moribidity. Little is known about the mechanisms of storage and release of toxins in algal species responsible for harmful algal blooms. Toxins have been thought to be exported from these unicellulars by an ill-defined “exudation” mechanism. Although secretion is a standard strategy of material export in plants and animal cells, regulated exocytosis has only recently begun to be explored in dinoflagellates (FEBS Letters 2006, 580:2201-2206). Results presented here using fluorescently-labeled antibrevetoxin antibodies show that brevetoxin is present in Karenia’s secretory vesicles and is released following blue light-stimulated exocytosis. The matrix of secretory granules functions as a caging polymer network that holds immobilized high payloads of active molecules including hormones, antibacterial peptides, or in this case toxins (Ann. Rev. Physiol 1990:52: 157-176). Upon release, Karenia’s vesicle matrix undergoes typical phase transition from condensed to solvated phase, with characteristic first order kinetics swelling, and release of its brevetoxin payload. These observations support the notion that Karenia b functions as a typical secretory cell, opening the way for a better understanding of Red Tide blooms dynamics.

1625-Pos Board B395
Influenza Virus Hemagglutinin Delays Endosomal Acidification - a Strategy for Successful Delivery of the Viral Genome?
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Upon endocytic uptake of influenza virus, acidification of the endosomal lumen triggers a conformational change of the virus spike protein hemagglutinin (HA) leading to fusion between the endosomal and the viral membrane. For efficient infection, release of the viral genome occurs in the vicinity of the nucleus to prevent lyosomal degradation. To study cellular antiviral response, we investigated the effect of cytoskeletal inhibitors on virus infection and fusion using a combination of single virus tracking and an intracellular fusion assay. In control cells, fusion mostly occurs in the perinuclear region. Inhibition of endosomal transport along microtubules by nocodazole did not change the numbers of fusion events, but randomized their sequence. As nocodazole also affects cellular and viral GTPases, we tested whether GTPase specific inhibitors would delay fusion. Using a glucose transporter, all mammalian cells take up glucose from the extracellular space for energy and metabolism. In this study we followed the dynamics of the surface distribution of insulin-regulated glucose transporter −4 (GLUT4), introduced as a photo-switchable GLUT4-EOS probe to learn how GLUT4 molecules diffuse and interact with GLUT4 domains resident in the PM. Using Fluorescence Photo-Activation Localization Microscopy (FPALM), we observed dynamic confinement and release of GLUT4 from these domains, and measured lifetime of GLUT4 molecules trapped inside the domains. Although, the average time molecules spend within the domain was several orders of magnitude longer than time expected as we cannot separate between these two in this measurement. The residence time distribution of channels that are on the cell surface from the beginning of our measurements has a median of 119 s, whereas for recycled channels the median is only 81 s, a 32% reduction (n = 334). In both instances it is surprising how short the residence time is on the cell surface of these channels. We propose that rapid channel turnover, via recycling pathways, helps the cell to maintain specialized regions in the membrane, which are entropically unfavorable. We investigate the role of actin in Kv2.1 trafficking using actin polymerization inhibitors. Upon the application of 5 μM cytochalasin D and 80 μM swinholide A, we observe that the residence times of both newly synthesized and recycled proteins are significantly reduced. In cells treated with actin inhibitors, channels are no longer sequestered into specific microdomains. Thus, channel recycling may function as an important factor in membrane compartmentalization and may be enhanced by stimuli that disrupt this organization.

1627-Pos Board B397
Rapid Cell Surface Kv2.1 Recycling Observed by Single Molecule Tracking
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We study the insertion and retrieval of voltage-gated potassium channels, Kv2.1, at the single molecule level. Kv2.1 channels are labeled with quantum dots (QDs) at an extracellular domain. We observe QDs being internalized by the cell and new QD-tagged channels being inserted into the membrane. Because labeling occurs solely on the cell surface, only recycled channels that were previously in the plasma membrane can carry emerging QDs. Controls with both GFP and QD labels indicate that newly arriving QDs are indeed Kv2.1 channels. Channels that are in the plasma membrane from the beginning of the experiment can be either recycled or newly synthesized channels, as we cannot separate between these two in this measurement. The residence time distribution of channels that are on the cell surface from the beginning of our measurements has a median of 119 s, whereas for recycled channels the median is only 81 s, a 32% reduction (n = 334). In both instances it is surprising how short the residence time is on the cell surface of these channels. We propose that rapid channel turnover, via recycling pathways, helps the cell to maintain specialized regions in the membrane, which are entropically unfavorable. We investigate the role of actin in Kv2.1 trafficking using actin polymerization inhibitors. Upon the application of 5 μM cytochalasin D and 80 μM swinholide A, we observe that the residence times of both newly synthesized and recycled proteins are significantly reduced. In cells treated with actin inhibitors, channels are no longer sequestered into specific microdomains. Thus, channel recycling may function as an important factor in membrane compartmentalization and may be enhanced by stimuli that disrupt this organization.

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Fence Model for Dynamic Exchange and Retention of GLUT4 in Plasma Membrane Domains
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Using a glucose transporter, all mammalian cells take up glucose from the extracellular space for energy and metabolism. In this study we followed the dynamics of the surface distribution of insulin-regulated glucose transporter −4 (GLUT4), introduced as a photo-switchable GLUT4-EOS probe to learn how GLUT4 molecules diffuse and interact with GLUT4 domains resident in the PM. Using Fluorescence Photo-Activation Localization Microscopy (FPALM), we observed dynamic confinement and release of GLUT4 from these domains, and measured lifetime of GLUT4 molecules trapped inside the domains. Although, the average time molecules spend within the domain was several orders of magnitude longer than time expected from free diffusion estimates, the molecules within the domain exhibited high mobility, and experienced multiple reflections from the boundaries of the domain. We further provide evidence that exocytosis serves as main route of delivery and protein-specific retention of GLUT4 in plasma membrane domains. Fusion of GLUT4 vesicles with existing domains resulted in selective retention of GLUT4, but not other proteins co-transported in the same vesicles. Interestingly, insulin-stimulated fusion of GLUT4 vesicles that took place outside of the domains resulted in complete dispersal and free diffusion of GLUT4 into the plasma membrane. Importantly, we show that for endocytosis, GLUT4 molecules had to redistribute back to the domains, as freely-diffusing GLUT4...