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REVIEW ARTICLE

Catalase-peroxidase (KatG): a potential frontier in tuberculosis drug development

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ABSTRACT

Mycobacterium tuberculosis (Mtb) depends on the bifunctional enzyme catalase-peroxidase (KatG) for survival within the host. KatG exhibits both catalase and peroxidase activities, serving distinct yet critical roles. While its peroxidase activity is essential for activating the frontline tuberculosis drug isoniazid, its catalase activity protects Mtb from oxidative stress. This bifunctional enzyme is equipped with a unique, protein-derived cofactor, methionine–tyrosine–tryptophan (MYW), which enables catalase activity to efficiently disproportionate hydrogen peroxide in phagocytes. Recent studies reveal that the MYW cofactor naturally exists in a hydroperoxylated form (MYW-OOH) when cell cultures are grown under ambient conditions. New findings highlight a dynamic regulation of KatG activity, wherein the modification of the protein cofactor is removable—from MYW-OOH to MYW—at body temperature or in the presence of micromolar concentrations of hydrogen peroxide. This reversible modification modulates KatG's dual activities: MYW-OOH inhibits catalase activity while enhancing peroxidase activity, demonstrating the chemical accessibility of the cofactor. Such duality positions KatG as a unique target for tuberculosis drug development. Therapeutic strategies that exploit cofactor modification could hold promise, particularly against drug-resistant strains with impaired peroxidase activity. By selectively inhibiting catalase activity, these approaches would render Mtb more vulnerable to oxidative stress while enhancing isoniazid activation—a double-edged strategy for combating tuberculosis.

HIGHLIGHTS

- KatG's catalase and peroxidase activities are inversely related, with inhibition of one enhancing the other.
- Isoniazid (INH) activation depends on KatG's peroxidase activity, which is often impaired in drug-resistant *M. tuberculosis* strains.
- The catalase activity of KatG is reliant on the crosslinked MYW cofactor, whereas the peroxidase activity operates independently of it.
- The MYW cofactor exhibits chemical accessibility, as shown by the natural occurrence of its indole oxygenated form (MYW-OOH), which suppresses catalase activity.
- Elevated temperatures or micromolar concentrations of hydrogen peroxide (H_2O_2) facilitate the reversible conversion of MYW-OOH to the catalytically active MYW form.
- A dual-purpose therapeutic strategy is proposed: Targeting and utilizing *M. tuberculosis* KatG simultaneously for anti-TB treatment.

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Catalase-peroxidase is a multifunctional enzyme

Catalase-peroxidase (KatG) is an enzyme widely distributed across archaea, bacteria, and lower eukaryotes (Njuma et al. 2014). Notable examples include KatG in Euryarchaeota organisms (Neira et al. 2021), *Mycobacterium tuberculosis* (Mtb) (Bernroitner et al. 2009), and *Magnaporthe grisea* (Zámocká et al. 2012; Gasselhuber et al. 2015, 2016), a rice blast fungus that devastates cereal crops (Boddy, 2016). KatG is particularly critical for bacterial pathogens such as *Acinetobacter baumannii* (Sun et al. 2016), *Bradyrhizobium japonicum* (Panek and O'Brian 2004), *Burkholderia pseudomallei* (Bernroitner et al. 2009), Mtb, and *Salmonella Typhimurium* (McLean et al. 2010). Even *Escherichia coli* harbors the *katG* gene (Gordon et al. 2011), and certain pathogenic strains utilize this enzyme in their virulence strategies.

KatG belongs to the heme-dependent peroxidase superfamily (Njuma et al. 2017). Its peroxidase facilitates the oxidation of a broad range of hydrogen donors, including polyphenols, thereby supporting pathogen survival and growth. A distinctive feature

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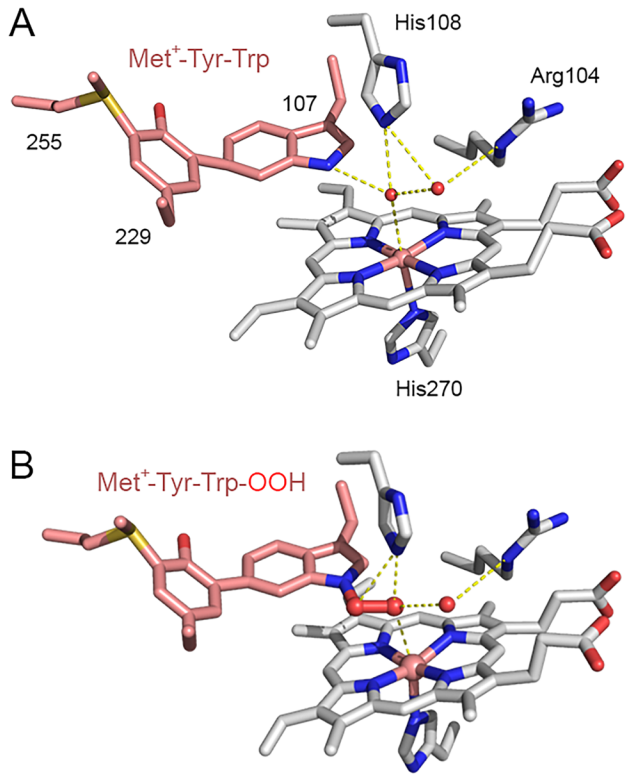


