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

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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

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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

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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*

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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?

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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

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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