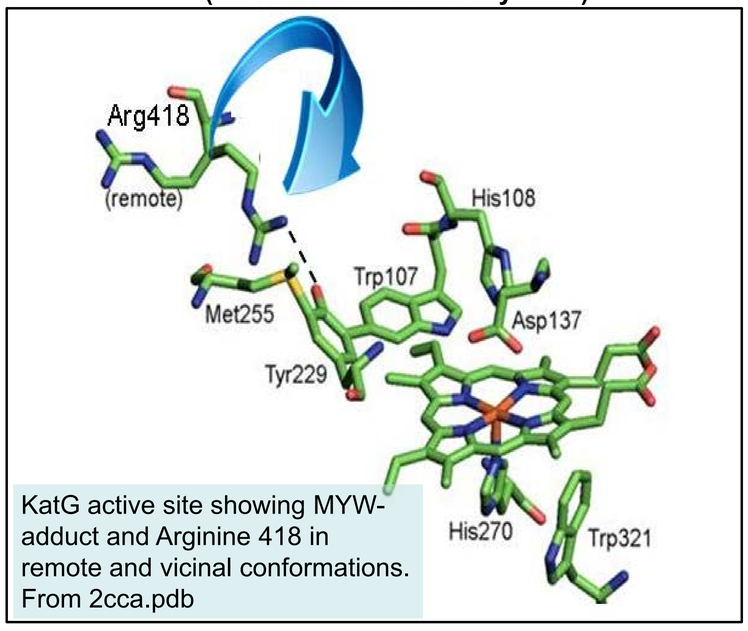
Stoichiometry of the MetTyrTrp-cofactor (MYW) radical in the KatG catalase reaction

Richard S. Magliozzo*, Xiangbo Zhao, Abdelahad Khajo, Bonnie Kruft, Andrzej Jarzecki
Brooklyn College CUNY, Brooklyn, NY and The Graduate Center CUNY (New York, NY)

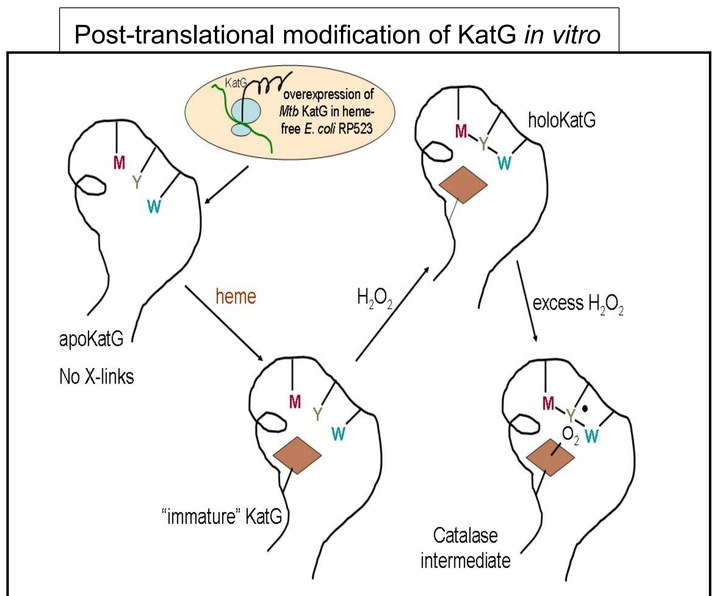
Abstract (**CONTROL ID:** 1708646)

