cells. Hemagglutinin (HA), the most abundant IAV membrane protein, is the primary target by the adaptive immunity and possesses well-studied protective epitopes both in its membrane-distal head domain and membrane-proximal stem. Although high conservation of the HA stem makes it an ideal target for long-lasting and broad immunity, the head region of HA tends to elicit more antibodies. Unfortunately, high mutation rates in the HA head necessitate annual vaccination against seasonal strains. To overcome the challenges in eliciting high titers of neutralizing antibodies against the conserved HA stem, a mechanistic understanding of the physical factors that limit its recognition by B cell receptors (BCRs) is needed. To address this gap in knowledge, we developed an *in vitro* imaging-based system to reconstitute the antigen extraction process using B cells genetically engineered to recognize one of several HA epitopes. We find that despite the ability of stem-specific BCRs to recognize membrane-proximal epitopes on whole virions, these BCRs are highly susceptible to inhibition by soluble antibodies that target either directly overlapping or non-overlapping epitopes. Thus, although stem-specific B cells are easily able to bind to HA under naïve conditions, our results suggest that their high sensitivity to epitope masking may contribute to the overall dominance of head-specific antibodies. We have also found that different epitopes have different sensitivity to epitope masking and the extent of epitope masking is likely dependent on the binding kinetics of the masking antibodies. Collectively, these results suggest that epitope accessibility—modulated by the presence or absence of competing antibodies—is a key factor for effective recognition of viral epitopes by B cells.

532-Pos

Stochastic model of extracellular vesicle-mediated immunosuppression implicates importance of biophysical parameters in T cell signaling

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Solid tumors represent up to ninety percent of all cancers and pose significant challenges in developing effective therapies due to the complex biochemical and biophysical barriers they develop. These barriers establish a tumor microenvironment capable of promoting tumor development and suppressing host immune responses against the tumor. Recent studies demonstrate that secreted extracellular vesicles (EVs) contribute to these microenvironments in part by carrying bioactive molecules capable of modulating the behavior and function of recipient immune cells. In particular, the checkpoint molecule PD-L1 was shown to contribute to EV-mediated immunosuppression of cytotoxic T cells, one of the main drivers of anti-tumoral activity. However, it is currently unknown what biophysical parameters mediate this immunomodulation and how they might interface with known biochemical parameters. To address this and gain a mechanistic understanding of how an EV affects cytotoxic T cell function, here we develop a novel model in which a Monte Carlo simulation of the EV/cell interface informs a stochastic model of T cell receptor (TCR) signaling. We find that various clustering patterns arise at the interaction interface as a result of binding affinities and biophysical parameters such as molecule length and flexural rigidity, leading to significantly different levels of phosphorylation of the transcription factor AKT, a critical player in cytotoxic T cell activation and development. These results bolster the growing evidence supporting the general importance of molecular clustering, a biophysical phenomenon, in regulating TCR signaling. Furthermore, these results provide novel quantitative and mechanistic insight into how EVs may serve to increase the local concentration of PD-L1 available to a cytotoxic T cell, thereby mediating tumor immunosuppression.

533-Pos

STED microscopy and single-molecule FRET to uncover membrane receptor-ligand interactions in apoptosis signal initiation

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The membrane receptor cluster of differentiation 95 (CD95), also known as FAS or Apo-1, plays a pivotal role in cancer cell apoptosis signaling initiation. However, some types of cancer show a deregulation of this apoptosis mechanism leading to proliferation rather than cell death. As the molecular conditions leading to proliferation or apoptosis are fundamentally not understood, we investigate the structural preconditions on the membrane as decisive factor to trigger a particular cell response. In our recent study, we show the primary monomeric and few dimeric CD95 undergoing conformational changes after CD95 ligand (CD95L) activation, with CD95 oligomerizing to dimers and trimers. The following downstream signal results in the activation of caspases 8, 3, and 7 and eventual cell death. Intriguingly, the efficiency of signaling activation appears to vary with CD95L presentation (membrane bound or in solution) and corresponding membrane protein stoichiometries are still unclear. Here, we design and purify structurally modified CD95 ligands and receptors, exhibiting different competency to trigger the apoptosis signal. Using stimulated emission depletion microscopy (STED) microscopy, we explore the relation between receptor-ligand complexes on the membrane relative to the signaling outcome. STED microscopy and single molecule FRET spectroscopy are further used to probe receptor/ligand interactions of particular protein domains. To this end, an imaging platform based on the biomolecules anchored to DNA origami with nanoscale precision is established.

534-Pos

Allosteric communication across the μ-opioid receptor-G_{i1} protein complex induced by ligands of varying efficacy

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Some of the most effective medications for severe pain are high-efficacy μ-opioid receptor (MOR) agonists (e.g., morphine). However, these drugs can also cause adverse effects, including potentially fatal respiratory depression from overdose, and addiction. Interestingly, several low-efficacy MOR agonists, including clinically used opioid drugs (e.g., buprenorphine) and atypical opioid chemotypes (e.g., mitragynine pseudoindoxyl (MP)), cause fewer or weaker adverse effects compared to full agonists. Available MOR-G_{i1} cryo-electron microscopy (cryo-EM) structures, bound to either the high-efficacy opioid lofentanil or the low-efficacy agonist MP, together with molecular dynamics (MD) simulation studies of the corresponding ligand-MOR systems (without G_{i1}), have suggested that the different efficacies of these opioids may result from variations in the binding site promoting distinct active-state conformations of the receptor at the interface with G_{i1}. In this work, we explore the possibility of variations within the G_{i1}, and offer atomic-level details of the ligand-specific allosteric communication across both the MOR and the G_{i1} to deepen our understanding of the molecular determinants that influence the different safety profiles of opioid ligands. Specifically, we built complete structures of various ligand-MOR-G_{i1} complexes by incorporating the absent G_{i1} alpha helical domain into the existing cryo-EM structures of MOR-G_{i1} complexes in a starting open conformation, using an available structural template. We then derived ligand-induced allosteric communication pathways across the entire ligand-MOR-G_{i1} complexes, applying robust statistical analysis tools to trajectories from MD simulations to analyze the causality of correlated motions in these systems. Our analyses unveiled statistically significant differences among the MOR residues that were the primary contributors to the allosteric communication in each simulated ligand-MOR-G_{i1} system. Some residues uniquely contributed to allosteric communication in certain systems, thereby offering testable hypotheses on the mechanistic basis of opioid ligand efficacy for G_{i1} signaling.

535-Pos

Drug-resistant EGFR lung cancer mutations promote tumor growth by stabilizing interfaces in ligand-free signaling-competent EGFR oligomers

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In non-small cell lung cancer (NSCLC), the epidermal growth factor receptor (EGFR) frequently undergoes mutations and has been successfully targeted with tyrosine kinase inhibitors. However, drug resistance eventually emerges as a significant hurdle in treatment. Overcoming this has been hindered by the poor understanding of how wild-type (WT)-EGFR elicits ligand-independent signals essential for cell homeostasis, which are later exploited by cancer mutations. Ligand-independent signaling cannot be explained by