Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

942-Pos

Elucidating allosteric communication in the human serotonin transporter

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The human serotonin transporter (hSERT) is a membrane protein that mediates the re-uptake of serotonin from the synaptic cleft into presynaptic neurons. Dysregulation in the SERT transport cycle has been associated with numerous neuropsychiatric disorders, highlighting the crucial role of the transporter in maintaining the brain neurotransmitter levels homeostasis. From a mechanistic point of view, the transport cycle can be described by the alternating-access model, in which structural rearrangements in the protein domains result in the alternated exposure of substrates to the extracellular or intracellular environment. So far, three conformational states have been resolved by X-ray or cryo-EM crystallography: outward-open, outward-occluded, and inward-open. Despite these structures provide important insights into the conformational changes of SERT, they represent static snapshots of a highly dynamic process. Our research focuses on the molecular mechanism of the transport cycle, together with allosteric communication between key residues and structural domains. Transporter function is intrinsically connected to its motions: through molecular dynamics simulations, it is possible to observe how the protein naturally samples its conformational space over time. Multiple all-atom systems of SERT in complex with substrate were simulated, for a total of 20 μs of unbiased simulations. The resulting trajectories were then analyzed in the context of dimensionality reduction: essential dynamics and cross-correlations maps allowed us to identify the principal modes of motion and communicative pathways within the transporter. Collectively, our results provide a framework for explaining how local interactions and motions couple to substrate occlusion, the initial step of the translocation process.

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Substrate-triggered occlusion in the human serotonin transporter unveiled a new and fully occluded structure

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The serotonin transporter (SERT; SLC6A4) is an important pre-synaptic protein involved in sodium-dependent re-uptake of serotonin (5HT), shaping the concentration of 5HT available for the post-synaptic receptor binding. Inadequate handling of synaptic monoamine levels have been associated with neuropsychiatric diseases like major depression disorder, affecting over 260 million people world-wide. SERT has therefore been implicated as an upmost important pharmacological target. High-resolution structures of SLC6 members including SERT confirmed its conserved 12 transmembrane helical architecture and the presence of the orthosteric binding site (S1) halfway through the membrane. Despite the importance of these structures for understanding the structure-function relationship, they represent averaged static snapshots of highly flexible proteins. The transport cycle described by the alternating access model proposes that several conformational states are visited during the translocation process, while ensuring that the S1 is alternatively accessibility from either sides of the membrane. Ions and 5HT binding to the S1 induces substrate occlusion by forcing the bundle domain to tilt towards the scaffold domain. Occlusion is mainly achieved by the rearrangements of extracellular parts of transmembrane helices, however, the molecular mechanism remains enigmatic. In this study, we used extensive bias-free all atom molecular dynamics simulation of the outward-open conformation to investigate the occlusion mechanism. We show i) the sequence of events necessary to trigger substrate occlusion, ii) the importance of water in the access path to the S1 determining the extracellular gate closure, and iii) we identified a new fully-occluded conformation not yet observed in structural studies.

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Characterizing antibody binding to influenza surface proteins at the virion level

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Influenza and other respiratory viruses cause seasonal epidemics and occasional pandemics with global mortality in the hundreds of thousands to millions each year. Antibodies play a crucial role in defending against viral infection, with many different functions at various points in the viral replication cycle. Most studies of antibody function are performed in bulk, and report results averaged across millions of viruses and cells. How antibodies may differ in their interactions with viruses at the single-particle level remains unknown. In traditional methods of measuring antibody binding affinities, the antigen is either studied in solution or is artificially bound to a surface to be presented to the antibodies. Aspects like the density, distribution, and orientation of the surface antigens may be quite distinct from that of antigens presented on the surface of a virus. This may be particularly important for viruses like influenza that vary considerably in their size, shape, and molecular composition. To address this challenge, our lab has developed a fluorescence-based assay for measuring antibody binding kinetics at the virion level. This assay allows us to characterize the binding of antibodies to viral antigens as presented on their native viral surface. Using this assay, we investigate the binding of antibodies specific to different regions of the influenza surface protein hemagglutinin. Our preliminary results indicate that the shape and curvature of the viral surface, together with the accessibility of the targeted epitope, influence rates and quantities of antibody binding. This approach could prove useful for investigating binding modes of antibodies with varying affinity and avidity to viral surface proteins.

945-Pos

Investigating SARS-CoV-2 ORF7a and BST-2 heterodimerization by molecular dynamics simulations

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ORF7a, a single-pass transmembrane protein, localizes to the endoplasmic reticulum-Golgi intermediate compartment. Its interaction with BST-2 associates to suppress host immune response against the virulence of SARS-CoV-2; however, a structural model of protein-protein interface within the heterodimer is unknown. This work aims to investigate the ORF7a heterodimerization with the wild-type (WT) and a naturally-occurring single-point mutation BST-2 through molecular dynamics (MD) and provide insights into molecular interactions to understand the effect of a naturally BST-2 mutation. A multi-scale MD simulation with coarse-grained (CG) and all-atom (AA) models was conducted to simulate the formation of dimer and identify the key dimer interactions. Initially separated monomers of the ORF7a and BST-2 were used to simulate dimers for five microsecond CG MD simulations by using the Martini force field (FF) with explicit solvent, and a bilayer of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipids. The structures obtained from the last step of CG MD simulations were used to covert to initial configurations for AA MD simulations using the CHARMM36 FF. The result of MD simulations shows variations of molecular conformations for the dimerization of ORF7a with the WT and I28S mutant of BST-2, whose residue-residue interactions were identified in different sections and intensities through hydrogen bonding and other hydrophobic contacts. Preliminary results indicate a consistently binding for ORF7a and the WT BST-2 heterodimerization, while a fluctuation of binding intensity and contact region for the dimerization of ORF7a and the I28S BST-2, which indicates less stable heterodimerization with the I28S mutant. This study matches the results from our experimental collaborators (Dr. Bryan Berger's Lab at UVA).

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Structural dynamics of prefusion spike protein of SARS-CoV-2 and its variants

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Over almost two years since late December 2019, a rapidly expanding and evolving pandemic has claimed many lives and crippled economies worldwide. SARS-COV-2, the virus behind the pandemic, uses its spike proteins to infect human cells by binding to ACE2 receptors on host cells. Due to the sheer volume of global infections, multiple mutations have arisen, among which there have been successful variants with increased transmissibility and capability for immune system evasions. Static information on the structure of wild-type spike proteins is not sufficient to understand the evolving process of infection by the coronavirus. Here we have used microsecond-level molecular dynamics (MD) simulations to study the active and inactive states of the spike proteins from the wild-type, Alpha, Beta, Gamma, Epsilon, and Delta variants of SARS-CoV-2, as well as an engineered spike protein associated with the Moderna vaccine. We have identified important mutations that contribute to changes in the structural and conformational dynamics of the spike protein. Our simulations reveal that certain mutations create significant instability in the spike protein. Notably, mutations shared by the Delta, Epsilon, and Beta variants appear to be responsible for the differential dynamic behavior observed in our simulations, which in turn could be linked to higher transmissibility and potential for immune evasion. This study provides insight into the dynamic behavior of the spike protein from different SARS-CoV-2 variants and