the coexpressed GPCRs EP2 or EP4 results in clearly distinguishable effects even though both receptors activate the Gs-cAMP signaling pathway. The mechanism regulating these distinct signaling outcomes remains unclear. Here we show that EP4, in contrast to EP2, dynamically localize in elongated areas of the plasma membrane that are dictated by the underlying cortical microtubule network. For EP4, this results in efficient but transient cAMP production throughout a range of PGE2 concentrations. In contrast, EP2 induces marginal but continuous cAMP levels. We propose a model where, differently from actin corrals and transient confienemtn zones, cortical microtubules form scaffolds for signaling hubs visited by receptor diffusing within the plasma membrane. This leads to efficient regulation of this GPCR signaling function, which is important to regulate cytoskeleton remodeling and migration in leukocytes and cancer cells.

**2642-Pos Board B334**

**Nanoarchitecture of Integrin Receptor Clusters on Very Soft Substrates**

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Sensing of extracellular matrix (ECM) physical properties is critical for several processes such as cell differentiation, cell migration in the developmental (haptotaxis) and disease (cancer metastasis). Clustering of matrix-activated integrins is an important step in the formation of matrix adhesions and clustering can occur by lateral association of activated integrin receptors. Using supported bilayers with fluid, lipid-linked RGD ligands, large clusters of activated integrins formed in bound mouse embryo fibroblasts (Yu et al., 2011. PNAS 108:20585). After cells spread on RGD bilayers for 15 minutes, integrin clusters were formed even in the presence of inhibitors of cytoskeletal assembly. We have quantified the cluster size, geometry and receptor distribution at a nanometer level using photoactivated light microscopy. When the temperature was decreased to 250C, the cluster formation was greatly diminished, indicating that the integrin cluster formation may be an entropically driven process. To identify the players crucial for this process, we observed that when Talin1 knockout cells spread, the cluster size and the position of the clusters around the cell was altered. This was rescued by a full length Talin construct, indicating that Talin is required for the early cluster formation. This was also rescued by Talin head domain and not by Talin rod domain, indicating that most likely in the absence of external force, the integrin activation by Talin head was required for cluster to form. This study shows that the integrin cluster formation proceeds in the absence of external traction force in a Talin1 dependent manner. Thus, we suggest that talin head binding is an important factor in integrin clustering.

**2643-Pos Board B335**

**Allosteric Regulation by Components of a Critical Membrane**

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We present a simple model of a membrane bound protein allosterically regulated by the local lipid composition. We are motivated by the experimental finding that the plasma membranes of mammalian cells are tuned close to a liquid-liquid critical point, where the sensitivity of many properties to perturbations is large. We consider a protein whose boundary conditions with the surrounding membrane are dependent on its functional state (i.e. conducting vs. non-conducting for an ion channel). For such a protein we show that small changes in the chemical potential of lipids can lead to dramatic functional changes near a critical point. This type of regulation becomes more potent as the protein becomes larger, and as the membrane gets closer to a critical point. Such a protein would also have its nanometer-scale localization correlated with its functional state. A cell could regulate such a protein by adjusting the composition either by changing the ratio of ordered to disordered lipids (experimentally probed by cholesterol depletion and loading) or by raising or lowering the critical temperature. Here we focus on perturbations that act to lower the critical temperature, like the liquid general anesthetics that have been shown to lower critical temperatures by ~4K at clinically relevant concentrations. We show that this change is sufficient to lead to changes in channel conductivity in line with what has been shown for a wide class of channels even without specific interactions between perturbing molecules and the channel itself.

**2644-Pos Board B336**

**Excitability of Guanylate Cyclase Signaling Pathway Mediating Chemotaxis**

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Chemotaxis, the directed migration of cells in chemical gradients, is a vital function in many biological processes, for example, morphogenesis and synapse formation. In chemotaxis of many eukaryotic cells, the localization of some key signaling mediators on the cell membrane regulates cell movements. Chemotaxis of Dictyostelium cells is mediated by some parallel signaling pathways like phosphoinositide-3-kinase (PI3K) pathway, soluble guanylate cyclase(sGC) pathway and so on. Previous work demonstrated that the response to chemoattractant, cyclic adenosine monophosphate (cAMP), of PI3K pathway has some characteristics of an excitable system. The theoretical model shows that this excitable pathway can regulate cell unidirectional movements in respond to a chemical gradient efficiently. However, what kind of property sGC pathway has remains to be clarified and it also remains unknown about the interaction between the sGC pathway and the PI3K pathway.

