

Supporting Information

Tuning, Inhibiting and Restoring the Enzyme Mimetic Activities of Pt-Apoferritin

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Materials and Methods:

Horse spleen apoferritin and all chemicals were obtained from Sigma.

Experimental Section

Preparation of Pt-apoferritin: After removing the dimers and trimers by size exclusion gel filtration, monomeric apoferritin was incubated with aqueous K_2PtCl_4 solutions with molar ratios of 1/15000 at pH 7.4 with phosphate buffered saline (PBS). The solution was mixed in darkness at 30 °C for 2 hours, before excessive salt was removed with the Zeba Desalting Column (40kDa MWCO, Thermo Scientific). In order to avoid the formation of metal particles outside the protein shell, apoferritin, desalted with the spin columns after the incubation with metal salts, was immediately mixed with 1/20 (v/v) 1 M $NaBH_4$ and stirred for 20 min at 30 °C. After the reduction, the apoferritin-containing platinum nanoparticles (Pt-apo) were purified twice with spin columns (40kDa MWCO).

All samples were again purified with a desalting column (7kDa MWCO) in advance of all activity assays. Before use, the desalting columns were extensively washed with water to remove all NaN_3 , which was added as preservative (0.05%) into liquid phase of the original column. After the preparation, the protein concentration was determined with the Pierce 660 nm Protein Assay Kit (Thermo Scientific). For multicycle experiments, Pt-apo was also separated from the reaction mixture with a spin column (7kDa MWCO) after each cycle.

Analysis of the catalase-activity: Samples were purified with the spin column before the assay. The hydrogen peroxide decomposition activity was determined in a solution containing 0.8 mg/ml protein and 330 μM H_2O_2 at 30 °C. The pH value was kept constant at 7.4 with phosphate buffered saline (PBS). The reaction was started with addition of H_2O_2 . A 10 μL aliquot was taken from the reaction solution after 0, 5, 10, 20 and 30 min. and its residual H_2O_2 was determined with PeroXOquant Quantitative Peroxide Assay Kit (Thermo Scientific) according to the manufacturer's manual.

Analysis of the SOD activity: The SOD activity of the nanoparticle-apoferritin composites was determined with the SOD Assay Kit-WST. The assay is based on the principle that the Dojindo's highly water-soluble tetrazolium salt, WST-1, forms the WST-1 formazan by reduction of O_2^- superoxide anions in the assay system. The SOD activity of the testing component can be quantified by measuring the decrease in the color intensity of the WST-1 formazan at 440 nm. The enzyme working solution and WST-working solution were prepared following the manufacturer's instructions. The mixtures in the plate were incubated for 20 minutes at 37 °C before measuring the absorbance with the microplate-reader (Victor X5, PerkinElmer).

X-ray photoelectron spectroscopy (XPS): Experimental Details Solutions of the analyzed composites were deposited on a piece of Si wafer and dried in air. The XPS spectra were acquired with a spherical analyzer from the type EA 125 (Omicron) and a non-monochromatic Al-K α (1486.6 eV) source. The measuring equipment was calibrated before use with the 4f line of a gold sample. All measurements were done at a working pressure less than 5×10^{-9} mbar and with a pass energy of 50 eV. The survey spectra were made with a step size of 0.5 eV and an exposure time of 0.5 s per measure point (not shown). The high resolution spectra of the Pt 4f lines were done with a step size of 0.1 eV and an exposure time of 10 times 0.5 s per measure point. To eliminate the charging effect of the surface, all spectra were corrected with the help of the C 1s line. The position and intensity of each XPS line was determined by a Voigt approximation with a linear background.

Gel filtration: Based on the particle size, the nanoparticle containing apoferritin can be separated from free nanoparticles with the size exclusion filtration (gel filtration). The Superdex 200 PC 3.2/30 column equilibrated with a buffer (10 mM Tris, 100 mM NaCl, pH 7.8) was used for the filtration. The separation process was carried out with a flow rate of 0.05 ml/min. and visualized by absorption at different UV wavelengths (protein at 280 nm, Pt particle at 450 nm). The eluted fractions (50 μ l) were collected automatically in 96-well plate. The desired fractions were pooled for further analysis.

