

Supporting Information

Atomic Ce sites promote a four-electron pathway of Pt as NADH oxidase mimics for in-situ coenzyme regeneration

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1.1 Instruments

X-ray Diffraction (XRD) characterization was obtained by a D8 ADVANCE (Bruker, Germany). Transmission electron microscope (TEM) images were acquired from FEI Talos F200X. X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) experiments on a Fe K-edge were obtained from the 1W1B station in Beijing Synchrotron Radiation Facility (BSRF). The content of each element in the samples was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6300). X-ray photoelectron spectroscopy (XPS) measurements were performed by Escalab Xi+ (Thermo ESCALAB QXi, United States). Electron paramagnetic resonance (EPR) spectra were obtained from a Bruker A300. *In situ* ATR-FTIR analysis was performed using a Nicolet iS50 FTIR spectrometer (Thermo) equipped with a diamond internal reflection element (IRE) (refractive index $n_{\text{diamond}} = 2.4$, incidence angle $\theta = 42^\circ$). All enzyme kinetics data and UV-vis spectra were obtained by a multimode reader (Tecan Spark, Switzerland).

1.2 Materials

Platinum(II) acetylacetonate ($\text{Pt}(\text{acac})_2$), cerium(III) acetylacetonate ($\text{Ce}(\text{acac})_3$), analytical grade poly(vinylpyrrolidone) (MW, 30,000), and N, N-dimethylformamide (99.5%) were purchased from Aladdin Chemical Reagent Co., Ltd. The reduced and oxidized nicotinamide adenine dinucleotides (NADH and NAD^+), lactate, lactate dehydrogenase (LDH), glucose, and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich. Alcohol dehydrogenase (ADH), alcohol dehydrogenase (ALDH), and glucose dehydrogenase (GDH) were ordered from Shanghai Yuanye Bio-Technology Co., Ltd. All chemicals were used as received without further purification. Ultrapure water was used throughout the experiments.

1.3 Computational methods

Density functional theory (DFT) calculations were performed using the Vienna Ab initio Simulation Package (VASP). The calculation of the electronic exchange-correlation term is described by the PBE of the generalized gradient approximation (GGA) method. The interaction between the atomic nucleus and the valence electron is described by the conjugated projection wave pseudopotential (PAW-PP). The cutoff energy for the plane-wave basis set was fixed at 450 eV. To achieve convergence, the Gaussian smearing method is used to use a bandwidth of 0.05 eV. In the spin-polarization treatment of the open-shell electron system, the Brillouin zone integral according to the special K-point method of the Monkhorst-Pack form and the K-point takes the value $1 \times 1 \times 1$. The convergence criterion of structure optimization and energy calculation is: energy convergence is 1.0×10^{-5} eV, and atomic force convergence is 0.05 eV/Å. A geometry optimization was considered convergent when the force change was smaller than 0.02 eV/Å. Grimme's DFT-D3 methodology was used to describe the dispersion interactions. The Brillouin zone integral uses the surface structures of Monkhorst-Pack K point sampling ($2 \times 2 \times 1$) for the Pt(111), and $\text{Ce}_1\text{Pt}(111)$ in a large size.

1.4 Synthesis of nanozymes

In a typical synthesis of Ce_1Pt , 10 mg of $\text{Pt}(\text{acac})_2$, 3 mg of $\text{Ce}(\text{acac})_3$, and 80 mg of poly(vinylpyrrolidone) (MW, 30,000) were dissolved in N, N-dimethylformamide (5 mL). Then, the mixed solution was stirred for 15 min and transferred into a Teflon-lined

stainless-steel autoclave. After the autoclave had been sealed, it was then heated at 180 °C for 12 h in an oven before it was cooled to room temperature. Then, the products were washed several times with ultrapure water and ethanol. Pt nanozyme was also synthesized using the same process without $\text{Ce}(\text{acac})_3$ as precursor.

1.5 The evaluation of oxidase-like activities

The nanozymes (1 mg/mL, 10 μL) were introduced into the HAc-NaAc buffer (0.1 M, pH 3.0, 100 μL). Then, TMB (1 mM, 100 μL) was added to the above solution, and the absorbance values of the reaction solution were obtained by a multimode reader after 10 min.

