KatG-mediated oxidation imparts reduced susceptibility of bacteria to kanamycin.

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Supplemental Information

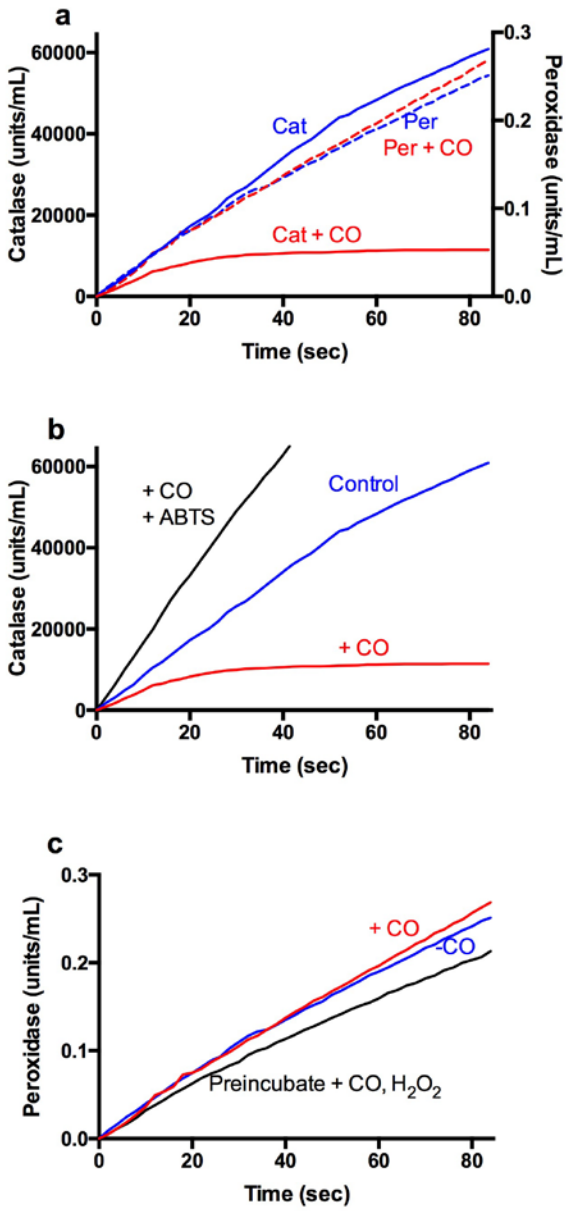

Figure S1. Effect of CO on catalase and peroxidase reactions of BpKatG. **Panel a**: The solid lines show catalase activity without (blue) and with (red) CO present in the buffer. The dashed lines show the peroxidase activity without (blue) and with (red) CO present. **Panel b**: Catalase activity is shown without CO (blue), with CO (red) and with CO and ABTS (black) in the buffer. **Panel c**: Peroxidase activity is shown without CO (blue), with CO (red) in the buffer. The trace in black shows the peroxidase activity following pre-incubation with CO and H_2O_2 for 2 minutes prior to the addition of ABTS and H_2O_2 to initiate the peroxidase reaction.

