Supporting Information for:

Regulating the Pro- and Anti-Oxidant Capability of Bimetallic Nanozymes for Detection of Fe$^{2+}$ and Protection of *Monascus* pigments

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Chemicals and Materials

Potassium tetrachloroplatinate (II) (K₂PtCl₄), Ruthenium (III) chloride hydrate (RuCl₃), polyvinylpyrrolidone K30 (PVP), glycine, 3,3’,5,5’-tetramethylbenzidine (TMB), ferrous sulfate hexahydrate (FeSO₄·6H₂O), hydrogen peroxide, salicylic acid, dimethyl sulfoxide (DMSO), p-nitrophenol were purchased from Sinopharm Chemical Reagent Co., Ltd. L-ascorbic acid (AA), sodium borohydride (NaBH₄), potassium superoxide (KO₂), superoxide dismutase (SOD) were obtained from Alfa Aesar. 1.1-diphenyl-2-bitteryl (DPPH), Xanthine, xanthine oxidase (XOD), superoxide dismutase (SOD), diethylenetriaminepentaacetic acid (DTPA), Spin trap 5-tert-butoxycarbonyl 5-methyl-1-pyrroline N-oxide (BMPO), and 18-crown-6 were purchased from Sigma-Aldrich. Milli-Q water (18 MΩ cm) was used for all the experimental preparations. All glassware and the autoclaves used in the following procedures were cleaned using an aqua regia solution (HNO₃/HCl = 1:3 v/v).

Synthesis of Pt and PtRu nanostructures

PtRu nanostructures were prepared according to previous method with some modifications. Typically, 20 mM Ru³⁺ and 20 mM PtCl₄²⁻ with desirable volumes were mixed into 6.7 ml water
in a 50 ml round bottom flask (according to the calculated Pt/Ru molar ratio of 3/1, 1/1, and 1/3). Next, into the above solution 180 mg glycine and 440 mg PVP were added and mixed ultrasonically till the solution became clear. The flask was then placed at a 60°C water bath and stirring was continued. After temperature equilibrium, 1.3 ml 1M AA solution (molar ratio of AA/(Ru$^{3+}$+Pt$^{2+}$)=25) was added into the homogenous solution under vigorous stirring. 2 hours later, the solution changed to dark grey suggesting the formation of PtRu bimetallic NPs. The same procedure was used to prepare Pt nanoparticles except for without adding Ru$^{3+}$. The samples were purified twice by centrifugation at 12000 rpm for 10 min and the precipitation was re-dispersed in water. We named here the PtRu NPs as Pt$_x$Ru$_{100-x}$ according to the measured Pt/Ru ratio.

**Characterization**

UV-vis-NIR absorption spectra were obtained using a UV-VIS-NIR Spectrometer (Varian Cary 5000) and a matched quartz cuvette with a path length of 1 cm. The crystal structures of the PtRu alloy nanoparticles were characterized by X-ray diffraction (XRD, Bruker D8 Advance diffractometer) using monochromatized Cu Kα radiation (λ = 1.5418 Å). Transmission electron microscopy (TEM) images were captured on a Tecnai G² F20 U-TWIN electron microscope with an accelerating voltage of 200 kV. That same microscope was used to perform high-resolution TEM (HRTEM). Elemental composition and element distribution were verified by scanning transmission electron microscope (STEM) and energy dispersive X-ray spectroscopy in the high-angle annular dark field (HAADF) mode. X-ray photoelectron spectroscopy was conducted using a Thermo ESCALAB 250XI multifunctional imaging electron spectrometer using 150 W Al Kα radiation and a base pressure of approximately 3×10$^{-9}$ mbar. The binding energies were
calibrated to the C 1s line at 284.8 eV.

**Electron Spin Resonance (ESR) measurements**

ESR spectra were obtained at ambient temperature with a Bruker EMX ESR Spectrometer (Billerica, MA). Each sample was mixed with reactants and transferred to a quartz capillary tube. The capillary tube was placed into the microwave cavity of the ESR Spectrometer. All ESR measurements were carried out using the following settings for detection of the spin adducts BMPO/•OOH: 10 mW microwave power; 100 G scan range and 1 G field modulation. The conditions for ESR oximetry using the spin label N15-PDT: 0.04 G field modulation, 5 G scan range, and 1 mW microwave power.

