leverages the wavelength-dependent properties of total internal reflection fluorescence microscopy. Dual-tagging a protein of interest with two spectrally separated fluorophores allows STAR to measure the intensity ratio and retrieve the z-position of the protein. Although the exponential decay of the evanescent wave is critical for STAR, it results in uneven excitation of the dual-tagged proteins. This might weigh down STAR measurements if dual-tagged proteins are distributed on a 3D object, such as a CCV. To understand the accuracy of STAR for studying vesicle formation, we used mathematical modeling. We represented the CCV as a monodisperse sphere and used the STAR equations to calculate vesicle height (Δz) and compared it to the theoretical center of mass (CM). We investigated the influence of vesicle formation, radius, the number and distribution of proteins, and the distance of the vesicle from the plasma membrane. In the initial formation of a 50-nm radius vesicle, the CM and Δz align until vesicle formation reaches 35% (CM = 33 nm). As vesicle formation continues, the calculated Δz was gradually less than the theoretical CM. This discrepancy was dependent on radius and elevation. We also investigated how photon noise influenced STAR measurements using Monte Carlo simulation. This mathematical model has allowed us to assess how the Δz measured by STAR compares to the ground truth. We will use these theoretical findings to improve STAR microscopy and develop a 3D dynamic model of the CCV formation from our experimental data.

362-Pos

Cell surface-bound La protein regulates the cell fusion stage of osteoclastogenesis

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Bone-resorbing osteoclasts are essential for life-long skeletal remodeling, and perturbations in their activity contribute to bone diseases afflicting >200 million. Treating resorptive bone diseases has focused on suppressing osteoclast development. Here we share a novel target at the cells' surface that shifts this paradigm by instead tuning the formation and function of osteoclasts at the mechanistic stage of cell-cell fusion. The fusion of precursors produces multinucleated osteoclasts, and each successive fusion event increases an osteoclast's resorptive capacity. Despite their importance, the mechanisms that control osteoclast fusion remain elusive. Here we report that the nuclear, RNA chaperon, La protein moonlights as a master fusion regulator at the surface of osteoclast precursors, controlling their size and resorptive capacity. Surprisingly, ubiquitous La disappears in precursors and, during osteoclast fusion, dramatically reappears as a cleaved, non-nuclear species. Cleaved La relocates to the surface of fusing osteoclasts and regulates membrane fusion by mechanisms independent of La's canonical RNA interactions. Finally, cleaved La is degraded in mature osteoclasts, and full-length La returns to the nuclei of mature cells. Raising or lowering La levels or surface activity promotes or inhibits fusion and bone resorption, respectively. Moreover, in a co-culture system where osteoclast formation is induced by osteoblasts, we found that suppression of surface La activity inhibits the formation of multinucleated osteoclasts in primary co-cultures. Surface La also controls ectopic osteoclast formation *in vivo*, in an inducible mouse model of fibrous dysplasia, and *ex vivo*, in murine marrow explants. Injections of anti-La antibodies suppressed bone loss *in vivo* and inhibited ectopic osteoclast formation *ex vivo*. We expect that targeting the La-dependent stage of osteoclast formation will lead to novel therapeutics and fill an unmet need in the treatment of resorptive bone diseases in the world's aging population.

363-Pos

Specific membrane lipids are required for different sub-reactions of SNARE-mediated membrane fusion

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Regulated intracellular membrane fusion requires SNARE proteins, Rab GTPases, and several chaperones including Sec17/αSNAP, Sec18/NSF, and SM proteins. In the model vacuolar membrane fusion system, it was recently found that the Rab Ypt7 stimulates the SM protein complex HOPS to catalyze SNARE complex assembly directly, and that this stimulation requires a certain lipid composition in the membrane. In this study, we present the effects of the 8 major lipids in the vacuolar membrane on several sub-reactions of membrane fusion. We show that phosphatidylinositol and phosphatidylinositol-3-phosphate are required for Ypt7-dependent activation of HOPS for trans-SNARE assembly, but they are not sufficient for membrane fusion. Rather, the lipids PE, PA, PS, and DAG each promote bilayer rearrangement for the final step of membrane fusion. Thus, regulated membrane fusion requires multiple sets of lipids for different steps in the fusion pathway.

