a partial inhibitor, but in mammals, arginine is an activator. We used Thermofluor methodology to determine if the effect of arginine on the thermal stability of NAGS parallels its effects on NAGS activity. Addition of arginine to bacterial NAGS, which is inhibited by arginine, resulted in a destabilized protein. Addition of arginine to the zebrafish and mouse NAGS stabilized both proteins, despite opposing effects of arginine on their enzymatic activity. We then used analytical gel chromatography to determine if changes in oligomerization state of NAGS could occur upon arginine binding. Our results indicate that bacterial and mammalian NAGS appear to be ensembles of molecules with different oligomerization states that are in rapid exchange with each other. Upon addition of arginine, the partition coefficient of both NAGS increased. The behavior of zebrafish NAGS was different. It eluted as two peaks suggesting two distinct oligomerization states. Upon addition of arginine to zebrafish NAGS the partition coefficients of both peaks decreased. These studies indicate that the effect of arginine on the biophysical properties of NAGS indeed changed during evolution and suggest that the inversion of the allosteric effect and stabilization effects of arginine on NAGS could be linked.

289-Pos Board B89
Heparin Activates PKR by Inducing Dimerization
Eric Anderson, Willythssa Pierre-Louis, C. Jason Wong, Jeffrey W. Lary, **James Cole**.
The RNA activated kinase PKR plays a key role in the innate immunity response to viral infection. PKR is induced by interferon in a latent form that is activated by binding double stranded RNA (dsRNA) or RNAs that contain duplex regions to undergo autophosphorylation. Activation by dsRNA is mediated by PKR dimerization. PKR can also be activated by heparin, a highly sulfated glycosaminoglycan. PKR activation by heparin does not require the presence of the dsRNA binding domain and heparin does not compete with dsRNA for binding to PKR, indicating that heparin and dsRNA bind at different sites. We have characterized the mechanism of PKR activation by heparin oligosaccharides of defined sizes. The smallest heparin capable of robust PKR activation is the hexasaccharide (dp6). PKR binding affinity is strongly dependent on both the heparin length and ionic strength. Dissociation constants measured in 75 mM NaCl vary from ~230 nM for dp8 to 32 uM for dp2. Sedimentation velocity measurements using interference and fluorescence detection indicate that dp8 binds to monomeric PKR and also increases PKR dimerization. The velocity data fit well to a classical linkage model where heparin binding is linked with PKR self-association with a coupling free energy of $\Delta G = -0.80$ kcal/mole. Thus, both dsRNA and heparin activate PKR by enhancing dimerization.

Physical Chemistry of Proteins and Nucleic Acids

290-Pos Board B90
Cooperativity in a Cluster of Carboxylic Groups in the Active Site of a Protein
Brian M. Doctrow, Carlos A. Castañeda, Carolyn Fitch, Ananya Majumdar, Jamie L. Schlessman, Maja Cieplak, Bertrand García-Moreno.
The active site of staphylococcal nuclease (SNase) has a cluster of four carboxylic residues, two with near normal pK_a values, one (Asp-19) with a low pK_a of 2.1, and one (Asp-21) with an unusually elevated pK_a of 6.5. Crystal structures provide little insight into why Asp-21 absorbs all the repulsive interactions while Asp-19 is actually stabilized in its charged form. To examine this unusual example of cooperativity the pK_a values of Asp and Glu residues were measured using NMR spectroscopy in variants in which ionizable groups in the active site were replaced with neutral ones. The low pK_a of Asp-19 reflects hydrogen bonds and a favorable Coulomb interaction whereas the elevated pK_a of Asp-21 reflects repulsive Coulomb interactions with the other carboxylic groups in the cluster. Even in the variant where all repulsive interactions within the cluster are eliminated, the pK_a of Asp-21 is elevated relative to the pK_a of Asp in water due to its ability to act as a hydrogen bond donor in the protonated state. The amplification of small perturbations is a hallmark of cooperative systems and is precisely why structure-based calculations of pK_a values in clusters of ionizable groups, such as those present in the active sites of many proteins, are extremely challenging. pK_a calculations with a variety of continuum electrostatics methods using static and MD-relaxed structures were performed to illustrate the difficulties inherent to pK_a calculations in cooperative systems, where calculations can fail owing to amplification of small and unavoidable inaccuracies in structure and in assumptions built into the calculations.

