

Electronic supplementary information

Unexpected photosensitivity of the well-characterized heme enzyme chlorite dismutase

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1. Nucleotide sequence synthetic AoCld gene codon optimized for *E. coli* including N-terminal his-tag

5' ATGGGCAGCAGCcatcatcatcatcatcaCAGCAGCGGCCTGGTGCCGCGCGGCAGCCAT
ATGCAACCGATGCAGGCGATGAAAATTGAACGCGGAACAATACTGACGCAACCGGGTGTCTT
TGGCGTTTTTACAATGTTCAAATTACGCCCCGATTGGAACAAAGTGCCAGCAATGGAACGTA
AGGGTGCCGCTGAAGAAGTTAAGAAATTGATTGAGAAGCATAAGGATAACGTTCTGGTGGAC
CTCTACCTGACACGTGGCCTGGAAACCAATTCCGACTTTTTCTTCCGCATCAACGCGTACGA
CTTGGCAAAAGCCCAGACCTTTATGCGCGAATTTTCGCTCGACTACTATCGGTAAAAATGCTG
ATGTGTTTCGAGACCCTTGTCGGTGTCAAAAACCCTTGAATTACATCAGCAAAGACAAGTCT
CCTGGGCTGAATGCGGGACTTAGTTCAGCGACCTATAGTGGGCCGGCTCCACGGTATGTGAT
AGTAATTCCCGTCAAAAAGAACGCGGAATGGTGGAACATGTCGCCGAAGAGCGTCTGAAAG
AGATGGAAGTTCATACGACACCTACCTTAGCTTATCTGGTGAATGTCAAACGCAAGTTGTAT
CACTCAACGGGCTTAGATGACACGGATTTTATTACCTATTTTGAAACTGATGATCTGACCGC
GTTTAACAACCTTAATGCTGTCTCTGGCACAGGTTAAAGAGAATAAGTTTCACGTGCGTTGGG
GTAGCCCAACTACGCTCGGCACCATTTCATTCGCCGAAGATGTGATCAAAGCCCTTGCCGAT
TAA-3'

2. Amino-acid sequence synthetic AoCld codon optimized for *E. coli* including N-terminal his-tag

MGSSHHHHHHSSGLVPRGSHMQPMQAMKIERGTILTQPGVFGVFTMFKLRPDWNKVPAMERK
GAAEEVKKLIEKHKDNVLVDLYLTRGLETNSDFFFRINAYDLAKAQTFMREFRSTTIGKNAD
VFETLVGVTKPLNYISKDKSPGLNAGLSSATYSGPAPRYVIVIPVKKNAEWWNMSPEERLKE
MEVHTTPTLAYLVNVKRKLYHSTGLDDTDFITYFETDDLTAFFNNLMLS LAQVKENKFHVRWG
SPTTLGTIHSPEDVIKALAD

3. Double exponential fit of the decay of the Soret peak at 402 nm of AoCld during the illumination experiment

$$A_{402} = A_1 e^{-k_{decay,1} \cdot t} + A_2 e^{-k_{decay,2} \cdot t} + A_0$$

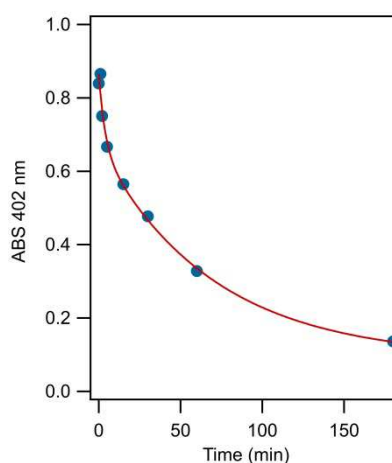
$$A_0 = 0.091 \pm 0.079$$

$$A_1 = 0.196 \pm 0.089$$

$$k_{decay,1} = 0.26 \pm 0.21 \text{ min}^{-1} = (0.43 \pm 0.35) \cdot 10^{-2} \text{ s}^{-1}$$

$$A_2 = 0.578 \pm 0.075$$

$$k_{decay,2} = 0.014 \pm 0.007 \text{ min}^{-1} = (2.3 \pm 1.2) \cdot 10^{-4} \text{ s}^{-1}$$



