in which we explore the complete assembly of capsid proteins during viral maturation and their disassembly during uncoating. Our simulations broadly suggest that capsid inhibitors accelerate capsid assembly, thereby undermining this necessarily-controlled process. As a result, several mechanisms of action are expressed, including malformed core formation and spontaneous core uncoating. Taken together, our results provide fundamental biophysical understanding into supramolecular protein assembly and elucidate a broad-spectrum biomedical strategy to combat viral infection.

**313-Pos**

***E. coli* High Throughput Assay Identifies Regulators of Endothelial Barriers**

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Regulation of endothelial barrier function is critical for vascular homeostasis, as dynamic and local control of vascular permeability permits macromolecular transport, immune surveillance, and deposition of a fibrin barrier to contain infection at sites of inflammation. Many of the signaling pathways promoting useful vascular permeability, however, are also triggered during disease, resulting in prolonged or uncontrolled vascular leak. Hyper-permeability triggered by inflammation or ischemia in the heart, brain, or lung promotes edema, exacerbates disease progression, and impairs recovery. Currently there is no clinical solution for the regulation of the endothelial barriers. A main reason for the lack of translational solutions is the technical difficulties (e.g. low throughput, and lengthy assays) to explore libraries of small compounds to begin drug development. Regulating endothelial barriers is crucial in the prevention of disease, disease progression, and the discovery and implementation of new therapies. Claudins, and occludin, are Tight Junction membrane proteins (TJ-MP) that seal the paracellular space creating the endothelial barriers.
Our laboratory has devised a strategy to express TJ-MPs in the outer membrane of *E. coli*. TJ-MP expression drives cell-cell interactions above the unicellular behavior of *E. coli*. TJ-MPs force this aggregation in a manner that correlates with their strength in the native endothelial barriers. The aggregation of *E.* coli can be quantified using flow cytometry. Our preliminary results suggest that our assay has sensitivity to detect hyper-permeability (barrier opening) as well as hypo-permeability (barrier closing or strengthening).

**314-Pos**

**Tetrameric Assembly of the Oncogenic C-Terminal Binding Proteins**

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C-terminal binding proteins (CtBP1 & 2) are co-transcriptional factors that have been implicated in progression of a number of cancers. CtBP possesses a D-isomer specific 2-hydroxyacid dehydrogenase (D2-HDH) domain that binds NAD(H) and provides an attractive target for small molecule intervention. Our crystal structures of CtBP1 and CtBP2 with bound substrate led to the design of an inhibitor (HIPP) with ~300nm binding affinity. Experiments using HIPP in $APC^{min}$ (colon cancer) mouse models suggest that CtBP inhibitors could be efficacious agents in a number of cancers. NAD(H) linked oligomeric assembly of CtBP has been suggested to contribute to co-transcriptional signaling, although the biologically relevant level of CtBP assembly has been unclear. Using multi-angle light scattering (MALS), we find that CtBP assembles from dimers into tetramers as a function of NAD(H) concentration with $EC_{50}$ values in the range of 50-150 nM, depending on the construct used for CtBP1 and CtBP2. Although crystal structures of CtBP1 and CtBP2 have been described in terms of dimers, due to extensive intradimeric interactions, examination of the distinct crystal lattices for CtBP1 and CtBP2 reveal a very similar tetrameric assembly in both paralogs. Site-directed mutations of residues involved in the observed lattice contacts support the lattice tetramer as the solution tetramer. We propose that this tetramer is the co-transcriptionally active form of CtBP and are investigating inhibitors that disrupt this tetrameric assembly as potential lead compounds for a therapeutically useful CtBP specific inhibitor.

