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to one large LES and two small non-LESs (that cannot satisfy the condition of local equilibrium). Furthermore, a significant fraction of the traces shows the transitions among the LES and the non-LESs. The fraction of the traces showing the transitions among the LES and the non-LESs in cytochrome *c* is larger than that of the traces of dye only. The difference can therefore be interpreted to arise from not the intensity fluctuation of the dye but the dynamics intrinsic to the protein. The long-time observation of a free single molecule is expected to reveal slow dynamic property of biological molecules, which could not be resolved by the other conventional methods.

1162-Pos Board B72
Time-Resolved Circular Dichroism Study of Protein Conformational Changes
Mai Thu Khuc, Lucille Mendonça, **François Hache**.
Circular dichroism (CD) is known to be a very sensitive probe of protein conformation, in particular in the far UV where characteristics signatures of secondary structures exist. We have developed new techniques aiming to measure CD in a pump-probe experiment and applied them to the study of the dynamics of conformational changes in proteins. Two techniques were implemented, either a straightforward probe polarization modulation technique or a more sophisticated pump-induced ellipticity measurement. Thorough study of the ultrafast dynamics following photodissociation of carboxy-myoglobin will be presented. Experiments in the visible (Soret band) as well as in the far-UV (helix band) display a strong transient CD feature. Thanks to a classical coupled oscillator calculation, we show that this signal comes from a distortion of the proximal histidine following the heme doming which relaxes on a 100 psec timescale. We have also introduced this transient CD technique in a T-jump experiment in a poly(glutamic acid) sample. A 6°C T-jump is obtained by directly exciting the water at 1.5 μm with a nanosecond pulse generated by a Nd:YAG pumped OPO. CD is measured at 225 nm on a microsecond timescale with a 12 ns time resolution. At pH 4.8, we observe a decrease of the CD signal with a 0.8 μs time constant that we assign to the dynamics of the alpha helix denaturation. This technique is expected to complement other time-resolved techniques based on IR absorption or fluorescence transfer with the advantage of being more straightforwardly quantitative in terms of protein helicity.

1163-Pos Board B73
Prediction of the Bio-Physical Chrateristics of Beta-Amyloid Mixture Based on Amino Acid Sequence
Hyobong Hong, Kibong Song, Chae Cheol Joo.
Amyloid beta (Aβ) is the ~4-k Da peptide originated from amyloid precursor protein (APP) by three different type of the secretases (α , β ,λ). Among them, two β and λ secretases are known to involve the production of the 40 residues ($A\beta_{40}$) and 42 residues ($A\beta_{42}$). $A\beta_{40}$ and $A\beta_{42}$ are main constituent of plaques found in Alzheimer's disease (AD). The shorted one $A\beta_{40}$ is found mainly in cerebrovascular amyloid. $A\beta_{42}$ is the major components in amyloid plaques core deposits and more neuro toxicity. The first 16 residues of $A\beta_{40}$ and $A\beta_{42}$ are largely hydrophobic and the rest of them are very hydrophobic. The most abundant $A\beta_{40}$ and $A\beta_{42}$ in a ratio are about 10:1. Although there are the small differences between two peptides, they show the significantly different biochemical characteristics. A lot of studies have suggested that the soluble oligomeric intermediates are more prone to be a cause of AD than insoluble fibrils including spherical particles and curvilinear structures called ''protofibrils'' . In addition to these interesting chemical characteristics and pathogenecity of Aβ, the relationship between the structure and composition ratio of Aβ and the occurrence of AD is also intensively studied. However, most of them are mainly focused on the one type of Aβ such as $A\beta_{40}$ or $A\beta_{42}$. In this study, we predicted the basic characteristic of Aβ mixture employing the computational chemistry and binding affinities of the small organic molecules including the very short peptides. The results from the calculation indicated that the change of the composition ratio of the Aβ caused the significant change at the characteristics and binding affinities.

