Multimerization of Solution-State Proteins by Anionic Porphyrins

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Protein-protein interactions and formation of multi-subunit protein complexes remain among the most challenging areas of modern Biophysics. Recently, surface binding and interactions of anionic porphyrins with cationic proteins gained a lot of attention as relevant models for protein surface molecular recognition and photoinitiated monomerization. However, interpretation of data in nearly all reports explicitly or implicitly assumed interaction of porphyrin with monodisperse proteins in solutions. Here, using small-angle X-ray scattering with solution phase samples, we demonstrate that horse heart cytochrome (cyt) c, tri-heme cytochrome c7 PpcA from Geobacter sulfurreducens, and hen egg lysozyme multimerize in the presence of several water-soluble porphyrins. Multimerization of cyt c induced by tetrakis(4-sulfonatophenyl)porphyrin showed a pH dependence with a stronger apparent binding affinity under alkaline conditions and was weakened in the presence of a high salt concentration. Ferric-cyt c formed a complex larger than those formed by ferro-cyt c. Free base TPPS and FeTPPS complexes with calcium concentrations larger than those of ZnTPPS. A number of carboxylated porphyrins induced multimer formation as well. No increase in protein aggregation state for cationic proteins was observed in the presence of anionic porphyrins or sulfonated anthraquinone. All-atom molecular dynamics simulations of cyt c and PpcA with free base TPPS corroborated X-ray scattering results and revealed a mechanism by which the tetrasubstituted charged porphyrins serve as bridging ligands nucleating multimerization of the complementarily charged protein. The final aggregation products suggest that multimerization involves a combination of electrostatic and hydrophobic interactions. The results demonstrate an overcomplexed in the design of multifunctional ligands for protein surface recognition.

Characterization of the Photophysical, Thermodynamic and Structural Properties of the Terbium(III)-KChIP3 Complex

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KChIP3 (K+ channel interacting protein 3) is a calcium binding protein and active modulator of Kv4 channels in neuronal cells as well as a novel Ca2+ regulated transcriptional modulator. KChIP3(calsenilin) may also be involved in Alzheimer’s disease through prevention of presenilin-2 fragmentation. Many of the KChIP3 interactions with its binding partners (Kvα4, calmodulin, DNA, and drugs) have been shown to be dependent on calcium. Therefore, understanding the structural changes induced by Ca2+ is of utmost relevance to elucidating the mechanism of calcium signal transduction. Here, we show that the fluorescence emission and excitation spectra of the calcium luminescent analog Tb3+ is enhanced upon binding to the EF-hand of KChIP3, likely due to a mechanism of energy transfer between Phe/Trp and Tb3+. We also observe that unlike Tb3+ bound calmodulin, the luminescence lifetime of terbium bound to KChIP3 decays as a complex multiexponential (ravelage ~ 1.8 ms) and is sensitive to the protein structure and drug (NS5806) binding. Using isothermal calorimetry we have determined that Tb3+ binds at least to four binding sites (Kd = 2.2 µM) and is able to displace bound Ca2+ through an entropically driven mechanism (ΔH~3 kcal/mol). Secondary structural analysis of KChIP3 using far-UV CD spectroscopy shows that binding of Tb3+ induces the formation of an intermediate structure with less alpha helical content than that induced by Ca2+. However, using the hydrophobic probe 1,8-ANS we show that the structural changes induced by Tb3+ are large enough to expose a hydrophobic surface on KChIP3 identical to Ca2+ bound protein. Similarly to Ca2+, terbium binding also induces the dimerization of KChIP3. Overall, these results support the use of Tb3+ as an alternative fluorescent label in the study of Ca2+ induced structural changes in KChIP3.

Simulations of Cosolute Effects on Protein’s Stability

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Cosolutes are small miscible organic molecules that affect the solubility of proteins. Many different kinds of cosolutes have been observed to affect the conformational equilibrium of proteins in solution. Of special consideration are cosolutes that are able to denature (urea), stabilize (TMAD), or prevent aggregation (arginine). We take under consideration a special case of cosolutes termed osmotlytes that have a sizable effect on the osmotic pressure of the solution. In particular, we are interested in how simple amino acids (Arg, Pro, Gly) increase the stability and solubility of proteins. We use molecular dynamics simulations to study the interactions of these cosolutes with proteins. The system under study is the mini protein trp cage, simulated in a variety of solutions consisting of different amino acids as cosolutes, in ensembles that are representative of the folded and the unfolded states. We calculate the osmotic pressure to measure the cosolute-cosolute interactions, and the preferential solute-functional group interactions to further assess the protein-cosolute interactions. These calculations provide information about the interactions of various amino acids with proteins. The calibration and validation with experimental data provide a way of refining force field parameters for modeling proteins in solution.
Plasmon resonance (SPR)-based assay. Several PPI inhibitors were identified and the inhibitory effect was further validated in a concentration-dependent SPR screen. Promising hits will be further tested in mosquitos. In vitro characterization of this interaction will provide a better understanding of pathogen-host interactions at a molecular level and allow the development of specific inhibitors of PGPAS0-hFactor H interaction in the mosquito.

1096-Pos Board B47
Phenylethanoids can Modulate Amyloid-β Aggregation Associated with Alzheimer’s Disease
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Alzheimer’s Disease (AD) is a common neurodegenerative disease and the 6th leading cause of death in the US. One neurological marker of AD is the deposition of extracellular plaques composed of aggregates of the amyloid-β (Aβ) protein. Aβ aggregation follows a nucleation-dependent pathway, beginning with monomer forming nuclei that grow into soluble aggregates and proceed to form the insoluble fibrils deposited in AD brain. As such, many therapeutic treatments target the inhibition of Aβ aggregation. It is hypothesized that compounds containing a phenol structure can interrupt aggregate β-sheet formation by disrupting π-π stacking at phenylalanine residues in the core of the protein. In this study, the phenylethanoid oleuropein, along with metabolites hydroxytyrosol and tyrosol, were studied for their effect on Aβ aggregation.

