The pancreatic β-cell can synthesize dopamine from the circulating precursor L-dopa. During glucose stimulated insulin secretion, co-secreted dopamine acts as an autocrine negative regulator of insulin secretion. It does so by activating type2 dopamine receptors (DRD2) - a member of the G-protein coupled receptor family. DRD2 are present on β-cell plasma membrane, and upon dopamine binding attenuate intracellular Ca2+ dynamics. In fact, the frequency of intracellular [Ca2+] oscillations that are triggered by 8 mM glucose is diminished by dopamine; the same effect is observed when islet dopamine content is increased prior to glucose stimulation.

We are interested in studying the molecular mechanism downstream of DRD2 activation that leads to the changes in intracellular Ca2+ dynamics. We propose that dopamine activation of DRD2 directly affects β-cell calcium influx via Gβγ subunit interaction with the L-type Ca2+ channel (CaV1.2). We use fluorescent protein labeled versions of the Gβγ subunit, and the CaV1.2 subunit α1C to perform FRET experiments on βTC-3 cells. Using a lock-in FRET approach, we can improve detection of FRET efficiencies and we can detect a small percentage of donor-acceptor interaction.

The understanding of dopamine signaling in the pancreatic β-cell is important. Since dopamine is known to inhibit insulin secretion, its signaling pathways may be targets for pharmacological treatments for type 2 diabetes.

968-Pos Board B754

High Definition Immunoassays (HDIA) with Spatially Resolved Detection

Qiaoqiao Ruan, Joseph P. Skinner, Sergey Y. Tetin.
Abbott Lab, Abbott Park, IL, USA.

Quantitative immunoassays are widely used in research and medical diagnostics. Often, analytes are captured with antibody coated microparticles and detected with signal generating conjugate. Total signal is collected from the sample after removal of the unbound conjugates through extensive wash. The drawbacks of this approach are non-specific binding and loss of reagents due to wash. To overcome these hurdles, we introduce the concept of fluorescence based high definition immunoassays (HDIA) which uses imaging microscopy for detection. The sources of fluorescence signals are examined pixel-by-pixel for each sample image. Algorithms are developed to differentiate signal from background, thus minimizing non-specific binding and reagent loss. Using Troponin and NGAL (Neutrophil Gelatinase Associated Lipocalin) as model analyte systems, we achieved both high sensitivity and wide dynamic range. We are also able to perform assays without any washing step. The HDIA can be used for both protein and nucleic acids testing.

969-Pos Board B755

Mapping Retinoids in Live P19 Cells with Autofluorescence Phasorflim Imaging

Giulia Ossato[1,2], Moreno Zamai[1,2], Alberto Roselló-Díez[1], Miguel Torres[1], Valeria Rosaria Caiolfa[1,2].
[1]CNIC, Madrid, Spain, [2]San Raffaele Scientific Insitute (HSR), Milan, Italy.

Recently a label-free FLIM imaging method has been proposed to distinguish metabolic states of germ cells (Stringari et al, 2011). This method, based on the phasor analysis of autofluorescence lifetime (AF-phasorFLIM), avoids some problems of state-of-art approaches due to multi-exponential fitting. The capability of phasorFLIM analysis to separate the different molecular species in a pixel lies on the rule of phasor addition. PhasorFLIM identifies molecules by their position in the phasor plot because every molecular species has a specific phasor that can be calibrated in reference samples.

We applied phasorFLIM to detect retinoids autofluorescence in live P19 embryonal carcinoma cells that undergo differentiation when treated with retinoic acid (RA).

RA and retinol are essential for cell-cell signaling during vertebrate organogenesis. RA is involved in stem cell differentiation and it is a morphogen. It is a diffusible signal that generates patterns in embryo because it induces different cellular responses depending on its local concentration. The presence of RA in tissues has been inferred through gene activation, and the RA average tissular concentration measured by HPLC. However, the spatial distribution in tissues and cells of the active molecule has never been observed or quantified directly.

We built a database of phasorFLIM standards of the major molecules that contribute to autofluorescence. The database allows assigning the contribution of fluorescent molecules to P19 autofluorescence.

P19 cells were treated with retinol to follow in time the effect of the molecule on cellular metabolism. PhasorFLIM analysis was performed using SimFCS and showed an increase of retinoids fluorescence after treatment. In cells treated with retinol, we observed retinol in plasma and nuclear membrane and an increase of RA in the nucleus.

AF-phasorFLIM allowed determining, distinguishing and localizing the contribution of retinoids to autofluorescence in live P19 cells.

