random. Domains were directly observed. The domain size depended on the type of the membrane anchor (HRas: 130 nm, KRas: 200 nm; NRas: 200 nm). Further we found that domain formation and disassembly was dynamic on a timescale of 5-30s.

1204-Plat

Super-Resolution Localization Microscopy Identifies Distinct Stages of Antigen-Induced IgE Receptor Cross-Linking and Immobilization in Rbl-2H3 Mast Cells

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Cross-linking of immunoglobulin E (IgE) bound to its receptor, FcεRI, by multivalent antigen initiates a transmembrane signaling cascade essential for mast cell activation and important for inflammatory immune responses and allergic disease. In this study, we apply super-resolution fluorescence localization microscopy to record receptor organization and dynamics on live RBL-2H3 mast cells undergoing antigen-mediated signaling, allowing us to measure nanoscale clustering and diffusion of FcεRI simultaneously. Through comparison of cross-linking-induced changes in these properties as a function of time, we are able to resolve two distinct temporal phases of receptor clustering and immobilization. Additionally, we correlate the time-dependence of the distinct phases with a functional signaling response, Ca^{2+} mobilization. In the first phase of receptor clustering and immobilization, receptors slow dramatically with a relatively small average increase in clustering, and individual receptors appear to transiently associate with small clusters. This first phase occurs before Ca^{2+} mobilization and concurrently with initial signaling steps. At later times, receptor-rich clusters become increasingly dense while receptors remain predominately immobilized. These latter behaviors are observed at times following the initiation of the Ca^{2+} response, and we conclude that although cross-linking is necessary for commencement of downstream signaling, receptor assembly into large, densely packed clusters at later times is likely associated with termination of the stimulated response. These findings motivate future study of the physical interactions that give rise to the observed changes in receptor organization and mobility, and how these translate into cellular functions. In ongoing experiments, we are exploring the requirements of signaling for receptor cross-linking through the use of antigens with controlled structure and valency, and we will correlate our observations with functional responses such as Ca^{2+} mobilization and receptor association with downstream signaling partners.

1205-Plat

The Actin Cytoskeleton Controls the Activation of Invariant Natural Killer T Cells by Fine-Tuning CD1d Nanoscale Aggregation on Antigen Presenting Cells

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Invariant Natural killer T (iNKT) cells are a subset of lipid-specific T cells, restricted by the MHC-I class like molecule CD1d. A recent study showed a correlation between the co-localization of lipid-loaded CD1d molecules with lipid rafts on the membrane of antigen presenting cells, and their capacity of eliciting secretion of Th1-cytokines by stimulated iNKT cells [1]. Thus, this study suggested that not only the structure of CD1d bound to an exogenous lipid could influence Cd1d-mediated immunity, but also its partitioning on the membrane. Here, we address the spatiotemporal behaviour of α-Galactosylceramide loaded CD1d complexes on the cell membrane of *human* myeloid cells using multiple colour high-speed single-particle tracking (100 Hz) combined with an iNKT T Cell Receptor-Qdot conjugate as imaging probe. Furthermore, we complement these studies using STED *nanoscopy* to obtain nanoscale images of CD1d spatial organization. Our results indicate a direct role of the actin cytoskeleton in actively segregating CD1d nanoclusters on the cell membrane resulting in an inhibition of the activation of iNKT cells [2]. As a whole, our work proposes a new paradigm of *biophysical* interaction between CD1d presenting cells and NKT cells which deviates significantly from classical MHCI/II complexes and CD8/CD4-T Cells interactions.

[1] J. S. Im et al., *Immunity*, **30**, 888, 2009.
[2] J. A. Torreno-Pina et al., in preparation.

1206-Plat

Multi-Color, Single-Molecule Fluorescence Imaging of GPCR Signalosomes

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G protein-coupled receptors (GPCRs) are the largest superfamily of membrane receptors in the human genome and they are targets for a quarter of all prescription drugs. Activation of a GPCR by an agonist ligand results in G protein-mediated downstream signaling, followed by kinase action and arrestin-mediated desensitization, internalization/sequestration, and recycling. Selective manipulation of these individual steps of the GPCR activation cycle is often desired when creating drugs targeting a given receptor. We are interested in the C-C chemokine receptor CCR5 that is the major HIV coreceptor used in person-to-person transmission. Globally, the HIV/AIDS pandemic has caused nearly 30 million deaths and a similar number of people are currently infected. Certain analogues of the chemokine RANTES/CCL5 are highly potent entry inhibitors against R5-tropic HIV-1 strains, in vitro and in vivo. Three such analogues, 5P12-, 5P14-, and 6P4-RANTES, are particularly interesting because while they differ only slightly in structure they show strikingly different pharmacological profiles (G protein-linked signaling activity, stimulation of receptor internalization). We have recently developed a general, simple, and robust method for stoichiometric, site-specific fluorescence labeling of expressed GPCRs. The method is based on bioorthogonal conjugation of a fluorescent reporter group to a genetically encoded azido group introduced into expressed GPCRs using amber codon suppression.[1] We have adopted a similar strategy for fluorescent labeling of chemokines with azido groups introduced by chemical synthesis. Here we present our progress towards automated, multi-color, single-molecule fluorescence studies of the compositional and conformational dynamics of GPCR signaling complexes ("signalosomes") using fluorescently labeled chemokines and receptors in biochemically defined systems.

