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

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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

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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.

3091-Pos Board B138

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

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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

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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

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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

induced by suramin binding. Equilibrium unfolding experiments monitored by fluorescence spectroscopy reveal that the D2 domain is significantly stabilized by suramin. ^{1}H-^{15}N chemical shift perturbation data shows that the suramin binding sites are mostly composed of residues located at the N- and C-terminal ends of the D2 domain and is supported by site-directed mutagenesis experiments. Interestingly, some of the residues that bind to suramin are located at the FGF-D2 domain interface. A structural model of the suramin D2 domain complex is generated from the experimental data. It appears that suramin inhibits cell proliferation activity of FGF by preventing its interaction with FGFR. The results of this study are expected to pave the way for a rational design of drugs against FGF-induced tumors.

3094-Pos Board B141

Rapid Discovery of Molecular Recognition Elements from Combinatorial Libraries of Peptoids

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Molecular Recognition Elements (MRE) are in the heart of bioassays for molecular diagnostics and biosensing. Antibodies are the gold standard of MRE for detection of proteins. Bioassays based on antibodies exhibit high specificity and affinity. However, natural antibodies are not reproducible and do not withstand temperature and other environmental factors. Synthetic MRE made of nonnatural sequence-specific heteropolymers is a valuable alternative to antibodies. Peptoid oligomers are of particular interest for creating synthetic MRE because of ease of synthesis and their chemical and biological stability. Several laboratories have shown a wide variety of potent biological activities of peptoids, including antibody-like molecular recognition functions. In this paper, we report on the development of novel method for the rapid discovery of synthetic MRE from one-bead-one-compound (OBOC) combinatorial libraries of peptoids. The approach employs total internal reflection fluorescence (TIRF) combined with electrochemistry and electric field control (TIRF-EC). Target protein is immobilized at the TIRF surface, and the OBOC library is injected into TIRF flow cell. TIRF allows for instantaneous detection of MRE-target interactions and real-time monitoring of their association and dissociation. EC allows for accelerating mass transfer of the beads and stimulating dissociation of bound beads for identification of the peptoids.

3095-Pos Board B142

Biophysical Characterization of FGF Signaling Complex

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Fibroblast growth factors are important heparin binding proteins involved in the regulation of key cellular processes such as angiogenesis, wound healing and differentiation. Heparin is believed to play a major role in the interaction of FGFs to their receptors, FGFRs. Most of the FGF binding sites are localized on the extracellular D2 domain of the receptor. In the present study, we characterize the structure of the minimalistic FGF-D2 domain interface using a variety of biophysical techniques, including multidimensional NMR spectroscopy and X-ray crystallography. Using sucrose octasulfate, a structural analogue of heparin, we examine the role of heparin in the formation of the FGF-receptor complex. Results of the isothermal titration calorimetry experiments indicate that the human acidic FGF-1 binds to the D2 domain with high affinity both in the presence ($K_{d(appa)} \sim 10^{-7}$ M) and absence of SOS ($K_{d(appa)} \sim 10^{-8}$ M). Far-UV CD and pulse proteolysis experiments, thermal denaturation experiments monitored by far-UV CD reveal that both FGF-1 and the D2 domain undergo subtle conformational changes upon binding and also reveal that SOS stabilizes a preformed 1:1 FGF-D2 domain binary complex. The X-ray structure of a minimalistic fibroblast factor signaling complex, consisting of D2 domain and FGF-1 and sucrose octasulfate (SOS) forms a 2:2:2 symmetrical ternary assemblage. Using ^{1}H-^{15}N chemical shift perturbation data, the SOS and the FGF binding sites on the D2 domain have been successfully mapped. NMR spectroscopy data is more consistent with the minimalistic ternary complex than the other crystallographic models of the FGF signaling complexes. Results of this study clearly suggest that the primary role of heparin in the FGF signaling process is merely limited to conferring stability to the FGF-receptor complex. Results obtained herein appear to challenge the existing view of the structural events leading to FGF-induced cell proliferation.