Atomic Absorption spectroscopy (AAS): All samples for the measurements had a protein concentration of 8 mg/ml. The measurements of the Pt concentration were carried out by the company BioSolutions (Halle, Germany) with a routine procedure.

Transmission electron microscopy (JEOL JEM-1010; FEI TITAN 80-300): A 10 μ L aliquot of Pt-apoferritin was dropped onto a carbon-coated copper grid (Plano). After 2 min. the excess liquid was removed with filter paper. For negative staining, 8 μ L of the staining

solution with 1.5% uranyl acetate and 250 µg/ml Bacitracin was dropped on the grid. After 30 s to 1 min. the excess solution was removed with filter paper and the sample was air-dried.

Table S1: Concentration of platinum measured with atomic absorption spectroscopy (AAS).

Sample	Cycle1	Cycle2	Cycle4	NaBH ₄
Platinum concentration (ppm)	288.48 ±16.11	281.15 ±25.04	199.09 ±37.76	207,36 ±17.31

* parts per million (ppm); the protein concentration of the samples was 0.8 mg/ml.

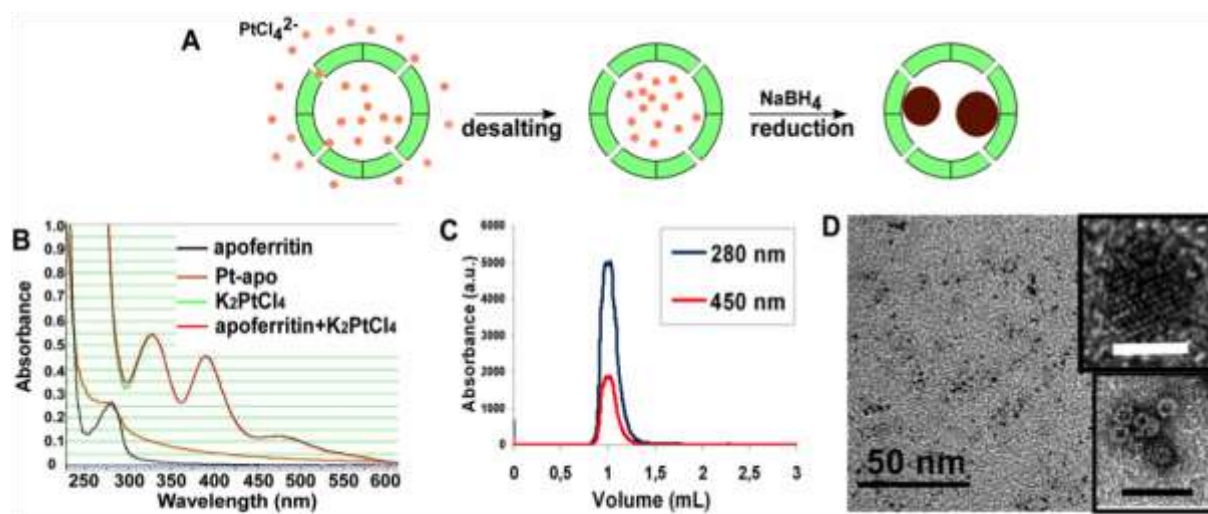


Figure S 1: (A) Schematic representation of the preparation of platinum nanoparticles within apoferritin (Pt-apo). (B) UV-vis absorption spectra of apoferritin, K₂PtCl₄ and Pt-apo. Apoferritin and Pt-apo were purified with a spin column before the measurements. The reference absorption spectrum was measured with purified water. (C) Co-elution of Pt (450 nm) and apoferritin (280 nm) with size exclusion chromatography. (D) TEM images of Pt-apo (Upper inset: high resolution electron micrograph of a platinum nanoparticle, scale bar: 2 nm; lower inset: TEM image of negatively stained Pt-apo, scale bar: 50 nm).

