by Ab40. Thus our results define a novel '*allosteric switch*' mechanism as the basis of protease inhibition by an allosteric antibody.

189-Pos
Allosteric Mechanism in Neurophysin
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Neurophysins are small two- domain proteins that exhibit ligand-facilitated dimerization and its reciprocal-stronger binding by dimer than by monomer. We have previously reported evidence of conformational differences between unliganded monomer and dimer, but had not established their identity, their origin, or how they affect binding affinity.
In the present study we have determined the relative structures of a neurophysin monomer and its dimer by NMR spectroscopy. The structures show that a number of neurophysin residues that interact with the essential tyrosine of the ligand peptide move from locations in the unliganded monomer that are unfavorable for binding to positions in the unliganded dimer that more closely resemble those of the liganded state, thereby contributing to the stronger binding by dimer. We have also identified the origin of the dimerization-induced conformational change; i.e., the same residues that form the principal inter-domain bonds in the monomer via side-chain interactions form the principal inter-subunit bonds in the dimer via backbone hydrogen bonding. Steric crowding upon dimerization leads to a modification of inter-domain interactions, a resultant change in the relative orientation of the two domains and associated changes in intra-domain interactions. The principal residues responsible for these interactions are the Cys residues of the 28-34 disulfide bridge (amino domain) and 74-79 disulfide bridge (carboxyl domain). Strong inter-domain van der Waals interactions between sulfur atoms appear to play important roles in determining the relative domain orientation.
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190-Pos
Lactose Binding To Galectin-1 Occurs With Negative Cooperativity and Attenuates Internal Motions Throughout the Protein
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Galectins are a sub-family of lectins with a conserved carbohydrate recognition domain (CRD) that interacts with beta-galactosides. By binding cell surface glycoconjugates, galectin-1 is involved in cell adhesion and migration processes and is an important regulator of tumor angiogenesis. Here, we used heteronuclear NMR spectroscopy and molecular modeling to investigate lactose binding to galectin-1 and to derive solution NMR structures of galectin-1 in the lactose bound and unbound states. Structure analysis shows that the beta-strands and loops around the lactose binding site which are more open and dynamic in the unbound state, fold in around the bound lactose molecule dampening internal motions at that site. Analysis of titration binding data reveal that lactose binds the two CRDs of the galectin-1 dimer with negative cooperativity. Molecular dynamics simulations provide insight into structural dynamics of the half-loaded lactose state and, together with NMR data, suggest that lactose binding at one site transmits a signal through the beta-sandwich and loops to the second binding site. Overall, our results provide new insight into galectin-1 structure-function relationships and to protein-carbohydrate interactions in general.

191-Pos
Conformational Plasticity in IL-2 Is Captured Via in Silico Fragment-Based Drug Design Using SILCS Fragmaps
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The IL-2 protein is an experimentally well-characterized example of conformational plasticity in a protein-protein binding interface. Whereas the original crystal structure of the IL-2 protein lacked a particular binding pocket at the interface, subsequent structures of the protein complexed with small-molecule inhibitors have shown the creation of a hydrophobic pocket in order to accommodate inhibitor binding poses. A new in silico fragment-based drug design methodology (SILCS FragMaps) was applied to the original IL-2 crystal structure and has been found to recapitulate this pocket observed in the complexes. Furthermore, the pocket is seen to have an affinity for hydrophobic fragments, consistent with experimental inhibitor binding poses. As the SILCS FragMaps methodology is based on explicit-water all-atom molecular dynamics simulations, the methodology is able to capture both the conformational plasticity in the IL-2 surface required for pocket formation as well as the selective partitioning of small hydrophobic fragments into the pocket from bulk solution.

192-Pos
Different Binding Modes of Compounds Affecting CETP Activity: Dalcetrapib and Torcetrapib
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Cholesteryl ester transfer protein (CETP) is a hydrophobic glycoprotein in plasma and facilitates the exchange of neutral lipids between various lipoproteins resulting in a net transport of cholesteryl ester from HDL to LDL. Decrease in CETP activity leads to an increase of HDL cholesterol which potentially reduces cardiovascular risk.
Human recombinant CETP (rhCETP) was purified to homogeneity by hydrophobic interaction chromatography and size exclusion chromatography (SEC). The protein showed an extraordinarily high thermodynamic stability against thermal as well as denaturant-induced unfolding.
The effects of CETP on lipoprotein profiles were characterized after *in-vitro* incubation of plasma with rhCETP followed by SEC and reverse phase protein arrays. Monitoring the distribution of apolipoproteins and the cholesterol profiles confirmed the transfer of cholesteryl ester from HDL to LDL induced by CETP. In the presence of the potent CETP inhibitor torcetrapib (1 μM) added CETP is bound to lipoproteins while free CETP can still be detected in presence of dalcetrapib (3 μM), a compound from a different chemical class.
^{14}C-torcetrapib and ^{14}C-dalcetrapib were bound to CETP immobilized on sepharose and incubated with excess of unlabelled compounds. ^{14}C-dalcetrapib bound as Dalcetrapib-thiol could only be displaced in the presence of reducing agent by an excess of unlabeled dalcetrapib but not by torcetrapib. Dalcetrapib did not compete for binding of ^{14}C-torcetrapib.
Interaction studies with Surface Plasmon Resonance confirmed reversible covalent binding of dalcetrapib-thiol and different binding sites for dalcetrapib and torcetrapib. In agreement with literature cysteine 13 of CETP was identified as attachment point of dalcetrapib-thiol. All data suggested that torcetrapib and dalcetrapib bind to different sites on CETP which may be related to differences observed in their pharmacological profiles.

193-Pos
Cholesteryl Ester Transfer Protein Penetrates Lipoproteins For Cholesteryl Ester Transfer
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Human cholesteryl ester transfer protein (CETP), a hydrophobic glycoprotein (476 amino acids, ~74 kDa), mediates the transfer of cholesteryl ester (CE) from high-density lipoproteins (HDL) to apo-B containing lipoproteins (low-density lipoproteins - LDL; very low density lipoproteins - VLDL). The crystal structure of CETP reveals a banana-shaped protein consisting of N- and C-terminal β-barrel domains, a central β-sheet, and a ~60-Å-long hydrophobic cavity. Since CETP deficiency is associated with elevated 'good-cholesterol' (HDL-associated) levels via delayed catabolism, as a result, CETP has become a high interest pharmacological drug target.
Here, we investigated the mechanism of CETP-mediated neutral lipid transfer by electron microscopy (EM), image-processing, and molecular dynamic simulations (MDS). We discovered that: 1) CETP bridges HDL and LDL/VLDL to form a ternary complex; 2) the CETP C-terminal beta-barrel domain penetrates partially into the LDL/VLDL surface; 3) the N-terminal beta-barrel domain penetrates through the HDL surface and interacts with the CE core; and 4) MDS analysis suggested that the penetrating regions of CETP are highly mobile and form pores that connect to cavities running through the central axis of the CETP molecule to generate a tunnel. The tunnel was mainly hydrophobic and connected the distal ends through its central cavities. Thus, we proposed CETP resembles a Chinese finger trap, in which both beta-barrel domains penetrate the lipoprotein surface, resulting in pressure from both ends. The pressure may result in the anti-twisting of the two domains, leading to opening of the tunnel for CE transfer between HDL and LDL. The distal end of N-terminal beta-barrel domain could be particularly important for the design of pharmacological inhibitors, as blockage of the N-terminal beta-barrel domain would prevent CE transfer with a low probability of off target effects on normal HDL metabolism.