simulation which is the most time demanding step. In this work we demonstrated a possibility to reduce time of conformational sampling using crystal environment simulation. Amber10 program and FF99SB/GLYCAM06 force fields combination were employed for MD simulations and free energy calculations. Six 3, 4 and 3,4-deoxy dimannose analogs were studied as ligands of the m4-P51G-CVN mutant of the potent anti-HIV carbohydrate binding agent Cyanovirin-N (1). The use of crystal over solution simulations results in at least 8 times faster generation of the equivalent length trajectories. Binding free energy ΔA estimated from crystal NVT simulation trajectories shows 0.93 and 0.94 correlation with ΔG from solution NPT simulations for MM/PBSA and MM/GBSA approaches, respectively. We also evaluated performance of the relatively new GLYCAM06 carbohydrate force field and found reasonable agreement between calculated ΔG and experimental value. Results of this study further support our earlier hypothesis about importance (for CVN specificity) of the eight-component H-bond interactions of dimannose and protein main chain atoms that also was recently observed in experiment. [1] Vorontsov and Miyashita (2009) Biophys. J., 97.

**210-Pos**

**Computational Prediction and Experimental Validation of a Novel Binding Site for Platelet Integrin AlphaIIb-Beta3**

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Fibrinogen mimetic drugs and a γ-chain peptide bind to both the αIIb and β3 subunits of integrin αIIbβ3, providing the final coordination site for the metal ion-dependent adhesion site (MIDAS) metal ion within the β3 I domain, and inducing a less compact conformation of the protein. We investigated the binding of a novel human-selective αIIbβ3 small molecular inhibitor (RUC-1) that we recently identified by high throughput screening, and predicted to bind preferentially to a pocket within the αIIb β-propeller domain by flexible ligand/rigid protein molecular docking studies. We first carried out both standard and enhanced molecular dynamics (MD) simulations of the proposed RUC-1-bound form of human αIIbβ3 integrin. The results of these studies pointed to an energetically preferred conformation of RUC-1 into the αIIb β-propeller domain that had no contact with the MIDAS metal ion or other sites in the β3 I domain. This binding mode of RUC-1 appeared to be stabilized by interactions with specific human αIIb residues, such as D224 (already known to contribute to αIIbβ3 binding), Y190 (F in both mouse and rat), and (through two water molecules) D232 (H in rat). Well-tempered metadynamics simulations of Y190F and D232H mutants supported the contribution of normal residues to the stabilization of RUC-1 in a specific binding mode and location. Functional experiments on recombinant cell lines expressing Y190FαIIbβ3 or D232HαIIbβ3 validated this hypothesis by showing a ~80-95% reduction in RUC-1 affinity. X-ray crystallography confirmed the RUC-1 binding pose suggested by MD simulations, while gel filtration and dynamic light scattering experiments showed that RUC-1 favored a compact αIIbβ3 conformation, in sharp contrast to the effect of fibrinogen-mimetic drugs.

**211-Pos**

**Membrane Binding and Lipid Extraction Studies of Gm2 Activator Protein (GM2AP)**

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GM2AP is an accessory protein that solubilizes the ganglioside GM2 from intralysosomal vesicles for hydrolytic cleavage by HexA to form GM3. The precise molecular interactions and method of extraction of GM2 from lipid vesicles are unknown. GM2AP also functions as a lipid transfer protein. This non-enzymatic protein contains three tryptophan residues (W5, W63, W131) with two of these (W63, W131) located in putative membrane binding loops. In order to investigate the possible role of the tryptophan (TRP) residues in membrane binding and lipid extraction, gel filtration and resorcinol absorption assays were used to investigate the extraction efficiency of GM2 by GM2AP in a series of TRP to ALA substituted constructs. GM2AP is shown to have two distinct substrate binding modes, one for the GM2 ganglioside and another for phospholipids. Fluorescence experiments were used to determine the orientation of dansyl-DHPE in the binding pocket of GM2AP. Quenching results suggest that dansyl-DHPE is oriented such that the head group of the lipid is located in the hydrophobic pocket of the protein, consistent with the binding mode of other phospholipids which were previously studied. Dansyl-labeled lipids were used to monitor the changes in the rates of lipid extraction and transfer by GM2AP from liposomes as a function of both pH and the TRP to ALA substituted constructs. The ability of GM2AP to bind and/or extract dansyl-labeled lipids from liposomes was affected with increased pH of the lipid environment. Additionally, removal of TRP from the putative membrane binding loops resulted in slower lipid extraction rates, suggesting that these residues are relevant for membrane binding and/or extraction of GM2AP.

