proposed. Interestingly the binding surface identified is concave that could force collagen to be bent.

1081-Pos Mechanism Of Calmodulin Association With Connexin32 Derived Peptides

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Board B57

Calmodulin (CaM) has been shown to modulate the chemical gating of gap junction channels[1]. Two calmodulin binding regions have been previously described in connexin32 (Cx32), one in the N-terminal and another in the C-terminal tail[1,2]. Using Ca^{2+} binding-deficient CaM mutants, CaM12 and CaM34, we have investigated the interaction of the N and C-lobe CaM Ca^{2+} binding sites with Cx32 derived peptides by stopped-flow kinetics at pH 7.0 at physiological ionic strength and 2 mM Mg^{2+}. Interaction with Cx32 1–19 was found to engage all 4 Ca^{2+} binding sites. The CaM C-lobe showed higher affinity (Ca^{2+} dissociation rate constant (k_{off}) $3.6 \pm 0.03\ s^{-1}$) than the N-lobe (k_{off} $56 \pm 1.8\ s^{-1}$). In contrast, Cx32 208–226 at 10 μM only affected the C-lobe CaM Ca^{2+} binding sites (k_{off} $6.1 \pm 0.1\ s^{-1}$). At 20 μM, however, 3 Ca^{2+} sites were involved: the Ca^{2+} binding sites of the C-lobe (k_{off1} $11.6 \pm 0.4\ s^{-1}$ and k_{off2} $1.67 \pm 0.05\ s^{-1}$) and an N-lobe site (k_{off} $11.6 \pm 0.4\ s^{-1}$). Dissociation constant K_d values were 1 and 3.4 μM CaM for Cx32 1–19 and 208–226, respectively. CaM conformation as measured by FRET[3] in the complexes was semi-compact which together with the Ca^{2+} kinetic data suggests separate functions for the C- and N-terminal lobes for CaM. Our data show that the C-terminal Cx32 208–226 peptide binds one CaM lobe only and suggest trans-domain CaM binding as a possible mechanism for chemical gating of gap junctions.

This work is supported by the Wellcome Trust.

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Protein Physical Chemistry

1082-Pos Entropy Definition of Non-Equilibrium Biological Macromolecules

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Board B58

Novel entropy definition for non-equilibrium biological macromolecules will be proposed in this paper and shown that non-equilibrium systems like biological macromolecules can form stable states. Also summary temperature of non-equilibrium systems will be defined and clarified that in such systems each sub-system has different temperature. Entropy of the system couldn't be *0* at $0\ ^0K$, i.e generally thermodynamics third low couldn't be right for non-equilibrium systems. Let take single biological macromolecule situated in a virtual closed box as a closed system and define entropy of such system. Let consider that one of the principal determinants of biomacromolecular structures' are intermolecular hydrogen bonds and discuss some sub-system where number of hydrogen bonds are n, assuming that in hydrogen bonds $A_iH—B_i$ ($i=1,2...n$) proton has two possible energetic levels E_0 and E (or two possible quantum states), first corresponds to non-formation of hydrogen bonds and second corresponds to involving of proton in hydrogen bonds with energy E. So there could be some kind of swinging of proton between two energetic negative atoms A_i and B_i by hole effect, where time of this swinging could be appropriate time for protons during the effect. The relaxation time of such hydrogen bonds will be endlessness thus proton will never stand on one energetic level in hydrogen bonds. If it is considered that such formalism of hydrogen bonds is correct then it could be said that:

1. it is non-equilibrium state;
2. degree $\hat{o}$ of non-equilibrium state of the sub-system is proportional to number of hydrogen bonds involved in this sub-system; and
3. entropy is a function of two parameters E and $\hat{o}$, $S=S(E,\hat{o})$.

Thus finally we will suggest conclusions that different parts of biological macromolecules could have different temperature and entropy could not be *0* at $0\ ^0K$.