291-Pos Board B91
Potential Role of Forbidden Disulfides in Zn Signalling
Dhakshinari V.K. Hulugalle, Naomi L. Haworth, Sara Ballouz, Jason Y. Liu, Samuel W. Fan, **Merridee A. Wouters**.
Expulsion of Zn^{2+} from proteins following oxidation of ligating Cysteine residues is an emerging area of the oxidative stress response. During a recent data mining survey of protein structures with pairs of thiols in both reduced and oxidized (disulfide bonded) states, we found two structural motifs repeatedly associated with Zn^{2+} binding (1). Forbidden disulfides are a canonical set of disulfides with abnormal stereochemistry associated with redox-activity. Here we show through systematic analysis of Zinc finger structures and sequences, that one of these motifs is extremely prevalent in Zinc fingers. We show that in around 50% of Zinc finger structures two of the Zn^{2+}-ligating thiols are embedded in a secondary structure similar to an anti-parallel β-diagonal disulfide-like motif (aBDD), located on the β-hairpin structure known as a Zinc knuckle. Formation of a disulfide by thiols of this motif has recently been characterized in the molecular chaperone Hsp33 and also demonstrated in several other transcription factors (2). Although other forbidden disulfide motifs are occasionally present in Zinc fingers, none are as ubiquitous as this aBDD-like motif. We show that the presence of this motif and its position in the structure is characteristic of different types of Zinc fingers, suggesting a functional relationship. As Zinc fingers comprise more than 17% of the human genome, this motif is likely important in Zn^{2+} signalling.
1. Fan SW, George RA, Haworth NL, Feng LL, Liu JY, Wouters MA. Conformational changes in redox pairs of protein structures. *Prot. Sci.* 18: 1745–1765, 2009.
2. Ilbert M, Horst J, Ahrens S, Winter J, Graf PCF, Lilie H, Jakob U. The redox-switch domain of Hsp33 functions as dual stress sensor. *Nat. Struct. Mol. Biol.* 14: 556–563, 2007.

292-Pos Board B92
Enzymatic Properties and Spectroscopic Studies of Photoresponsive Lysozyme
Taeko Inada, Ayuri Kamei, Koichi Kikuchi.
Photochromic compounds such as azobenzene derivatives change their chemical and physical properties reversibly with their structural change by photo irradiation. This character may be useful for regulation of functions of biomaterials, analysis of their reaction mechanism, and development of function materials. Previously, we have reported that photoresponsive lysozymes were modified with azobenzene derivatives at a position-specific residue (Lys33, or Lys116) on hen egg white lysozyme, and their enzymatic properties were reversibly modulated according to photoisomarization of azobenzene moiety. In this study, hen egg white lysozyme was modified with 4-(phenylazo) aniline at Asp 87, which forms an amphiphilic helix with hydrophilic residues directed toward the solvent. The enzyme reaction, and the fluorescence, transient absorption, and circular dichroism spectroscopies were studied to clarify the photoregulation mechanism. In MALDI TOF mass analysis, azobenzene moiety binds at Asp 87 through amide binding was revealed. The enzyme kinetics parameters of the modified lysozyme were modulated reversibly according to isomerization of azobenzene moiety: the catalytic efficiency for cell wall of Micrococcus lysodeikticus of trans form was more than tenfold that of cis form. The binding constant with a substrate analogue (tri-N-acetyl-D-glucosamine) was also reduced by isomerization from trans form to cis form. Moreover the efficiency of energy transfer to Eosin Y modulated reversibly by photo irradiation; in absence of the substrate analogue, the efficiency of trans form was higher than that of cis form, while in the presence of the substrate analogue, this efficiency shows opposite behavior. The fluorescence and circular dichroism spectroscopic studies suggest that the modulation of enzymatic properties is mainly induced by a steric hindrance due to the conformational changes in the vicinity of modification position.

293-Pos Board B93
Quantification of Photosensitized Singlet Oxygen Production by a Fluorescent Protein
Xavier Ragàs, Laurie P. Cooper, John H. White, Santi Nonell, **Cristina Flors**.
Fluorescent proteins are increasingly becoming actuators, rather than just sensors, in a range of cell biology techniques. One of those techniques is chromophore-assisted laser inactivation (CALI), which is employed to specifically inactivate the function of target proteins or organelles by producing photochemical damage [1]. CALI is achieved by the irradiation of dyes that are able to produce reactive oxygen species (ROS). The combination of