

recombination rates and yields are the same for the two chains, consistent with recent results with  $^{15}\text{N}$ -heme isotopomer hybrids. The spectra also reveal systematic shifts in the deoxy-heme  $\nu_4$  and  $\nu\text{Fe-His}$  RR bands, which are anti-correlated. These shifts are resolved for the successive intermediates in the protein structure, as previously determined from time-resolved UVRR spectra. Both chains show Fe-His bond compression in the immediate photoproduct, which relaxes during the formation of the first intermediate,  $R_{\text{deoxy}}$  (0.07  $\mu\text{s}$ ), in which the proximal F-helix is proposed to move away from the heme. Subsequently, the Fe-His bond weakens, more so for the  $\alpha$  than the  $\beta$  chains. The weakening is gradual for the  $\beta$  chains, but abrupt for the  $\alpha$  chains, coinciding with completion of the R-T quaternary transition, at 20  $\mu\text{s}$ . Since the transition from fast- to slow-rebinding Hb also occurs at 20  $\mu\text{s}$ , the drop in the  $\alpha$  chain  $\nu\text{Fe-His}$  supports the localization of ligation restraint to tension in the Fe-His bond, at least in the  $\alpha$ -chains. The mechanism is more complex in the  $\beta$  chains.

#### 2246-Pos Board B216

##### Production Of Bioactive NO From The Reaction Of Met-nitrite Hemoglobin With NO: Use Of Glassy Matrices And Sol-gels

Mahantesh Navati, Camille R. Roche, Joel M. Friedman.

Albert Einstein College of Medicine, Bronx, NY, USA.

There is growing evidence that nitrite-mediated reactions of Hb can generate bioactive forms of nitric oxide. The nitrite reductase reaction in which deoxy Hb reacts with nitrite has been studied from this perspective; however, this reaction generates NO under conditions where it can be easily scavenged. In contrast the proposed reaction of NO with nitrite bound to ferric heme derivatives to generate  $\text{N}_2\text{O}_3$  is a much more promising mechanism. This follows because  $\text{N}_2\text{O}_3$  can rapidly react with thiol containing peptides such as glutathione to produce S-NO derivatives. These species are likely to be the relatively long lived bioactive forms of NO that produce positive physiological effects in the vascular endothelium as occurs for blood substitutes derived from PEGylated Hb. We present results obtained using Hb samples encased in either glassy matrices or sol-gels that directly support this model. In particular there is spectroscopic evidence for the formation of a ferrous intermediate resulting from the direct reaction of NO with met-nitrite derivatives that precedes the formation of either ferrous NO or ferrous CO derivatives. This intermediate is attributable to a ferrous  $\text{N}_2\text{O}_3$  complex.

#### 2247-Pos Board B217

##### Novel Catalytic Antioxidative Activity of Nitroxide Radicals in the Heme/H<sub>2</sub>O<sub>2</sub> system

Uri Samuni.

Queens College, Flushing, NY, USA.

Deleterious reactive oxygen species (ROS), are involved in cellular damage, dysfunction and diseases. Cell-permeable stable nitroxide radicals (RNO $\cdot$ ), effectively protect against ROS-induced injury in cell-free, cellular, isolated organs models as well as in whole animals. Unlike common antioxidants, which operate stoichiometrically, and are progressively depleted particularly under oxidative stress, RNO $\cdot$  can provide cytoprotection even at extremely low concentrations suggesting a catalytic mechanism. RNO $\cdot$  antioxidative activity involves 1-electron exchange between its reduced (RNOH) and oxidized (RNO $^+=\text{O}$ ) states, which allows its recycling. However, in spite of the significant therapeutic potential of nitroxides still, their protective mechanism(s) from ROS are not fully clear. A better mechanistic understanding of nitroxides activity in particular in the presence of heme proteins is essential for better selection of desired nitroxides, improvement of their efficacy, and minimizing potential adverse effects. Our objective is to elucidate the catalytic mechanism of nitroxides reaction under oxidative stress in the presence of heme proteins. We have studied RNO $\cdot$  reaction in the heme/H<sub>2</sub>O<sub>2</sub> system and have found the kinetics to be far more complex than previously assumed. Our recent electron paramagnetic resonance (EPR) spectrometry results indicate transient oscillatory changes in [RNO $\cdot$ ] during the catalytic dismutation of H<sub>2</sub>O<sub>2</sub> and detoxification of the deleterious oxidized heme species such as heme protein radical and ferryl. Our results suggest that a key element in the catalytic antioxidative activity of nitroxides in the presence of heme/H<sub>2</sub>O<sub>2</sub> is a Belousov-Zhabotinsky like reaction mechanism, involving two reaction pathways (possibly ionic and radical).

#### 2248-Pos Board B218

##### Conformational Changes Of Ferricytochrome c Induced by pH and Temperature

Jonathan Soffer.

Drexel University, Philadelphia, PA, USA.

