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Supporting Information

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Supporting Information

for

New Approach for Local Structure Analysis of Tyrosine Domain in Proteins by Using Site-Specific and Polarity-Sensitive Fluorescent Probes

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Labeling of tyrosine

The labeling of tyrosine alone as a model compound (Figure S1) was also attempted by using a similar procedure to that in the modification of SOD. A 2-mL solution of 125 μM tyrosine (5 equiv) in 0.1 M sodium phosphate buffer (pH 8.6) was mixed with 10 μL of 5 mM DBHA (1 equiv) and 10 μL of the catalyst/ligand solution [8 equiv of $Pd(OAc)_2$ and 96 equiv of TPPTS]. The mixture was stirred for 1 h at 25 ºC. The reaction mixture was then extracted with 2 mL of ethyl acetate. After removal of the solvent by rotary evaporation, the residue was subjected directly to ESI-MS analysis. As shown in Figure S1B, besides the peaks of DBHA (*m/z* 475.3 $[M + H]^+$ and *m/z* 497.3 $[M + Na]^+$), the expected labeling product of tyrosine (*m/z* 596.1 $[M + H]^+$) is indeed detected. The apparent low labeling efficiency may be ascribed to the poor solubility of DBHA in water.

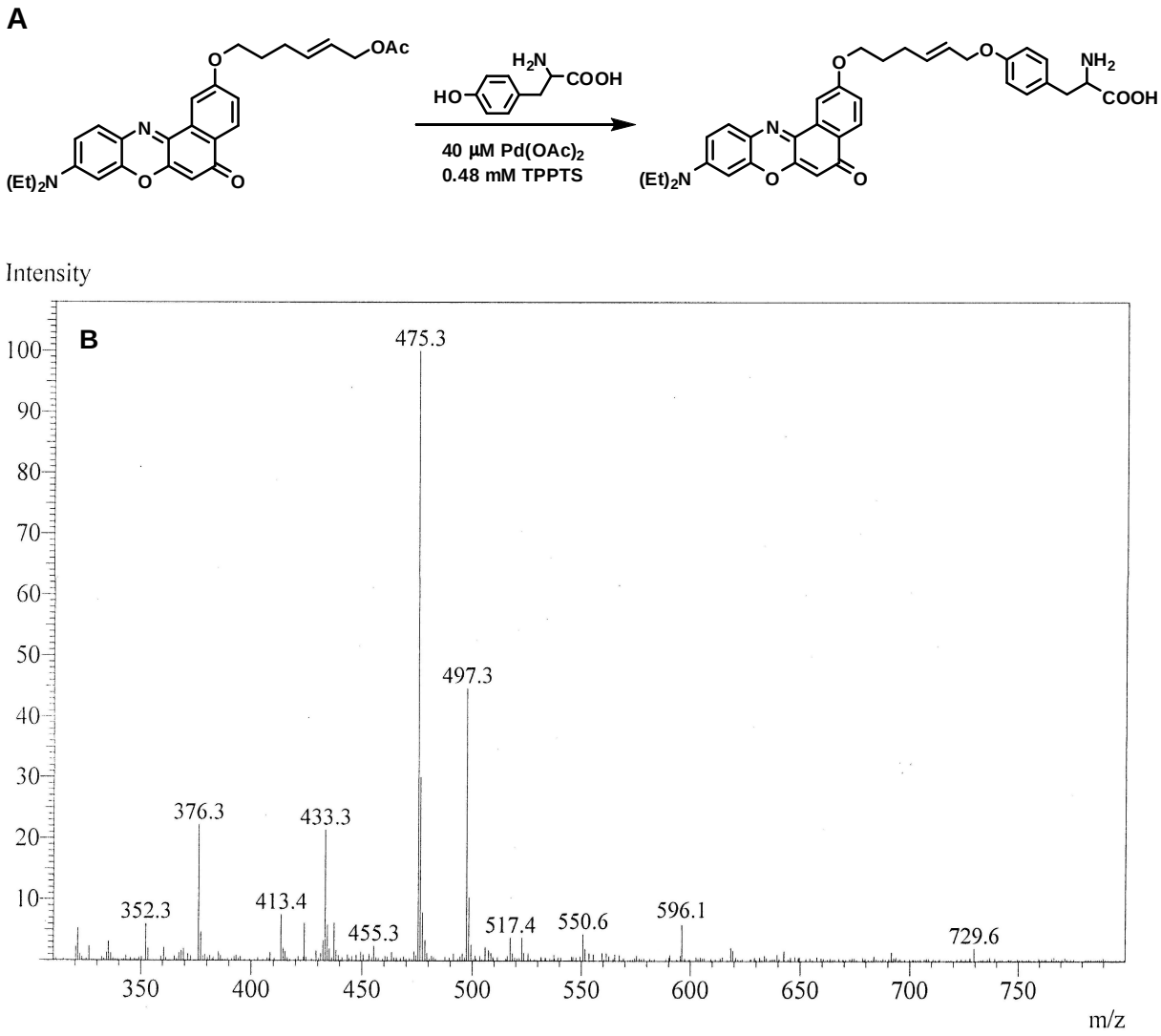

Figure S1. A) Modification of tyrosine with DBHA. B) ESI mass spectrum of the reaction products of DBHA with tyrosine.

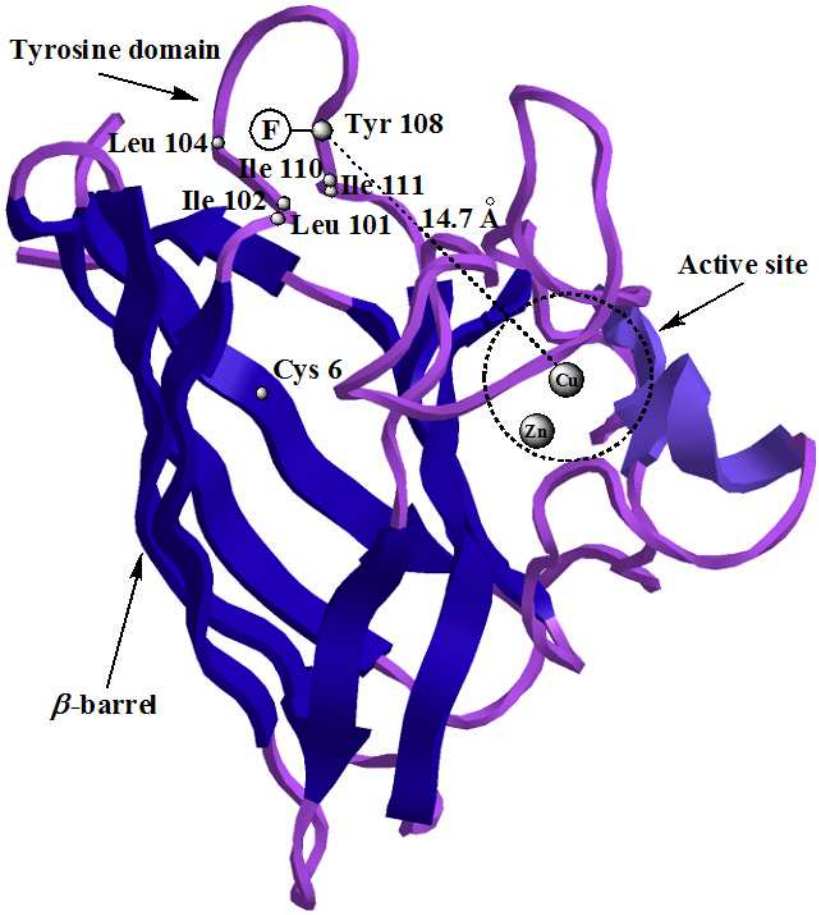

Figure S2. Crystallographic model of SOD (PDB ID: 1cb4, http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/). Distance from Tyr108 to copper ion was calculated with RasTop 2.0.3 software (USA). F denotes the DBHA probe.

