role of the dynamin GTPase activity in controlling fusion pore expansion and post-fusion granule membrane topology was investigated using the powerful membrane curvature-imaging technique of polarized TIRF microscopy and amperometry. A dynamin-1 mutant with increased GTPase activity resulted in faster fusion pore widening and flattening of the granule membrane after exocytosis; dynamin-1 mutants with decreased activity slowed fusion pore widening by stabilizing post-fusion granule membrane curvature. The experiments indicate that the GTPase activity of dynamin functions as a timer determining the rapidity of fusion pore expansion from 10's of milliseconds to seconds after fusion, in addition to its role in endocytosis. These findings expand the membrane-sculpting repertoire of dynamin to include the regulation of immediate post-fusion events in exocytosis that control the rate of release of soluble granule contents.

159-Plat
Inhibition of F-Actin Cycling in Rbl Mast Cells Prevents Endosome Acidification but not Internalization of Antigen-Crosslinked IgE Receptor Complexes
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Previous studies have implicated roles for the actin cytoseleton in endocytosis via clathrin-coated pits, as well as in antigen-stimulated endocytosis of immunoglobulin-E (IgE) receptors (Ra et al., Eur. J. Immunol. 19:1771, 1989). To monitor endocytosis in real time, we use a fluorescence assay that reports pH-dependent quenching of FITC-IgE. We find that micromolar concentrations of either cytochalasin D or latrunculin A, inhibitors of actin polymerization, as well as jasplakinolide, a stabilizer of F-actin polymerization, all prevent this antigen-stimulated FITC quenching due to endosomal acidification. Under these conditions, crosslinked IgE receptors appear localized in endosomes by confocal imaging, despite the lack of acid-dependent FITC quenching. A quantitative flow cytometry assay for surface-available IgE shows that greater than 60% of these IgE receptor complexes are internalized in response to antigen crosslinking in the presence or absence of these inhibitors of actin polymerization cycling. After 20 min at 37oC with antigen, we observe partial co-localization of IgE receptor-containing endosomes with a lysosomal marker, indicating normal endosomal trafficking of these complexes in the presence or absence of these agents. Addition of latrunculin A or cytochalasin D after antigen stimulated endocytosis causes rapid, partial reversal of endosomal acidification monitored by FITC-IgE, suggesting dynamic regulation of this process by the actin cytoskeleton. In contrast, secretory lysosomes labeled with FITC-dextran by fluid-phase pinocytosis maintain normal acidification in cells treated with these agents. In ongoing experiments we are investigating the molecular mechanism for cytoskeletal regulation of endosomal acidification.

160-Plat
Understanding the Fundamentals of Platelet Granular Storage and Release at Single Cell Level
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Platelets are traditionally recognized as critical cells in hemostasis and thrombosis but have also recently been identified to play a significant role in many diseases, including bacterial infections, cancer, and allergic asthma. Platelets store and release important messenger molecules via exocytosis from two populations of granules (α- granules with adhesive protein species and δ- granules with small molecule/ion species). However, very little known about how chemical messengers are stored in these granules and the driving forces for secretion into the blood stream, thus limiting the development of new therapeutic approaches to manage the role of platelets in many physiological events. Carbon-fiber microelectrode amperometry (CFMA) enables both quantal and kinetic analysis of the exocytotic event of single cell with sub-ms time resolution but is limited to measuring electroactive species from δ-granules. Localized surface plasmon resonance (LSPR) spectroscopy is a complementary tool that offers an outstanding sensing ability by measuring the extremely small changes in the sensing media. This work aims to overcome the gap in fundamental knowledge about platelet granular storage and secretion by detecting serotonin secretion from δ- granules using CFMA and Platelet factor 4 (PF4) secretion from α- granules using LSPR. These measurements are performed while exposing platelets to different stimulants, including ionomycin, adenosine diphosphate (ADP), and thrombin. Significant differences are seen in both α- and δ-granule secretion with varied stimulant exposure, and these combined measurements yield biophysical insight into the mechanism behind these secretory behaviors.