1083-Pos A Geometric Construction To Aid In Qualitative Analysis Of Ternary Mixture Phase Separation Conditions

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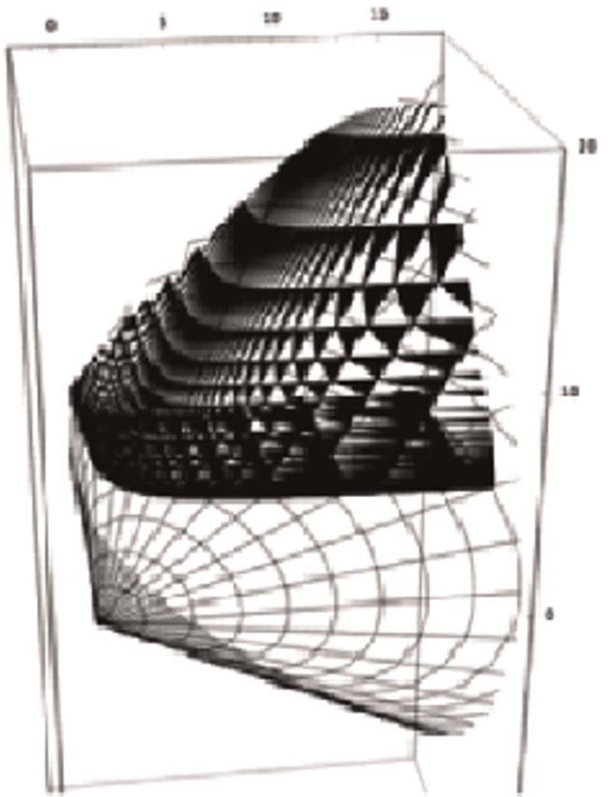
Board B59

Understanding the molecular origins of the phase boundaries of eye lens protein solutions can help us understand loss of transparency of the eye lens in cataract disease, the leading cause of blindness. It has recently been found that eye lens alpha and gamma crystallin mixtures need finely tuned interactions to avoid opacity, in that thermodynamic stability of their highly concentrated mixtures depends non-monotonically on alpha/gamma interaction strength[1]. Experiment and theory have long shown that ternary mixture phase separation typically depends non-monotonically on molecular interaction strengths. Here we present a geometric construction to aid qualitative analysis of phase separation conditions, in the space of the three independent components of the Hessian of the intensive Gibbs free energy. In the simplest mean-field models, a generic non-monotonic dependence of stability on interaction strengths is associated with conic sections formed by the intersection of planes with the elliptical cone of spinodal conditions. A similar construction can help in analyzing more complicated, realistic free energy models.



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1084-Pos Amyloid Fibrillogenesis of Hen Egg White Lysozyme in Acidic Solutions

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Board B60

Amyloidoses are a group of human disorders characterized by deposits of insoluble protein fibrils, with Alzheimer's and Parkinson's disease being the most prominent examples. Recent findings suggest that cell toxicity associated with amyloidosis is mediated, at least in part, by intermediate aggregates formed during the assembly of amyloid monomers into insoluble fibrils. Hence, understanding the kinetics of fibril formation by amyloid proteins is important for unraveling the causes of cellular toxicity.

We have investigated the kinetics of fibril formation and the morphology of intermediate aggregates during fibrillogenesis of the small (MW = 14.3 kD) enzyme hen egg white lysozyme. Amyloid fibrils readily form during incubation of lysozyme in acidic solutions (< pH 4) at 50 °C. The presence of amyloid fibrils was confirmed by shifts in the absorption spectrum of the indicator dye Congo Red and by direct observation with atomic force microscopy.

