Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information:

In-situ colorimetric tracking of protein structural evolution based on the distance-dependent light scattering of embedded gold nanoparticles

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Contents

Supplementary Methods

1. SOD1 purification.

Human SOD1 genes encoding the wild type were cloned into the pET23b(+) (Novagen) vector and the proteins were expressed in *E. coli* BL21(DE3)pLysS.[1] Cultures were induced by treatment with 0.5 mM isoprophyl b-D-thiogalactopyranoside for 3 to 6 h at 30 ^{o}C, and the cells were lysed by sonication in a buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF). Solid $(NH_4)_2SO_4$ was added to the cell extracts to ~ 50 % saturation (0.313 g/ml), and after centrifugation, the supernatant proteins were loaded on a phenyl-sepharose 6 Fast Flow high sub hydrophobic column (Amersham Biosciences). Proteins were eluted with a linear gradient of ammonium sulfate (0.75 - 0 M) in 50 mM sodium phosphate (pH 7.0), 150 mM NaCl, 0.1 mM EDTA and 0.25 mM DTT. Wild type SOD1 was released with a high specificity from the column between 1.3 and 0.8 M ammonium sulfate.

2. Preparation of Au nanoparticles (GNPs).

Colloidal Au nanoparticles (20-nm diameters) were prepared by the reduction of $HAuCl_4 \cdot 3H_2O$ with citrate as previously described.[2] Optical spectra were recorded using a Hewlett-Packard 8453 spectrophotometer. Aqueous suspensions of the particles render the color of the suspension red.

3. Preparation of SOD1/GNP conjugate

The SOD1 monomer contains four cysteine residues, at positions 6, 57, 111, and 146 of which residues 57 and 146 form a disulfide linkage in the native state. Because SOD1 contains the free cysteine residues, this feature can be utilized to self-assemble SOD1 onto the GNP (via a gold-thiol conjugation technique). We initially prepared different samples of varying ratios between the SOD1 solution (0.1 mg/mL) and the synthesized GNP solution, ranging from 100:1 to 1:100 by volume. The optimum ratio determined for the GNP in this study is 1:30, and nanoparticles coated at this ratio were used in all experiments.

Supplementary Figures

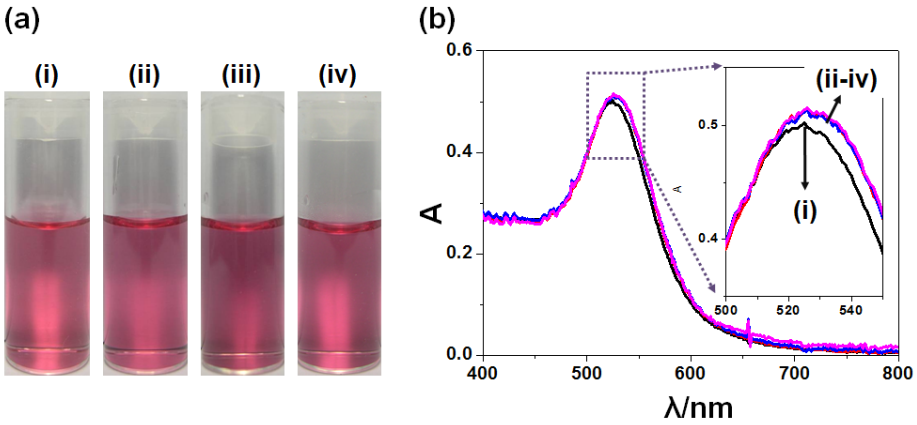

Fig. S1. Stability of the SOD1-GNP mixture solution. (a) and (b) Colors and spectra of the mixture solution at each step. (i) Bare GNP solution, (ii)-(iv) 0 hr, 2 hr , and 24 hr after SOD1 addition to the GNP solution.

