Supplementary Materials

High Resolution Crystal Structure of Copper Amine Oxidase from *Arthrobacter globiformis*: Assignment of Bound Diatomic Molecules to O_2

Takeshi Murakawa, Hideyuki Hayashi, Tomoko Sunami, Kazuo Kurihara, Taro Tamada, Ryota Kuroki, Mamoru Suzuki, Katsuyuki Tanizawa, and Toshihide Okajima

Re-data-collection of $AGAO_{RT}$ and $AGAO_{GL}$

To accurately compare the crystal parameters, X-ray diffraction data of $AGAO_{RT}$ and $AGAO_{GL}$ were recollected using the same detector and beamline station as those for $APAO_{PG}$. AGAO crystals were obtained by the micro-dialysis method as described in the main text. For $AGAO_{GL}$, after sufficient growth of the crystals, the dialysis buttons were transferred into new reservoirs where solutions were supplemented with 45% (v/v) glycerol as a cryoprotectant and kept at 16 °C for 24 h for cryo-protection. The crystals were mounted on thin nylon loops (ϕ, 0.4–0.5 mm) and frozen by flash cooling to 100 K in a cold N_2 gas stream. For $AGAO_{RT}$, the single crystal generated in a normal atmosphere was mounted in a quartz capillary.

X-ray diffraction data were collected with the synchrotron X-radiation (λ = 0.9 Å) using an MX-225HE detector (Rayonix, Evanston, IL, USA) in the beamline station BL44XU at SPring-8 (Hyogo, Japan) at 100 K and 298 K for $AGAO_{GL}$ and $AGAO_{RT}$, respectively. The data was collected with a single crystal-to-detector distance, 160 mm. The sets of the diffraction data collected for the crystal were processed and scaled using HKL2000. Both $AGAO_{GL}$ and $AGAO_{RT}$ were found to belong to the same space group *C*2 with cell dimensions as reported previously (1IU7 and 1AV4, respectively). The crystal parameters of data collection are summarized in Supplementary Table S1.

Table S1. Conditions and parameters of AGAO crystals belonging to the small *C*2 cells.

AGAO crystals (PDB code)	$AGAO_{RT}$ [a]	$AGAO_{GL}$ [a]	$AGAO_{GL2}$ (1W6G)	$AGAO_{PG}$ (3WA2)
Data collection temperature	298 K	100 K	100 K	100 K
Crystallization method	microdialysis	microdialysis	hanging drop	microdialysis
Composition of the crystallization solution	25 mM HEPES, pH 6.8, 1.05 M tartrate NaK	25 mM HEPES, pH 6.8, 1.05 M tartrate NaK	100 mM MES, pH 6.5, 1.6 M ammonium sulfate, 12% (v/v) dioxane	25 mM HEPES, pH 6.8, 1.05 M tartrate NaK
Cryoprotectant	none	45% (v/v) glycerol	30% (v/v) glycerol	35% (v/v) PEG200
Resolution (Å)	2.11	1.70	1.55	1.08
Space group	*C*2	*C*2	*C*2	*C*2
Unit cell dimensions				
a, *b*, *c* (Å)	158.4, 64.8, 93.3	192.9, 62.8, 158.0	157.8, 63.2, 92.0	157.7, 62.4, 92.1
β (deg)	112.5	117.6	112.0	112.1
Number of molecules per asymmetric unit	1	2	1	1
Cell volume/subunit ($Å^3$)	885,000 (100)[b]	848,000 (95.8)[b]	851,000 (96.2)[b]	840,000 (94.9)[b]
Solvent content (%)	60.8	59.1	59.2	58.7
B factor from Wilson plot ($Å^2$)	23.7	18.0	19.7	9.9

[a]Recollected data (not deposited to PDB). [b]Percent cell volume relative to that of $AGAO_{RT}$ is shown in parenthesis.

