Dengue virus (DENV) is an enveloped virus that infects millions of people worldwide. The only commercially available vaccine has low efficacy and safety issues. DENV is ~50 nm in diameter, and contains 90 envelope (E) and membrane (M) protein dimers embedded within a lipid envelope, encapsulating an RNA genome. There are four closely related DENV serotypes, which hamper broad neutralization by host antibodies and can result in antibody dependent enhancement worsening the clinical symptoms of dengue infections. During its life cycle, DENV adopts numerous conformations in response to environmental factors such as pH and temperature changes, which result in varying degrees of exposure of its accessible epitopes. These factors make vaccine development challenging both from the immunological and structural points of view. Virus-like particles (VLPs) represent promising platforms as novel vaccine alternatives because they are non-infectious and highly immunogenic. DENV VLPs are ~30 nm in diameter, containing 30 E/M protein dimers on their surface, likely leading to significant differences in their conformational dynamics compared to the virus itself. Notably, these VLPs exhibit a significant ''groove'' at the E protein dimeric interface, yielding more exposed epitopes than those present on the typical DENV particle. This factor is likely to be critical to the efficacy of resultant vaccines. Here, we employed an integrative modeling and multiscale simulation approach supported by various experimental measurements to understand the differences in conformational dynamics between both mature and immature DENV and its VLPs, to assess epitope exposure and particle stability. Similar tools were also used to guide several site-directed mutations that may guide the improvement of such VLPs, and hence represents a platform for novel vaccine development strategies.

2474-Pos

Lipid modulation of membrane protein conformational equilibria does not require lipid immobilization in long-lasting protein-lipid complexes

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Lipid regulation of membrane proteins is widely rationalized as a process whereby lipid molecules become immobilized in a complex with the protein, supposedly due to strong, specific interactions. While this conventional paradigm might apply to some regulatory processes, we posit that it is not a universal mechanism. As a counterexample, we examine the mechanism by which lipids modulate the dimerization reaction of the bacterial CL^-/H^+ antiporter CLC-ec1. Using molecular simulations, we have shown that monomeric CLC-ec1 induces a pronounced defect in the morphology of the surrounding membrane, including thinning of the hydrophobic core due to drastic lipid tilting and entanglement of the two bilayer leaflets. Upon dimerization, this interface becomes buried, and the morphological defect in the membrane is completely eliminated. Using advanced simulation methods, we have shown that the free-energy gain resulting from the elimination of this defect accounts for much of the free-energy of CLC-ec1 association measured experimentally. These experiments, based on single-molecule microscopy, also demonstrate that introduction of short-chain lipids drastically destabilize dimerization, but the concentration dependence of this inhibitory effect is incoherent with a conventional competitive ligand-binding process. To examine the microscopic mechanism underlying these intriguing observations, we calculate a continuous 40-microsecond molecular dynamics trajectory of all-atom monomeric CLC-ec1 embedded in a 70:30 mixture of POPC and DLPC lipids. This simulation shows that DLPC molecules become enriched within the thinned-membrane defect, alleviating its energetic cost and hence inhibits dimerization. However, we find that this enrichment does not stem from the immobilization of these lipids in a long-lasting protein-lipid complex, but from subtle yet statistically significant differences in the residence times and exchange frequency of PO and DL lipids within the first solvation shells.

2475-Pos

Predicting protein-lipid interactions through machine learning methods employing new tokenization techniques

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Machine learning (ML) methods have had a broad and tremendous impact on structural and dynamical studies of different classes of proteins. Nonetheless, the application of ML models to protein-membrane interactions have received less attention. Given their crucial importance to several aspects of cell communication, we focus our attention to peripheral proteins. In this work, we develop a novel tokenization algorithm for protein-membrane complexes. This method accounts for spatial relationships between the membrane-bound phase of a protein and the lipid bilayer. With this tokenization method, we prepare an autoencoder-based ML workflow for predicting contact maps between residues in a peripheral membrane-binding protein and select key lipids in a lipid bilayer. Our ML model is trained with a series of molecular dynamics (MD) simulations including different PIP-binding proteins and lipid bilayers including PIP lipids. Then, we seek to predict the contact map of PIP-binding proteins, not present in the training set, and verify the predictions of the ML model by MD results. A key element of our tokenization scheme is the embedding of distances, between the protein's membrane-bound phase residues and lipids in the bilayer; we believe this provides a productive feature space for our ML workflow. Details of the ML workflow and results will be discussed. The tokenization method we employ for protein-membrane interactions may facilitate developing applications of previously un-tested ML models in problems regarding protein-membrane interactions. Furthermore, our results for contact map prediction serve as a motivation to further explore ML applications for systems containing a lipid bilayer.

