Neutralizing the Detrimental Effect of Glutathione on Precious Metal Catalysts

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Supporting Information

General Considerations 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline, glutathione, Maleimide, 2-bromo-1-phenylethanone, Oxone, Phenylvinyl sulfone, diamide, 3-phenyl-2-propynenitrile, benzoquinone and $K_3Fe^{III}(CN)_6$ were purchased from commercial suppliers and used as received. Streptavidin (Sav) mutants were produced, purified and characterized as previously described [1]. The Sav used in this work and on which all variants were based was T7-tagged core Sav described by Gallizia *et al.* [2] and herein we refer to it as wild-type Sav. The corresponding ATHase is also referred to as wild-type (WT). For a detailed synthesis procedure of [Cp*Ir(Biot-*p*-L)Cl], see reference [3]. HPLC measurements were performed on Agilent machines equipped with modules from the 1100 and 1200 series and diode array detectors. HPLC columns were used with the appropriate guard columns.

Stock solutions and buffers MOPS/formate buffer: 3-(*N*-morpholino)propanesulfonic acid and sodium formate were dissolved in milliQ water to the desired concentrations (see below) and the pH was adjusted by addition of NaOH.

Ir-complex: [Cp*Ir(biot-*p*-L)Cl)] was dissolved in DMF to a final concentration of 2 mM. This solution was prepared freshly for each experiment.

Substrate: 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline (164.2 mg) was dissolved in water (2 ml) to a final concentration of 400 mM.

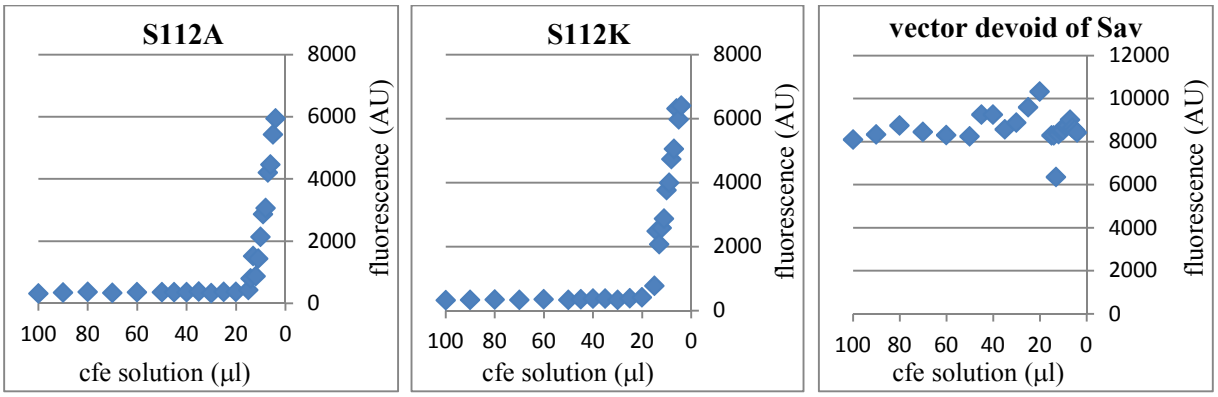
Glutathione: Reduced glutathione was dissolved in milliQ water to the desired final concentration (see below). This solution was prepared freshly for each experiment.

GSH neutralizing agents: Maleinimide, 2-bromo-1-phenylethanone, Phenylvinyl sulfone, diamide, 3-phenyl-2-propynenitrile and benzoquinone were dissolved in DMF to a final concentration of 200 mM.
Oxone and $K_3Fe^{III}(CN)_6$ were dissolved in milliQ water to a final concentration of 200 mM.
These solutions were prepared freshly for each experiment.

