

Supplementary Information

Rh Single-Atom Nanozymes for Efficient Ascorbic Acid Oxidation and Detection

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1. Experimental section

Chemical and Reagents. Rh(acac)₃, Zn(NO₃)₂, 2-methylimidazole, Nafion solution (5% wt/wt) were purchased from Alfa Aesar Chemical Co. Carbon black (Vulcan XC-72) was purchased from Carbot Corporation. Methanol was obtained from Sinopharm Chemical Reagent Co. NaH₂PO₄, Na₂HPO₄, oleylamine, lactic acid (LA), uric Acid (UA), dopamine (DA), L-ascorbic acid (AA), potassium chloride (KCl) and sodium chloride (NaCl), commercial Pt/C (30wt.%) and Pd/C (10wt.%) were purchased from Macklin. Silver conductive ink (LOCTITE EDAG PE 409) was purchased from Henkel Investment Co., Ltd. (Dusseldorf, Germany). Carbon screen ink (CH-8 MOD2) and insulating ink (IN-15M) were purchased from Jujo Chemical Co., Ltd. (Tokyo, Japan). Vitamin C tablets, chewable tablets, and effervescent tablets were purchased from supermarket. The composition of different formulations is shown in Table S3. Milli-Q deionized water was used throughout. 0.1 M PBS (pH 7.4) consisting of NaH₂PO₄ and Na₂HPO₄ was employed as the supporting electrolyte in the electrochemical experiments.

Characterization Methods

XRD patterns were recorded with a Rigaku D/max 2500Pc X-ray powder diffractometer with monochromatized Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$). TEM and HRTEM images were recorded using a JEM-F200 instrument and an FEI Tecnai G2 F20 S-Twin working at 200 kV. HAADF images were taken on a JEM-ARM200F atomic-resolution analytical microscope operated at 300 kV. ICP-OES was measured by a Thermo Fisher IRIS Intrepid α system. XAFS measurements were carried out at the BL14W1 station in Shanghai Synchrotron Radiation Facility (SSRF, 3.5 GeV, 250 mA maximum, Si (311) double crystals). The Rh K-edge spectra of SA-Rh/CN

catalysts were collected in fluorescent mode using a seven-element Ge detector. Reference spectra were recorded in transmission mode using an ionization chamber.

Electrochemical characterization

In the electrochemical experiments on the glassy carbon electrode (GCE), the modified GCE was used as the working electrode. Platinum foil and Ag/AgCl were used as the counter electrode and the reference electrode, respectively. The electrochemical experiments were performed on a CHI 760 Electrochemical workstation (Shanghai, China). GCE were modified by drop-casting an ink containing catalyst powder. Typically, 5 mg Rh SAzymes, 1 ml water/ isopropanol (1:1) and 0.01 ml 5 wt% Nafion were ultrasonicated to form an ink. Then 2.2 μ l of ink solution (containing 0.11 μ g Rh) was loaded onto the GCE ($d = 0.3$ cm) for the further electrochemical testing in 0.1 M PBS (pH = 7.4) with the presence of ascorbic acid. The Rh/C, RhO_x/C, Pt/C, and Pd/C electrodes were modified using a similar process, except that the loading of precious metal on the electrodes was 0.55 μ g.

Detection performance evaluation

The limit of detection (LOD) is defined as the lowest concentration tested that has a peak height that is greater than or equal to the average of a blank sample (no analyte) plus three standard deviations of the blank. LOD was calculated according to the following equation:

$$\text{LOD} = 3 \delta / S$$

where δ is the standard deviation of blank samples in repeated examination, and S represents the slope of the calibration curve.

To assess the stability of the Rh SAzymes sensor, cyclic voltammetry was conducted

over a period of 28 days under the same testing environment. The measurements were performed in the solution of 8 mM AA and 0.1 M PBS (pH = 7.4) with the scan rate of 50 mV s⁻¹. The long-term stability of the sensor was evaluated based on the recorded peak current responses.

The reproducibility of Rh SAzymes sensor was evaluated by the peak responds of 10 mM AA in 0.1 M PBS (pH = 7.4) with seven identical working electrodes decorated by the same Rh SAzymes suspensions.

Screen-printed Sensor Fabrication and Modification

Screen printing technique was used to fabricate the glucose biosensor. In general, the fabrication process is as follows: 1) The polyethylene terephthalate (PET) substrate was washed thoroughly with acetone and water to remove impurities. 2) The electrode patterns designed with AutoCAD software were overprinted by hand screen printing machine. The silver conductive ink was first printed on PET according to the electrode patterns. The carbon paste was then overprinted on Ag/PET as the working electrode and counter electrode. Every printing was cured at 60 °C for 30 min. 3) The Ag/AgCl reference electrodes were obtained by immersing the Ag film into 0.1 M FeCl₃ solution for 1 min. Finally, the VC sensors were obtained after the WE were modified with Rh SAzymes.