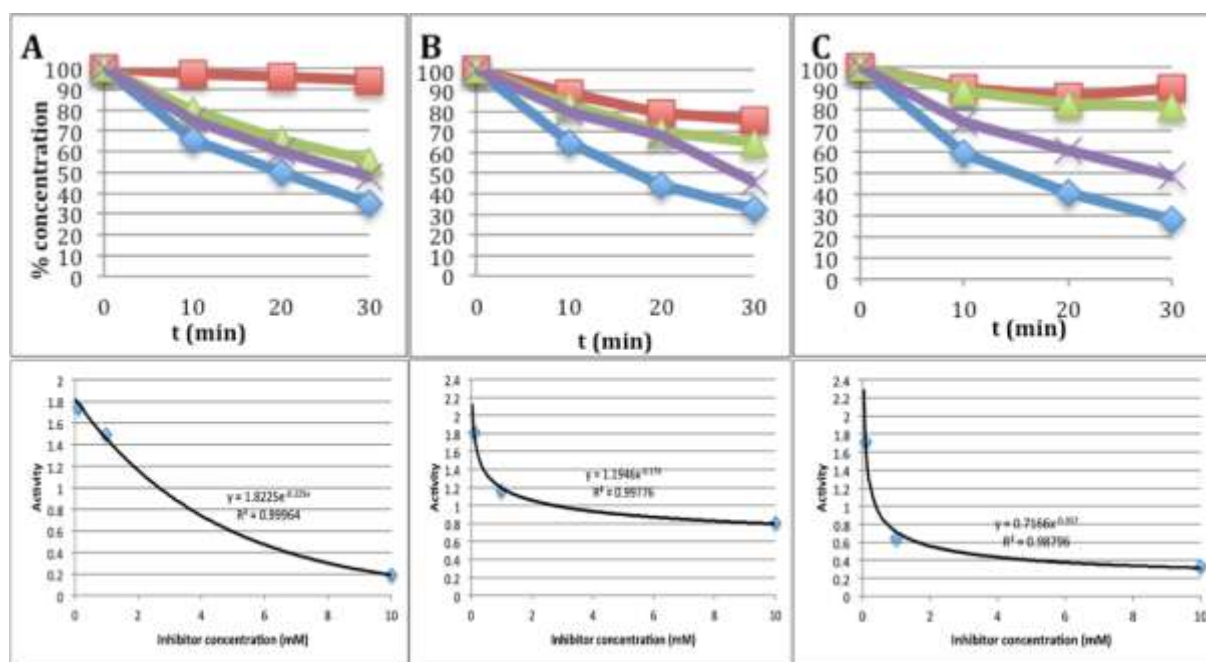


Figure S 2 Peroxide decomposition activities in the presence of inhibitors: A)3AT. B) NaN₃. C) Hg²⁺. Common assay conditions: 30 °C, PBS pH 7.4, 1.5 mg/ml Pt-apo and 2 mM peroxide. Blue curve: without inhibitor; red curve: 10 mM inhibitor; green curve: 1 mM inhibitor; purple curve: 0.1 mM inhibitor. The lower plots presented the average activity (decomposed H₂O₂/min) vs the concentration of the corresponding inhibitor.