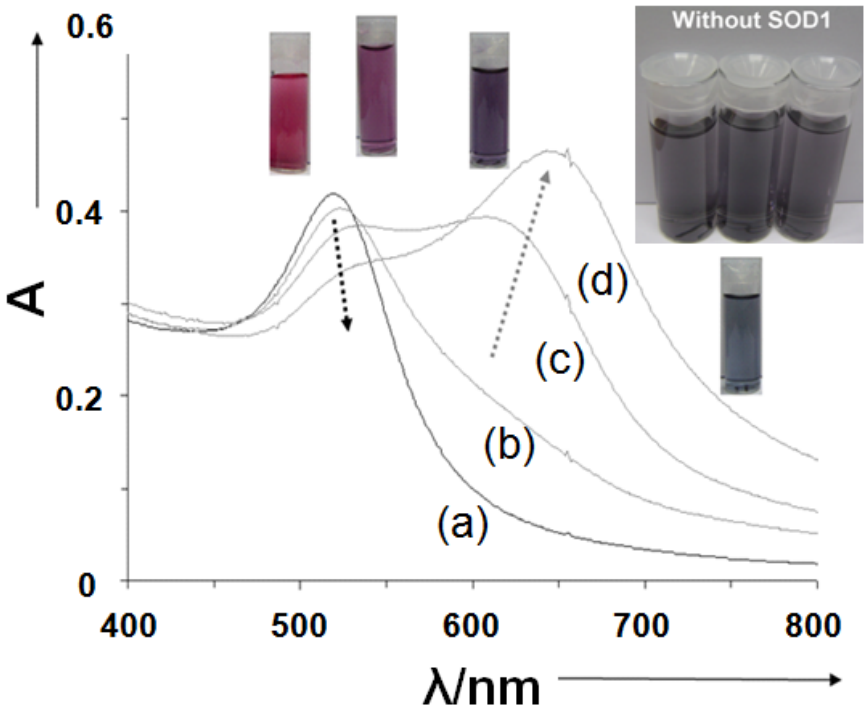

Fig. S2. Representative colorimetric response and corresponding spectral traces from the solutions consisting of free SOD1 and SOD1/GNP conjugates: (a) "As-made" and (b)-(d) "Under aggregation-promoting conditions" with increasing amounts of Cu^{2+} at constant H_2O_2. Cu^{2+} concentrations in samples (b)-(d) are 12.5, 25, and 50 μM, respectively. Inset: Color changes (control experiments) observed upon exposing bare GNP solutions to Cu^{2+} ions (the concentrations of Cu^{2+} in vials from left to right correspond to that of (b)-(d).

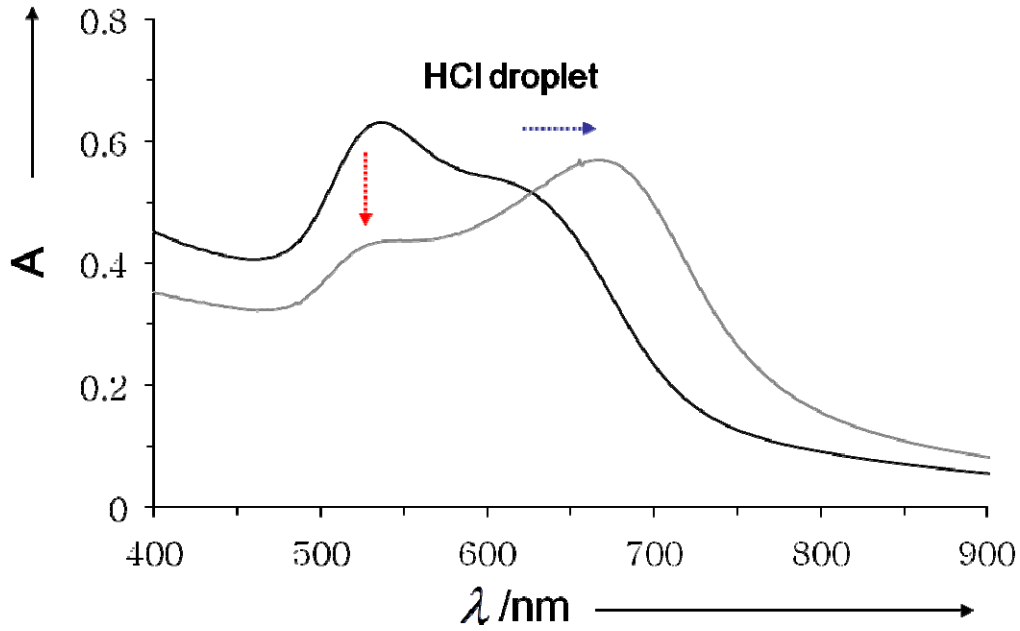

Fig. S3. UV-vis spectra of the SOD1-GNP solution after exposure with 30 μM Cu^{2+} (black solid line) and after adding 20 μL of 1 M HCl (gray solid line).