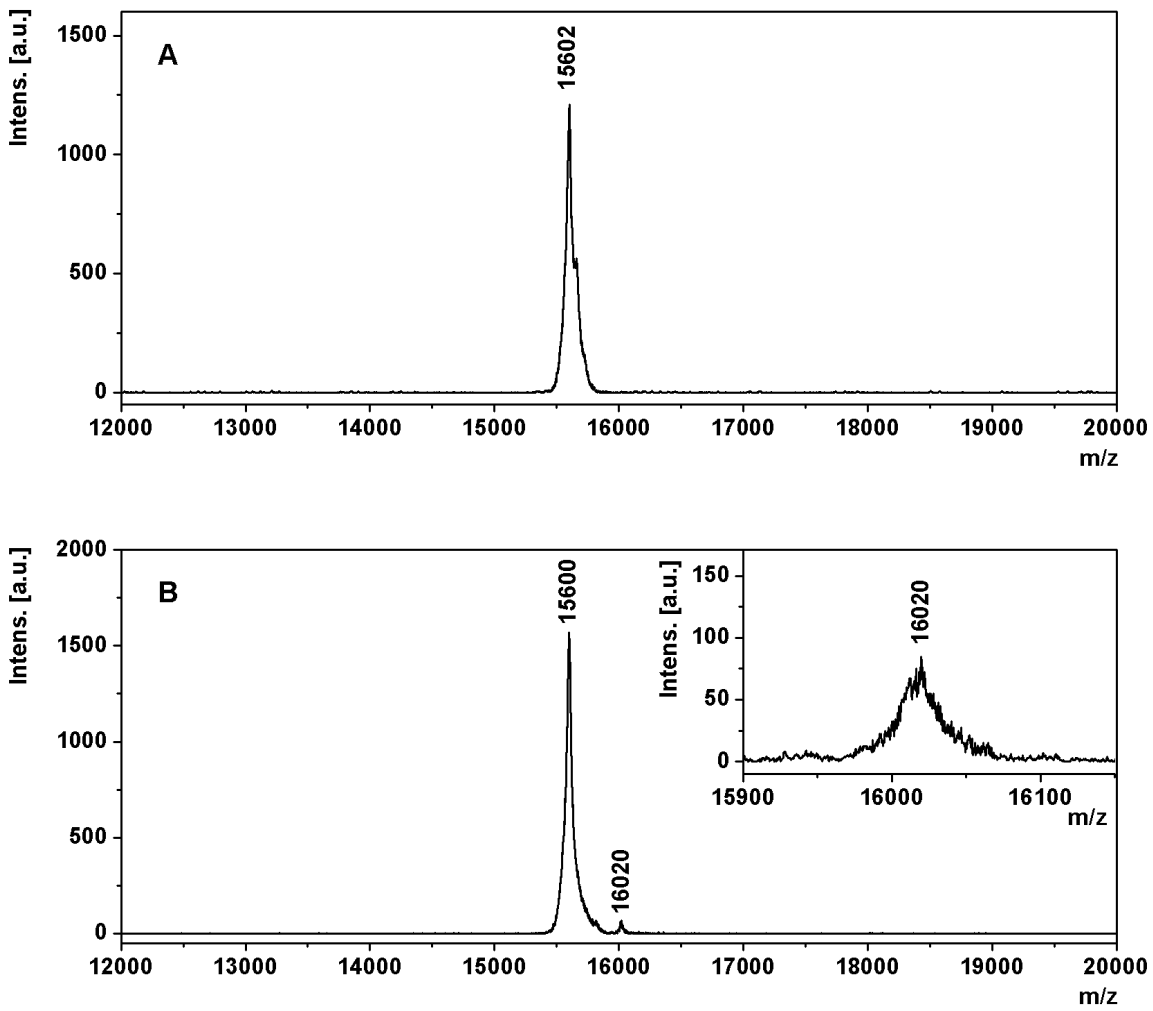

Figure S3. MALDI-TOF mass spectra of native (A) and DBHA-labeled SOD (B) measured on a Bruker BIFLEX III instrument in linear mode. The inset is the magnified mass spectrum of DBHA-labeled SOD in the *m/z* range of 15900 to 16150.