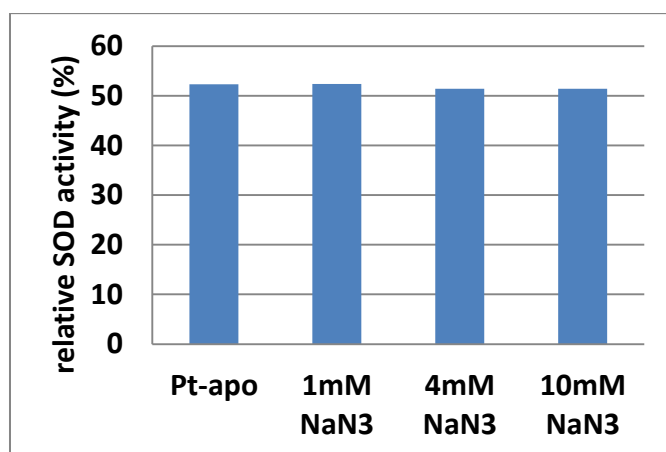


Figure S 3 . Inhibition effects on the SOD activity of Pt-apo with NaN₃.

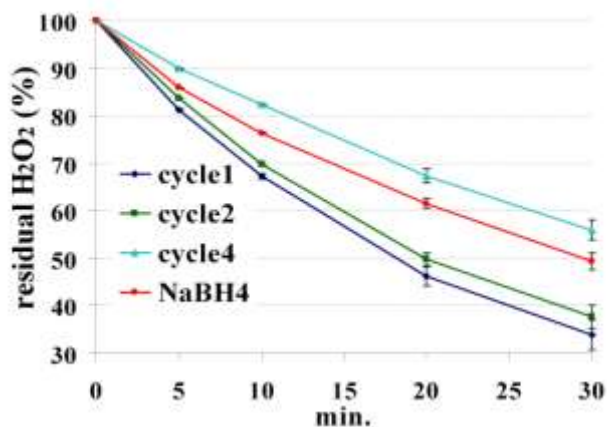
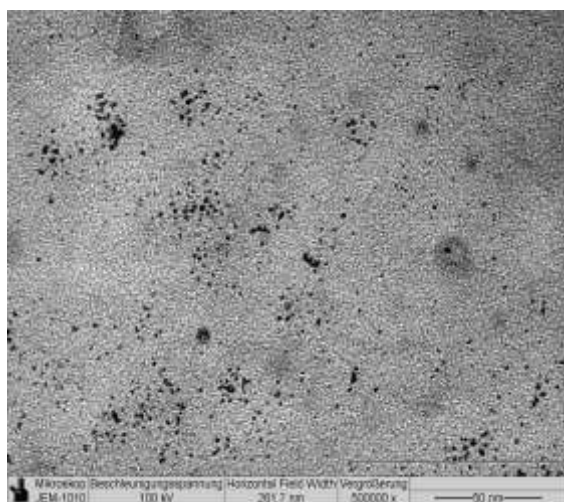
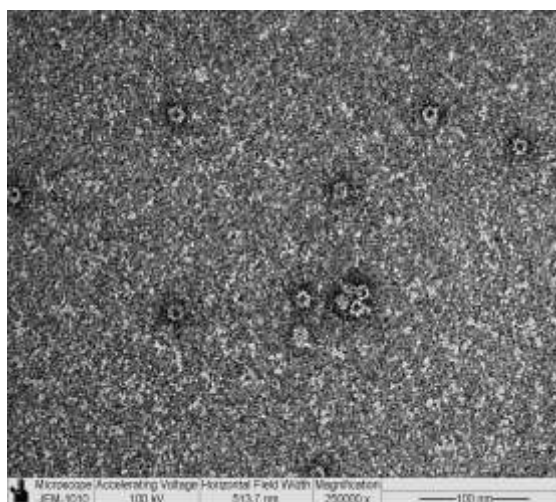


Figure S 4. The change of the catalytic activity of Pt-*apo* after multiple reaction cycles and the treatment with NaBH₄ (red line, not normalized with the Pt amount). After each cycle the Pt-*apo* was separated from the reaction mixture with a spin column.



A



B

Figure S 5: TEM images of A) Pt-*apo* after the peroxide decomposition reaction. B) The intact protein shell was visible after negative staining with 1.5% uranyl acetate.

