

polarizable force fields is 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).

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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.

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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. This work is supported by NIH GM47022.

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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.

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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-Galactosidase 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

(5), and sorbitol (6), in which the number in parentheses indicate the number of hydroxyl groups present in each molecule. At concentrations of 7 M, except for sorbitol (6), all polyols showed various degrees of deactivation of the enzyme. It is interesting to note that this effect showed no correlation with the number of hydroxyl groups. The increasing order in deactivation was glycerol (3), xylitol (5), erythritol (4). Sorbitol (6) showed no impact on the activity of beta-Galactosidase at any concentration. These results will be discussed in terms of the effect of co-solutes on the structure of water as well as on the structure-function correlation of the enzyme.

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Molecular Dissection of an Allosteric Protein by Using Ionic and Non-Ionic Co-Solutes and Their Impact on the Protein Function

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We have studied the impact of co-solutes not considered allosteric effectors, both ionic and non-ionic, on the oxygenation function of human hemoglobin (Hb). In the presence of NaI, the oxygenation characteristics of Hb showed a complex behavior depending on the concentration of the halide salt. At concentrations of 0.1 M NaI or below, the oxygenation affinity for the first oxygen, K_1 , decreased as the concentration of the halide salt was increased. At concentrations between 0.1 M and 0.5 M NaI, the oxygenation affinity for the last oxygen, K_n , decreased slightly, while K_1 was unaffected. At concentrations between 0.5 M and 1 M NaI, K_1 values increased gradually with increasing concentrations of the salt, while K_n values remained practically constant. Compared to Hb under stripped solution conditions, the oxygenation curve for Hb in the presence of 2 M NaI showed a decreased affinity for oxygen and a reduced cooperativity, being its symmetric shape the most striking feature, i.e., the curve resembled that of an allosterically linked two-oxygen binding site derivative. Size exclusion chromatography revealed that Hb was mostly dimerized under the above-mentioned solution condition. The retention of cooperativity contrast greatly with the widely accepted knowledge that dimers: (1) do not show cooperativity, and (2) exhibit high oxygen affinity for oxygen. Oxygenation experiments carried out using non-ionic amphiphatic solutes of low molecular weight showed characteristics that were similar to those exhibited in the presence of NaI, especially in the presence of co-solutes that show moieties with high hydrophobicity. Since these features are not observed in the presence of NaCl, we conclude that the dimerization is caused by the iodide ion altering the characteristics of water.

3106-Pos Board B153

Characterizing pH Induced Conformational Changes in the ProSegment of Prorenin with Site-Directed Spin Labeling

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Renin is an aspartic protease enzyme that catalyzes the reaction of angiotensin I to the vasoactive angiotensin II, and plays an essential role in controlling hypertension and electrolyte balance. However, the inactive zymogen prorenin is found in higher concentrations in the body. Structurally, prorenin contains a 43 amino acid pro-segment, which can be enzymatically cleaved to form renin. The pro-segment also undergoes acid induced conformational changes that expose the active site, thus producing acid activated prorenin. Here, site-directed spin labeling (SDSL) is utilized to probe the acid induced conformational changes of the pro-segment. Specifically, a series of single cysteine (CYS) mutants have been generated for SDSL in regions identified as structurally important, i.e. the 'gate' and 'handle' regions. Structural and functional assays confirm proper folding and function of the spin labeled CYS mutant. Monitoring the motions of MTSL by the EPR spectrum suggests that under low pH levels the pro-segment undergoes a conformational change which exposes the active site of renin. This implies that the pro-segment of prorenin has enzymatic control.

