High throughput single-molecule technology

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transfer imaging. By coupling this approach with nucleic acid barcoding, microfluidic handling systems, and advanced data processing, we aim to automate and simplify multiperspective single-molecule studies to accelerate the emerging field of dynamic structural biology.

1480-Plat
High throughput single-molecule technology
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Single-molecule fluorescence resonance energy transfer (smFRET) has been an indispensable tool to probe the structure of biomolecules on the nanometer scale. The intrinsic low-throughput nature of single molecule observation has, however, prevented the technique to be used to study a large pool of samples. Here, we present a highly parallel smFRET measurement scheme by combining the conventional smFRET measurement with a next generation sequencing method. A library of DNA molecules, each of which carries one of the thousands of different sequences of interest, was prepared and measured in a one pot recipe. By using this high-throughput approach, we experimentally determine the sequence-dependent end-to-end distance of single stranded DNA in various native aqueous buffer conditions.

Symposium: Viral Recognition, Entry, and Egress

1481-Symp
Herpesviruses deform membrane during capsid nuclear export by lipid ordering and protein scaffolding
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Herpesviruses are large viruses that infect nearly all vertebrates and some invertebrates and cause lifelong infections in most of the world's population. A critical step in their replication is the export of the viral capsids from the nucleus into the cytoplasm, which occurs by an unusual mechanism termed nuclear egress. Too large to fit through nuclear pores, capsids instead bud at the inner nuclear membrane to form perinuclear enveloped virions, which then fuse with the outer nuclear membrane, releasing the capsids into the cytoplasm. This process is mediated by the virus-encoded nuclear egress complex (NEC) that deforms the membrane around the capsid. To understand how the NEC generates negative membrane curvature, we reconstituted the membrane budding process *in vitro* using the NEC from herpes simplex virus 1, a prototypical herpesvirus that causes cold sores. To probe its mechanism, we employed a combination of confocal microscopy, electron spin resonance, and structural biology. We found that the NEC uses clusters of positively charged residues to insert into the lipid headgroups, which increases lipid order. We also found that the NEC oligomerizes into a membrane-bound hexagonal coat on the inner surface of the budded vesicles. We propose that the NEC combines lipid ordering and oligomerization to mold the membrane into a spherical shape. Whereas lipid ordering generates negative membrane curvature locally, the NEC oligomerization into a hexagonal scaffold is necessary to achieve negative membrane curvature over a large membrane area. Similar principles may underlie membrane deformation in other systems. Our findings provide a biophysical explanation for the phenomenon of virus-induced nuclear budding.

1482-Symp
Dissecting host/pathogen interactions in the gastrointestinal epithelium
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Human intestinal epithelial cells (hIECs) are arranged as a monolayer of cells and provide the first line of defense against invading pathogens. Upon viral infection hIECs upregulate type I and III interferons to control the infection. These interferons lead to the induction of hundreds of interferon stimulated genes (ISGs) that aid in the clearance of the virus and protection of uninfected cells. Upon infection hIECs preferentially upregulate type III interferons to clear the infection. We and others have shown that when added in *trans* both type I and type III interferons act on hIECs as key antiviral cytokines against several enteric viruses, however they achieve their antiviral results using unique patterns of ISGs. Importantly, while hundreds of ISGs have been described using mainly enterocyte models, whether all cell types in the human gastrointestinal tract upregulate the same ISGs following enteric virus infection has yet to be addressed. Using human intestinal mini-gut organoids, multiplex RNA FISH and single cell RNA sequencing (scRNA-Seq), we have been able to determine the intestinal cell type specific response to virus infection. Our analysis showed that each intestinal cell type in the human gastrointestinal tract upregulated a unique pattern of ISGs to combat enteric virus infection. Further investigations of these ISG expression pattern will help us understand virus tropism and virus clearance mechanisms used by hIECs.

1483-Symp
Structure, function and dynamics of alphavirus capping pores
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Positive-sense single stranded RNA viruses such as coronaviruses, flaviviruses or alphaviruses, carry out transcription and replication inside virus-induced membranous organelles within host cells. Remodelling of the host cell membranes for formation of these organelles is coupled to the membrane association of the viral replication complexes and RNA synthesis. These viral niches allow for concentration of metabolites and proteins for the synthesis of viral RNA, also preventing its detection by the cellular innate immune system. I will present the cryo-EM structural characterization of the chikungunya virus non-structural protein 1, the viral protein responsible for RNA capping and membrane binding of the viral replication machinery, and the capping mechanism. The structures show the enzyme in its active form assembled in a monotopic membrane-associated dodecameric rings. It provides the structural basis for the coupling between membrane binding and allosteric activation of the capping enzyme. We also show different stages of the reaction pathway carried out by the enzyme, showing how nsP1 pores recognize the substrates of the methyl-transfer reaction, GTP and SAM, how it reaches a metastable post-methylation state with SAH and m^7GTP in the active site, the subsequent covalent transfer of m^7GMP to nsP1 and post-reaction conformational changes triggering the opening of the pore. In addition, we biochemically characterize the capping reaction, demonstrating specificity for the RNA substrate and the reversibility of the cap transfer resulting in decapping activity and the release of intermediates of the reaction. Our data identify the molecular determinants allowing each transition, provide explanation for the need for the SAM methyl donor all along the pathway and new clues about the conformational rearrangements associated to the enzymatic activity of nsP1. Together our results set new ground for the structural and functional understanding of alphavirus RNA-capping and the design of antivirals.

1484-Symp
Conformational dynamics of viral fusion proteins at single-molecule resolution
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The dynamic events that occur during viral membrane fusion during entry into cells have evaded elucidation. Envelope glycoproteins reside on the surface of viral particles, recognize cellular receptors, and promote the fusion of viral and cellular membranes. We have developed multiple single-molecule Förster resonance energy transfer (smFRET) assays to visualize the conformational changes in the Ebola virus envelope glycoprotein (GP) in its native membranous environment on the surface of viral particles. In parallel experiments, we have used fluorescence correlation spectroscopy (FCS) to correlate GP conformation with the ability to interact with a target membrane. Previous studies have identified proteolytic cleavage of GP by host proteases, binding to a cellular receptor, and the chemical environment of the late endosome as being critical during fusion. But the molecular mechanisms by which these events and variables trigger the necessary conformational changes in GP are not known. As a result, a complete and specific model of Ebola fusion, which integrates host factors, environmental conditions, and GP conformational changes currently does not exist. Here, we sought to specify the conformational changes undergone by GP during exposure to the factors and cues that Ebola encounters in the late endosome. Our results support a working model in which acidic pH and Ca^{2+} stabilize a GP conformation capable of binding a membrane. Receptor binding stabilizes an extended intermediate, which is sufficient for lipid mixing, but insufficient to trigger formation of the post-fusion GP conformation. Additional endosomal cues are currently being investigated for a potential role in promoting conversion to the post-fusion state.