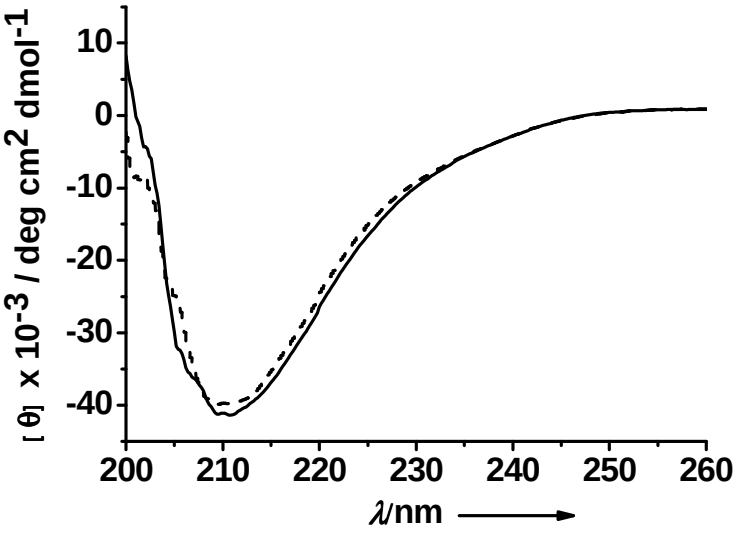

Figure S4. Circular dichroism spectra of native SOD (solid line) and DBHA-labeled SOD (dash line) at 2.5 μM in 0.05 M sodium phosphate buffer (pH 7.4).

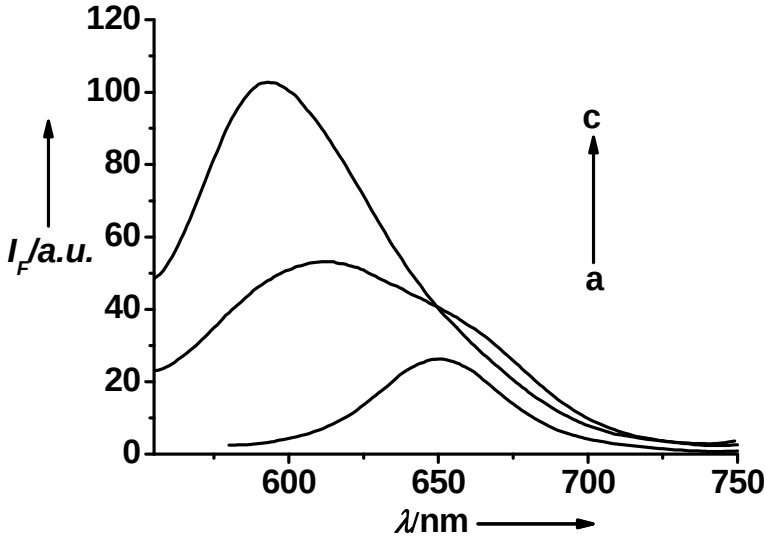

Figure S5. Fluorescence emission spectra of various sample solutions. a) 0.1 μM DBHA itself; b) DBHA (0.1 μM) plus native SOD (5 μM); c) DBHA (0.1 μM) plus the pretreated SOD (5 μM) by heating at 70 °C for 60 min. Note that in the binding experiment a much lower concentration of DBHA than SOD was used for the complete binding of the probe to SOD, so that an elimination of fluorescence interference from the free DBHA could be achieved. All the spectra were recorded at room temperature with λ_{ex} = 530 nm.

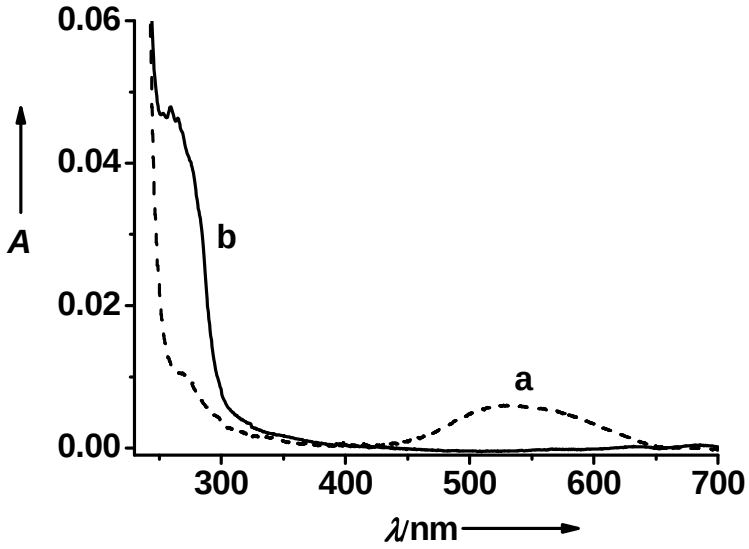

Figure S6. Absorption spectra of a) DBHA itself (0.1 μM) and b) SOD separated out from the simple mixture of SOD (5 μM) plus DBHA (0.1 μM) by a Sephadex G-25 gel column with the phosphate buffer as eluent.

