Supporting Information for:

Structural Basis for Oxygen Sensing and Signal Transduction of the Heme-based Sensor Protein Aer2 from *Pseudomonas aeruginosa*

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Expression and purification of Aer2

The *aer2* gene from *Pseudomonas aeruginosa* PAO1 was synthesized by GeneScript, the codon of which were optimized for the expression in *Escherichia coli*. *E. coli* BL21(DE3) and pET-15b (Novagen) were used for a host and an expression vector for the expression of PH-Aer2, respectively. For the expression of full-length Aer2, pKK223-3 and *E. coli* DH5α were used as an expression vector and a host, respectively. The expression vectors were designed as PH-Aer2 and full-length Aer2 are expressed with a His_6 tag at the N-terminus and C-terminus, respectively. The QuickChange site-directed mutagenesis kit (Stratagene) was used to prepare mutants. The DNA sequences were determined using a DNA sequencer ABI PRISM 310 (Applied Biosystems).

Several colonies of transformed *E. coli* cells carrying the expression vector on LB agar plates containing 30 μg/ml ampicillin and 2 % (w/v) glucose were inoculated in 30 ml LB broth containing 100 μg/ml ampicillin and 2 % (w/v) glucose to be cultivated for 5 hours at 37 °C with shaking at 180 rpm for preculture. For the expression of full-length Aer2, 3 ml of preculture solution was inoculated into 300 ml TB broth containing 100 μg/ml ampicillin, 0.5 mM δ-aminolevulinic acid, and 0.3 mM IPTG. The cells were grown for 15 hours at 37 °C with shaking at 80 rpm. For the expression of PH-Aer2, 3 ml of preculture solution was inoculated into 300 ml TB broth containing 100 μg/ml ampicillin, 0.5 mM δ-aminolevulinic acid to be cultivated at 37 °C with shaking at 140 rpm. After 4 hours, 0.3 mM IPTG was added, and then the cultivation was allowed to continue for 15 hours at 20 °C with shaking at 80 rpm. After cultivation, the cells were collected by centrifugation.

The cells were suspended in buffer A (50 mM MOPS/Na (pH 6.8) containing 500 mM NaCl, 10 mM imidazole, 0.5 mg/ml lysozyme) and sonicated on ice. The supernatant isolated by centrifugation at 100,000 xg for 1 hour at 4 °C was applied to a HisTrap FF column (GE Healthcare). The column was then washed with buffer A containing 50 mM imidazole. The adsorbed proteins were eluted with buffer A containing 250 mM imidazole. The fractions containing Aer2 were combined and then loaded to a CHT ceramic hydroxyapatite (Bio-Rad) column that was equilibrated with buffer B (50 mM MOPS/Na (pH 6.8) containing 500 mM NaCl). The column was washed with buffer B, and the adsorbed proteins were eluted with buffer B containing 300 mM potassium phosphate (pH 6.8). The fractions containing Aer2 were combined and diluted by 10 times with buffer C (50 mM MOPS/Na (pH 6.8)). The diluted sample was applied to a HiTrap SP FF (GE Healthcare) column equilibrated with buffer C. After washing the column with buffer C containing 100 mM NaCl, the adsorbed proteins were eluted with buffer C containing 750 mM NaCl. The fractions containing Aer2 were combined and concentrated with Amicon Ultra-0.5 30K filter unit (Millipore). Aer2 was purified as the O_2-bound form.

Deoxy form was prepared by adding sodium dithionite to a final concentration of 1 mM under N_2 atmosphere. Optical absorption spectra were measured on an Agilent 8453 spectrophotometer at room temperature. The met (ferric) form was prepared by adding an excess amount of potassium ferricyanide.

Excess ferricyanide was removed by a PD-10 column (GE Healthcare) using buffer C containing 750 mM NaCl. Oxy form was prepared by removing excess dithionite from the deoxy Aer2 solution using a PD-10 column under aerobic conditions. Optical absorption spectra were recorded on an Agilent 8453 spectrophotometer at room temperature.

The as-purified Aer2 showed the same spectrum as that of the oxy form in Fig. 4. When CO gas was bubbled into the as-purified sample of wild-type Aer2 without any reductant, the resulting sample showed the same absorption spectrum as that of CO-bound Aer2 produced by the reaction of deoxy Aer2 with CO, indicating that the heme-bound O_2 in the as-purified Aer2 is replaced by CO. The CO-bound Aer2 showed the Soret, α, and β peaks at 422, 571, and 538 nm, respectively. These results indicate that the protein is purified in the oxygen-bound form.

