TriC to restore unfolded γ Crystallin to a native fold investigated using size exclusion chromatography. In collaboration with fellow members of the Center for Protein Folding Machinery, we are investigating the possibility of visualizing the crystallin substrate in the chaperonin/substrate complex by Cryo-EM.

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2235-Pos Board B205

HαB-Crystallin Suppresses The Aggregation Upon Refolding Of Its Physiological Substrates HγD-, HγC- And HγS-Crystallin

Ligia I. Acosta-Sampson, Jonathan King.
MIT, Cambridge, MA, USA.

The passive chaperone α-crystallin, a small heat shock protein, is one of the ubiquitous crystallins in vertebrate lenses, along with the βγ-crystallins. It is composed of two subunits (~ 20 kDa) αA- and αB-crystallin (αA- and αB-crys), which form an hetero-oligomeric, polydisperse complex of ~ 800 kDa in the lens. Aggregates isolated from mature-onset cataracts, the major cause of sight loss worldwide, contain damaged and misfolded forms of βγ-crystallins, as well as α-crystallins. We have studied the chaperone function of Human αB-crystallin interacting with its physiological Human γ-crystallin substrates. Human γD-crystallin (HγD-crys) and γC-crystallin (HγC-crys) are stable and long-lived mammalian γ-crystallins localized in the lens nucleus. Human γS-crystallin (HγS-crys) is abundant in the lens outer cortex. All three γ-crystallins can refold in vitro to their native state after unfolding in high concentrations of GdnHCl. However, at very low denaturant concentrations (< 1 M GdnHCl) aggregation of refolding HγC- and HγD-Crys intermediates competes with productive refolding. Diluting unfolded HγC-, HγD-, or HγS-crys to low GdnHCl concentrations (100 μg/ml, 37°C) resulted in the protein population partitioning between productive refolding and aggregation pathways. HγD-, HγC- or HγS-Crys protein was allowed to refold and aggregate in the presence of HαB-Crys homo-oligomers at different monomer-to-monomer ratios of γ-Crys to αB-Crys. HγD- and HγC-Crys aggregation was suppressed to similar levels, whereas HγS-Crys aggregation was not suppressed as strongly in assays measuring solution turbidity at 350 nm. SEC chromatograms of the products of suppression reactions showed the presence of a high molecular weight complex containing the chaperone-substrate complex in ratios of 1γC:5αB and 1γD:5αB chains. This complex was still present 4 days after the suppression reaction was initiated. These results provide a model for how α-crystallin interacts with aggregation-prone substrates in vivo.

2236-Pos Board B206

Interaction between Molecular Chaperone Prefoldin with Group II Chaperonin in the Presence of Nucleotides: Implication for Substrate Transfer Mechanism from Prefoldin to Chaperonin

Tamotsu Zako[1], Yosuke Murase[1], Ryo Iizuka[2], Taro Kanzaki[3], Masafumi Shimizu[4], Masafumi Yohda[3], Mizuo Maeda[1].
[1]RIKEN Institute, Saitama, Japan, [2]The University of Tokyo, Tokyo, Japan, [3]Tokyo University of Agriculture and Technologu, Tokyo, Japan, [4]Tokyo University of Technology, Tokyo, Japan.

Prefoldin (PFD) is a molecular chaperone that captures a protein-folding intermediate and transfers it to a group II chaperonin (CPN) for correct folding. However, mechanism of substrate transfer from PFD to CPN remains to be elucidated. Previous studies showed that CPN has a helical protrusion as a built-in-lid, and uses ATPase cycling to promote the conformational change necessary to open and close the lid. In this study, we have examined interaction between archaeal PFD and CPN in the presence of various nucleotide analogs. Affinities between fluorescein-labeled Pyrococcus PFD (PhPFD) and Thermococcus CPN (ThCPN) in the absence or presence of ADP and AMPPNP were examined by fluorescence anisotropy measurement. In the presence of ADP and AMPPNP, ThCPN was shown to take open and closed conformation, respectively.

The affinity of PhPFD to ThCPN was weakest in the presence of AMPPNP, which suggests that PFD does not bind to CPN in closed-state. In contrast, PhPFD bound more tightly to ThCPN (nucleotide free or ADP) in open-state. Interestingly, affinity of PhPFD to ADP-ThCPN was higher than nucleotide free-ThCPN, even though both take open conformations. This result also implies that these open conformations are different, which is supported by other experiments indicating that ADP-ThCPN can suppress thermal aggregation of citrate synthase more efficiently than nucleotide free-ThCPN. Our data implicates that substrate protein is delivered from PFD to CPN of the open conformation selectively in ADP bound-state rather than nucleotide free-state.

