combined computational\cite{ref1, ref2} and experimental\cite{ref3, ref4} studies have demonstrated binding of the juxtamembrane (JM) region of EGFR to anionic lipids within the inner leaflet of the plasma membrane bilayer. This interaction is thought to be functionally significant for receptor dimerization and activation.

Using a multiscale molecular dynamics approach, we have explored interactions of all 58 human receptor tyrosine kinases (RTKs) with anionic membrane lipids. Our results reveal that the JM regions of these receptors are able to induce clustering of anionic lipids into ordered ring-like patterns around the transmembrane helix in both simple asymmetric bilayers, and more complex physiological bilayers which mimic the additional complexity found in vivo\cite{ref5}. The observations we have driven primarily via electrostatic interactions between basic residues within the JM region, and negatively charged lipid head groups. The insights these simulations provide are of interest both for understanding RTK structure and function, and in the wider organisation of proteins and lipids within bilayers.


1305-Pos Board B256
The Dipole Potential Influences the Clustering of ErbB Proteins
Tamas Kovacs, Agnes Szabo, Janos Szollosi, Peter Nagy
Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary.
ErbB proteins are receptor tyrosine-kinases and their signal transduction mechanisms play significant roles in the pathogenesis of tumors. Their homo- and heteroassociations lead to activation of signaling. The role of the extracellular domain is well-known in regulating receptor clustering but the transmembrane domain may also play a role. Dipole potential is a large positive potential barrier at the membrane midplane created by inward-pointing molecular dipoles at the interface planes and it might influence the functions of transmembrane domains of receptors.

6-ketocholestanol and phloretin were used to increase and decrease, respectively, the dipole potential. Their effect was evaluated by the dipole potential sensitive fluorescent dye, di-8-ANEPPS. The homo- and heteroassociations of ErbB proteins in serum-starved and EGF-stimulated SKBR-3 cells were measured by flow cytometric fluorescence resonance energy transfer and fluorescence microscopy using number\&brightness analysis. The functional effect of changing dipole potential was determined in both starved and stimulated cells using flow cytometry by indirect labeling of tyrosine-phosphorylated proteins by PY99, phosphorylated ErbB2 by Ab18 and phosphorylated ErbB1 by anti-pEGFR antibodies.

The dipole potential was successfully increased by 6-ketocholestanol and decreased by phloretin in SKBR-3, JIMT-1 and CHO cell lines. An increased dipole potential resulted in a significant increase in ErbB2-ErbB2 homoassociation both in starved and EGF stimulated samples, it increased ErbB1-ErbB1 homo- and ErbB1-ErbB2 heteroassociation in stimulated cells, while decreasing the dipole potential caused a non-significant decrease in most samples. The effect on homoassociations was confirmed by number\&brightness analysis. Kethocholestanol increased and phloretin decreased ErbB2-, ErbB1-specific and general tyrosine phosphorylation in EGF stimulated cells.

The dipole potential may play an important role in controlling the homo- and heteroassociation of transmembrane receptors. Intentional or accidental modification of the dipole potential by drugs might result in modified signal transduction processes.

1306-Pos Board B257
Homo and Hetero Dimerization of Receptor Protein Tyrosine Phosphatases
Elizabeth Dembier, Maxwell Watkins, Damien Thevenin.
Chemistry, Lehigh University, Bethlehem, PA, USA.
Many cell-signaling events are regulated through reversible tyrosine phosphorylation of proteins, which is controlled by the counterbalanced actions of two key enzyme families: Protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Interestingly, both families include transmembrane receptor-like enzymes, namely the receptor tyrosine kinases (RTKs) and the receptor-like PTPs (RPTPs). While the regulation and actions of many RTKs are well characterized, the mechanisms controlling the enzymatic activity of RPTPs and how they interact with their substrates remain to be fully explained. Thus, understanding how these receptors function and interact will give fundamental insights into how tyrosine phosphorylation is finely tuned in cells, and how it can be modulated.