970-Pos Board B756

Mechano-Biology of Chondrocytes in Intact Joints Under Muscular Loading

Ziad Abusara, Walter Herzog.
University of Calgary, Calgary, AB, Canada.

It is well documented that onset and progression of OA is directly related to the mechanical loading of joints, and the associated mechano-biology of chondrocytes. However, work on the mechano-biology of chondrocytes has been performed with isolated cells or tissue explants subjected to confined and unconfined loading, conditions which neglect the role of the matrix and ignores the boundary conditions of cartilage-on-cartilage contact, and fails to reproduce physiological loading conditions.

We developed a novel approach which allows quantification of chondrocyte mechanics, signaling and analysis of proteins, cytokines and fluid borne stem cells contained within the synovial fluid of intact knees in live mice while the joint is loaded using controlled muscular stimulation.

Chondrocytes and their nuclei deform on average 18-25% for sub-maximal muscular loading in the intact mouse knee. Deformation occurs ''instantaneously'' upon loading, but requires minutes for full recovery (Figures 1a). High muscular loading of the mouse knee caused an increase in total protein content in the synovial fluid (Figure 1b) and an increase in PRG4 (also known as lubricin). When ''high'' intensity load conditions were repeated, the PRG4 and total protein release was stopped (Figure 1b).

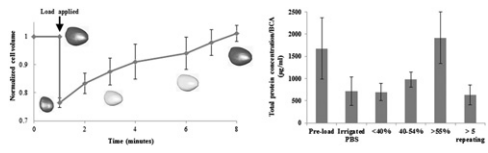

Figure 1: (a) Normalized cell volume changes as a function of time. Upon loading, the cell immediately looses between 18-25% of its volume which is only fully recovered after about 7 minutes following load removal. (b) Total Increases in loading of the mouse knee caused an increase in the total protein content of the synovial fluid (means ± 1 SD; n=9), while repeat "intense" loading conditions showed a decreasing amount of protein with each repeat bout. Pre-load is the synovial fluid prior to any loading. Irrigated PBS refers to the resting protein content of the irrigated joint fifteen minutes following irrigation. The percentage values shown below the vertical bars indicate the load relative to the maximal possible muscle load (100%). The last bar shows measurements of repeat loading at "high" intensities (>55% of maximal).

971-Pos Board B757

Rifampicin - Independent Interactions Between the Pregnane X Receptor Ligand Binding Domain and Peptide Fragments of Co-Activator and Co-Repressor Proteins

Punya Navaratnarajah, Bridgett L. Steele, Matthew R. Redinbo, Nancy L. Thompson.
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

The pregnane X receptor (PXR), a member of the nuclear receptor family, regulates the transcription of target genes whose products are involved in the metabolism of endogenous and exogenous compounds, including many pharmaceutical compounds. The conventional view of nuclear receptor action is that ligand binding enhances the receptor's affinity for co-activator proteins, while decreasing its affinity for co-repressors. Here, we report the use of steady-state total internal reflection fluorescence microscopy (TIRFM) and total internal reflection with fluorescence recovery after photobleaching to measure the thermodynamics and kinetics of the interaction between the PXR ligand binding domain (PXR-LBD) and a fluorescently labeled, peptide fragment of the steroid receptor co-activator 1 (SRC-1) in the presence and absence of the established PXR agonist, rifampicin. Equilibrium dissociation and dissociation rate constants of about 5 μM and 2 s^{-1}, respectively, were obtained both in the presence and absence of rifampicin. Additionally, TIRFM was used to examine the interaction between PXR-LBD and peptide fragments of the co-repressor proteins, nuclear receptor co-repressor (NCoR) and silencing mediator for retinoid or thyroid receptors (SMRT), in the presence and absence of rifampicin. Equilibrium dissociation constants of about 170 μM and 70 μM were obtained for NCoR and SMRT, respectively, in the presence and absence of rifampicin. These results indicate that rifampicin does not alter the affinity of PXR-LBD for co-activator and co-repressor fragments, thereby suggesting that the mechanism of PXR action differs markedly from that of other nuclear receptors.

972-Pos Board B758

Chondrocyte Deformation Under Extreme Tissue Strains

Ryan Madden, Walter Herzog.
University of Calgary, Calgary, AB, Canada.

Articular cartilage cells (chondrocytes) are responsible for maintaining the health and integrity of the tissue extracellular matrix (ECM). Chondrocyte activity under mechanical loading has been linked to changes in joints leading to osteoarthritis (OA). The purpose of this study was to investigate the deformation behavior of chondrocytes in their native environment using a novel in situ experimental approach.