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morbidity. 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 favorably occurs in the vicinity of the nucleus to prevent lysosomal degradation of the viral RNA and activation of the cellular antiviral response. How influenza viruses ensure optimal duration of endosomal residence to avoid premature as well as delayed fusion and release of the genome is not understood.
The tight packing of HA in the viral envelope represents a remarkably high intra-endosomal protein concentration with high buffering potential for incoming protons. By using pH sensitive fluorescent markers we could show for the first time that the presence of a virus inside an endosome drastically slows down the acidification kinetics. We investigated the effect of cytoskeletal inhibitors on virus fusion and infection 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 localization within the cell. Interestingly, this dislocation correlates with strongly reduced infection efficiency, confirming that the site of virus-endosome fusion indeed plays an important role in the delivery of the viral genome.
Taken together, our results demonstrate that influenza virus HA delays the endosomal acidification to ensure timely as well as locally optimal release of its genome. This suggests a general function of the high-density packing of spike proteins that is characteristic of enveloped viruses infecting via the endocytic route.

1626-Pos Board B396
Role of Rac1-GTPase in Glucose Inhibition of Glucagon Secretion
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The significance of glucagon in glucose homeostasis is becoming ever clearer, yet the mechanisms underlying its secretion from pancreatic α-cells remain poorly understood. Clinical studies show that drugs that suppress glucagon secretion can restore normoglycemia in diabetic patients, suggesting a possible alternative to insulin treatments. Two classes of models have been proposed to explain glucose inhibition of glucagon secretion (GIGS), but both share a requirement for Ca2+ triggering. In contrast, data from our lab show that changes in α-cell Ca2+ activity do not correlate with GIGS. This suggests that glucagon secretion is suppressed downstream of membrane depolarization, potentially at the level of exocytosis. The F-actin modifying Rac1-GTPase can regulate insulin secretion from β-cells by direct action on the exocytotic machinery. It has been shown that cAMP can regulate Rac1 activation in β-cells through PKA activation. Additionally, glucose metabolism activates the cAMP pathway independently of Ca2+ oscillations, though the complete role of metabolic activity in exocytosis is unknown.
Since the α-cell and β-cell are closely related, we hypothesize that GIGS depends on cAMP signaling that leads to deactivation of Rac1 and down-regulation of exocytosis. To identify the role of Rac1 in regulating GIGS, we have developed EGFP-tagged dominant negative and constitutively active Rac1 viral constructs that we use to infect murine islets with RFP-labeled α-cells. This allows us to study directly the effects of Rac1-GTP and Rac1-GDP on α-cell glucagon secretion, Ca2+ activity, and glucose metabolism to determine their relative roles in Rac1-mediated GIGS. Additionally, we are characterizing the glucose dose-response of Rac1 activation in α-cells. Finally, to determine the role of cAMP signaling we are using cAMP and PKA agonists at low and high glucose to identify their effects on Rac1 activation state and glucagon secretion.

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.

1628-Pos Board B398
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

molecules did not accumulate in clathrin-coated pits that were not associated with GLUT4 domains.
Based on these data we propose fence-based model for retention and accumulation of GLUT4 at specific domains of the plasma membrane responsible for active delivery and internalization of GLUT4 distinctly from other proteins undergoing constitutive recycling.

1629-Pos Board B399
Microtubule-Driven Migration of Clathrin-Coated Pits Towards Vesicle Fusion Sites for Rapid Recycling in Pancreatic Beta Cells
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Due to the long time required for de novo formation of deeply invaginated clathrin-coated pits that mostly appear at sites different from exocytosis before fission, clathrin-dependent vesicle recycling in secretory cells is usually regarded as a slow process. Here we show that regulated exocytosis in pancreatic beta cells is associated with glucose-dependent recruitment of clathrin and dynamin-1 puncta at the release sites shortly or long after fusion pore opens. The subsequent disassembly of these clathrin puncta mediates internalization of synaptotagmin VII clusters in the plasma membrane. The fast clathrin-dependent endocytosis contributes significantly to the total vesicle recycling process, and involves microtubule-dependent linear movement of preformed clathrin-coated pits to vesicle release sites prior to fission. These results have revealed an unexpected close spatio-temporal coupling of clathrin-dependent endocytosis to vesicle fusion in pancreatic beta cells, and highlighted a novel pathway to replenish the ‘‘readily retrieval pool’’ of clathrin that sustains fast clathrin-dependent endocytosis under intense stimulation.

