Supplementary Information for:

Understanding the roles of strictly conserved tryptophan residues in O_2 producting chlorite dismutases

Beatrice Blanc, Kenton R. Rodgers, Gudrun S. Lukat-Rodgers,* Jennifer L. DuBois*

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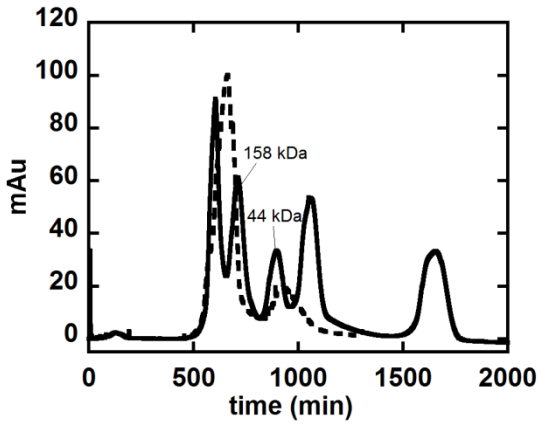

Fig. S1. Analytical gel filtration for molecular weight estimations for WT and mutant *Da*Clds. Separation of the standard mixture (solid curve) and W155F (dashed curve) on an 120 cm long, 3 cm radius gel filtration column containing 600 mL resin (Sepharyl S-200 GE Biosciences) run at 0.4 mL/min. The globular standards bovine thyroglobuline, bovine γ-globuline, chicken ovalbumine, horse myoglobin and vitamin B12 standards have molecular weight of 670 kDa, 158 kDa (labeled), 44 kDa (labeled), 17 kDa and 1.35 kDa respectively. Based on these results, the apparent molecular weight of W155F (if globular) is 54 kDa, suggesting a possible dimer.

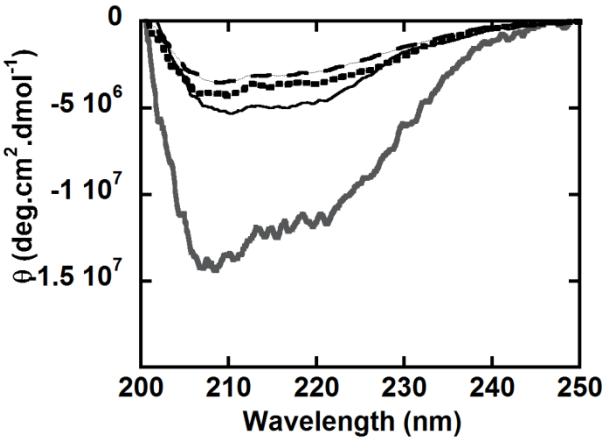

Fig. S2. CD spectra of 3 µM of W155F (—), W156F (- - -) and W227F (— —) mutants compared to WTCld (—) at pH 6.8 in 100 mM phosphate buffer at 20 ◦C.