2237-Pos Board B207

Networks of Functional Residues in GroEL and GroES

Riina Tehver, Jie Chen, D. Thirumalai.
University of Maryland, College Park, MD, USA.

The chaperonin GroEL and its cofactor GroES make up a molecular machine that rescues aggregation-prone misfolded proteins. The GroEL functional cycle consists of a series of large-scale allosteric transitions between the T, R, R′ and R″ states. The corresponding large structural rearrangements facilitate substrate protein capture, refolding, and release, and are thus essential for the proper operation of the chaperonin. Using a C_α-sidechain elastic network model-based structural perturbation method, that probes the response of a local perturbation at all residue sites, we have studied the molecular details of the T -> R and R″ ->T transitions and determined the key mechanical residues that support the allosteric cycle - the allostery wiring diagram. We provide a molecular level interpretation for the intraring positive cooperativity and interring negative cooperativity as well as the role of GroES in the GroEL allosteric cycle.

2238-Pos Board B208

ClpXP Degradation of the DNA-Protection Protein Dps Requires Auto-Tethering to the Enzyme

Anne S. Meyer, Julia M. Flynn, Tania A. Baker.
Massachusetts Institute of Technology, Cambridge, MA, USA.

Dps is a dodecameric bacterial protein that acts to prevent the formation of hydroxyl radicals and condenses cellular DNA to form ''biocrystals'' under stressful conditions, protecting the chromosome from damage. During exponential growth, Dps is continually synthesized but rapidly degraded by the AAA+ protease ClpXP, resulting in a low cellular concentration. Dps degradation is rapidly turned off when cells respond to nutritional or oxidative stresses, allowing Dps to accumulate swiftly and counteract the damaging effects of the stressors. This environmental regulation of degradation is highly specific; stressors such as hydrogen peroxide result in the stabilization of Dps, while the degradation of other ClpXP substrates is not affected by this treatment. Maintenance of genomic integrity then crucially depends upon selective proteolysis of Dps by ClpXP only during non-stress conditions. The molecular mechanism of Dps recognition and degradation by ClpXP was probed through a combination of *in vivo* and *in vitro* techniques. Dps degradation exhibits an absolute requirement for the N-terminal domain of ClpX, a region that mediates interaction with substrate-delivery proteins called adaptors. The characterized ClpX adaptor SspB as well as a peptide representing only the ClpX-binding region of SspB are each able to compete efficiently with ClpXP for Dps degradation. The N-terminus of Dps seems to interact with ClpX, primarily on its N-domain. An extended region or multiple regions within the N-terminus of Dps are required for efficient competition of Dps degradation by ClpXP. Thus, Dps functions analogously to an adaptor protein by using its unstructured N-terminus to tether itself to ClpX during the degradation process. This mechanism may increase the affinity of Dps for ClpX by allowing the two proteins to engage in multiple contacts simultaneously.

2239-Pos Board B209

Controlling oligomerization through protein engineering: in vivo analysis of Hsp90

Natalie Wayne, Dan Bolon.
University of Massachusetts Medical School, Worcester, MA, USA.

Many homo-oligomeric proteins are vital for biology including ion-channels, the p53 tumor suppressor, and the essential kinase-associated chaperone Hsp90. Mutational analyses of these homo-oligomeric systems in vivo is complicated by cross-oligomerization between wild-type and mutant subunits. We have devised a generalizable thermodynamic strategy to prevent cross-dimerization. Appending an oligomerization domain to the mutant subunits reduces the free energy of homocomplexes relative to wild-type/mutant heterocomplexes. We have used this strategy to engineer super-stabilized Hsp90 dimers that do not cross-oligomerize with wild-type Hsp90. Super-stabilized Hsp90 supports yeast viability and is fully active in the maturation of v-src kinase. Thus, our stabilization strategy does not disturb the biochemical function of Hsp90.

We have used superstabilized Hsp90 to address a fundamental and long-unanswered question regarding Hsp90: what clients or substrate proteins depend on Hsp90 ATPase activity in vivo. The identification of ATP dependent Hsp90 substrates has been a major challenge both in vitro and in vivo. In vitro studies are complicated by the large number of co-chaperones required for Hsp90 to function efficiently. In vivo studies are complicated both because ATPase deficient Hsp90 mutants do not support viability and because when different Hsp90 variants are co-expressed they form a mixture of different dimer species. We have used our engineered super-stabilized Hsp90 to developed a yeast system to identify clients that rely on Hsp90 ATPase activity. Using this approach,

we find that Hsp90 mutants deficient for ATP binding or hydrolysis have differential impacts on the activation of kinase and hormone receptor clients in vivo. These results provide a rationale for understanding anti-cancer drugs that competitively bind to the ATPase site of Hsp90.

