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A Live Cell Tirf-Microscopy Based Screening Assay for Fast Analysis of Receptor Tyrosine Kinase Modulators
Peter Lanzerstorfer[1], Shin-Ichiro Takahashi[2], Otmar Höglinger[1], Julian Weghuber[1].
[1]University of Applied Science, Wels, Austria, [2]University of Tokyo, Tokyo, Japan.
Receptor tyrosine kinases (RTKs) are cell membrane receptors with intrinsic tyrosine kinase activity that trigger signal transduction. RTKs are known key regulators of essential cellular processes and have a critical role in the development of many types of cancer. Here we introduce an assay using micro-patterned surfaces in combination with TIRF microscopy for the analysis of EGF-receptor (EGFR) and insulin/insulin-like growth factor-receptor (IR/IGFR) downstream signaling. In addition, we applied the technique to validate the efficacy of medically relevant RTK modulators. We used micro-patterning to force bait-EGFR/IR/IGFR molecules into microscopic domains on the surface of living cells, while monitoring co-recruitment of a fluorescent intracellular prey molecule (Grb2/IRS), respectively. First, micropatterning of HeLa cells expressing the prey Grb2 enabled us to quantify the EGFR-Grb2 interaction, and we could show that Grb2 was recruited to the EGFR in a phosphorylation dependent manner. Pretreatment with pharmacologically active ingredients used for the treatment of human cancers significantly reduced the inducibility of the signaling system. Based on these results we could set up a dose-response relationship in a live cell context. Second, we performed fluorescence recovery after photobleaching (FRAP) experiments with RKT class II receptors (IR and IGFR) and different cytosolic insulin receptor substrate (IRS) proteins. Our results indicate prominent differences in the interaction strength of IRS1 and IRS2 to the IR/IGF1-R, compared to the one of IRS3. Taken together the results approve the power of the micropatterning technique to study the interaction properties of plasma-membrane localized receptors. This method enables a fast analysis of the samples with high sensitivity in a live cell context. We could quantify the molecular effects of different RTK modulators and we will use the system in the near future to study further active pharmaceutical ingredients.

3643-Pos Board B371
The FRET Signatures of Non-Interacting Proteins in Cellular Membranes
Christopher R. King[1], Sarvenaz Sarabipour[2], Patrick Byrne[3], Daniel Leahy[4], Kalina Hristova[2].
[1]Biophysics, The Johns Hopkins University, Baltimore, MD, USA, [2]Materials Science and Engineering, The Johns Hopkins University, Baltimore, MD, USA, [3]Biophysics, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, [4]Biophysics and Biophysical Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.
Förster resonance energy transfer (FRET) experiments are often used to study interactions between integral membrane proteins in cellular membranes. However, in addition to the FRET of sequence specific interactions, these experiments invariably record a contribution due to proximity FRET, which occurs when a donor and an acceptor approach each other by chance within distances of 100 Å. This effect does not reflect specific interactions in the membrane and is rarely appreciated in the field, despite the fact that its magnitude can be significant. Here we develop a comprehensive computational description of proximity FRET, simulating the cases of proximity FRET when the fluorescent proteins are used to tag monomeric, dimeric, trimeric and tetrameric membrane proteins, as well as membrane proteins existing in monomer-dimer equilibrium. We also perform rigorous experimental measurements of this effect by identifying membrane receptors that do not associate in mammalian membranes. We measure the FRET efficiencies between YFP and m-Cherry-tagged versions of these receptors in plasma membrane derived vesicles, as a function of receptor concentration. Finally, we demonstrate that the experimental measurements are well described by our predictions. The work presented here should bring much-needed rigor in FRET-based studies of membrane protein interactions, and should have broad utility in membrane biophysics research.

