the IC3 loop and Carboxyl terminus of ETA and ETB. Additionally, we have generated a structural model of the ETA Carboxyl terminus (Residues 373-427) fit to NMR data both in solution and in the presence of DPC micelles. We hope that with these tools we can soon identify and develop targeted inhibitors of vasocostruction through high throughput screening and rational design.

545-Pos  Board B300  Investigating EGF Receptor Signaling Dynamics with Patterned Ligand Surfaces  
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Receptor mediated signaling is often precisely regulated in both space and time, which necessitates the application of methods for simultaneously capturing both aspects with a high level of detail. The epidermal growth factor receptor (EGFR) is a prominent example as numerous, dynamic interactions exist between this receptor and its downstream effectors. To advance spatiotemporal insights on EGFR signaling, we have developed a technique by which lithographic processes and chemical modification are used to pattern micron-sized features of EGF on silicon or glass. Fluorescently labeled streptavadin, covalently attached to these surfaces, binds biotinylated EGF for the presentation of immobilized receptor ligand. NIH-3T3 cells stably over-expressing EGFR are allowed to settle on these surfaces and are subsequently fixed. Fluorescence microscopy is then used to visualize specific cellular proteins concentrating at patterned EGF features and correlation coefficient analysis measures the extent of colocalization. We have observed and quantified the recruitment of various EGFR signaling components, including Ras, MEK, and phosphorylated Erk in an F-actin and phosphoinositide synthesis-dependent manner. Further, we find that paxillin-GFP becomes visibly clustered at patterned EGF features. Pretreatment of cells with the EGFR inhibitor fressa significantly reduces this paxillin clustering behavior and establishes the dependence of paxillin on tyrosine kinase activation. These studies reveal the formation of multi-protein EGFR signaling complexes in response to spatially defined growth factor. To measure dynamics of EGFR protein redistributions in living cells, we have recently extended this patterning technique to coverslip-thick glass substrates and two-color total internal reflection fluorescence microscopy (TIRFM). This is providing for the real time investigation of signaling proteins and their recruitment to patterned EGF. We are also applying alternative analysis methods for quantifying the redistribution of cellular components to take full advantage of this patterned ligand approach.

546-Pos  Board B301  Deciphering Regulatory Mechanism of the Juxtamembrane Region in Thrombopoietin Receptor Activation  
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Abstract: The thrombopoietin receptor (TpoR/c-MPL) plays an important role in megakaryocyte differentiation, platelet formation and hematopoietic stem cell renewal. Its intracellular membrane-proximal region, which constitutively binds to JAK2, regulates activation of the receptor and signalling of the JAK-STAT pathway. However, how the activation of this molecular level is not understood. In our report, cell proliferation assay, fluorescence, circular dichroism and NMR spectroscopy are employed to elucidate the role of the juxtamembrane (JM) region in TpoR activation. We find that secondary structure and membrane affinity of the JM region are strongly controlled by the juxtamembrane (JM) region in TpoR activation. We find that secondary structure and membrane affinity of the JM region are strongly controlled by the combination of ligands targeting both receptors could elicit functional crosstalk. In our report, cell proliferation assay, fluorescence, circular dichroism and NMR spectroscopy are employed to elucidate the role of the juxtamembrane (JM) region in TpoR activation. We find that secondary structure and membrane affinity of the JM region are strongly controlled by the combination of ligands targeting both receptors could elicit functional crosstalk.

The JAK-STAT pathway. However, how the activation of this molecular level is not understood. In our report, cell proliferation assay, fluorescence, circular dichroism and NMR spectroscopy are employed to elucidate the role of the juxtamembrane (JM) region in TpoR activation. We find that secondary structure and membrane affinity of the JM region are strongly controlled by the combination of ligands targeting both receptors could elicit functional crosstalk. In our report, cell proliferation assay, fluorescence, circular dichroism and NMR spectroscopy are employed to elucidate the role of the juxtamembrane (JM) region in TpoR activation. We find that secondary structure and membrane affinity of the JM region are strongly controlled by the combination of ligands targeting both receptors could elicit functional crosstalk.