Homo-dimerization has been shown to regulate the activity of several RPTPs. Even though the transmembrane (TM) and the juxtamembrane (JM) domains have been proposed to be involved in this process, there is no clear structure-based proposal for the role of these regions. Moreover, several RPTPs have been identified as candidate regulators of RTKs. For instance, the phosphorylation of the epidermal growth factor receptor (EGFR) is reduced upon expression of PTEN or PTPI. Particularly, EGFR was shown to physically interact with PTEN and PTPI at the cell surface. However, the contact interface between these receptors is unknown.

Here, using a dominant-negative transcriptional activator-based assay (DN-AraTM), and mutagenesis, we show that: (1) several RPTPs have a tendency to homo- and heterodimerize, (2) PTPI heterodimerizes with EGFR through TM-TM interactions, and (3) these interactions are mediated by specific residues. This work represents the first structure-function study of RPTP-RTK interaction.

1307-Pos Board B258
In vivo Thermodynamics of RTKs in the Cell Membrane: Quantitative Spectral FRET
Christopher R. King, Kalina Hristova.
Biophysics, Johns Hopkins University, Baltimore, MD, USA.
Here, we introduce a new quantitative FRET method (qs FRET) that allows for the measurement of apparent FRET efficiency and the concentrations of donor and acceptor-tagged membrane-bound proteins in cell-derived vesicles and live cells. This new method utilizes two-photon excitation and spectral imaging technology, where the complete emission spectrum of each pixel in an image is acquired. We utilize this method to measure the equilibrium association constant of fluorescent protein-tagged Receptor Tyrosine Kinases in live cells.

1308-Pos Board B259
Comparison of EGFR Dimer Stabilities in the Presence and Absence of the Ligand EGF
Matt Salotto1, Deo R. Singh1, Chris King2, Pat Byrne3, Daniel Leahy2, Kalina Hristova1.
1Materials Science and Engineering, Johns Hopkins University, Baltimore, MD, USA, 2Molecular Biophysics, Johns Hopkins University, Baltimore, MD, USA, 3Biophysics and Biophysical Chemistry, Johns Hopkins University, Baltimore, MD, USA.
EGFR is a member of the ErbB family of receptors. EGFR consists of a glycosylated extracellular domain, a single pass transmembrane segment, and an intracellular domain with kinase activity. Mutations or increased expression of this receptor have been linked to human cancers. EGFR is a ligand that binds and activates EGFR. It is generally accepted that EGF binding increases the stability of EGFR dimers. However, the magnitude of this stabilizing effect has not been quantified yet. Here, we used spectral FRET, combined with two-photon microscopy, to study the dimerization of EGFR in plasma membranes. We observed significant stabilization of the EGFR dimer in the presence of the EGF ligand. These data allowed us to quantify the increase in EGFR dimer stability upon EGF binding.

1309-Pos Board B260
Label-Free Characterization of a Novel Early-Stage Drug Discovery Platform
Edward Esposito1, Verna Frasca1, Kevin Mattison2.
1Microlot products group, Malvern Instruments Ltd., Northampton, MA, USA, 2Malvern Instruments Ltd., Westborough, MA, USA.
Template-Directed Assembly (TDA) of membrane-associated proteins is a discovery tool that has been in use for some time. There is an abundance of data that suggests that recombinant proteins cloned without their transmembrane or membrane anchoring domains do not behave as they do in the cell. TDA technology utilizes the common affinity tag (multi-histidine) to assemble recombinant fragments of membrane targets (single pass cytoplasmic or extracellular) onto liposomes incorporating Ni-chelating headgroups. In many cases, these assembled recombinant proteins demonstrate cell-like activity and selectivity. Despite the abundance of activity data that exists, there is a relative paucity of biophysical characterization of these systems. Importantly, it has been proposed that TDA-assembled molecules are conformationally distinct from the unassembled cloned fragments and more cell-like in character. Using differential scanning calorimetry (DSC), we present data comparing the thermal stability of TDA-assembled cytoplasmic domains of transmembrane receptor tyrosine kinases to unassembled RTK domains. We also compare thermal stability of the RTK domains in conditions previously