161-Plat
Low Physiological Level of Stimulation Elicits Exocytosis, not by Ca^{2+} Influx, but by Suppression of Ca^{2+} Syntillas in Mouse Adrenal Chromaffin Cells
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Catecholamine and neuropeptide exocytosis from adrenal chromaffin cells (ACCs) is controlled by sympathetic autonomic discharge. It has been thought that sympathetic discharge accomplishes this task exclusively by regulating Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs), which serves as a direct trigger for exocytosis. But our studies on spontaneous exocytosis in ACCs revealed the presence of Ca^{2+} syntillas, focal cytosolic transients mediated by ryanodine receptors (RYRs), and these had the surprising effect of *inhibiting* spontaneous exocytosis (Lefkowitz et al., 2009).
Here we examine the role of syntillas under physiologic stimulation in ACCs using simulated action potentials (sAPs) designed to mimic native input at a frequency associated with basal sympathetic tone: 0.5 Hz. Stimulation at this frequency induces a general increase in the frequency and size of amperometric events comparable to that observed when syntillas were suppressed under spontaneous release conditions. Unexpectedly, we found that sAPs delivered at 0.5 Hz completely abolished Ca^{2+} syntillas within two minutes. Ca^{2+} arising from VGCCs was not enough to elevate the global $[Ca^{2+}]_i$ as measured with fura-2. Hence, it appears that *inhibition* of syntillas by action potentials in ACCs is responsible for the increase in exocytosis at this level of stimulation.
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Lefkowitz, J.J., K.E. Fogarty, L.M. Lifshitz, K.D. Bellve, R.A. Tuft, R. ZhuGe, J.V. Walsh, Jr., and V. De Crescenzo. 2009. Suppression of Ca^{2+} syntillas increases spontaneous exocytosis in mouse adrenal chromaffin cells. *J Gen Physiol* 134(4):267-280.

162-Plat
Complexin Facilitates Coupling of Secretory Vesicles and Voltage-Gated Calcium Channels
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The SNARE complex mediates fusion of vesicle and plasma membranes in exocytosis. Complexin (Cplx) binds with high affinity and 1:1 stoichiometry to the SNARE complex and is believed to regulate SNARE function. However, how Cplx functions is still controversial. Using mouse neuromuscular junctions (NMJs) and adrenal chromaffin cells, we tested the hypothesis that Cplx, in addition to enlarging the pool of primed vesicles, enhances exocytosis and neurotransmission by increasing the coupling between vesicles and voltage-gated calcium channels (VGCC). Only Cplx 1 is expressed at adult mouse NMJs. Cplx 1 knockout NMJs displayed not only a diminished endplate potential (EPP) amplitude, but also a loss of synchronicity ("jitter"). Furthermore, whereas in response to high-frequency stimulation, wildtype NMJs displayed marked depression of EPP size upon successive stimulations, knockout NMJs showed facilitation. These observations suggest that in Cplx knockout NMJs, vesicles and calcium channels are uncoupled. To further test this hypothesis, we recorded capacitance measurements from mouse adrenal chromaffin cells. We used short depolarizations to elicit fusion of vesicles close to the calcium channels (IRP) and longer depolarizations to elicit fusion of the remaining primed vesicles (RRP). Cplx 2 KO chromaffin cells have a significantly smaller IRP/RRP ratio, and we can rescue the phenotype by heterologous expression of complexin in KO cells. Our work in two separate model systems supports a role for complexin in coupling vesicles to VGCC.

163-Plat
Complexin and Synaptotagmin Genetically Interact in vivo Controlling the Rates of Spontaneous and Nerve-Evoked Vesicle Fusion
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Presynaptic vesicle fusion is a highly regulated process that requires Synaptotagmin (SYT) and Complexin (CPX) proteins. Current data suggest that SNARE binding by the two proteins may be co-regulating the last step of release. However, their precise roles in synaptic function are still under debate. Here, we present a thorough analysis of synaptic transmission of