Electronic Supplementary Information

3D domain swapping of azurin from *Alcaligenes xylosoxidans*

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Contents

Table S1. Statistics of data collection and structure refinement of the azurin dimer (PDB ID: 6L1V).

Data collection	
X-ray source	SPring-8 (BL26B1)
Wavelength (Å)	1.0000
Space group	$C222_1$
Unit cell parameters	
a, *b*, *c* (Å)	107.45, 167.81, 116.99
α, β, γ (º)	90.0, 90.0, 90.0
Resolution (Å)	49.17−2.25 (2.32−2.25)
Number of unique reflections	50385 (4598)
R_{merge}[a]	0.079 (1.145)
Completeness (%)	99.9 (100.0)
$\langle I/\sigma(I)\rangle$	12.3 (1.6)
$CC_{1/2}$	(0.739)
Redundancy	6.8 (7.2)

Refinement	
Resolution (Å)	49.17−2.25 (2.31−2.25)
Number of reflections	47826 (2534)
R_{work}[b]	0.20277 (0.327)
R_{free}[b]	0.23021 (0.323)
Completeness (%)	99.9 (100.0)
Number of atoms in an asymmetric unit	
Protein	3916
Water	269
Cu	4
Average *B* factors (Å^2)	
Protein	49.6
Water	49.6
Cu	42.4
Ramachandran plot (%)	
Favored	99.0
Allowed	1.0
Outlier	0

Statistics for the highest-resolution shell are given in parentheses.
[a] $R_{merge}=\Sigma_{hkl} \mid I - \langle I\rangle \mid (\Sigma_{hkl} \mid I \mid)^{-1}$.
[b] $R_{work}=\Sigma_{hkl} \mid \mid F_{obs} \mid - k \mid F_{calc} \mid \mid (\Sigma_{hkl} \mid F_{obs} \mid)^{-1}$, k: scaling factor. R_{free} was computed identically, except where all reflections belong to a test set of 5 % of randomly selected data.

Table S2. Root-mean-square deviation (RMSD) values for the Cα atoms between the structures of the azurin monomer (PDB ID: 1RKR) and domain-swapped dimer (PDB ID: 6L1V).

unit	RMSD (Å)			
	monomer 1	monomer 2	monomer 3	monomer 4
residues 1-36 of protomer 1 and residues 41-129 of protomer 4	0.79	0.81	0.81	0.79
residues 1-36 of protomer 2 and residues 41-129 of protomer 3	0.86	0.89	0.88	0.87
residues 1-36 of protomer 3 and residues 41-129 of protomer 2	0.81	0.83	0.84	0.81
residues 1-36 of protomer 4 and residues 41-129 of protomer 1	0.81	0.83	0.84	0.81

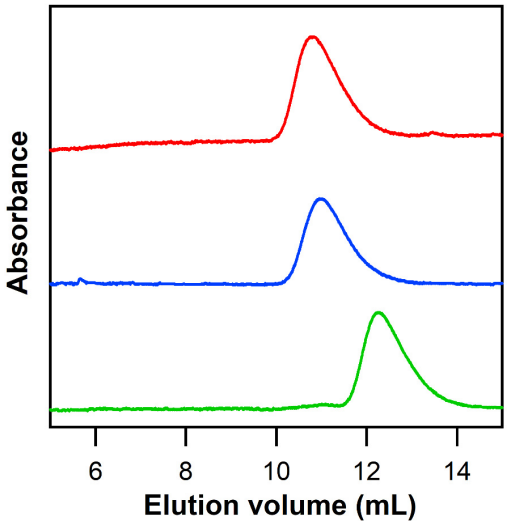

Fig. S1 Size exclusion chromatograms of the solution obtained before (red) and after incubation of the Cu(II)-azurin dimer in potassium phosphate buffer, pH 7.0, at 60 °C (blue) and 70 °C (green) for 1 h. Measurement conditions: column, Superdex 75 10/300 GL; azurin concentration: 20 μM (copper unit); buffer: 50 mM potassium phosphate buffer, pH 7.0; monitoring wavelength: 280 nm.