Heme Proteins

2240-Pos Board B210

Reactive Vibrational Dynamics of Iron in Heme

Alexander Barabanschikov[1], J. Timothy Sage[1], W. Robert Scheidt[2], Chuanjiang Hu[2], Minoru Kubo[3], Paul M. Champion[1], Jiyong Zhao[4], Wolfgang Sturhahn[4], E. Ercan Alp[4].
[1]Northeastern University, Boston, MA, USA, [2]University of Notre Dame, Notre Dame, IN, USA, [3]University of Hyogo, Hyogo, Japan, [4]Advanced Photon Source, Argonne National Lab, Argonne, IL, USA.

Nuclear resonance vibrational spectroscopy (NRVS) measurements supported by DFT calculations identify vibrational modes of the iron atom in halide derivatives of iron porphyrins. These compounds capture many essential aspects of heme geometry and vibrations. The smaller (porphine) models simplify the vibrational spectrum and enable accurate analysis using DFT methods. NRVS identifies both doming and Fe-halide stretching components of the reaction coordinate with confidence. Correlation analysis between 4-coordinate and 5-coordinate compounds suggests significant mixing between Fe-ligand and heme modes. Measurements and calculations on larger porphyrins reveal the effect of peripheral groups. Measurements on oriented porphine halide crystals definitively identify the contribution of in plane and out of plane Fe motion. The frequency of heme doming vibrations varies in a systematic manner with peripheral substitution and halide mass, which will allow us to evaluate their contributions to vibrational signals that follow femtosecond laser excitation. Such measurements will ultimately enable quantitative estimates of the energetics of molecular distortions that modulate reaction rates in heme proteins.

2241-Pos Board B211

Low frequency dynamics of Cystathionine beta-synthase

Karunakaran Venugopal[1], Yuhan Sun[1], Zhenyu Zhang[1], Abdelkrim Benabbas[1], Sangita Singh[2], Ruma Banerjee[2], Paul M. Champion[1].
[1]Northeastern University, Boston, MA, USA, [2]University of Michigan, Ann Arbor, MI, USA.

Femtosecond coherence spectroscopy is used to study the low frequency dynamics of cystathionine beta-synthase (CBS). CBS is a pyridoxal-5′-phosphate-dependent heme enzyme with cysteine and histidine axial ligands that catalyzes the condensation of serine and homocysteine to form cystathionine. Resonance excitation near the maximum of the ferric state Soret band reveals a mode near ~40 cm^{-1} (phase ~pi/2). The phase indicates that the initial non-equilibrium coherent wavepacket for this mode is dominated by a momentum displacement. This is consistent with doming of the ferric five-coordinate species and suggests photolysis of the histidine ligand. When exciting on the red side of the Soret band, a mode near ~25 cm^{-1} is observed that exhibits a phase jump of ~pi for blue-side excitation. This mode may involve the response of an unphotolyzed fraction of hot ferric six-coordinate species, subsequent to ultrafast non-radiative decay. A strong correlation between the ''detuned'' coherence spectrum (which reveals higher frequencies) and the Raman spectrum is also demonstrated. Normal coordinate structural decomposition of the ferric heme crystal structure predicts strong saddling, doming, and ruffling modes and they are observed in the coherence spectra. The relative intensities of these modes are monitored as a function of pH in order to explore the potential correlation between redox equilibria, pH, and protein-induced heme structural perturbations. The low frequency spectra of ferrous CBS and its NO-bound complex were also obtained, along with the CO rebinding kinetics. The geminate rebinding of CO to CBS was found to be unusually fast and similar to that of CooA.

2242-Pos Board B212

Proximal Ligand Switch Triggered by Carbon Monoxide in Inducible Nitric Oxide Synthase

Joseph Sabat, Denis L. Rousseau, Syun-Ru Yeh.
Albert Einstein College of Medicine, Bronx, NY, USA.