Here, we studied that the sGC pathway also is an excitable system by analyzing the spatiotemporal dynamics of sGC. We found that focal sGC-enriched domains were spontaneously generated in a no external stimulus condition. The sGC-enriched domain generated spontaneously had same property with the domains induced by cAMP stimulation. These features are characteristics of an excitable system like the PI3K pathway. We will report that the dynamics of the PI3K pathway is influenced by modulation of the sGC pathway.

The chemotactic signal transduction consists of multiple excitable pathways. This structure of signaling system may increase efficiency of respond to the steep gradient of cAMP.

**2645-Pos Board B337**

**Investigating Phosphatidylinositol 3,4-Bisphosphate 3-Phosphatase Activity of Ci-VSP in Xenopus Laevis Oocytes and Cho Cells using Fluorescent Phosphoinositide Probes**

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The voltage sensitive phosphatase Ci-VSP is generally considered to be a $PI(4,5)P_2/PI(3,4,5)P_3$-5-phosphatase *in-vivo*, while 3-phosphatase activity could be demonstrated *in-vitro* using the isolated catalytic domain of Ci-VSP. However, a recent study demonstrates $PI(3,4)P_2$-3-phosphatase activity of Ci-VSP expressed in *Xenopus laevis* oocytes (Kurokawa et al, 2012, PNAS). This is in contrast to our previous findings obtained in Chinese hamster ovary (CHO) cells (Halaszovich et al., 2009, JBC).

We set out to do a careful re-evaluation of Ci-VSP's specificity in oocytes as well as CHO cells. TAPP1-PH-GFP was used as the $PI(3,4)P_2$ sensor. In some experiments, the resting level of PI-3-phosphates was increased using either co-expression of a constitutively active PI-3-kinase or stimulation of endogenous IGF-receptors with insulin. Membrane binding of TAPP1-PH-GFP as a measure of $[PI(3,4)P_2]$ was quantified using fluorescence microscopy (confocal LSM for oocytes, TIRF microscopy for CHO cells). The membrane voltage of oocytes was controlled using the two-electrode voltage-clamp technique, for CHO cells the patch-clamp technique was used.

We failed to demonstrate 3-phosphatase activity against $PI(3,4)P_2$ in oocytes but always detected production of this phosphoinositide species, which presumably reflects $PI(3,4,5)P_3$-5-phosphatase activity of heterologously expressed Ci-VSP as well as endogenous VSPs. However, in Ci-VSP expressing CHO cells we could detect a reduced increase, but not a decrease in TAPP1-PH-GFP binding to the cell membrane at high voltages. This reduction probably reflects $PI(3,4)P_2$-3-phosphatase activity and not reduced $PI(3,4,5)P_3$-5-phosphatase activity.

In conclusion, our findings show an overall production of $PI(3,4)P_2$ over the whole voltage range studied. Therefore we presume that under physiological conditions the $PI(3,4,5)P_3$-5-phosphatase activity of Ci-VSP outweighs its $PI(3,4)P_2$-3-phosphatase activity.

