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

polarizable force fields id greatly facilitated by extensive use of quantum mechanical data for both fitting and testing purposes.

3099-Pos Board B146
Single Molecule Reaction Kinetics within Femtoliter Volume Containers
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We create and observe controlled single molecule chemical reactions within femtoliter containers called hydrosomes. Hydrosomes are stable aqueous nanodroplets suspended in a low index-of-refraction fluorocarbon medium. The index of refraction mismatch between the nanodroplets and fluorocarbon is such that individual hydrosomes can be optically trapped. Using optical tweezers, the hydrosomes are held within a confocal observation volume, and we interrogate the encapsulated molecule by means of fluorescence excitation. Hydrosome encapsulation has an important advantage over liposome encapsulation techniques in that hydrosomes fuse on contact, thereby mixing the encapsulated components. Optical tweezers are used to manipulate the hydrosomes and to induce a fusion event. Custom fabricated microfluidic channels are used to sort the hydrosomes containing different molecule species. We demonstrate the use of hydrosomes as microreactors by fusing two hydrosomes, each containing a complementary single strand of DNA, and observing the subsequent hybridization via FRET (Fluorescence Resonance Energy Transfer).

3100-Pos Board B147
Photoexcitation Spectrum of Protonated Tyrosine in Vacuum and Theoretical Interpretation
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Photodepletion spectrum of gaseous protonated tyrosine molecules was obtained at 150 K by UV laser spectroscopic technique in conjunction with mass spectrometry and interpreted by theoretical methods. The spectrum exhibits distinct three bands separated each other by about 800 . Four stable conformers of the molecular ion were determined by the quantum mechanical density functional theory and their electronic transition energies were obtained by a semi-empirical quantum chemistry calculation. The whole pattern of the spectrum was reproduced very well by a combination of theoretical methods, the second order cumulant expansion, ZINDO/S calculation, molecular dynamics simulation, and a semi-classical time-correlation function approach. The three spectral bands turned out to arise from the vibronic transition of two vibrational modes constituted by the ''benzene breathing'' mode and a torsional mode of the amino acid backbone. It is suggested that the major factor of the spectral broadening is not conformational disorder but the thermal fluctuation of the most stable conformer. Excellent agreement between the experimental and theoretical spectra exemplifies the validity of the theoretical methods applied for the present molecular system.

3101-Pos Board B148
Prediction of Hofmeister ion effects on biopolymer processes
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At moderate to high concentrations, salt ions exert a wide range of effects on protein folding and other protein processes, from extremely destabilizing (GuH^+, SCN^-) to very stabilizing (SO_4^{2-}). The Hofmeister series is a qualitative ranking of these effects, originally based on the effectiveness of salts as protein precipitants and subsequently observed for other processes including creating air-water surface and dissolving hydrocarbons in water. Recently surface spectroscopy, molecular dynamics simulations and molecular thermodynamic analysis of surface tension and model compound solubility data have all provided evidence that local accumulation or exclusion of individual salt ions, relative to bulk concentrations, is responsible for their Hofmeister effects. In particular, application of a novel two-domain salt ion partitioning model (SPM) to analyze effects of Hofmeister salts on the surface tension of water and on hydrocarbon and peptide solubility (Pegram & Record, J. Phys. Chem. B 112, 9428 (2008); 111, 5411 (2007)) provides a quantitative molecular thermodynamic description of the individual partitioning of salt cations and anions at uncharged interfaces, with predictive capability. This analysis shows that the Hofmeister rank order of ions arises from their interactions with nonpolar surface. Surface-bulk partition coefficients of ions obtained from hydrocarbon and amide model compound solubility data, together with a coarse-grained description of functional groups that make up molecular surfaces, allow the quantitative prediction of Hofmeister (noncoulombic) salt effects on micelle formation, protein folding, protein crystallization and DNA helix formation.
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3102-Pos Board B149
Hofmeister Effects on Beetle Antifreeze Protein Activity Enhancement
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Antifreeze proteins (AFPs) noncolligatively depress the nonequilibrium freezing point of a solution. A difference between the melting and freezing points is termed as thermal hysteresis (TH). Some low-molecular-mass solutes can affect the TH values of AFPs. The TH enhancement effects of series low-molecular-mass enhancers on an AFP from the beetle *Dendroides canadensis* (DAFP) have been extensively investigated. However, the mechanisms of the enhancement effects of the enhancers are still not well understood. The adsorption-inhibition mechanism is a generally accepted mechanism of AFPs. In this work, the effect of a series of neutral salts on salting out DAFP on ice is treated by a simple classical theory. In the presence of these anions, DAFPs are salted out on ice, which may lead a larger surface DAFP coverage than that in the absence of these anions. Therefore, the TH value of DAFP will enhance in the presence of a series of simple anions. The TH values of DAFP in the presence of the series of anions assessed by Differential Scanning Calorimetry (DSC) are in an excellent agreement with the theoretical results from the classical theory. Our results show that the Hofmeister effect may be one of the potential mechanisms for AFP enhancers. Besides Hofmeister ion interactions, the anion enhancers may also interact with DAFP/water/ice through other ways. Further discussions are given.

3103-Pos Board B150
Solvation in Solvent-Cosolvent Mixtures
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The volumetric properties of solutes, including their partial molar volume, compressibility, and expansibility, are known to be determined by and, therefore, sensitive to the entire spectrum of solute-solvent interactions. However, applications of volumetric measurements to study of solvation of solutes in binary solvents consisting of the principal solvent and a cosolvent are almost nonexistent. This deficiency reflects the lack of an adequate theoretical framework to rationalize the measured volumetric observables in terms of solute-principal solvent and solute-cosolvent interactions. To address this deficiency, we present in this work a simple statistical thermodynamic model describing solute thermodynamics in binary solvents. Based on the model, we derive relationships that link the partial molar volumetric properties of solutes with the extent and intensity of interactions of a solute with the principal solvent and the cosolvent. If the approximation of uniform solvation is introduced, the derived formalism can be simplified to a form that can be readily employed for practical treatment of experimental volumetric data on small solutes and/or individual, chemically homogeneous atomic groups. As an example, we use this simplified formalism to rationalize the volumetric properties of the zwitterionic amino acid glycine in concentrated urea solutions. In general, the theoretical development presented in this work opens the way for systematic applications of volumetric measurements to quantitative characterization of solute-solvent interactions in complex solvents.

3104-Pos Board B151
Effect of Ionic and Non-ionic Co-Solutes on the Activity of β-Galactosidase
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The hydrolysis of the lactose analog o-Nitrophenyl-beta-D-Galactopyranoside into o-Nitrophenol and beta-D-Galactose by the action of beta-Galatosidase is a well known enzymatic reaction. We have studied this reaction with varying concentrations of substrate in the presence of several ionic and non-ionic cosolutes considered chaotropes and kosmotropes, and examined their effects on the enzyme activity. In the presence of increasing concentrations of up to 1 M NaCl, beta-Galactosidase showed an activity that was barely affected by the salt concentration and was basically similar to that under stripped conditions. In contrast, in the presence of NaI, while at concentrations of 0.1 M NaI and below the effect on the enzyme activity was comparable to that of 0.1 M NaCl, in the presence of 1 M NaI the enzyme showed a marked deactivation. This fact suggested that the difference in effects was caused by the presence of iodide ions. On the other hand, the activity of this enzyme was also investigated in the presence of several polyols differing in the number of hydroxyl groups. The kosmotropes used were glycerol (3), erythritol (4), xylitol