Platform AM: Protein-Ligand Interactions

2210-Plat

Conformational Transitions Upon Ligand Binding: Holo Structure Prediction from Apo Conformations

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Biological function of proteins is frequently associated with the formation of complexes with small-molecule ligands. Experimental structure determination of such complexes at atomic resolution, however, can be time-consuming and costly. Computational methods for structure prediction of protein/ligand complexes, particularly docking, are as yet restricted by their limited consideration of receptor flexibility, rendering them not applicable for predicting protein/ligand complexes if large conformational changes of the receptor upon ligand binding are involved. Accurate receptor models in the ligand-bound state (holo structures), however, are a prerequisite for successful structure-based drug design.

Hence, if only an unbound (apo) structure is available distinct from the ligand-bound conformation, structure-based drug design is severely limited.

We present a method to predict the structure of protein/ligand complexes based solely on the apo structure, the ligand and the radius of gyration of the holo structure. The method is applied to ten cases in which proteins undergo structural rearrangements of up to 7.1 A backbone RMSD upon ligand binding. In all cases, receptor models within 1.5 A backbone RMSD to the target were predicted and close-to-native ligand binding poses were obtained for eight of ten cases in the top-ranked complex models.

The developed protocol is expected to enable structure modeling of protein/ligand complexes and structure-based drug design for cases where crystal structures of ligand-bound conformations are not available.

2211-Plat

Design and Characterization of Small Molecule Inhibitors of the PICK1 PDZ Domain with Binding Free Energy Calculations

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PDZ domains are scaffolding proteins that assemble and regulate many cellular signaling pathways by recognizing specific C-terminal type II peptide sequences. Consequently, these domains are associated as well with human disease and represent putative targets for new pharmacotherapeutics. The PICK1 (Protein Interacting with C Kinase 1) contains a N-terminal PDZ for which the first small molecule inhibitor (FSC231) was identified from fluorescent polarization assay screening in the lab of Ulrik Gether (Thorsen TS, Madsen KL, Rebola N, Rathje M, Anggono V, Bach A, Moreira IS, Stuhr-Hansen N, Dyhring T, Peters D, Beuming T, Huganir R, Weinstein H, Mulle C, Strømgaard K, Rønn LCB, Gether U - submitted). To identify the binding modes of FSC231 in both the wild type and a K83H mutant for which FSC231 exhibited higher affinity, we undertook computational docking of the compound to the crystal structure of the PDZ domain and subsequent refinement by Molecular Dynamics simulations. Based on the structure of FSC231, a library of over 1000 novel small molecules were designed by employing Ligbuilder GROW strategy and were docked to the same PICK1 PDZ domain. The top-ranked molecules from the docking results were subjected to binding free energy calculation using a potential of mean force (PMF) simulation method (Woo and Roux, PNAS 2005) with restraining potentials. This method samples the physical path of the protein-ligand binding, involving the decomposition of the binding process to several stages, and had proven successful in predicting the binding affinity for a variety of peptide-PDZ domains complexes. The results from the modeling and free energy simulation work characterized the molecular interaction network of PICK1 PDZ domain and guide the efficient rational design of new lead compounds.

2212-Plat

Computational Design of Protein Interfaces with Receptor Flexibility

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In this work, we computationally graft the binding epitope of various small proteins obtained from the RCSB database to bind to barnase, lysozyme, and trypsin using a previously derived and validated algorithm. In an effort to probe the protein complexes in a realistic environment, all native and designer complexes were subjected to a total of nearly 400 ns of explicit-solvent molecular dynamics (MD) simulation. The MD data led to an unexpected observation: some of the designer complexes were highly unstable and decomposed during the trajectories. In contrast, the native and a number of designer complexes remained consistently stable. The unstable conformers provided us with a unique opportunity to define the structural and energetic factors that lead to unproductive protein-protein complexes. To that end we used free energy calculations following the MM-PBSA approach to determine the role of non-polar effects, electrostatics and entropy in binding. Remarkably, we found that a majority of unstable complexes exhibited more favorable electrostatics than native or stable designer complexes, suggesting that favorable electrostatic interactions are not prerequisite for complex formation between proteins. However, non-polar effects remained consistently more favorable in native and stable designer complexes reinforcing the importance of hydrophobic effects in protein-protein binding. While entropy systematically opposed binding in all cases, there was no observed trend in the entropy difference between native and designer complexes. A series of alanine scanning mutations of hot-spot residues at the interface of native and designer complexes showed less than optimal contacts of hot-spot residues with their surroundings in the unstable conformers, resulting in more favorable entropy for these complexes. Finally, disorder predictions revealed that secondary structures at the interface of unstable complexes exhibited greater disorder than the stable complexes.