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**2646-Pos Board B338**

**In-Situ Description of the Role of $PtdIns(3,4,5)P_3$ and PtdSer on PDK1 Regulation in Human Cancer Cells by Advanced Quantitative Microscopy**

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3'-phosphoinositide dependent kinase-1 (PDK1) plays a central role in the transduction of signals downstream phosphoinositide 3-kinase (PI3K), a pathway often found deregulated in tumours. PDK1 regulation is known to respond to phosphatidylinositol(3, 4, 5)trisphosphate ($PtdIns(3,4,5)P_3$) levels and it has been recently shown that its PH domain specifically binds to

phosphatidylserine (PtdSer) at the PM [1]. Previous studies have shown that PDK1 homodimerisation is essential for its regulating activity [2].
Our goal is to elucidate the role of PtdIns(3,4,5)$P_3$ and PtdSer in the homodimerisation mechanism of PDK1. Measuring Förster Resonance Energy Transfer (FRET) by Fluorescence Lifetime Imaging Microscopy (FLIM) we have studied the spatial and temporal distribution of the homodimeric population of wild-type and point mutants of PDK1 with decreased affinity for PtdIns(3,4,5)$P_3$ and PtdSer on individual human breast cancer cells (SK-BR-3) and non-cancerous mouse fibroblasts (NIH-3T3) before and after growth factor stimulation. Based on the statistical comparison of these populations, we have established the critical and cooperative role of both phospholipids for the recruitment of this kinase at the PM and its subsequent regulation through the intermolecular organisation of homodimers.
1. Lucas N and Cho W (2011) Phosphatidylserine Binding Is Essential for Plasma Membrane Recruitment and Signaling Function of 3-Phosphoinositide-dependent Kinase-1. J Biol Chem 286: 41265-41272.
2. Masters TA, Calleja V, Armoogum DA et al (2010) Regulation of 3-Phosphoinositide-Dependent Protein Kinase 1 Activity by Homodimerization in Live Cells. Science Signaling 3(145): ra78.

**2647-Pos Board B339**
**HER2 Overexpression Induces Membrane Deformation that Increases Cell Motility**
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Approximately 20% of breast cancers (BC) are characterized by the gene amplification and overexpression of HER2, a member of the ErbB/HER receptor family. While targeted therapies against HER2 effectively delay disease progression in this BC subtype, details of how overexpressed HER2s drive these tumors to malignancy are still unclear. To better understand this process, we investigated cellular responses to HER2 overexpression in individual live cells. We developed novel single receptor diffusion analyses to probe the spatial distribution of HER2s and determine their activation status by their diffusivity. Surprisingly, we found that HER2 overexpression induced deformation of basal cell membranes, which depended only on the HER2 density, regardless of the receptor signaling activity. Moreover, this membrane deformation lowered the focal adhesion coverage on the cell surface, which reduced cell adhesion and increased cell motility. These findings suggest that there is a signaling-independent role of HER2 overexpression in disease progression of HER2 positive BCs.

**2648-Pos Board B340**
**CD44-Based Adhesion and Mechanotransductive Signaling on Engineered Hyaluronic Acid Matrices**
**Yushan Kim**, Sanjay Kumar.
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Glioblastoma multiforme (GBM) is the most clinically aggressive primary brain tumor and is characterized by diffuse infiltration of glioma cells into brain parenchyma, which is rich in the glycosaminoglycan hyaluronic acid (HA). The extracellular matrix of GBM tumors is associated with aberrant HA secretion, overexpression of the HA receptor CD44, and tissue stiffening. Here we combine matrix engineering and genetic manipulation of GBM tumor cells to determine the role of HA/CD44 adhesion in the outputs of cell adhesion, migration, and invasion. We show that HA/CD44 adhesion contributes to the mechanosensing and invasive motility of GBM tumor cells, both intrinsically and in the context of RGD/integrin adhesion. We also find that three-dimensional cell invasion is maximized in an HA-rich environment, but reduction of CD44-based adhesion or addition of integrin-adhesive peptides to the matrix suppresses invasion. While comparatively much is known about cell-matrix mechanosensing through integrins and the role it may play in tumor progression, our findings reveal a previously under-appreciated role of CD44 and possibly other HA-specific receptors in this process. These findings have broad implications for the field's fundamental understanding of how cancer cells interact with the tumor microenvironment and suggest new therapeutic strategies.

