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motions and intraring interactions that support them, the stability of the oligomeric states of the enzymes, and potential modes of interaction between enzymes and a microtubule leading to first steps in severing. I will discuss the results in the context of existing models, experimental probes of microtubule severing, and data regarding the action of other AAA+ motors.

Platform: Structural Virology

137-Plat

A cryptic pocket in Ebola VP35 allosterically controls RNA binding

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Many proteins are difficult to target with drugs because their primary function is to participate in protein-protein interactions (PPIs) and protein-nucleic acid interactions (PNIs) through flat interfaces that are less conducive to small molecule binding. These interactions are found in many viral proteins that interact with host factors and viral nucleic acids in order to evade the host immune system. We focused our study on viral protein 35 (VP35), from the highly lethal Ebola virus. VP35 plays essential roles in Ebola's replication cycle, including binding the viral RNA genome to block a host's innate immunity. However, there are no FDA approved drugs targeting VP35. One promising opportunity for discovering and designing new drugs is cryptic pockets, or pockets that are absent in available protein structures but form due to protein dynamics. Identifying and exploiting cryptic pockets remains challenging as most known pockets were discovered alongside the identification of a small molecule effector. Here, we applied adaptive sampling simulations to sample states in VP35's conformational landscape with large pocket volumes which revealed a potentially druggable cryptic pocket. Then, using allosteric network detection algorithms, we predicted that VP35 harbors a cryptic pocket that is allosterically coupled to its RNA-binding interface. Thiol labeling experiments along with dsRNA binding experiments suggest the VP35 cryptic pocket is present and that stabilizing this pocket in its open form allosterically disrupts RNA binding. We then conducted an experimental high throughput screen for dsRNA binding inhibitors targeting VP35 that yielded a number of hits. These results demonstrate the potential of cryptic pockets to allosterically affect PPI and PNIs presenting new therapeutic opportunities for targeting these prevalent interactions.

138-Plat

Architecture and antigenicity of the nipah virus attachment glycoprotein

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Nipah virus (NiV) and Hendra virus (HeV) are highly pathogenic zoonotic henipaviruses (HNVs) responsible for recurrent outbreaks of encephalitis and respiratory illness. As the current COVID-19 pandemic is gradually waning, attention is now being refocused on the nature of the next viral pandemic might be and what preparedness measures could be taken. Among almost all these discussions, the NiV Bangladesh strain (NiV-B) has been at the forefront of such concern by the scientific community, including the WHO and the Coalition for Epidemic Preparedness Innovations (CEPI). As another bat-borne virus, NiV infections are associated with human case fatality rates ranging from 40-95% and NiV-B is associated with significant human-to-human transmission. Currently, HNV G protein is both a vaccine antigen of interest for humans and a major viral target of monoclonal antibody therapy. Still, since the emergence of HeV in 1994 and NiV in 1998, no licensed treatments or vaccines for human use are yet available, but an approved equine vaccine is in use in Australia based on a soluble version of the HeV attachment G glycoprotein. However, the lack of structural information for any HNV G besides the receptor-binding head domain limits our understanding of viral infection and of host immunity. In this study, we successfully captured a high resolution Cryo-EM structure of NiV G protein ectodomain, revealed the intertwined architecture of this key therapeutic target and defined additional neutralization epitopes along with the potent antigen site, which all together providing a new platform for understanding the immunogenicity of HNV attachment protein as well as why it is such an important viral target of the immune response. These new findings and structural information will aid the development of both monoclonal antibody-based treatments and vaccine development strategies against these important zoonotic viral threats.

139-Plat

The SARS-CoV-2 spike protein reversibly samples an open-trimer conformation exposing novel epitopes

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Current COVID-19 vaccines and many clinical diagnostics are based on the structure and function of the SARS-CoV-2 spike ectodomain. Using hydrogen deuterium exchange mass spectrometry (HDX-MS), we have uncovered that in addition to the prefusion structure determined by cryo-EM, this protein adopts an alternative conformation that interconverts slowly with the canonical prefusion structure. This conformation—an open trimer—contains easily accessible RBDs and exposes the conserved trimer interface buried in the prefusion conformation, thus exposing potential epitopes. We have shown that a synthetic pan-coronavirus antibody with neutralization activity, 3A3, specifically binds the open-trimer state, demonstrating the potential therapeutic opportunity of targeting the open trimer. The dynamic nature of this conformational state appears to make it refractory to cryo-EM analysis, highlighting the utility of HDX-MS approaches. The population of this state and kinetics of interconversion are modulated by temperature, receptor binding, antibody binding, and sequence variants observed in the natural population. Knowledge of the structure and populations of this conformation will help improve existing diagnostics, therapeutics, and vaccines.

140-Plat

A tethered ligand assay to probe SARS-CoV-2:ACE2 interactions

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SARS-CoV-2 infections are initiated by attachment of the viral receptor-binding domain (RBD) to angiotensin-converting enzyme-2 (ACE2) on human host cells. This critical first step occurs in the dynamic environment of the respiratory tract, where external forces constantly act on the binding partners, making stable attachment crucial for effective infection. Understanding the molecular mechanisms of this process would enable targeted interventions. Thus, there is an urgent need for assays that can quantitate SARS-CoV-2 interactions with ACE2 under mechanical load. Here, we introduce a tethered ligand assay that comprises the RBD and the ACE2 ectodomain joined by a flexible peptide linker. Using magnetic tweezers and atomic force spectroscopy as highly complementary single-molecule force spectroscopy techniques, we investigate the RBD:ACE2 interaction over the whole physiologically relevant force range. We combine the experimental results with steered molecular dynamics simulations and observe fully consistent unbinding and unfolding events across the three techniques, which we assign to their molecular origin. By studying the RBD:ACE2 bond at constant forces of 2-5 pN, we can fully quantify the force dependence and kinetics of the bond. We show that the SARS-CoV-2 RBD:ACE2 interaction has higher mechanical stability, larger binding free energy, and a lower dissociation rate in comparison to SARS-CoV-1. By studying how free ACE2 outcompetes tethered ACE2, we show that our assay is sensitive to the prevention of bond formation by external binders. We anticipate the value of our assay for studying how blocking agents for targeted pharmaceutical intervention, such as antibodies or small molecule binders, affect physiologically loaded bond characteristics.