1.6 The evaluation of NOX-like activities

For NADH oxidation, 1 mg/mL catalysts (0.25 mL) and 1 mM NADH (0.25 mL) were added into PBS buffer (10 mM, pH 7.4) and incubated for 30 min. The supernatant was obtained by centrifuging. Then, the absorption spectra were recorded for further analysis. To determine the influence of different gas environments (N_2 , O_2 , and air), the corresponding atmospheric condition was maintained before and during measurements. The rapid color formation from the reaction can be conveniently measured on a spectrophotometer.

1.7 Monitoring the catalytic process of catalysts by in-situ FTIR

10 mg/mL nanozymes (5 μL) were first dropped on the diamond internal reflection element (IRE). The PBS buffer (10 mM, pH 7.4) was added, and the FTIR spectrum was recorded as a reference. Then, 100 mM NADH was further introduced, and the spectra were recorded every 2 min for further analysis.

1.8 Electrochemical measurement

An electrochemical workstation (CHI-760E) was employed for the electrochemical measurements at room temperature. A saturated calomel electrode acted as the reference electrode, and a Pt wire was used as the counter electrode. A glassy carbon electrode (GCE) with a diameter of 3 mm was polished with 1.0 μm alumina powder and washed with Milli-Q water. Then, the working electrode was obtained by dropping 1 mg/mL of catalyst solution (5 μL) onto the clean GCE and drying it at 50 °C.

1.9 Ethanol detection

All experiments were performed in accordance with the Guidelines "Declaration of Helsinki", and approved by the ethics committee at "the China Resources & Wisco General Hospital, Wuhan University of Science and Technology (Wuhan, P.R. China)". Informed consents were obtained from human participants of this study. All human samples were de-identified of all identifying information. Informed and permitted consent was obtained from each subject in all clinical experiments of this manuscript. Human serum samples were diluted 50 times for the test of ethanol and then added to the proposed nanozyme sensing system. Thereafter, serum samples were spiked with different designated concentrations of ethanol for testing.

1.10 Nanozyme sensing system in a microfluidic device

The microfluidic device was fabricated using photosensitive resin three-dimensional printing, with the injection pump connected via polypropylene tubing. Nanozyme was immobilized in the second reaction chamber. First, the sample, along with PBS buffer (pH 7.4), was pumped into the first microfluidic reaction chamber through inlets I and II, respectively, and incubated for 10 min at 37 °C. Then, the NaAc-HAc buffer (pH 3.0) was pumped into the next reaction chamber from inlet III, together with the mixture of the previously incubated solution, and retained for 5 min at 37 °C. Simultaneously, TMB was injected from the inlet IV. Finally, the solution was collected from the outlet and measured by a UV-vis spectrophotometer.

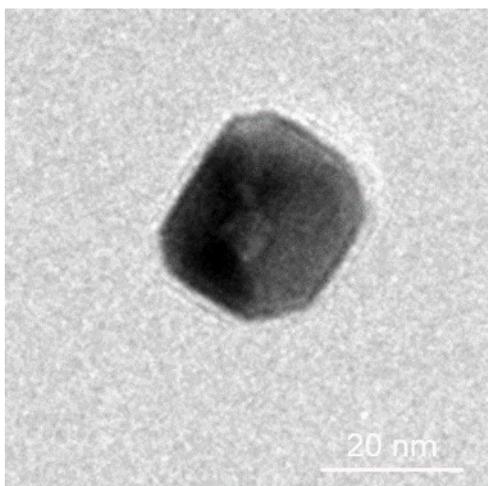


Fig. S1: TEM image of Pt.

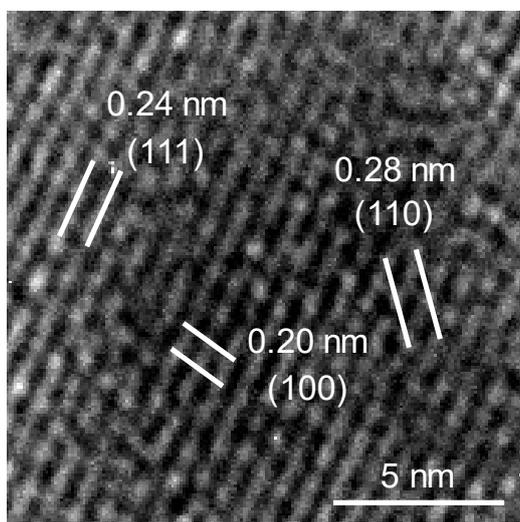


Fig. S2: HRTEM image of Pt.

