neurotransmitter signaling. While protein targets for many drugs are well-characterized, drug effects on membrane lipids, which are responsible for regulating signaling, are less clear. We used small angle neutron scattering, a novel technique to study phase separation in small (100-30 nm) vesicles made from mixtures of lipids, to study the effect of antidepressants on model raft forming lipid mixtures. Using as our staring point the raft-mimicking lipid mixture: 1:1:1 DOPC:DPPC:Cholesterol, we systematically replaced cholesterol with the drug Escitalopram and its clinically-inactive isomer, Rcitalopram. Escitalopram and Rcitalopram differ only by their opposing chiralities; the former is an effective antidepressant drug while the latter, Rcitalopram, is not. There is a clear difference in the behavior of domain size and composition when cholesterol is replaced by both Escitalopram and Rcitalopram, however, these changes are similar. These results suggest that drugs like Escitalopram do affect the membrane environment, potentially facilitating drug action, but ultimately, Escitalopram's chirality is important for protein interaction.

**3139-Pos Board B294**

**Revealing the Relationship between Fibroblast Growth Factor Receptor-Like 1 (FGFRL1) and Free Zinc in Pancreatic Beta-Cells using Quantitative Fluorescence Microscopy**

**Pamuditha N. Silva**, Dawn M. Kilkenny, Jonathan V. Rocheleau.
University of Toronto, Toronto, ON, Canada.

Type 2 diabetes is characterized by a loss of normal pancreatic beta-cell function and mass. In beta-cells, tyrosine kinase Fibroblast Growth Factors Receptors (FGFRs) modulate insulin processing, fatty acid metabolism and cell survival. However, a deeper understanding of mechanisms that regulate function of these receptors will be necessary to use this pathway therapeutically. We have recently identified beta-cell expression of Fibroblast Growth Factor Receptor-like 1 (FGFRL1), a newly-identified member of the FGFR family. FGFRL1 shares the canonical extracellular domain of FGFRs but uniquely exhibits a short C-terminal histidine-rich zinc-binding domain rather than intracellular catalytic kinase domains. Zinc is a second messenger normally found at picomolar concentration in the cytosol that mediates phosphatase activity to regulate Mitogen-Activated Protein Kinase (MAPK) signaling. We determined that FGFRL1 co-localizes with insulin secretory granules where zinc accumulates at micromolar concentrations. We therefore postulate that FGFRL1 alters beta-cell MAPK signaling by chelating zinc and regulating its intracellular concentration. To measure intercellular zinc, we imaged living murine beta-cells expressing full-length and truncated fluorescent protein variants of FGFRL1 co-labeled with cell-permeable zinc indicators FluoZin-3 and RhodZin-3. Our data confirm that FGFRL1 reduces free intracellular zinc via the unique histidine-rich region. We further show that zinc induces FGFRL1 receptor dimerization at the cell membrane using homo-fluorescence resonance energy transfer (Homo-FRET) imaging. Dimerization of FGFRL1 and association with insulin secretory granules suggest receptor activity is tightly regulated by zinc and likely associated with glucose-stimulated insulin secretion. Elucidating novel signaling mechanisms that regulate FGFR-activity in beta-cells will improve our understanding of how this pathway can be used therapeutically to treat diabetes.

**3140-Pos Board B295**

**Cells must Accumulate Interleukin-4 Receptor Subunits within Cortical Signaling Endosomes to Drive Complex Formation and Signal Transduction**

Kristina Kurgonaite[1], Hetvi Gandhi[2], Remigiusz Worch[3], Benjamin Obermann[2], Martin Hintersteiner[4], Sophie Pautot[1], Sigrun R. Hofmann[5], Christian Bökel[1], Petra Schwille[6], **Thomas Weidemann**[6].
[1]Technical University Dresden, Center for Regenerative Therapies Dresden, Germany, [2]Technical University Dresden, BIOTEC, Germany, [3]Polish Academy of Sciences, Warsaw, Poland, [4]Novartis Institutes for BioMedical Research, Basel, Switzerland, [5]Technical University Dresden, University Hospital Carl Gustav Carus, Germany, [6]Max Planck Institute of Biochemistry, Munich, Germany.