Using dynamic light scattering changes in the size distribution of lysozyme can be closely followed during aggregation. Following a prolonged latency period without any discernable changes in particle size or particle interactions, aggregation commences abruptly with a transient "burst" in particle size. A small population of large particles with apparent radii of many hundreds of nanometers transiently appears, followed by a similarly abrupt collapse down to 20 nm. These smaller aggregates then slowly and steadily grow in size from around 20 to 40 nm, and their number increases until the onset of fibril formation. Atomic force microscopy confirms that the small, intermediate aggregates of 20 nm size have a spherical morphology reminiscent of micelles.

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1085-Pos Salt Specific Effects on Direct and Hydrodynamic Interactions of Lysozyme

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Board B61

Among the important aspect of protein crystallization are the kinetics of phase nucleation, and how they are affected by the choice of precipitant. As a step toward addressing this issue, we have systematically investigated salt-specific effects on direct and hydrodynamic interactions of hen egg white lysozyme in solution. Static and dynamic light scattering data for lysozyme solutions at pH = 4.5 for five different salts were collected. The Rayleigh ratio R_υ and the collective diffusion coefficient D_c vs protein concentration (c_p) were determined for different salt concentration (c_s) and solution temperatures (T). Changes in protein hydration with solution conditions were obtained by extrapolating D_c to its value at $c_p = 0$ and by using the Stokes-Einstein relation with the correct values for solution viscosity $\eta = \eta(T,c_s)$. The slope k_S of Debye plots (Kc_p/R_υ vs. c_p) for static scattering data indicates the magnitude and sign of direct protein interactions. The initial slope k_d of D_c *vs.* c_p from dynamic light scattering, in turn, arises from a combination of hydrodynamic (fluid-mediated) and direct protein pair-interactions. Combining both data sets, we were able to separate the differential effects of salt identity on either direct and hydrodynamic protein interactions under comparable solution conditions and over a wide range of temperatures. The results of our measurements will be compared with measurements of lysozyme solubility and available cloud point data.

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1086-Pos On the pH Dependence of the Second Virial Coefficient of Bovine Eye Lens Alpha Crystallin

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Board B62

The solution properties of eye lens proteins can help understand the molecular origins of cataract disease. Alpha crystallin is a prevalent eye lens protein comprising numerous 20kDa subunits. Mixtures of alpha with gamma crystallin, a 21kDa phase-separating lens protein, show phase separation that is sensitive to alpha/gamma interaction. We use static light scattering to measure the second virial coefficient of bovine eye lens protein alpha crystallin as a function of pH and ionic strength, to help in identifying molecular sources of alpha/gamma interaction. With use of 100mM sodium phosphate buffer in the pH range 6.8 to 7.4, we find an alpha crystallin molecular weight between 490,000 and 540,000 grams per mole, and a positive second virial coefficient of $1.5 \times 10\text{^}(-8)$ (mL/mg)(mole/g), that increases with increasing pH. We examine potential molecular origins of the observed second virial coefficient.

1087-Pos Correlation Between Binding Driving Forces And Structural Details In The Interactions Between BLIP And TEM-1 β-Lactamase

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Board B63

β-lactamase inhibitor protein (BLIP) interacts with TEM-1 β-lactamase (TEM1) through 23 contact residues, of which ten are hotspot residues grouped into 2 distinctive locales. Our extensive isothermal titration calorimetry (ITC) measurements of the binding between TEM1 and 20 BLIP mutants of alanine substituted contact residues show that Y51A and W150A have distinctive TEM1-binding thermodynamics (Wang J., et al. *JBC*. 2007; *282*:17676–84). The binding heat capacity changes for Y51A/TEM1 and W150A/TEM1 interactions are −809±37 cal/mol/K and −275±28 cal/mol/K, respectively (−667±51 cal/mol/K for the wild type), while the W150A TEM1 interaction is ∼3000 fold weaker than the wild type. We calculated the pure "hydrophobic free energy" of the binding based on Baldwin's equation using our experimentally determined binding heat capacity changes (Baldwin RL. *PNAS*. 1986; *83*:8069–72). The calculations suggest that the binding interactions of these complexes are driven by a large "hydrophobic force" and counter balanced by a large "nonhydrophobic forces" resulting in moderate affinities. These calculations further suggest that more negative binding heat capacity changes tend to have larger "hydrophobic forces" resulting in stronger complexes. To understand the structural basis of the altered thermodynamic properties, we crystallized and determined structures of the complexes of the Y51A/TEM-1, W150A/TEM-1 and W112A mutant using x-ray crystallography. The weak-binding complex of W150A/TEM-1 shows significant structural rearrangement around the mutated residue W150A and more water molecules trapped in the interface. Our crystal structures show the correlation between the structural rearrangement and hydration of the interface and the binding heat capacity changes. This correlation appears to extend to the affinities.

