

The structural analysis of the pro-oxidant copper-binding site of denatured apo-H43R SOD1 and the elucidation of the origin of acquisition of the pro-oxidant activity

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Supporting Information for Review Only

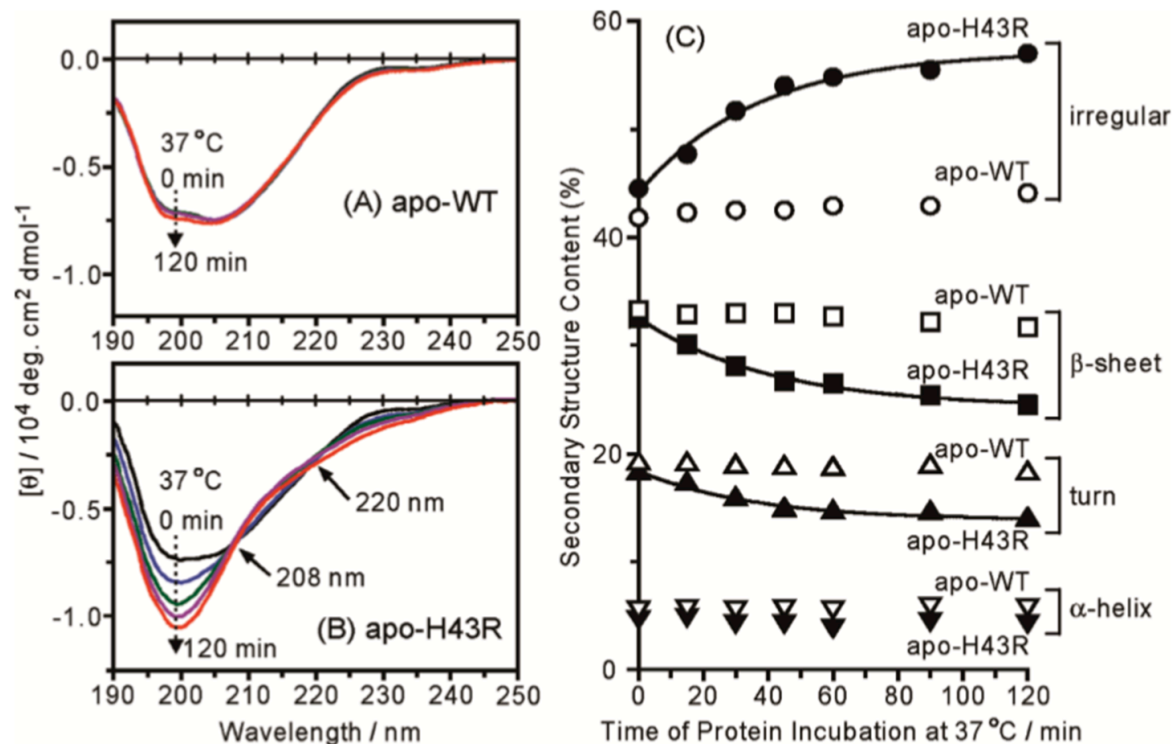


Figure S1. CD spectral and secondary structural changes during the incubation of SOD1 at 37 °C: (A) CD spectral change of apo-WT, (B) CD spectral change of apo-H43R, and (C) changes in secondary structure content calculated from the CD spectra for apo-WT (empty symbols) and apo-H43R (filled symbols). The spectra were recorded after incubation at 37°C for 0 (no incubation), 15, 30, 45, 60, 90, and 120 min, but those at 45 and 90 min are not shown in panels A and B for the sake of clarity. Reprinted with permission from ref. 25. Copyright 2011, American Chemical Society.