The heme enzyme catalase-peroxidase (KatG) is responsible for oxidative stress management through its robust catalase activity ($2H_2O_2 = 2H_2O + O_2$). In *M. tuberculosis*, KatG is the only catalase. Rapid turnover of H_2O_2 depends on a unique amino acid adduct containing covalently linked Met255, Tyr229 and Trp107 formed by post-translational autoprocessing. We assigned a radical associated with catalase turnover to the MYW adduct and are investigating a specific role for this radical in a new catalase mechanism. Rapid freeze-quench EPR results show a maximum radical yield less than 0.5 spin/heme during catalysis. We sought to demonstrate a stoichiometric equivalence of MYW-adduct radical and catalase-active enzyme to confirm its role. KatG lacking the MYW adduct was prepared by isolation of apoenzyme and its reconstitution with heme, followed by treatment with peroxide to catalyze MYW-adduct formation in increasing amounts. Large amounts of pure apoKatG were produced using a λDE3-lysogenized *E. coli* strain for overexpression of His-tagged KatG under the T7 promoter. The strain (RP523(DE3)) lacks heme biosynthesis due to a hemB mutation. Growth under anaerobic conditions affords completely heme-free apoKatG lacking the cross links of the adduct found in holoenzyme overexpressed in the presence of heme. Reconstitution of apoKatG was efficiently achieved through a freeze-thaw method, to give holoenzyme having optical and CD spectra equal to holoenzyme. Titration of the reconstituted enzyme with small excesses of H_2O_2 allowed us to monitor catalase activity along with the stoichiometry of MYW-adduct radical previously characterized in WT KatG. The quantitative results demonstrate for the first time that the specific activity of KatG catalase turnover depends directly on the steady-state concentration of MYW-adduct radical. Investigation of radical formation in response to alkyl peroxide or H_2O_2 in the reconstituted enzyme shed light on the mechanism for MYW cross-link formation as well as the concomitant changes in heme iron from ferric to hypervalent (ferryl) forms.

Goals: prove catalytic competence of a unique cofactor radical in catalase-peroxidase; apply DFT to examine steady-state intermediate (MYW-adduct radical/oxyheme)



KatG active site showing MYW-adduct and Arginine 418 in remote and vicinal conformations. From 2cca.pdb