3644-Pos Board B372
Resolving the Brassinosteroids Signal Transduction Mechanisms by Single-Molecule Assays
Song Song[1], Haijiao Wang[2], Xue-Lu Wang[2], **Yan-Wen Tan**[1].
[1]Physics, Fudan University, Shanghai, China, [2]School of Life Sciences, Fudan University, Shanghai, China.
Brassinosteroids (BRs) are the sixth class of plant hormones that involved in numerous plant development processes such as leaf expansion, shoot elongation and pollen tube formation. Once the signal transduction is initiated by the membrane receptor kinase BRI1 (brassinosteroid insensitive 1), the signal transmits from the cytoplasm to the nucleus and a number of genes will be regulated. The downstream signaling pathway is realized by three proteins: BIN2 (brassinosteroid insensitive 2), BES1 (BRI1 ems suppressor1) and a kind of 14-3-3s protein . BRs signaling pathway have been extensively studied via genetics, proteomics, genomics and cell biology techniques. However, these bulk methods can't follow the transduction process in situ or resolve molecular details at a rate matching the true signaling time-scale. Here we use a single molecular assay based on Total-Internally Reflected Fluorescence (TIRF) microscopy to observe the interaction of these three proteins. The result shows that BIN2 can phosphorylate BES1 on the order of seconds, and the dimeric 14-3-3s can only bind with BES1 in its phosphorylated form. In addition, we have, for the first time, found that the interaction between BIN2 and BES1 is oxygen dependent. This result may have implications on BRs signaling pathway's involvement of stress acclimation in plants.

3645-Pos Board B373
Fast and Local Mechanotransduction Control via Magnetic Nanoparticles: Mechanical Stimulation of Auditory Cells
Michael Lévy[1], Jae-Hyun Lee[2], Albert Kao[1], Ji-wook Kim[2], Seung-hyun Noh[2], Yung Ji Choi[2], Dolores Bozovic[1], Jinwoo Cheon[2].
[1]Department of Physics and Astronomy, UCLA, Los Angeles, CA, USA, [2]Department of Chemistry, Yonsei University, Seoul, Korea, Republic of.
Converting a mechanical force into an electrochemical signal is a fundamental physiological process that underlies a number of senses, including touch, balance, proprioception, and hearing. Mechanosensitive ion channels play a crucial role in this process, switching into the open state when subjected to a mechanical stimulus which can take the form of stretch, pressure, or twist, and thus allowing an influx of ions into the cell. Although there has been much interest in understanding and controlling the mechanotransduction process, the development of appropriate tools with precise spatiotemporal control has posed challenges.
We develop a technique to actuate mechanosensitive cells in a fast, reversible, and localized fashion and we test this method on auditory hair cells from the Bullfrog's sacculus. Cube-shaped magnetic nanoparticles are conjugated to Concanavalin A to bind to the hair bundle's surface. An electromagnetic probe then applies a calibrated magnetic force on pN scale on the particles, inducing a mechanical entrainment of the hair bundle at frequencies up to 10kHz. Such mechanical stimulation triggers the switching of ion channels from open to closed state, with the concomitant Calcium influx into the cell during the open state. Such influx is observed using fluorescent Calcium indicators. Moreover, the magnetic force stimulus can be designed to be complex and non-periodic, in order to probe specific nonlinear properties of the biological system.
Our technique is applicable not only to the auditory system but to a broad range of sensory systems with mechanosensitive channels. The utilization of a controlled magnetic field, to which biological tissue is transparent, can be beneficial for remote and non-invasive stimulation of a wide range of biological targets.

3646-Pos Board B374
Role of Calcium Signaling in Endothelial Barrier Function
Judith A. Stolwijk[1], Christian W. Renken[2], Mohamed Trebak[1].
[1]College of Nanoscale Science and Engineering, University at Albany, State University of New York, Albany, NY, USA, [2]Applied Biophysics Inc., Troy, NY, USA.
Intact endothelia lining the vasculature play a crucial role in tissue homeostasis and organ function. Various blood borne and tissue released mediators influence endothelial barrier function under physiological conditions and become critical under common pathologies such as inflammation and sepsis, known to be associated with increased vascular leakage. Prominent inflamatory mediators such as Thrombin and Histamine transiently disrupt the endothelial barrier via activation of G-protein coupled receptors (GPCRs), while other GPCR agonists, including Sphingosine-1-phosphate (S1P), enhance endothelial barrier function.
The barrier disruptive activities of Thrombin and Histamine were repeatedly proposed to be associated with these agonists ability to increase intracellular Calcium (Ca^{2+}) concentrations ($[Ca^{2+}]_i$) *via* activation of Gq-associated signaling involving phospholipase C (PLC) activation, production of inositol-1,4,5 trisphosphate (IP_3), Ca^{2+} entry through store-operated calcium channels (SOCs) and initiation of Ca^{2+}-dependent endothelial contractility through myosin light chain kinase (MLCK) activation. Here, we use Electric Cell-Substrate Impedance Sensing (ECIS) to challenge this view. We determined barrier function upon stimulation with various barrier modulating agonists in primary human dermal microvascular endothelial cells (HDMECs). We noted obvious discrepancies in the effective agonist concentrations able to evoke