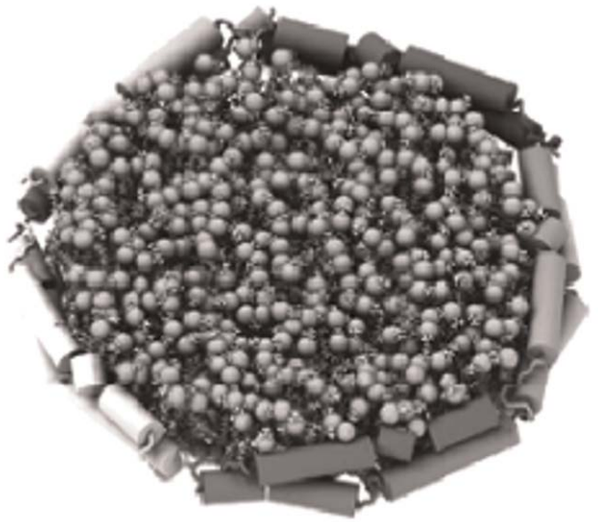

Platform V: Protein-Ligand Interactions

929-Plat Reduced complexity of T cell recognition: Gamma Delta T cells recognize the MHC ligand T22 using a single CDR loop

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Reduced complexity of T cell recognition: Gamma Delta T cells recognize the MHC ligand T22 using a single CDR loop. Antigen recognition by the adaptive arm of the immune system is mediated by receptors expressed on T cells or antibody producing B cells that are generated through a process of genomic rearrangement. The diversity inherent in the rearrangement process, at the protein level, is concentrated in the receptor's six Complementary Determining Region (CDR) loops and endows these receptors with the ability to recognize a diverse array of ligands. We show that a unique subpopulation of T cells that express rearranged T cell receptors (TCR) composed of a gamma and delta chain as opposed to the more ubiquitous alpha and beta chains, recognize their ligand, the non-classical MHC class I molecule T22, through only one CDR loop, CDR3delta. Fusion of this loop from two T22 reactive gamma delta TCRs onto a naïve TCR transfers reactivity, with similar affinities to those of the wildtype receptors. The reactivity of these fusion constructs to ligand confirms that the recognition "glue" is solely through their CDR3delta loop. However, amino acid and length variation exists between the two loops that were examined; alanine scanning confirms that ligand binding is accomplished through use of different registers of amino acids in these loops. The reduced recognition complexity of T22 reactive gamma delta TCRs therefore contrasts with alpha beta TCR or antibody recognition of antigen, which involves a combination of the six CDR loops inherent in these receptors structure. However, the use of different amino acid residues within these loops for ligand binding suggests within each loop there exists complexity that is a result of a convergent recognition strategy.

930-Plat Aberrations in the Mechanism of Protein Kinase A Mediated Phosphorylation Caused by Phospholamban Mutants

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Protein phosphorylation is fundamental in the modulation of myocardial contractility. One mechanism which controls this modulation occurs through alterations of Ca^{2+} flux formed across the sarcoplasmicreticulum (SR) membrane, which has profound dependence on the interactions of three proteins: protein kinase A (PKA), sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA), and a single pass transmembrane protein, phospholamban (PLN). Phosphorylation of PLN by PKA is associated with an augmented rate of SR Ca^{2+} uptake and relaxation of the myocardium. Mutants of PLN (PLN-R9C and PLN-R14-Delete) have been shown to be linked with forms of the fatal hereditary disease, dilated cardiomyopathy. We have proposed that this form of the disease is largely attributed to abnormalities in protein phosphorylation of these mutants of PLN. Here, we examine the structural perturbations, thermodynamics of substrate binding, and kinetics of phosphorylation for the interactions between the full catalytic subunit of PKA and peptides corresponding to the cytoplasmic region of PLN ($PLN_{1–19}$) and its mutants ($R9C_{1–19}$ and $R14Del_{1–19}$) using NMR spectroscopy and coupled enzyme kinetic assays. We found that the binding thermodynamics for $PLN_{1–19}$ or $R9C_{1–19}$ are nearly identical ($K_d \sim 14\ \mu M$), but the kinetics of phosphorylation for $R9C_{1–19}$ are drastically reduced (k_{cat} is decreased 5-fold for $R9C_{1–19}$). By using TROSY-based NMR techniques, we also mapped the residue specific differences in the amide fingerprint of C-subunit PKA (PKA-C) during substrate binding. Our results suggest that changes in the perturbation of key allosteric residues in the enzyme may implicate a decrease in the ability of $R9C_{1–19}$ to become phosphorylated. Finally, we present a model of the protein-protein complexes formed, using a novel technique which measures residual dipolar couplings of the backbone amides for PKA-C in alignment media containing phospholamban embedded in the matrix.

