(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.

3105-Pos Board B152

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

Antonio Tsuneshige[1], Yuki Yashiro[2].
[1]Department of Frontier Bioscience, Faculty of Bioscience and Applied Chemistry, Tokyo 184-8584, Japan, [2]Department of Chemical Science and Technology, Faculty of Bioscience and Applied Chemistry, Hosei University, Tokyo 184-8584, Japan.

We have studied the impact of co-solutes not considered allostetic 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_4, 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_4 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 Inducted Conformational Changes in the ProSegment of Prorenin with Site-Directed Spin Labeling

Lisette M. Fred.
University of Florida, Gainesville, FL, USA.

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

Vicente M. Aguilella, Antonio Alcaraz, Andreu Andrio, Marcel Aguilella-Arzo.
Universitat Jaume I, Castellón, Spain.

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

Elena García-Gimenez, Vicente M. Aguilella, **Antonio Alcaraz**.
University Jaume I, Castellon, Spain.

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.

3109-Pos Board B156

Interfacial Properties Of The Fluorescent Protein B-phycoerythrin Extracted From The Red Microalga *Rhodosorus Marinus*

Guadalupe Adriana Básaca[1], María Guadalupe Burboa[1], Luis E. Gutièrrez-Millán[1], Francisco M. Goycoolea[2], **Miguel A. Valdés**[1].
[1]University of Sonora, Hermosillo, Mexico, [2]University of Santiago de Compostela, Santiago de Compostela, Spain.

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 *Rhodosorus marinus. Rhodosorus marinus* 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 inmunomodulating and anti-carcinogenic activities The spectroscopic characterization was performed by UV, fluoresence and cicrular 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 a maximum at 578 nm, and a secondary structure almost stable with pH changes The surface properties of B Phycoerythrin were analised with a Langmuir Balance. Diferent 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 domins at the air-water interface. AFM images were obtained for diferent 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

Serdal Kirmizialtin, Ron Elber.
The University of Texas Austin, Austin, TX, USA.

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

mobile cations. Binding of one type of a mobile cation is affected significantly by the presence of a second type. While magnesium ions are tighter binders than sodium ions they can be partially repelled from RNA with a moderately high concentration of monovalent ions. This finite system behaves more as a strong electrolyte consistent with Debye Huckel arguments than as a polyelecrolyte that condenses ions around it.

3111-Pos Board B158

The Correlation Between Folding And Activity Of The 10-23 Deoxyribozyme Studied By 3-color ALEX-FRET

Jiwon Jung, Hye Ran Koh, Seong Keun Kim.
Department of Chemistry, Seoul National University, Seoul, Republic of Korea, Korea.

The 10-23 deoxyribozyme is one of the most well-known deoxyribozymes with RNA-cleaving activity, whose folding is typically controlled by the concentration of Mg^{2+} ions. We carried out a systematic study of folding vs. activity of this enzyme and found that they are strongly correlated. We also investigated the effect of single base mutation on folding and activity, and found that the core region plays an important role in the folding and the enzymatic activity of the 10-23 deoxyribozyme.

3112-Pos Board B159

Transition Metal Complexes and the B-to-Z DNA Transition: Investigating the Role of Geometry and Hydration

Richard S. Preisler, Bao N. Ha, Alan J. Pribula.
Towson University, Towson, MD, USA.

A combination of charge-charge interactions with the DNA backbone and site-specific hydrogen bonds to phosphates and base pairs accounts for the unusual ability of $[Co(NH_3)_6]^{3+}$ to drive the B-to-Z transition. We have used circular dichroism (CD) spectroscopy to analyze effects of other stable cobalt, chromium and platinum complexes on the conformation of the DNA copolymer poly[d(G-C)]. We previously reported that a number of octahedral complexes, with hydrogen bonding ligands similar to those in $[Co(NH_3)_6]^{3+}$, also induce the transition. Cationic charge plays a major role in determining their effectiveness, with transition midpoints $\leq$ 10 μM for +3 complexes, but $\geq$ 500 μM for a +1 complex. A series of new +2 complexes with octahedral or square planar geometry have been tested. The transition midpoint was about 100 μM for the octahedral $[Co(NH_3)_5NO_3]^{2+}$. However, the square planar $[Pt(NH_3)_4]^{2+}$ and $[Pt(en)_2]^{2+}$ failed to induce the transition even at 1500 μM, supporting the hypothesis that hydrogen bonding groups in three mutually *cis* positions facing the DNA molecule are required. A number of prior studies have highlighted the importance of hydration in the B-Z conformational equilibrium and osmotic stress measurements in our lab and Donald Rau's showed that addition of an osmolyte such as sucrose induced the transition at even lower $[Co(NH_3)_6]^{3+}$ concentrations. New results indicate that the transition mediated by $[Co(NH_3)_5Cl]^{2+}$ is even more osmotically sensitive. We will continue this line of investigation to explore why more water molecules appear to be displaced by the binding of the +2 complex compared to the +3 complex. Supported by a Towson University Faculty Development and Research Committee grant, a Towson University Undergraduate Research Grant (to B. Ha) and by the Towson University Department of Chemistry.

3113-Pos Board B160

Real-time Optical Assay For Monitoring Nucleic Acid Strand-exchange And Cleavage

Besik I. Kankia, Karin Musier-Forsyth.
The Ohio State University, Columbus, OH, USA.

