Supporting Information for

# Electronic structures of blue copper centers of amicyanin and azurin in solution state

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**Comparisons of the protein structures before and after geometrical optimizations**

Figures S1(a) shows the conformations of amicyanin before (crystal structure) and after the geometry optimization without the solvent effect (isolated structure). Structural changes throughout the protein were anticipated. In particular, a helical structure changed to an unordered structure (arrow in Fig. S1(a)). Compared to the isolated structure with the structure optimized with the solvent effect (solution structure) (Fig. S1(b)), the solvent effect preserved the helical structure observed in the crystal structure (arrows in Figs. S1(a) and S1(b)). For the isolated structure, the distances between copper and each ligand atom were almost the same with those of solution structure.

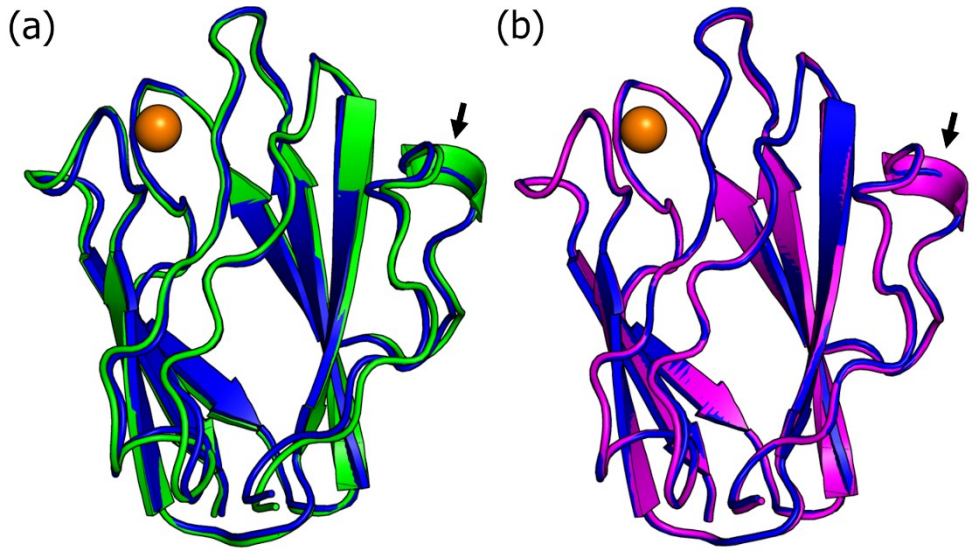


Fig. S1. (a) The structures of amicyanin before (crystal structure; green) and after the optimization without solvent effect (isolated structure; blue). (b) The structures of amicyanin optimized with (solution structure; magenta) and without solvent effect (isolated structure; blue). A software, PyMol (https://www.pymol.org/), was used.

Figures S2(a) shows the crystal and isolated structures of azurin. Secondary structural changes were induced by the geometrical optimization (arrows in Fig. S2(a)). Compared to the isolated and solution structures of azurin (Fig. S2(b)), the whole structures slightly

changed though the alteration of secondary structures was not observed. The distances between copper and each ligand atom in the isolated structure were almost the same with those of solution structure.

Thus, it was predicted that the geometrical optimization and solvation effect induced the structural changes of amicyanin and azurin. Those structural differences affected calculations of XANES spectra as shown below. It again suggests the protein coordinates used in the calculations should be selected properly following experimental situations.

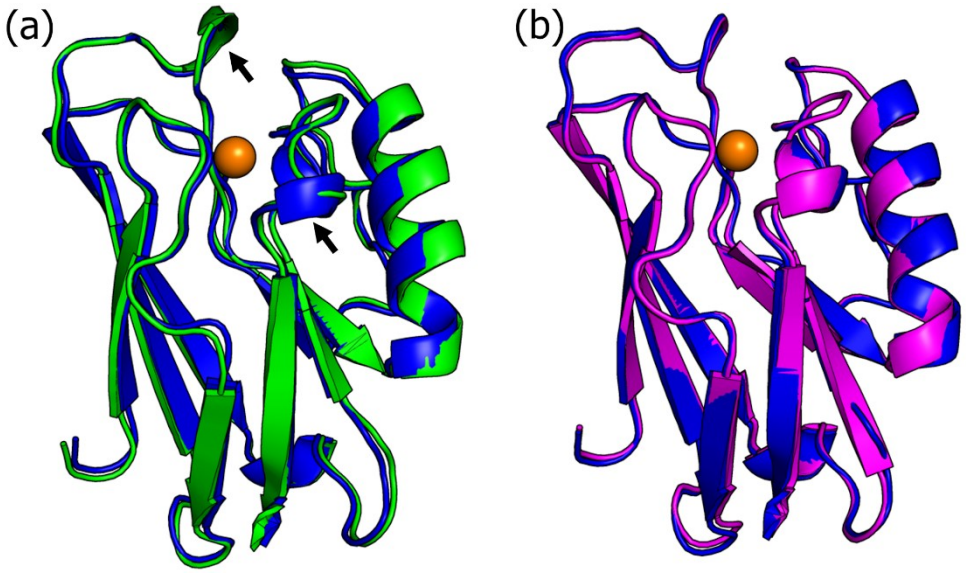


Fig. S2. (a) The structures of azurin before (crystal structure; green) and after the optimization without solvent effect (blue). (b) The structures of azurin optimized with (magenta) and without solvent effect (blue). The PyMol was used.

