studies have revealed several components of this process. There is now little doubt that these functional mechanisms involve a combination of changes both in the structure of the receptor and in its intermolecular interactions. However, it has been difficult to put together a coherent and unified mechanism of ErbB receptor activation. The principal reason for this difficulty is that our current understanding is derived from the study of isolated fragments of receptor. Here, we propose to overcome this difficulty by taking advantage of a new residue-level coarse-grained (CG) molecular representation and simulation approach, termed ELNEDYN, which is being developed in our laboratory to investigate these mechanisms in the structural context of ''full'' length receptor constructs. One of our long-term objectives is to identify structural and dynamical mechanisms (e.g. structural rearrangements and modes of receptor-receptor association) that underlie or regulate the signaling function of ErbB receptors. Here we summarize results from these CG 3D-modeling studies that show how the conformational equilibrium properties and structural changes of the extracellular domain modulate the conformational equilibrium of the transmembrane and tyrosine kinase domains of the receptor, thereby providing insight into intramolecular factors that govern and regulate the activation and outside-in signaling mechanism of ErbB receptors.

1890-Plat

A Biophysical Mechanosensor Model for T-Cell Receptor Signaling

Zhen-Yu J. Sun[1], SunTaek Kim[2], Koh Takeuchi[1], Maki Touma[2], Jiahuai Wang[2], Gerhard Wagner[1], Ellis L. Reinherz[2].
[1]Harvard Medical School, Boston, MA, USA, [2]Dana Farber Cancer Institute, Boston, MA, USA.

T-cells (cytolytic, helper and others) are vital components in adaptive mammalian immune defense systems. T-cell receptor (TCR) signaling which follows recognition of a peptide antigen bound to an MHC molecule (pMHC) on an antigen presenting cell (APC) is critical for T-cell activation, differentiation, and proliferation. For decades, the mechanism for TCR signaling across the T-cell membrane by this multi-subunit receptor has remained a mystery. Based on our recently obtained structural data on stimulatory and non-stimulatory anti-TCR antibodies and their binding footprint to TCR components obtained by NMR, we present here a simple but all-inclusive ''dynamic torque transducer'' model for TCR signaling. Extracellular mechanical torque can result in quaternary structure changes between the pMHC-binding TCR alpha/beta chains and the non-convalently linked CD3 chains within the TCR complex, triggering downstream signaling via ITAMs in the CD3 cytoplasmic tails. In this process, the pMHC behaves as a detachable effort arm of this complex-lever system; its binding is determined by the specificity of an individual TCR but is not sufficient to mediate signal transduction per se. An external torque is additionally required to provide the energy for this signaling. Such a torque can be generated either via a shear force or vertical pressure between the opposing APC and T cell membranes. The former "bind-and-tug" mode may be responsible for initial signaling during T-cell scanning of APCs, while the latter ''bind-and-bend'' mode is responsible for sustained signaling inside immune-synapses during T-cell activation. Our model can also explain T-cell signaling mediated by antibody or multimeric pMHC cross-linking. This mechanism of converting mechanical energy to a biochemical signal, mediated and controlled by a detachable interface, may be generally applicable in other cell-cell signaling systems both within and outside of the immune system.

1891-Plat

Mechanical Forces in T Cell Triggering

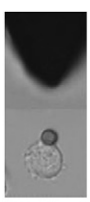
Benoit Carpentier[1], Claire Hivroz[2], **Nelly Henry**[1].
[1]Institut Curie/CNRS, Paris, France, [2]Institut Curie/INSERM, Paris, France.

T cell is one of the main player of mammalian immune response. It ensures antigen recognition at the surface of antigen presenting cells (APCs) in a complex highly sensitive and specific process where encounter of T cell receptor with agonist peptide associated with major histocompatibility complex triggers T cell activation. Despite its central role, the mechanism of TCR triggering is still unclear. Several models involving receptor oligomerisation, kinetic proofreading, serial triggering are currently under debate.

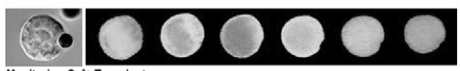
We present here a work aimed to explore experimentally the role of mechanical cues in T cell activation.