Preparation of cell free extracts (cfe) and cell lysates *E. coli* cells (BL21(DE3)pLysS) containing either a pET11b-T7Sav-plasmid including the gene for Sav-S112A or -S112K, respectively, were grown in LB medium containing 34 µg/ml chloramphenicol and 100 µg/ml ampicillin (1 L culture) at 37 °C until an OD_{600} of 0.8-1 was reached. Expression was induced by adding IPTG (400 µ M final concentration). The cultures where shaken for 3.5 hours at 37 °C, and cells were harvested by centrifugation (3846 x *g*) for 10 minutes at 4 °C. To the pellet (between 2.5 and 3 g per liter) was added Tris-HCl buffer (20 mM, pH 7.4) containing 1 mM PMSF (5-10 ml according to have a final concentration of 28.6 µM tetrameric Sav) as well as DNAseI (Roche, small tip of spatula, some 1000 U) before shaking at room temperature for 1 h. The resulting cell lysate was stored at -20 °C.
Cfe was prepared by centrifugation of the cell lysate for 10 minutes and lyophilizing the frozen supernatant to obtain beige to brown powder which was stored at 4 °C. The content of Sav was determined using a biotin-4-fluorescein titration assay (see below). [4]

Biotin-4-fluorescein assay Cfe was dissolved in 100 mM phosphate buffer pH 7 (5 mg/ml) and decreasing volumes of this solution (100 to 4 µl) were added to microtiter plate wells (Nunclon) each containing 10 µl of a 40 µM biotin-4-fluorescein solution (prepared by dilution of a 0.6 M stock solution in DMSO with phosphate buffer). The final volume of 120 µl was adjusted by addition of phosphate buffer in each well before the fluorescence was detected in a plate reader (Tecan Saphire, λ_{ex} = 485 nm, λ_{abs} = 520 nm). The fluorescence was then plotted as a function of the amount of cfe solution added to each well and the concentration of biotin binding sites was calculated from the volume at the equivalence point (where the concentration of Sav free binding sites is equal to the biotin-4-fluorescein concentration, see below). The content of Sav in cell lysates was determined from diluted (1:2-1:4) supernatants of centrifuged samples as described above.

Figure SI1 Determination of Sav biotin biding capacity of cell free extraxts using biotin-4-fluorescein. Upon incorporation within Sav isoforms, the fluorescence is quenched. Once all biotin-binding sites are saturated, the excess biotin-4-fluorescein leads to fluorescence.



Ellman's assay 2.5 ml of a reaction buffer containing 100 mM phosphate (pH 8) and 1 mM EDTA was treated with 50 µl of a 5,5'-dithio-*bis*-(2-nitrobenzoic acid) solution (10 mM in reaction buffer) as well as 250 µl cfe solution (20 mg/ml in reaction buffer) and incubated for 30 min at RT before the absorbance at

412 nm was measured. The thiol concentration was calculated using a molar extinction coefficient of 14150 $M^{-1}cm^{-1}$. [5]

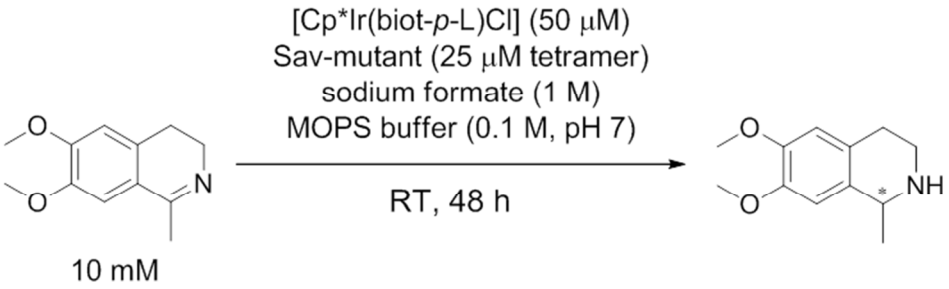
General set up for reactions using purified Sav mutants Lyophilized Sav was dissolved in MOPS/formate buffer containing 0.2 M MOPS and 2 M sodium formate (pH 7) to a final concentration of 50 µM tetrameric Sav (the average number of free binding sites per Sav tetramer was determined with a biotin-4-fluorescein assay [4]). 100 µl of this solution were added to a glass tube equipped with a mechanical stirrer followed by addition of water (50 µl), glutathione stock solution (16.7 µM, 30 µl), DMF (between 0 and 10 µl) and GSH neutralizing agent stock solution (between 0 and 10 µl corresponding to the desired final concentration). This mixture was then stirred for 2 or 15 hours at room temperature before the Ir-complex stock solution was added (5 µl). After 5 minutes, the reaction was initiated by adding the substrate stock solution (5 µl), and the mixture was stirred for 48 hours at room temperature (final volume: 200 µl; final concentrations: 50 µM [Cp*Ir(biot-*p*-L)Cl)], 25 µM Sav, 0.1M MOPS, 1 M formate, 2.5 mM glutathione, between 1.25 and 10 mM glutathione neutralizing agent, 7.5 % (v/v) DMF). The set up for reactions without Sav was identical, except that MOPS/formate buffer containing no Sav was used.