**212-Pos**

**Extracellular pH and Regulation of Integrin-Ligand Interactions**

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In the tumor and wound microenvironments, the extracellular matrix often exhibits acidic extracellular pH. It is well known that acidic pH can strongly affect processes such as cell adhesion and migration. However, the molecular mechanisms governing these effects have not been established. Here, we consider the hypothesis that acidic extracellular pH directly alters the interactions between cell surface integrin receptors and ECM ligands, which are critical to cell adhesion and migration. We employed Multi-Conformation Continuum Electrostatics to predict amino acid pKa values in the integrin avb3 headpiece, and conducted molecular dynamics simulations at acidic and physiological pH to examine the effect of pH on integrin conformational states. Our results suggest that acidic pH promotes opening of the avb3 headpiece, an important step in activation that can enable more effective ligand-receptor association interactions. This has important implications for downstream cell processes in the cancer and wound environments. We also conducted molecular-level experimental approaches, including flow cytometry and atomic force microscope-enabled force spectroscopy, to further examine the role of pH in regulating integrin-ligand interactions. These molecular-level results are connected to cell-level measurements of adhesion and migration at different pH levels, providing a detailed, multi-scale understanding of how acidic extracellular pH affects cell behavior.

**213-Pos**

**Effect of Molecular Sway on the Recognition of Peptide/MHC Complex by T Cells**

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CD4+ T cell responses require the recognition of specific peptide-MHC complexes displayed by APC. It is important to determine how antigen presentations affect the ensuing T cell response. Immunizations of B10.BR mice with naturally processed peptide 48-61 of Hen egg lysozyme elicit two different types of T cell responses. First type of T cell (type A termed by Unanue et. al) respond to APC pulsed with either peptide or whole HEL protein. Second type of T cell (termed type B) respond to APC incubated with peptide but showed no response to APC pulsed with whole protein. Reactivity of the type A T cell clones correlated well with the affinity of the peptide to the MHC molecules. However, some type B T cell clone exhibit better response to the low affinity truncated (52-61) peptide than to high affinity peptide (48-61). Since weak MHC binding peptides form unstable complex, we hypothesize that type B T cells respond to the transitional conformations generated by unstable peptide/MHC complex. To test this hypothesis, we analyzed the movement of peptide/MHC complex at the single molecular level by using diffracted X-ray tracking (DXT) method. It was found that movement of the low affinity peptide/MHC complexes was different from that of high affinity peptide/MHC complexes. Moreover, comparison of the movement of a series of low affinity peptide/MHC complexes revealed clear correlation between magnitude of twisting movement of peptide and T cell recognition. Thus, our results clearly demonstrated that movement of peptide in MHC strongly affects to T cell recognition and some but not all T cells recognize a transitional conformation generated by weak binding peptides.

**214-Pos**

**A Force Spectroscopy-Based Protein-Ligand Interaction Assay**

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Binding of small molecules are crucial to the function and folding of many protein machineries inside cell. Thus it is of fundamental importance to measure the binding affinity of small molecule ligands to proteins and reveal the binding mechanism. Here we report a force spectroscopy based single-molecule binding assay that is capable of determining the binding affinity as well as the binding mechanism of ligands to proteins at the single-molecule level. This assay is based on the difference in the mechanical stability of the given protein upon ligand binding. As a proof-of-principle, we use the binding of metal ions, Ni2+, to an engineered metal binding protein, G6-53, as a model system to establish this method. The apo-G6-53 and Ni2+-bound G6-53 exhibit distinct mechanical stability: apo-G6-53 unfolds at around 120 pN while Ni2+-bound