4. Supplementary tables and figures

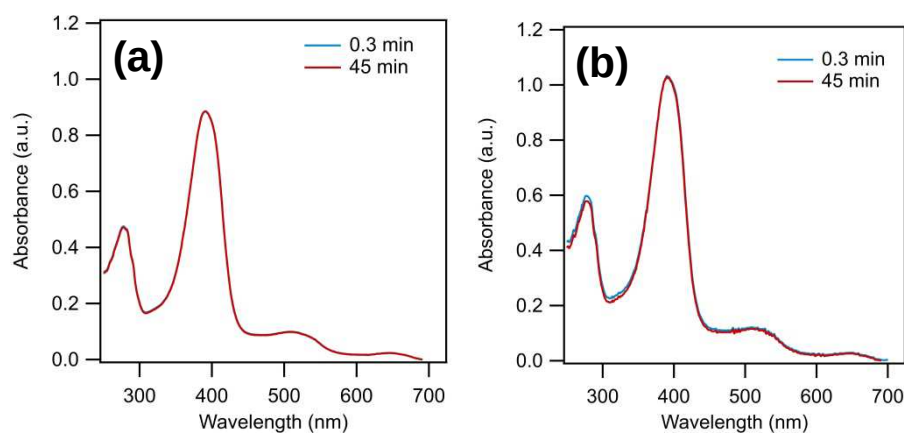


Fig. S1 UV-visible spectral changes of AoCld in a Cary60 UV-vis spectrophotometer.

The spectrophotometer contains a Xenon pulse lamp and a Czerny-Turner monochromator. Spectra were recorded every 0.3 min for 45 min at 21 °C while stirring. No significant change of the spectrum was observed for **a** 9.0 μM AoCld in 100 mM KPi pH 7.0 or **b** 11 μM AoCld in 50 mM KPi pH 7.0. Only the first and last spectrum are shown.

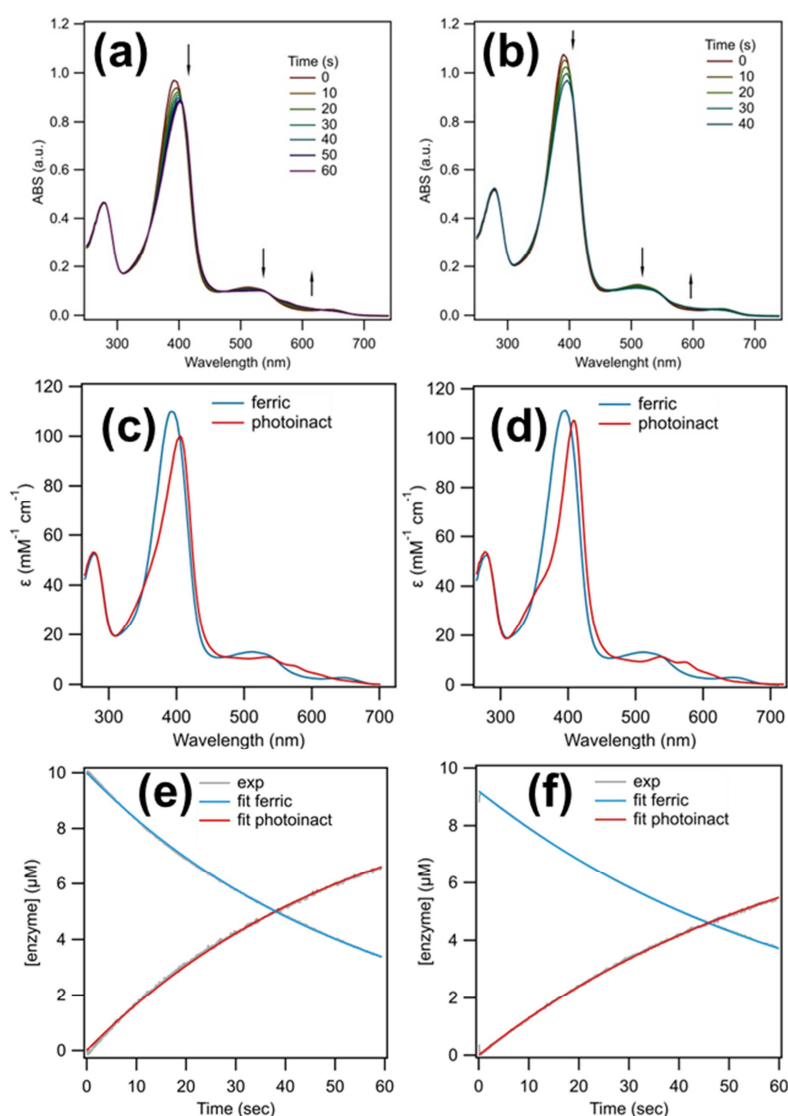


Fig. S2 UV-visible spectral changes of AoCld with and without chlorite measured with stopped-flow (SF) spectroscopy