364-Pos

Modulation of membrane fusion using a novel fusogenic system composition

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Cell membranes are believed to be heterogeneous and to exhibit lipid domains of coexisting phases, which can govern cellular functions and processes. Different factors can affect membrane fusion efficiency, such as the membrane phase state. In this work, we proposed a new composition of the fusogenic system preferentially in the gel phase at room temperature but fluid in physiological temperatures; additionally, membrane fusion efficiency was also investigated. The fusogenic system was based on large unilamellar vesicles (LUVs, 100-150 nm) and composed of an equimolar mixture of neutral and positively charged gel lipids, whereas the GUVs were composed of a mixture of zwitterionic and/or negatively charged fluid lipids. Initially, the fusogenic system was characterized using dynamic light scattering (DLS), zeta potential, anisotropy and differential scanning calorimetry (DSC). Then, fusion efficiency was characterized using microscopy-based fluorescence assays (lipid and content mixing). The DLS and zeta potential measurements exhibited a size average of ≈120 nm and a surface charge of ≈42 mV, respectively. The anisotropy and DSC measurements showed that the lipid mixture was in the gel phase at room temperature. The incubation of the fusogenic system with neutral GUVs did not lead to significant lipid mixing in any incubation condition and therefore no/low membrane fusion. However, negatively charged GUVs exhibited high lipid and content mixing. Interestingly, the highly membrane fusion efficiency in high temperature incubation induced phase-separation in the GUVs. We demonstrated that membrane fusion can be controlled by modulating the membrane charge of the system despite the membrane phase state of the fusogenic system. These findings can play an important role in regulating the interaction between cells and liposomes used in drug delivery systems.

365-Pos

Fusion peptide from SARS-2 spike transforms into a wedge inserted in a bilayer leaflet, and thins the opposite leaflet

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Activation of SARS-CoV-2 Spike deploys its fusion peptide to a membrane of the host cell to infect it. NMR in solution demonstrates that this fusion peptide transforms from intrinsic disorder in solution into a wedge-shaped structure inserted in bilayered micelles. According to NOEs and proximity to a nitroxide spin label deep in the membrane mimic, the globular fold of three helices contrasts the open, extended conformations observed in compact prefusion states. In the hydrophobic, narrow end of the wedge, helices 1 and 2 contact the fatty acyl chains of phospholipids. 50 of the resulting paramagnetic NMR relaxation enhancements and 6 lipid-protein NOEs provided ambiguous distances as collective variables (colvars) to bias and guide MD simulations. Simulations in NAMD using the CHARMM36 forcefield included colvars for 130 medium- and long-range NOEs to maintain the equilibrium structure. In the gently NMR-biased simulations, the fusion peptide maintained its insertion of helices 1 and 2 within a single leaflet while helix 3 remained exposed. A cation occasionally visited the anionic side chains in the loop joining helices 2 and 3 or at the N-terminal end of helix 1. The unoccupied leaflet is thinned and distorted opposite the fusion peptide. The thinning could be related to the fusion peptide promoting formation of the hemi-fusion intermediate in the process of viral-cell fusion. Supported by NSF Rapid award 2030473. See Koppisetti et al. (2021, DOI: 10.1021/jacs.1c05435).

366-Pos

Elucidating the distinct structural features of the SARS-CoV-2 spike protein fusion domain

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The spike protein of SARS-CoV-2, the primary antigenic target of COVID-19, has been studied extensively since the emergence of the virus in late 2019. With most research focused on receptor binding the immediate next step in the viral lifecycle, membrane fusion, remains poorly understood. Viral glycoproteins often contain a short stretch of highly conserved and predominately hydrophobic residues known as the fusion domain (FD), which is widely accepted as the initiator of membrane fusion. A greater understanding of the