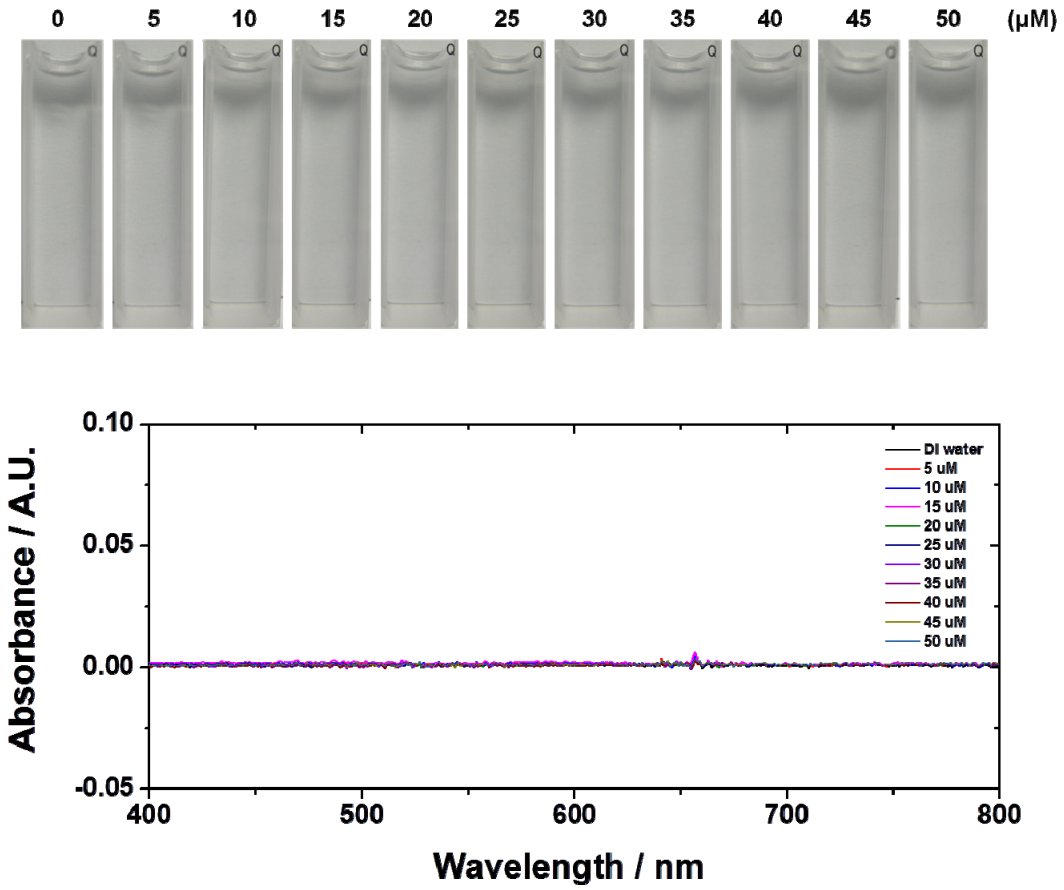

Fig. S4. Control experiments showing the Cu^{2+} ion solutions induce no significant color change in the concentration range (0-50 μM), where we investigated the protein aggregation.: Top: the color of D.I. water during the addition of Cu^{2+} ion solution (which is the exact same amount/concentration as Fig. 3), reading from left to right, are 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 μM. Bottom: Corresponding UV-Visible spectra.

