Supplementary Material (ESI) for Chemical Communications


# Protein scaffold of a designed metalloenzyme enhances the chemoslectivity in sulfoxidation of thioanisole

*Jun-Long Zhang, Dewain K. Garner, Lei Liang, Qian Chen and Yi Lu**

## Supporting Information

*Computer Modeling and Protein Design*

The X-ray crystallographic coordinates of an achiral salen with an axial imidazole ligand (Cambridge structural database ID SAJLIY[1]) were used as a base model for **1**. The linking arms (to the sulfide only) were added using the molecule builder in Molecular Operating Environment suite [2] and the atoms of the coordinating imidazole removed. After energy minimization of the arms with the Charmm22 force field, **1•Mb** was modeled into the energy minimized Mb heme pocket (oxymyoglobin, 1.6 Å PDB ID 1MBO[3]). Placement of the artificial cofactor was modeled to mimic the native cofactor positioning by aligning the metals and the metal coordinating atoms of the native porphyrin and the model salen complex. The heme atoms were removed and the geometry of the linking arms was adjusted by optimizing their dihedral energies. The model was then manual inspected to identify residues pointing into the Mb pocket and near the predicted location of the sulfide group of the linking arm. Using the base model generated for selection of anchor points, point mutations were introduced using the sequence editor. Each sulfide of the Cys residue was covalently attached to the corresponding sulfide at the end of the linking arms. The position of the Mn atom, atoms directly coordinated to it and residues further than 10Å from the Mn were fixed and the structure minimized using the Charmm22 force field until the RMS gradient of the potential energy was less than 0.05 kJ $mol^{-1}$ $Å^{-1}$.

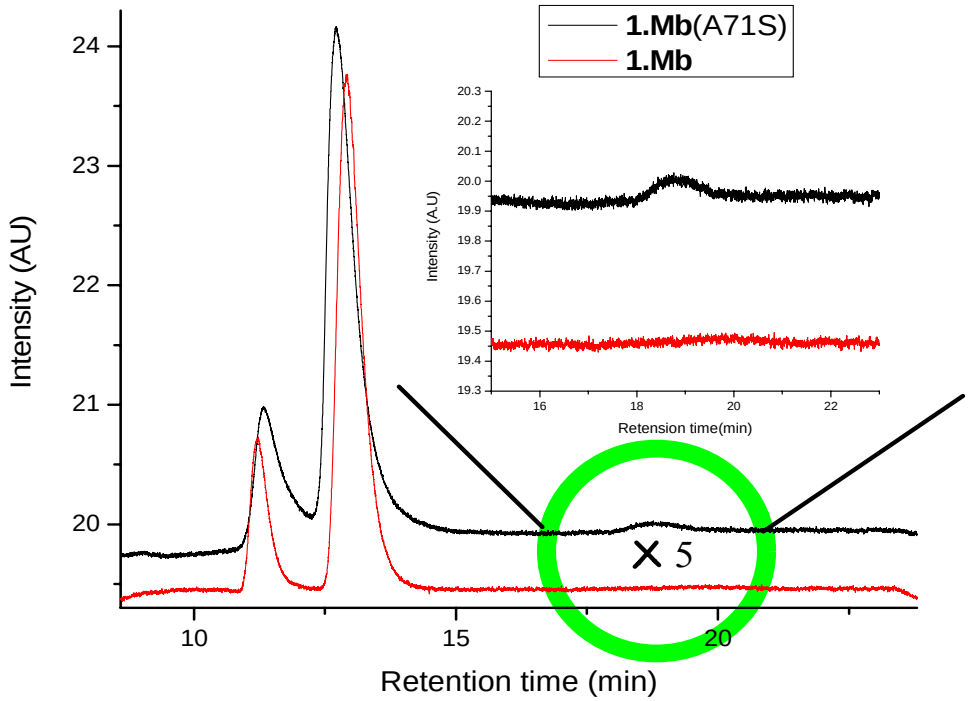
*Protein Expression and Purification*

Introduction of Cys residues at the desired locations in the Mb scaffold was accomplished via quick change mutagenesis and the variant proteins were expressed and purified as reported previously.[4,5] Generation of the heme free apo variants was accomplished via a combination of the methods of Teale[6] and Fisher[7]. Addition of a solution of **1** in dimethylformamide to the apo Mb variant resulted in 100% conversion to the dually anchored Mn salen artificial biocatalysts **1•**.Mb and (**1•**.Mb(A71S). Electrospray Mass Spectrometry was performed using a Quattro II Quadrupole Mass Spectrometer (Micromass, UK). Mass Scale was calibrated using sperm whale Mb in water (25pmoles/ul). Samples were of the artificial enzyme post incorporation of the Mn Salen cofactor and after exchange to 50mM ammonium acetate buffer at pH 5.1.

**1•Mb**: ESI MS: 17792.0 ±2.1Da. calcd: 17794.4; UV/Vis: λmax ($\varepsilon_{mM\text{-}1cm\text{-}1}$) 292 nm (33.95).

**1•Mb(A71S)**: ESI MS: 17812.5±2.1Da. calcd: 17810.5; UV/Vis: λmax ($\varepsilon_{mM\text{-}1cm\text{-}1}$) 292 nm (33.95).

Sulfoxidation activity of all of the four artificial enzymes were assessed at pH 5.1 as reported previously for the Mn**•1•**.apoMb(Y103C/L72C) artificial enzyme.[5]




**FigS1.** GC traces of sulfoxidation of thioanisole catalyzed by **1·Mb(A71S)** and **1·Mb**. Insert shows an expansion of the area within the green circle corresponding to the retention time of sulfone.

[1] N. Matsumoto, N. Takemoto, A. Ohyosia, H. Okawa, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2984.
[2] *MOE 2005.06*, C.C.G., Inc., 1255 University St., Suite 1600, Montreal, Quebec, Canada, H3B 3X3, **2005**
[3] S. E. V. Phillips, *J. Mol. Biol.* **1980**, *142*, 531.
[4] B. A. Springer, S. G. Sligar, *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8961; J. A. Sigman, H. K. Kim, X. Zhao, J. R. Carey, Y. Lu, *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3629.
[5] J. R. Carey, S. K. Ma, T. D. Pfister, D. K. Garner, H. K. Kim, J. A. Abramite, Z. Wang, Z. Guo, Y. Lu, *J. Am. Chem. Soc.* **2004**, *126*, 10812.
[6] F. W. J. Teale, *Biochim. Biophys. Acta* **1959**, *35*, 543.
[7] W. R. Fisher, H. Taniuchi, C. B. Anfinsen, *J. Biol. Chem.* **1973**, *248*, 3188.

Supplementary Material (ESI) for Chemical Communications