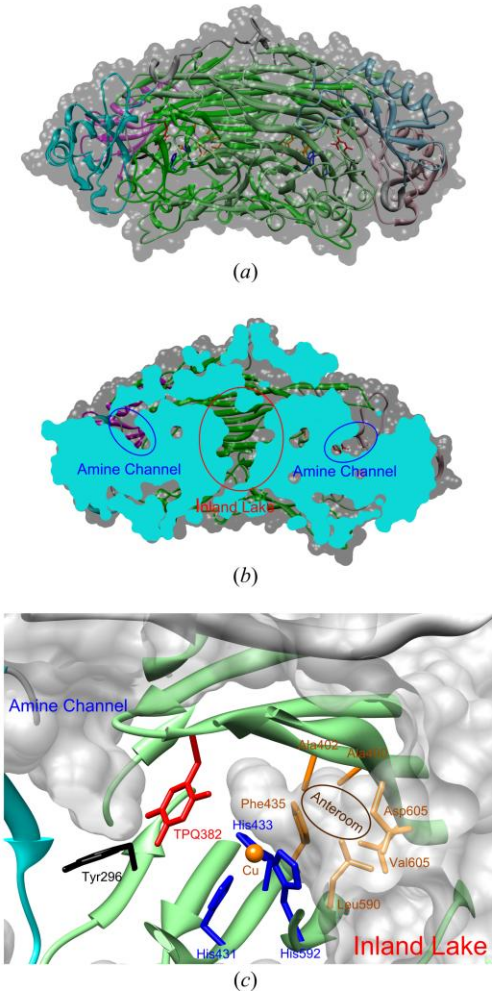

Figure S1. X-ray crystal structure of AGAO. (*a*) Overall shape of the AGAO dimer (1IU7) is shown as gray shade. Domains are depicted with a colored ribbon model: D2, sky blue and faded sky blue; D3, pink and faded pink; D4, green and faded green; and domain-connecting loops, gray. (*b*) The model of (*a*) is sliced so that the cross section is across the amine channels and the inter-subunit space, the 'inland lake'. (*c*) The active site residues are shown by stick models with ribbon models of nearby main-chains and surfaces of the amine channel and the inland lake. A brown circle represents the anteroom proposed previously as a dioxygen- (pre-) binding site (Johnson *et al*., 2007).