1088-Pos Distribution Of Water Molecules In Hydration Shell Around A Small Globular Protein Calculated By Using Molecular Dynamics

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Board B64

Distribution of water molecules around a small globular protein, bovine pancreatic trypsin inhibitor (BPTI), is calculated by using molecular dynamics. Space of the solvent around the protein is divided with Voronoi polyhedrons where generating points are centers of protein atoms on the surface. Calculated results show that distribution of water molecules in a hydration shell around a hydrophobic atom is similar to that in a hydration shell around a methane molecule. Number of water molecules in the first hydration shell of the solute atom directly affects hydration free energy of the solute in the framework of scaled particle theory. Distribution of water molecules around a hydrophobic atom has a second peak corresponding to the distance mediated by one water molecule. However, in the case of hydrophilic atom, distribution does not have a clear second peak. Calculated distributions of water molecules for the surface atom in different chemical group are compared. Widely known an empirical method using accessible surface area proposed by Ooi et al., which reproduces hydration free energies of proteins, uses different weight values of accessible surface area for different chemical group. Our results suggest that the weight difference is caused by the number of water molecules in a first hydration shell inside the Voronoi polyhedron which belongs to a surface atom.

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1089-Pos Ice-Surface Adsorption Enhanced Colligative Effect of Antifreeze Proteins in Ice Growth Inhibition

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Board B65

This manuscript describes a mechanism to explain antifreeze protein's function to inhibit the growth of ice crystals. We propose that the adsorption of antifreeze protein (AFP) molecules on an ice

surface induces a dense AFP-water layer, which can significantly decrease the mole fraction of the interfacial water and, thus, lower the temperature for a seed ice crystal to grow in a super-cooled AFP solution. This mechanism can also explain the nearly unchanged melting point for the ice crystal due to the AFP's ice-surface adsorption. A mathematical model combining the Langmuir theory of adsorption and the colligative effect of thermodynamics has been proposed to find the equilibrium constants of the ice-surface adsorptions, and the interfacial concentrations of AFPs through fitting the theoretical curves to the experimental thermal hysteresis data. This model has been demonstrated by using the experimental data of serial size-mutated beetle *Tenebrio molitor* (Tm) AFPs. It was found that the AFP's ice-surface adsorptions could increase the interfacial AFP's concentrations by 3 to 4 orders compared with those in the bulk AFP solutions.

1090-Pos Characterization of Recombinant *durum* Metallothionein and Implications for the Native Protein

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Board B66

Metallothioneins (MTs) are small, cysteine-rich proteins with high binding capacity for metals like Zn, Cu and Cd. MTs exist in a wide range of organisms and are classified in one super-family according to the distribution of cysteine motifs in their sequences. Recent studies indicate that all members of this family do not have a single unifying function; while some MTs participate in metal homeostasis others play a role in detoxification of heavy metals (e.g. Cd and As). Despite the large literature on MT sequences and functional roles, lack of direct biochemical and biophysical data on purified proteins hinders a comprehensive understanding of sequence, structure, metal-binding and function relationships in MTs from different sources.