3107-Pos Board B154

Dielectric Saturation of Water in a Protein Channel

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Water molecules in confined geometries like nanopores and biological ion channels exhibit structural and dynamical properties very different from those found in free solution. Protein channels that open aqueous pores through biological membranes provide a complex spatial and electrostatic environment that decreases the translational and rotational mobility of water molecules, thus altering the effective dielectric constant of the pore water. By using Booth

equation, we study the effect of the large electric field created by ionizable residues of an hour-glass shaped channel, the bacterial porin OmpF, on the pore water dielectric constant, ϵ_w . We find a space-dependent significant reduction (down to 20) of ϵ_w that may explain some *ad hoc* assumptions about the dielectric constant of the protein and the water pore made to reconcile model calculations with measurements of permeation properties and pKa's of protein residues. The electric potential calculations based on the OmpF protein atomic structure and Booth field-dependent dielectric constant show that protein dielectric constants ca. 10 yield good agreement with Molecular Dynamics simulations as well as permeation experiments

3108-Pos Board B155

Negative Cooperativity in a Protein Ion Channel Revealed by Current Noise, Conductance and Selectivity Experiments

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Cooperativity is a phenomenon of universal importance in biophysics and has been extensively reported in many systems including enzymes, protein receptors and protein ion channels, among others. The concept of positive cooperativity appeared in the study of oxygen uptake by hemoglobin to explain that binding of a molecule of oxygen makes it easier the subsequent binding of other molecules. In contrast, negative cooperativity is found when the presence of the first molecule makes the binding of the second molecule more difficult. In particular, we study here the pH titration of the OmpF channel through measurement of current noise amplitude, conductance and ion selectivity. The steep pH dependence found both in channel conductance and Reversal Potential, together with the wide peak found in current noise amplitude are analyzed in terms of the Hill formalism. In all cases, Hill coefficients lower than unity are found, suggesting a negative cooperative behavior. Although OmpF porin is a trimer, previous studies demonstrate that each monomer is identical and both structural and functionally independent. The origin of cooperativity in each monomeric is subtle and does not necessarily demand the existence of different binding domains or subunits. Finally, experiments performed at different electrolyte concentrations evidence that salt cations play a major role in the observed channel features.

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Interfacial Properties Of The Fluorescent Protein B-phycoerythrin Extracted From The Red Microalga *Rhodospirillum rubrum*

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Fluorescent proteins have been used as biomarkers long time ago. In this work we show the extraction, spectroscopic characterization and some interfacial properties of B-Phycoerythrin obtained from the red microalga *Rhodospirillum rubrum*. *Rhodospirillum rubrum* showed three types of phycobiliproteins: Phycoerythrin, phycocyanin and allophycocyanin. However the highest proportion was for B-Phycoerythrin. It is widely used as a fluorescent probe, analytical reagent, natural dye in foods and cosmetics, in the development of biosensors and also has been shown to have a therapeutic value due to their immunomodulating and anti-carcinogenic activities. The spectroscopic characterization was performed by UV, fluorescence and circular dichroism. The purified B-phycoerythrin showed a $A(545)/A(495)$ ratio of 4.8, peaks at 540, 562 nm with a 498 shoulder, a fluorescence emission maximum at 578 nm, and a secondary structure almost stable with pH changes. The surface properties of B Phycoerythrin were analysed with a Langmuir Balance. Different pH conditions in the subphase were used. The protein monolayers were very stable with an acidic subphase. Brewster angle microscopy was used to visualise the protein domains at the air-water interface. AFM images were obtained for different pH conditions in the subphase. Very stable and ordered Langmuir-Blodgett protein monolayers were measured.

3110-Pos Board B157

Ionic Mixtures and Distributions Around RNA: Atomically Detailed Simulations with Replica Exchange

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Atomically detailed distributions of ions around RNA are computed. Different mixtures of monovalent and divalent ions are considered explicitly. Studies of tightly bound ions and of diffusive (but bound) ions around RNA molecule of 25 base pairs are conducted in a single computational framework. Replica exchange simulations provide detailed equilibrium distributions with moderate computing resources (9 nanoseconds of simulation using 64 replicas). Binding constants are in qualitative agreement with ion condensation theory. Negative mobile ions can be found near the RNA but must be assisted by proximate and