Analysis of AA in human saliva

Saliva samples were collected from a healthy male volunteer (27 years old) by the already reported “passive drool method”. The volume of saliva collected is around 2 ml. Before collection, the mouth was thoroughly cleaned with water to remove food residue. The collected saliva was used directly without any further purification, where

the volume of saliva dropped onto electrode was 0.1 mL. The following i-t electrochemical measurements were conducted on screen printing electrode by applying a constant potential of 0.2 V (*vs.* Ag/AgCl).

Analysis of AA in nutritional products

The different nutritional products were purchased from local stores and their respective oral formulations are detailed in Table S3. The crushed samples were dispersed in 40 mL of 0.1 M PBS, and dissolved using sonication. The supernatant from dispersed samples of number 8 and 9 needs to be diluted to one-tenth concentration before testing. After static layering, 0.1 mL of supernatant was dropped onto the printed electrode for further i-t testing with the applied potential of 0.2 V (*vs.* Ag/AgCl). It should be noted that the pH value of above supernatants is also 7.4.

Electrocatalyst Synthesis

Synthesis of Rh SAzymes. Rhodium(III) 2,4-pentanedionate (0.36 mmol) and zinc nitrate (6.3 mmol) were dissolved in 40 mL methanol with vigorous string for 2 h. 2-Methylimidazole (16.0 mmol) was added into the solution with continuing stirring for 10 min. The solutions were poured into a 50 mL Tefon-lined stainless-steel autoclave and heating under 120 °C for 4 h. After cooled to room temperature, the product (Rh-ZIF-8) was collected by centrifugation and washed three times with DMF and methanol. Rh SAzymes are obtained by pyrolyzing Rh-ZIF-8 under 1000 °C for 6 h in the N₂ atmosphere without further purification.

Synthesis of Rh/C and RhO_x/C. Rh/C was synthesized according to the reference with some modifications.¹ In a typical synthesis of Rh nanocrystals, Rh(acac)₃ (60 mg), ascorbic acid (215 mg) and oleylamine (15 mL) were mixed in a glass vial

(volume: 50 mL). After the vial was capped, the mixture was ultrasonicated for around 10 min and then immersed into an oil bath of 185 °C. The reaction was maintained at 185 °C for 4 h with magnetic stirring (300 rpm) before it was cooled down to room temperature. The Rh nanocrystals were collected by centrifugation and washed several times with n-hexane. RhO_x nanocrystals were obtained by calcining Rh nanocrystals in air at 300 °C for 3 h according to literature.² Finally, Rh/C (RhO_x/C) can be easily obtained by loading the synthesized Rh (RhO_x) onto Vulcan carbon at a 5 wt.% loading.

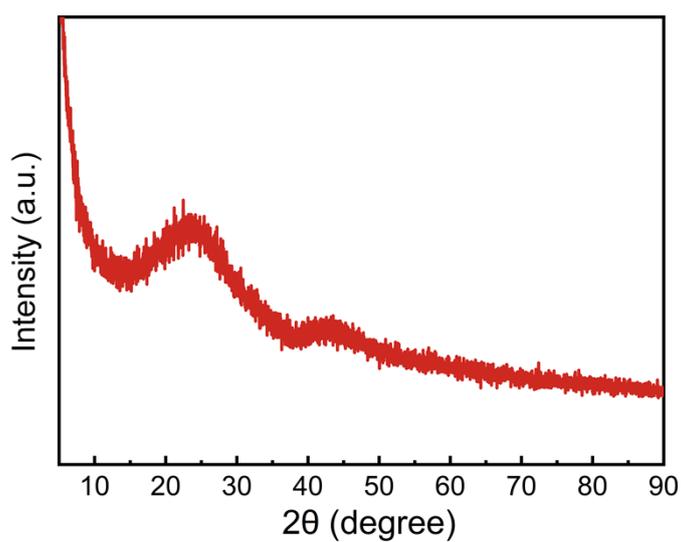


Figure S1 XRD pattern of Rh SAzymes.