Figure 1. Two forms of the protein-derived cofactor, MYW (A) and MYW-OOH (B) are present in the as-isolated wild-type *M. tuberculosis* KatG proteins, which are posttranslationally modified from Met255, Tyr229, and Trp107 in an autocatalytic process mediated by the adjacent heme. The active site structures are depicted from the recently reported crystal structures of the enzyme (PDB entry: 8CZP and 8W1W, respectively).

of KatG is its crosslinked covalent triad, methionine$^+$-tyrosine-tryptophan (Met$^+$-Tyr-Trp, also known as MYW), located in the distal pocket of the prosthetic heme group (Figure 1). This conserved cofactor has been identified across structurally characterized KatG proteins, starting with *Haloarcula marismortui* (Yamada et al. 2002), and later on KatG from *Burkholderia pseudomallei* (Carpena et al. 2003), Mtb (Bertrand et al. 2004), and an eukaryotic source (Zámocká et al. 2012). The MYW cofactor confers catalase activity to KatG, rendering it nearly comparable to stand-alone single-functional catalase enzymes.

KatG's catalase and peroxidase activities both utilize hydrogen peroxide (H_2O_2) as a substrate but are inherently competing processes. Inhibition of one activity often enhances the other, highlighting their interdependence. Although the catalase and peroxidase activities are mutually antagonistic, synergistic cooperation between these activities is observed with certain peroxidatic electron donors also enhancing the catalase activity of KatG (Njuma et al. 2017).

KatG's catalase activity demonstrates significantly higher catalytic efficiency than its peroxidase activity in H_2O_2 turnover, suggesting that catalase function predominates under most physiological conditions. However, when catalase activity is inhibited—whether through genetic mutations or chemical agents—the enzyme's peroxidase activity can be upregulated. Notably, this upregulation is not directly proportional to the suppression of catalase activity, and the precise mechanisms governing this interplay remain to be elucidated.

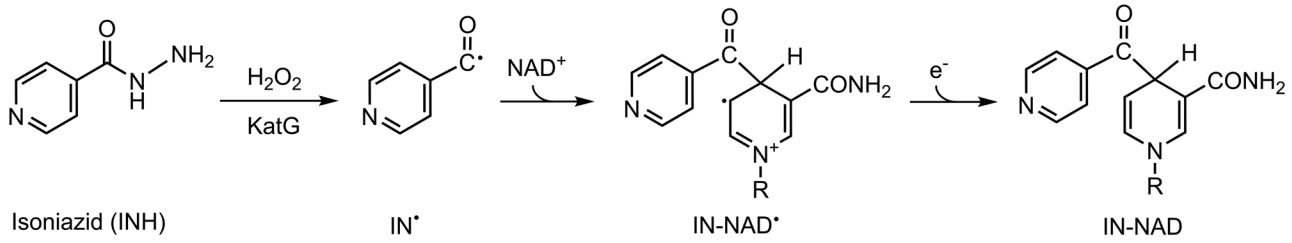
Beyond its hallmark catalase and peroxidase functions, KatG exhibits additional catalytic activities, including isoniazid (INH) lyase and NADPH oxidase activities (Carpena et al. 2006). It also performs peroxynitritase activity, broadening its functional repertoire (Wengenack et al. 1999; McLean et al. 2010). These multifunctional properties underscore KatG's complexity and its critical role in the physiology and virulence of various pathogens.

KatG as a prodrug activation utility in tuberculosis treatment

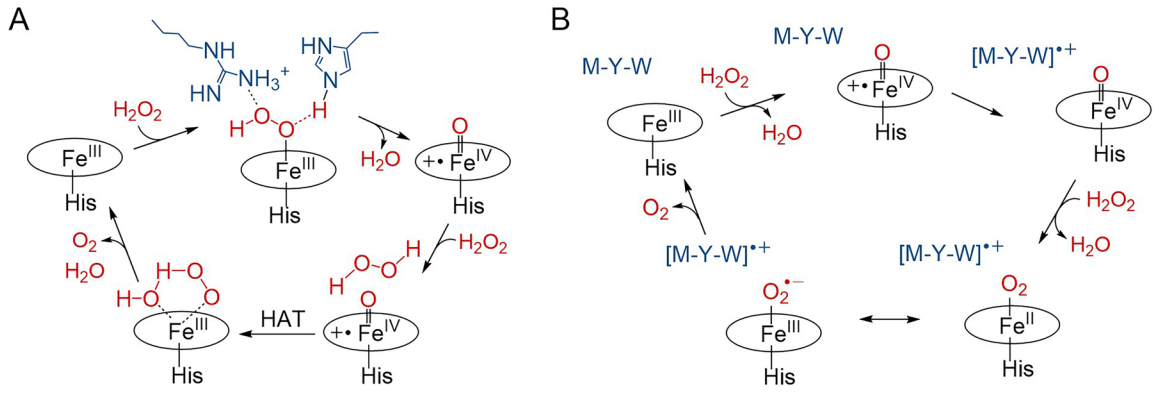
KatG is best known for its role in activating INH, a potent antituberculosis (TB) prodrug. Since its FDA approval in 1952, INH has remained a cornerstone of TB treatment, standing out as one of the most clinically successful and widely used medications in anti-TB regimens (Chakraborty and Rhee 2015; O'Connor and Brady 2021). INH relies on the enzymatic activity of KatG in Mtb, the causative agent of TB, for its activation. This critical role in drug efficacy has made KatG a frequent focus in anti-TB research as a utility.