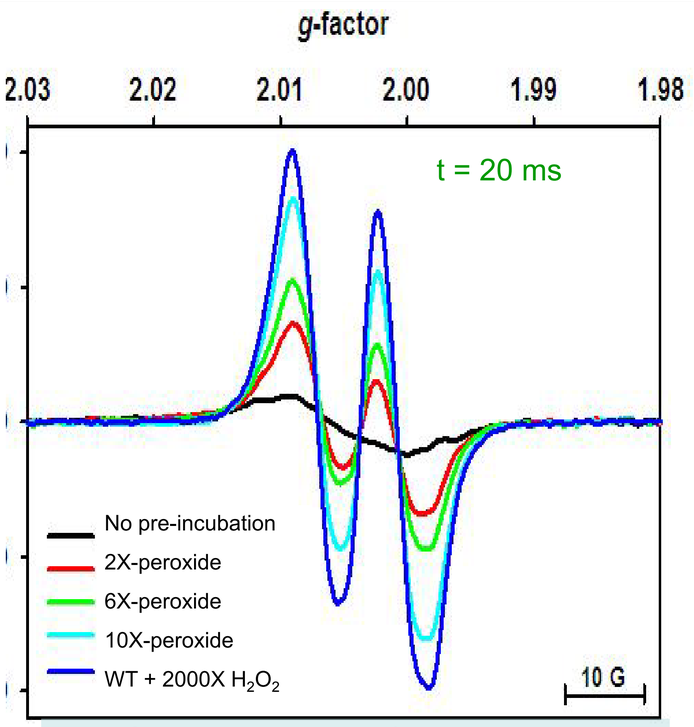


ApoKatG → holoKatG (immature) → native KatG

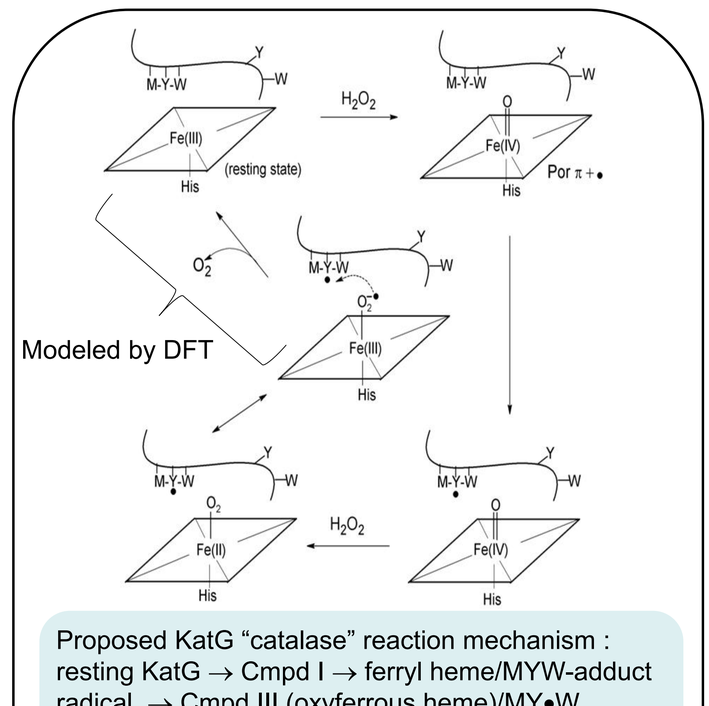
The reconstituted enzyme gains catalase activity after treatment with H_2O_2 or ROOH

H_2O_2 Pre-treatment (equiv/heme)	*Catalase Activity (U/mg)*	*MYW-radical conc. (spin/heme)*
0	1058 (26%)	0.065 (22%)
5	2795 (69%)	0.130 (44%)
8	3389 (83%)	0.216 (73%)
10	3741 (92%)	0.267 (91%)
(WT KatG)	4060	0.294

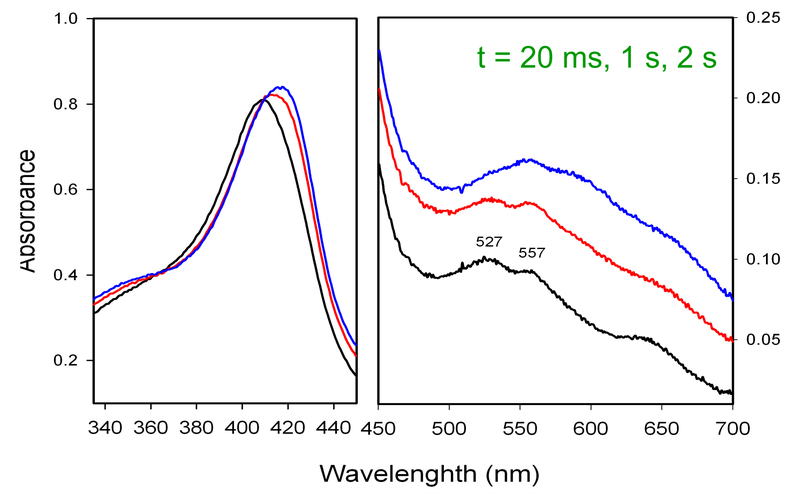
ROOH Pre-treatment (equiv/heme)	*Catalase Activity (U/mg)*	*MYW-radical conc. (spin/heme)*
0.5	-	0.089 (30%)
1	2705 (67%)	0.127 (43%)
2	3882 (95%)	0.245 (83%)
3	4016 (99%)	-
4	3379(83%)	0.225 (76%)
6	2520 (62%)	0.188 (64%)
(WT KatG)	4060	0.294



Rapid freeze-quench EPR of reconstituted "immature" KatG treated with 2000X H_2O_2. The narrow doublet EPR spectrum is from the MYW-adduct radical present during steady-state turnover.