General set up for reactions using cell free extracts Cfe was dissolved in MOPS formate buffer containing 0.686 M MOPS and 3.429 M sodium formate (pH 7) to a concentration of 22.9 mg/ml (corresponds to 28.6 µM tetrameric Sav). 175 µl of this slightly turbid solution was added to a glass tube equipped with a mechanical stirrer followed by addition of water (5 µl), DMF (between 0 and 10 µl) and GSH neutralizing agent stock solution (between 0 and 10 µl corresponding to the desired final concentration). The mixture was then stirred for 15 hours and the reaction was started as described above (final volume: 200 µl; final concentrations: 50 µM [Cp*Ir(biot-*p*-L)Cl)], 25 µM Sav, 0.6 M MOPS, 3 M formate, between 1.25 and 10 mM glutathione neutralizing agent, 7.5 % v/v DMF).

General set up for reactions using lysed cells To cell lysates was added MOPS and sodium formate to a concentration of 0.686 M and 3.429 M, respectively, and the mixture was stirred by means of a vortex mixer until the MOPS and formate were completely dissolved. The pH was adjusted to 7 by addition of NaOH, and reactions were performed with 175 µl of the resulting mixture as described for catalysis with cfe (see above). Final volume: 200 µl; final concentrations: 50 µM [Cp*Ir(biot-*p*-L)Cl)], 25 µM Sav, 0.6 M MOPS, 3 M formate, 17.5 mM Tris, 0.875 mM PMSF, between 0.1 and 10 mM glutathione neutralizing agent, 7.5 % (v/v) DMF)

Work up and analysis To the reaction mixtures milliQ water (500 µl) was added followed by addition of 20% NaOH (50 µl). The mixture was then extracted two times with dichloromethane (1 ml), the combined organic fractions were collected in a PP tube containing anhydrous sodium sulfate, centrifuged (18800 x *g*) for 5 minutes and the supernatant was analyzed by chiral HPLC using a Chiralpak IC column (5 µm, 4.6 mm · 25 mm) and dichloromethane containing 1% isopropanol and 0.06% diethylamine as an eluent; 1 ml/min; 25 °C, 280 nm, T_R 8.5 min ((*S*)- 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline), 9.8 min (6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline), 14.6 ((*R*)- 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline, referred to as salsolidine). Yields were calculated under consideration of a response factor of 1.95.

Table SI1 Reproduction of catalysis with purified Sav spiked glutathione, and the GSH neutralizing agents



