# Toward Engineering Oxygenase Activity into the Electron Transfer Protein Azurin

Nathan Sieracki Final Seminar July 28, 2010

Structural knowledge of the metal environment is important in understanding the function of metalloproteins and is critical to harnessing their power. Modeling within a protein scaffold offers a pathway to rational design of desired function using Nature's toolbelt; to both gain novel insights into metal-mediated processes, and demonstrate true understanding through re-creation.[1] We seek specifically to model the function of the complex enzyme PHM (peptidylglycine α-hydroxylating monooxygenase) into a simple cupredoxin scaffold (see Figure 1).[2]

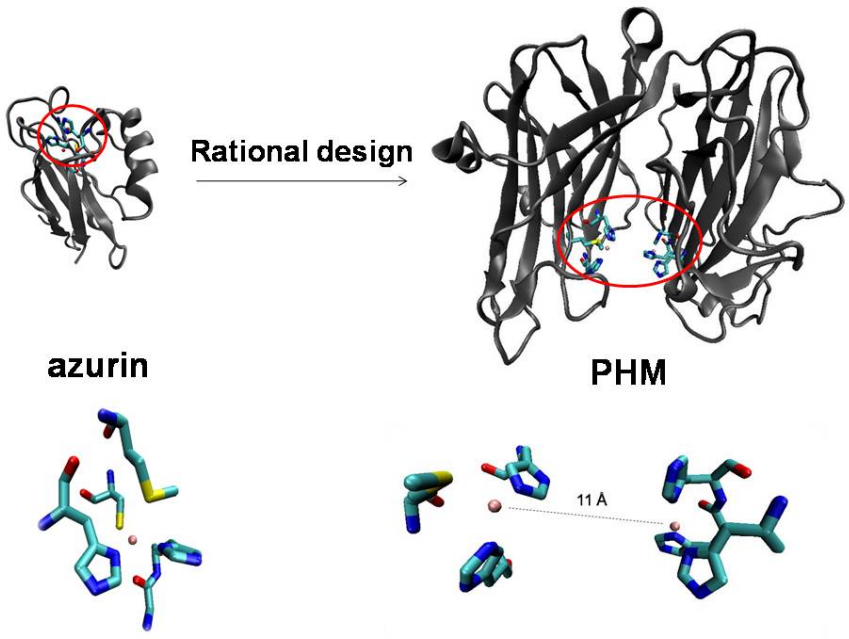


**Figure 1**: Engineering oxygenase activity into the electron transfer protein azurin (PDB code: 4AZU) through rational design inspired by PHM (PDB code: 1SDW).

The cavity mutant approach to small molecule activation within the azurin scaffold is demonstrated through description of the first reported Cu(II)-sulfenic acid species (see Figure 2). This species, prepared in high yield through copper mediated reduction of hydrogen peroxide, represents the first report of small molecule processing with a 'converted' electron transfer protein. Furthermore, it is the first report of a synthetic sulfenic acid functionality within the context of a protein. The latter is an important contribution to the field of protein design, as precise knowledge and control of cysteine oxidation state is an important parameter in dictating function in cysteine-containing native and designed peptides and proteins.[3]

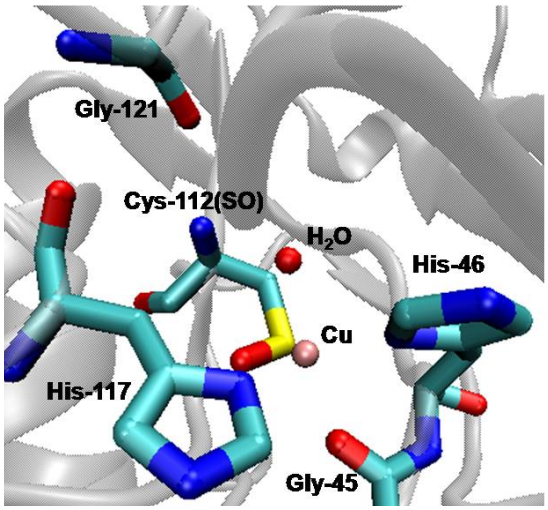


**Figure 2:** Computer model of Cu(II)SO-M121G azurin; generated using crystal parameters for Cu(II)-M121G azurin and crystal parameters for a coordinated sulfenate group from Co-containing thiocyanate hydrolase (PDB code: 2ZZD).

Second, a series of Type-2 azurin variants is reported in which the anionic redox 'chameleon' Cys-112 is replaced with neutral His-112, in an effort to develop a robust proteinic scaffold for modeling of PHM activity.[4] A series of intramolecular substrates (offering an accessible H-atom) were generated through site directed mutagenesis at the axial Met-121 position above the trigonal plane. The spectral characterization of the series is described, and mutant-dependent reactivity with added $O_2$ and ascorbic acid is described.

Low temperature effects on the pH of biological buffer solutions have been long established.[5] If left unaccounted for, these changes could lead to incorrect conclusions about the behavior of biological molecules at physiological conditions. The development of a temperature independent pH (TIP) buffer is described, along with brief demonstration of utility in low-temperature antibiotic storage, and low temperature spectroscopy.[6]

## References

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