

Supplementary Information

Access channel residues Ser315 and Asp137 in *Mycobacterium tuberculosis* catalase-peroxidase (KatG) control peroxidatic activation of the *pro*-drug isoniazid

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Experimental section.

1. Overexpression of recombinant *M. tuberculosis* (*M.tb*) WT KatG and its mutant enzymes, and their purification were performed as described in previous reports;¹ the heme precursor δ -aminolevulinic acid (200 μ M) was added to culture media immediately after inducing to promote formation of holo-enzyme. KatG concentration is expressed as the heme concentration based on the pyridine hemochromogen assay.

2. The rates of IN-NAD adduct generation with varying concentrations of INH were examined directly in a spectrophotometric assay according to our previous protocol² with minor modifications. All reactions were performed at room temp in 20mM potassium phosphate buffer (pH 7.2) containing 0.1 mM EDTA. The assay mixture contains: KatG (1 μ M), NAD^+ (200 μ M), Glucose Oxidase (\sim 15 mU/mL, generating 3.0 μ M/min H_2O_2), and glucose (5 mM). To monitor adduct formation, the absorbance at 326 nm was recorded for 10 min after the addition of glucose. The concentration of IN-NAD adduct was calculated according to its extinction coefficient ($\epsilon_{326\text{ nm}} = 6900\text{M}^{-1}\text{cm}^{-1}$).^{3,4} The reference cuvette contained all components except NAD^+ to correct for background. The rate of adduct formation was computed from the absorbance change recorded during the first 10 min.

3. KatG[Asp137Ser] and KatG[Arg418Leu] were crystallized under conditions similar to those previously reported.⁵ The crystals were grown by sitting-drop vapor diffusion with the crystallization solutions containing 100 mM sodium acetate, pH 4.6, 6% PEG 4000, and 0.17 mM n-dodecyl β -D-maltoside. The crystallization drop was a 1:1 mix between the crystallization solution and \sim 20 mg/mL KatG solution.

4. For X-ray diffraction experiments, single crystals were transferred into a cryo-solution for 10-20 seconds before being flash-frozen in liquid nitrogen in nylon loops (Hampton Research). The cryo-solutions were made by including 30% glucose as a cryoprotectant in the crystallization solutions. For the KatG[Asp137Ser] crystal, the diffraction data were collected at beam line X10SA at the SLS (Swiss-Light-Source), Villigen, Switzerland, while for the KatG[Arg418Leu] crystal, the diffraction data were collected at beamline ID29 at the ESRF (European Synchrotron Radiation Facility), Grenoble, France. Both data sets were collected at 100 K with a 6M Pilatus detector (DECTRIS, Ltd.). The diffraction data were processed and scaled with MOSFLM⁶ and SCALA⁷ or AIMLESS, and the space group was confirmed with POINTLESS through the CCP4 software suite^{8,9}. The tetragonal structures have two KatG molecules in the asymmetric unit, and the structures were solved by molecular replacement with PHASER¹⁰ based on the structure of WT *M.tb* KatG (PDB entry 2CCA). Refinement was performed through multiple cycles of restrained refinement in REFMAC¹¹ using NCS restraints, and model building and addition of water molecules in COOT.¹² No restraints were used for the Fe-N_{Heme}, Fe-N_{His}, Fe-H₂O, C η ₂_{Tyr107}-C ϵ ₁_{Tyr229} or C ϵ ₂_{Tyr229}-S δ _{Met255} distances. The program RADDOSSE^{13,14} was used to calculate the absorbed X-ray doses ($\text{Gy} = \text{J}\cdot\text{kg}^{-1}$) of the different crystals during crystallographic data collection. The access channel in the KatG variants was calculated with HOLLOW¹⁵ using a 1.4 \AA probe radius. The backbone C α root mean square deviation (RMSD) values were calculated by use of PDBeFold (<http://www.ebi.ac.uk>) to be 0.33 \AA between the WT KatG and KatG[Asp137Ser] structures and 0.35 \AA between the WT KatG and KatG[Arg418Leu] structures. Structure figures were made using PyMOL.¹⁶

References

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Table S1 – Crystal data, data collection and refinement statistics.

