

SUPPLEMENTAL DATA

TABLE S1: Data collection and refinement statistics.

	Triple mutant-GTN	WT-thionitrate
PDB entry	4FR8	4FQF
Data collection		
Beamline	ESRF ID 14-4	DESY X13
Wavelength (Å)	1.0044	0.8123
Unit cell	$a=101.7$ Å $b=175.9$ Å $c=102.3$ Å $\beta=95.0^\circ$	$a=136.9$ Å $b=148.4$ Å $c=176.0$ Å
Space group	$P2_1$	$C222_1$
Twin fraction / twin law	0.21 / l,-k,h	
Resolution range (Å)	37.1 – 2.20 (2.32 – 2.20)	19.8 – 2.28 (2.39 – 2.28)
Completeness (%)	99.8 (99.9)	99.1 (99.9)
Redundancy	3.4 (3.4)	6.4 (6.4)
R_{sym}	0.085 (0.357)	0.082 (0.295)
I/σ_I	11.1 (4.1)	14.5 (5.7)
Unique reflections	180585	80015
Refinement		
R / R_{free}	0.132 / 0.167	0.170 / 0.209
r.m.s.-deviations		
bond length (Å)	0.012	0.012
bond angle (°)	0.857	0.889
Number of atoms		
protein	30566	15210
GTN or NO ₂	15	12
cofactor/additive/metal ions	302	208
water	2856	889
B-factors (Å ²)		
protein	20.8	20.0
GTN or NO ₂	26.5	34.4
cofactor/additive/metal ions	29.3	25.0
water	25.3	24.2

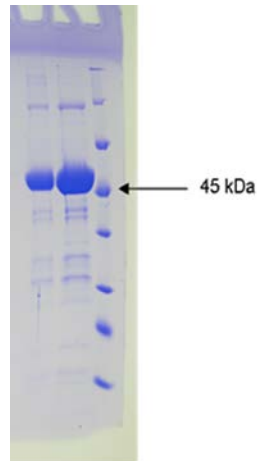


FIGURE S1. SDS-PAGE gel with 2 μ l and 4 μ l of the concentrated enzyme purified with the additional ion exchange purification step. Lane 3 shows markers in the range from 14.4 to 116 kDa. The arrow indicates the 45 kDa marker band.

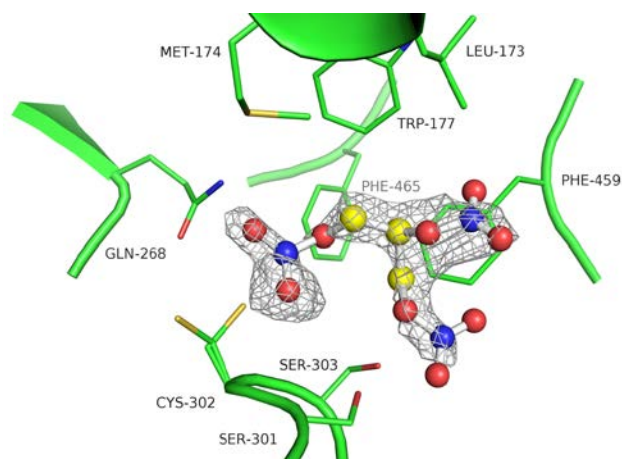


FIGURE S2. Electron density for GTN. Active site of the triple mutant with the residual density prior to inclusion of GTN in the model. The 2Fo-Fc map is contoured at 0.8 σ .

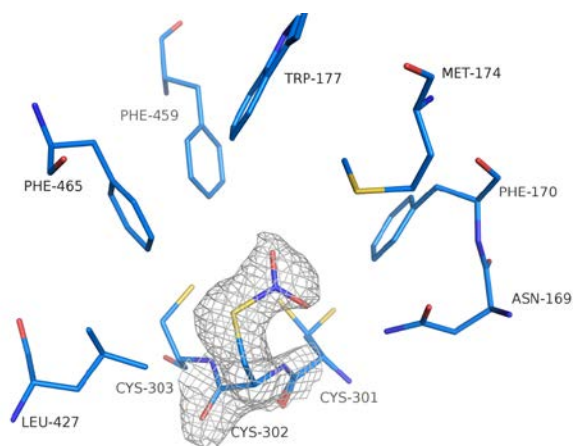


FIGURE S3. Electron density for the thionitrate adduct. Active site of the wild-type with the density at Cys302 prior the inclusion of the thionitrate in the model. The 2Fo-Fc map is countered at 1σ.