[1] H Tian, TP Sakmar, & T Huber (2013) Site-specific labeling of genetically encoded azido groups for multi-color, single-molecule fluorescence imaging of GPCRs. Methods in Cell Biology, 117, in press.

1207-Plat

Dengue Virus Infection Mediated by DC-SIGN

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DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) is a pattern recognition receptor which binds to the mannose or fucose structures present on a variety of pathogens and stimulates diverse immune responses. DC-SIGN forms nanodomains on cell surfaces which are entry portals for viruses including HIV, Ebola, dengue and hepatitis C. In particular, dengue is a mosquito-borne viral infection and has become a rapidly growing global health threat. Many reports have shown that ectopically expressed DC-SIGN enhances productive dengue infection in different human cell types; however, detailed molecular-level studies on interactions between DC-SIGN membrane assemblies and dengue virus (DENV) at the initial binding and internalization stages are lacking. By employing immunostaining, confocal imaging, super-resolution direct stochastical optical reconstruction microscopy (dSTORM) and flow cytometry assays, we show that cell surface DC-SIGN nanodomains are sufficient to capture the small sized DENV (50 nm), leading to efficient virus internalization and productive infection of the host cells. At the initial binding stage, DENV is highly colocalized with cell surface DC-SIGN domains. Internalization of DENV was observed within a few minutes after incubating DENV with cells expressing DC-SIGN, and massive viral particle synthesis was observed at 24h after infection. In contrast, no virus replication was observed on control cells even after 72h of incubating with DENV. The results indicate that DC-SIGN capturing of DENV leads to rapid internalization of the viruses and productive infection thereafter. Furthermore, superresolution dSTORM shows that a single DC-SIGN nanodomain is sufficient to capture single DENV particles. Supported by NIH GM 41402 and NIAID RO1-AI107731.

1208-Plat

How Talin Head Domain and Soluble Ligand Contribute to Integrin αIIbβ3 Activation

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Integrin αIIbβ3 is widely known to regulate the process of thrombosis via activation at its cytoplasmic side by talin and interacting with soluble fibrinogen. Three groups of interactions regulate integrin activation: a set of salt bridges

in the cytoplasmic side of the transmembrane domain of integrin α- and β-subunits named as the inner membrane clasp, a hydrophobic packing of a few transmembrane residues on extracellular side between α- and β-subunits that is termed as the outer membrane clasp, and the key interaction group of βA domain and βTD domain. Molecular details of this key interaction group as well as events that lead to detachment of βTD and βA domains have remained ambiguous.
Full-length structure of integrin αIIbβ3 embedded in a patch of lipid bilayer was used to simulate its interactions with three soluble RGD ligands as well as talin, using a molecular dynamics software. We showed that talin's interaction with the membrane-proximal and membrane-distal regions of integrin cytoplasmic-transmembrane domains significantly loosens the inner membrane clasp as well as an additional salt-bridge (R734-E1006), which facilitates integrin activation through the separation of integrin's α- and β-subunits. Also, it is shown that interaction of the Arg of the RGD sequence may activate integrin via disrupting the key interaction group between Lys346 on βA domain and Ser663/Ser664 on the βTD. Interestingly, we observed the full dissociation of βA and βTd domains when this interaction group was disrupted and was eventually dissociated as a result of a competition between the Arg of the RGD peptide with Ser664. Consequently, we proposed a mechanistic scenario as a potential mechanism for outside-in activation of integrin αIIbβ3 by soluble RGD ligand that reconciles the switchblade and dead-bolt models for integrin activation.

1209-Plat
Single Molecule Imaging of Human Epidermal Growth Factor Receptors
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The human epidermal growth factor receptor (HER/ErbB) family of receptor tyrosine kinases encompasses transmembrane signaling proteins important for cellular growth, differentiation, and survival. The four members of this family (EGFR, HER2, HER4 and the HER3 pseudokinase receptor) are able to bind to numerous different growth factor ligands, leading to their homo and hetero-oligomerization and subsequent activation. This combinatorial potential gives rise to a diverse signaling output, which is strongly affected by misregulation of any one receptor. For example, mutations in EGFR and HER2 overexpression are primary mechanisms driving lung and breast cancer, respectively. Under these conditions, heterodimerization of these receptors with the HER3 pseudokinase confers resistance to tumors treated with EGFR and HER2-targeted therapeutics. The molecular basis for these heterodimeric interactions and their regulation by growth factors remains poorly understood. HER receptor heterodimers have never been observed directly, and their existence remain inferred from the analysis of downstream signaling. To understand the scope and specificity of heterodimeric interactions between the HER3 pseudokinase receptor and its active HER homologs, we seek to directly investigate the underlying receptor activation mechanisms using high resolution fluorescence microscopy. We quantify the extent of heterodimeric interactions in response to ligand binding using both live cell single molecule tracking and stochastic optical reconstruction microscopy (STORM). These techniques allow us to probe the timescales of receptor interactions as a function of ligand binding, the dependence on receptor density at the membrane surface, and the specificity of heterodimer formation. This work aims to contribute to the fundamental understanding of the activation mechanism of HER receptors and to help advance development of new therapeutics targeting aberrant cross-talk among HER receptors in human disease.

