2200-Pos Board B337
Simultaneous Measurements of Intracellular $[Ca^{2+}]i$ and [cAMP]i in Intact Islets to Study the Mechanism Underlying Dopaminergic Inhibition of Insulin Secretion
Alessandro Ustione, David W. Piston.
Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA.
Pancreatic islets secrete multiple hormones, including insulin, that are required to maintain euglycemia while meeting the energy demand of the body during everyday activities. We are interested in understanding the interplay between molecular mechanisms that precisely regulate these secretions. One specific paracrine modulator, dopamine, functions as a negative regulator of insulin secretion in the context of the pancreatic islet. It is secreted by the insulin-producing β-cells, activates an autocrine negative feedback that decreases the frequency of glucose-stimulated $[Ca^{2+}]_i$ oscillations, and in turn, inhibits insulin secretion. The G-protein coupled dopamine receptors are present in islet cells, but it is not clear how activation of the these receptors results in the observed changes in $[Ca^{2+}]_i$ in intact pancreatic islet cells. We are using an mTurquoise-Based cAMP biosensor with an improved dynamic range (*Klarenbeek, J.B., et al., PLoS One, 2011*), along with organic and genetically encoded Ca^{2+}-indicator dyes. Labeled cells are studied by live imaging using perfused pancreatic islet. Spectral unmixing is used to extract the fluorescence emissions. This experimental setup allows us to monitor the effect of specific dopamine receptor agonists and antagonists on the two main cellular second messengers. Also, islets from mice with a genetic target mutation of the DRD3 (D3-KO) are used to measure how the deletion of the dopaminergic feedback changes the second messenger dynamics. With the same approach we are measuring the effects of the overexpression of dopamine receptor D3 in wild-type and D3-KO islets. The information from these experiments will help elucidate the mechanism of dopamine signaling in the pancreatic islet.

2201-Pos Board B338
Determining the Dopaminergic Feedback Pathway in Pancreatic β-Cells with Fluorescence Fluctuation Spectroscopy
Brittany Caldwell[1], Alessandro Ustione[2], David Piston[1,2].
[1]Biomedical Engineering, Vanderbilt University, Nashville, TN, USA, [2]Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA.
Tight regulation of insulin and glucagon, two hormones secreted by the pancreatic islet, allows the body to maintain glucose homeostasis. Insulin resistance coupled with insufficient insulin secretion leads to type 2 diabetes. Insulin secretion from pancreatic β-cells is regulated by multiple signaling inputs including the neurotransmitter dopamine. Upon treatment with dopamine, the amplitude and frequency of intracellular free calcium oscillations in β-cells decrease, leading to a concomitant decrease in insulin section. We hypothesize that activation of the dopamine receptor (DRD3) by dopamine releases the Gβγ complex to stimulate a G protein-coupled inwardly-rectifying potassium channel (GIRK3). This inward current keeps the cell polarized, and reduces currents through voltage-gated calcium channels (CaV1.2). This model predicts numerous protein interactions that may only be transient in nature, in particular, we expect close interactions between the DRD3 and GIRK3 as well as each of these with the Gβγ subunit. To test this hypothesis, we are utilizing fluorescence fluctuation spectroscopy to determine protein interactions in stable β-cell lines. Fluorescence fluctuation spectroscopy provides a method to determine interaction between two proteins in a live cell that surpasses many of the limitations of FRET. Single and two color studies allow us to determine diffusion constants of each protein before and after dopamine stimulation and the cross-correlation, or amount of interaction, between two proteins. Additionally, brightness analysis can be used to determine homo- and heterodimerization of the tagged proteins. Diffusion constants, cross-correlation rates, and protein clustering among the DRD3, GIRK3, and Gβγ subunit will be presented. Through this analysis, the dopamine signaling pathway in pancreatic β-cells can be determined, which will reveal novel potential therapeutic targets that can increase insulin secretion during diabetes mellitus.

Other Channels

2202-Pos Board B339
Statistical Analysis of Multichannel Signals
Rishabh Kumar[1], Prashant Srinivasa[2], **Horia I. Petrache**[1].
[1]Physics, Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, [2]Physics, California Polytechnic State University, San Luis Obispo, CA, USA.
As opposed to single channel statistics, the analysis of superimposed signals is complicated due to signal overlap and loss of channel identity. We present a statistical method that uses a newly defined concept of multichannel events. These events can be easily differentiated and analyzed to provide information on single channel parameters such as ON and OFF times.

