conformational changes involved in the centrin-Sfi1p$_{21}$ complex formation by FT-IR spectroscopy, two dimensional correlation spectroscopy and isothermal titration calorimetry. The binding was exothermic and the thermodynamic data for Hcen1-Sfi1p$_{21}$ was the following: N 1.33 ± 0.0165, Ka 1.59 x10^7 ± 2.48 x10^6 M, ΔH −1.72 x10^4 ± 301.1 kcal/mol and ΔS −23.8 kcal/mol. We have also established the relative stability of these proteins by differential scanning calorimetry. Our experiments address key questions underlying the molecular basis of this complex interaction.

3089-Pos Board B136

Unraveling Integrin Antagonists' Target-Recognition Mechanisms

Roy R. Hantgan[1], Samrat Dutta[2], Martin Guthold[2].
[1]Wake Forest University School of Medicine, Winston-Salem, NC, USA, [2]Wake Forest University, Winston-Salem, NC, USA.

Background: Pharmaceutical blockade of the platelet αIIbβ3 integrin receptor has reduced mortality from cardiovascular disease. However, gaps in mechanistic understanding limit clinical efficacy and delay new drug development.
Objectives: Integrating surface plasmon resonance (SPR) and dynamic force spectroscopy (DFS), we aim to measure the strength of integrin:ligand bonds, determining their weakest link and identifying new therapeutic intervention routes.
Methods: cHarGD, a cyclic peptide structurally similar to eptifibatide, a widely used antiplatelet drug, served as a model ligand, one readily coupled to biosensors and AFM tips. SPR provided kinetic, equilibrium, and transition state thermodynamic parameters for αIIbβ3:cHarGD complex formation, while DFS measured their mechanical stability. cHarGA, lacking the aspartate required αIIbβ3 binding, served as a negative control.
Results: SPR demonstrated that integrin binding to immobilized cHarGD was rapid (k_{on} ~ 7 × 10^3 L/mol-sec at 25 °C), readily reversible (k_{off} ~ 10^{-2} sec^{-1}), and specific (100-fold smaller signals with cHarGA). Eyring and van't Hoff analyses indicated that after overcoming an entropic barrier ($\Delta G_a^{o\ddagger}$ 12 kcal/mol), both enthalpy and entropy favored assembly of the αIIbβ3:cHarGD complex (ΔG° - 8 kcal/mol). Preliminary DFS experiments (12 nN/sec loading rate) indicated that the rupture force of cHarGD:αIIbβ3 is about 300 pN. In control experiments, where the tip was functionalized with cHarGA or albumin, lower rupture forces of 225 pN and 170 pN were observed.
Conclusions: Our SPR data indicate that entropy plays a major role in target recognition by integrin antagonists, a property shared by ~25% of drug:receptor interactions. Our DFS data suggest that integrin:ligand interactions are stabilized by multivalent contacts between clustered receptors and pharmaceutical inhibitors.This study will provide the first complete picture of the landscape for integrin:ligand interactions, using temperature and force as thermodynamic variables to determine the energetics and nm scale on which bond disruption occurs.

3090-Pos Board B137

Human Liver Fatty Acid Binding Protein: Solution Structure and Ligand Binding

Jun Cai[1], Christian Lücker[2], Zhongjing Chen[1], Elena Klimtchuk[1], Ye Qiao[1], James A. Hamilton[1].
[1]Boston University, Boston, MA, USA, [2]Max Planck Research Unit for Enzymology, Halle, Germany.

Liver Fatty Acid Binding Protein (L-FABP), a small (14 kDa) abundant cytosolic protein, may perform several functions in cells, including intracellular transport of fatty acids, nuclear signaling, and regulation of intracellular lipolysis. Among the members of the intracellular lipid binding protein (iLBP) family, L-FABP is very unique in its ability to bind two molecules of FA and a variety of other bulky ligands such as bilirubin. To help understand the promiscuous binding and transport properties of L-FABP, we have applied multi-dimensional homonuclear and heteronuclear NMR spectroscopy for studies of its structure and ligand binding. The overall conformation of human L-FABP, as determined from NOE-derived distance restraints, shows a β-clam motif comprised of a 10-stranded anti-parallel β-sheet that is covered by 2 short nearly parallel α-helices. Ligand binding to L-FABP is being studied by NMR titration experiments with two types of ligands. In the case of oleic acid, which is the primary physiological ligand of L-FABP, 2D HSQC spectra with different binding stoichiometries showed two binding sites with different affinities. In addition, two 13C-labeled bilirubin analogs are being studied to assess binding of bulky ligands. We hypothesize that the unique binding of bulky hydrophobic ligands enables the L-FABP to undergo a conformational change that is different from the other FABPs.

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A Novel Domain Implicated in the Interactions between pre-mRNA Splicing Factors

Ankit Gupta, Clara L. Kielkopf.
University of Rochester, Rochester, NY, USA.

