Activating Water with an Artificial Metalloprotein

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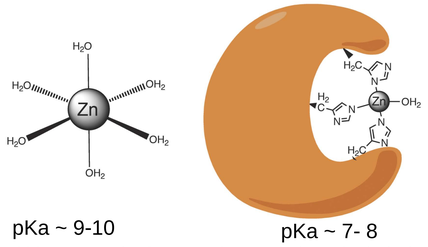
Background Information

Metalloproteins

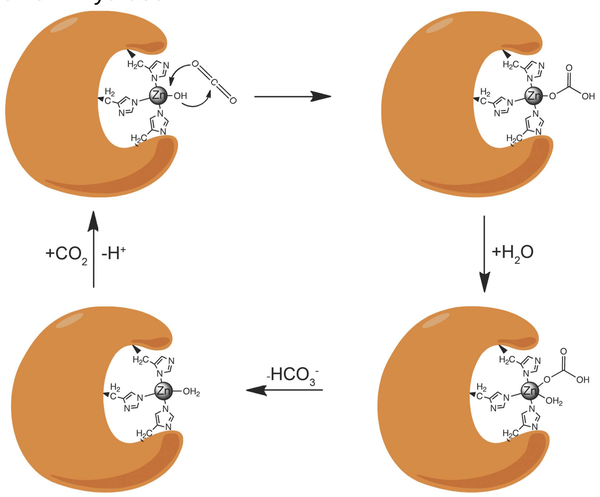
Metalloproteins are proteins with metal cofactors. We worked with both HPhen1 and MBPPhen2. The phenanthroline (phen) compound on the proteins is a bidentate ligand that binds to metal ions. The metal ions can then be used in the hydrolysis of other molecules.

Activated Water



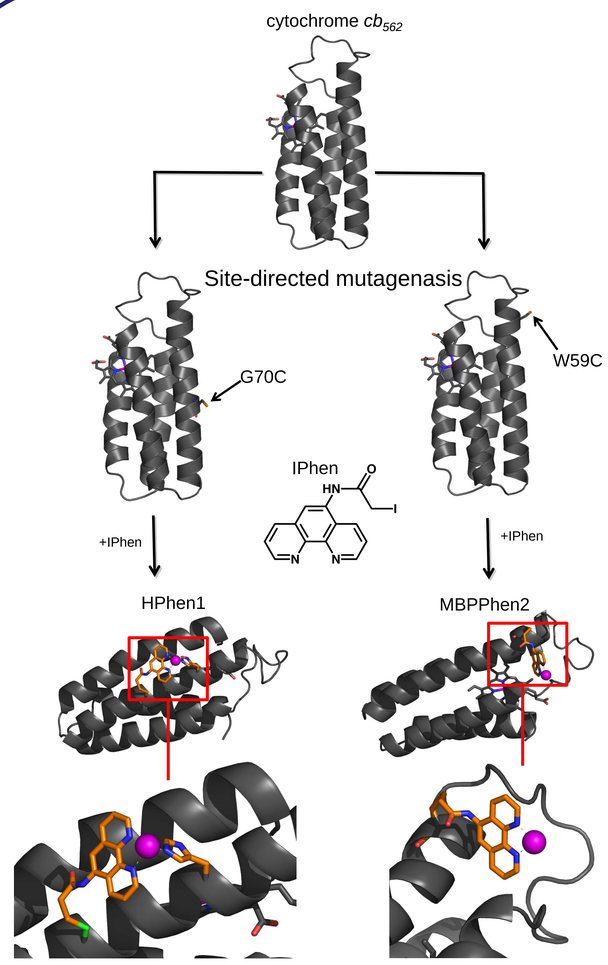
The metal ions within a protein framework can induce hydrolysis by creating activated water. The high Lewis acidity of the metal ion in a metalloprotein, such as carbonic anhydrase, lowers the pKa of the coordinated water making OH^- more favored.

Carbonic Anhydrase



Carbonic anhydrase is a physiological example of a metalloprotein. It is found in red blood cells and helps produce bicarbonate buffer. The protein has three histidines that are bound to a zinc molecule which is used to convert CO_2 into HCO_3^-.

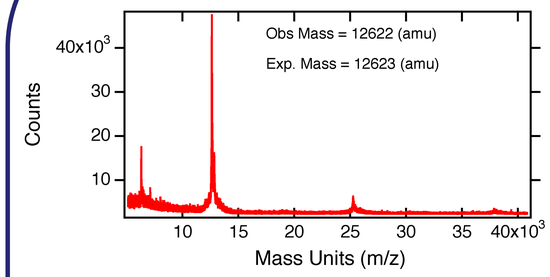
Creating the Model Systems



HPhen1 is the phen labeled G70C mutation of cytochrome cb_{562}. MBPPhen2 is the phen labeled W59C mutation of cytochrome cb_{562}. HPhen1 was chosen due to the stability of the engineered three coordinate metal binding motif. We worked with MBPPhen2 because phen has previously been shown to be located in a hydrophobic pocket. Both three coordinate metals and hydrophobic environments are common features in metalloproteins with the ability to activate water and these proteins allowed us to explore the reaction in a modular fashion.