G6-53 unfolds at ~250 pN. Using their characteristic unfolding forces as a reporter, we were able to directly quantify the partitioning of G6-53 between the apo and Ni2+ bound states at different Ni2+ concentration and measure the binding affinity of Ni2+ to G6-53. The distinct unfolding forces of apo and holo forms of G6-53 also allow us to discriminate different species in the process of folding and Ni2+ binding and measure their kinetic evolution. We unfolded G6-53 by force and waited to allow it to fold and bind with Ni2+. We found that the unfolded G6-53 folds to apo form before incorporating Ni2+. The folding rate of G6-53 is independent of Ni2+ concentration, while the binding rate of Ni2+ to apo form of G6-53 is directly proportional to the Ni2+ concentration. Our kinetic data can be fully described using a ''folding before binding'' model. We anticipate that this novel assay will find unique applications in the study of various protein-ligand interactions.

**215-Pos**

**Binding of Antimicrobial Lactoferricin Peptides to Targets in the Angiogenesis Pathway**

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Peptides derived from lactoferricin B (LfB25; F*KC*<u>*RRWQWR*</u>*MKK*LGAPSITCVRRAF; +8), a 25-residue cationic innate immunity peptide released from bovine lactoferrin, exhibit broad spectrum antimicrobial and anti-angiogenic properties. An increase in drug-resistant bacteria and the role of angiogenesis in promoting tumor growth make LfB peptides attractive candidates for future drug development. An important principle for the design of peptide drugs is to reduce the number of amino acids and the sequence complexity, while maintaining maximal activity and minimal toxicity. LfB25 is proposed to inhibit angiogenesis, the formation of new blood vessels, by competing with fibroblast growth factor (FGF) for binding to negatively charged heparin sulfate proteoglycans on endothelial cell surfaces. Previously we used isothermal titration calorimetry (ITC) to characterize the binding of LfB25 and LfB6 (RRWQWR-$NH_2$; +4; underlined above), the 'antimicrobial core' of LfB25, to heparin and sucrose octasulfate (SOS), a heparin analogue. The binding of LfB25 with SOS and heparin was found to fit a two site model, with Kd values on the order of $10^{-6}$ and $10^{-7}$; whereas the isotherms for LfB6 fit a single binding site model, with Kd values on the order of $10^{-6}$.

We now report ITC binding assays for two new LfB peptides, LfB25-$Ala^{3,20}$ and LfB11-$Ala^{2,9}$ (K<u>A</u>RRWQWR<u>A</u>KK-$NH_2$; +7;sequence *italicized* above). To remove the disulfide bond, the two cysteines were replaced to give LfB25-$Ala^{3,20}$. To reduce sequence length and complexity, in LfB11-$Ala^{2,9}$ a cysteine and a methionine were changed to alanine, and the sequence was reduced to 11 residues. The binding of both LfB25-$Ala^{3,20}$ and LfB11-$Ala^{2,9}$ to SOS and heparin fit a single-site model, with Kd values on the order of $10^{-6}$, similar to those for LfB6. Results from antimicrobial and hemolytic assays will be presented.

## Physical Chemistry of Proteins & Nucleic Acids

**216-Pos**

**Salt-Dependence of DNA-Protein Binding: A Study of Four DNA-Binding Families**

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Long-range salt-mediated electrostatic interactions are crucial for DNA-protein complex formation and stability. The DNA backbone has a strong anionic character, while the DNA-binding proteins here studied display a large positive surface potential patch due to positively charged amino acids facing the DNA-binding site. A linear relationship between ln(Kobs) and ln[M+], where [M+] is the 1:1 salt concentration, is often interpreted as an indication of electrostatic effects and it is named SKobs. This parameter is usually equated to the number of ion pairs found in the complex. We determined the electrostatic binding free energy as a function of 1:1 salt concentration with the non-Linear Poisson-Boltzmann (NLPB) equation to predict SKobs. We investigated four families of DNA-binding proteins: (i) Homeodomains, (ii) High Mobility Group (HMG)-Box proteins, (iii) Interferon Regulatory Factors, and (iv) basic-region Leucine Zippers for which there is experimental binding data from the same laboratory. We correlated structural features to charge distribution, and determined surface accessibility of residues. We found a qualitative relationship between our NLPB predictions of SKobs and the experimental SKobs for homeodomains and for HMG proteins, but not for families in which protein and DNA suffer severe bend and conformational changes. This observation indicates SKobs is sensitive to conformational adaptability and thus this effects have to be accounted in order to improve NLPB predictions of SKobs. We did not find a relationship between SKobs and number of ion pairs, but we found that SKobs is better correlated with the Coulombic interaction energies between molecules of the complex.