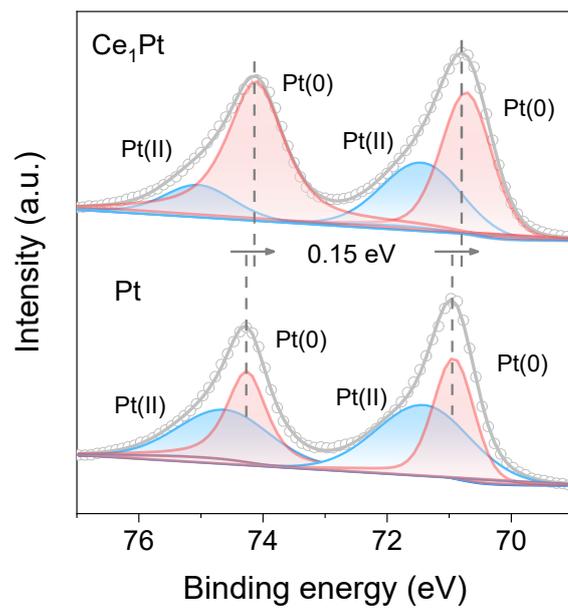


Fig. S3: Pt 4f XPS spectra of nanozymes.

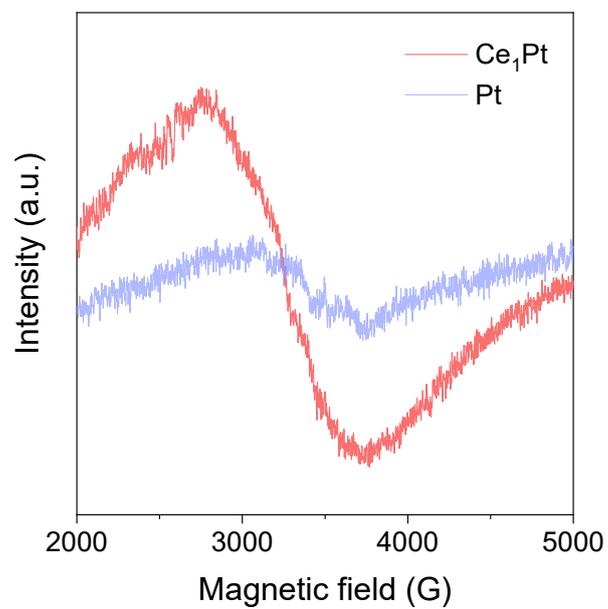


Fig. S4: EPR signals of nanozymes.

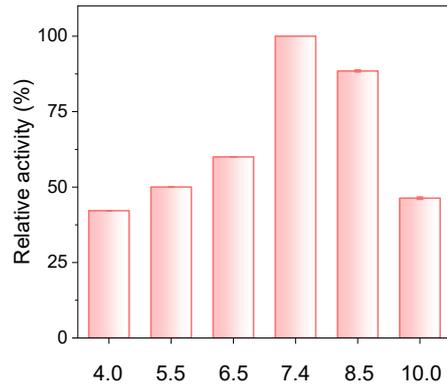


Fig. S5: The effect of pH on NOX-like activity of nanozymes.

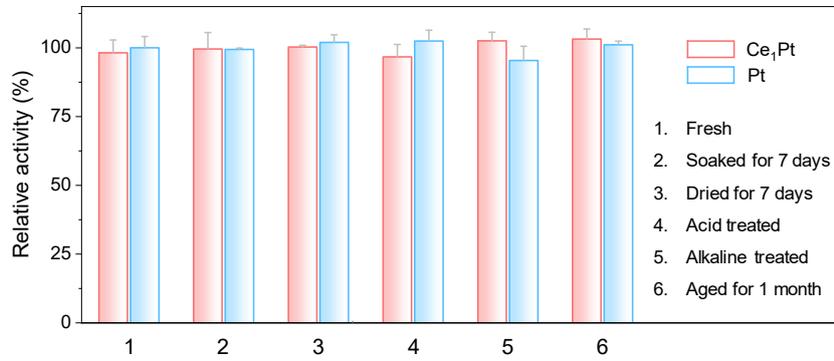


Fig. S6: Stability testing of nanozymes.