KatG	D137S	R418L
Crystal data		
Space group	P4 ₂ 2 ₁ 2	P4 ₂ 2 ₁ 2
<i>a</i> , <i>b</i> , <i>c</i> (Å)	150.6 / 150.6 / 157.4	150.7 / 150.7 / 157.1
$\alpha\beta\gamma$ (°)	90 / 90 / 90	90 / 90 / 90
Crystal size (μm ³)	120×120×10	100×70×15
Data collection		
X-ray source	SLS-X10SA	ESRF-ID29
Wavelength (Å)	1.0000	0.97627
Detector	Pilatus 6M	Pilatus 6M
Temperature (K)	100	100
Beam size (μm ²)	100×100	30×30
Flux (photons/sec)	620·10 ⁹	309·10 ⁹
Absorbed X-ray dose (MGy)	2.2	19.8
Resolution range (Å)	75.3-2.5 / 2.56-2.50	44.1-3.10 / 3.27-3.10
Completeness (%) [*]	99.9 / 100.0	99.7 / 99.8
Redundancy [*]	6.2 / 6.5	7.7 / 7.3
<i>I</i> /sd(<i>I</i>) [*]	8.7 / 3.9	11.4 / 4.5
<i>R</i> _{sym} ^{*†}	13.0 / 38.7	15.7 / 46.7
Total observations	389770	2588312
Unique reflections	62942	33364
Mosaicity	0.42	0.51
Refinement Statistics		
<i>R</i> _{work} (%) [†]	26.6	16.3
<i>R</i> _{free} (%) [#]	31.1	21.4
Mean overall isotropic B-factor (Å ²)	34.3	39.7
Ramachandran plot: ration in most favoured / other allowed regions / generously allowed regions (%)	88.8 / 10.9 / 0.2	87.1 / 12.1 / 0.8
Estimated overall coordinate error based on <i>R</i> _{work} / Maximum Likelihood (Å)	0.59 / 0.31	- / 0.28
RMSD bond (Å) / angles (°)	0.012 / 1.4	0.012 / 1.5
Solvent content (%)	56.3	56.5
Molecules per asymmetric unit	2	2
Added water molecules	82	6
Volume not occupied by model (%)	46.6	46.8
Unmodelled residues	1-24	1-23
PDB code	4C50	4C51

^{*} The value before the backslash is for all data, and the value after the backslash is for the data in the highest resolution shell

[†] $R_{\text{sym}} = \sum |I - \langle I \rangle| / \sum I$

[†] $R_{\text{work}} = \sum (|F_{\text{obs}}| - |F_{\text{calc}}|) / \sum |F_{\text{obs}}|$

[#] *R*_{free} is the *R*_{cryst} calculated on the 5% reflections excluded for refinement.