The mechanism of INH activation involves the peroxidase activity of Mtb KatG, which oxidizes INH into its isonicotinic acyl radical. This radical subsequently combines with NAD to form the isoniazid-NADH adduct (Scheme 1), a potent inhibitor of the Mtb enoyl reductase, InhA (Rawat et al. 2003). By blocking InhA, a key enzyme in the synthesis of type II fatty acids, including mycolic acid, the adduct disrupts the bacterial cell wall—a critical target for TB treatment (Rawat et al. 2003; He et al. 2007). Despite KatG's indispensable role in activating this prodrug, it has not been considered a direct therapeutic target due to the benefits it provides in facilitating INH efficacy.

Mutations in the *katG* gene are the primary mechanism of INH resistance in multidrug-resistant (MDR) TB strains. The most common mutation, Ser315→Thr (S315T), significantly impairs INH activation (Mokrousov et al. 2002; Kapetanaki et al. 2003, 2005; Zhao et al. 2006; Ranguelova et al. 2008; Zhao et al. 2013; Unissa et al. 2015; Torres Ortiz et al. 2021; Valafar 2021; Vazquez-Chacon et al. 2022; Napier et al. 2023). In wild-type KatG, the Ser315 residue forms two hydrogen bonds with the heme-propionate group,



Scheme 1. Activation of the pro-drug isoniazid (INH) by KatG through its free radical-forming peroxidase function.



Scheme 2. Current understanding of the heme-dependent catalase mechanisms. (A) Typical Mono-functional catalases utilize a heme, as well as a distal arginine and histidine pair, to facilitate the O–O bond cleavage of H_2O_2 and hydrogen atom transfer (HAT), and (B) Protein cofactor-mediated catalase pathway in KatG.

contributing to the electronic structure and catalytic efficiency of the enzyme. The S315T mutation disrupts this interaction, reducing INH activation efficiency by approximately fivefold (Saint-Joanis et al. 1999; Cade et al. 2010). Despite this reduction, MDR strains often retain some peroxidase activity to support their growth (Lukat-Rodgers et al. 2000, 2001; Wengenack et al. 2004; Zhao et al. 2006; Morey-Leon et al. 2022).

Unfortunately, understanding these mutations' structural and functional effects has provided limited therapeutic solutions, as reversing such genetic changes is not currently feasible. Given the persistent clinical reliance on INH and rifampin (Chakraborty and Rhee 2015; Saini et al. 2022), novel strategies are urgently needed to address KatG's reduced peroxidase activity in MDR strains.

TB remains a global health crisis. According to the latest World Health Organization (WHO) report, TB causes over one million deaths annually, with more than 10 million people continuing to fall ill with TB every year, and the number has been rising since 2021 (WHO 2024). The COVID-19 pandemic has further exacerbated challenges in TB treatment, including delays in diagnosis and care. These pressing issues highlight the importance of exploring KatG's molecular mechanisms to devise innovative strategies for overcoming INH resistance in evolving MDR strains.

Harnessing oxidative power: the role of protein-derived cofactor

KatG's primary catalytic functions—catalase and peroxidase—both depend on a prosthetic heme cofactor that utilizes H_2O_2 as a substrate. The oxidative power generated at the heme center is distributed and finely regulated by an intricate, protein-derived cofactor, which operates through novel mechanisms.

While KatG's peroxidase activity, particularly its isonicotinic-NAD synthase function, has garnered significant attention due to its critical role in activating INH, the catalase activity plays an equally vital role in bacterial survival. The catalase function protects Mtb against oxidative stress imposed by host immune defenses, such as H_2O_2, during infection. Together, these activities illustrate the enzyme's dual importance: catalase activity for survival within the host and peroxidase activity for promoting growth.

Unlike standalone catalases, which use a conserved Arg-His pair in the distal heme pocket to achieve highly efficient H_2O_2 deprotonation (Scheme 2A) (Regelsberger et al. 2000; Jakopitsch et al. 2003; Meunier 2003; Ranguelova et al. 2008; Kudalkar et al. 2015), KatG employs a distinct, radical-based catalase mechanism (Chouchane et al. 2002; Jakopitsch et al. 2004; Ranguelova et al. 2007; Singh et al. 2007; Suarez et al. 2009; Zhao

et al. 2009; Colin et al. 2010; Zhao et al. 2010, 2012; Kruft et al. 2015). This mechanism is enabled by a unique protein-derived cofactor: the MYW covalent triad. The MYW cofactor forms through an autocatalytic crosslinking reaction mediated by the adjacent heme center in cells.

As outlined in Scheme 2B, the catalase activity in KatG begins when an H_2O_2 molecule reaches the heme center, oxidizing ferric heme to a high-valent ferryl intermediate (Chouchane et al. 2002, Singh et al. 2007). This intermediate transfers one of its two oxidizing equivalents to the adjacent MYW cofactor, generating a one-electron oxidized MYW radical. This radical has been experimentally observed and characterized by electron paramagnetic resonance (EPR) spectroscopy, with its identity confirmed through specific isotope labeling of Tyr229, the central component of MYW (Zhao et al. 2010). A second H_2O_2 molecule then acts as a reducing agent, donating an oxygen atom to the ferryl heme to form Fe(IV)=O and subsequently generating a ferrous heme-bound dioxygen complex. Next, radical-ion coupling ultimately regenerates the resting ferric heme state and releases O_2 as the catalytic product (Scheme 2B). This interplay between the heme center and the MYW cofactor exemplifies KatG's atypical and sophisticated catalase mechanism.