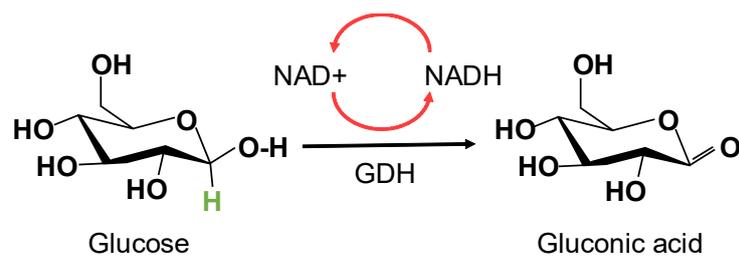


Fig. S7: Schematic illustration of the glucose metabolic processes.

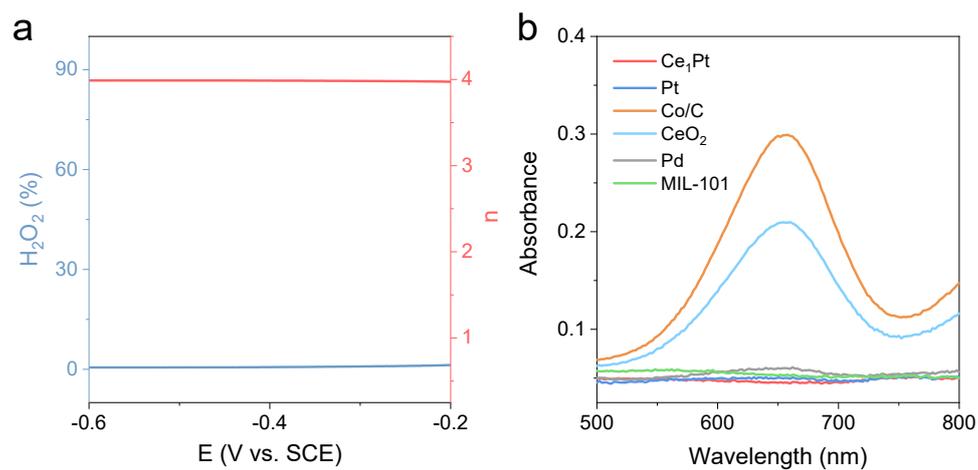


Fig. S8: (a) Electron transfer number and H₂O₂ selectivity versus the potential for Ce₁Pt. (b) Absorbance spectra of the NADH oxidation catalyzed by nanozymes in the HRP-TMB system.

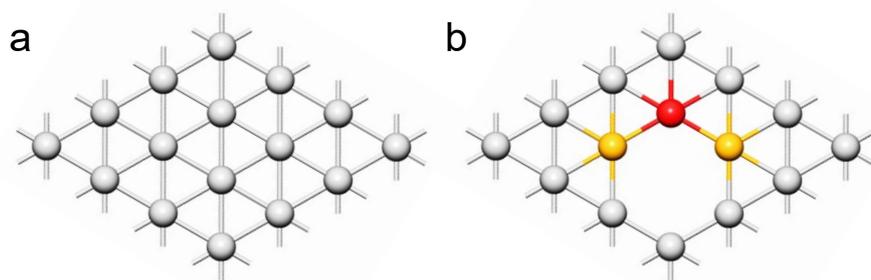


Fig. S9: Models of (a) Pt and (b) Ce_1Pt for DFT calculation. The grey, red, and yellow spheres represent Pt, O, and Ce atoms.