Resonance Raman spectra of Aer2

Resonance Raman spectra were measured by the excitation at 410 nm using a frequency-doubled diode laser (Matsushita Electric Industrial CO., Ltd.). The sample solution was contained in a 10 mm NMR tube ad spun with a spinning cell device designed to minimize off-centre deviation during rotation. The Raman scattering light was collected and focused onto the entrance slit of a Czerny-Turner configured Littrow prism prefilter (Bunkoukeiki) coupled to a single spectrograph with a focal length of 550 mm (iHR550, HORIBA Jobin Ybon) by two achromatic doublet lenses. The dispersed light was detected with a liquid nitrogen cooled CCD camera (SPEC-10:400B/LN-SN-U, Roper Scientific). The spectra were calibrated using the standard Raman spectra of indene, cyclohexane, and carbon tetrachloride.

Table S1 Data collection and refinement statistics.

	PH-Aer2
Data collection	
Space group	$P6_522$
Cell dimensions	
a, *b*, *c* (Å)	83.02, 83.02, 107.76
α, β, γ (°)	90.00, 90.00, 120.00
Resolution (Å)	50.00-2.40 (2.49-2.40)*
R_{sym}	0.048 (0.569)*
$I / \sigma I$	66.5 (5.7)*
Completeness (%)	99.9 (100.0)*
Redundancy	21.5 (22.0)*
Refinement	
Resolution (Å)	30. 0-2.4
No. reflections	9,073
R_{work} / R_{free}	0.2233 / 0.2677
No. atoms	
Protein	1,067
Heme	43
Water	11
R.m.s. deviations	
Bond lengths (Å)	0.020
Bond angles (°)	2.008
Ramachandran plot	
Favored / allowed / outlier (%)	94.4 / 5.6 / 0.0

*Numbers in parentheses are for the highest-resolution shell.
$R_{sym} = \Sigma_{hkl}\Sigma_i | I_i(hkl) - \langle I(hkl)\rangle | / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the average intensity of the *i* observations.
$R_{work} = \Sigma_{hkl} | |F_{obs}(hkl)| - |F_{calc}(hkl)| | / \Sigma_{hkl} |F_{obs}(hkl)|$. R_{free} is calculated for 5 % of reflections randomly selected and excluded from refinement. R_{work} is calculated for the remaining 95 % of reflections used for structure refinement.