In the presence of the MYW cofactor, the catalase activity overwhelmingly dominates in KatG, consuming H_2O_2 with remarkable efficiency. Studies have shown that KatG from *Mtb* exhibits a turnover rate (k_{cat}) of $6{,}000 \pm 70\,s^{-1}$ and an efficiency (k_{cat}/K_M) of $2.40 \pm 0.03 \times 10^6$ $M^{-1}s^{-1}$—33,000 times more efficient than its peroxidase activity under similar conditions (Ghiladi et al. 2005). This efficiency ensures that the catalase function outcompetes the peroxidase function in terms of H_2O_2 turnover.

When the MYW cofactor is disrupted by mutations in the covalently crosslinked triad-forming residues, the catalase activity is nearly abolished, dropping to a minimal basal level. Interestingly, this loss of catalase function is accompanied by a significant enhancement of the peroxidase activity—often by an order of magnitude—transforming the bifunctional KatG into a predominantly peroxidase enzyme (Regelsberger et al. 2000, 2001; Jakopitsch et al. 2003; Yu et al. 2003; Ghiladi et al. 2005; Ghiladi et al. 2005; Cade et al. 2010).

MYW is a home-made cofactor formed through heme-dependent oxidations

The MYW covalent triad is among the most intricate protein-derived cofactors known (Okeley and van der Donk 2000; Davidson 2011; Klinman and Bonnot 2014; Davidson 2018; Fujieda 2020). Its complexity arises not only from the crosslinking of three amino acid residues—methionine, tyrosine, and tryptophan—but also from the presence of a sulfonium ion in its C-S^+ bond. This feature is highly unusual, distinguishing it from the charge-neutral C-S bonds typically observed in other protein cofactors, such as cysteine–tyrosine (Cys-Tyr) linkages (Okeley and van der Donk 2000; Davidson 2011; Hromada et al. 2017; Davidson 2018). The formation of this cofactor through an autocatalytic, heme-dependent process represents a significant biochemical challenge for study.

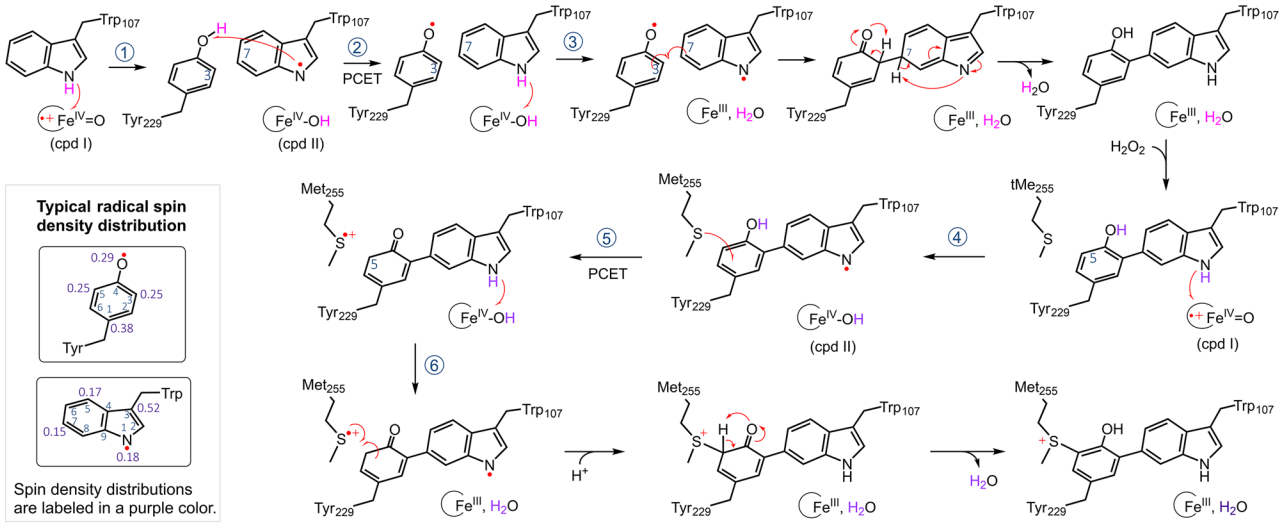
Traditional approaches to studying protein-derived cofactors, such as site-directed mutagenesis, often fall short when applied to MYW. By disabling residues critical for cofactor formation, these methods essentially "knock out" the cofactor entirely, precluding investigations into its biogenesis. As a result, the detailed mechanism underlying the autocatalytic formation of MYW has remained elusive. A notable large effort has generated wild-type Mtb KatG lacking the Met–Tyr–Trp through overexpression of apoprotein followed by heme reconstitution. Subsequent MYW cofactor formation is achieved in the holo-KatG lacking the cofactor after adding peroxide (Ghiladi et al. 2005; Ghiladi et al. 2005). This work demonstrates that MYW cross-link is a result of the heme- and peroxide-dependent reactions.

Scheme 3 presents a revised mechanism for MYW biogenesis, building upon previous hypothetical models (Ghiladi et al. 2005; Ghiladi et al. 2005; Njuma et al. 2014) and incorporating insights from recent studies on protein-bound tryptophanyl (Yukl et al. 2013; Davis et al. 2018) and tyrosyl radical spin density distributions in other systems (Bender et al. 1989; Hoganson et al. 1996; Lendzian et al. 1996; Hulsebosch et al. 1997; Stubbe and van der Donk, 1998; Rokhsana et al. 2006). The process begins with KatG's intrinsic peroxidase activity, leveraging high-valent heme intermediates as oxidative agents.