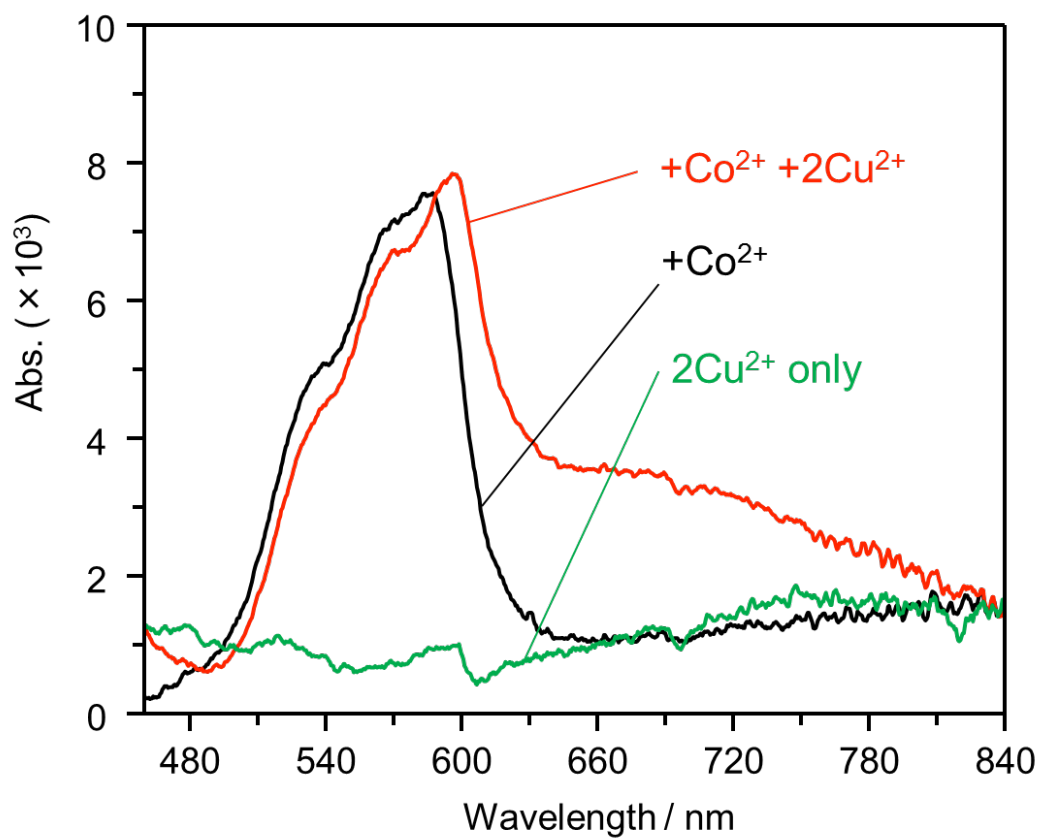


Figure S2. Effect of the addition of Cu²⁺ on the d–d absorption spectra of Co²⁺-bound native apo-H43R in phosphate buffer. The concentrations were 18 μM in monomer units (native apo-H43R), 18 μM (CoCl₂) and 36 μM (CuCl₂). The spectra were measured by the addition of 1 equiv. Co²⁺ (black line) and 2 equiv. Cu²⁺ after 1 equiv. Co²⁺ (red line). The absorption spectrum of CuCl₂ (36 μM) in phosphate buffer without apo-H43R (green) is also shown for comparison.

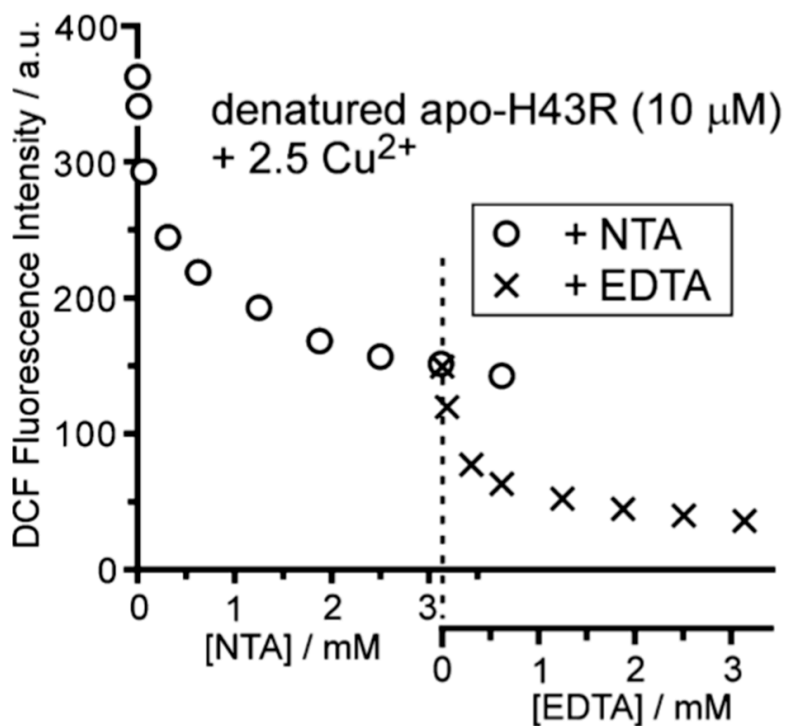


Figure S3. Effect of Cu^{2+} depletion on the pro-oxidant activity of Cu^{2+} -bound denatured apo-H43R. The pro-oxidant activity was measured as the fluorescence intensity from DCF produced by the oxidation of DCFH (50 μM) in the presence of denatured apo-H43R (10 μM), CuCl_2 (10 μM), H_2O_2 (50 μM), and one or two chelators (NTA and/or EDTA, 0–6.2 mM). The DCF fluorescence was excited at 495 nm and observed at 523 nm. NTA was added first and then EDTA was added. Reprinted with permission from ref. 26. Copyright 2013, American Chemical Society.