a 20 μM AoCld in 100 mM KPi pH 7.0 was mixed (1:1) in the SF with water at 20 $^{\circ}\text{C}$ and spectra were recorded every 60 ms for 60 s. **b** 18 μM AoCld in 100 mM KPi pH 7.0 was mixed (1:1) in the SF with 20 μM chlorite in water at 20 $^{\circ}\text{C}$ and spectra were recorded every 60 ms for 60 s. **c** reconstructed spectra after SVD analysis of the SF data of AoCld versus water, in which ferric enzyme (blue) and photoinactivated species (red). **d** reconstructed spectra after SVD analysis of the SF data of AoCld versus 1 eq. chlorite, with ferric enzyme (blue) and photoinactivated species (red). **e** time traces of the two spectral species after SVD analysis (grey) of the AoCld versus water SF data and fits to first-order irreversible decay of ferric enzyme (blue) and increase of photoinactivated species (red) with $k_1 = (1.82 \pm 0.03) \text{ s}^{-1}$. **f** time traces of the two spectral species after SVD analysis (grey) of the AoCld versus 1 eq. chlorite SF data and fits to first-order irreversible decay of ferric enzyme (blue) and increase of photoinactivated species (red) with $k_1 = (1.51 \pm 0.05) \text{ s}^{-1}$.

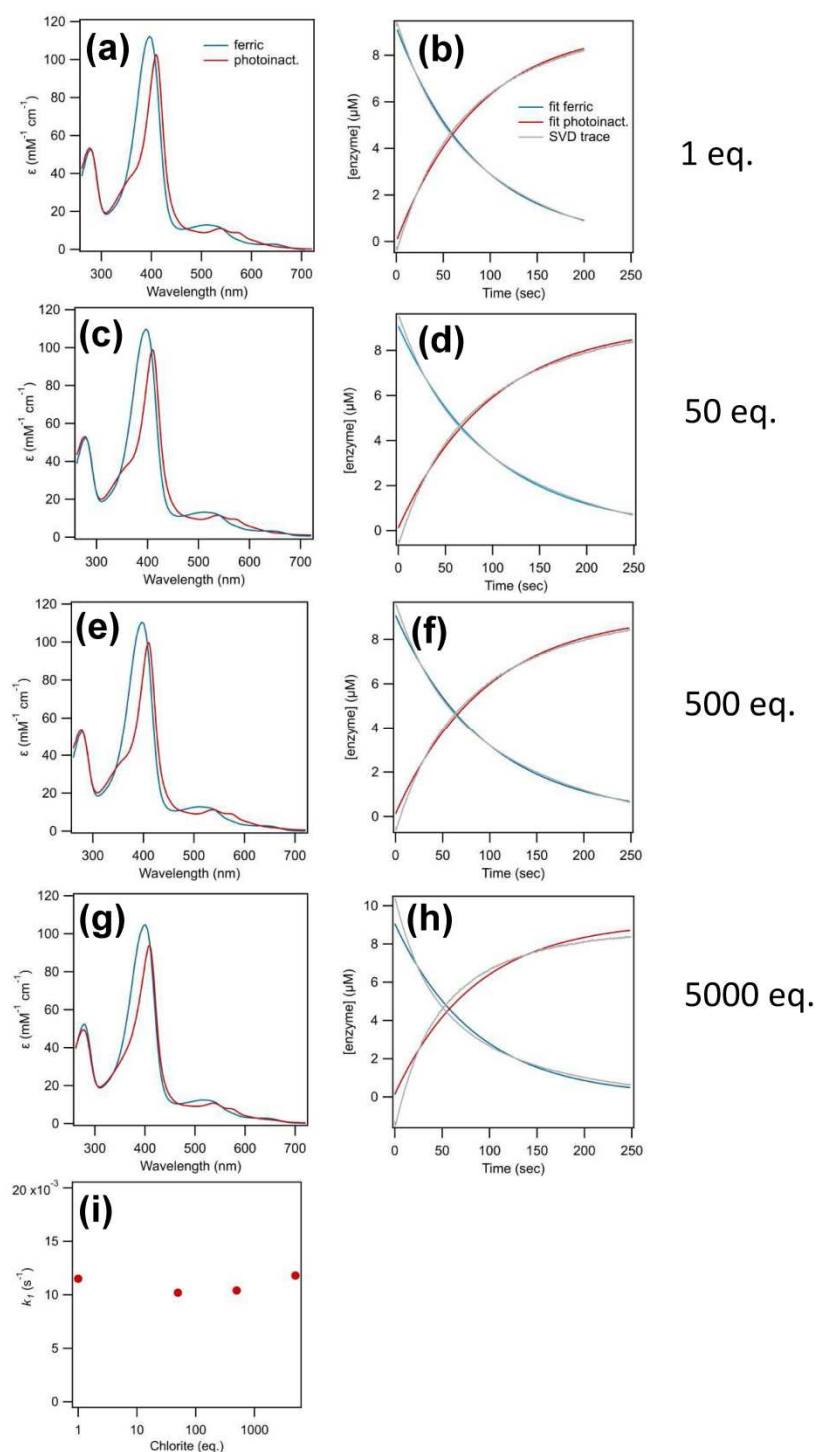
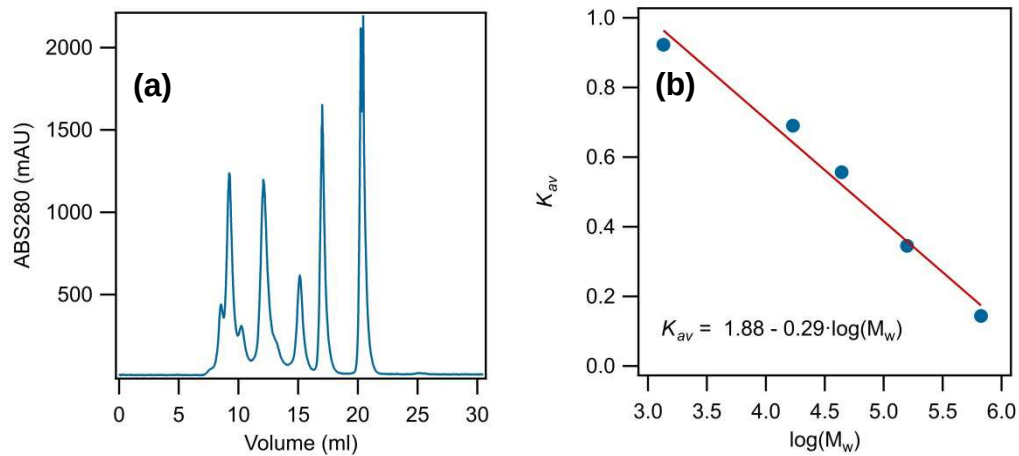