The mechanism unfolds as follows:

1. Trp Oxidation: The indole nitrogen of Trp107, positioned only 4.3 Å from the heme iron, is the initial site of oxidation. This proximity ensures that Trp107 is the first residue to form a radical species ($Trp107^{\bullet}$).
2. Tyr Oxidation via protein-coupled electron transfer (PCET): The $Trp107^{\bullet}$ radical oxidizes Tyr229, which is located 10 – 11 Å away from the iron ion, via a PCET. This step regenerates Trp107 and produces a neutral $Tyr229^{\bullet}$ radical.
3. Crosslink Formation: A second oxidation of Trp107 by Fe(IV)-OH generates another $Trp107^{\bullet}$ radical, which couples with Tyr229 to form a new C–C bond, creating the first crosslink.

The formation of the C–S^+ crosslink between Trp107-Tyr229 and Met255 involves additional oxidative steps:



Scheme 3. Proposed MYW production through two rounds of heme-mediated hydrogen peroxide oxidation. KatG functions primarily as a peroxidase in the absence of MYW. The autocatalytic process generates a high-valent Fe-bound oxidant carrying two oxidizing equivalents in each round, which oxidizes Trp107 in the first round and Tyr229-Trp107 in the second round. The radical spin density delocalization across fused and conjugated rings and proton-coupled electron transfer (PCET) play a critical role during the process of crosslink. The inset shows typical spin density distributions of protein-bound tyrosyl and tryptophanyl radicals.

4. Tyr-Trp Radical Formation: The Tyr229-Trp107 intermediate is further oxidized by the heme compound I species (Fe(IV)=O), generating a Tyr-Trp radical with spin density partially delocalized to Tyr229's C5 position.
5. Met255 Activation: A PCET transfers an electron and a proton from Met255 to the Tyr229-Trp radical, producing a Met255 cation radical.
6. C–S$^+$ Bond Formation: Subsequent oxidation by Fe(IV)-OH creates a Trp-Tyr radical, which undergoes radical coupling with Met255 to form the sulfonium ion. Since the sulfur in Met255 lacks a proton, an external proton donor is required for re-aromatization of the Tyr229 ring, completing the MYW cofactor.

This proposed mechanism highlights the remarkable coordination between heme redox chemistry and radical intermediates, emphasizing KatG's unique biochemistry. The autocatalytic formation of MYW underscores the enzyme's sophistication and adaptability, playing a pivotal role in its bifunctional catalytic activities.

Discovery of MYW-OOH as a natural form of the protein-derived cofactor

The MYW cofactor is a key regulator of KatG's oxidizing power, intricately controlling its catalytic balance. Its regulation is further modulated by a distant arginine residue, Arg418 (~20 Å from the heme), which acts as a molecular switch (Carpena et al. 2005). This arginine alternates between two conformations: one interacting with the tyrosyl moiety of the MYW cofactor, adding positive charges to influence its behavior, and the other more remote. This regulatory mechanism underscores the dynamic interplay necessary for distributing oxidizing power between KatG's catalase and peroxidase activities. However, this mobile Arg is not the sole regulator of the MYW cofactor.

An oxygenated form of the MYW cofactor, MYW-OOH, has been observed in crystalline KatG. Interestingly, MYW-OOH emerges under specific conditions, such as when *Burkholderia pseudomallei* KatG crystals, initially grown at pH 5.6, are soaked in a pH 8.5 buffer. This observation suggests the possibility of an autocatalytic conversion of MYW to MYW-OOH (Carpena et al. 2006). MYW-OOH has also been reported in mutant KatG proteins from various organisms (Deemagarn et al. 2005; Carpena et al. 2006; Loewen et al. 2014; Uribe-Vázquez et al. 2024), highlighting its potential significance.

A computational study proposed a mechanism where MYW donates an electron to ferric heme, forming a MYW cation radical and ferrous heme, which then binds O_2, resulting in oxygenation of MYW (Vidossich et al. 2011). However, this hypothesis remains contested because ferric heme and MYW are air-stable in all KatG proteins, making the proposed pathway theoretically valid but unlikely in practice.

The original report of MYW-OOH did not identify the oxygen source for the hydroperoxyl group. However, studies on protein-derived cofactors, such as

tryptophan tryptophylquinone (TTQ), suggest that H_2O_2 from cryoprotectants like polyethylene glycol could be the source of peroxide to initiate chemical oxidations inside crystals (Yukl et al. 2013). This slow peroxide-releasing agent accelerates oxidation at higher pH levels, coinciding with the KatG observation.

MYW-OOH was initially proposed as a catalytic intermediate in the catalase pathway based on studies of *B. pseudomallei* KatG (Loewen et al. 2014). However, this interpretation is debated (Li et al. 2024). The exceptionally efficient H_2O_2 disproportionation catalyzed by KatG challenges the notion that MYW-OOH represents a long-lived intermediate, as such stabilization would reduce catalytically active forms of the enzyme, contradicting steady-state kinetic observations. Further complicating this hypothesis is the detection of MYW-OOH in the as-isolated wild-type *M. grisea* KatG and Mtb KatG crystals, even without active catalase turnover prior to crystallization.