547-Pos  Board B302  FGFR1 and FGFR2 Induced FGFR3 Dimerization in Plasma Membrane Derived Vesicles  
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FGF2 and FGF4 are mitogenic factors for a variety of cells in the plasma membrane and regulate cell survival, differentiation and angiogenesis. EGFR growth factor receptor 3 (FGFR3) is a receptor tyrosine kinase which resides in the plasma membrane and regulates cell survival, differentiation and angiogenesis. EGFR growth factor receptors (fgfrs) are a family of secreted protein ligands which bind to FGFRs to potentiate signaling. FGFR3 functions via lateral dimerization in the cellular plasma membrane. It has been shown that FGFR3 forms dimers in the absence of fgf ligands, however it is not clear how FGF3 affects FGFR3 dimerization. Previously we have developed a quantitative imaging FRAT (Q-FRET) technique to study membrane protein interactions in a cell-derived model system. Here we have used this method to measure thermodynamics of FGFR3 interactions in the presence of fgf1 and fgf2 in Chinese Hamster Ovary (CHO) cell derived vesicles. These measurements provide novel mechanistic insights into the role of ligand binding in receptor tyrosine kinase interactions.

548-Pos  Board B303  Voltage Affects the Dissociation Rate Constant of the M2 Muscarinic Receptor  
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G-protein coupled receptors (GPCRs) comprise the largest protein family and mediate the vast majority of signal transduction processes in the body. Until recently G-protein coupled receptors were not considered to be voltage dependent. Newly it was shown for several GPCRs that the first step in GPCR activation, the binding of agonist to the receptor, is voltage sensitive: Voltage shifts the receptor between two states that differ in their binding affinity. Here we show that this shift involves the rate constant of dissociation. We used the m2 muscarinic receptor (m2R) a prototypical GPCR and measured directly the dissociation of [3H]Ach from m2R expressed Xenopus oocytes. We show, for the first time, that the voltage dependent change in affinity is implemented by voltage shifting the receptor between two states that differ in their rate constant of dissociation. Furthermore, we provide evidence that suggest that the above shift is achieved by voltage regulating the coupling of the GPCR to its G protein.

549-Pos  Board B304  Cross-Signaling between the Metabotropic Glutamate 2 Receptor and the Serotonin (5-HT) 2A Receptor in Hek-293 Cells  
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Atypical antipsychotics drugs targeting the Gq-coupled 5-HT2A receptor (2AR) are widely used in the treatment of schizophrenia and psychosis. Recent studies point to a new class of potential antipsychotic drugs targeting the Gi-coupled metabotropic glutamate 2 receptor (mGlur2). We recently reported that a heteromeric complex formed between these two GPCRs integrates the actions of secondmtergic and glutamatergic drugs, modulating the balance between Gi and Gq signaling and allowing us to predict the psychoactive properties of these drugs (Fribourg et al. 2011, Cell. 147(5):1011-23). Our results uncover a unifying mechanism of action of two families of antipsychotic drugs and pave the road for the development of assays for high-throughput screening of serotonergic and glutamatergic drugs. While developing such an assay, we needed to address a controversy raised by a recent study, in which co-expression of the two receptors in HEK-293 cells had no significant effect on either Gi or Gq signaling in response to several serotonin and glutamatergic drugs (Delille et al., 2012, Neuropharmacology 62(7):2184-91). Since in Xenopus oocytes the degree of functional crosstalk between the two receptors depended on their expression levels, we generated several clones of HEK-293 cells expressing different levels of the two receptors in the background of the G protein in question. How various GPCRs respond to expression levels of co-expressed receptors is currently unknown. Since in Xenopus oocytes the degree of functional crosstalk between the two receptors depended on their expression levels, we generated several clones of HEK-293 cells expressing different levels of the two receptors in the background of the G protein in question. How various GPCRs respond to expression levels of co-expressed receptors is currently unknown. Since in Xenopus oocytes the degree of functional crosstalk between the two receptors depended on their expression levels, we generated several clones of HEK-293 cells expressing different levels of the two receptors in the background of the G protein in question. How various GPCRs respond to expression levels of co-expressed receptors is currently unknown. Since in Xenopus oocytes the degree of functional crosstalk between the two receptors depended on their expression levels, we generated several clones of HEK-293 cells expressing different levels of the two receptors in the background of the G protein in question. How various GPCRs respond to expression levels of co-expressed receptors is currently unknown.