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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,