speculation that these glycans can modulate the conformational change of the spike protein. Here we built the glycosylated SARS-CoV-2 spike protein in both the down and up conformations based on high-resolution cryo-EM structures and mass spectrometry data. We ran metadynamics from both conformations using two carefully chosen collective variables to visualize the transition between the two states, especially the dynamics of the hinge. From the structures generated by the metadynamics simulations, we determined the free energy change and dominant pathway between the two states. The down conformation is found to be of lower energy than the up conformation, but the energy barrier between them is low enough to permit spontaneous hinge opening. The results were also compared to the protein without glycosylation, and we found the glycans to be essential in stabilizing each of the conformations independently.

**73-Pos**

**Coronavirus Pathogenicity is Determined by Stability of the Spike Protein Open Conformation**

**Josiah Bones**[1], Ben Corry[2].
[1]Research, Australian National University, Bruce, Australia, [2]Research, Australian National University, Canberra, Australia.

SARS-CoV-2, the virus inducing the COVID-19 illness, has claimed over 1 million lives since emerging in December 2019, while disrupting many more. It is the seventh coronavirus to emerge in human populations. However, what makes one coronavirus more deadly than another is not well understood. Furthermore, no tools exist to reliably assess pathogenic potential in coronavirus strains threatening future emergence from animal populations. Here, we attempt to answer why one coronavirus is more pathogenic than another and describe a tool for future predictive uses. Coronaviruses invade host cells using a spike glycoprotein. To bind to the host receptor and initiate fusion with the host membrane, the spike protein's receptor binding domain (RBD) must first transition from a closed to open conformation to avail itself for binding. We propose that the proportion of time spent in the open receptor-ready conformation, related to this conformation's stability, indicates the pathogenic potential of the coronavirus strain. To test this hypothesis, we compared the spike protein RBDs from three human ACE2 binding coronavirus strains with varying pathogenic potential: SARS-CoV-1, SARS-CoV-2, and hCoV-NL63, using molecular dynamics simulations. We employed both long equilibrium simulations and umbrella sampling to derive closed to open transition free energy profiles. Our results suggest a strong correlation between the ability of the RBDs to transition to the open state and pathogenic potential. with additional testing, our technique may prove useful in assessing animal coronaviruses' pathogenic potential, highlighting them as a high or low-risk strain before they can emerge into human populations.

**74-Pos**

**An Integrative MD Simulation and Network Analysis Approach to Study Glycosylation of Spike in SARS-CoV-2**

**Mahdi Ghorbani**[1], Bernard R. Brooks[2], Jeffery B. Klauda[1].
[1]Dept Chemical and Biomolecular Engineering, University of Maryland, College Park, MD, USA, [2]National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA.

The ongoing SARS-COV-2 pandemic continues to have devastating consequences on human public health and global economy. A major characteristic of all coronaviruses is a spike glycoprotein on the surface of the virus, which mediates host cell entry and is the target of all current antibody design efforts. A receptor binding domain in the spike protein is responsible for binding to host cell receptor when the spike protein is the open conformation. The spike protein of coronavirus is shielded by an array of glycans, providing a sugar-coated barrier to help the virus evade the human immune response. To evaluate the dynamic motion of glycans in the spike protein, microsecond-long molecular dynamics (MD) simulations were performed on the spike protein in two different states (open or closed) based on variation in the receptor binding domain (RBD). MD simulation of the open state revealed a scissoring motion on the N-terminal domain of neighboring monomers in the trimeric spike. To uncover the role of glycans in providing effective shield, we used network analysis in graph theory, where measures such as betweenness centrality revealed the importance of glycans in the apex of spike. Glycan microdomains featuring high degree of interaction were identified. Most known antibodies bind to regions between these microdomains. Next, an antibody overlap analysis identified the glycan microdomains and specific glycans that restrict the access of antibody to the epitopes on spike surface. Overall, the results of this study provide detailed understanding of the spike glycan shield, which may be utilized for any therapeutic efforts against this crisis.