1164-Pos Board B74
Ion Pairs in the Hydrophobic Interior of a Protein: How Do Proteins Dissolve Salt in Oil?
Aaron Robinson, Carlos Castaneda, Jamie Schlessman, Bertrand Garcia-Moreno.
Internal ionizable groups in proteins are essential for many important biochemical processes. Despite their importance, their properties are poorly understood. Buried pairs of acidic and basic residues are an especially important functional motif in proteins that perform energy transduction. These pairs are assumed to be charged, but this is usually not known. If proteins behaved as a material with a dielectric constant of 2 to 4, as assumed in most continuum electrostatics models, it would be impossible to bury an ion pair in the interior of a protein (i.e. the groups would be buried as a neutral pair). To examine this aspect of dielectric properties of proteins experimentally we engineered a Glu-Lys pair in a hydrophobic pocket in a highly stable variant of staphylococcal nuclease. In the crystal structure of the V23E/L36K variant the ionizable moieties of Glu-23 and Lys-36 are very close to each other and surrounded by hydrophobic matter save for two internal water molecules. NMR spectroscopy confirmed that the V23E/L36K double variant is folded and stable over a wide range of pH in solution. The pKa values of the internal Glu-23 and Lys-36 in the single-site variants are 7.1 and 7.2, respectively. In the V23E/L36K double variant the pKa values of both ionizable groups are shifted back towards normal values, demonstrating that a stable ion pair can exist in the hydrophobic interior of proteins. The experiments demonstrate that structural adaptations are not required to accommodate the charged, paired moieties. Continuum electrostatics calculations were unable to self-consistently reproduce the dehydration and Coulomb energy experienced by the charges in the ion pair using a single protein dielectric constant. This illustrates a serious shortcoming of continuum electrostatic theory as applied to proteins.

1165-Pos Board B75
Effects of Macromolecular Crowding on Oligomeric Protein Unfolding: Case Study with Human Co-Chaperonin Protein 10 (cpn10)
Ximena Aguilar, Pernilla Wittung-Stafshede.
In vivo, proteins fold and associate in highly crowded environments. Previousexperiments on several monomeric proteins have shown that macromolecular crowding stabilizes towards heat perturbation and also can modulate native-state structure. To assess the effects of macromolecular crowding on unfolding of an *oligomeric* protein, where denaturation involves both unfolding and dissociation, we here tested the effects of the synthetic crowding agent Ficoll 70 on cpn10, a heptameric protein consisting of seven identical β-barrel subunits assembling into a ring. The stability of the heptamer is dominated by subunit-subunit interactions and the individual subunits have low stability on their own. Using far-UV circular dichroism (CD), tyrosine fluorescence and cross-linking experiments, we investigated thermal and chemical stability without and with crowding agent. We find that cpn10 is thermally stabilized by about 5°C in 300 mg/ml Ficoll 70 as compared to in buffer. Whereas there is a large effect of Ficoll 70 on the urea-induced unfolding reaction (midpoint shifts by 2 M), a smaller effect is seen when GuHCl is used as the chemical denaturant (midpoint shifts by 0.6 M). Kinetic unfolding experiments show that the higher equilibrium stability found for cpn10 in crowded conditions can be explained in part by slower unfolding rates.

1166-Pos Board B76
Developing Solutes as Quantitative Probes of Protein and DNA Processes
Emily J. Guinn, Laurel M. Pegram, Michael W. Capp, Michelle Pollock, Joanne Tsarouha, Dana Bellissimo, Xiaoyi Qu, M.T. Record.
To develop the use of urea, trifluoroethanol, KGlutamate, proline and other biochemical solutes as probes of interface formation and large scale conformational changes in protein and DNA processes, we are quantifying the thermodynamics of the competition between these solutes and water to interact with different types of biopolymer surface (e.g. aliphatic and aromatic C, polar and charged O and N). Model compounds displaying one or more of these surface types are used to obtain this information. Interactions of solutes with these model compounds are quantified from excess osmolalities (ΔOsm) from vapor pressure osmometry (VPO) or from the solute's effect on model processes like solubility or micelle formation. These experiments yield chemical potential derivatives (μ_{23}) which are dissected in an analysis based on water accessible surface area (ASA) assuming additivity of interactions. From this analysis we determine a set of interaction potentials ($\mu_{23}/RT(ASA)_i$) for the interaction of a solute with a unit area of each significant type of biopolymer surface (i). These solute interaction potentials are interpreted using the solute partitioning model (SPM) to obtain K_p, the partition function describing the distribution of the solute between local (hydration) water and bulk water. Effects of these solutes on protein folding, DNA helix formation or other biopolymer processes (m-values = $\Delta\mu_{23}$) are interpreted or predicted using this quantitative information together with structural information about the change in amount of each type (i) of water accessible biopolymer ΔASA_i in the process. Also, the large effects of urea , KGlutamate, trifluoroethanol and glycine betaine on the rate constant for dissociation of RNA polymerase-promoter DNA open complexes are interpreted in terms of folding and assembly of a jaw/clamp on downstream duplex DNA after opening of the initiation bubble in open complex formation.
Supported by NIH GM47022