virus (DENV2) NS1 dimers were obtained, which have aided interpretation of low-resolution cryo-electron microscopy reconstructions of the hexameric structure of secreted NS1. This along with biochemical and NMR analyses of the NS1 lipid cargo was used to build and simulate models of the DENV2 NS1 hexamer with and without a lipid cargo. Coarse-grained molecular dynamics simulations showed that the glycosylation of NS1 is essential for hexamer stability, with the glycans compensating for the paucity of protein-protein contacts between dimers. Lipid loaded NS1 hexamers can pick up lipids from their environment and deposit lipids into membranes but are not able to pick up lipids from membranes, whereas NS1 hexamers without a lipid cargo are not effective at picking up environmental lipids. The lipid cargo forms a dumbbell shape, with polar lipids towards the outside of the bulbs and non-polar lipids in the centre of the bulbs and bar of the dumbbell. These results add to our somewhat scarce understanding of the otherwise enigmatic function of a protein whose presence is an important biomarker for dengue fever, but whose role in infection has been controversial and not well understood.

1499-Plat
Structural basis for mTORC1-dependent regulation of the lysosomal and autophagic transcription factor EB
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The transcription factor TFEB is a master regulator of lysosomal biogenesis and autophagy. The phosphorylation of TFEB by the mechanistic target of rapamycin complex 1 (mTORC1) is unique in its mTORC1 substrate recruitment mechanism, which is strictly dependent on the amino-acid-mediated activation of the RagC GAP FLCN. TFEB lacks the TOR signaling (TOS) motif responsible for the recruitment of other mTORC1 substrates. We used cryo-electron microscopy (cryo-EM) to determine the structure of TFEB as presented to mTORC1 for phosphorylation. Two full Rag-Ragulator complexes present each molecule of TFEB to the mTOR active site. One Rag-Ragulator complex is bound to Raptor in the canonical mode seen previously in the absence of TFEB. A second Rag-Ragulator complex (non-canonical) docks onto the first via a RagC GDP-dependent contact with the second Ragulator complex. The non-canonical Rag dimer binds the first helix of TFEB in a RagCGDP-dependent aspartate clamp in the cleft between the Rag G domains. Mutation of the clamp drives TFEB constitutively into the nucleus whilst having no effect on mTORC1 localization. The remainder of the 108-amino acid TFEB docking domain winds around Raptor and then back to RagA. This structure presents the phosphorylatable Ser residues of TFEB to the mTORC1 active site in a suitable geometry for their phosphorylation. The double use of RagC GDP contacts in both Rag dimers explains the strong dependence of TFEB phosphorylation on FLCN and the RagC GDP state.

1500-Plat
The ATPase cycle of the AAA-ATPase protein transporter Bcs1 is highly concerted
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Bcs1, a homo-heptameric transmembrane AAA-ATPase that belongs to the superfamily of AAA proteins, acts as translocation machinery for the folded