**Comparisons of theoretical XANES spectra**

Figures S3(a) and S3(b) show theoretical Cu $L_3$-edge XANES spectra of amicyanin and azurin, respectively, in their crystal, isolated, and solution structures. The spectra in the crystal and solution structures are the same as those shown in the main text. The spectral shapes in the isolated structures were similar to those in the solution structures, but the peak positions and ratios differed. Those differences originated from the structural

differences. In this work, we adopted the theoretical spectra in the solution structures for comparisons with the experimental spectra because those suited for the experimental situation and reproduced the experimental spectra better, especially for the azurin.

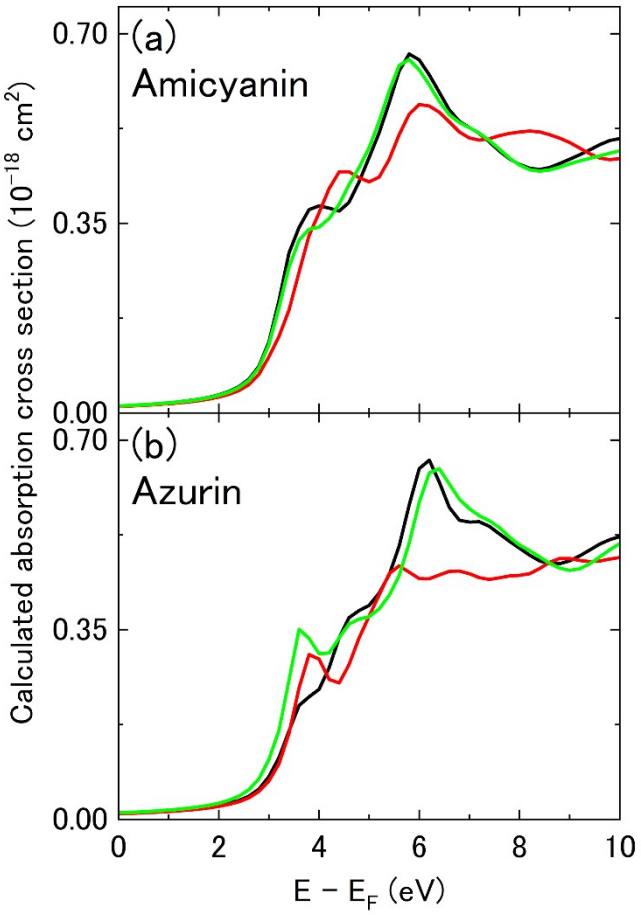


Fig. S3. Theoretical Cu $L_3$-edge XANES spectra of amicyanin (a) and azurin (b) in crystal (red), isolated (green), and solution (black) structures.

## Comparisons of experimental XANES spectra

Figure S4 shows experimental XANES spectra of amicyanin and azurin for ease of comparison. The detailed comparisons and discussion are described in the main text.

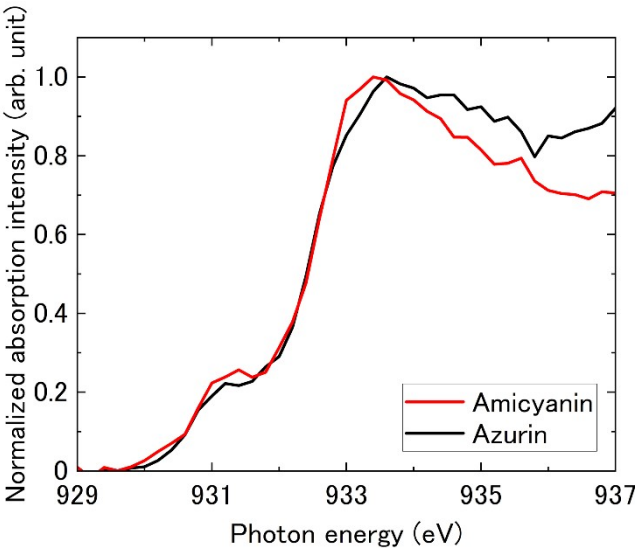


Fig. S4. Comparison of experimental XANES spectra of amicyanin and azurin.