Sav-mutant	[GSH] (mM)	GSH neutralizing agent	[GSH neutralizing agent] (mM)	incubation time (h)	conv. (%)	ee (%)	abs. conf.
no	2.5	**MalIn**	2.5	15	24	0	*rac*
no	2.5	**MalIn**	5	15	20	0	*rac*
no	2.5	**MalIn**	10	15	7	0	*rac*
S112A	2.5	**MalIn**	2.5	15	12	80	(*R*)
S112A	2.5	**MalIn**	5	15	2	75	(*R*)
S112A	2.5	**MalIn**	10	15	2	59	(*R*)
S112K	2.5	**MalIn**	2.5	15	5	46	(*S*)
S112K	2.5	**MalIn**	5	15	4	46	(*S*)
S112K	2.5	**MalIn**	10	15	5	20	(*S*)
no	0	**MalIn**	2.5	15	100	0	*rac*
no	0	**MalIn**	5	15	99	0	*rac*
no	0	**MalIn**	10	15	85	0	*rac*
S112A	0	**MalIn**	2.5	15	22	65	(*R*)
S112A	0	**MalIn**	5	15	15	60	(*R*)
S112A	0	**MalIn**	10	15	8	56	(*R*)
S112K	0	**MalIn**	2.5	15	26	26	(*S*)
S112K	0	**MalIn**	5	15	20	20	(*S*)
S112K	0	**MalIn**	10	15	13	13	(*S*)
no	2.5	**BroPheOne**	2.5	15	92	0	*rac*
no	2.5	**BroPheOne**	5	15	77	0	*rac*
no	2.5	**BroPheOne**	10	15	49	0	*rac*
no	2.5	**BroPheOne**	2.5	2	94	0	*rac*
no	2.5	**BroPheOne**	5	2	95	0	*rac*
no	2.5	**BroPheOne**	10	2	88	0	*rac*
S112A	2.5	**BroPheOne**	2.5	15	71	84	(*R*)
S112A	2.5	**BroPheOne**	5	15	65	86	(*R*)
S112A	2.5	**BroPheOne**	10	15	58	87	(*R*)
S112A	2.5	**BroPheOne**	2.5	2	68	85	(*R*)
S112A	2.5	**BroPheOne**	5	2	68	86	(*R*)
S112A	2.5	**BroPheOne**	10	2	62	87	(*R*)
S112K	2.5	**BroPheOne**	2.5	15	40	63	(*S*)
S112K	2.5	**BroPheOne**	5	15	34	59	(*S*)

S112K	2.5	**BroPheOne**	10	15	29	51	(*S*)
S112K	2.5	**BroPheOne**	2.5	2	34	58	(*S*)
S112K	2.5	**BroPheOne**	5	2	24	54	(*S*)
S112K	2.5	**BroPheOne**	10	2	29	51	(*S*)
no	0	**BroPheOne**	2.5	15	96	0	*rac*
no	0	**BroPheOne**	5	15	74	0	*rac*
no	0	**BroPheOne**	10	15	54	0	*rac*
S112A	0	**BroPheOne**	2.5	15	72	83	(*R*)
S112A	0	**BroPheOne**	5	15	74	84	(*R*)
S112A	0	**BroPheOne**	10	15	67	83	(*R*)
S112K	0	**BroPheOne**	2.5	15	45	58	(*S*)
S112K	0	**BroPheOne**	5	15	45	53	(*S*)
S112K	0	**BroPheOne**	10	15	37	41	(*S*)
no	2.5	**PheViSul**	2.5	15	98	0	*rac*
no	2.5	**PheViSul**	5	15	99	0	*rac*
no	2.5	**PheViSul**	10	15	98	0	*rac*
no	2.5	**PheViSul**	2.5	2	79	0	*rac*
no	2.5	**PheViSul**	5	2	97	0	*rac*
no	2.5	**PheViSul**	10	2	86	0	*rac*
S112A	2.5	**PheViSul**	2.5	15	78	80	(*R*)
S112A	2.5	**PheViSul**	5	15	86	64	(*R*)
S112A	2.5	**PheViSul**	10	15	91	55	(*R*)
S112A	2.5	**PheViSul**	2.5	2	71	79	(*R*)
S112A	2.5	**PheViSul**	5	2	69	73	(*R*)
S112A	2.5	**PheViSul**	10	2	44	59	(*R*)
S112K	2.5	**PheViSul**	2.5	15	61	58	(*S*)
S112K	2.5	**PheViSul**	5	15	88	21	(*S*)
S112K	2.5	**PheViSul**	10	15	63	28	(*S*)
S112K	2.5	**PheViSul**	2.5	2	43	52	(*S*)
S112K	2.5	**PheViSul**	5	2	63	38	(*S*)
S112K	2.5	**PheViSul**	10	2	53	29	(*S*)
no	0	**PheViSul**	2.5	15	100	0	*rac*
no	0	**PheViSul**	5	15	99	0	*rac*
no	0	**PheViSul**	10	15	100	0	*rac*
S112A	0	**PheViSul**	2.5	15	64	83	(*R*)
S112A	0	**PheViSul**	5	15	52	84	(*R*)
S112A	0	**PheViSul**	10	15	10	83	(*R*)
S112K	0	**PheViSul**	2.5	15	65	58	(*S*)
S112K	0	**PheViSul**	5	15	38	53	(*S*)
S112K	0	**PheViSul**	10	15	18	41	(*S*)
no	2.5	**Ox**	2.5	15	0	0	-
no	2.5	**Ox**	5	15	0	0	-
no	2.5	**Ox**	10	15	27	0	*rac*
S112A	2.5	**Ox**	2.5	15	0	0	-
S112A	2.5	**Ox**	5	15	0	0	-
S112A	2.5	**Ox**	10	15	55	80	(*R*)
S112K	2.5	**Ox**	2.5	15	0	0	-
S112K	2.5	**Ox**	5	15	0	0	-
S112K	2.5	**Ox**	10	15	22	43	(*S*)
no	0	**Ox**	2.5	15	100	0	*rac*
no	0	**Ox**	5	15	100	0	*rac*