Splicing Factor 1 (SF1) and U2 snRNP auxiliary factor (U2AF^{65}) form an essential protein complex that recognizes the 3′ splice site during the initial stages of pre-mRNA splicing. A ~100 amino acid domain of SF1 located between an N-terminal region that is necessary and sufficient U2AF^{65}-interactions, and a C-terminal RNA-binding domain. Despite high sequence conservation from yeast to mammals, the structure and function of this SF1 'mystery' domain is currently unknown. Here, we demonstrate that the SF1 'mystery' domain participates in the SF1 / U2AF^{65} interface by comparing heat capacity changes and chemical shift differences for U2AF^{65} association with deletion variants of SF1. Heat capacity changes for association of SF1 with the U2AF^{65} interacting domain (UHM) are significantly greater than those observed for association with a SF1 peptide composed of the minimal U2AF^{65}-interacting region. In contrast, the heat capacity changes for SF1 peptide/U2AF^{65}UHM association closely matched those predicted from the buried surface area of the complex. Given that heat capacity changes often correlate with the amount of surface area buried by complex formation, one possible explanation for this difference was that additional regions of SF1 participate in the U2AF^{65}UHM interface. To investigate this possibility, the HSQC spectra of ^{15}N-labeled U2AF^{65}-UHM in complex with SF1 C-terminal deletion variants were compared. Chemical shift differences imply that residues from conserved 'mystery' domain of SF1 participate in the U2AF^{65}UHM interface. The influence of this SF1 domain on affinity and cooperativity of pre-mRNA recognition by the SF1 / U2AF^{65} is further investigated by calorimetry and fluorescence anisotropy. These studies aid in elucidating the structural and thermodynamic means for 3′ splice site recognition by the essential SF1 and U2AF^{65} complex.

3092-Pos Board B139

A Molecular Approach to Ligand-Receptor Interaction

Matteo Ceccarelli[1,2], Francesca Collu[1], Paolo Ruggerone[1,2].
[1]University of Cagliari, Monserrato, Italy, [2]CNR-SLACS, Cagliari, Italy.

We have studied a human delta-opioid receptor interacting with two agonists, Clozapine and Desmethylclozapine. Delta-opioid receptors belong to the family of G protein-coupled receptors, that transduce an intracellular biological signal upon activation via interaction with a ligand in the transmembrane domain. Although Clozapine and Desmethylclozapine only differ by a methyl group, experimental data have evidenced a more efficient action of Desmethylclozapine in the treatment of refractory schizophrenia. A molecular analysis may help to clarify issues related this difference. Molecular Dynamics simulations help to elucidate the microscopic mechanism of the interactions between the ligand and the receptor identifying features barely seen in experiments. However, as in our case, the time scale of the processes of interest is often too long to be approached by standard MD techniques. Thus, for our study we have used a recent technique, the metadynamics, that accelerates MD runs extending simulation times. Our results pointed out different routes of the drugs inside the receptor: Clozapine touches a larger number of competing minima far from the putative receptor active zone than Desmethylclozapine. This latter spends most of its time inside the receptor close to the residues of the active zone, inducing noticeable structural modifications. Additionally, the simulation of the entrance has provided evidence of a stronger interaction with the receptor of Desmethylclozapine than Clozapine, resulting in a more frequent entrance of the former. Clozapine exhibits a preferential interaction with the membrane because of its enhanced hydrophobicity. The free energy surfaces extracted from the simulations have been used for kinetic Monte Carlo simulations to obtain reliable residence times of the drugs inside the receptor. The whole results helps to understand how microscopic details can remarkably affect efficiency and activity of compounds, supporting the idea of a bottom-up strategy in the drug design.

3093-Pos Board B140

Understanding the Mechanism of the Anti-angiogenic Activity of Suramin

Karuppanan M. Kathir, Khalil Ibrahim, Thallapuranam Krishnaswamy Suresh Kumar.
University of Arkansas, Fayetteville, AR, USA.

Angiogenesis is a cellular process that involves the sprouting of new blood vessels from pre-existing ones. Fibroblast growth factors (FGFs) play a crucial role in the regulation for angiogenesis and tumor metastases. Therefore, intensive research efforts are on to develop drugs that can specifically inhibit FGF-induced angiogenesis. FGFs exhibit their cell proliferation activity by binding to the extracellular D2 domain of their cell surface receptor. Suramin has been previously shown to inhibit FGF-induced tumors. In this context, in the present study, we investigate the interaction of suramin with the extracellular D2 domain of the FGF receptor (FGFR). Results of the isothermal titration calorimetry (ITC) experiments suggest that suramin binds to the D2 domain of FGFR with a reasonably high affinity (K_d ~ 10^{-6} M). ITC experiments, carried out at various salt concentrations, show that suramin-D2 domain interaction is mostly stabilized by ionic interactions. Limited trypsin digestion experiments and ANS binding experiments reveal conformational changes in the D2 domain