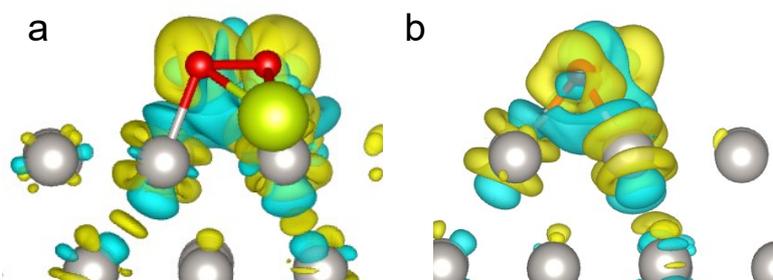


Fig. S10: Calculated charge density differences study the bonding interactions and the charge transfer of (a) Ce_1Pt and (b) Pt among Pt, Ce, and O atoms. The yellow/blue isosurfaces denote an increase/decrease of electron density, the grey, red, and yellow spheres represent Pt, O, and Ce atoms, respectively.

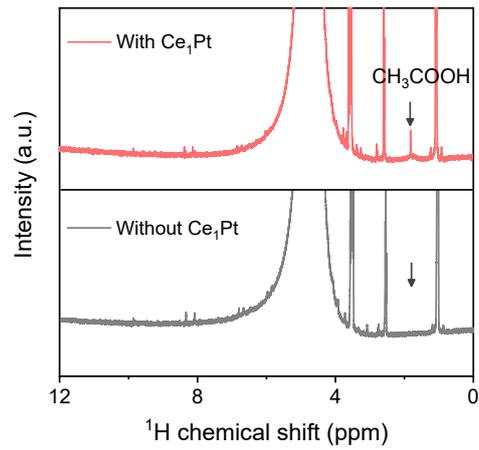


Fig. S11: ^1H -NMR spectra of ethanol with and without Ce_1Pt .

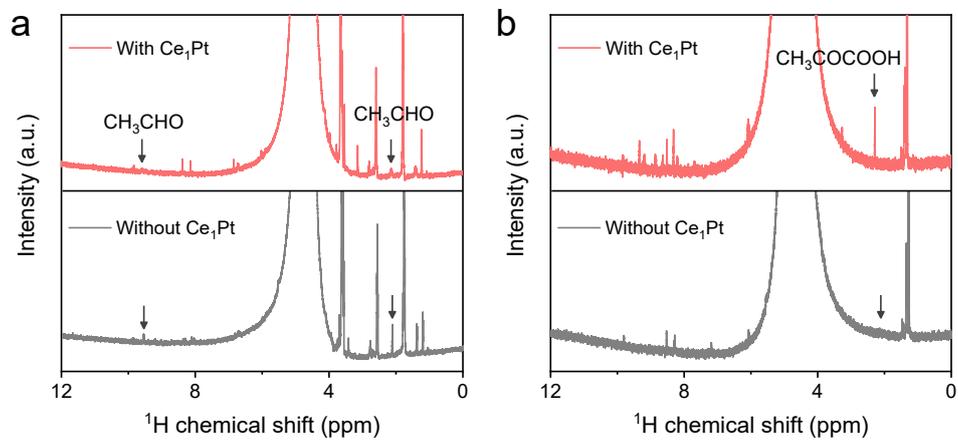


Fig. S12: ^1H -NMR spectra of (a) aldehyde and (b) lactate metabolic reaction with and without Ce_1Pt .

Table S1. Kinetic parameters of catalysts with NOX-like activity.

Catalysts	Substrate concentration	V_{max} ($\times 10^{-5}$ M min ⁻¹)	K_m (μ M)
Ce1Pt (1 mg/mL)	10-1000 μ M	6.81	249.94
Pt (1 mg/mL)	10-1000 μ M	4.66	328.28
Ir-N ₅ SA ¹	50-250 μ M	1.06	24.28
Ir-N ₄ SA ¹	50-250 μ M	0.82	39.11
Co/C(800°C) ²	10-250 μ M	1.39	70.50
Co/C(700°C) ²	10-250 μ M	1.12	76.60
Co/C(900°C) ²	10-250 μ M	1.01	91.20
Co/C(600°C) ²	10-250 μ M	0.87	95.70
CdS ³	0-2000 μ M	0.13	418.81
Superphane 1 (10 μ M) ⁴	25-250 μ M	1.71	70.50
Natural NOX (40 μ g/mL) ⁴	25-600 μ M	16.70	192.90

Notes and references

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