3096-Pos Board B143

Designing Fibroblast Growth Factor with Higher Heparin Binding Affinity

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Fibroblast growth factors (FGFs) play significant roles in the regulation of cell proliferation, angiogenesis, differentiation, tumor formation, embryonic growth, invasion, inflammation, and tissue repair. FGFs are also able to improve wound healing caused by metabolic diseases such as obesity, diabetes, infection, chronic liver failure, malnutrition, and second-degree burns. Studies have indicated that FGFs produce biological responses by binding to two types of receptors on the cell surface. The first class is a high-affinity family of transmembrane tyrosine kinase receptors called FGFRs. The second class of receptors is the family of heparan sulfate proteoglycans (HSPGs), which have a low affinity for FGFs. The macromolecular interactions of the growth factors, HSPGs, and FGFRs that lead to signal transduction are key to signaling by this important class of molecules. Proteoglycans, such as heparin, are required for the cell proliferation activities of FGFs. Additionally heparin helps to increase the affinity and half-life of the FGF-FGFR complex, which is crucial for signal transduction. The three-dimensional structure of the FGF-heparin complex show that the sulfate groups in the proteoglycans contribute significantly to binding. Residues involved in heparin binding correspond to amino acid 126 to 142 of the human FGF-1 sequence. In this context, we examined the role of additional lysine residues in the putative heparin-binding region of wild-type FGF-1, as introduced by single and double -site mutagenesis on binding, stability, and structure using various biophysical techniques including multi-dimensional NMR spectroscopy. The results clearly indicate that the introduction of lysine residues in three different positions in the heparin-binding pocket significantly increases binding to sucrose octasulfate (SOS, a heparin analog) and conformational stability. In addition, results of this study provide a valuable basis for novel therapeutics targeting this interaction.

3097-Pos Board B144

Quantitative Analysis of Water Dynamics in and near Proteins

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Proteins exist in aqueous solution. Hydration can be viewed as a description of how the protein disturbs the structure and dynamics of water. Water molecules in the vicinity of proteins are generally seen as either external or internal water molecules. Internal water molecules occupy cavities, exchange on a time-scale of 0.1–10 microseconds with bulk water, are almost as conserved as amino acids, and are therefore likely to be important for function. External water molecules tend to be found in protein crevices and are typically not conserved, even between crystal structures of the same protein. We introduce a method to analyze the behavior of water molecules in molecular dynamics (MD) simulations in terms of graphs. The graph encodes a simple hopping model: Nodes in the graph correspond to hydration sites, typically defined from the density in computer simulations or observed water sites in crystal structures. Directed edges correspond to transitions (''hops'') between sites, with transition rates computed from MD simulations. We apply this analysis to the water-filled cavity of intestinal fatty acid binding protein (I-FABP) in its apo and holo (palmitate-bound) state. This demonstrates how ligand binding influences the well-defined set of hydration sites in and around the protein's cavity. The ligand displaces a number of hydration sites but does not affect others close by. The parameters extracted from the network model allow us to model the movement of water molecules with a Markov Chain Monte Carlo model. The graphical construct reproduces the average site occupancy found in the MD simulations and the fluctuations of the occupancy.

This approach suggests new types of sampling and analysis that can be applied to extend the range of molecular dynamics models and the role of water in ligand binding.

Physical Chemistry of Protein & Nucleic Acids

3098-Pos Board B145

Polarizable Force Fields for Protein Simulations

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Computer simulations have become a widely used tool in biophysical and biochemical research. Fixed-charges empirical force fields have the advantage of computational speed, but explicit treatment of electrostatic polarization as a way to represent many-body interactions is often necessary if accurate energetic results are desired. Examples of such results include protein-ligand binding energies and acidity constants. We have demonstrated that using a polarizable force field permits achieving a ca. 0.6 pH units accuracy in calculating protein pKa values and qualitatively successful predictions of protein-ligand complex stabilities which are predicted as unstable by fixed-charges force fields. Moreover, we are developing a fast version of a complete polarizable force field for proteins, which is expected to speed up these accurate calculations by about an order of magnitude. Furthermore, the development of our