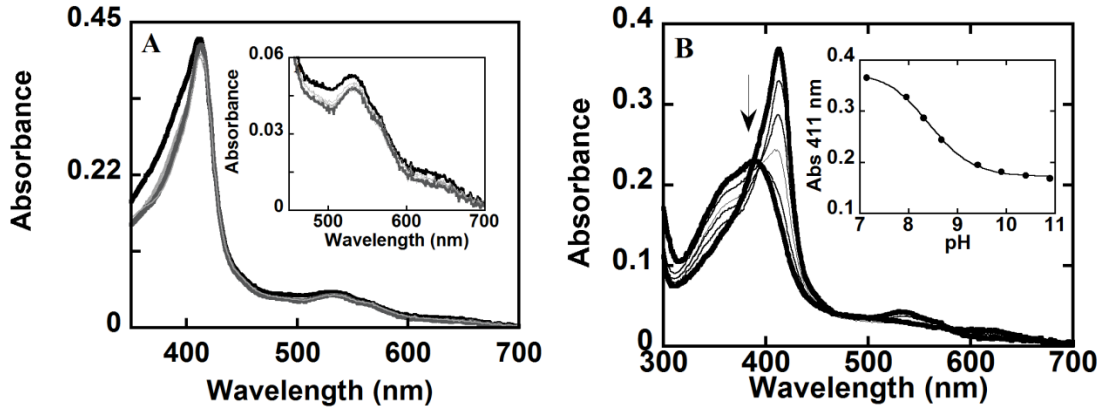

Fig. S3 UV/Vis titration of W156F in 100 mM phosphate buffer. (left) Spectra at pH 6 (—), 6.3, 6.5, 7 (—) and 7.5 are shown. The alkaline transition is likely between 6 and 7 due to the decrease of the Soret after this pH. (right) Spectra were measured at increments of 0.4 pH units from pH 7.1-10.9. Spectra at pH 7.1, 7.9, 8.31, 8.7, 9.4 and 10.9 are shown. Insets show the fitting of one sigmoidal curve at 411 nm. Transition was measured at pH 8.5.

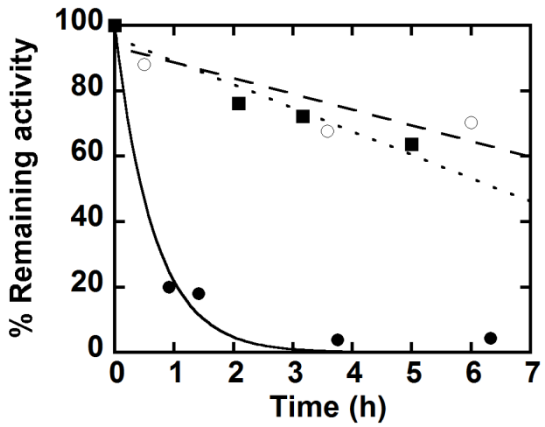

Fig. S4 Remaining activity following dilution by 1000, 50 and 50 for WT, W227F and W156F respectively. (○) 0.7 μM WTCld, (●) 2.6 μM W156F and (■) 2.6 μM W227F at pH 6 over time in 100 mM phosphate buffer, 20 °C. The plots were fitted to an exponential curve for W156F (—) and to a line for WT (- - -) and W227F (. . .)

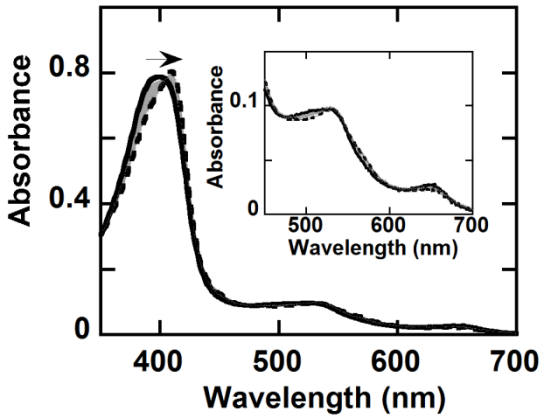

Fig. S5 UV/Vis spectra of concentrated (8 μM, pH 6.8) monomer of W156F after incubation in buffer at pH 6.8 for 1h. The spectrum at time 0 is shown as a black line and at 1h as a dashed line. These spectra show pH-independent and dilution-dependent equilibration of W156F.

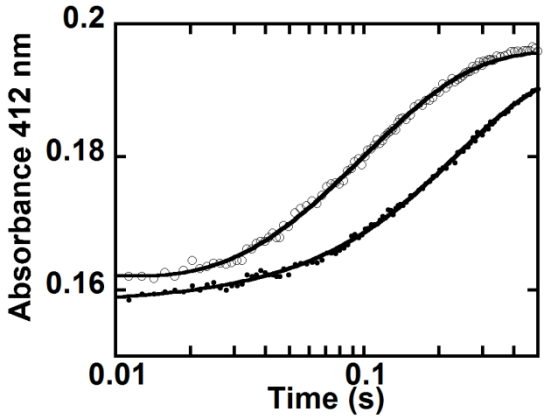

Fig. S6 Kinetic traces at 412 nm for the reaction of 2 μM W227F and 50 μM PAA (●) and 100 μM PAA (○) at pH 6 and 20^{0}C. The black curves represent two exponential fits at 412 nm for 50 μM and 100 μM reactions. The two exponential fits are consistent with the appearance of two distinct spectral species following mixing, as predicted by SVD (Figure 10). Kinetics constants determined by fitting a sum of two exponentials to each equation were strongly consistent with constants determined by SVD carried out for the entire spectral data set: k_1 = 10.8 s^{-1}, k_2 = 5.4 s^{-1} (50 μM); k_1 = 89 s^{-1}, k_2 = 10 s^{-1} (100 μM).