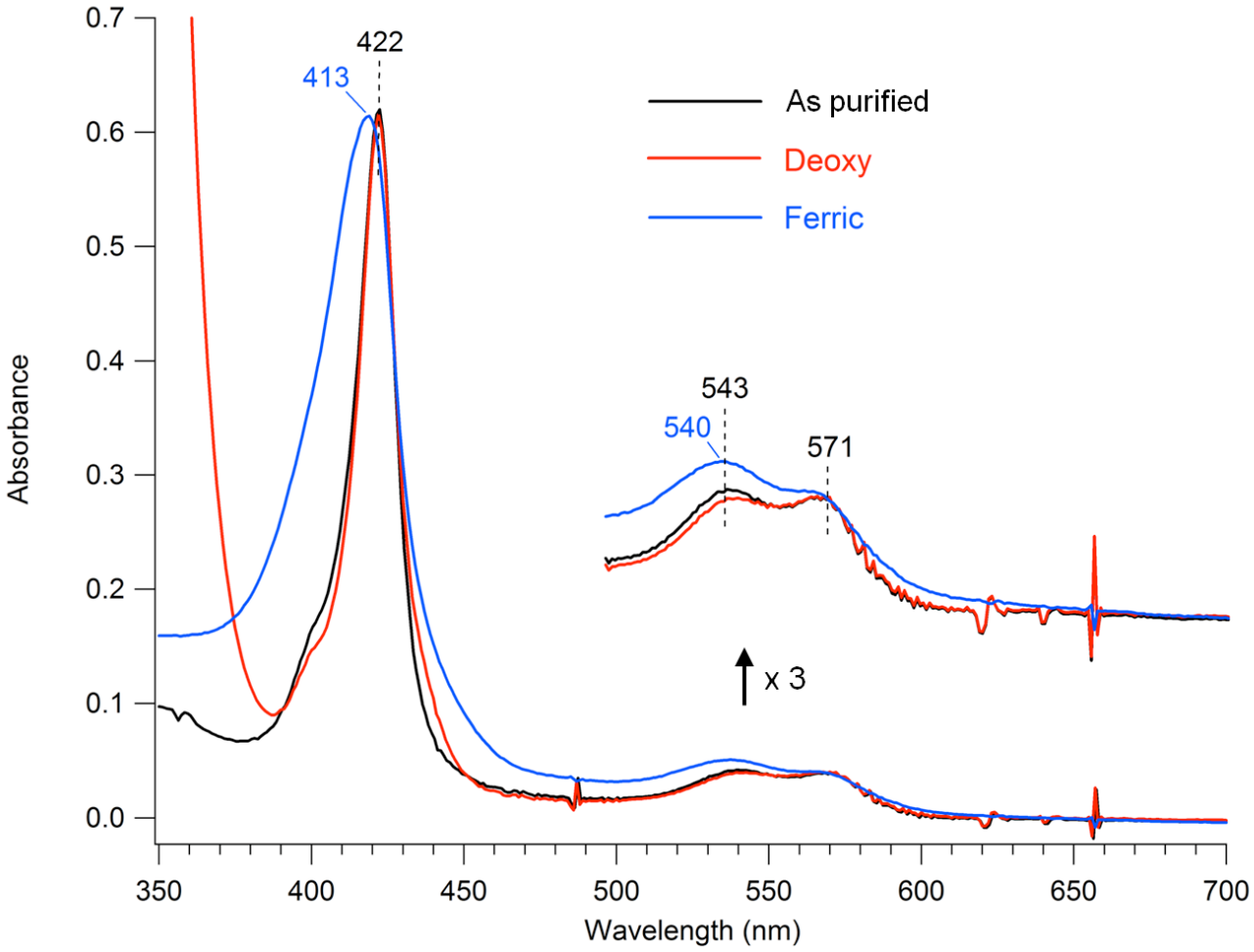

Fig. S1 Optical absorption spectra of W283L mutant in full-length Aer2.

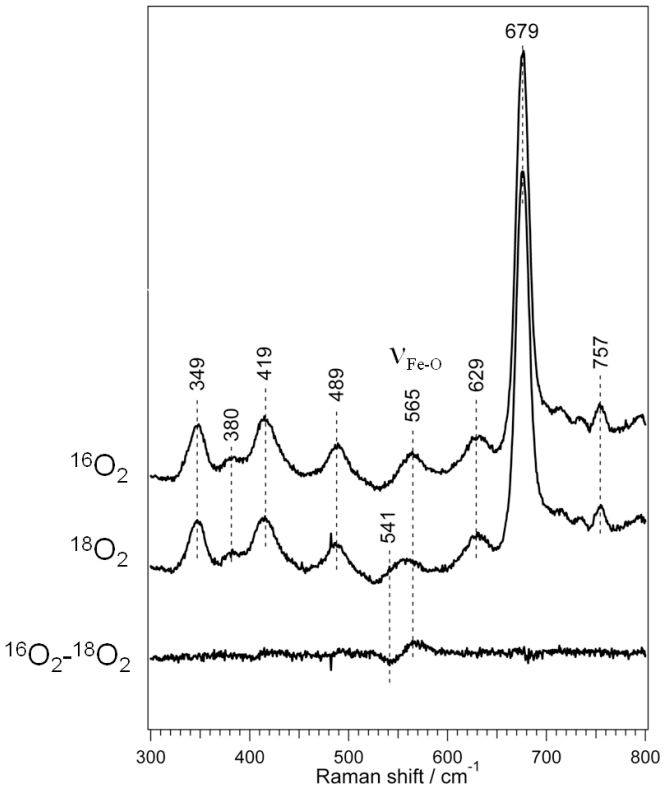

Fig. S2 Resonance Raman spectra of of Aer2 in the oxy form.
The spectra of $^{16}O_2$-bound form and $^{18}O_2$-bound form are shown at the top and middle, respectively. The bottom spectrum is the difference spectrum between them. The $\delta(C_\beta C_c C_d)$ band, the bending mode of heme propionate group, was observed at 380 cm^{-1}. The stretching mode of Fe-O_2 (ν_{Fe-O}) was observed at 565 cm^{-1}.