no	0	**Ox**	10	15	99	0	*rac*
S112A	0	**Ox**	2.5	15	87	78	(*R*)
S112A	0	**Ox**	5	15	89	77	(*R*)
S112A	0	**Ox**	10	15	90	77	(*R*)
S112K	0	**Ox**	2.5	15	57	49	(*S*)
S112K	0	**Ox**	5	15	57	44	(*S*)
S112K	0	**Ox**	10	15	53	40	(*S*)
no	2.5	**Fe^{3+}**	2.5	15	0	0	-
no	2.5	**Fe^{3+}**	5	15	0	0	-
no	2.5	**Fe^{3+}**	10	15	0	0	-
S112A	2.5	**Fe^{3+}**	2.5	15	0	0	-
S112A	2.5	**Fe^{3+}**	5	15	0	0	-
S112A	2.5	**Fe^{3+}**	10	15	0	0	-
S112K	2.5	**Fe^{3+}**	2.5	15	0	0	-
S112K	2.5	**Fe^{3+}**	5	15	0	0	-
S112K	2.5	**Fe^{3+}**	10	15	0	0	-
no	0	**Fe^{3+}**	2.5	15	0	0	-
no	0	**Fe^{3+}**	5	15	0	0	-
no	0	**Fe^{3+}**	10	15	0	0	-
S112A	0	**Fe^{3+}**	2.5	15	0	0	-
S112A	0	**Fe^{3+}**	5	15	0	0	-
S112A	0	**Fe^{3+}**	10	15	0	0	-
S112K	0	**Fe^{3+}**	2.5	15	0	0	-
S112K	0	**Fe^{3+}**	5	15	0	0	-
S112K	0	**Fe^{3+}**	10	15	0	0	-
no	2.5	**PhePropNit**	2.5	15	0	0	-
no	2.5	**PhePropNit**	5	15	94	0	*rac*
no	2.5	**PhePropNit**	10	15	87	0	*rac*
S112A	2.5	**PhePropNit**	2.5	15	0	0	(*R*)
S112A	2.5	**PhePropNit**	5	15	57	82	(*R*)
S112A	2.5	**PhePropNit**	10	15	40	84	(*R*)
S112K	2.5	**PhePropNit**	2.5	15	0	0	-
S112K	2.5	**PhePropNit**	5	15	30	52	(*S*)
S112K	2.5	**PhePropNit**	10	15	13	39	(*S*)
no	0	**PhePropNit**	2.5	15	98	0	*rac*
no	0	**PhePropNit**	5	15	99	0	*rac*
no	0	**PhePropNit**	10	15	68	0	*rac*
S112A	0	**PhePropNit**	2.5	15	73	83	(*R*)
S112A	0	**PhePropNit**	5	15	74	84	(*R*)
S112A	0	**PhePropNit**	10	15	33	86	(*R*)
S112K	0	**PhePropNit**	2.5	15	54	60	(*S*)
S112K	0	**PhePropNit**	5	15	46	57	(*S*)
S112K	0	**PhePropNit**	10	15	23	50	(*S*)
no	2.5	**BQ**	1.25	15	2	0	*rac*
no	2.5	**BQ**	2.5	15	2	0	*rac*
no	2.5	**BQ**	5	15	1	0	*rac*
no	2.5	**BQ**	10	15	2	0	*rac*
S112A	2.5	**BQ**	1.25	15	6	77	(*R*)
S112A	2.5	**BQ**	2.5	15	5	73	(*R*)
S112A	2.5	**BQ**	5	15	5	63	(*R*)
S112A	2.5	**BQ**	10	15	12	46	(*R*)