This investigation is a comparison between the native and non-native states of ferricytochrome c as it is adopted between a pH of 1 to 13 and temperature of 278K to 353K. Visible electronic circular dichroism [ECD] and absorption spectroscopy were employed to probe the changes occurring at low ionic

strength within the heme environment. The data presented is of the Soret band with the Q-band data and charge transfer band profile currently being gathered. At pH 2, state I, shows the emergence of a negative band, which is believed to represent the dissociation of both ligands from the heme. At this pH as the protein becomes unfolded and the iron is in a high spin state (Dyson, H, J.Biol.Chem, 1982), as the protein environment was acidified there was an emergence of a Cotton band in the CD spectra, also the intensity of the bands decreased, starting at pH 4. Approaching state III, as the iron enters a low spin state, there is a stronger couplet that emerges reflecting band splitting which is predominantly caused by a combination of electronic and vibronic perturbations (Reinhard Schweitzer-Stenner, J. Phys. Chem. B, 2008) and is maintained below 343K. This is suggesting that there is a conformational transition from the native state, into a thermally activated intermediate state, affecting the internal electric field causing moderate rearrangements of the heme, until it enters its thermally unfolded state. This unfolded state of the protein consistently becomes populated at higher temperatures across the pH range. This couplet remains into pH 9 which could reflect an intermediate transition of state III into state IV, moving to more alkaline states this couplet disappears. This study has overall shown significant heterogeneity of the protein throughout the pH range.

#### 2249-Pos Board B219

##### Investigation Of The Role Of Neuroglobins In Globin Redox Reactions

Priscilla Tosqui, Marcio Colombo.

unesp, Sao jose do rio preto, Brazil.

Neuroglobin (Ngb) is a recently identified member of the globins family. In the absence of external ligands, it is hexa-coordinated with a bis-histidyl heme at physiological conditions. This novel protein may protect the cell from hypoxia, since the protein is expressed whereas there is a raise in ROS (reactive oxygen species), typical of this condition. Unlike other hemoproteins such as Hemoglobins (Hbs) and Myoglobins (Mbs), Ngbs are not oxidized by hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). In this work we have investigated by UV-Vis spectroscopy the influence of mouse ferric Ngb on the redox reactions between human ferrous and ferric Hb with  $\text{H}_2\text{O}_2$ , a former of ROS species. The reaction of  $\text{H}_2\text{O}_2$  with ferrous Hb produces the oxidation of the heme to the ferric metaHb ( $\text{Fe}^{+3}$ ) state and X-ferryl-Hb ( $\text{Fe}^{+4}$ ) state; the latter state is an intermediate species which reacts with Hb- $\text{O}_2$  ( $\text{Fe}^{+2}$ ) exchanging electrons to produce metaHb. We show in this work that the amount of  $\text{H}_2\text{O}_2$  required to induce the formation of ferryl and met-Hb, and from there to oxoferryl species, increases in the presence of Ngb. We have studied the effect of other known ROS scavengers, such as superoxide-dismutase and catalase, to compare with that observed in the presence of Ngb. Taken together the results of these studies suggest that Ngb might participate in the redox reactions, probably acting as a protector of globins from ROS. Further studies are in course to elucidate the mechanism of these reactions.

#### 2250-Pos Board B220

##### Conformational Heterogeneity of Cytochrome c Probed by Resonance Raman Spectroscopy as a function of pH and Temperature

Maria Alessi<sup>1</sup>, Andrew Hagarman<sup>1</sup>, Reinhard Schweitzer-Stenner<sup>2</sup>.

<sup>1</sup>Drexel university, Philadelphia, PA, USA, <sup>2</sup>Drexel University, Philadelphia, PA, USA.

The oxidized state of cytochrome c is a subject of continuous interest due to the multitude of conformations the protein adopts. Despite numerous studies, native and non-native states of ferricytochrome c have not been comprehensively analyzed regarding the influence of solvent conditions on structure, function, and thermodynamic equilibrium. Compared to the oxidized state, the reduced state of cytochrome c is rather stable since it adopts one conformation over a broad pH (2-12) and temperature ( $\sim 100^\circ\text{C}$ ) range. In the current study, we have analyzed the high frequency (1200-1800 $\text{cm}^{-1}$ ) Soret and Q-band resonance Raman spectra of oxidized and reduced horse heart cytochrome c (hbc) in terms of depolarization ratios and normalized Raman intensities, as a function of increasing pH and temperature. Initial analysis of our data collected for the Soret band resonance indicates that the depolarization ratios of  $A_{1g}$  modes  $\nu_2$ ,  $\nu_3$ , and  $\nu_4$  of the native state III and the alkaline state IV are practically identical. They deviate from the respective  $D_{4h}$ -value, indicating that  $B_{1g}$  (trigonal) or  $B_{2g}$  (rhombic) type distortions affect the Raman tensor. For state III, the depolarization ratios of  $B_{1g}$  modes  $\nu_{10}$ ,  $\nu_{11}$ , and  $\nu_{13}$  deviate substantially from the expectation value indicating that these modes are affected by a large  $B_{1g}$  type distortion. The alkaline III $\rightarrow$ IV transition moves the depolarization ratios of these  $B_{1g}$  modes closer to their  $D_{4h}$ -value of 0.75, indicating a substantial decrease of the rhombic  $B_{1g}$ -type deformation. Data from pH 12, which favor the population of the V-state, suggest a substantial increase of rhombic deformations, apparently caused by the replacement of a lysine by a hydroxyl ligand. Currently we are analyzing the depolarization ratios for the Q band resonance and the high temperature data for both resonances.