931-Plat Live Cell Single Molecule Imaging and Micropatterning of CD4 reveals Novel Binding Mechanisms to Lck

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Detection and quantification of protein interactions represent the cornerstone of proteomic research. Current screening platforms, however, rely on measuring protein interactions in highly artificial systems rendering the results difficult to confer on the in vivo situation. We present here a method to identify and characterize interactions between a fluorescently labeled protein (“prey”) and a membrane protein (“bait”) in living mammalian cells. Cells transfected with a fluorescent protein fusion of the prey are plated on micropatterned surfaces functionalized with specific antibodies to the exoplasmic domain of the bait. Bait-prey interactions are assayed via the concomitant redistribution of the fluorescent prey. We applied the technology for characterizing the interaction between CD4, the major co-receptor in T cell activation, and Lck, the protein tyrosine kinase essential for early T cell signaling. Equilibrium associations were quantified by semi-automatic analysis of the Lck-micropatterns; information on interaction dynamics was obtained by combining the platform with photobleaching experiments and single molecule imaging. In addition to the known zinc clasp, we identified additional Lck domains contributing to CD4 binding. The membrane-anchor was found to have a strong impact on the interaction: it mediates direct binding, and further stabilizes the interaction of other Lck domains; in total, membrane-anchorage increases the interaction lifetime by two orders of magnitude. In addition, multiple membrane-distal domains were found to modulate the binding affinity in a subtle way. The broad range of affinities contributed by different Lck domains render this molecule amenable to fine-tuning of its binding capabilities, thereby providing additional means for regulating Lck recruitment in cellular activation processes.

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932-Plat A Functional Single-molecule Binding Assay Via Force Spectroscopy

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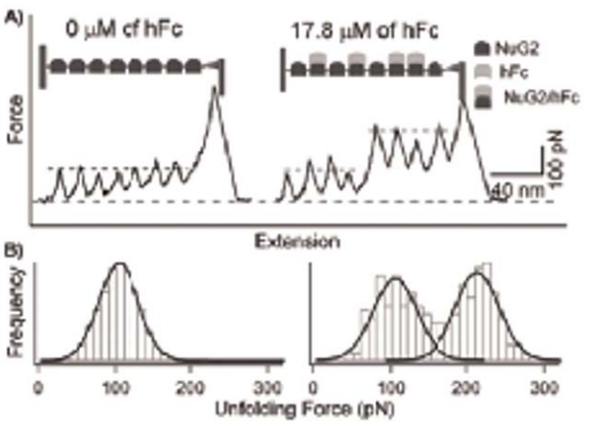
Protein-ligand interactions are ubiquitous in biology. A wide range of methodologies have been developed for quantitative analysis of protein-ligand interactions. However, most of them do not report direct functional/structural consequence of ligand binding. Thus, important information about the functional state of protein-ligand complexes cannot be obtained directly. Here we report a functional single molecule binding assay that uses force spectroscopy to directly probe the functional consequence of ligand binding. As a proof of principle, we used NuG2 mutant of protein G and Fc-fragment of IgG as a model system. Binding of Fc to NuG2 does not induce major structural changes of NuG2 but results in significant enhancement of its mechanical stability (Figure). Using mechanical stability of NuG2 as an intrinsic functional reporter, we directly distinguished and quantified Fc-bound and Fc-free forms of NuG2 on a single-molecule basis, and accurately determined the dissociation constant. This single molecule functional binding assay is label-free and nearly background-free, and can detect functional heterogeneity, if any, among protein-ligand interactions. This novel methodology opens up new avenues of studying protein-ligand interactions in a functional context and will find broad applications in diverse protein-ligand systems.