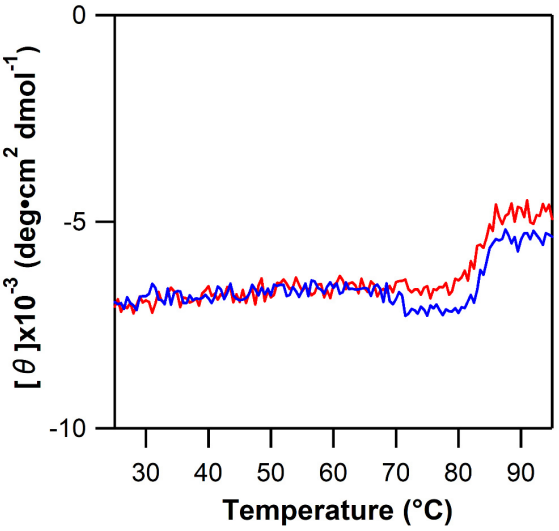

Fig. S2 CD ellipticities at 222 nm of Cu(II)-azurin monomer (red) and dimer (blue) at 25–95 °C. Measurement conditions: azurin concentration: 5.0 μM (copper unit); buffer, 50 mM potassium phosphate buffer, pH 7.0; monitoring wavelength 222 nm; scan rate, 1 °C/min.