Posters: Membrane Receptors and Signal Transduction

2476-Pos

Building an affinity panel of synthetic polyclonal T-cell receptor ligands that mimic native peptide-MHC

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T cells accurately and sensitively discriminate between foreign- and self-peptide antigens presented in Major Histocompatibility Complexes (pMHC). T cells can achieve near single molecule sensitivity to agonist foreign peptides, even in a background of self-peptides that may outnumber agonists more than 1000:1. Each individual T cell receptor (TCR) discriminates its particular agonist ligands from all others based on the binding dwell time of the pMHC:TCR complex, with agonists binding for a few seconds or longer. Overall, even successful pMHC:TCR binding pairs tend to be rather weak binders. A native population of T cells from an organism consists of a polyclonal repertoire of millions of distinct TCRs, each with its own ligand specificity for foreign antigen. Thus from an investigative, or therapeutic perspective, it is not possible to activate TCR, in a polyclonal manner, through the same binding epitope as native pMHC. Additionally, native pMHC ligands do not activate T cells from solution unless cross linked, in which case they yield a different activation profile then native membrane-associated, monovalent pMHC. Our interest here is to construct a monovalent polyclonal TCR activator that exhibits similar activation behavior to pMHC (monomeric, inactive from solution, and single molecule active on membranes). We synthetically couple a monovalent anti-TCR OKT3 Fab', which strongly binds human T cells, to a supported lipid bilayer (SLB) using short DNA handels. By purifying different OKT3 Fab' ligands with varying binding affinities we develop a panel of synthetic polyclonal TCR ligands spanning a potency range from null/self to foreign/agonist peptides. This modular OKT3 Fab'-DNA ligand can bind and universally activate human T cells in polyclonal populations.

2477-Pos

Computational characterization of the binding mode and mechanism of action of tricyclic small molecules at 5-HT1E and 5-HT1F receptors

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5-hydroxytryptamine (serotonin) 1E and 1F receptors (5-HT_{1E} and 5-HT_{1F}, respectively) are highly expressed human G protein-coupled receptors with high sequence identity among themselves and other 5-HT_1 receptor subtypes. Although the physiological role of 5-HT_{1E} and 5-HT_{1F} is not fully understood, they are molecular targets of drugs that are effective to treat migraine, depression, and schizophrenia, albeit not without also producing adverse effects. Notably, no selective or high-affinity drugs have been reported for the 5-HT_{1E} receptor to date. Thus, there is widespread interest in understanding at an atomic level of detail how small molecules bind and activate these receptors for the ultimate purpose of designing improved therapeutics. Through computational studies including docking calculations, metadynamics rescoring, and molecular dynamics simulations of cryo-electron microscopy structures, we predict the binding mode and mechanism of action of tricyclic

small molecules that are capable of simultaneously activating the 5-HT_{1E} and 5-HT_{1F} receptors. Our results provide testable hypotheses of ligand-receptor interactions and ligand-induced allosteric modulation that may be used to improve the efficacy of these small molecules towards specific biological endpoints.

2478-Pos

Dissecting the contributions of epitope accessibility in B cell activation

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Antibody-mediated immunity is highly specific and is activated by B cell exposure to pathogens or antigens. Antigen bound to the B cell receptor (BCR) triggers signaling cascades that can lead to B cell activation, antigen phagocytosis, and the eventual secretion of antibodies. Although our understanding of the biochemical and biophysical events involved in B cell activation have advanced considerably in recent years, the specific features of antigens that contribute to BCR engagement, B cell activation, and ultimately the production of antibodies are not well-defined. Among the factors that may contribute, the accessibility of particular epitopes on the antigenic surface is thought to play an important role. In this line of thinking, accessible epitopes are more easily bound by the BCR, directing the adaptive immune response to these sites. However, because different epitopes also differ in their sequence and structure, decoupling the contributions of accessibility from other contributing factors has been challenging. To address this challenge, we have developed an experimental system to specifically dissect the contributions of epitope accessibility in BCR engagement and B cell activation. Using engineered Ramos B cells that express BCRs specific to different epitopes on the fusion protein of respiratory syncytial virus (RSV) that can be presented in either membrane-proximal or membrane-distal conformations, we are able to compare the efficiency of BCR engagement and B cell activation to a single epitope while varying its accessibility. We track these processes using fluorescence microscopy combined with site-specific labeling of viral antigens and the BCR. These experiments will help to identify physical constraints imposed on BCR binding and B cell activation which may contribute to the immunodominance of particular epitopes on RSV and other viral pathogens.

