(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 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.

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Characterizing pH Inducted 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.

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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

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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 *Rhodosorus Marinus*

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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 *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.

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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