We identified an *mt* gene in Cd resistant durum wheat and the recombinant protein (dMT) was over-expressed in *E. coli* as GST-fusion (GSTdMT). Due to their aggregation propensity, sensitivity to oxidation and proteolytic attack and difficulties in quantification, MTs cannot be purified using standard procedures. GSTdMT purification required strict anaerobic conditions and critical parameters included Cd and reducing agent concentration and choice of buffers. Results of characterization of purified GSTdMT by size exclusion chromatography, SDS- and native-PAGE, UV-VIS absorption spectrophotometry, atomic absorption spectroscopy, dynamic light scattering, circular dichroism and small-angle solution X-ray scattering will be presented. Results show that the metal content and structure of dMT in the fusion protein are preserved and structural parameters of dMT can be inferred from those of GSTdMT fusion protein. These studies help to resolve some of the ambiguities encountered in structural studies of dMT and provide additional data for structural models.

1091-Pos Investigation of dilute aqueous solutions of Aβ1-40 and DMSO via Fluorescence Correlation Spectroscopy

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Board B67

Amyloid-β (Aβ), a 39–42 amino acid peptide, is produced throughout the body during normal cleavage events. However, under certain conditions, Aβ aggregates in brain to form the long, thin fibrils that are the hallmark of Alzheimer's disease (AD). It is proposed that small oligomeric species formed during the early stages of this aggregation process may be the primary mechanism of toxicity in AD. In addition to oligomers, some studies indicate that Aβ may form micelles during the early stages of aggregation. The existence of Aβ micelles is controversial, and there are conflicting reports on whether micelles, if they exist, act to promote aggregation or simply sequester monomeric Aβ.

Here, we use multiphoton fluorescence correlation spectroscopy to study dilute solutions of Aβ1–40. We observe only monomeric Aβ or small (< 10 nm radius) oligomers under normal conditions. However, the addition of as little as 1% v/v dimethyl sulfoxide (DMSO) induces phase separation of Aβ into micelle-like particles 50–100 nm in diameter. Thioflavin-T binding experiments show that these DMSO-Aβ micelles slow aggregation, indicating that the micelles act to sequester Aβ and are not nucleation centers. Transmission electron micrographs also show differences in the fibril growth process in the presence and absence of DMSO. Since DMSO is a common solvent used in peptide handling and storage, these results indicate that peptide processing may affect the observed *in vitro* aggregation pathway. Moreover, similar self-sequestering of Aβ into favorable environments may occur *in vivo* and possibly impact the development of AD.

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1092-Pos Characterization of Attractive and Repulsive Protein-Protein Interactions in Highly Concentrated Solution Via Measurements of Static Light Scattering

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Board B68

We introduce a new static light scattering method for characterization of the association state of proteins in highly concentrated solution. The instruments required for setting up this method are a light scattering detector, a programmable dual-syringe pump and a stirring system. The dual-syringe pump is used to sequentially dilute

an initially highly concentrated solution of protein contained in a continuously stirred light-scattering cell mounted within the light scattering detector. With this apparatus we obtain a gradient of solute concentration that decreases exponentially with each dilution step, and measure the relative intensity of light scattered at each solute concentration. A concentration gradient may be acquired automatically in less than one hour, and protein is conserved for later study if desired. Initial results obtained from BSA solutions at high concentration (120 g/l) are quantitatively accounted for by an effective hard particle model without significant self-association at concentrations up to this value. The results of analysis of nonideal behavior resulting from nonspecific repulsive interaction between BSA molecules (steric + electrostatic repulsion) agree well with those previously obtained from analysis of the concentration dependence of sedimentation equilibrium and osmotic pressure of BSA solutions under the same experimental conditions.