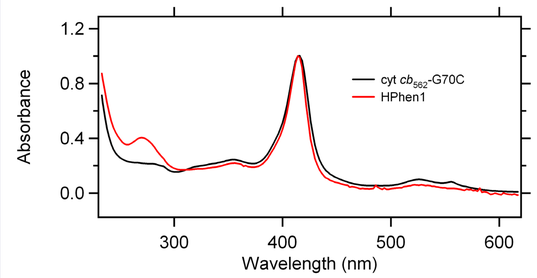
Protein Purification

MALDI Mass Spectrometry



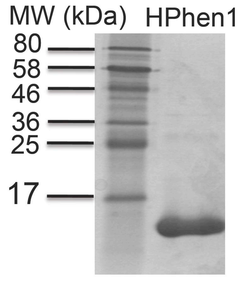
Matrix- assisted laser deprotonization/ ionization (MALDI) mass spectrometry of HPhen1 showed a main peak with an amu of ~12622 indicating that the protein was correctly labeled and purified.

UV Visible Spectrometry



The heme group in both proteins creates a peak in absorbance at 415nm that can be used to monitor protein concentration. We were able to determine if the protein was labeled because the phen group created a peak in absorbance at 270nm.

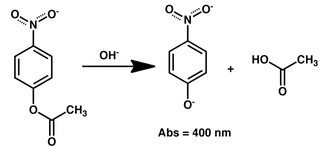
SDS-PAGE

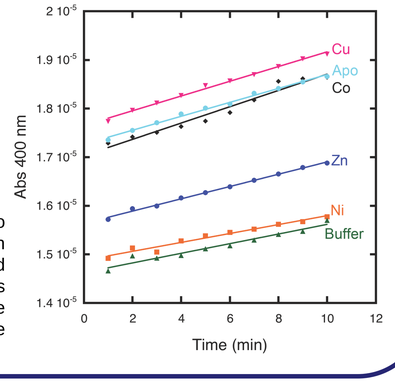


Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in order to test for any impurities in the protein. The dark color of the protein band is indicative of the high concentration of the protein and the lack of other bands indicates that it has few impurities.

Testing for Activated Water

Activity Assays

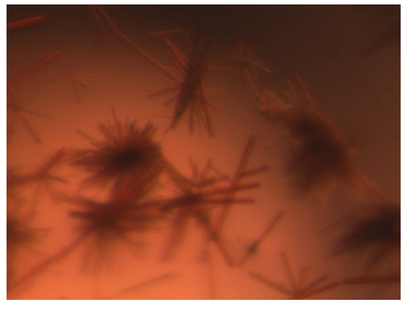




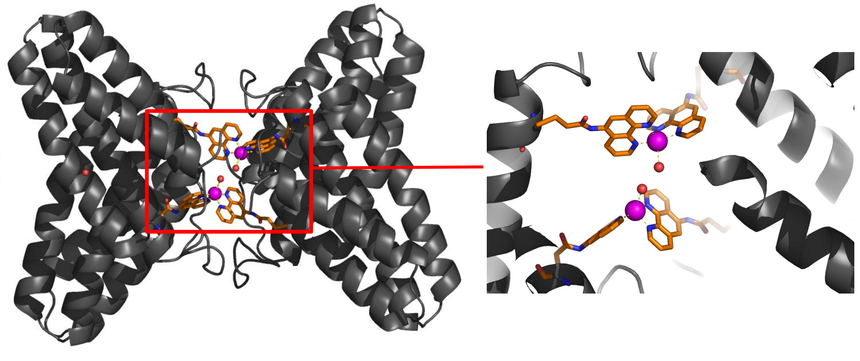
p-nitrophenyl actate (PNPA) was added to metal bound MBPPhen2 and the formation of *p*-nitrophenoxide was monitored sepctroscopically at 400 nm. The slopes remained consistent (~2 x 10^{-9}) so the metal ions did not seem to increase the rate of the reaction.

Future Work

MBPPhen2 Crystals



The preliminary results indicate that a combination of both a three cooridnate zinc ion as well as a properly engineered environment are necessary for water activation. In the future we will engineer a glutamate to act as an acidic residue to further lower the pKa of the reaction. Additionally the interesting structure of MBPPhen2 (right) might indicate its possibility for heterogeneous water activation.



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