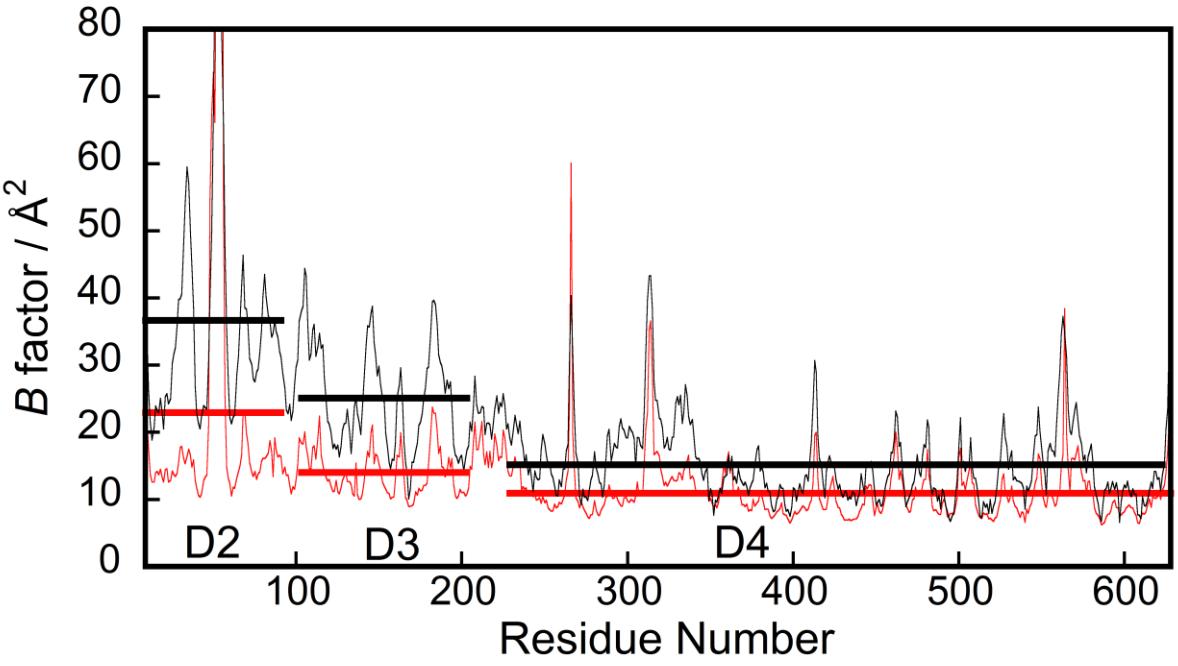

Figure S2. Distribution of isotropic *B* factors. The average *B* factors of the main chain atoms in $AGAO_{GL}$ and $AGAO_{PG}$ are plotted with black and red lines, respectively, against the residue number. The horizontal lines indicate the average *B* factors of $AGAO_{GL}$ (black) and $AGAO_{PG}$ (red) within D2, D3, and D4 domains.

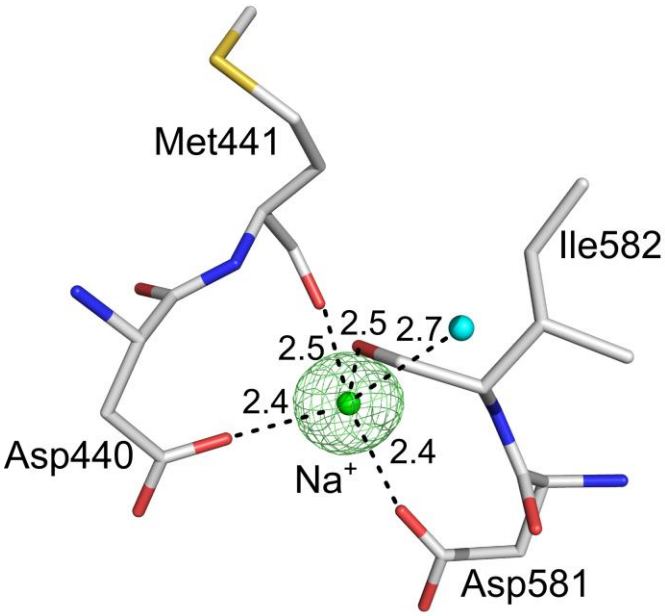

Figure S3. Na^+-binding site of $AGAO_{PG}$. (c) The green and cyan spheres represent a Na^+ ion and a water molecule, respectively. Broken lines and numbers indicate the coordination and distances (in Å) from the Na^+ ion. An $F_o - F_c$ omit map calculated for Na^+ ion was contoured at +5σ (green mesh).

