

Cp149/Cp183. ProCharTS absorption in near the UV-Visible region (250–800nm) is observed when photoinduced electron transfer occurs from HOMO of COO<sup>-</sup> in glutamate (donor) to LUMO of the NH<sub>3</sub><sup>+</sup> in lysine or polypeptide backbone (acceptor). Alternatively it can also occur from polypeptide backbone to lysine cation. It is frequently observed among charge clusters that are in close proximity in folded proteins. Reports of intrinsic absorbance in aggregating protein/peptides has been encouraging in this aspect. In HBV capsids where Cp homodimers are assembled, new contacts between charge residues are made, amplifying ProCharTS absorption. We observe time-dependent increase in ProCharTS absorbance in HBV capsids as they assemble compared to dimeric HBV Cp. Further this absorption is sensitive enough to track HBV capsid assembly-disassembly kinetics. We conclude that ProCharTS can serve as a label-free tool to characterize HBV dimer to capsid transition.

#### 1611-Pos

##### Unravelling structural organization of individual $\alpha$ -Synuclein oligomers grown in the presence of phospholipids

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Parkinson's disease (PD) is a severe neurological disorder that affects more than 1 million people in the U.S. alone. A hallmark of PD is the formation of intracellular  $\alpha$ -synuclein ( $\alpha$ -Syn) protein aggregates called Lewy bodies (LBs). Although this protein does not have a particular localization in the central neural system,  $\alpha$ -Syn aggregates are primarily found in certain areas of the midbrain, hypothalamus, and thalamus. Microscopic analysis of LBs reveals fragments of lipid-rich membranes, organelles, and vesicles. These and other pieces of experimental evidence suggest that  $\alpha$ -Syn aggregation can be triggered by lipids. In this talk, I will demonstrate how atomic force microscope infrared spectroscopy (AFM-IR) can be used to investigate the structural organization of individual  $\alpha$ -Syn oligomers grown in the presence of two different phospholipids vesicles. AFM-IR is a modern optical nanoscopy technique that has single-molecule sensitivity and subdiffraction spatial resolution. Our results show that  $\alpha$ -Syn oligomers grown in the presence of phosphatidylcholine have a distinctly different structure than oligomers grown in the presence of phosphatidylserine. We infer that this occurs because of specific charges adopted by lipids, which in turn governs protein aggregation. We also found that the protein to phospholipid ratio has a substantial impact on the structure of  $\alpha$ -Syn oligomers. These findings demonstrate that  $\alpha$ -Syn is far more complex than expected from the perspective of the structural organization of oligomeric species.

#### 1612-Pos

##### Architecture and self-assembly of the jumbo phage nucleus-like compartment

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"Jumbo phages" are an emergent and ubiquitous class of large (>200 kb) dsDNA bacteriophages—viruses that infect bacteria—that construct a selectively-permeable, proteinaceous compartment around their replicating genome during infection. This micron-scale compartment, termed the "phage nucleus", forms *de novo* and grows with the replicating viral DNA. The phage nucleus allows export of viral mRNA and import of select DNA replication and transcription proteins, while excluding host ribosomes and restriction enzymes such as CRISPR-Cas. Here, by performing cryo-focused ion beam milling and electron tomography of jumbo phage infected cells, we have visualized the native architecture of the phage nucleus shell by subtomogram averaging. Furthermore, we have determined the high-resolution *in vitro* structure of the major shell protein, chimallin, by single-particle cryo-electron microscopy. By integrating these data with simulations, biophysical, and cell biological experiments, we propose molecular bases of self-assembly and structural plasticity of the phage nucleus shell, as well as provide potential insights into the selective-permeability of the compartment.

#### 1613-Pos

##### Deciphering the molecular organization of Get pathway chaperones through native top-down dissociation of multi-protein complexes

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From regulating membrane trafficking to the release of neurotransmitters at the neuronal synapses, C-terminal tail-anchored membrane proteins (TAPs) play a central role in eukaryotic biology. Trafficking of TAPs to their target physiological membrane follows the usual anterograde trafficking route from the endoplasmic reticulum (ER), where they are first inserted. Nevertheless, unlike other membrane proteins, their insertion into the ER membrane is not mediated via the signal recognition particle (SRP) pathway. Instead, a significant portion of TAPs, with hydrophobic C-terminal helix, gets inserted into the ER membrane via the Get pathway. Get3/4/5 chaperone complex is responsible for targeting C-terminal tail-anchored membrane proteins (TAPs) to the endoplasmic reticulum (ER). Despite the availability of several crystal structures of independent proteins and partial structures of subcomplexes, different models of oligomeric states and structural organization have been proposed for the protein complexes involved. Here, using native mass spectrometry (nativeMS) coupled with top-down dissociation, we show that Get4/5 exclusively forms a tetramer using both Get5/5 and a novel Get4/4 dimerization interface. Addition of Get3 to this leads to a hexameric (Get3)<sub>2</sub>-(Get4)<sub>2</sub>-(Get5)<sub>2</sub> complex with closed ring cyclic architecture. We further validate our claims through molecular modeling and mutational abrogation of the proposed interfaces. NativeMS has become a principal tool to determine the state of oligomeric organization of proteins. The work demonstrates that for multiprotein complexes, top-down nativeMS, coupled with molecular modeling and mutational perturbation, can provide an alternative route to render a detailed view of both the oligomeric states as well as the molecular interfaces involved. This is especially useful for large multiprotein complexes with large unstructured domains that make it recalcitrant to conventional structure determination approaches.

#### 1614-Pos

##### The spike-ACE2 interaction underlying SARS-CoV-2 infection and inhibition is enhanced by intermolecular cross linking

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The interaction between the SARS-CoV-2 spike trimer with the host dimeric angiotensin-converting enzyme 2 (ACE2) receptor is the first step in virus entry. Consequently, efforts aimed at therapeutic intervention have focused on blocking spike-ACE2 binding by designing ACE2-based decoys. The multivalency of ACE2 and spike is fundamental to our understanding of the molecular mechanism underlying ACE2-spike affinity, manifested by the observation of much tighter binding of dimeric ACE2 to spike compared with monomeric ACE2. While structural evidence suggests that intra-spike avidity is unlikely to explain these observations, the alternative, intermolecular avidity mechanism through ACE2-mediated crosslinking, is challenging to detect and quantify owing to the inherent heterogeneity of crosslinked oligomeric complexes. Mass photometry, single-molecule mass measurement, provides accurate quantification of binding stoichiometries and affinities by detecting biomolecular complexes in solution. Using mass photometry, we quantified the free energies for the spike-ACE2 interaction building blocks; the spike receptor binding domain and soluble monomeric or dimeric ACE2. Similarly, we quantified the interaction between monomeric ACE2 and trimeric soluble spike. In all cases, intermolecular avidity was not possible, and the interaction free energies were similar, additive, and consistent with previously reported affinities on the order of K<sub>D</sub>=20nM. For dimeric ACE2 and trimeric spike, we found that ACE2 coexists with spike trimer occupied by zero, one or two ACE2 dimers. In addition, however, the mass balance was shifted, predominantly favoring high-mass oligomers with increasing ACE2 concentration. Our results show that in solution, ACE2 and spike can form crosslinking interactions, which coupled with the enhanced inhibitory effect of dimeric ACE2 compared to monomeric ACE2 on viral infection, suggests that intermolecular ACE2-spike avidity may enhance spike-ACE2 interaction. These are relevant not only for our understanding of the cell-virus interaction, but also for the search and design of therapeutics.