**2649-Pos Board B341**
**Regulation of the HER3/ErbB3 Pseudokinase Domain by an ATP-Competitive Inhibitor**
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HER3 (or ErbB3) is a member of the human epidermal growth factor receptor (HER/EGFR) family of receptor tyrosine kinases. HER3 carries inactivating mutations in its kinase domain and therefore is denoted as a pseudokinase. Despite lacking catalytic activity, the pseudokinase domain of HER3 plays an important role in activating EGFR and HER2 kinases, resulting in potent downstream signaling. Recent advancements in cancer research identify HER3 as an important therapeutic target in breast, lung, gastric, ovarian and colorectal cancers, but due to HER3's pseudokinase status, its inhibition remains a major challenge. Our past work demonstrated that the pseudokinase domain of HER3 tightly binds ATP. This finding suggested an exciting opportunity - it might be possible to regulate HER3 signaling through modulation of its nucleotide-binding pocket. However, due to lack of available compounds, this idea was left untested. Here we are reporting that the allosteric activator function of HER3 can be modulated through this site via the ATP-competitive inhibitor bosutinib. We present a first structure of the HER3 pseudokinase domain with an inhibitor, bosutinib, bound in the nucleotide-binding site. To our knowledge, this is the first structure of any pseudokinase bound to an inhibitor. We then perform in vitro assays to show for the first time that HER3 function can be modulated through small molecule binding to the nucleotide-binding pocket. Although we show that bosutinib has the opposite effect from what is desired in cancer therapies, our study provides the proof of principle for small molecule regulation of the HER3 function. This finding opens an exciting direction for the generation of a new class of HER3-specific therapeutics that directly target the pseudokinase domain.

**2650-Pos Board B342**
**Archazolid-B Provides Alternative Therapy for Trastuzumab-Resistant ErbB2 Positive Breast Cancer**
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Trastuzumab is the most commonly used humanized monoclonal antibody for specific therapy of breast cancer. Even though its clinical introduction was a breakthrough, a large fraction of the treated tumors are or become resistant. It is well known that receptor internalization and recycling are crucial for ErbB2 mediated signal transduction. Accordingly, inhibition of its molecular machinery could provide alternatives for tumor treatment. Vacuolar $H^+$-ATPase (V-ATPase) is involved in the regulation of endocytotic/recycling pathways. Archazolid-B was described as a potent V-ATPase inhibitor, which induces apoptosis and impairs migration of tumor cells. Based on these observations we investigated the effect of Archazolid on trastuzumab-resistant Jimt-1 cells *in vitro* and *in vivo*. 10nM of Archazolid caused decreased membrane ErbB2 expression, which was accompanied by the reduced relative phosphorylation on Y1248 and intracellular accumulation of the receptors. As an *in vivo* model, SCID mice were transplanted with Jimt-1 xenografts and treated with Archazolid. Following administration of the V-ATPase inhibitor there was a significant decrease in tumor growth compared to control tumors. Confocal microscopic images of tumor sections showed heterogeneous distribution of the proliferation marker Ki67 positivity. Tumor areas with low Ki67 nuclear expression showed intracellular accumulation of ErbB2 molecules similarly to the *in vitro* experiments, compared to high Ki67 expressing areas with prominent membrane ErbB2 expression. This heterogeneity might be due to diverse tumor-vascularization. Our results demonstrate that Archazolid can be used for *in vitro* and *in vivo* tumor growth inhibition based on its interference with ErbB2 recycling.

## Exocytosis and Endocytosis II

**2651-Pos Board B343**
**Rescue of Dopamine Release and Behavior by Transplanted Neural Stem Cells in a Rat Model of Parkinsonism**
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Parkinson's disease (PD) is a neurodegenerative disorder due to reduced dopamine (DA) in the striatum and loss of DA neurons in the substantia nigra pars compacta. Embryonic stem cells (ESCs) are an optimal source for cell therapy for PD. We recently developed a fast (one-week) protocol using small molecules that effectively induces human ESCs to become primitive neural stem cells (pNSCs), which are then differentiated into DA-like neurons *in vitro*. As pNSCs are infinitely expandable, this approach offers a strategy to readily generate DA neurons on a large scale. But whether these pNSC-differentiated DA (pNSC-DA) neurons can functionally integrate into the damaged brain is unknown.