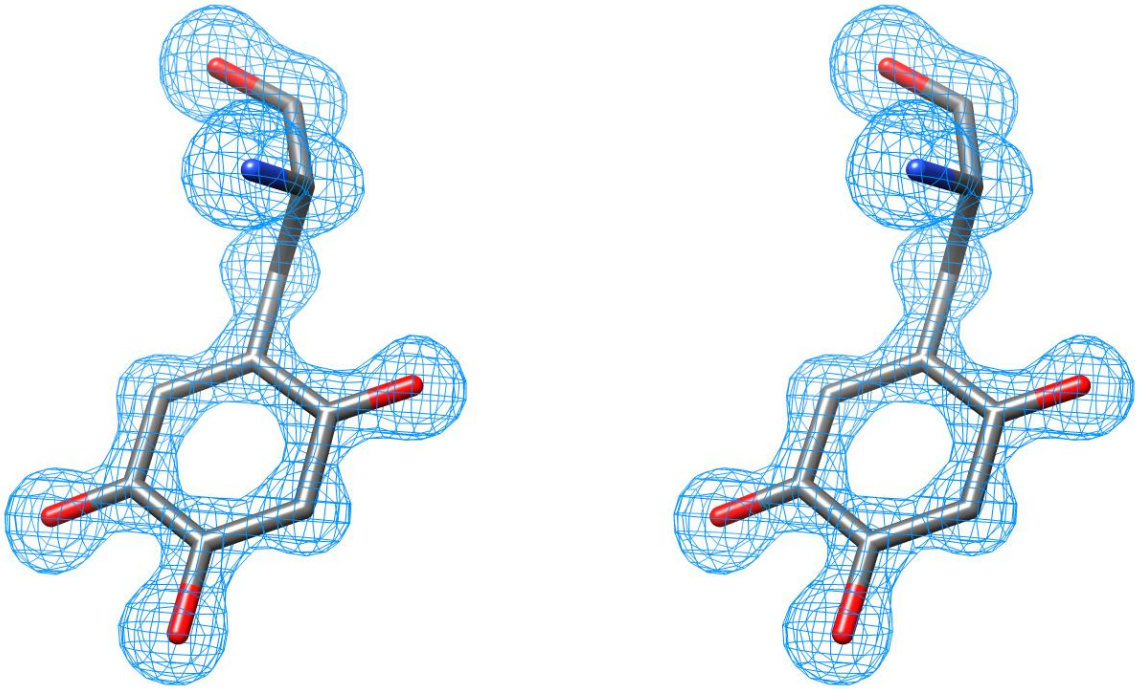

Figure S4. Stereo diagram of the electron density map of TPQ. The refined model of TPQ382 is shown with the $F_o - F_c$ omit map contoured at 6σ with sky blue mesh.

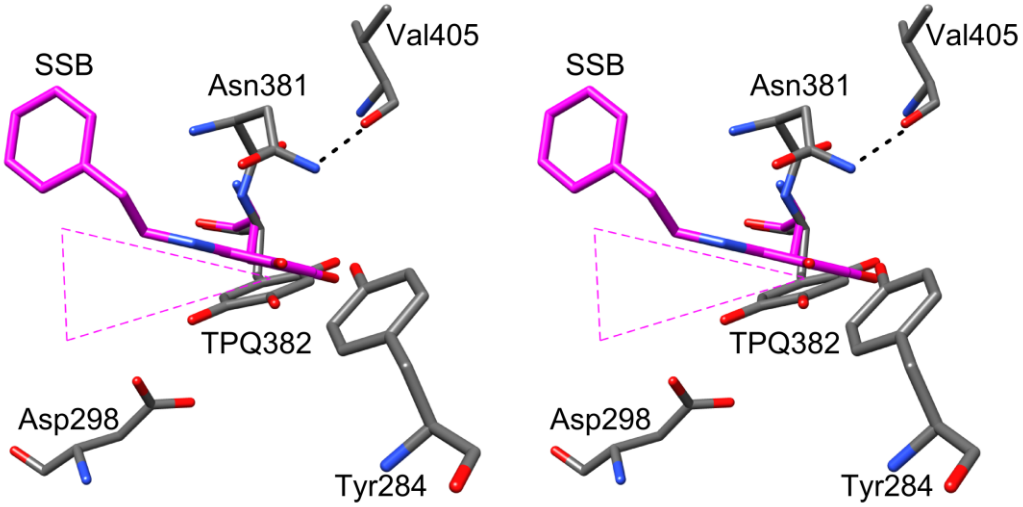

Figure S5. Stereo diagram of the wedge-shaped movement of the TPQ ring upon substrate Schiff-base formation during catalysis. Stick models of the active-site residues around TPQ382 of $AGAO_{PG}$ (gray) are shown with the substrate Schiff-base (SSB, pink) formed with phenylethylamine (2CWU) viewed from the direction of the C4 hydroxyl group of TPQ. A wedge-shaped space between the TPQ rings is represented by a pink dotted line triangle. A hydrogen bond between the side chain ND2 atom of Asn381 and the main chain carbonyl group of Val405 is indicated by a black dotted line.