S112K	2.5	**BQ**	1.25	15	6	51	(*S*)
S112K	2.5	**BQ**	2.5	15	5	48	(*S*)
S112K	2.5	**BQ**	5	15	4	18	(*S*)
S112K	2.5	**BQ**	10	15	11	10	(*S*)
no	0	**BQ**	1.25	15	14	0	*rac*
no	0	**BQ**	2.5	15	13	0	*rac*
no	0	**BQ**	5	15	14	0	*rac*
no	0	**BQ**	10	15	17	0	*rac*
S112A	0	**BQ**	1.25	15	16	55	(*R*)
S112A	0	**BQ**	2.5	15	20	46	(*R*)
S112A	0	**BQ**	5	15	20	32	(*R*)
S112A	0	**BQ**	10	15	39	21	(*R*)
S112K	0	**BQ**	1.25	15	17	14	(*S*)
S112K	0	**BQ**	2.5	15	21	4	(*S*)
S112K	0	**BQ**	5	15	30	2	(*S*)
S112K	0	**BQ**	10	15	39	2	(*S*)
no	2.5	**DiAm**	1.25	15	33	0	*rac*
no	2.5	**DiAm**	2.5	15	22	0	*rac*
no	2.5	**DiAm**	5	15	16	0	*rac*
no	2.5	**DiAm**	10	15	14	0	*rac*
no	2.5	**DiAm**	1.25	2	27	0	*rac*
no	2.5	**DiAm**	2.5	2	12	0	*rac*
no	2.5	**DiAm**	5	2	8	0	*rac*
no	2.5	**DiAm**	10	2	7	0	*rac*
S112A	2.5	**DiAm**	1.25	15	64	84	(*R*)
S112A	2.5	**DiAm**	2.5	15	64	85	(*R*)
S112A	2.5	**DiAm**	5	15	63	84	(*R*)
S112A	2.5	**DiAm**	10	15	58	83	(*R*)
S112A	2.5	**DiAm**	1.25	2	65	82	(*R*)
S112A	2.5	**DiAm**	2.5	2	60	82	(*R*)
S112A	2.5	**DiAm**	5	2	53	82	(*R*)
S112A	2.5	**DiAm**	10	2	45	82	(*R*)
S112K	2.5	**DiAm**	1.25	15	24	55	(*S*)
S112K	2.5	**DiAm**	2.5	15	18	53	(*S*)
S112K	2.5	**DiAm**	5	15	13	50	(*S*)
S112K	2.5	**DiAm**	10	15	9	47	(*S*)
S112K	2.5	**DiAm**	1.25	2	17	49	(*S*)
S112K	2.5	**DiAm**	2.5	2	7	37	(*S*)
S112K	2.5	**DiAm**	5	2	4	30	(*S*)
S112K	2.5	**DiAm**	10	2	3	19	(*S*)
no	0	**DiAm**	10	15	100	0	*rac*
S112A	0	**DiAm**	10	15	71	82	(*R*)
S112K	0	**DiAm**	10	15	45	60	(*S*)

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