2203-Pos Board B340
Novel Step Detection Algorithms for Photobleaching Analysis of Protein Complexes with Many Subunits
Nathan C. Deffenbaugh[1], Yalei Chen[1,2], Charles T. Anderson[2,3], William O. Hancock[1,2].
[1]Department of Biomedical Engineering, Penn State University, University Park, PA, USA, [2]Cell and Developmental Biology, Huck Institutes of the Life Sciences, Penn State University, University Park, PA, USA, [3]Department of Biology, Penn State University, University Park, PA, USA.
Single molecule photobleaching of fluorescently labeled protein complexes is an effective technique for counting constituent subunits and has been applied to determine the stoichiometry and oligomerization of several different transmembrane proteins including voltage- and ligand-gated ion channels, as well as the makeup of the cellulose synthesis complex. Fluorophore bleaching occurs as a random process, resulting in discrete intensity drops over time. Step detection algorithms can be used to identify these steps within noisy signals in order to determine the total number of fluorescent subunits within a protein complex. Reliable identification of steps can become difficult however, when high numbers of subunits are present. Here, we present two step detection algorithms, one based on modified t-testing and another based on the Bayesian Information Criterion (BIC), that perform at higher levels of precision than existing algorithms and account for temporal changes in variance within a signal, which is expected in photobleaching experiments. We also present a method for determining a unitary step amplitude associated with bleaching of a single fluorophore based on a Gaussian Mixture Model.

2204-Pos Board B341
Single-File Water Permeation through Aquaporin Channels
Peter H. Nelson.
Physics and Biology, Benedictine University, Lisle, IL, USA.
A diffusive model of osmosis is presented that explains currently available experimental data. It makes predictions that distinguish it from the traditional convective flow model of osmosis, some of which have already been confirmed experimentally and others have yet to be tested. It also provides a simple kinetic explanation of Raoult's law and the colligative properties of dilute aqueous solutions. The diffusive model explains that when a water molecule jumps from low to high osmolarity at equilibrium, the free energy change is zero because the work done pressurizing the water molecule is balanced by the entropy of mixing. It also explains that equal chemical potentials are required for particle exchange equilibrium in analogy with the familiar requirement of equal temperatures at thermal equilibrium. These are topics that should be considered for inclusion in the redesign of introductory physics courses for the life sciences (IPLS). The diffusive model also makes detailed predictions for the unidirectional fluxes through single-file aquaporins that can be tested experimentally or via molecular dynamics simulation. Predictions are made for both non-equilibrium and equilibrium simulations in which there may or may not be a water chemical potential difference across the membrane. The effects of both osmolarity and hydrodynamic pressure differences are included in the model.
DUE-0836833 http://circle4.com/biophysics

2205-Pos Board B342
Making an Aquaporin Water-Tight: Structural Basis of Selectivity in Plant Nodulin 26 Intrinsic Proteins
Zachary G. Beamer[1], Tian Li[2], Jerome Baudry[1], Daniel M. Roberts[1].
[1]Biochemistry and Cellular and Molecular Biology, The University of Tennessee, Knoxville, TN, USA, [2]Genome Science and Technology, The University of Tennessee, Knoxville, TN, USA.
The evolution of land plants led toan amplification and diversification of the aquaporin superfamily of membrane channels. Among the subfamilies of plant specific aquaporin-like changes are the nodulin-26 intrinsic proteins (NIPs) which are multifunctional transporters of water, ammonia, glycerol and metalloid nutrients that participate in a number of osmoregulatory and metabolic functions. NIPs share the canonical hourglass fold of the aquaporin family, but possess substitutions within the aromatic arginine (ar/R) selectivity filter. The nine proteins of the NIP subfamily in the model plant *Arabidopsis thaliana* can be subdivided into two ar/R pore subgroups: the NIP subgroup I, which form aquaglyceroporins that are permeated by glycerol, water and ammonia, and the NIP subgroup II, which form metalloid transporters which are lack aquaporin activity and are essentially "water tight". These two NIP pore families differ principally by the substitution of a conserved alanine (NIP subgroup II) for a conserved tryptophan (NIP subgroup I) in the helix 2 position (H2) of