2213-Plat

The Two Enantiomers of Citalopram Bind to the Human Serotonin Transporter in Reversed Orientations

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The human serotonin transporter exhibits substantial selectivity in the binding affinities of the two enantiomeric forms of the antidepressant citalopram. Previous studies of the structural determinants of *S*- and *R*-citalopram binding revealed residues Tyr95 and Ile172 as involved in the discrimination between the two enantiomers. However, the overall orientation of the ligand in the binding site and the precise nature of protein-ligand interaction remain unknown. In this study the binding of *S*- and *R*- citalopram to a homology model of the human serotonin transporter are extensively examined via Induced Fit Docking (IFD), QM-polarized Ligand Docking (QPLD), and GRID calculations. This resulted initially in two binding models for the *R*-enantiomer and two models for the *S*-enantiomer; however, we were able to propose one for each enantiomer through computational methods. These proposed binding modes were validated with biochemical experiments using a large battery of twelve different human serotonin transporter mutant proteins. These mutants in combination with six different optically pure citalopram analogs were utilized in a Paired Mutant Ligand Analogue Complementation study. By this strategy important protein-ligand interaction points could be traced hereby validating a binding model for each enantiomer by itself. This provided a detailed picture of how the high-affinity serotonin selective reuptake inhibitor, *S*-citalopram, and its low-affinity enantiomer occupy the binding site of human serotonin transporter. The results show that the two enantiomers have contrasting orientations of the *para*-fluorophenyl and 1,3-dihydroisobenzofuran-5-carbonitrile groups of citalopram. In the validated model for *S*-citalopram the fluoro-group is located in close proximity to the pocket lined by Ala173 and Thr439 and the cyano-group is found close to Phe341 and *vice-versa* for the *R*-enantiomer

2214-Plat

T Cell Receptor Cross-Reactivity Directed by Antigen Dependent Tuning of Peptide-MHC Molecular Flexibility

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T cell receptor recognition (TCR) of an antigenic peptide presented by a major histocompatibility complex protein (MHC) is required for a cellular immune response. TCRs are intrinsically cross-reactive, capable of recognizing multiple peptide/MHC complexes. TCR cross-reactivity is necessary for the normal functioning of the immune system, but has also been implicated in numerous pathologies. Multiple mechanisms have been postulated for TCR cross-reactivity, including molecular mimicry, where ligands share crucial chemical and structural features, and conformational adaptability, where flexibility allows receptors to 'adapt' to different ligands.

The human TCR A6 recognizes the Tax peptide derived from HTLV-I when presented by the class I MHC HLA-A2. However, A6 also recognizes the Tel1p peptide from S. cerevisia. To investigate how A6 cross-reacts between these two ligands, we determined the structures of the A6-Tel1p/HLA-A2 ternary complex, the unligated A6 TCR, and the unligated Tel1p/HLA-A2 complex. The structures revealed that cross-reactivity occurs via conformational rearrangements on both sides of the interface, including the receptor, the peptide,

and the HLA-A2 peptide binding domain. Although the conformational changes are consistent with a classical induced fit mechanism, further investigations using fluorescence anisotropy, proline mutagenesis, and molecular simulations revealed that the conformational changes were triggered instead by the presence of peptide-specific conformational dynamics in the HLA-A2 protein as well as flexibility in the TCR CDR loops. Overall, the results indicate that A6 recognition of the Tel1p peptide requires the mutual adaptation of two flexible molecules, with the degree of flexibility in the peptide/MHC complex dependent on the nature of the presented peptide. These findings have implications for the nature of TCR binding and cross-reactivity and shed new light on how structural diversity can be presented to and accommodated by receptors of the immune system.

2215-Plat

Mechanical Unbinding of Leukocyte Function-Associated Antigen-1 with ICAM-1 and ICAM-3 Complexes involves a Single Energetic Barrier

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Integrin belongs to a family of proteins that play crucial roles in both cell adhesion and signal transduction. Integrins on leukocytes (leukocyte function-associated antigen-1 or LFA-1) bind to intercellular adhesion molecules (ICAMs) to facilitate the adhesion and the migration of the cell to an inflammatory site. Recently, Moy et al. probed the unbinding of LFA-1 with ICAM-1 and ICAM-2 using AFM at the single-molecule level. They observed two separate regimes where the most probable unbinding force depended linearly on the logarithm of the loading rate and interpreted the two-regime behavior as the crossing of two free energy barriers. In the present work, we used coarse-grained Brownian Dynamics simulation to study the mechanical unbinding of LFA-1 from ICAM-1 and ICAM-3. We observed that the force-loading rate curves also displayed the fast and slow loading regimes, and the extracted kinetic parameters according to the Bell and Evans models were in quantitative agreement with those extracted from the experimental data. Moreover, employing the force-clamp mode, we found only a single energetic barrier and the two regimes resulted from an abrupt change in the transition state position. We expect similar results for the LFA-1/ICAM-2 complex, whose PDB structure is thought to be similar but not yet available.