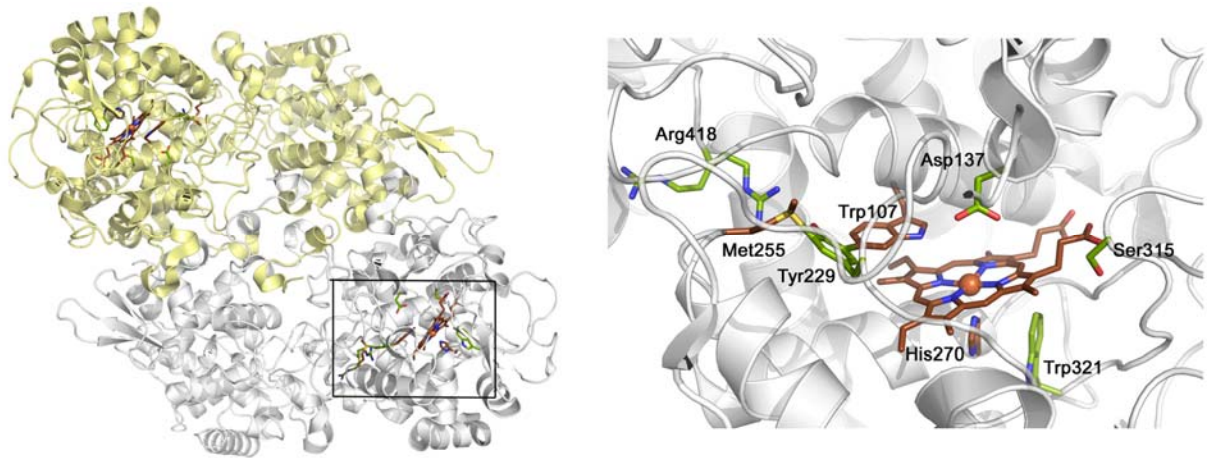


Fig. S1. Structure of the *M.tb* KatG dimer (2CCA.pdb) shown on the left. On the right, the residues mutated for this study are colored in green. The figure was generated using PyMOL.¹⁶