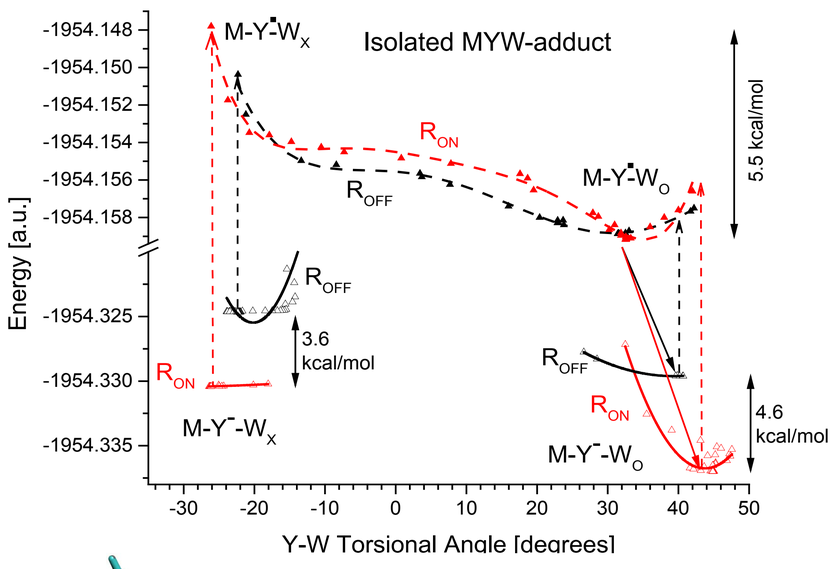
Proposed KatG "catalase" reaction mechanism : resting KatG → Cmpd I → ferryl heme/MYW-adduct radical → Cmpd III (oxyferrous heme)/MY•W ***Return to the resting state with release of O_2 was modeled using DFT calculations shown below.***

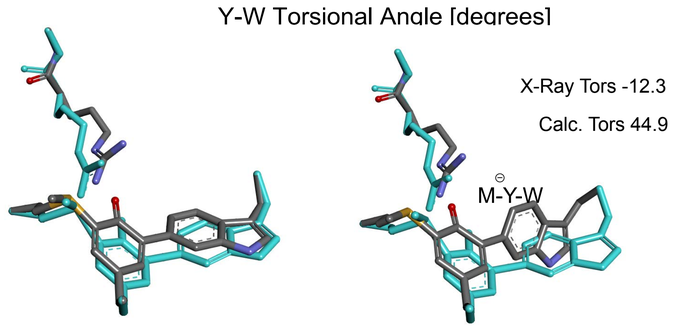


Optical stopped-flow spectra show formation of ferryl heme upon treatment of reconstituted "immauture" KatG with peroxide.

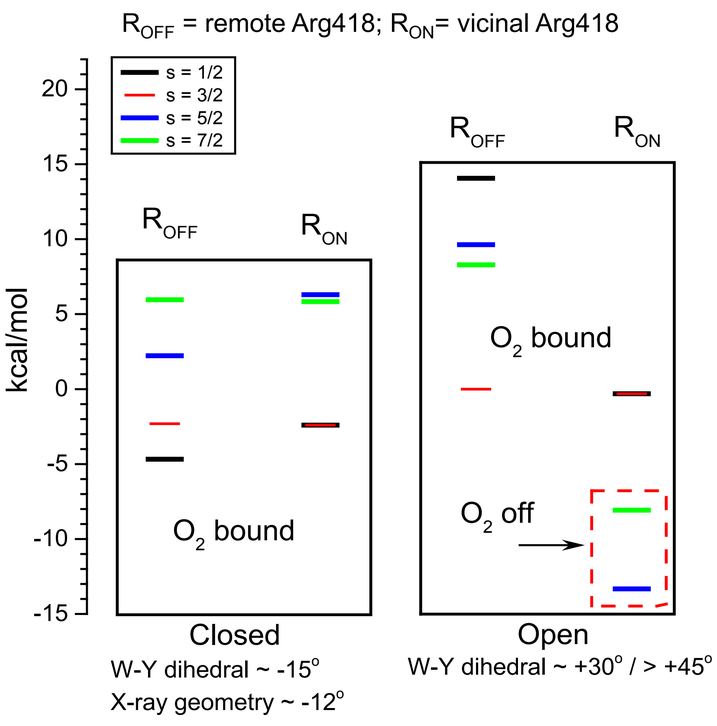
DFT calculations predict steric and electronic features leading to quenching of the steady-state (experimentally observed) MYW-adduct radical/oxyheme species.