We will show the first results of mechanical engagement of TCR in Jurkat cell line using magnetic particles and related cell response as reported by intracellular Ca^{2+} transient.

We will discuss then how these results could provide a mechanistic solution to the TCR triggering puzzle.



Pulling on TCR



Monitoring Ca^{2+} Transient

1892-Plat

Adenosine A_1 Receptor Signaling Unraveled By Particle Image Correlation Spectroscopy (PICS)

Stefan Semrau, Piet Lommerse, Margot Beukers, Thomas Schmidt.
University Leiden, Leiden, Netherlands.

The adenosine A_1 receptor is a typical example of a G protein coupled receptor (GPCR). Despite a wealth of biochemical data the general mechanism of GPCR signaling has not been fully clarified. Whether GPCR signaling takes place in membrane microdomains, and whether the respective G proteins are precoupled, is still heavily debated. Both mechanisms would explain the fast receptor G protein interaction that is observed in experiments. Using single-molecule microscopy in live CHO cells and our recently developed analysis technique (PICS, Semrau, Schmidt., Biophys. J., 2007) we unraveled the first steps of the A_1 receptor signaling. We found that at least 7% of the receptors are precoupled to the G protein already before stimulation with an agonist. Furthermore, 9% of the receptors translocate to membrane microdomains upon agonist stimulation. These domains, which are about 150 nm in size, are related to the cytoskeleton. We believe that this knowledge about the molecular mechanisms of GPCR signaling will open up new ways to manipulate GPCRs and develop new, potent drugs.

1893-Plat

Spontaneously Formed EGFR Dimers Are Primed For Activation

Inhee Chung[1,2], Derek Toomre[2], Joseph Schlessinger[3], Ira Mellman[1,2].
[1]Genentech, South San Francisco, CA, USA, [2]Department of Cell Biology, Yale School of Medicine, New Haven, CT, USA, [3]Department of Pharmacology, Yale School of Medicine, New Haven, CT, USA.

The Epidermal Growth Factor Receptor (EGFR) plays a central role in normal biological processes and disease states such as cancer. Considerable effort has thus been devoted to understanding the mechanisms that control its activation. The conventional model of EGFR activation is that ligand binding induces a conformational change in the receptor, which then leads to dimerization and activation of the intrinsic kinase. Structural studies have identified a key loop protruding from domain II (dimerization arm) of the EGFR ectodomain as a crucial mediator of dimerization. However, a precise mechanistic picture of receptor activation requires time dependent probing of individual molecules on living cells. Here, we report quantum dot (QD)-based optical tracking of single receptor movements on the membranes of living cells. In the absence of ligand, receptors underwent reversible dimerization, indeed dependent on their dimerization arms. However, the dimer concentration is low in cells with normal EGFR expression due to a relatively high dissociation constant (~10-40 μM). Ligand binding stabilized spontaneously formed dimers by reducing the dissociation rate constant, which leads to sustained kinase activation. We found that spontaneously formed dimers can also initiate kinase activation without ligand binding. Moreover, we found that EGFR dimer density was higher in the periphery of the cell versus the center. This difference was reflected in both spatial and temporal heterogeneity of EGFR signaling, where the periphery of the cell serves as an early response site for EGFR activation. Our findings suggest that therapeutic antibodies may be more effective if they increase the dissociation rate constant of EGFR dimers to weaken the activation capability of primed dimers.

1894-Plat

Modeling and Simulation Of A Protein Tertiary Complex: Study of the Interaction Interface and Conformational Dynamics of Dark State Rhodopsin in complex with Transducin

Nikolaos G. Sgourakis, Angel E. Garcia.
Rensselaer Polytechnic Institute, Troy, NY, USA.

We report the first all-atom Molecular Dynamics simulation of a transmembrane protein tertiary complex composed of the G-protein coupled receptor (GPCR) rhodopsin and its G-protein intracellular counterpart transducin in a mixed DOPC membrane/water environment. Based on the analysis of our μsec-timescale simulation trajectory starting from a docked conformation of the complex, we characterize the dynamics present in the dark-adapted state and their influence in the properties and stability of the interaction interface