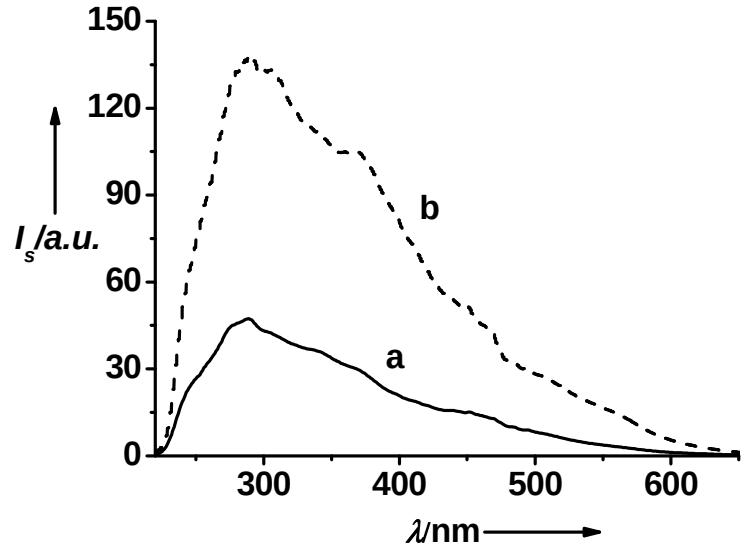

Figure S7. Synchronous scattering spectra of a) native SOD (5 μM) and b) heat-treated SOD (5 μM) at 70 °C for 60 min. Each of the spectra was recorded with both excitation and emission slit widths of 5 nm, and a 400 V PMT voltage.

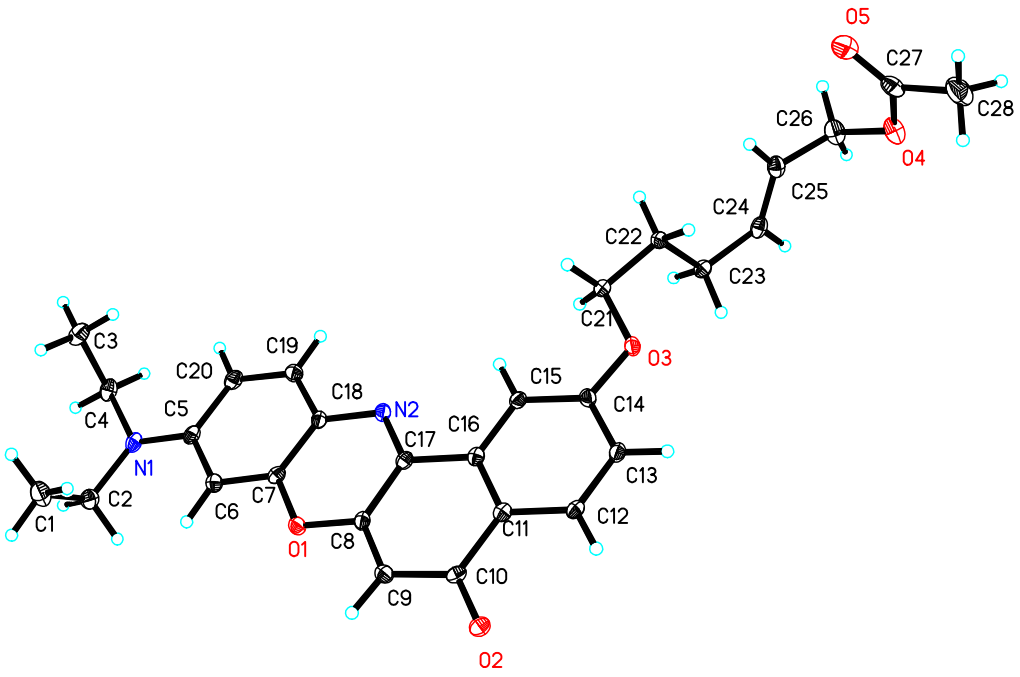

Figure S8. X-ray crystal structure of DBHA.