A simple, real-time optical assay has been developed to monitor nucleic acid strand-exchange and DNA/RNA cleavage reactions. The method takes advantage of the property of some guanine-reach oligonucleotides to adopt monomolecular quadruplex conformations in the presence of certain cations. The quadruplex structure is characterized by a significant absorption signal in the long-wavelength range of the ultraviolet region where other secondary structures are transparent. The ''signal'' oligonucleotide is incorporated into a reactant duplex, which is released into solution upon catalysis. The release is accompanied by fast quadruplex formation and the reaction is monitored by optical methods. We describe the use of this assay to monitor (i) strand exchange catalyzed by the HIV-1 nucleocapsid protein (NC), (ii) RNA cleavage by a DNAzyme in the presence of NC and (iii) DNA cleavage by restriction endonucleases. The reactions were studied as a function of temperature, ionic strength and the concentration and sequence of the substrate molecules. The strand-exchange data were analyzed in terms of activation energies and two alternative pathways (''dissociative'' and ''sequential displacement''). The role of NC in strand exchange and RNA secondary structure invasion by the DNAzyme were evaluated. Principles involved in selection of specific recognition sites by DNA-binding proteins will also be discussed.

3114-Pos Board B161

Adsorption Of DNA And PAMAM Dendrimers - At Silica Surfaces And Model Membranes

Marie-Louiseöberg Ainalem, Tommy Nylander.
Physical Chemistry 1, Lund, Sweden.

The objective in non-viral gene delivery is to enable the passage of DNA over membranes using *e.g.* cationic agents as a way of replacing viral vectors as gene carriers. The study presented here forms part of a larger project, Neonuclei that aims to design a module for DNA packaging, *e.g.* a transcription competent DNA-based particle. The cationic agent used for *in vitro* condensation of DNA is the PAMAM dendrimer of generation 4, highly monodisperse in both size and constitution and with primary amines as functional groups. Upon mixing of the two, DNA undergoes a transition from a semi-flexible coil to a more compact globule due to the electrostatic interaction present, providing protection against DNase activity and also inhibiting the genetic expression.

Neonuclei aims not only to design a module for DNA packaging but also to reveal how this module interacts with the cell and its membranes. The eukaryotic nucleus is surrounded by a double lipid membrane and the intranuclear space itself also contains phospholipids, not in connection with the nuclear envelope. Lipids within nuclei are thought to play a role in cellular signaling and to be linked to the function of the nucleus, possibly stabilizing the chromatin structure.

Here, the interaction between cationic PAMAM dendrimers and DNA is studied with regard to the presence of macroscopic surfaces using *in situ* null ellipsometry, quartz crystal microbalance with dissipation as well as neutron reflectometry. In addition to using bare silica surfaces as substrates, measurements were performed using model membranes composed of deposited DOPC bilayers on solid surfaces. The adsorbed amount, solvent content as well as the layer thickness and the lateral molecular distribution of an adsorbed film exemplifies the important information obtained.

3115-Pos Board B162

Static and Dynamic Light Scattering applications in Protein Crystallogenesis

Isabel Yepes-Ochoa[1], Ariel E. Mechaly[1], Jon Agirre[1], Augusto Bellomio[2], Aintzane Cabo-Bilbao[3], Juan M. Gonzalez Mañas[1], **Diego M.A. Guérin[1]**.
[1]Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain, [2]INSIBIO (CONICET-UNT), Tucuman, Argentina, [3]CIC-BioGune, Derio, Spain.

In this communication we give many examples about different uses of Static (SLS) and Dynamic Light Scattering (DLS) in a protein crystallography laboratory. Although the advantages of both techniques are well documented in the literature their use in the crystallographic community is almost limited to determining the sample polydispersity index (PDI). Nevertheless, both dispersion techniques can be powerful tools in helping protein crystallogenesis. Here we illustrate some applications of both techniques to test protein, virus, and protein/lipid/detergent solutions. We illustrate how useful and easy is to determine -aside from the PDI- the oligomerization state, the molecular weight, to find pH-dependent aggregations, to predict crystallization conditions through the Second Viral Coefficient, and to measure detergent's CMC and lipid-detergent micelles sizes. In sum, we encourage crystallographers to exploit their DLS/SLS equipments in order to maximize the information about the state and conditions of the protein solution prior to set-up crystallization experiments.

[&] INSIBIO (CONICET-UNT). Chacabuco 461. 4000 San Miguel de Tucumán, Tucumán, Argentina.
[#] Present address Structural Biology. CIC BioGUNE, Biological Park of Bizkaia, Build. 800, 48160 Derio, Bizkaia, Spain.

Membrane Structure III

3116-Pos Board B163

Cholesterol Reverts The Relative Susceptibility Of Sphingomyelin And Phosphatidylcholine To Solubilization By Triton X-100. A P31-NMR Study

Hasna Ahyayauch[1], M.-Isabel Collado[1], Félix M. Goñi[2], **Dov Lichtenberg[3]**.
[1]Unidad de Biofísica (CSIC-UPV/EHU), Bilbao, Spain, [2]Unidad de Biofísica (CSIC-UPV/EHU), Bilbao, Spain, [3]Tel Aviv University, Tel Aviv, Israel.

Solubilization of bilayers made of sphingomyelin (SM) requires much less Triton X-100 (TR) than solubilization of bilayers made of phosphatidylcholine (PC). By apparent contrast, partial solubilization of biomembranes results in PC-rich mixed micelles, in which the SM/PC ratio is lower than 1.0, in coexistence with detergent-resistant membranes (DRM), rich in SM (SM/PC>1.0) and cholesterol. Regardless of the question of whether DRM are