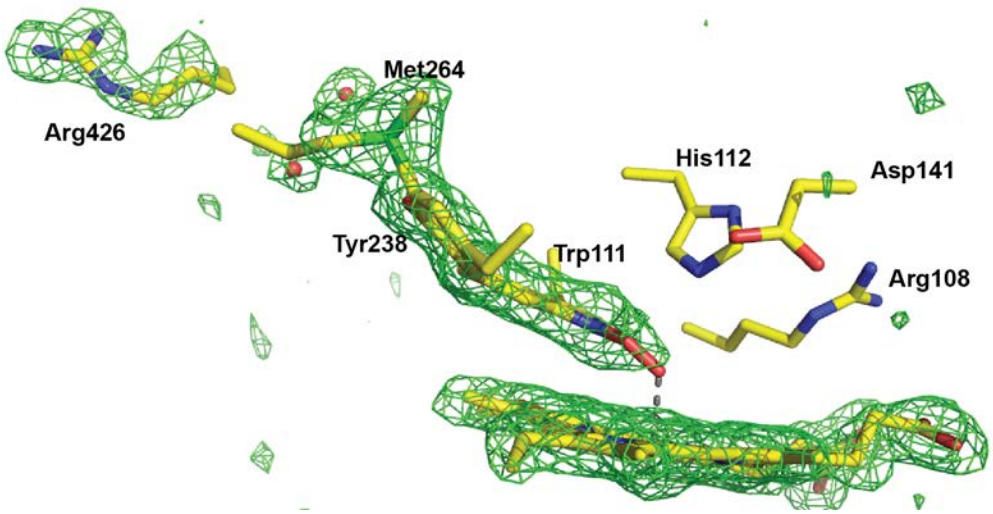

Figure S2. Heme and surrounding residues of WT BpKatG after soaking of the crystal in CO-saturated mother liquor containing 5 mM H_2O_2. The F_o-F_c omit electron density map at 5.0 σ was calculated without the heme, Trp111, Tyr238, Met264 or Arg426 in the model. The absence of an iron ligand in the heme distal side appears unquestionable eliminating, in particular, the coordination of a CO molecule. Instead, the tryptophan of the adduct Trp111shows a high occupancy of the perhydroxy modification, while the mobile arginine Arg426 is mostly in the **out** position, an unlikely intermediate in the catalase cycle [S1].

S1. Loewen, P. C., Carpena, X., Vidossich, P., Fita, I. & Rovira, C. An ionizable active-site tryptophan imparts catalase activity to a peroxidase core. *J. Am. Chem. Soc.* **136**, 7249-7252 (2014).

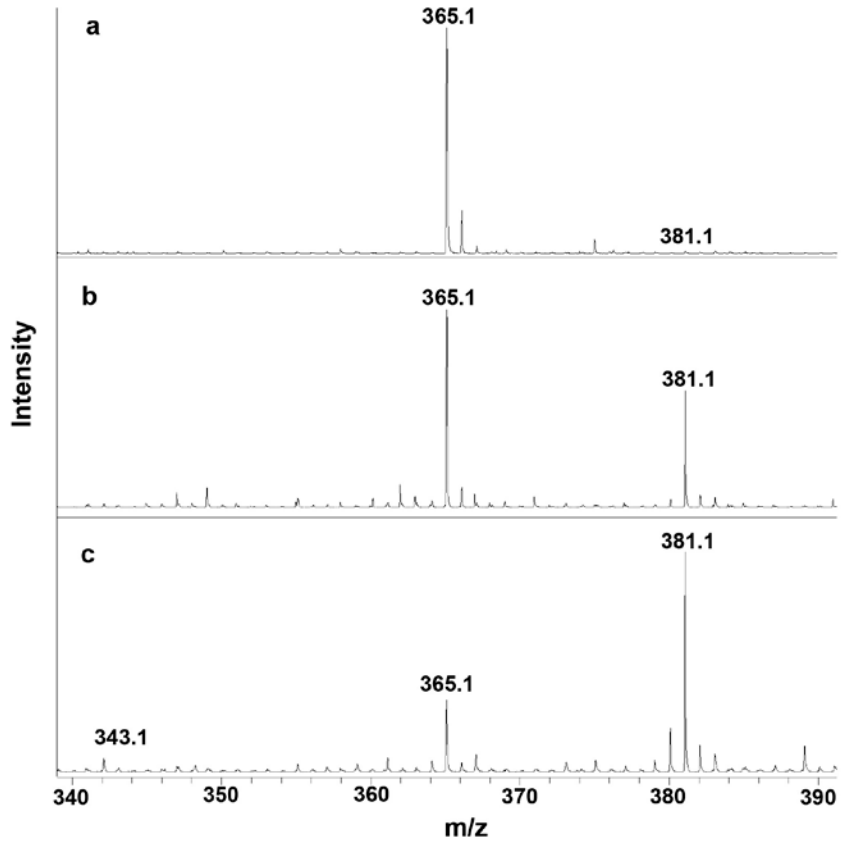

Figure S3. Mass spectrometry analysis of the oxidation products of maltose. Panel **a** contains the spectrum of maltose before treatment with KatG or glucose oxidase. The predominant ion has an associated sodium ion, 343.1 + 22.0 = 365.1. The sample in panel **b** was incubated with 1 µg/mL KatG for 15 minutes and the sample in panel **c** was incubated with 1 µg/mL KatG supplemented with 5 mg/mL glucose oxidase and 5 mM glucose for 15 minutes.

