

achieved by these methods. Statistical analysis has been revealed at least six fine individual structures for HSA. Each structures present individual and exclusive function. It can be concluded that, albumin unique features and functions are due to its switching capability to different possible fine structures with the lowest energy exchange.

**Keywords:** Human Serum Albumin, Fine Structures, Structural And Functional Alteration.

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**Abstract No.287**

**Mixed Quantum Mechanical/ Molecular Mechanical (QM/MM)  
Study of the S-nitrosylation Reaction 4-phenyl-3-  
Furoxan carbonitrile as Inhibition of Thioredoxin Glutathione  
Reductase (TGR)**

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Combined quantum mechanics/molecular mechanics (QM/MM) methods allow computations on chemical events in large molecular systems. We present a theoretical study of a mechanism for the S-nitrosylation Reaction 4-phenyl-3-furoxan carbonitrile or furoxan belongs to an oxadiazole-2-oxide class whose therapeutic effects are largely due to their inhibition of thioredoxin glutathione reductase (TGR) for the control of schistosomiasis bases in QM/MM calculations at the DFT RB3LYP/6-31G+(d)//SCC-DFTB AMBER level. The activity of the oxadiazole 2-oxides was associated with a donation of nitric oxide. We first attempted to dock inhibitor in the active site of enzyme available in the Protein Data Bank, (PDB codes: 3H4K), to see how intact inhibitor would bind. Since our interest was focused on the region where reaction should take place, all molecules that might potentially be involved in the mechanism of nucleophilic attack, namely furoxan, residues Cys159, and nearby water molecules, were treated by QM, while the rest of the protein, as well as the bulk water, were treated by traditional molecular mechanics. As soon as a preliminary minimization of complex was carried out on TGR using the hybrid QM/MM potential, migration of the Cys159 to the carbon 3 furoxan was observed. We were quite surprised by this outcome, while the formation of the tetrahedral intermediate might be a reasonable explanation for location of the initial nucleophilic attack shows this mechanism: The potential

energy of the system with respect to the initial state ( $\Delta U$ ) along the minimum energy reaction path (MERP) is also reported with the 3D structures of relevant intermediates. The results are consistent with the available experimental data and provide new insight into the detailed mechanism of this important reaction.

**Keywords:** Quantum Mechanic, Molecular Mechanical, Thioredoxin, Glutathione Reductase.

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**Abstract No.288**

**Effect of Glycine, as Chemical Chaperone, on Catalase  
Glycation by Glucose**

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Oxidative mechanisms are thought to have a major role in several biological phenomena, including cataract formation and diabetic complications. Glycation of catalase, as a powerful antioxidant enzyme, by glucose alters its structure and function and increases the risk of cataract formation in diabetic patients. Glycine is a chemical chaperones that inhibit protein glycation and stabilize them against thermal and chemical denaturation. Therefore, we investigated the effects of this chemical chaperone on catalase glycation. Catalase solution, pH 7.4, was incubated separately with glucose in the presence or absence of glycine at 37°C in a shaker incubator. The aliquots were collected every week and stored at -80°C. Then, they were analyzed by fluorescence spectroscopy, circular dichroism spectroscopy (CD) and polyacrylamide gel electrophoresis (PAGE). The activity of catalase in each sample was also determined. Structure and activity of catalase were changed due to the glycation with glucose. Glycation caused an increase in the fluorescence emission and electrophoretic mobility and a decrease in the alpha helix content and catalase activity. Glycine inhibited this phenomenon and the fluorescence emission, alpha helix content, electrophoretic mobility and activity of catalase were closed to the normal values. Our results indicated a decrease in the catalase glycation by glucose in the

presence of glycine. It implies the usefulness of this\e chemical chaperone in reduction of diabetic complications such as cataract.

**Keywords:** Catalase, Chemical Chaperone, Glycation, Glycine.

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#### Abstract No.289

##### Thermodynamic Aspects of Disulfide Bridge Introduction and Bioluminescence Color Shift in Firefly Luciferase

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Relationship between stability, color shift and position of flexible loop in firefly luciferase is investigated. Bioluminescence reaction, which uses luciferin, Mg<sup>2+</sup>-ATP and molecular oxygen to yield an electronically excited oxyluciferin, is carried out by the luciferase and emit visible light. Multi-color bioluminescence is developed using introduction of single/double disulfide bridges in firefly luciferase through structural stabilization and displacement of functional loops. The bioluminescence color of firefly luciferases is determined by the luciferase structure and assay conditions. Single and double disulfide bridges are introduced into firefly luciferase to make separate mutant enzymes. By introduction of disulfide bridges using site-directed mutagenesis in *Photinus pyralis* luciferase the color of emitted light was changed to red or retained. Multicolor bioluminescence is accompanied with displacement of critical loops in red-emitter luciferases without significant changes in green emitter mutants. Thermodynamic analysis revealed that among mutants, L306C-L309C mutant with a single disulphide bridge shows a remarkable stability against urea denaturation and also considerable increase in kinetic stability with changes in emission spectra towards red.

**Keywords:** Bioluminescence, Disulfide Bridges, Luciferase, Red-Emitter, Site-Directed Mutagenesis.

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#### Abstract No.290

##### Biotechnological Applications of Peroxidases

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Peroxidase enzymes that may find ubiquitously in animals, plants and microorganisms, are able to oxidize a wide range of reductants in the expense of peroxides as acceptors[1,2]. Most part of our knowledge on the structure–function relationships and catalytic mechanisms of peroxidases is indebted on horseradish peroxidase. In classical peroxidases, ferric protoporphyrin IX is the prosthetic group and imidazole the fifth iron ligand. The characteristic activity of peroxidases is two electron oxidation, which proceeds through formation of compounds I and II. Compound I is reduced back to the ferric resting state either by a two sequential one-electron transfer processes from peroxidase to substrates or by two-electron oxidation process associated with the ferryl oxygen transfer to substrates[3]. The radicals produced in the reaction generally evolve nonenzymatically to nonradical products by pathways characteristic of each substrate (coupling, dismutation etc.). Chloroperoxidase (CPO) is unique among the peroxidases because it contains a cysteinic thiolate as the fifth axial ligand of the heme instead of the imidazole ligand. For this reason, many of its spectroscopic and chemical properties are similar to those of cytochrome P-450. CPO is unusually versatile: it catalyzes not only the reactions typical of peroxidases but also those of catalases and monooxygenases, and it is also almost unique in catalyzing halogenation reactions in the presence of halide ions and H<sub>2</sub>O<sub>2</sub>. Peroxidase-catalyzed reactions exist in four categories[4]: 1. Oxidative dehydrogenation 2. Oxidative halogenations 3. H<sub>2</sub>O<sub>2</sub> dismutation 4. Oxygen-transfer reactions: Benzylic/allylic hydroxylation, Alcohol oxidation, Epoxidation, Indole oxidation, Heteroatom oxidation (Sulfur oxidation, Nitrogen oxidation) Present work reviews and discusses recent biotechnological developments and applications of peroxidase family including homogeneous, heterogeneous, artificial peroxidases and miniperoxidases.

**Keywords:** Peroxidase, Chloroperoxidase, Application, Reaction.

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