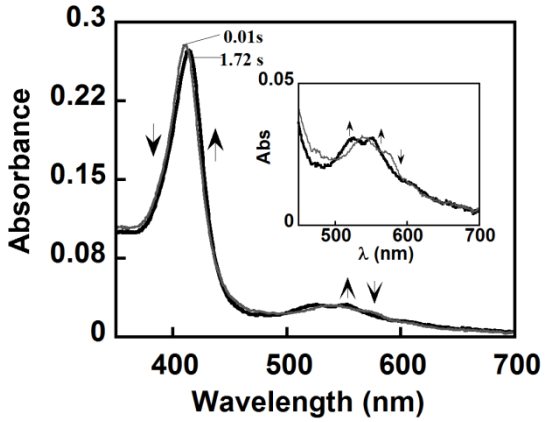

Fig. S7 Transient intermediates formed upon reaction of W227F with 25 eq PAA at pH 8. Approximately 4 μM W227F (2 μM final) was mixed with 100 μM peracetic acid (50 μM final) in 0.2 M citrate-phosphate buffer at pH 8 (20 ^{0}C). The initial spectrum is shown in grey and the spectrum for Cpd II formed after ~1.72 seconds in black. Inset: visible bands shown on an expanded scale for clarity.

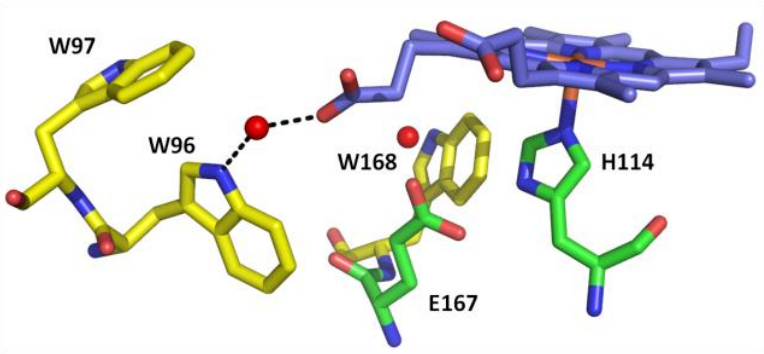

Fig. S8 Crystal structure of *Nitrobacter winogradskyi* Cld *(Nw*Cld)[1] with water molecule represented as red spheres. The Trp residues are represented in carbon-yellow and the His 114 and Glu 167 in carbon green (PDB 3QPI). W96 and W97 align with W155 and W156 from *Da*Cld, respectively.

Mass spectrometry

Mass spectrometry (MS) measurements on a microQ-ToF II instrument were attempted in order to determine the oligomerization state of WTCld and mutants. The experiments were performed under positive ion mode using soft electrospray (no fragmentation). The pressure of the source was 6 mbar. Assessment of the oligomerization state was successfully performed via a similar method for *Azospira oryzae* Cld (AzCld).[2] Following this procedure, WTCld was diluted into 50 mM ammonium formate at pH 6.8 at ~25 μM. Only traces of the monomer were detectable. The pressure in the source of our MS is ~6mBar. According to Tahallah et al.[3], large intact proteins can only be studied by raising the pressure in the source. De Geus et al added a special apparatus to the MS in order to modify and control the pressure. Unfortunately, we didn't have this kind of set up in our instrument.

References

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2 D.C. De Geus, A.J. Thomassen, P.L. Hagedoorn, N.S. Pannu, E. Van Duijn, J.P. Abrahams, *J. Mol. Biol.*, 2009, **387**, 192-217.

3 N. Tahallah, M. Pinkse, C.S. Maier, A.J. Heck, *Rapid. Commun. Mass Spectrom*, 2001, **15**, 596-601