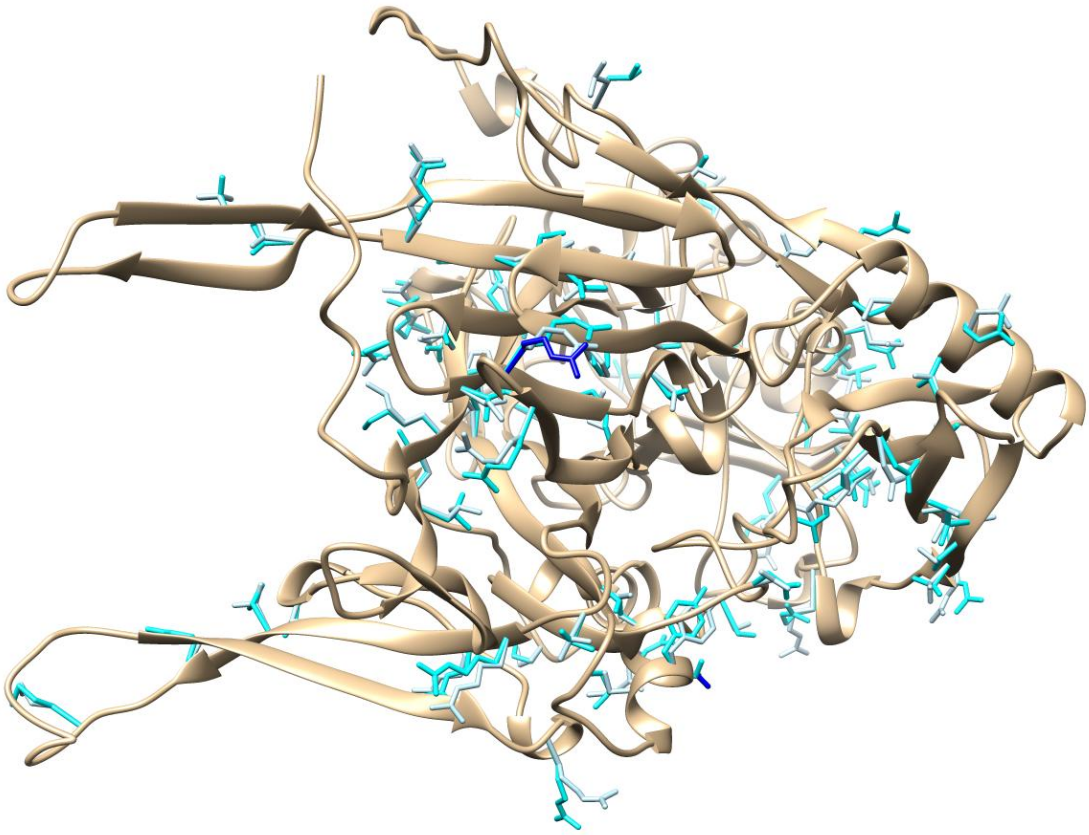

Figure S6. Alternative conformers of $AGAO_{PG}$. Residues with alternative conformers are shown as stick models on the overall main chain models in light blue, cyan, and dark blue for conformers *a*, *b*, and *c*, respectively.

