reflection and confocal excitation. By using a FRET pair with dyes at the ends of the DNA substrates we are able to follow the bending dynamics of the substrates at the single molecule level. We carried on experiments with duplex DNA 15 bp and 18 bp long to determine binding affinity and potential binding of multiple units to the substrate. DNA substrates containing bulges were used for comparison to study the effect of pre-bent structures on binding affinity and conformational dynamics. Bending of the DNA substrates is observed by changes in FRET efficiency allowing determining the conformational dynamics of the system in real time with temporal resolution in the order of milliseconds.

1885-Plat
Slide into Action: Dynamic Shuttling of HIV Reverse Transcriptase on Nucleic Acid Substrates
Shixin Liu[1], Elio A. Abbondanzieri[1], Jason W. Rausch[2], Stuart F.J. Le Grice[2], Xiaowei Zhuang[1,3].
[1]Harvard University, Cambridge, MA, USA, [2]National Cancer Institute, Frederick, MD, USA, [3]Howard Hughes Medical Institute, Cambridge, MA, USA.
The reverse transcriptase (RT) of human immunodeficiency virus (HIV) catalyzes a series of reactions to convert single-stranded viral RNA into double-stranded DNA for host cell integration. This process requires a variety of enzymatic activities, including DNA polymerization, RNA cleavage, and strand displacement synthesis. As a major target for anti-HIV therapy, RT has been the subject of extensive research. Nonetheless, how the enzyme-substrate complex acquires specific functional configurations and switches between different functional modes remains unclear. Here, we used single-molecule fluorescence resonance energy transfer to probe the interactions between RT and nucleic acid substrates in real time (1). Surprisingly, RT was observed to slide on nucleic acid duplexes, rapidly shuttling between opposite termini of the duplex. Sliding kinetics were regulated by cognate nucleotides and non-nucleoside RT inhibitors, a major class of anti-HIV drugs, which stabilized and destabilized the polymerization mode, respectively. These long-range translocation activities facilitate multiple stages of the reverse transcription pathway. First, sliding allows RT to target the polymerization site by one-dimensional search. Remarkably, upon reaching the polymerization site, an RT molecule originally bound in the opposite orientation can spontaneously flip into the polymerization orientation without dissociation, enhancing the target search efficiency. Furthermore, sliding helps RT to actively disrupt secondary structures on the substrate and kinetically access the polymerization site, thereby facilitating strand displacement synthesis. It is remarkable that an enzyme could have such large-scale translational and orientational dynamics. This type of dynamic flexibility may be a general design principle for multi-functional enzymes, helping them to rapidly access different configurations required for different functions.
Reference:
1. S. Liu, E.A. Abbondanzieri, J.W. Rausch, S.F.J. Le Grice, X. Zhuang, ''Slide into action: dynamic shuttling of HIV reverse transcriptase on nucleic acid substrates'', Science (in press).

1886-Plat
Single Molecule Study Of the RNA Degradation and Polyadenylation Activities of the Archaeal Exosome
Gwangrog Lee[1], Sophia Hartung[2], Karl-Peter Hopfner[2], Taekjip Ha[1,3].
[1]Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Munich Center for Advanced Photonics, Department of Chemistry and Biochemistry, Gene Center, Ludwig-Maximilians-University Munich, Munich, Germany, [3]Howard Hughes Medical Institute, Urbana, IL, USA.
RNA biosynthesis involves a certain frequency of errors due to inherent inaccuracies of enzymes involved. Although DNA damage is repaired, RNA errors are rapidly eliminated. In particular, the degradation of 3' poly A tail is very important in RNA metabolism to maintain the fidelity of mRNA synthesis in the cell. A key enzyme involved in both RNA processing and RNA degradation is the exosome complex. The archaeal exosome can function in reverse reactions as either an RNA polymerase or an RNA exoribonuclease. In the presence of free inorganic phosphate (P_i), the exosome phosphorolytically degrades RNA substrates from the 3' end, whereas in the presence of ADP, it polymerizes and extends the 3' end. The dynamics of the RNA degradation and polymerization reactions of the exosome are not well characterized and thus warrant further investigation in order to better understand the exosome function. Here, we measured the real time activities of the archaeal exosome using single molecule Fluorescence Resonance Energy Transfer (smFRET). Both degradation and polyadenylation activities were highly processive and followed the Michaelis-Menten kinetic parameters over a range of substrate concentrations. Using the combination of smFRET and gel-based assays, we examined these reactions in order to understand the reversibility between 3'→5' nuclease activity and 5'→3' polyadenylation under an equilibrium condition of ADP and P_i. We found that the enzyme can switch the direction rapidly between both reactions back and forth at the condition where both speeds of polymerization and degradation are equivalent.