To verify the ability of NPs to scavenge superoxide, xanthine and xanthine oxidase (XOD) were mixed together in a PBS buffer (pH 7.4) to generate superoxide and BMPO was used to trap superoxide in the form of spin adduct BMPO/•OOH. The control sample contained 25 mM BMPO, 0.05 mM DTPA, 1mM xanthine and 0.2 U/mL XOD in 10mM pH 7.4 PBS, to which SOD or PtRu NPs was additionally introduced to scavenge radicals. The reaction was initiated by adding XOD. For testing the oxygen generation, samples contained 0.1 mM PDT, 0.5 mM H2O2 mixed without or with NPs or catalase in pH 7.0 PBS buffer, ESR spectra were collected after 4 min of incubation.

**Peroxidase-like and oxidase-like activities assays**

The reaction kinetics for the catalytic oxidation of TMB was studied by recording absorption spectra at selected time intervals in a scanning kinetics mode. Unless otherwise noted, reactions were performed at room temperature. For testing peroxidase-like activity, 20 μl of 20 mM TMB solution and 20 μl of 0.1M H2O2 were mixed in 3 ml H2O, then, a suspension of 10 μl
2.5 mg/ml PtRu NPs (with different Pt/Ru ratio) or Pt NPs was added to initiate the oxidation of TMB. The reaction was also performed in the absence of hydrogen peroxide to detect oxidase activity and the PtRu NPs increase to 20 μl. For the measurement of ferroxidase-like activity, briefly, 20 μl of NP catalyst was added into the mixture of 2.5 ml of 6 mM salicylic acid and 0.5 ml of 60 mM FeSO₄ to initiate the reaction. The reaction rates were calculated by recording the spectra along with time at 2 min intervals. For the free radical scavenging experiment, 7 μl of 2.5 mg ml⁻¹ PtRu NPs with different Pt/Ru ratio were added into 2.5 ml 0.1mg ml⁻¹ DPPH ethanol solution, then the UV-visible absorption spectroscopy was recorded at selected time intervals.

**Protection of Monascus pigments from oxidation of H₂O₂**

*Monascus* pigments were extracted in our lab. Typically, 10 gram of *Monascus*-fermented rice sample was transferred to a tube containing 100 mL of 75% ethanol, mixed and stirred for 5 min at room temperature, sonicated in an ultrasonic bath for 60 min at 40 °C, and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the debris was re-extracted twice using the same procedure. The obtained pigment extract was concentrated to 5 ml in ethanol as stock solution. To evaluate the capability of Pt or PtRu NPs to prevent the *Monascus* pigments degradation, we mixed 2.0 ml aliquot of *Monascus* pigments ethanol solution (OA, λ₄₉₃nm = 2.5) and 0.1 mL 30% H₂O₂. Then we added a 20 μl 2.5 mg ml⁻¹ suspension of one type of NP per each quartz cell. The mixture was placed in the dark at ambient temperature, the UV-vis spectra were recorded at different intervals for calculation of degradation rates in the absence and presence of NPs.
Figure S1. HRTEM image of PtRu NPs obtained under adding Pt$^{2+}$/Ru$^{3+}$ molar ratio of 3/1, and the FFT images from the selected area in the image.
Figure S2. STEM-HAADF image (a) of PtRu NPs, STEM-EDS element mappings of Pt (b), Ru (c), and their layered image (d).
Figure S3. TEM images of Pt (a) and PtRu NPs obtained under different Pt$^{2+}$/Ru$^{3+}$ atomic ratio of 3/1 (b), 1/1 (c) and 1/3 (d).
Figure S4. High resolution XPS spectra of Pt 4f (b) and Ru 3p (b) from PtRu NPs obtained under Pt$^{2+}$/Ru$^{3+}$ atomic ratio of 3/1.
Figure S5. The relationship between measured and calculated Ru content in different PtRu NPs.
Figure S6. Peroxidase-like activity of Pt and PtRu NPs. The absorbance change (at 650 nm) with time during the TMB oxidation in the presence of H$_2$O$_2$ catalyzed by different catalysts. Conditions: 0.67 mM H$_2$O$_2$+0.13 mM TMB+8.3µg/ml NPs in 3 ml H$_2$O.
Figure S7. The DPPH scavenging activity. The photographs of 0.1mg/ml DPPH taken at 10 min after mixing with or without different NPs (6.0 µg/ml).