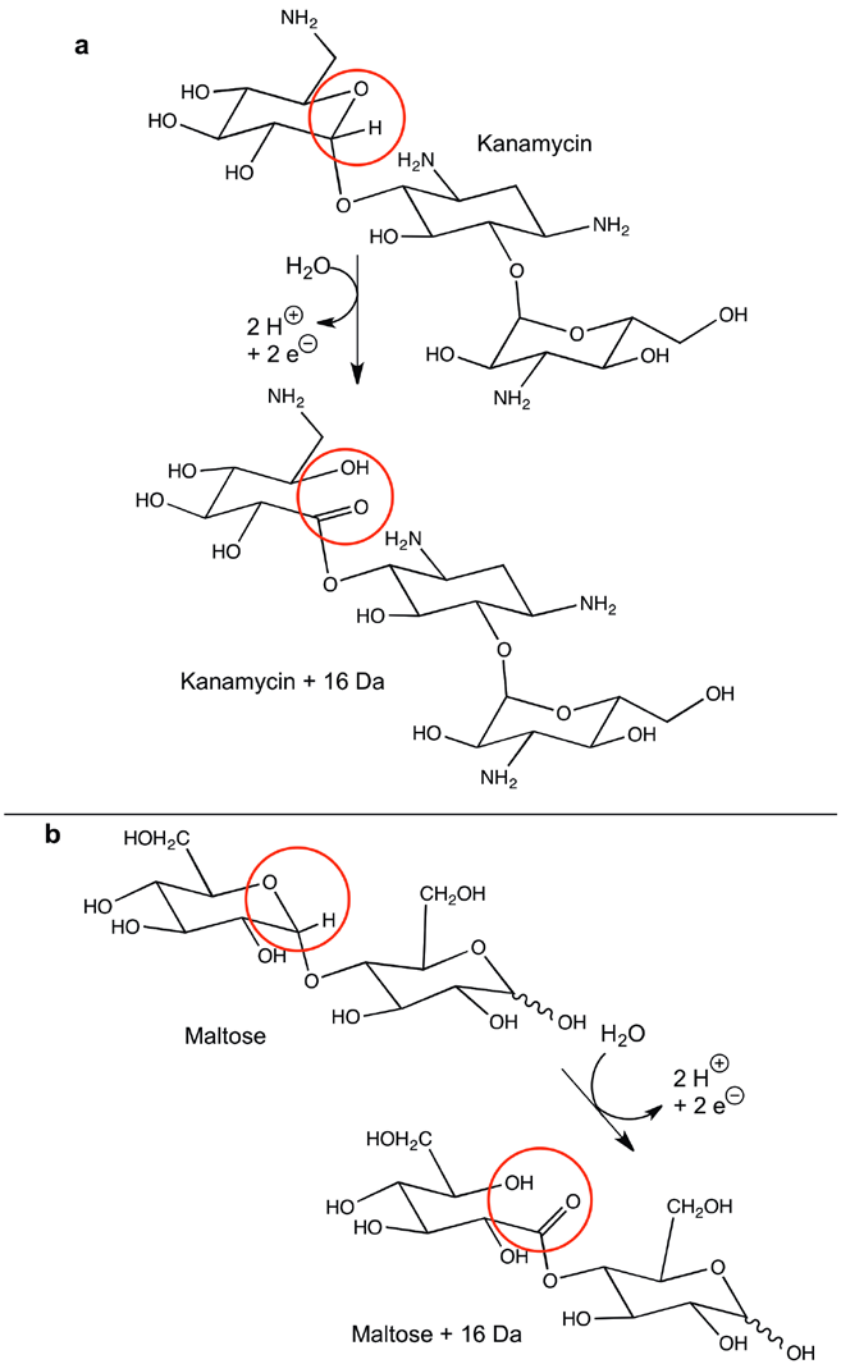

Figure S4. Schemes for the oxidation of kanamycin (panel **a**) and maltose (panel **b**) that are most consistent with the experimental data.

Table S1: Bacterial strains and plasmids used

Bacterial Strains	Relevant characteristics	Source
E. coli MP180	*thi-1* HfrH	Ref. S2
E. coli UM120	As MP180 but *katE12*::*Tn10*	Ref. S3
E. coli UM122	As MP180 but *katF13*::*Tn10*	Ref. S3
E. coli UM202	As MP180 but *katG17*::*Tn10*	Ref. S4
E. coli CSH7	*lacY rpsL thi-1*	Cold Spring Harbor Collection
E. coli CSH57a	*leuB6 proC83 purE42 trpE38 his-208 argG77 ilvA681 met-160 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 azi-6 rpsL109 tonA23 tsx67 supE44 malA38 xthA*	Cold Spring Harbor Collection
E. coli UM1	CSH7 *katE1 katG14* (^{415}C→T resulting in ^{138}Leu→Phe in KatG)	Ref. 2 & S5
E. coli UM2	CSH57a katE2 katG15 (^{356}G→A resulting in ^{118}Gly→Asp in KatG)	Ref. 2 & S5
A. baumannii ATCC17978		
A. baumannii AB155	ATCC17978:Δ*katG*	This study
A. baumannii AB188	ATCC17978:Δ*katE*	This study
A. baumannii AB189	AB115:Δ*katE*	This study
pMO130	Km^r, suicide plasmid	Ref. 23
pFLP2	Ap^r, *flp*-recombinase	Ref. S6
pBluescript KS-	Ap^r	
pBpKatG	pBluescript KS expressing *katG* from *Burkholderia pseudomallei*	Ref 16

S2. Pearson, M. L. The role of adenosine-3'-5'-cyclic mono- phosphate in the growth of bacteriophage lambda. Virology **49**, 605-609 (1972).

