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Secretory granules transit through a series of highly regulated associations and dissociations of molecular interactions enroute to membrane fusion and exocytotic release of the granule content. The ability to sequence these protein-protein interactions as they occur in living cells in real-time, with high spatial resolution, is paramount to furthering our understanding of how they relate to the functional state of the secretory granule as it transits the regulated exocytotic pathway. In the present study we have taken both an experimental and a theoretical approach to gain a quantitative understanding of the effects of evanescent illumination on sensitized-emission FRET calibrations and measurements, under a variety of conditions that mimic differing subcellular localizations of interacting molecules. Our results demonstrate that the TIRF-FRET method is straightforward for simple situations in which both donor and acceptor are on the same molecule and localized to the plasma membrane. By comparison when donor and acceptor molecules are localized to multiple intracellular compartments and where one compartment may be mobile, additional considerations must be taken into account. Our results define several of the parameters that are critical to the quantitative application of this method in living cells. Moreover, we demonstrate use of TIRF-FRET to visualize and quantify a specific set of bi-molecular interactions on insulin secretory granules in Min6 cells as they occur in time and subcellular space within the cell and we correlate these to the secretory event. This work supported by NIH, NINDS 039914 and NIDDK 053978.

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Patterning Single Cell-Electrode Pairs for Electrochemical Measurement of Quantal Exocytosis on Microchips

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We are developing transparent multi- electrochemical electrode arrays on microchips in order to automate measurement of quantal exocytosis. Design goals are that one and only one cell be positioned directly over each electrode and working electrodes have μm-scale dimensions in order to resolve pA-level currents. Patterning of cell-adhesion molecules in register with electrodes using conventional photolithographic approaches is problematic because organic solvents can disable sensitive biomolecule films. We report the parylene ''dry liftoff'' approach pioneered by Ilic and Craighead (Biomed Microdev 2: 317, 2000) can be used to pattern single cell-electrode pairs on the chip. A 1 μm-thick parylene C film is deposited on the multi-electrode array and S1813 photoresist is spin coated onto the device and patterned. The unprotected parylene over the electrodes is then removed using Reactive Ion Etch. Poly-l-lysine (PLL) is then added to promote cell attachment. Chromaffin cells are loaded on the chip in standard culture media and left in an incubator overnight. Finally, the parylene film is peeled off to remove excess cells and PLL, leaving tightly adhered chromaffin cells at the desired locations. Importantly, we find that promoting cell attachment with PLL films does not passivate the electrochemical electrodes. Experiments are in process to explore an alternative approach whereby PLL is patterned using the dry liftoff approach but cells are added after peel off of the parylene. With this approach, cell attachment to inactive areas of the chip is blocked by using ''cytophobic'' materials such as Teflon AF. This alternative approach may allow efficient targeting of cells at lower cell densities as cells migrate from cytophobic areas to the electrode binding sites (Supported by NIH BRP grant RO1 NS048826).

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Endophilin N-BAR Domains-induced Membrane Remodeling Revealed by Molecular Dynamics Simulations

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Endophilin N-BAR domains play a critical role in membrane remodeling (e.g., endocytosis, synapses) due to their membrane sculpting abilities. Presently, roles of the amphipathic helices and the positively charged concave surface on the crescent dimer in membrane remodeling are still not well understood. In addition, the endophilin N-BAR domain has one additional inserted helix on each of the monomers, thus making it unique in the entire BAR superfamily. Both the structure and the function of this additional helix are unknown up to now. Interestingly, the tubulated structures of endophilin N-BAR domains are much larger than the corresponding amphiphysin N-BAR domains. It is important to investigate the effect of the inserted helices in order to fully understand the mechanism of endophilin N-BAR domain protein driven liposome tubulation. Large scale all-atom molecular dynamics simulations are used to examine the details of the endophilin mediated membrane remodeling process. By comparing the results of different possible arrangements of the protein and membrane, we predict the optimum location of the additional helix. These results will facilitate in understanding the overall mechanism of endophilin N-BAR domains membrane oligomerization and remodeling.

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Chronic Palmitate Exposure Inhibits Insulin Secretion By Dissociation of Ca^{2+}-Channels From Secretory Vesicles

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Type-2 diabetes, characterized by insufficient insulin secretion, shows a strong correlation with obesity and elevated plasma levels of free fatty acids (FFA). Long-term exposure of pancreatic islets to FFAs results in marked suppression of glucose-induced insulin secretion. Although the latter effect has been extensively characterized, the cellular mechanisms remain enigmatic. We have examined the effect of long-term exposure of pancreatic β-cells to palmitate using a combination of elecrophysiology and evanescent field microscopy. Here we show that rapid exocytosis in β-cells requires discrete microdomains of Ca^{2+}-entry close to the secretory vesicles and that this arrangement becomes disrupted following palmitate exposure. This culminates in the selective suppression of insulin release during brief (<50 ms) action potential-like stimulation whereas exocytosis evoked by unphysiologically long (>300 ms) pulses is unaffected. Additionally, inclusion of the slow Ca^{2+}-buffer EGTA (10 mM) in the electrode solution reversed the restored secretion observed during long pulses. Prolongation of the β-cell action potential by pharmacological maneuvers which expand the $[Ca^{2+}]_i$ microdomains corrects the FFA-induced secretion defect in both mouse and human islets. We propose that the FFA-induced dissociation of Ca^{2+}-entry from vesicles in β-cells selectively impairs the readily-releasable pool of vesicles but leaves vesicle docking with the membrane unaffected. This finding may represent an evolutionarily preserved mechanism to abate insulin secretion during nutrient deprivation when normoglycaemia is maintained by mobilization of lipids from fat depots.

Endoplasmic Reticulum & Protein Trafficking

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Conformational transition of the Sec translocon induced by channel partner: A molecular dynamics study

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Sec translocon is a highly conserved heteromeric membrane protein complex, which functions as a protein-conducting channel. In bacteria, the Sec translocon (SecYEG) achieves the translocation of polypeptides across the membrane by binding of the channel partner, SecA ATPase. However, little is known about the atomically detailed mechanism on the translocation. Recently, a new crystal structure of the SecYE translocon bound with an anti-SecY Fab fragment has been determined. It contains a large hydrophobic crevasse open to the cytoplasm (the pre-open form) and differ from the crystal structure of SecYEβ from *Methanococcus jannaschii* in the closed form, suggesting that the binding of a channel partner induces a large conformational change of the Sec translocon in the initial step of the polypeptide translocation. To investigate the role of channel-partner binding to the SecYE translocon, we performed all-atom molecular dynamics simulations of SecYE with and without a Fab fragment in explicit membrane. During a 100-ns simulation, SecYE undergoes a large conformational transition toward the closed form in the absence of a Fab fragment, whereas the structure keeps the widely opened crevasse in the simulation of SecYE with a Fab fragment. In the transition, protein-lipid interaction around the lateral gate region of SecYE is changed greatly, indicating that there is a competition of interactions between the protein and phospholipid molecules, which is controlled by the binding of the channel partner to the SecYE transolon.

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Simulations of Multi-protein Complexes: Structure, Binding Affinity, and Dynamics of Vps27/hse1 Bound to Membrane-tethered Ubiquitin

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Protein-protein interactions play an essential role in many cellular functions. While biophysical and structural characterizations have traditionally focused on strong binary complexes, the biological importance of weakly bound multi-protein complexes is increasingly recognized. Such complexes typically contain various proteins, with different folded domains held together in part by flexible linkers. Further increasing the complexity, many multi-protein