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 N2+ bound states at different N2+ concentration and measure the binding affinity of N2+ 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 N2+ binding and measure their kinetic evolution. We unfolded G6-53 by force and waited to allow it to fold and bind with N2+. We found that the unfolded G6-53 folds to apo form before incorporating N2+. The folding rate of G6-53 is independent of N2+ concentration, while the binding rate of N2+ to apo form of G6-53 is directly proportional to the N2+ 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
Geri E. Burkett, Nicole McClellan, D. Rajalingam, Anna E. Daily, Thallapuranam Suresh Kumar, Denise V. Greathouse.
University of Arkansas, Fayetteville, AR, USA.
Peptides derived from lactoferricin B (LfB25; FRRWQWR-MKKKLGAP-SITCVYRRAF-38), 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 anticancer drugs is 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, Interkalation and Cooperativity: the Modes of Interaction of Actinomycin to DNA
Andre L. Galo, Marcio F. Colombo.
São Paulo State University, São José do Rio Preto - SP, Brazil.
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 cationic 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 interaction 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 magnitude measured upon intercalation.

218-Pos Urea Destabilization of DNA and RNA Double Helices: Preferential Interactions with Nucleobase Conjugated Pi-Pi-Systems
Jeffrey J. Schwinefus, Joe McDevitt.
St. Olaf College, Northfield, MN, USA.
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'-NMP) 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.

Physical Chemistry of Proteins & Nucleic Acids

216-Pos Salt-Dependence of DNA-Protein Binding: A Study of Four DNA-Binding Families
Cristina Russo, Erin Asbury, Meredith Wall, Marcia O. Fenley.
Florida State University, Tallahassee, FL, USA.
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[C0], 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, and we found that SKobs is better correlated with number of ion pairs, but we found that SKobs is better correlated with the Coulombic interaction energies between molecules of the complex.

219-Pos The Effect of Site-Specific Modifications of DNA on Thermodynamic Stability, Ion Binding and Hydration
Manjori Ganguly1, Ruowen Wang1, Feng Wang2, Michael P. Stone2, Luis A. Marky3, Barry I. Gold1.
1University of Pittsburgh, Pittsburgh, PA, USA, 2Vanderbilt University, Nashville, TN, USA, 3University of Nebraska Medical Center, Omaha, NE, USA.
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/O6 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 N7 position in the major groove of purines (7-deaza-guanine, 7-aminoethyl-7-deazaguanine, 7-hydroxyethyl-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-aminoethyl-7-deazaguanine