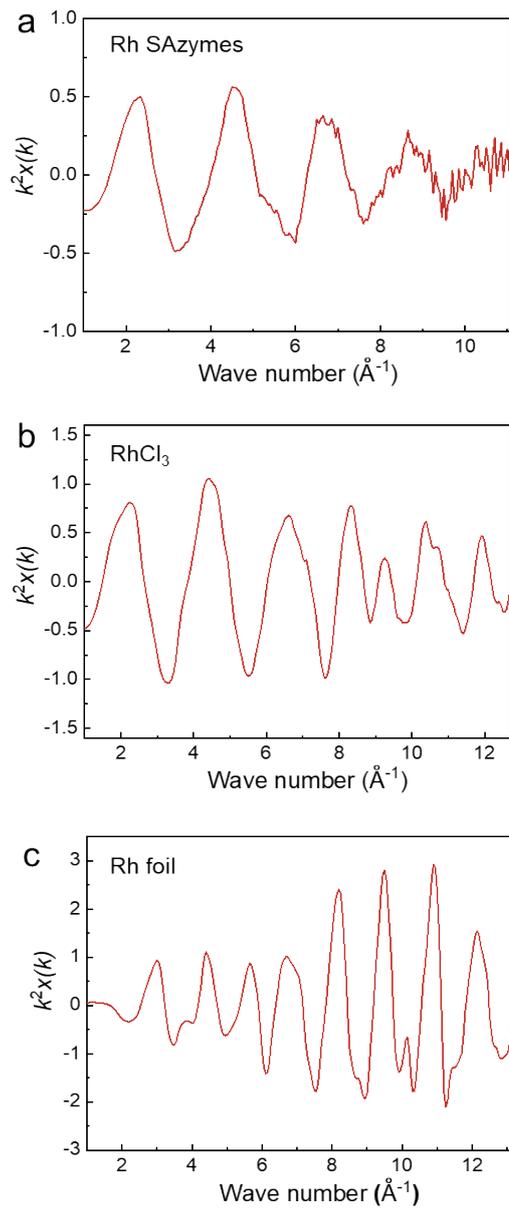


Figure S2 The k spaces curves for Rh SAzymes, RhCl₃ and Rh foil.

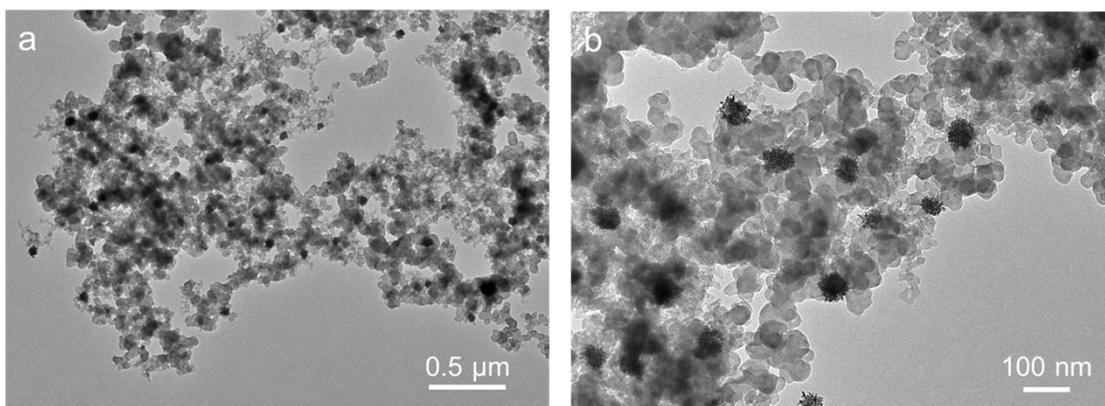


Figure S3 TEM images of Rh nanocrystals on Vulcan carbon.

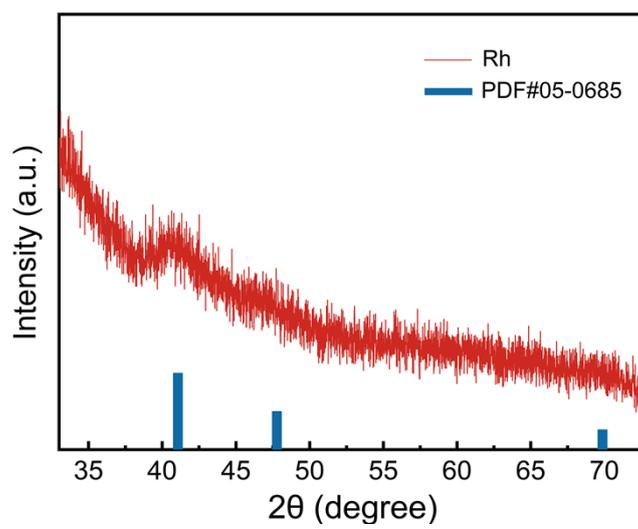


Figure S4 XRD pattern of Rh NP.