**75-Pos**

**Structures of Capsid and Capsid-Associated Tegument Complex Inside the Epstein-Barr Virus**

**Wei Liu**[1], Yanxiang Cui[2], Caiyan Wang[2,3], Zihang Li[2], Danyang Gong[4], Xinghong Dai[5], Guo-Qiang Bi[1], Ren Sun[6], Hong Zhou[2].
[1]Center for Integrative Imaging, Hefei National Laboratory for Physical Sciences at the Microscale, and School of Life Sciences, University of Science and Technology of China, Hefei, China, [2]California NanoSystems Institute, University of California-Los Angeles, Los Angeles, CA, USA, [3]International Institute for Translational Chinese Medicine, Guangzhou University of Chinese Medicine, Guang Zhou, CA, USA, [4]Department of Therapeutics Discovery, Amgen Research, Amgen Inc., Thousand Oaks, CA, USA, [5]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, USA, [6]Department of Molecular and Medical Pharmacology, University of California-Los Angeles, Los Angeles, CA, USA.

As the first discovered human cancer virus, Epstein-Barr virus (EBV) causes Burkitt's lymphoma and nasopharyngeal carcinoma. Isolating virions for determining high-resolution structures has been hindered by latency—a hallmark of EBV infection—and atomic structures are thus available only for recombinantly expressed EBV proteins. in the present study, by symmetry relaxation and subparticle reconstruction, we have determined near-atomic-resolution structures of the EBV capsid with an asymmetrically attached DNA-translocating portal and capsid-associated tegument complexes from cryogenic electron microscopy images of just 2,048 EBV virions obtained by chemical induction. The resulting atomic models reveal structural plasticity among the 20 conformers of the major capsid protein, 2 conformers of the small capsid protein (SCP), 4 conformers of the triplex monomer proteins and 2 conformers of the triplex dimer proteins. Plasticity reaches the greatest level at the capsid-tegument interfaces involving SCP and capsid-associated tegument complexes (CATC): SCPs crown pentons/hexons and mediate tegument protein binding, and CATCs bind and rotate all five periportal triplexes, but notably only about one peri-penton triplex. These results offer insights into the EBV capsid assembly and a mechanism for recruiting cell-regulating factors into the tegument compartment as 'cargoes', and should inform future anti-EBV strategies.

**76-Pos**

**A Combined HDX-MS and MD Simulation Approach to Identify Potential Druggable Regions in the NS5 Protein of the Dengue Virus Serotype 2**

**Juliet O. Obi**, Daniel J. Deredge.
Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland-Baltimore, Baltimore, MD, USA.

The number of people infected with the dengue virus has increased steadily in recent decades due to global trends including global travel, human population growth, and an increase in urbanization. This increase in incidence is caused by a geographical expansion of mosquito vectors, including those that cause dengue fever (DF), making DF a major global health concern. There is no specific treatment for dengue fever currently available, therefore research efforts need to be intensified in the development of antiviral drugs and design of vaccines against the dengue virus. The nonstructural protein 5 (NS5) is highly conserved among flaviviruses, including the dengue virus. The dengue virus serotype 2 (DENV2) NS5 is a multifunctional protein with an N-terminal methyltransferase, and a C-terminal RNA-dependent-RNA-polymerase (RdRp). The dual enzyme activity of this NS5 protein, and its essential role in dengue viral RNA replication, makes it an attractive antiviral target for the treatment of dengue infection.Hydrogen deuterium exchange coupled to mass spectrometry (HDX-MS) has proven to be a powerful biophysical approach, used to study protein structure and dynamics. We employed solution HDX-MS analysis, and molecular dynamics (MD) simulations to probe solvent accessibility and assess the conformational landscape of the DENV2 NS5 protein. We characterized regions with high deuterium uptake in the DENV2 NS5 apo structure and corroborated our findings with MD simulations. The results obtained from this combined biophysical approach will enable us to identify solvent accessible regions in the DENV2 NS5 protein, which will be further explored with the aim of identifying novel binding site(s) for small molecule inhibitors against the dengue virus.

**77-Pos**

**Cooperative Dynamics of REC-Nuc Lobes Prime Cas12a for DNA Processing**

**Aakash Saha**[1], Pablo R. Arantes[1], Rohaine Hsu[1], Yogesh B. Narkhede[1], Martin Jinek[2], Giulia Palermo[1].
[1]Department of Bioengineering, University of California-Riverside, Riverside, CA, USA, [2]Department of Biochemistry, University of Zurich, Zurich, Switzerland.