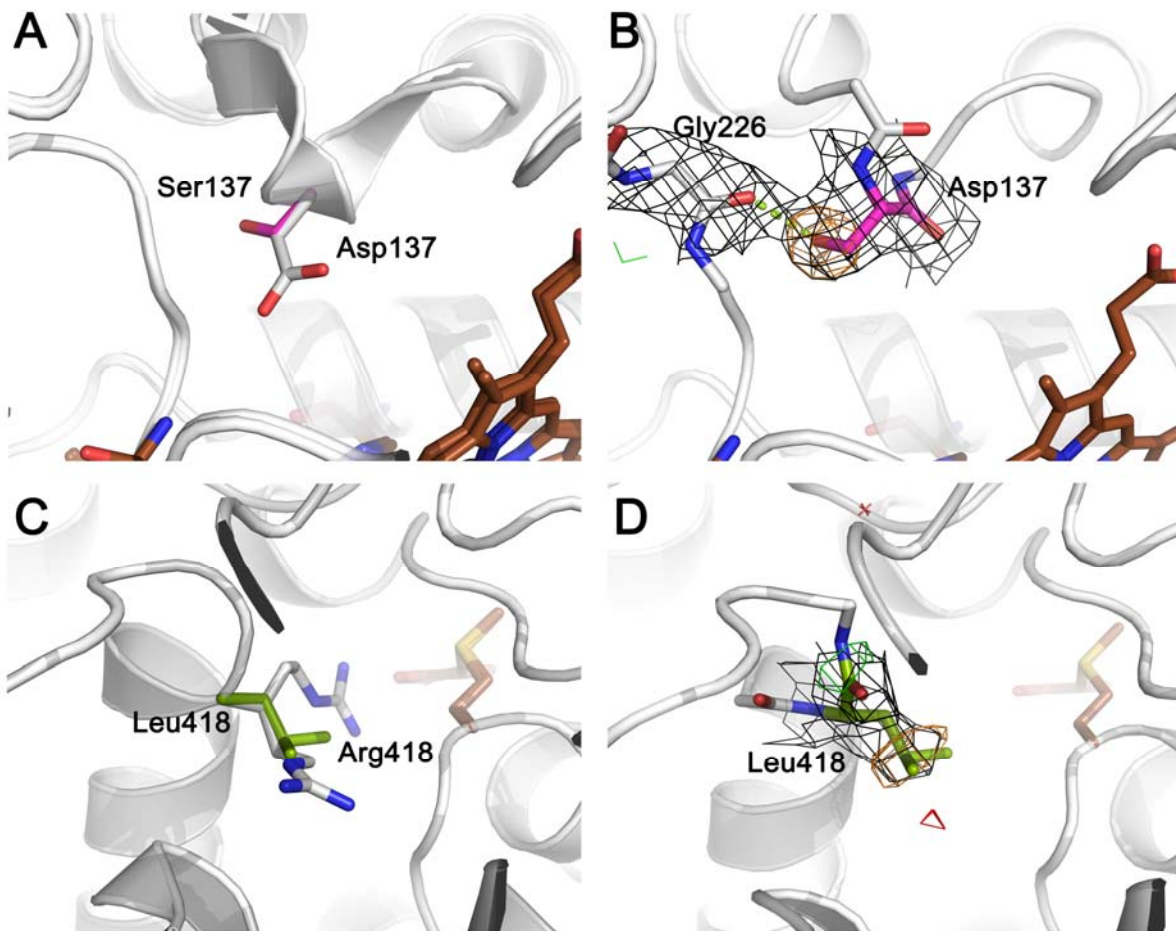


Fig. S2. Structures of the Asp137Ser and Arg418Leu mutants of *M.tb* KatG. **A.** Overlay of WT (2CCA.pdb) and Asp137Ser mutant structures; **B.** Asp137Ser mutant structure showing Ser137 and Gly226 with electron density; **C.** Overlay of WT (2CCA.pdb) and Arg418Leu mutant structures; **D.** Arg418Leu mutant structure showing Leu418 with electron density. The $2F_o-F_c$ electron density maps (black) are contoured at $+1\sigma$, the F_o-F_c electron density difference maps are contoured at -3σ (red) and $+3\sigma$ (green), and the F_o-F_c OMIT-map of the mutated residues are contoured at $+4.7\sigma$ (orange). The figure was generated using PyMOL.¹⁶

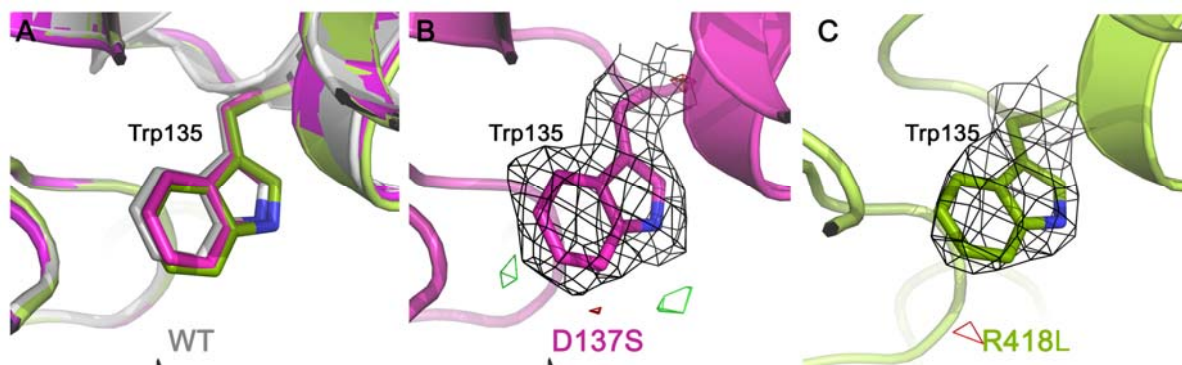


Fig. S3. Structures of *M.tb* WT KatG, KatG[Asp137Ser], and KatG[Arg418Leu] mutants showing the Trp135 residue. **A.** Overlay of WT (2CCA.pdb) and Asp137Ser and Arg418Leu structures; **B.** Asp137Ser mutant structure showing Trp135 with electron density; **C.** Arg418Leu mutant structure showing Trp135 with electron density. The $2F_o-F_c$ electron density maps (black) are contoured at $+1\sigma$, and the F_o-F_c electron density difference maps are contoured at -3σ (red) and $+3\sigma$ (green). The figure was generated using PyMOL.¹⁶