Fig. S3 The effect of catalytic turnovers on the photosensitivity of AoCld.

Kinetic analysis of SF spectroscopy experiments of 9 μM (final concentration) AoCld in 50 mM KPi pH 7.0 and different equivalents of chlorite at 20 °C and spectra were recorded every 0.2 s for 200 or 250 s. **a, c, e, g** reconstructed spectra after SVD analysis showing two species: ferric enzyme (blue) and photoinactivated species (red). **b, d, f, h** time traces of the two spectral species after SVD analysis (grey) and fits to first-order irreversible decay of ferric enzyme (blue) and increase of photoinactivated species (red). **i** first-order rate constant k_1 at different initial chlorite equivalents. In all cases the chlorite was completely consumed within less than 1 sec.

Table S1 Specific activity (U mg^{-1}) of illuminated and non-illuminated AoCld

Illumination time (min)	Specific activity (10^3 U mg^{-1})
Non-illuminated	8.53 ± 0.16
30 min	2.32 ± 0.05
60 min	1.99 ± 0.11
180 min	0.126 ± 0.006

**Fig. S4** Size exclusion chromatography (SEC) calibration

a SEC chromatogram of the protein standards. **b** SEC calibration curve. Protein standards SEC with Thyroglobulin (670 kDa), γ -globulin (158 kDa), Ovalbumin (44 kDa), Myoglobin (17 kDa) and Vitamin B₁₂ (1.35 kDa). SEC conditions: column Superdex S200 10/300 GL, flow rate 0.5 ml/min, and running buffer 100 mM KPi pH 7.0.

Table S2 Molecular weight estimation of AoCld

Peak	V_e (ml)	K_{av}	Mw calculated (kDa)	Apparent oligomeric state
AoCld native, major peak	12.5	0.373	140	pentamer
AoCld illuminated, major peak	15.9	0.613	21.3	monomer
AoCld illuminated, minor peak	14.3	0.500	51.7	dimer

V_e is the elution volume. Void volume $V_o = 7.2$ ml, total column volume $V_t = 21.4$ ml. Gel-phase distribution coefficient $K_{av} = \frac{V_e - V_o}{V_t - V_o}$. The Mw of AoCld 30.3 kDa per subunit.