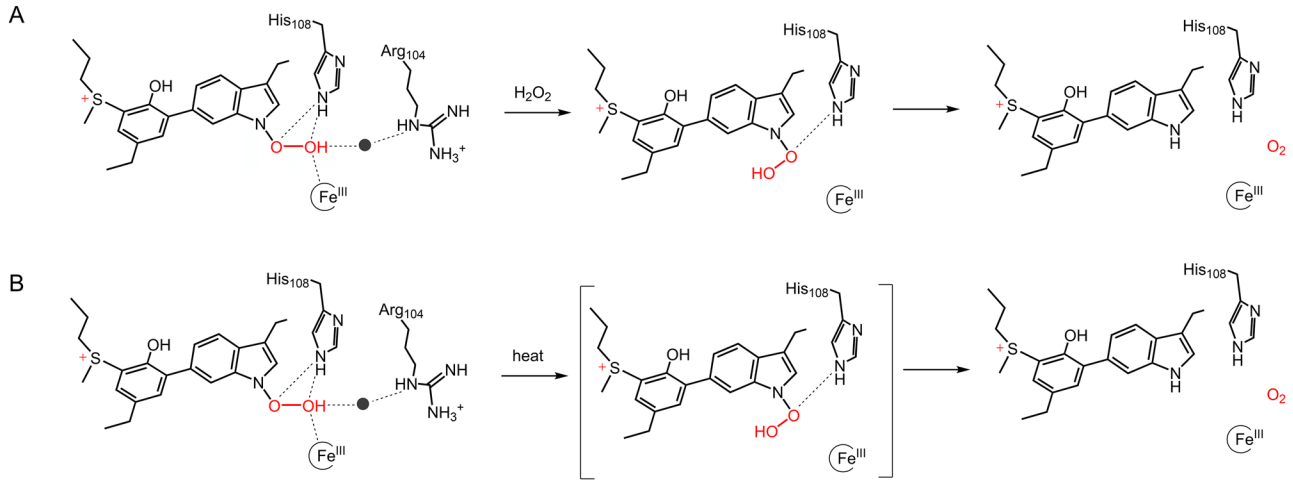
Recent studies provide evidence of MYW-OOH as a natural cofactor form in solution (Li et al. 2024). In Mtb KatG, MYW-OOH was observed in as-isolated full-length wild-type proteins expressed under ambient temperatures without overexpression inducers. This form is spectroscopically distinguishable from MYW due to its effect on the distal heme environment and electronic structure. Proteins isolated under these conditions exhibited MYW-OOH both in solution and in crystals. In contrast, proteins expressed at body temperature primarily contained MYW (Li et al. 2024).

Temperature appears to be a critical factor for stabilizing MYW-OOH. Heat treatment or exposure to micromolar concentrations of H_2O_2 converts MYW-OOH back to MYW, as evidenced by spectroscopic changes and *in crystallo* observations (Li et al. 2024). Interestingly, peroxide binding destabilizes MYW-OOH, likely by disrupting stabilizing interactions with the heme iron and distal histidine (Scheme 4). An intermediate form of the hydroperoxyl group, lacking stabilization from the heme, has been visualized in crystal structures under these conditions, along with molecular oxygen in the distal heme pocket, suggesting peroxide-driven hydroperoxyl displacement (Li et al. 2024).

These latest findings suggest MYW-OOH represents an alternate, yet unstable, natural cofactor form that can interconvert with MYW depending on environmental conditions. At high peroxide concentrations, competition between peroxide and MYW-OOH for interaction with the heme iron likely drives the observed conversion. The discovery of MYW-OOH expands the understanding of KatG's cofactor dynamics and highlights its complex interplay with environmental factors.

Impact of MYW-OOH on KatG functions

The discovery of MYW-OOH in solution has provided the first opportunity to analyze the functional implications of the hydroperoxyl adduct on the MYW cofactor. MYW-OOH inhibits catalase activity while preserving a reduced yet significant level of peroxidase function, which operates at a slower, steady rate (Li et al. 2024). This analysis became possible only after conditions for isolating Mtb KatG with MYW-OOH in solution were



Scheme 4. Diagram of conversion of MYW-OOH to MYW upon meeting micromolar concentration of hydrogen peroxide (A) or increased temperature (B). MYW-OOH is stabilized by the heme, His108, and Arg104 *via* a water molecule (PDB entries 8W1W). Adding a competing external ligand, such as hydrogen peroxide, forces the hydroperoxyl group to adopt a new conformation with fewer stabilization interactions (PDB entry 8W1X). The filled circle represents an ordered water molecule in the active site. Square brackets report the proposed intermediate state.

established. Enzymatic assays of KatG with MYW-OOH revealed a characteristic lag phase of 1 – 2 s before activity initiation (Li et al. 2024). This delay contrasts sharply with the rapid catalase turnover rate of ~6,000 s^{-1} observed with the MYW cofactor (Ghiladi et al. 2005).

Environmental stimuli, such as elevated temperatures or increased peroxide concentrations, trigger a rapid conversion of MYW-OOH to the catalase-active MYW form. This dual-cofactor system creates a finely tuned regulatory mechanism, allowing KatG to alternate between a catalase-dormant state (MYW-OOH) and a highly active catalase state (MYW) for better supporting its life cycle (Figure 2). Such adaptability ensures efficient catalase activity is reserved for conditions where it is most needed, optimizing metabolic efficiency for Mtb.