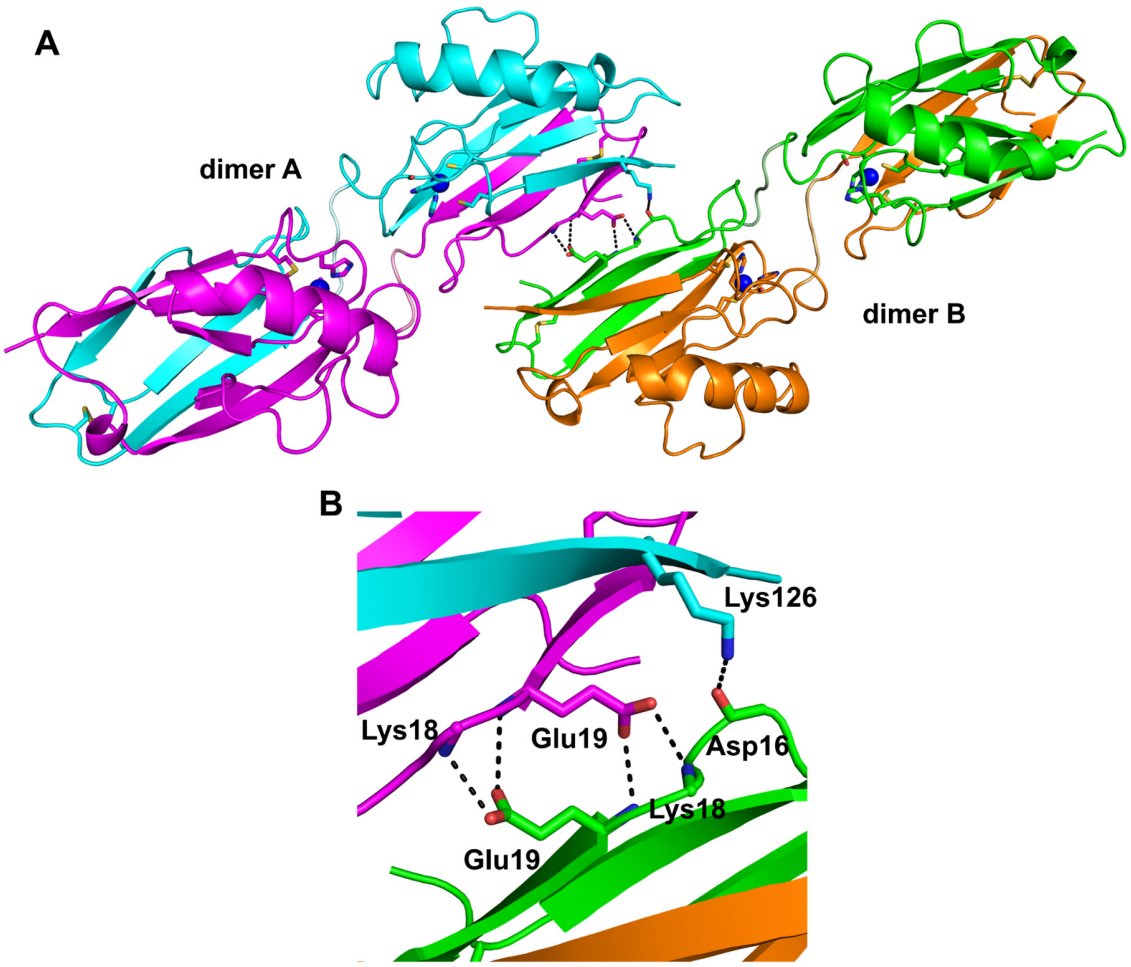

Fig. S3 Structures of azurin dimers in the unit cell (PDB ID: 6L1V). (A) Protomer 1 (magenta) domain swapped with protomer 4 (cyan) in dimer A, whereas protomer 2 (green) domain swapped with protomer 3 (orange) in dimer B. The copper ions are shown as sphere models. The main chain of Gly45 and the side chains of Cys3, Cys26, His46, His117, Cys112, Met121, and Gly45 are shown as stick models. (B) Hydrogen bonds between dimer A and dimer B. The hydrogen bonds are depicted in black broken lines. The Gly37–Ala40 residues (hinge loop) are depicted in pale colors. The side chains of Asp16, Lys18, Glu19, Lys126 and the main chain carbonyl of Asp16 are shown as stick models. The nitrogen atoms of the main chains of Lys18 and Glu19 and the side chains of His46, His117, and Lys126 are depicted in blue, the oxygen atoms of the main chains of Asp16 and Gly45 and the side chains of Glu19 are depicted in red, and the sulfur atoms of Cys3, Cys26, Cys112, and Met121 are depicted in yellow.

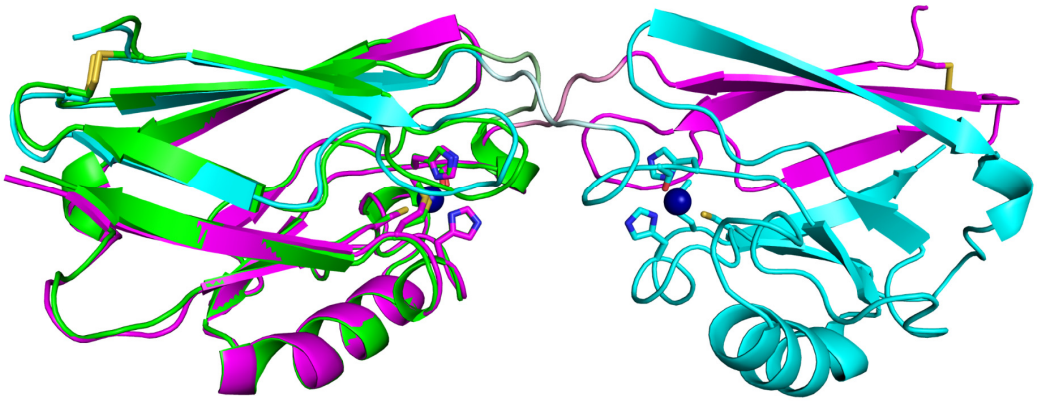

Fig. S4 Superimposed structures of the azurin monomer and dimer: monomer (PDB ID: 1RKR; green) and dimer (PDB ID: 6L1V; magenta and cyan). The copper ions are shown as sphere models. The main chain of Gly45 and the side chains of Cys3, Cys26, His46, His117, Cys112, Met121, and Gly45 are shown as stick models. The Gly37–Ala40 residues (hinge loop) are depicted in pale colors. The nitrogen atoms of the side chains of His46 and His117 are depicted in blue, the oxygen atom of the main chain of Gly45 is depicted in red, and the sulfur atoms of Cys3, Cys26, Cys112, and Met121 are depicted in yellow.