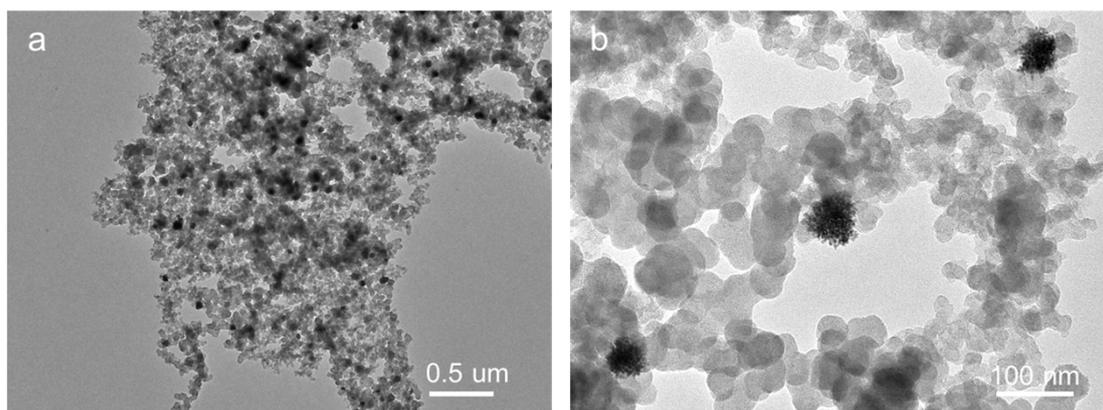


Figure S5 TEM images of RhO_x nanoclusters on Vulcan carbon.

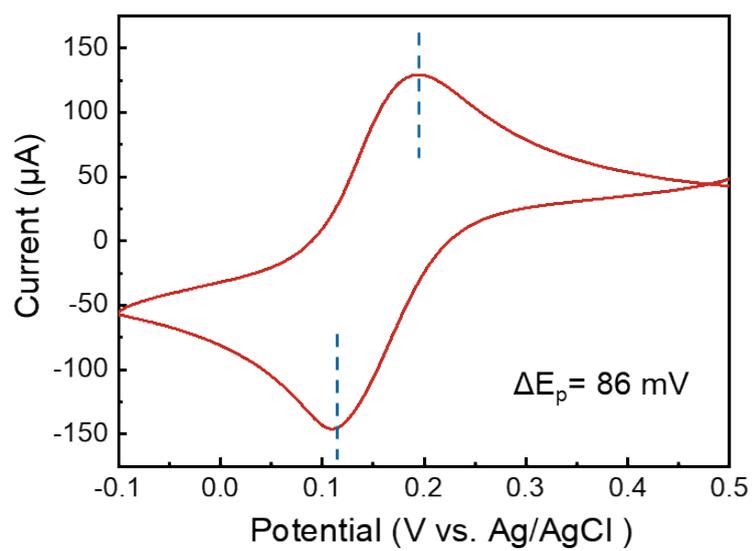


Figure S6 CV of the screen-printed electrode in potassium ferricyanide solution.

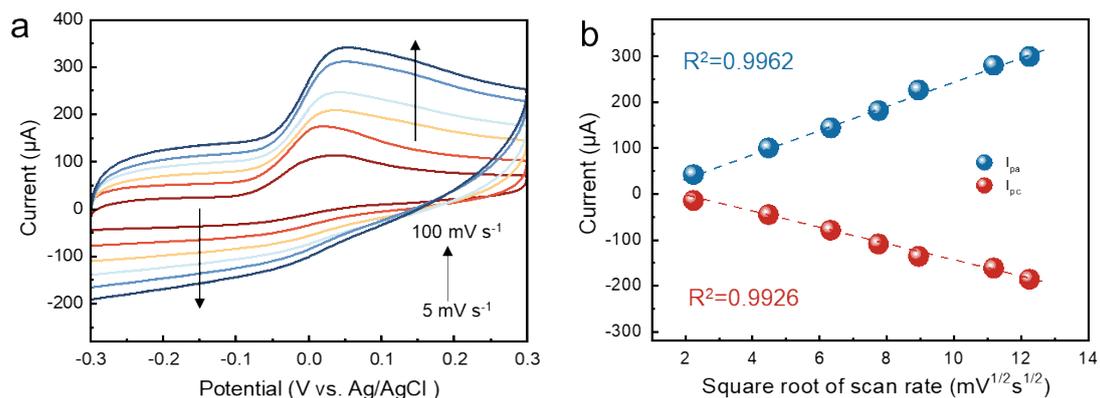


Figure S7 (a) CVs of Rh SAzymes in 0.1 M PBS with 10 mM vitamin C at different scan rates (5, 25, 50, 75, 100, 125, 150 mV s^{-1}). (b) The corresponding calibration curve of the amperometric response to $v^{1/2}$.