While it's true that Mtb spends most of its life cycle inside the human host at body temperature, its survival and transmissibility during its brief or extended time outside the host (in air droplets, contaminated food, or water) are critical for its spread. This outside-host phase exposes the pathogen to fluctuating environmental conditions, including varying temperatures. Notably, Mtb demonstrates remarkable resilience and virulence in extracellular environments (Rickards et al. 1909). Studies have shown Mtb can survive in sputum, carpets (up to 19 days), and soil for extended periods—up to four weeks in some cases (Kramer et al. 2006; Norby et al. 2007; Velayati et al. 2015). This persistence is crucial for its survival outside the host and supports the hypothesis that the MYW-OOH form of KatG functions as a catalase-dormant state, primed for activation only when necessary.

One plausible biological rationale is that during Mtb's slow growth in ambient environments, full catalase activity is unnecessary. Instead, KatG's primary function may shift to peroxidase activity, which is sufficient to maintain viability in the presence of low endogenous peroxide levels. However, when Mtb encounters the peroxide-rich environment of an infected host, the rapid activation of KatG's catalase function becomes critical for neutralizing host defenses (Ng et al. 2004).

The MYW-OOH cofactor appears preconfigured to protect the heme pocket from inhibitory molecules during dormancy, allowing KatG to remain poised for activation. The catalase-dormant state is readily switched to the active form under heightened peroxide conditions, as depicted in Scheme 4. This activation

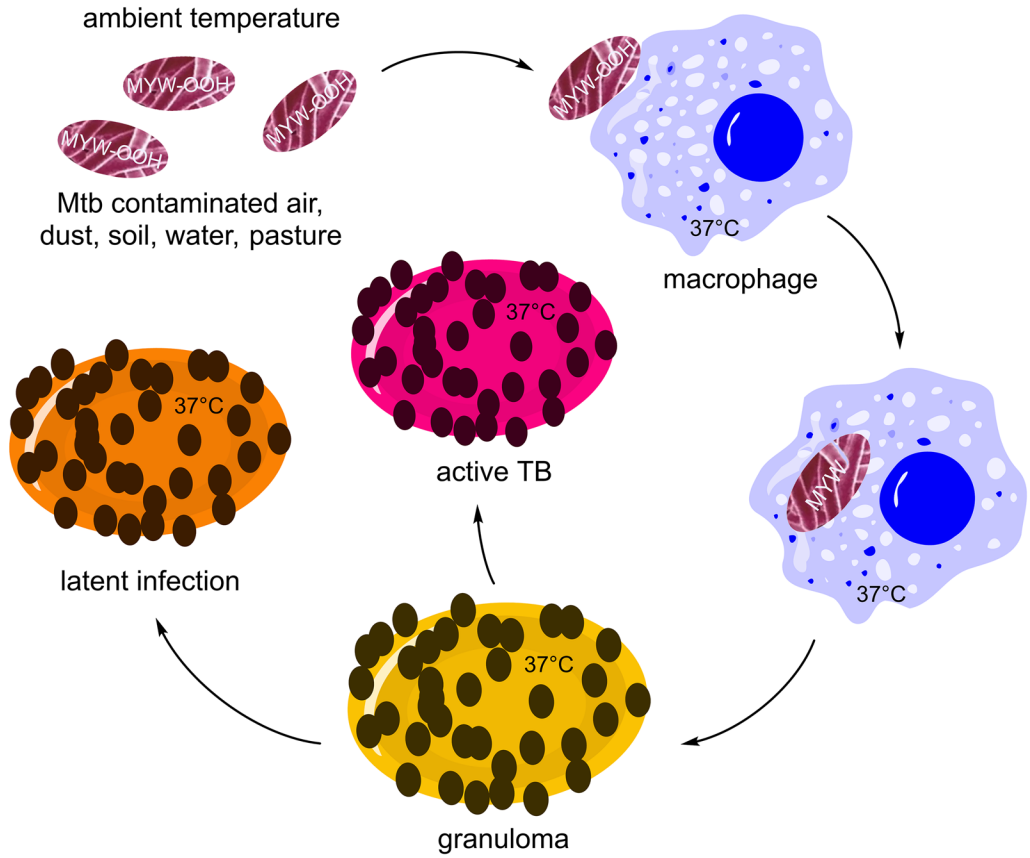


Figure 2. Potential role of dual cofactor forms of KatG in the life cycle of *M. tuberculosis*.

requires at least two equivalents of H_2O_2 and ensures KatG is fully functional upon entering the host's oxidative environment.

The discovery of two interconvertible forms of the protein-derived cofactor—MYW and MYW-OOH—-suggests a potential adaptive mechanism in KatG, a key bifunctional enzyme of Mtb. This regulatory "off switch" modulates catalase activity at ambient temperatures, promoting peroxidase activity under low peroxide conditions while conserving catalase potential for high-stress environments. Such versatility likely contributes to Mtb's ability to persist and thrive across a range of environmental and host conditions.

Reimagining KatG as a dual-propose enzyme: from prodrug activator to therapeutic target, a double benefit

The discovery of the MYW-OOH as an alternate natural form of protein-derived cofactor in Mtb KatG has significantly advanced our understanding of indole N-OOH chemistry, enzyme catalysis, and the oxidizing power regulation mechanisms of Mtb (Li et al. 2024). This finding also presents new therapeutic opportunities, as it demonstrates that the MYW crosslink is chemically accessible and modifications to this cofactor can alter the enzyme's catalytic function. These revelations prompt a reevaluation of KatG's biomedical role, particularly its potential as an anti-TB therapeutic target.