2479-Pos

Quantitative fret studies of EphA2-Lyn interactions

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The regulation of the receptor tyrosine kinase (RTK) signal transduction pathways is critical for proper cellular function. RTK activation occurs in response to ligand binding, which induces RTK dimerization or oligomerization to allow cross-phosphorylation of specific tyrosines in the kinase domains of the RTK. Downstream signals are then propagated upon the recruitment of soluble kinases or adaptor proteins to these phoshorylated tyrosines. The EphA2 receptor tyrosine kinase is a member of the largest RTK family and has been reported to initiate downstream signaling by recruiting Lyn, a member of the Src family of non-receptor tyrosine kinases. Contrary to expectations, quantitative FRET experiments demonstrate that ligand-induced EphA2 tyrosine phosphorylation does not lead to Lyn recruitment to EphA2. In control experiments, Lyn is efficiently recruited by a different RTK, EGFR, upon ligand stimulation. The differential interactions of Lyn with EphA2 and EGFR may be a means of signal diversification. Current work is focused on the mechanism behind the unexpected lack of interactions between EphA2 and Lyn upon EphA2 clustering and phosphorylation.

2480-Pos

The impact of endoplasmic reticulum morphology on IRE1 protein clustering

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The Unfolded Protein Response (UPR) is a signaling network that responds to an increased load of unfolded proteins within the Endoplasmic Reticulum (ER), referred to as ER stress. ER stress activates the transmembrane signaling protein IRE1, which subsequently forms oligomers and clusters with signaling activity that modifies target gene expression to reestablish ER homeostasis. Experiments have shown that IRE1 clusters can have complex shapes that form along the ER tube structure, including wrapping around an ER tube, and suggest that IRE1 protein clusters tend to be found on narrower ER tubes. However, the influence of ER morphology on the dynamics of these IRE1 clusters remains largely unexplored. We quantitatively modeled the dynamics of IRE1 protein clusters on a tubular surface, using a kinetic Monte Carlo algorithm that treats the IRE1 proteins as particles undergoing stochastic diffusion. We show that cluster shape exhibits a phase transition from approximately round clusters to clusters that wrap around the ER tube as a cluster becomes sufficiently large or on sufficiently narrow tubes. These wrapped clusters, which easily form on narrow tubes, evaporate much slower compared to circular clusters of equal size that form on larger tubes. The threshold concentration between cluster growth and decay is also lower for wrapped clusters compared to circular clusters, exhibiting greater stability of wrapped clusters under varying cellular concentrations of IRE1. This modeled behavior aligns with experimental observations and suggests cluster wrapping around the ER tube is important to understanding IRE1 cluster dynamics and corresponding signaling. UPR malfunction is implicated in the development of many diseases including neurodegeneration and cancer. By furthering understanding of IRE1 protein clustering, we gain insight into how ER morphology controls the IRE1 behavior and impacts UPR signaling

2481-Pos

Understanding the spatial and functional link between the IL-2R and TCR at the immunological synapse

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The immunological synapse is the molecular platform for signal integration that controls T cell activation in response to antigen presentation. Interleukin 2 (IL-2) is an essential signal for effective T cell activation and proliferation after T cell receptor (TCR) engagement. However, the molecular mechanisms that link IL-2 signaling with TCR activation are not fully understood. Here, we investigate the spatial and temporal relationship between the IL-2 receptor (IL-2R) and the TCR function by visualizing their localization and activity during the formation of a synaptic contact. We use a supported lipid bilayer (SLB) system to mimic the surface of a virus-infected cell and we visualize antigen-specific CD8+ T cells forming a contact with it. The SLB system allows us to manipulate the two-dimensional accessibility of IL-2R and TCR ligands at the contact. For this purpose, we use the novel biologic therapeutics Immuno-STATs™. The full Immuno-STATs™ framework consists of a Fc-formatted peptide-HLA complex and a modified IL-2 with reduced affinity. Using structural variants of these compounds, we can manipulate the spatial link between IL-2 and TCR signaling pathways and evaluate its relevance for an efficient synapse formation. Our data sheds light into the mechanisms of early IL-2 action and IL-2R assembly during the immunological synapse.

2482-Pos

Phosphatidyl serine preconditions the A_{2A}AR for G-protein coupling via a conserved motif on TM6 and TM7

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^{19}F NMR of A_{2A}AR in nanodiscs of defined composition indicates that negative charge modulates activation of the receptor. The fully active state is obtained either when bound to G-protein, or without G-protein but in a membrane environment containing negatively charged headgroups. Mutagenesis indicates that a trio of residues on the intracellular ends of TM6 and TM7 are responsible for the lipid-dependent activity. In order to determine the mechanism, a series of molecular dynamics simulations were performed. Comparison of simulations of the inactive and the active, G-protein bound state shows that a glutamic acid on the helix of the G-protein coordinates interactions between these three residues. This suggested a hypothesis in which a negatively charged headgroup can stand-in for the glutamic acid side chain, coordinating the ends of TM6 and TM7 and preconditioning the receptor for G-protein coupling. To test this idea, simulations of the fully active state were performed with and without phosphatidyl serine