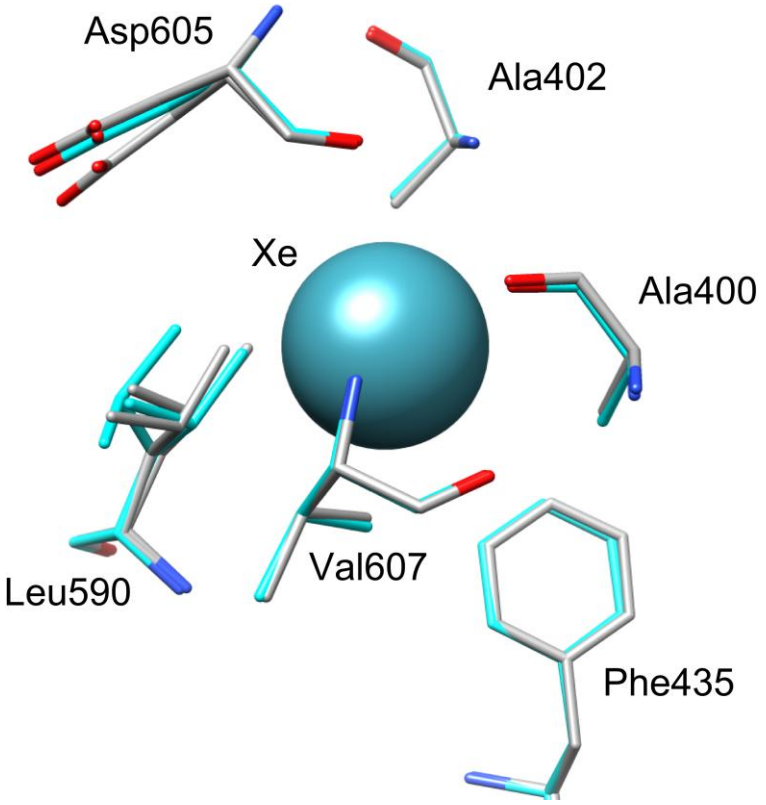

Figure S7. Conformations of the residues constituting the anteroom. Stick models of the anteroom residues (sky blue) in the Xe (blue sphere)-bound AGAO (1RJO) are overlaid with the corresponding residues (white) in $AGAO_{PG}$.

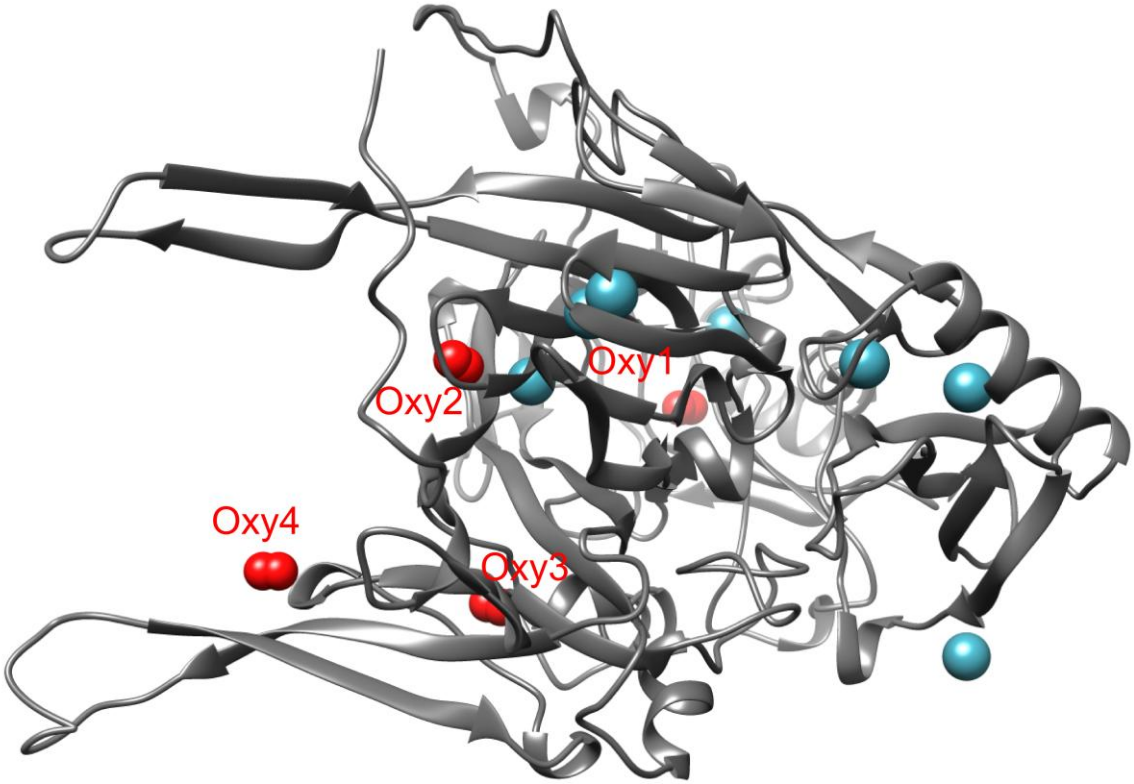

Figure S8. Locations of identified O_2-like diatomic molecules and Xe atoms found previously in AGAO (Duff *et al*., 2004). Oxy1–4 molecules (red spheres) and xenon atoms (lime spheres) of Xe-bound AGAO (1RJO) are depicted on the $AGAO_{PG}$ overall structure (this study).