Inducible Nitric Oxide Synthase (iNOS) is one of three isoforms of NOS, responsible for the oxidation of L-Arginine to L-citrulline and nitric oxide (NO). This isoform is implicated in the pathophysiology of several inflammatory disorders including arthritis, atherosclerosis, and transplant rejection. iNOS is unique among the isoforms in that it is not regulated by the intracellular calcium concentration. Instead, iNOS is exclusively regulated at the transcriptional and molecular levels. One molecular regulator of iNOS is Carbon Monoxide (CO) generated by heme oxygenase (HO-1), an inducible enzyme that produces CO and is known to mediate anti-inflammatory effects. It has been shown that CO binding to iNOS promotes its gradual conversion to an inactive ''p420'' form. On this basis, we hypothesize that the cross-talk between HO-1 and iNOS plays an important role in attenuating the activity of iNOS and modulating inflammatory responses in vivo. The structure of the iNOS p420 has been a subject of debate, as the proximal ligand has been proposed to be either a histidine residue or a protonated, neutral thiol form of the native cysteine thiolate. In this work, we use resonance Raman Spectroscopy to explore the properties of the p420 derivative of iNOS in order to resolve this issue. We show that the nanosecond time-resolved Raman spectrum of iNOS p420 exhibits a band consistent with an iron-histidine stretching mode. To evaluate the identity of the proximal ligand of iNOS p420, we measured the Raman spectra of the H93G cavity mutant and organic model compounds with a neutral thiol coordinated to them as reference systems. On the basis of these studies, we postulate a novel reversible ligand-switching mechanism that may be critical for the in vivo regulation of iNOS activity involving endogenous CO.

2243-Pos Board B213

Resonance Raman Investigation of the R481 Mutants of Cytochrome *c* Oxidase from *R. sphaeroides*

Tsuyoshi Egawa[1], Hyun-Ju Lee[2], Robert B. Gennis[2], Syun-Ru Yeh[1], Denis L. Rousseau[1].
[1]Albert Einstein College of Medicine, Bronx, NY, USA, [2]The University of Illinois, Urbana, IL, USA.

The enzymes of the heme-copper oxidase superfamily have a highly conserved arginine residue (R438, R481, and R473 of the bovine, *R. sphaeroides*, and *P. denitrificans* cytochrome *c* oxidases, respectively), which is located close to the heme-propionate substituents of the heme *a* and heme a_3 moieties. To explore the structural and functional implications of this conserved arginine, we used resonance Raman spectroscopy to study the heme vibrational spectra of the R481 mutant proteins (R481H, R481Q, and R481L) of cytochrome *c* oxidase from *R. sphaeroides*. All the mutants showed significant down-shifts in the C=O stretching vibrational frequencies of the heme *a* and a_3 formyl substituent groups in the fully oxidized state, while they showed up- and down-shifts of the *a* and a_3 formyl C=O stretching modes, respectively, in the fully reduced state. On the basis of these observations, the role of the conserved arginine will be discussed.

2244-Pos Board B214

Indoleamine 2, 3- Dioxygenases 2: The Missing Link For The 1-methyl-D-trp Mechanism Of Action?

Laura B. Granell-Ortiz, Syun- Ru Yeh.
Albert Einstein College of Medicine, Bronx, NY, USA.

Indoleamine 2, 3-dioxygenase (IDO1) is one of the only two heme-containing enzymes that catalyze the first and rate-limiting step of the kynurenine pathway of L-Trp metabolism. IDO1 has been implicated in the escape mechanism of cancer cells from immune surveillance. Consequently, IDO1 has been recognized as an important anticancer drug target. Recent studies showed that an IDO1 inhibitor, 1-methyl-Trp (1-M-Trp), triggers antitumor immunity and can be used to improve the efficacy of traditional chemotherapeutic drugs. Preclinical studies showed that the D stereoisomer of 1-M-Trp exhibits superior antitumor activity; however, it is less potent for the purified enzyme. On this basis, a second isoform of IDO1 had been suspected. It was not until last year that this second isoform of IDO1, named IDO2, was identified. To understand the missing link for the D-1-M-Trp mechanism of action, we have cloned, expressed and purified recombinant human IDO2. Resonance Raman and optical absorption spectroscopic studies showed that IDO2 exhibits structural features slightly different from IDO1. Furthermore, an activity assay with D- or L-Trp shows that IDO2 does not produce N'-Formyl-kynurenine as IDO1 does; instead, a new product with an absorption maximum at 344 nm was produced. Stopped-flow measurements show that IDO2 binds O2 in a similar fashion as IDO1. Together these data indicate novel function and action mechanisms of IDO2 that are distinctive from IDO1.

2245-Pos Board B215

Linking Heme Activation to Conformation Change in Hemoglobin Via Chain Selective Time-resolved Resonance Raman Spectroscopy on Mesoheme Hybrids

Gurusamy Balakrishnan[1], Mohammed Ibrahim[1], Piotr J. Mak[2], Jessica Hata[2], James R. Kincaid[2], Thomas G. Spiro[1].
[1]Univ Washington, Seattle, WA, USA, [2]Marquette Univ, Milwaukee, WI, USA.

Time-resolved Resonance Raman spectra are reported for Hb tetramers, in which the α and β chains are selectively substituted with mesoheme. Hb function is unaffected by the substitution, but the Soret absorbtion band shift in meso- relative to proto-heme permits chain-selective excitation of heme RR spectrum. The evolution of these spectra following HbCO photolysis show that geminate