Aggregation of SEC-purified Aβ was initiated via agitation in the presence of a 5-fold excess of compound and monitored using thioflavin-T to detect aggregate β-sheet structure. To examine the earliest stages of aggregation, oligomerization was induced by combining DMSO-solubilized Aβ with a 10-fold excess of inhibitor and diluting into PBS. Oligomer formation was monitored via SDS-PAGE and Western blotting to quantify oligomer size. Distinct correlations were observed between compound structure and the effect on oligomer size and the formation of larger aggregates. Hydroxytyrosol, a metabolite of oleuropein, exhibited the most effective inhibition among these compounds in aggregation and oligomerization. Thus, effectiveness of phenylethanoid compounds in Aβ inhibition is influenced by the substitutions present on the ring. In contrast, the structure with only one hydroxyl group, tyrosol, has little effect. Further study will elucidate the effect that these changes in Aβ aggregation have upon Aβ neurotoxicity.

1097-Pos Board B48
Probing the Role of Cytoglobin’s Extended Termi
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Cytoglobin (Cygb) is a non-heme, hexacoordinated globin protein that is expressed ubiquitously in vertebrate tissues. The physiological function of Cygb remains unknown; however it has been proposed to have a role in oxygen sensing, lipid peroxidation and NO metabolism. Structurally, Cygb has a unique feature among vertebrate globins including extended, disordered N- and C- termini. Although the function of the terminal extensions remains to be determined, their role in promoting intracellular interactions has been proposed. In order to probe the impact of the N- and C- termini on the functional and structural properties of Cygb, human Cygb (hCygb) with truncated N-terminal (hCygb ΔN), C-terminal (hCygb ΔC) and N- and C- termini (hCygb ΔNΔC) were studied using various spectroscopic techniques. The presence of the N- termini increases the stability of Cygb by 13°C. In addition, all studied forms of Cygb bind 1-anilino-8-naphthalene sulphonate hydrophobic probe, although the affinity for 1,8-ANS increases in the absence of the N- and C- termini suggesting that the deletion of the N- and C-termini leads to the exposure of the hydrophobic sites on the protein surface. Furthermore, the impact of the extended N- and C-termini on Cygb interactions with small gaseous ligands will be discussed.

Protein Assemblies I

1098-Pos Board B49
Statistical Thermodynamics of One-To-Many Molecular Recognition Accompanied by Partner-Dependent Folding: In the Case of a Tumor Suppressor Protein p53
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A tumor suppressor protein p53 recognizes over 100 biomolecules. Here we investigate the mechanism of its molecular recognition accompanied by partner-dependent folding of its partial peptide, the p53 C-terminal domain (p53-CTD). The p53-CTD lacks a well-defined tertiary structure without a partner, form a helix in binding to S100ββ, a sheet in binding to sirtuin, and a coil with two distinct backbone conformations in binding to CBP or cyclin A. We calculate changes in thermodynamic quantities upon these binding processes using a statistical-mechanical approach combined with molecular models for water. The hydration entropy is calculated by our hybrid method in which the angle-dependent integral equation theory applied to a multipolar water model is combined with the morphometric approach. The three-dimensional reference interaction site model theory is employed for calculation of the hydration energy. This approach is the cutting edge of studies on the biomolecule hydration and on a variety of biological self-assembly processes. It is shown that a common driving force for the p53-CTD binding is a large gain of the water entropy, which originates primarily from an increase in the total volume available to the translational displacement of water molecules in the system and the reduction in the water crowding. We discuss the several characteristics of the one-to-many molecular recognition by p53-CTD from the viewpoint of hydration thermodynamics.

1099-Pos Board B50
Computer Simulations of Viral Capsid Assembly with Patchy Particle Model
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Most viruses use the power of self-assembly to protect and transport their genomic material with the help of a protein capsid. Different computational approaches have been employed to understand the dynamics of the assembly pathway, ranging from molecular dynamics simulations with atomistic detail through Brownian dynamics simulations with coarse-grained interactions to statistical or thermodynamic approaches without any explicit representation of space. We have used coarse-grained Brownian dynamics simulations of a patchy particle model to represent the physically correct assembly dynamics in time and space and at the same time to achieve sufficiently long simulation times to study the statistics of complete capsid self-assembly. In particular, we have developed algorithms that account for the anisotropic translational and rotational diffusion of assembly intermediates and ensure detailed balance for reversible patchy particle models (Klein et al., J. Chem. Phys. 140:184112, 2014). We report on studies of two biologically important questions that can be addressed only in such a computer time-efficient framework: the role of event-driven reactivity in capsid self-assembly (Baschek et al., BMC Biophysics 5:22, 2012) and the role of the rate of capsomere addition (Boetcher et al., preprint 2014). We find that hierarchical assembly is more favorable than direct assembly in the case of stable bonds and that slower capsomere supply is more favorable in order to attain a steady state of continuous capsid assembly.

1100-Pos Board B51
Characterizing the MCT-1:DenR Complex, a Translational Enhancer for Lymphoma Survival
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MCT-1 is an oncogene that is highly expressed in human lymphomas and has been linked to increased cell proliferation and decreased apoptosis through the enhanced translation of survival-related transcripts. Acting on mRNAs that possess the p53-CTD binding is a large gain of the water entropy, which originates primarily from an increase in the total volume available to the translational displacement of water molecules in the system and the reduction in the water crowding. We discuss the several characteristics of the one-to-many molecular recognition by p53-CTD from the viewpoint of hydration thermodynamics.