**315-Pos**

**Should Virus Capsids Assemble Perfectly? A Simple Equilibrium Model for Defects**

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Although published structural models of virus capsids generally exhibit perfect symmetry, defects might be expected for several reasons including the finite-temperature, fluctuating environment in which capsids assemble and, in some cases, the requirement for disassembly during the infection cycle. Different lines of evidence also suggest the presence of defects: the observation of defective structures in computer simulations, and the presence of imperfect capsids in single-particle cryo-EM studies. To begin to quantify the conditions under which defects might be expected, we develop a simple equilibrium theory for capsids based on a lattice model allowing for both ideal and defective sites. Both analytical and numerical calculations with the model show that a significant population of defects appears below a fairly sharp threshold value of a key parameter: the difference in effective pair-binding affinities between ideally oriented and mixed ideal/defective pairs of interacting sites. The generality of the model enables us to estimate threshold values for a wide range of virus capsids, providing potential guidance for future experimental studies. Beyond furthering our understanding of potential structural heterogeneity in virus capsids, the findings suggest a novel strategy for assembly inhibition by enhancing 'off-lattice' interactions among capsid proteins via adaptor molecules.

**316-Pos**

**Structures and Functions of the HIV-1 Pre-Integration Complexes**

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After retroviral infection of a target cell, during the early phase of replication, the HIV-1 genomic RNA is reverse transcribed by the viral RT to generate the double-stranded DNA that interact with viral and cellular proteins to form the pre-integration complex (PIC). Viral integrase (IN) is the key component of the PIC and is involved in several steps of replication notably in reverse transcription, nuclear import, chromatin targeting and integration. Viral components such as IN cannot perform these functions on their own and need to recruit host cell proteins to efficiently carry out the different processes. IN is a flexible protein, property allowing its interaction with multiple partners and enabling its multiple functions. To study the molecular mechanisms of viral integration we use a bottom - up strategy by assembling in vitro and/or in cellulo multiprotein complexes around the integrase protein (core protein of the PIC) and DNA. This strategy enabled us to solve cryo-EM structures of the IN/LEDGF and IN/LEDGF/INI1 complexes at low resolution [1, 2]. With the recent progress of the cryo-EM techniques and our improvement in the complex preparations [3] new cryo-EM datasets on the IN/LEDGF/DNA and IN/LEDGF/nucleosome complexes are collected which will enable us to increase the structure quality to near atomic resolution for the IN/LEDGF/DNA complex. Moreover, the efficient production of the proteins in mammalian cells allowed us to identify PTMs on HIV integrase. Their effect on viral replication and 3' processing are analyzed. The diverse structures and functions of HIV-1 integrase induced by protein and DNA interactions as well as by PTMs will be presented and discussed. [1] Michel et al. (2009) EMBO J., 28, 980-991. [2] Maillot et al. (2013) PLoS ONE 8(4): e60734. [3] Levy et al. (2016) Nature comm. 7 : 10932.

**317-Pos**

**Probing and Differentiating the Shell and Enzyme Proteins of the Bacterial Microcompartment by Thermal Shift Assay**

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Bacterial microcompartments are prokaryotic organelles, distributed across bacterial species and are involved in several metabolic pathways. These are proteinaceous in nature and have a distinct shell-core structure. The core is made up of enzymes which are encapsulated by an outer coat made up of shell proteins. The shell proteins self-assemble to form an extended sheet which folds to form a closed shell. The shell protein units have vivid concave and convex surfaces. These two surfaces differ in the distribution of hydrophobic and hydrophilic residues. In this work, we employ a thermal shift assay to distinguish between the shell proteins and the internal enzyme clusters of bacterial microcompartment. Thermal shift assay is a well-known technique for studying protein unfolding using a fluorophore. We observe a few interesting differences between the signature fluorescence spectra of enzymes and shell proteins as probed by fluorescent dyes. Unlike the encapsulated globular enzymes, these shell proteins show high initial fluorescence. The enzyme clusters being globular in nature give their characteristic transition peaks at their melting temperatures, interestingly not observed in case of shell proteins. This difference is because of the variation in the distribution of hydrophobic