1630-Pos Board B400
Clathrin Self-Assembly into Polyhedral Cages Studied by Computer Simulations
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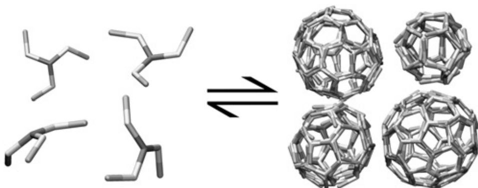
Clathrin is a three-legged proteins that self-assembles into polyhedral cages with regulatory and mechanical functions in the formation of cargo-laden vesicles at the cell-membrane and trans Golgi network. The essential features of self-assembly are innate to clathrin, as cages are also formed in purified slightly acid solutions. Our simulations of this process using a highly coarse-grained clathrin model reveal that a non-uniform distribution of interactions over clathrin's surface, rather than its characteristic shape, holds the key to self-assembly [1]. The cages are polydisperse, with a strong preference for a small subset of all possible configurations with twelve pentagonal and a variable number of hexagonal faces. Based on the experimental critical assembly concentration, we deduce an average binding energy of ~23kT per clathrin [2]. The simulations also answer the long-standing question of how a flat purely hexagonal clathrin lattice can produce a cage with twelve pentagons: the introduction of spontaneous curvature through a change of hub and/or knee puckers causes tensions that result in the release of dome-shaped fragments, which may subsequently grow into full cages by recruiting cytosolic clathrin [2].



[1] Biophys. J. 99, 1231 (2010).
[2] Traffic 12, 1407 (2011).

1631-Pos Board B401
Stochastic Nature of Clathrin-Coated Pit Assembly
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The dynamics of clathrin coated pit (CCP) formation, observed through total internal reflection fluorescence microscopy, shows considerable diversity. Foremost is fate divergence, which leads to ‘‘abortive’’ and ‘‘productive’’ pits, i.e., structures which, respectively, do or do not mature into clathrin coated vesicles (CCVs). Also, there is notable heterogeneity in the lifetimes of abortive pits and the apparent time to the completion of productive CCPs. We explore the extent to which the stochastic nature of CCP growth can explain these observations. For this purpose we analyze a simple model that includes a kinetic scheme for CCP assembly and a related functional form for free energy of CCP formation. Using this model, we calculate the lifetime distribution of abortive pits (via Monte Carlo simulation) and fit it to experimental data to determine the exact effective potential experienced by CCPs. We show that the CCPs without cargo are energetically unstable, and that the binding of cargo might stabilize a CCP and thereby facilitate CCV formation. Finally, we estimate how variation in the time of CCV formation is linked to the stochastic associations and dissociations of coat components.

1632-Pos Board B402
Dynamin I Regulates Activity-Dependent Fusion Pore Dilation via a Calcineurin-Dependent Pathway in Mouse Adrenal Chromaffin Cells
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Chromaffin cells of adrenal medulla utilize two modes of exocytosis in order to achieve a proper response to sympathetic input under basal tone as well as the sympathetic stress response. Under sympathetic tone, modest synaptic excitation drives chromaffin cells to selectively secrete modest levels of catecholamine through a restricted fusion pore. In contrast, elevated sympathetic activity experienced under acute stress results in dilation of fusion pore to achieve maximal catecholamine release and to facilitate release of co-packaged peptide transmitters. Therefore, the dilation of fusion pore is the key control point for the activation of the sympatho-adrenal stress response. Despite the physiological importance of this process, the molecular mechanism for how it is achieved is still unclear. Here, we employ electrophysiological, electrochemical, and fluorescence based approaches to investigate hypothesized signaling pathway for the regulation of activity-mediated fusion pore expansion. We show that dynamin I is dephosphorylated by calcineurin only under high stimulation. Calcineurin-mediated dephosphorylation of dynamin I leads to the recruitment of syndapin-N-WASP. Disruption of each step of this cascade results in limited fusion pore dilation. Our results suggest that fusion pore dilation is regulated by a calcineurin-dependent dephosphorylation of dynamin I.

1633-Pos Board B403
Structural Analysis of Dynamin Reveals Power Stroke
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D Dynamin family members are large GTPases involved in membrane fission and fusion events throughout the cell. The founding member, dynamin, plays a major role in vesiculation events during endocytosis, synaptic membrane recycling, and membrane trafficking. The current model predicts dynamin wraps around the necks of coated pits and upon GTP hydrolysis dynamin constricts and disassociates from the lipid, which then leads to membrane fission. In support of this model, purified dynamin self-assembles into spirals around lipid, generating dynamin-lipid tubes that constrict, twist and fall off upon GTP addition. To determine the conformational changes that occur during GTP hydrolysis, we calculated 3D maps of dynamin by cryo-electron microscopy methods. Here we present our latest 3D map of ΔPRD-dynamin (resolution of 12 Å) with three crystal structures docked into our map, the GMP-PCP GG domain (GTPase domain-GED fragment), the stalk domain from another dynamin family member, MxA, and the PH domain from dynamin. Based on the docking results, we predict the location and interactions between the domains. In addition, comparison between the GTP-bound state (GMP-PCP) and transition state (GDP.AlF4-) within the GG construct suggests that the conformational change induced by GTP hydrolysis drives a large swing of the BSE (bundle signaling element). We predict that the BSE movement is dynamin's power stroke that results in a significant twist and constriction of the underlying lipid bilayer leading to membrane fission. Recently, we have calculated a 3D map of full-length dynamin in a further constricted state, with a resolution of ~15Å. The inner lumenal diameter of this structure is ~2-4 nm, a range that is compatible with spontaneous lipid fusion. Currently we are docking the crystal structures into our K44A-dyn map to identify changes within dynamin domains that leads to maximum constriction and ultimately membrane fission.