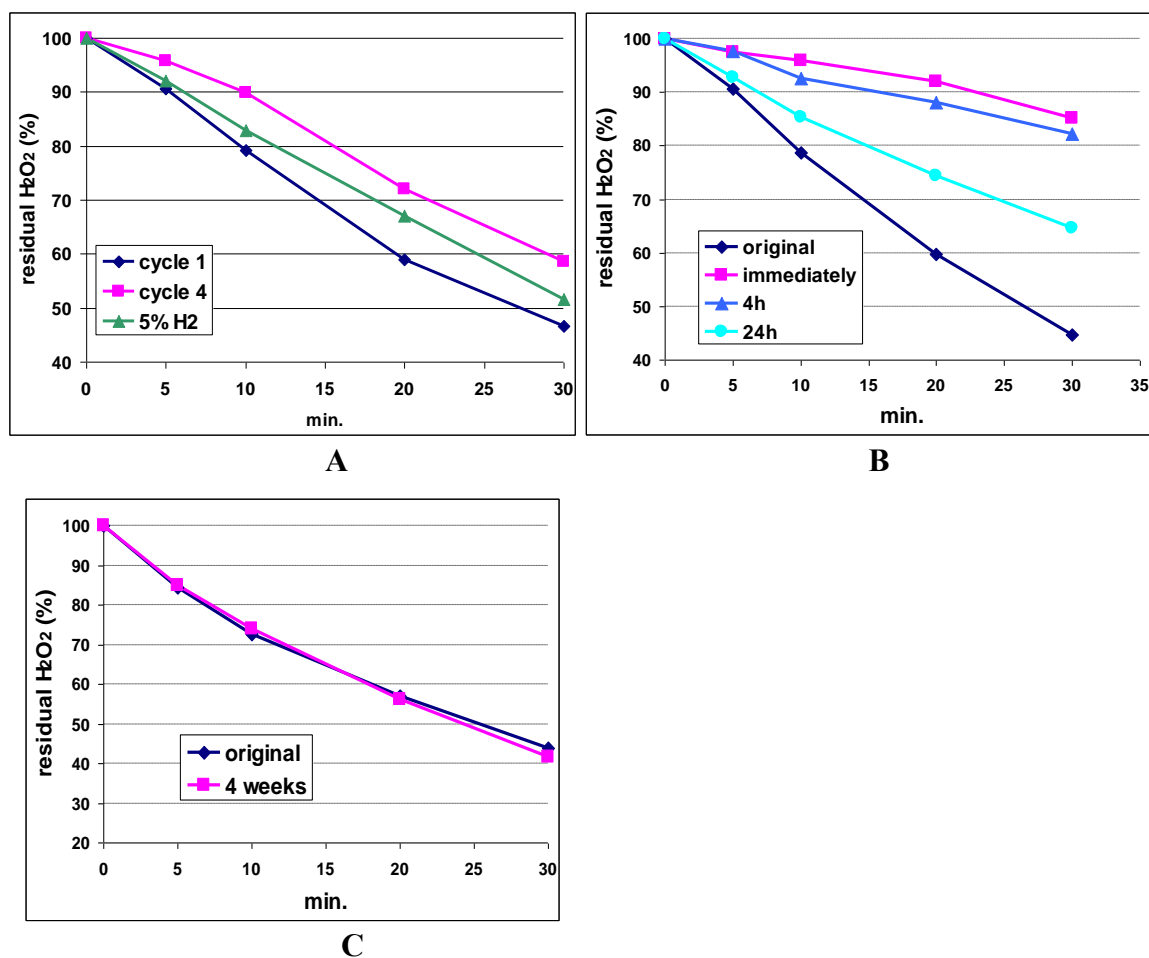


Figure S 6: A) Recovery of the activity of Pt-*apo* for the peroxide decomposition reaction with hydrogen gas (5% in Argon). B) Activity change after the treatment with NaBH_4 . The legend shows the storage time after the treatment and before the activity assay. C) Activity change of Pt-*apo* after storage in water at 4 °C for 4 weeks.