The MYW-adduct radical species allows indole ring rotation

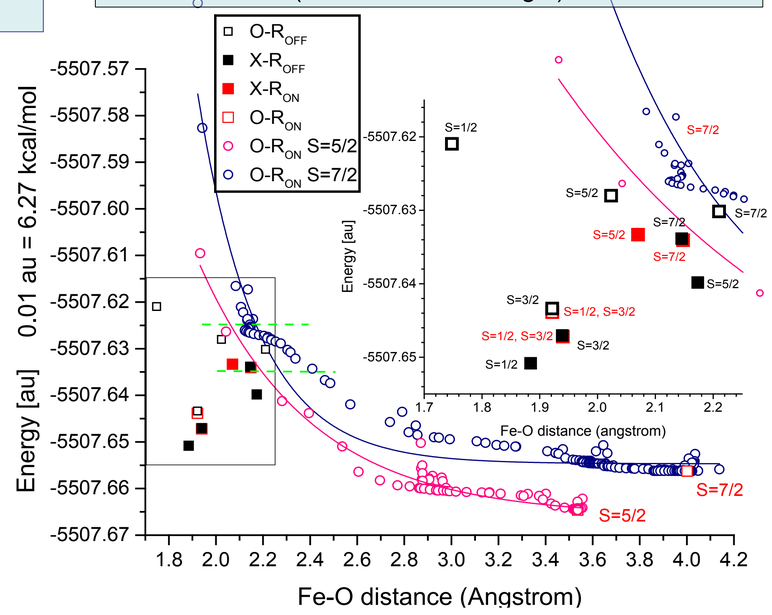


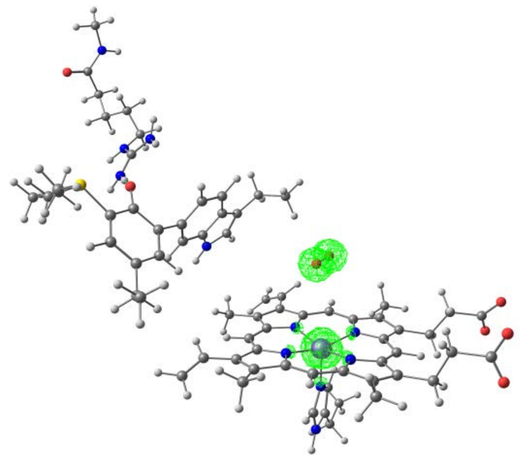


Release of triplet oxygen and radical quenching occur for geometry with Trp ring rotated away from oxy ligand, the side-chain of Arg418 hydrogen bonded to the phenolic oxygen of Tyr229. with total spin state ≥ 5/2



Energy landscape shown as function of Fe-O_2 distance (and Y-W torsion angle)





Experimental Methods:

- Overexpress *M. tuberculosis* apo-KatG in lysogenized heme-free strain of *E. coli* anaerobically.
- Purify (metal-affinity) apo-enzyme, add heme, add increasing amounts of peroxide (PAA or H_2O_2) to generate MYW-adduct X-links in limiting yields
- Use quantitative EPR to evaluate concentration of MYW-adduct (radical) during steady-state catalase turnover (>1000-fold H_2O_2)
- Correlate radical concentration with catalase specific activity (Table 1).

Calculations

- Density Functional Theory (B3LYP functional combined with 6-311G** basis sets) in Gaussian 09 with Polarizable Continuum Model (PCM); natural population analysis of Kohn-Sham orbitals.
- initial MYW-adduct model geometry optimized from *M. tuberculosis* KatG x-ray coords (2CCA.pdb)
- Positions of C-alphas and propionate carboxylates were fixed.
- Arg-418 residue in vicinal and remote conformations and backbone of Asp149 included
- MYW-adduct radical + oxyheme model included the proximal His-270. The MYW-adduct radical was produced by removal of a hydrogen atom from the hydroxyl group of Tyr-229.
- Relative energies were computed for total spin states of the MYW-oxyheme model from S=1/2 to S=7/2 in steps of S = 1.

Acknowledgment$: NIH/NIAID Award: 2R56AI060014-06A1

NSF Award: 1058116