Historically, KatG's role as a prodrug activator, particularly in the activation of the FDA-approved antibiotic INH, has overshadowed its potential as a therapeutic target. While INH has been a cornerstone of TB treatment for over 70 years, multidrug-resistant (MDR) TB strains often reduce KatG's peroxidase activity to diminish INH activation (Saint-Joanis et al. 1999; Lukat-Rodgers et al. 2000, 2001; Mokrousov et al. 2002; Wengenack et al. 2004; Zhao et al. 2006; Cade et al. 2010; Unissa et al. 2015; Torres Ortiz et al. 2021; Valafar, 2021; Morey-Leon et al. 2022; Napier et al. 2023). This has led to a focus on optimizing INH derivatives to enhance potency or improve drug delivery rather than targeting KatG directly (Martins et al. 2014; Hu et al. 2017; Farad and Jagdale 2020; Ridahunlang et al. 2023; Alghamdi et al. 2024). In parallel, considerable efforts have been devoted to designing direct inhibitors of InhA as an alternative strategy (Menendez et al. 2012; Manjunatha et al. 2015; Spagnuolo et al. 2017). However, a paradigm shift is emerging: **could KatG be leveraged both as a target to disrupt bacterial defenses and as a utility to enhance prodrug efficacy?**

KatG's dual enzymatic functions—catalase and peroxidase—offer a unique therapeutic opportunity. Its catalase activity protects Mtb from oxidative damage by disproportionating host-derived H_2O_2, while its peroxidase activity is essential for activating INH. Theoretically, inhibiting KatG's catalase activity with exogenous compounds would weaken Mtb's oxidative defenses, leaving it more vulnerable to the host immune response. Simultaneously, this inhibition could enhance peroxidase-mediated prodrug activation by reducing the competition between these two catalytic functions, as demonstrated by the increased peroxidase activity in cofactor-disrupted KatG mutants (Regelsberger et al. 2000, 2001; Jakopitsch et al. 2003; Yu et al. 2003; Ghiladi et al. 2005; Ghiladi et al. 2005). This dual-targeting strategy could enhance INH efficacy while increasing the susceptibility of MDR TB strains to oxidative stress during infection.

Several lines of evidence support this emerging strategy. First, MDR strains frequently exhibit reduced peroxidase activity, leading to diminished INH activation. Furthermore, studies demonstrate that mutations reducing KatG's catalase function can concomitantly increase peroxidase activity, highlighting the potential benefits of selectively disarming catalase activity. Second, KatG is the primary catalase-active protein in Mtb, playing a critical role in neutralizing host-derived oxidative stress (Suarez et al. 2009). Gene knockout studies in TB mouse models reveal that losing KatG's catalase function impairs Mtb's ability to counteract host defenses (Ng et al. 2004).

Selectively inhibiting KatG is inherently challenging due to its shared active site for catalase and peroxidase activities. However, the MYW cofactor presents a unique opportunity for targeted intervention. Unlike other Met, Tyr, and Trp residues, the MYW triad exhibits distinct redox properties conferred by covalent crosslinking, which alters its electron distribution and reactivity. This structural distinction renders MYW chemically unique, providing a vulnerability for selective targeting.

Two primary strategies emerge: (1) designing small molecules that form covalent adducts with MYW residues—such as targeting tyrosine or the indole nitrogen—to disrupt catalysis and inhibit catalase activity; and (2) developing electrophilic compounds that preferentially react with the oxidized, radical form of MYW, impairing radical transfer and selectively disrupting KatG's function without broadly affecting other redox-active residues.

To facilitate inhibitor development, computational approaches, including molecular dynamics simulations and structure-based drug design, can help identify and optimize molecules with high affinity for MYW. Additionally, high-throughput screening against the

MYW active site could accelerate the discovery of promising candidates. Rigorous *in vitro* and *in vivo* validation will be essential to confirm efficacy and selectivity.

Leveraging the unique chemical properties of the MYW cofactor offers a novel strategy for KatG inhibition, distinct from traditional active-site blockade. Further exploration of MYW's accessibility in drug development could enhance existing antimicrobial therapies, mitigate oxidative stress in pathogenic microorganisms, and address drug resistance in tuberculosis and other infectious diseases.

Concluding remarks

The decades-long study of KatG and its MYW cofactor have deepened our understanding of how Mtb adapts to oxidative stress, unveiling new opportunities for drug development. The characterization of MYW-OOH in Mtb KatG provides a detailed framework for exploring how this bifunctional enzyme modulates its activity to support bacterial survival. Targeting the MYW cofactor to inhibit catalase activity while enhancing peroxidase function represents a promising therapeutic avenue to combat TB, particularly MDR strains.

By reimagining KatG as both a therapeutic target and a utility, researchers can leverage this enzyme's dual functionality to develop innovative strategies against Mtb infection. Modifying the MYW cofactor to inhibit catalase activity selectively could enhance host-mediated oxidative killing while retaining or augmenting peroxidase activity for prodrug activation. Computational modeling, high-throughput screening, and structural studies are essential next steps to identify small molecules capable of achieving this dual-targeting effect. This approach underscores the broader potential of protein-derived cofactors as critical targets in antimicrobial drug development, offering hope for more effective interventions against TB and other challenging pathogens.

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No potential conflict of interest was reported by the author.

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