Enzymes

1093-Pos Mechanism of Enzymatic Activity of β-subunit of the Voltage-Gated Potassium Channels

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Board B69

Oxygen sensitive Kv channels (Kv1.5 and Kv4) bind the auxiliary β-subunits which belong to the aldo-keto reductase (AKR) superfamily. Kvβ1–3 subunits differentially alter the gating properties of the Kv channel and impart or accelerate Kv inactivation when bound to NAD(P)H and abolish inactivation in presence of $NAD(P)^+$. In pulmonary arteries, β-subunits are expressed in a gradient manner and possibly regulate the perfusion ventilation system. We found that Kvβ displays catalytic activity with several aldehydes and ketones. Substituted benzaldehydes and acetophenones were among the substrates with highest catalytic activity (k_{cat}~0.2 min^{-1}) and Michaelis constants in submillimolar range (K_m for p-nitrobenzaldehyde is 162±33 μM; K_m^{NADPH} is 32±11 μM). Structure-activity relationship studies with a series of substituted benzaldehydes and acetophenones revealed importance of substituent in the para-position of benzene ring (nitro-group being best) and acceptance of ketones (p-nitroacetophenone) as substrates. Endogenous aromatic e.g., vitamin K3 as well as aliphatic lipid peroxidation products such as 4-oxo-nonenal, POVPC and eicosanoids such as PGJ_2 were good substrates with K_ms in the micromolar range. Reduction was found to be the preferred direction for Kvβ2 enzyme; as no oxidation of p-nitrobenzylalcohol was detected. Substitution of Tyr90 which is homologous to the catalytic tyrosine in other AKRs, for Phe abolished enzymatic activity of Kvβ2 suggesting that catalytic mechanism of this enzyme is similar to other AKRs. Measurements of the kinetic isotope effects using NADPD showed a $^Dk_{cat}$ for p-nitrobenzaldehyde and nitroacetophenone of 2.0±0.1 suggesting that the hydride transfer step is partially rate-limiting. Limited kinetic isotope effect on k_{cat}/K_m and cofactor binding studies suggest that the release of the cofactor and substrate/product(s) also contribute to rate-limitation. Discovery of high-affinity endogenous substrates (or ligand) suggests that intracellular metabolism maybe kinetically coupled to Kv channel gating by Kvβ subunits.

1094-Pos Crystal Structures Of Human Orotidine-5′-monophosphate Decarboxylase - Implications For Drug Design And Catalysis

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Board B70

The human UMP synthase (UMPS) catalyzes the last two steps of *de novo* pyrimidine nucleotide synthesis and is a malaria and cancer drug target. The C-terminal domain of UMPS harbors the orotidine-5′monophosphate decarboxylase (OMPD) activity, which does not require any cofactors, yet is among the most proficient enzymes known. Based on biochemical and structural data of OMPDs from various micro-organisms several non-covalent mechanisms for decarboxylation through high energy intermediates have been formulated. We describe several crystal structures of the human OMPD in complex with substrate, product, and several novel nucleotide inhibitors. These first structures of a mammalian OMPD outline the requirements potential drugs must meet to maximize ligand efficiency and minimize cross-species activity. Chemical mimic by iodide identified a binding site for the product CO_2, which can be exploited for drug design. Unexpectedly, simple compounds can replace the natural nucleotide and induce a closed conformation of the enzyme, defining a tripartite catalytic site. Structural plasticity of catalytic residues and a covalent OMPD-UMP complex prompt a re-evaluation of the prevailing decarboxylation mechanism in favor of a covalent intermediate. This mechanism can also explain the observed catalytic promiscuity of OMPD.

1095-Pos Are Enzyme Active Sites Built in Multiple Layers?

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Board B71

The importance of "second shell" residues in enzyme active sites is addressed. Much experimental evidence has established the importance of specific residues in the catalytic and recognition capabilities of hundreds of particular enzymes. Nearly all of the residues that have been studied are in direct contact with the reacting substrate in the "first coordination shell" of the substrate molecule in the bound enzyme-substrate complex. Computational evidence, in addition to a very limited set of previous experimental data, suggests that remote residues, particularly those in the "second shell" around the reacting substrate, also contribute to catalytic activity. Residue