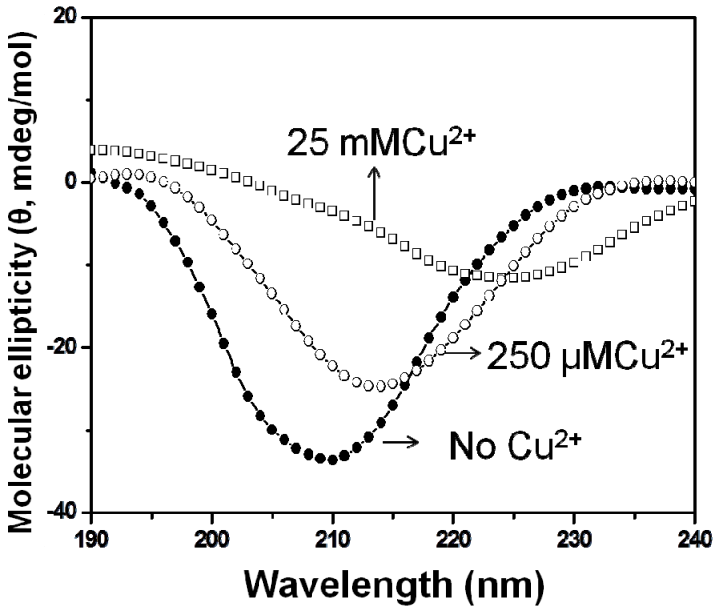

Fig. S5. Circular dichroism (CD) spectra of the structural evolution Cu^{2+} induced SOD1 aggregation.; It was not until 250 μM Cu^{2+} concentration (where is at least 50 times higher than what we used in the experiment) that we observed the changes in the CD spectra.

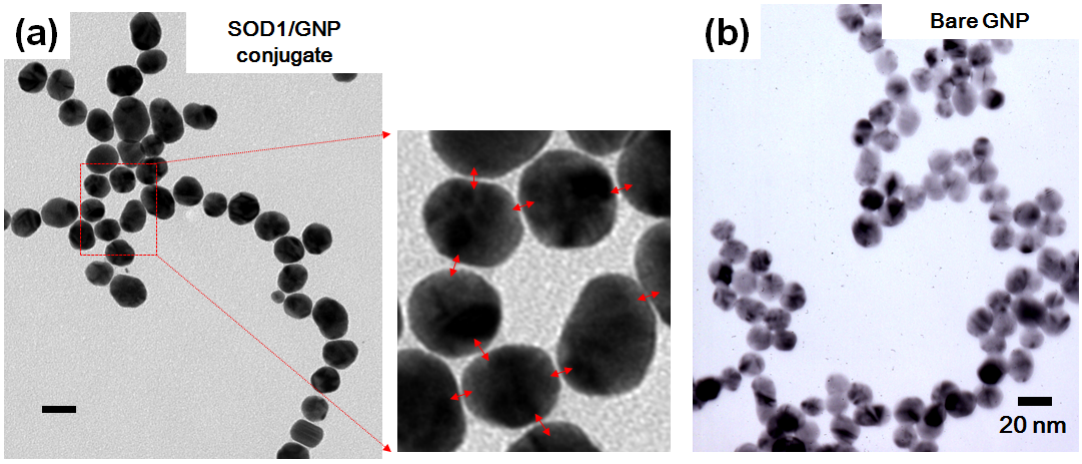

Fig. S6. (a) TEM image of a SOD1/GNP from the SOD1-GNP mixture solution before exposure to the denaturant. Although the GNPs were gathered by drying procedure during the TEM sample preparation, inset clearly shows the nano-sized gap observed between individual nanoparticle, which is due to the conjugation of SOD1 to the GNP. (b) TEM image of the bare GNP (without SOD1) solution after exposure wih 50 μM of Cu^{2+} and 5 μM of H_2O_2.

References

1. Boissinot, M.; Karnas, S.; Lepock, J. R.; Cabelli, D. E.; Tainer, J. A. ; Getzoff, E. D. ; Hallewell, R. A. *EMBO J.* **1997**, *16*, 2171.

2. (a) Handley, D. A. Methods for Synthesis of Colloidal Gold. *In Colloidal Gold: Principles, Methods and Applications*; Hayat, M. A., Ed.; Academic Press: San Diego, 1989. (b) Chah, S.; Fendler, J. H.; Yi, J. *J. Colloid Interface Sci.* **2002**, *250*, 142.