2216-Plat

Predicting the Interactions between PDZ Adapter Domains and Disordered Peptides

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PDZ domains, one of the most ubiquitous and important scaffolding modules in human proteins, bind the disordered C-terminus of plasma membranes, mediating protein-protein interactions. Experiments have demonstrated that dissimilar C-terminal peptides bind to the same PDZ domain and different PDZs can bind the same peptides. Crystallographic studies revealed that binding to the PDZ domains requires a four residue long strand anchored by a C-terminal hydrophobic residue. Based on this information, we developed a novel semi-flexible docking method to model the peptide-PDZ complex structure and estimate its absolute affinity. The method has been tested on a set of 126 15-residue long natural peptides binding to PDZ3 of PSD95. The resulting sensitivity and specificity rates were 90.91%/79.13% by defining a kinetic and a thermodynamic threshold. Moreover, complex structures of 5 different peptides bound to PDZ domains were successfully recovered as the top ranked predicted models. This general structure-based technology is the first de novo approach to dock disordered peptides, providing a needed complementarity to proteomic assays to mine GeneBank for new targets of scaffold proteins and to predict novel protein-protein interactions. Our findings also reveal that the four-residues C-terminal recognition motif leads to only a weak non-specific binding intermediate complex, while an extended network of contacts established by the next three to five unconstrained residues determines the high specificity of the complex.

2217-Plat

Statistics and Physical Origins of pK and Ionization State Changes Upon Protein-Ligand Binding

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We investigate statistical prevalence and overall physical origins of changes in charge states of receptor proteins upon ligand binding. These changes are explored as a function of ligand type (small molecule, protein, and nucleic acid), and distance from the binding region. Standard continuum solvent methodology is used to compute pK changes upon binding for a total of 5899 ionizable residues in 20 protein-protein, 20 protein-small molecule, and 20 protein-nucleic acid high resolution complexes. The size of the dataset combined with an extensive error and sensitivity analysis allows us to make statistically justified conclusions: in 60% of all protein-small molecule, 90% of all protein-protein, and 85% of all protein-nucleic acid complexes there exists at least one ionizable residue that changes its charge state upon binding at physiological conditions (pH=6.5). Considering the most biologically relevant pH range of 4 to 8, the number of ionizable residues that experience substantial pK changes (> 1.0) due to ligand binding is appreciable: on average, 6% of all ionizable residues in protein-small molecule, 9% in protein-protein, and 12% in protein-nucleic acid complexes experience a substantial pK change upon ligand binding. Most of the change occurs in the immediate binding interface region, where about one out of five ionizable residues experiences substantial pK change regardless of the ligand type. However, the physical origins of the change differ between the types: in protein-nucleic acid complexes, the pK values of interface residues are predominantly affected by electrostatic effects, whereas in protein-protein and protein-small molecule complexes structural changes due to the induced-fit effect play an equally important role. In protein-protein and protein-nucleic acid complexes, there are a statistically significant number of substantial pK perturbations, due to the induced-fit structural changes, in regions far from the binding interface.

Platform AN: Regulatory Networks & Systems Biology

2218-Plat

Probing Multicellular Dynamics in *xenopus Laevis* Embryonic Development through Microfluidic Feedback Control

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Long-term spatiotemporal regulation of chemical environments in and around cells or tissues is critical to understand developmental signaling where dynamic responses to chemical factors control the subsequent coordinated events in development. Although progress has been made in the manipulation of single cell environments, both long-term and high-speed regulation of multicellular stimulation in developmental organisms is still challenging. We have developed a novel microfluidic feedback control system that allows long-term and high-speed manipulation of a laminar flow interface in a microfluidic channel for probing developmental systems. Our approach enabled long-term spatiotemporal manipulation of chemical environments of Animal Cap (AC) explants during the gastrulation stage in *Xenopus laevis* embryonic development. We present time and frequency responses of AC explants to periodic stimulation of steroid hormone dexamethasone (DEX) by tracking a hormone-activated nuclear-localizing green fluorescent protein tagged glucocorticoid receptor (GR) that can report the localized activity of DEX in the explants. We examine the sensitivity of GR-complex translocation to DEX concentration and frequency of stimulation. Concentration and frequency are critical factors when analyzing multicellular developmental systems such as *Xenopus*. We believe that our approach will be useful in diverse areas including biophysics, embryonic development, and engineering spatiotemporally integrated biological responses.

2219-Plat

Cells Respond Digitally to Variation in Signal Intensity via Stochastic Activation of NF-κB

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Cells detect and process spatiotemporal signals and activate gene regulatory pathways in response. Here we use high-throughput microfluidic cell culture, quantitative gene expression analysis and mathematical modeling- to investigate how mammalian cells detect external concentrations of the signaling molecule TNF-α and relay information to the gene expression programs via the transcription factor NF-κB. We measured NF-κB activity in thousands of fluorescently labeled live cells with single-cell resolution with a temporal resolution of 6 minutes and for durations up to 12 hours under TNF-α concentrations covering 4 orders of magnitude. TNF-α induced mRNA levels of 23 genes were measured and quantified at the same concentration range and duration, linking the transcription factor dynamics to the gene expression. A stochastic model was developed that reproduces the single-cell dynamics and gene expression profiles at all measured conditions, constituting a broadly applicable model for TNF-α induced NF-κB signaling. We find, in contrast to population studies, that the activation is a discrete process at the single cell level with fewer cells responding at lower doses. Nevertheless, the activated cells respond robustly