S3. Loewen, P. C. & Triggs, B. L. Genetic mapping of *katF*, a locus that with *katE,* affects the synthesis of a second catalase species in *Escherichia coli.* J. Bacteriol. **160**, 668-675 (1984).

S4. Loewen, P. C., Switala, J. & Triggs-Raine, B. L. Catalases HPI and HPII in *Escherichia coli* are induced independently. *Arch. Biochem. Biophys.* **243,** 144–149 (1985).

S5. Loewen, P. C. Isolation of catalase-deficient *Escherichia coli* mutants and genetic mapping of *katE,* a locus that affects catalase activity. J. Bacteriol. **157**, 622-626 (1984).

S6. Choi, K. H. & Schweizer, H. P. An improved method for rapid generation of unmarked *Pseudomonas aeruginosa* deletion mutants. BMC Microbiol. **5**:30 (2005).

Table S2: Oligonucleotides used in this study

Primer	Sequence	Source	Product size
katG Up Forward	TTACTAAACCAAAAATCGGGAT	This study	1021 bp
katG Gm Up Reverse	AGGAACTTCAAGATCCCCAATTCGTGAC ATGATTATATTCTCTCTGG	This study	
katG Gm Down Forward	TCAGAGCGCTTTTGAAGCTAATTCGGACT TAGCTTAATTTAAGCCAAA	This study	1027 bp
katG Down Reverse	TGACACCCGTTTTTATAATCA	This study	
katE Up Forward	GTCATGCCTATAAAGCCATT	This study	1019 bp
katE Gm Up Reverse	AGGAACTTCAAGATCCCCAATTCGAATTC GAATACGCTCAGGTATACGCT	This study	
katE Gm Down Forward	TCAGAGCGCTTTTGAAGCTAATTCGCGCT TTCAGGGCTTTTTCTA	This study	925 bp
katE Down Reverse	TAGAGGTAGTCTAGACATGG	This study	
katG qRT Forward	GGCGATGAAAAAGAATGGTTA	Ref. S7	185 bp
katG qRT Reverse	ATTTCTTCATCATCCATTGCC	Ref. S7	
katE qRT Forward	AACTTTGACTTCGATTTGCTGGA	Ref. S7	205 bp
katE qRT Reverse	TGTATGAAAATAGTCGGGCTTGT	Ref. S7	

S7. Sun, D., Crowell, S. A., Harding, C. M., De Silva, P. M., Harrison, A., Fernando, D. M., Mason, K. M., Santana, E., Loewen, P. C., Kumar, A. & Liu, Y. KatG and KatE confer *Acinetobacter resistance* to hydrogen peroxide but sensitize bacteria to killing by phagocytic respiratory burst. *Life Sci.* **148**, 31-40 doi:10.1016/j.lfs.2016.02.015 (2016).

Table S3 - Data collection and refinement statistics of BpKatG with maltose bound (6B9B) and after treatment with H_2O_2 in CO-containing buffer (5KT9).

A. Data collection statistics	6B9B	5KT9
Space group	$P2_12_12_1$	$P2_12_12_1$
a (Å)	100.42	100.85
b (Å)	115.09	113.79
c (Å)	174.98	174.63
α, β, γ (°)	90	90
Resolution[a]	48.026 - 1.80	47.67 - 1.88
	(1.90 - 1.80)	(1.98 - 1.88)
Unique reflections	184,467 (27,052)	162,264 (23,554)
Completeness %	98.7 (99.8)	99.5 (99.7)
R_{merge}	0.067 (0.568)	0.90 (0.570)
R_{pim}	0.038 (0.332)	0.046 (0.293)
$\langle I/\sigma I \rangle$	12.9 (2.4)	10.7 (2.2)
CC(1/2)	0.998 (0.803)	0.998 (0.826)
Multiplicity	3.8 (3.8)	4.4 (4.6)
B Model refinement statistics		
No. reflections	175,184	153,938
R_{cryst} (%)	13.6	17.7
R_{free} (%)	16.2	21.3
Non-H atoms	12,765	12,633
Water Molecules	1,452	1,415
Average B-factor Å²		
Protein	25.0	27.5
Heme	17.9	20.3
Waters	33.8	36.4
Other		
Coor. err. Å[b]	0.058	0.101
rms dev. bonds Å	0.028	0.028
rms dev. angles °	2.29	2.24

[a] Values in parentheses correspond to the highest resolution shell

[b] Based on maximum likelihood