**217-Pos**

**Sorption, Intercalation and Cooperativety: the Modes of Interaction of Actinomycin to DNA**

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The interaction of Actinomycin-D to DNA has been long investigated given it inhibits the synthesis of ribonucleic acid, inhibits the growth of cancer cells and induces apoptosis. So far, thermodynamic and structural studies have demonstrated that Actinomycin-D intercalates to DNA double helix preferentially to G-C pairs. There is also evidence that binding affinity is modulated by nearest base pairs flanking the intercalation site. However, the mechanism of Actinomycin-D interaction to DNA, and thus its energetic, is still ill understood. While some studies show evidence that ActD intercalation to natural DNA occurs via a mechanism consistent with a model of one independent and equivalent sites, other studies show evidence of the existence of two classes of independent binding sites; other yet show that the binding at low saturation is cooperative. In this work we measured the binding of Actinomycin-D to calf thymus DNA by optical titration and dialysis equilibrium under different solution conditions. Thus, we have found the conditions where the different kinds of binding reported in the literature can be reproduced. Through the analysis of the data correlating experimental design and solutions conditions, we were able to characterize the complexity of ActD interactions with DNA. In this work we show experimental evidences that intercalation of ActD at low drug/DNA ratio is cooperative; that the strong binding site is a consequence of cooperative binding; and that ActD not only intercalates to the DNA double helix but it also binds to the helix surface with a affinity which is in the same order of that measured upon intercalation.

**218-Pos**

**Urea Destabilization of DNA and RNA Double Helices: Preferential Interactions with Nucleobase Conjugated Pi-Pi-Systems**

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Thermal denaturation transition temperatures of AT (adenine-thymine)- and AU (adenine-uracil)-rich double helices decrease to a greater extent in aqueous urea solutions than GC (guanine-cytosine)-rich double helices. The work presented here seeks to identify the chemical functional groups urea preferentially interacts with to account for the greater destabilization of AT- and AU-rich double helices. Vapor pressure osmometry was used to determine the preferential interaction coefficients of urea with nucleoside 5′-monophosphates (5′-NMPs) to quantify the accumulation of urea near the 5′-NMP solvent accessible surface areas. Additionally, molecular dynamics (MD) simulations of the 5′-NMPs in explicit water and 1 molal urea predict urea preferential interactions above and below the nucleobase plane through pi-pi interactions. These MD simulation results are supported by the strong correlation between the fraction of accessible surface area devoted to the base conjugated pi-system and the preferential interaction coefficients determined from vapor pressure osmometry. Implications for urea destabilization of DNA and RNA double helices are discussed.

**219-Pos**

**The Effect of Site-Specific Modifications of DNA on Thermodynamic Stability, Ion Binding and Hydration**

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Cations, which associate with DNA in both the major and minor grooves, play a significant role in determining DNA conformation. In the major groove, cations are associated with the $N7/O^6$ edge of guanines, while in the minor groove they are found at A-T pairs. Both G-C and A-T have potential cation binding sites that when modified should result in the reorganization of salts and water, which in turn would affect local conformation and stability. We report herein the biophysical characterization of DNA duplexes in which we altered the N-7 position in the major groove of purines (7-deaza-guanine, 7-aminomethyl-7-deazaguanine, 7-hydroxymethyl-7-deazaguanine and 7-deaza-adenine) and at N-3 position of adenine in the minor groove (3-deazaadenine and 3-methyl-3-deazaadenine). These modifications alter the electronic properties of the heterocyclic bases and specifically eliminate DNA cation binding sites in the different grooves, or in the case of 7-aminomethyl-7-deazaguanine