1887-Plat
Direct Observation of NS3 Substeps at Single Base Pair Resolution
Wei Cheng, Ignacio Tinoco, Carlos Bustamante.
UC Berkeley, Berkeley, CA, USA.
Helicases are a ubiquitous class of nucleic acid motor proteins. They utilize ATP to catalyze the opening of double-stranded nucleic acids. Previous crystallographic and single molecule fluorescence studies have implied that a single ATP molecule would be used for every base pair (bp) being opened by a helicase. However, this has not been directly observed. The hepatitis C virus encodes NS3, an RNA helicase that is essential for viral RNA replication. Previous single molecule optical tweezers studies on NS3 unwinding have revealed an 11-bp periodicity with 3.6 bp substeps on average. By building an optical tweezers instrument with high spatial resolution and low drift, we were able to monitor the discrete unwinding substeps of NS3 on RNA at single base pair resolution. We show that under conditions where ATP binding limits the motor dwell time between successive substeps, single base pair substeps are clearly visible. We present a distribution of these substep sizes and the implication of this result on mechanisms of NS3 catalyzed RNA base pair opening and strand release. The distribution of motor dwell times between substeps gave us a direct measure of the coupling ratio between ATP and base pair opening, and we propose an integrated model to rationalize all these observations.

1888-Plat
Visualizing an RNA-dependent RNA Polymerase at Work: Polymerization And Strand-switching
Igor D. Vilfan[1], Wiecher Kamping[1], Andrea Candelli[1], Susanne Hage[1], Antti P. Aalto[2], Minna M. Poranen[2], Dennis H. Bamford[2], Nynke H. Dekker[2].
[1]Technical University of Delft, Delft, Netherlands, [2]University of Helsinki, Helsinki, Finland.
RNA-dependent RNA polymerases (RdRP) are responsible for the transcription and replication of the genomes of many RNA viruses. These enzymes have also emerged as an integral part of the RNA interference mechanism in plants. RdRPs share many of the structural features with other types of polymerases, However, the ability of certain viral RdRPs to perform both replication and transcription sets them apart from more commonly known polymerases such as DNA-dependent RNA polymerases.
We have applied magnetic tweezers to study the transcription kinetics of a model RdRP from Bacteriophage Phi6 (Phi6 RdRP), a double-stranded RNA (dsRNA) virus. During *in vivo* transcription, Phi6 RdRP binds the 3'-end of the antisense RNA strand within the dsRNA genome and polymerases a new sense RNA strand. Concurrently, the original sense RNA strand is displaced. In magnetic tweezers, we measure the transcription kinetics by following the length of the displaced sense RNA strand. As a result, we measure a rate of transcription of ~20 nt/s, which is comparable with our bulk experiments. However, in approximately 30% of transcription events in the magnetic tweezers, we also observe for the first time a conversion of the displaced sense RNA strand back to double-stranded form. By elimination of other possibilities, we attributable this to strand-switching of the RdRP. This is surprising given the 'closed' structure of the RdRP predicted from crystallography. However, it is a phenomenon that has also been observed for DNA helicases and provides a potential mechanism for RNA recombination observed in RNA viruses.

Platform AD: Membrane Receptors & Signal Transduction

1889-Plat
Coarse-grained Modeling And Simulation Of ErbB Receptors: Intramolecular Factors That Govern Outside-in Signaling And Activation
Marco Ceruso, Marco Cavalli.
The City College of New York, New York, NY, USA.
Epidermal growth factor receptors (ErbB receptors) mediate a number of critical cellular processes including cell proliferation, differentiation, migration and apoptosis. These receptors have received a lot of attention because they have been implicated in a number of human cancers including breast, colo-rectal and prostate cancer. ErbB receptors are also important targets in cancer therapy. The activation and regulation of ErbB receptor function is known to be a highly regulated and multilayered process. A number of elegant structural

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

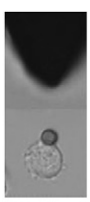
Benoit Carpentier[1], Claire Hivroz[2], **Nelly Henry**[1].
[1]Institut Curie/CNRS, Paris, France, [2]Institut Curie/INSERM, Paris, France.

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.

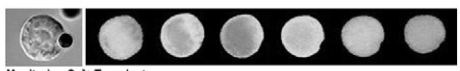
We present here a work aimed to explore experimentally the role of mechanical cues in T cell activation.

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