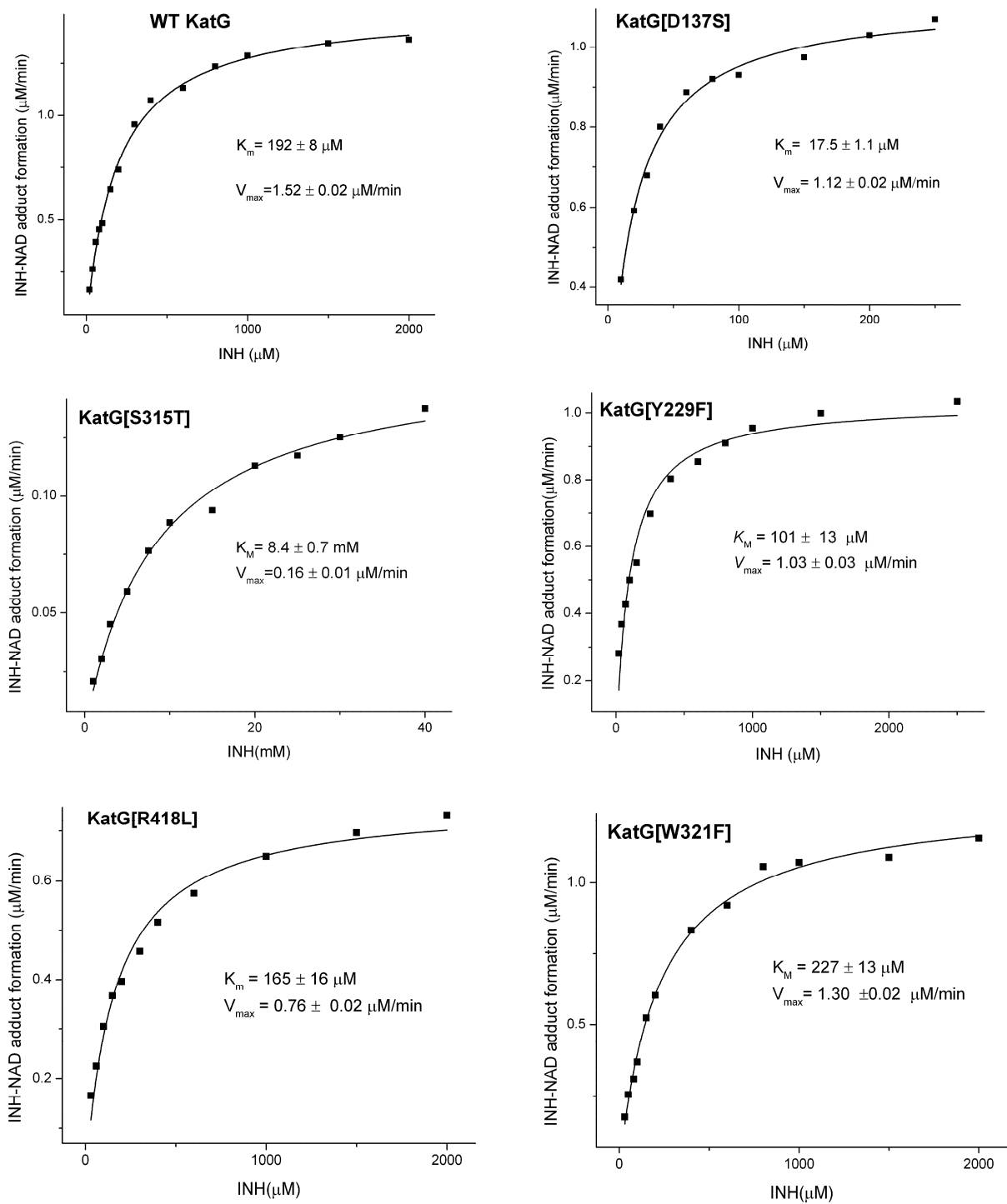


Fig. S4. Fitting of kinetics to Michaelis-Menten equation.