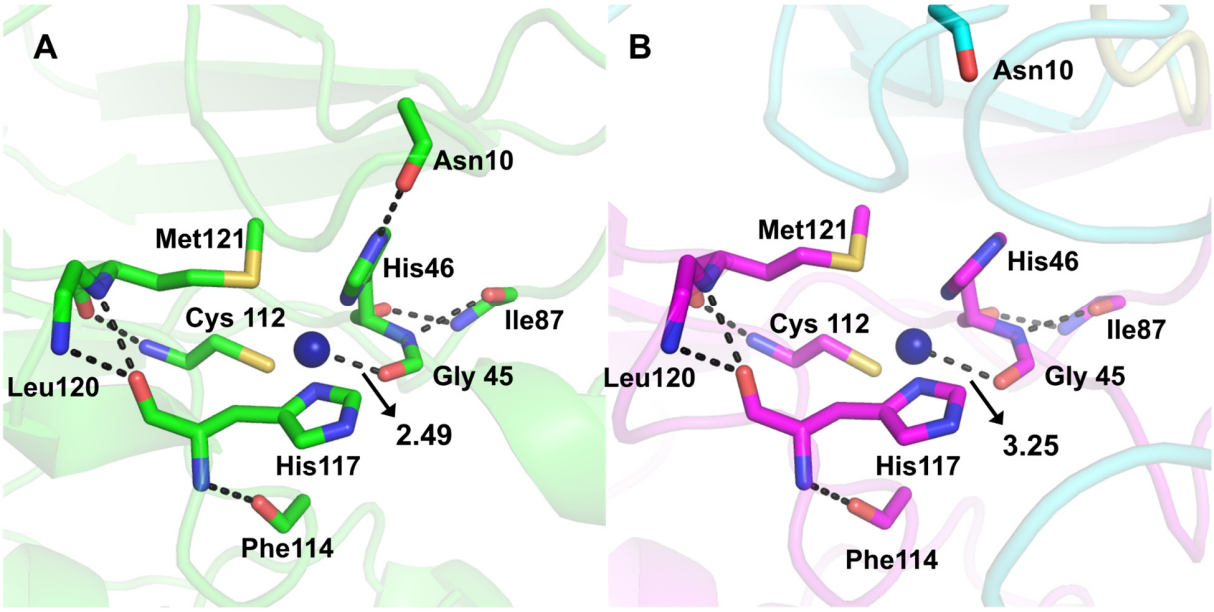

Fig. S5 Structures of azurin monomer and dimer around the copper active site: (A) monomer (PDB ID: 1RKR; green) and (B) dimer A (PDB ID: 6L1V; magenta and cyan). The copper ions are shown as sphere models. The two protomers of the dimer are depicted in magenta and cyan, respectively. The main chains of Asn10, Gly45, His46, Ile87, Cys112, Phe114, His117, Leu120, and Met121 and the side chains of His46, Cys112, His117, and Met121 are shown as stick models. Hydrogen bonds are depicted in black broken lines. The Gly37–Ala40 residues (hinge loops) are depicted in pale colors. The nitrogen atoms of the main chains of His46, Ile87, Cys112, His117, Leu120, and Met121 and the side chains of His46 and His117 are depicted in blue, the oxygen atoms of the main chains of Asn10, Gly45, His46, Ile87, Phe114, and His117 are depicted in red, and the sulfur atoms of Cys112 and Met121 are depicted in yellow.