The cytokines Interleukin-4 (IL-4) and IL-13 provide cues for many important immune functions and their upregulation is associated with disorders like allergy and asthma. Signaling requires ligand mediated dimerization of single-pass transmembrane receptors. We recently established epithelial HEK293T cells as a model to characterize ectopically expressed IL-4R subunits with single- and dual-color fluorescence correlation spectroscopy [1]. Here we report an improved experimental setup employing hexahistidine specific dyes and demonstrate for the first time ligand-induced IL-4R complex formation in a native plasma membrane. Furthermore, we quantified the two-dimensional affinity constants for all three combinations of receptor dimers with 120 (IL-4:IL-4Rα/IL-13Rα1), 510 (IL-13:IL-13Rα1/IL-4Rα), and 825 (IL-4:IL-4Rα/IL-2Rγ) receptor molecules per $\mu m^2$. However, considering physiological surface expression levels of several 100-1000 receptor molecules per cell, such low affinities challenge the traditional view that signaling productive complexes self-assemble in significant numbers in the plasma membrane. Instead, we mount several lines of evidence that signal transduction requires the accumulation of the receptor subunits within a novel class of early endosomes. These cortical signaling endosomes are stably anchored within the actin cortex just beneath the plasma membrane and carry markers of both the early sorting (EEA1, Rab5) and recycling compartments (Rab11). The IL-4R subunits show Rac1/Pak-dependent trafficking from the surface into these endosomes with a time constant of 6-9 min. Using fluorescence lifetime imaging / Förster resonance energy transfer (FLIM/FRET) microscopy, we could demonstrate ligand-dependent complex formation within the cortical signaling endosomes. Importantly, specific inhibition of the endocytosis machinery with drugs abrogates both receptor trafficking and phosphorylation of the downstream signal transducer STAT6. In summary, our findings suggest a unique thermodynamic function for endocytosis upstream of JAK/STAT pathway activation.

[1] Weidemann, T., *et al.* (2011) *Biophys. J.***101:** 2360-69.

**3141-Pos Board B296**

**The Neck Region Regulates Spatiotemporal Organization and Virus-Binding Capability of the Pathogen Recognition Receptor DC-Sign**

Juan A. Torreno-Pina[1], Carlo Manzo[1], Carl G. Figdor[2], Alessandra Cambi[2], **Maria F. Garcia-Parajo**[1,3].
[1]ICFO-Institute of Photonic Sciences, Castelldefels, Barcelona, Spain, [2]Nijmegen Centre for Molecular Life Sciences, Nijmegen, Netherlands, [3]ICREA-Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.

Receptor nanoclustering *prior* to ligand activation is rapidly emerging as an essential feature common to most cell membranes. Yet, the mechanisms governing this distinct spatial patterning and functional role are still poorly understood. We used a combination of biochemical and advanced biophysical techniques, including optical superresolution and single particle tracking, to investigate the spatiotemporal organization of DC-SIGN, a pathogen recognition receptor that homo-oligomerizes in-vitro. We found an intrinsic nanoclustering capacity of DC-SIGN (*ca.* 180 nm in size) far beyond basal tetramerization, which strictly depended on its molecular structure. DC-SIGN nanoclusters exhibited Brownian diffusion on the cell membrane with values of the order of $10^{-2}$ $\mu m^2/s$. Truncation of the neck region, known to abrogate tetramerization, significantly reduced nanoclustering and concomitantly increased lateral diffusion. Importantly, DC-SIGN nanocluster dissolution compromised DC-SIGN binding to nanoscale size pathogens. As such, our results underscore a direct relationship between spatial nanopatterning, driven by intermolecular interactions between the neck regions, and receptor diffusion to provide DC-SIGN with the exquisite ability to dock pathogens at the virus length-scale. We suggest that protein-protein interactions facilitated by structural molecular motifs might represent a so far underestimated but general mechanism to pre-organize receptors on the cell membrane for efficient action under stimulated conditions.

**3142-Pos Board B297**

**Lysophosphatidic Acid Signalling in Red Blood Cells**

**Lars Kaestner**[1], Jue Wang[1], Lisa Wagner-Britz[2], Anna Bogdanova[3], Kathrina Wiesen[1], Qinghai Tian[1], Ingolf Bernhardt[2], Stephan Philipp[1], Marc Freichel[4], Veit Flockerzi[1], Peter Lipp[1].
[1]Saarland University, Homburg/Saar, Germany, [2]Saarland University, Saarbrücken, Germany, [3]University of Zürich, Zürich, Switzerland, [4]Uniersity of Heidelberg, Heidelberg, Germany.

Red blood cells are among the most intensively studied cells in natural history, elucidating numerous principles and ground-breaking knowledge in cell biology. Morphologically, red blood cells are largely homogeneous, and most of the functional studies have been performed on large populations of cells, masking putative cellular variations. We studied human and mouse red blood cells by live-cell video imaging, which allowed single cells to be followed over time. In particular we analysed functional responses to hormonal stimulation with lysophosphatidic acid, a signalling molecule occurring in blood plasma, with the calcium sensor Fluo-4. Additionally, we developed an approach for analysing the calcium responses of red blood cells that allowed the quantitative characterisation of single-cell signals. In red blood cells, the lysophosphatidic acid-induced calcium influx showed substantial diversity in both kinetics and amplitude. Also the age-classification was determined for each particular red blood cell and consecutively analysed. While reticulocytes lack a calcium response to lysophosphatidic acid stimulation, old red blood cells approaching clearance generated robust lysophosphatidic acid-induced signals, which still displayed broad heterogeneity. We revealed the intracellular signalling from the lysophosphatidic acid receptors to the calcium channels.