933-Plat Measuring interactions between the Antennapedia Homeodomain and DNA using Fluorescence Cross-Correlation Spectroscopy with a biarsenical ligand

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Recombinant proteins containing a tetracysteine motif CCPGCC on C-terminus can be covalently labelled with a biarsenical fluorescein derivate called FlAsH. Here we use this approach to label the homeodomain of the transcription factor Antennapedia from Drosophila. In this study, we use Fluorescence Cross-Correlation Spectroscopy (FCCS) to analyse the DNA binding properties of this transcription factor with different DNA fragments. We developed, for the first time, a reproducible in vitro fluorescence assay, based on the FlAsH technology, at the single molecular level. The obtained dissociation constants for homeodomain DNA interactions are in total agreement with previous data from gel shift assays. Binding data from the DNA fragment Ultrabithorax carrying three specific binding targets was analysed by equilibrium binding experiments based on FCCS resulting in a dissociation constant, Kd, of 31 ± 2 nM. The Kd of this interaction was further reduced if the binding assay is performed as an equilibrium competition experiment, Kd = 23 ± 5 nM, and even further if the DNA length is 1 kb, Kd = 5 ± 8 nM. Accordingly, the combination of FCCS, FlAsH labelling, and an interaction study in competition mode is well

suited for fast and efficient screening to study new transcription factor interactions. It is a prerequisite to unravel the molecular mechanisms of gene regulation.

934-Plat Predicting protein-ligand binding free energies

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We discuss recent work calculating accurate absolute and relative protein-ligand binding free energies in several different binding sites, in some cases predictively. These sites range from model binding sites to proteins which are targets of current drug discovery effort. We discuss insights gained into the contributions of multiple ligand orientations and protein conformational changes to protein-ligand binding. Conformational changes prove especially important: even small protein conformational changes at the amino acid side chain level in Interleukin 2 and a lysozyme model binding site can contribute several kcal/mol to binding free energies. Smaller conformational changes prove important, as well: When the protein is treated as rigid, RMS errors relative to experiment are extremely large; even a small amount of protein flexibility can substantially improve results.

935-Plat Agonist Binding and Allostery in NR3A Receptors

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N-methyl-D-aspartate (NMDA) receptors are heteromultimeric ligand-gated ion channels involved in cognitive processes such as learning, memory and, in dysfunctional cases, mental illness, stroke, and neurodegeneration. A less-characterized NMDA receptor subunit, NR3A, has been discovered to act as a dominant negative regulator of receptor activity that, upon binding of glycine, attenuates calcium permeability and magnesium sensitivity in the receptor.

Recent crystal structures of NR3A bound to glycine or the partial agonist ACPC show very few structural differences despite binding affinities that differ by four orders of magnitude. To elucidate the cause of this difference and investigate potential allosteric mechanisms, molecular dynamics and quantum chemical calculations were conducted.

In contrast to previous, conventional simulations of other NMDA receptor subunits, results from our replica exchange MD simulations showed increased order in the binding site residues of the NR3A-ACPC complex. In the NR3A-glycine complex an allosteric conformational change distant to the binding site was observed that was absent in the apo or ACPC-bound states. This change is transmitted through a hydrophobic cluster beginning with a tyrosine residue directly involved in ligand binding, and leads to 30 degree tilting of a helix in an NR3A-specific portion of the receptor. We hypothesize that this helix is involved in allosteric interactions with other domains of the NR3A receptor or other portions of the multimeric assembly.

Similar simulations of the apo state allowed the modeling of a hypothesized, but as yet not crystallographically resolved, "open" state in which the ligand binding site is fully exposed to solvent.

Free energy perturbation simulations exchanging glycine and ACPC in the binding site revealed the role of specific atomic interactions in binding affinities as well as allosteric coupling.

936-Plat Watching Insulin Dimers Dissociate using Ultrafast IR Spectroscopy and MD Simulation

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Insulin homodimer dissociation releases the monomers necessary to initiate glucose regulation by binding the insulin receptor. Understanding and controlling the interplay between association and internal coordinates in the monomer-dimer equilibrium would contribute to a paradigmatic protein-protein interaction with profound public health implications. We report the combination of two-dimensional infrared spectroscopy (2D IR) and molecular dynamics (MD) simulations as a new tool to study the con-formational changes associated with insulin dimer dissociation. By spreading a vibrational spectrum over two frequency axes, amide I 2D IR spectra can display cross-peak ridges between different secondary structure-sensitive protein vibrations. Spectral blueshift of the band center and cross-peak ridges formed only during close contacts of the intermolecular β-sheet are identified as markers for insulin dimer dissociation. During thermal dissociation, these markers are used to probe the depletion of interfacial structure. The 2D IR spectra are directly calculated from MD simulations of the dissociation reaction to provide an atomistic model of the changes.

Platform W: Voltage-Gated K Channels, K-Channel, Structure & Dynamics

937-Plat Ligand-dependent Conformational Dynamics in a Potassium Channel Ca^{2+}-binding Domain

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TvoK is a prokaryotic Ca^{2+}-activated K^+ channel whose ligand-dependent gating is regulated by its cytoplasmic RCK domain