unknown. In this study we have used atomically detailed molecular dynamics simulation approaches to uncover the mechanism by which lipid A is transferred to, interacts with and binds to a hypothesized amino-terminal pocket in CD14. We modelled the interactions and dynamics of CD14 in the presence of a range of lipid ligands, including control fatty acid systems, and lipid A in monomeric and aggregate/micelle forms. These simulations were run in order to observe the spontaneous ligand binding process, and have subsequently been extended to establish the thermodynamics of ligand recognition. Our results emphasise the dynamic nature of the amino-terminal pocket which allows it to adapt its volume to widely varying ligand size, consistent with the broad specificity of CD14. We have also identified a possible ligand gating mechanism consistent with available NMR data, and key sites that may be essential for LPS/lipid A binding which may ultimately be targeted by novel anti-septic drugs.

1762-Plat
Inside-Out Signaling of Oncogenic EGFR Mutants Promotes Ligand-Independent Dimerization
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Mutations within the epidermal growth factor receptor (EGFR/erbB1/Her1) are often associated with carcinogenesis. Specific mutations common in non-small cell lung cancer (NSCLC), including EGFR-L858R and EGFR-ΔL747-P753, lead to ligand-independent phosphorylation, however the molecular mechanism by which these mutations in the EGFR kinase domain confer constitutive activity remain unknown. Here, using multiple sub-diffraction-limit imaging modalities, we reveal the altered behavior of NSCLC-associated EGFR mutants within the plasma membrane_including altered receptor dimerization, dynamics, and structure_which collectively dysregulate receptor activity. Using multi-color single particle tracking (SPT) and Hidden Markov Model analysis, EGFR mutants are shown to form stable dimers in the absence of ligand and exhibit a slower mobility that is consistent with receptor signaling. These results were confirmed using two-color, single-molecule super-resolution microscopy (dSTORM) to visualize the spatial distribution of receptors. Receptor clustering was quantified by localization-based cross-correlation analysis to show ligand-induced aggregation of EGFR as well as ligand-independent aggregation of EGFR mutants. Since the receptor ectodomain is known to play a critical role in dimerization, live cell FRET measurements between the EGFR N-terminus and the plasma membrane was used to quantify changes in ectodomain structure. We found that unliganded EGFR mutants are more readily found in the extended conformation, similar to the ligand bound wild type receptor. Therefore, mutation within the kinase domain biases the structural equilibrium of the extracellular domain toward a dimer-competent state.
Collectively, these data support a model where oncogenic signaling from NSCLC-associated EGFR mutants is a result of productive dimerization between non-ligand bound receptors. Furthermore, because these mutations are found in the kinase domain, this work introduces the concept that oncogenic EGFR signaling may be controlled in part by a form of ''inside-out'' signaling.

1763-Plat
Mechanisms of Autoinhibition and Dimerization of the EGF Receptor Family
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The epidermal growth factor receptor (EGFR/ErbB) family of receptor tyrosine kinases initiates cell signaling in response to growth factors, thereby regulating cell growth, migration and division. Four members comprise this family (EGFR/HER1/ErbB1, ErbB2, ErbB3 and ErbB4), and activating mutations in each protein are associated with severe cancers of the lung, colon, head and neck.
ErbB proteins consist of an extracellular ligand binding domain, a single transmembrane alpha-helix, and an intracellular kinase domain. Structural and functional studies of isolated domains of the EGFR family have revealed details of the complex mechanism of receptor regulation. Somewhat lacking, however, is our knowledge of the full-length receptor in its native membrane environment. Ligand binding is known to promote receptor dimerization and activation, but it is unclear how receptor behavior is altered by the two-dimensional environment of the plasma membrane. In particular, the mechanism by which receptor domains cooperate to transmit signals across the membrane is not well understood.
Toward this end, we employed quantitative FRET microscopy to study dimerization of EGFR family proteins in plasma membrane-derived vesicles. This method measures FRET efficiency as a function of two-dimensional receptor concentration in the membrane. We investigated four receptor pairs: homodimers of ErbB1, ErbB2, and ErbB3, as well as heterodimers containing ErbB2 and ErbB3. We first observe that the isolated transmembrane (TM) domains have an intrinsic propensity to associate. Next, we demonstrate that the extracellular domains function to prevent TM domain interactions. Finally, we show that the intracellular domains alone are sufficient to drive receptor dimerization in the absence of ligand. Dimerization occurs in a concentration-dependent manner, and ligand shifts this threshold to low receptor concentration. Importantly, both of these dimerization events occur over a physiologically relevant concentration range.

Platform: DNA Structure

1764-Plat
Solid-To-Fluid DNA Transition Inside HSV-1 Capsid Close to the Temperature of Infection
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DNA in the human Herpes simplex virus type 1 (HSV-1) capsid is packaged to a tight density. This leads to tens of atmospheres of internal pressure responsible for the delivery of the herpes genome into the cell nucleus. In this study we show that despite its liquid crystalline state inside the capsid, the DNA is fluid-like which facilitates its ejection into the cell nucleus during infection. We found that the sliding friction between closely packaged DNA strands, caused by interstrand repulsive interactions, is reduced by the ionic environment, mimicking that of epithelial cells and neurons susceptible to herpes infection. However, variations in the ionic conditions corresponding to neuronal activity can restrict DNA mobility in the capsid, making it more solid-like. This can inhibit intranuclear DNA release and interfere with viral replication. In addition, the temperature of the human host (37°C) induces a disordering transition of the encapsidated herpes genome which reduces interstrand interactions and provides genome mobility required for infection.

1765-Plat
Nanopore Sensors for Analysis of Circular DNA Topology
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Over the course of its life cycle, a cell's DNA undergoes many carefully orchestrated topological changes, which facilitate vital cellular processes such as replication and transcription. Consequently, unresolved conformational defects in the structure can interfere with critical interactions, and may result in genetic anomalies that culminate in cell death. As a result, DNA has become the target for numerous anti-cancer treatments which seek to induce structural changes to inhibit tumor growth [1, 2]. The development of innovative anti-cancer treatments can be greatly enhanced by high-throughput low-cost methods to characterize their effects on DNA topology [3]. In this study, we use Si_3N_4 solid-state nanopores to investigate changes in circular DNA topology induced by intercalation of ethidium bromide (EtBr). Our measurements reveal three distinct current blockade levels and a six-fold increase in translocation times for EtBr treated circular DNA as compared to untreated circular DNA. We attribute these increases to changes in the supercoiled topological state hypothesized to be branched or looped structures formed in the circular DNA molecule. Further evidence of the conformational changes is demonstrated by qualitative atomic force