Platform: Structure and Dynamics of RNA in Biology

1210-Plat
Deciphering Ribosomal Frameshifting Dynamics
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Ribosomes programmed by specific messenger RNA (mRNA) sequence elements can switch translation reading frames and synthesize different polypeptides from a single template. The *Escherichia coli dnaX* mRNA encodes two DNA polymerase III subunits, τ and γ, synthesized from 0-frame and probabilistic −1-slip across the slippery sequence: AAAAAAG. When further enhanced by structural barriers situated around the slippery sequence-an internal Shine-Dalgarno sequence and a stable hairpin stem loop, an 80% (= γ/(γ+τ)) frameshift efficiency is attained. Here, we attempt to determine the frameshift timing within one translation cycle by following a single ribosome translating a frameshift-promoting mRNA held on optical tweezers. In parallel, by mass spectrometry (MS), we survey the synthesized polypeptides to resolve where on the mRNA the ribosome has slipped.
From the mass spectra of polypeptides terminated at the −1-frame stop codon, we learned that the ribosome −1-slips from more than one codon position around the slippery sequence. Some −1-frameshifted polypeptides were found to bear an extra amino acid, or to lack one, indicating that slipping sizes of −4 and +2-nt also occurred. Similarly, distinctive large-scale fluctuating translocation dynamics were seen in our real-time single-ribosome translation trajectories. This reveals that a translocating ribosome can explore a broad range of frameshift pathways. Frequently adopted frameshift pathways, i.e. the more abundant frameshifted species resolved by MS, exhibit a preference for minimizing codon:anticodon base-pair mismatches on the ribosome after a slip. Mismatch-containing ribosomes can be prematurely terminated by release factors, resulting in release of incomplete peptides. Indeed, we observed higher yields of incomplete peptides that are terminated at frameshift sites where significant mismatches were encountered. These species coincide with the prematurely stalled ribosomes recorded in the translation trajectories. Collectively what emerges from our results is a versatile ribosomal frameshifting scheme during mRNA translocation, facilitating branching of frameshift pathways.

1211-Plat
Unraveling the Mystery of Ribosome Induced RNA Unfolding
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During translation, the ribosome encounters various structures in the mRNA from simple hairpins to more complex tertiary RNA folds. These structures must be unwound for accurate decoding and translation to proceed. To probe the unwinding activity of the ribosome, we prepared dye labeled RNA transcripts and dye labeled ribosomes to directly report on the folded/unfolded states of the RNA at various stages along the translational elongation cycle. Consistent with previous studies, we found that the ribosome was sufficient to resolve RNA structures in the absence of any additional factors. Surprisingly, significant unwinding was observed in the absence of translocation or motion of the ribosome along the mRNA and can be attributed to dynamic motions within and between the two ribosomal subunits. This suggests that thermal energy resident within these ribosomal motions is sufficient to lower the energetic barrier of unfolding. The extent of unwinding was observed to depend on the relative stability of the RNA structure used and the number of tRNAs present in the ribosome. Differences between unfolding of these RNA structures in the presence and absence of the ribosome will be discussed.

1212-Plat
Single-Molecule Profiling of Ribosome Translational Phenomena
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Translation elongation is a heterogeneous process, involving multiple compositional factors stochastically binding to the ribosome to direct protein synthesis, which in turn regulates the conformation of the ribosome. The rate of translation is often regulated by the underlying messenger RNA (mRNA) sequence and structure. Here, we use single-molecule fluorescence resonant energy transfer (FRET) and colocalization with zero-mode waveguides (ZMWs) to correlate directly ribosome conformations and compositions of thousands of ribosomes simultaneously during multiple rounds of elongation. This allows us to profile global translational rates while delineating mechanistic details of the dynamics with codon resolution. We first studied translation of a canonical mRNA with uniform translation rates to establish the tight interplay between compositional factors and conformational dynamics of the ribosome during elongation. We then determined how mRNA sequences and structures, such as hairpins and possible ribosome-mRNA base pairing, as expressed in the the dnaX −1 frameshifting sequence, perturb the basal elongation process. Our results show how mRNAs can modulate and uncouple ribosomal conformational and compositional dynamics.

1213-Plat
The Ribosome Uses Cooperative Conformational Changes to Maximize the Efficiency of Protein Synthesis
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Execution of individual steps in the multi-step functional cycles of large, multi-component molecular machines such as the ribosome almost universally