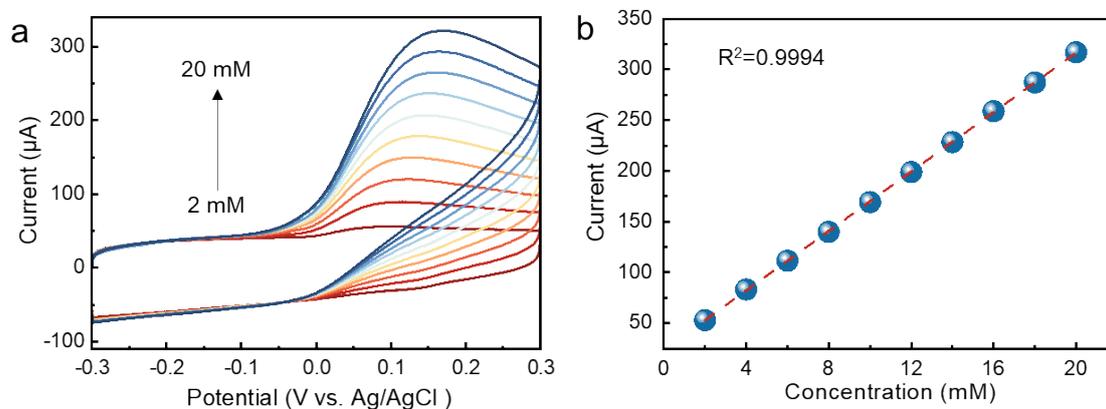


Figure S8 (a) CVs of Rh SAzymes in 0.1 M PBS with vitamin C of different concentrations (2, 4, 6, 8, 10, 12, 14, 16, 18, 20 mM). (b) The corresponding calibration curve of the amperometric response to the concentration.

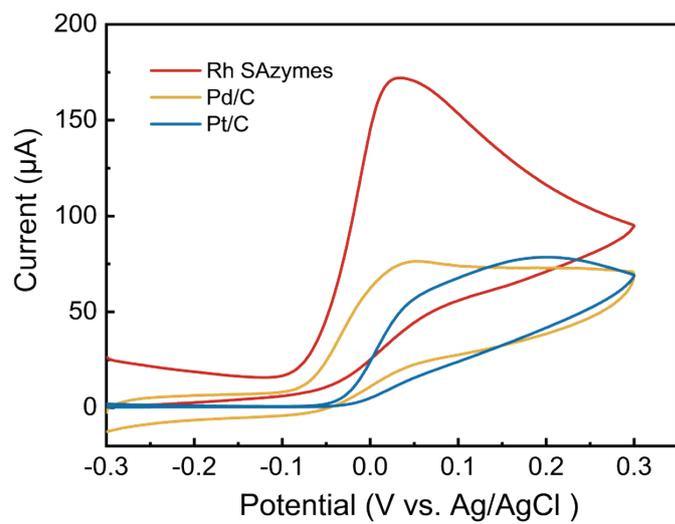


Figure S9 Background-corrected CV curves of Rh SAzymes, Pd/C, and Pt/C at a scan rate of 50 mV/s in 10 mM AA and 0.1 M PBS.

Table S1 Comparison of the analytical performance of the Rh SAzymes electrode with that of reported.

| Catalyst | Linear range (μM) | Limit of detection (μM) |
|--------------------------------|--------------------------------|--------------------------------------|
| Rh SAzymes | 10-53090 | 0.26 |
| S,N-GQDs ³ | 10–500 | 1.2 |
| pTS-/PPy MIP/GEC ⁴ | 5–1000 | 19 |
| Pt@NP-AuSn/Ni/CFP ⁵ | 200-2000 | 5.51 |
| Co-CQDs ⁶ | 600–1600 | 18 |
| CFME/GO–CNT ⁷ | 10-1500 | 0.73 |
| PANI/RGO ⁸ | 0-8000 | 18.3 |
| HNP-PtTi ⁹ | 200–1000 | 24.2 |
| NPCC ¹⁰ | 20–375 | 1.04 |
| Au nanoparticles ¹¹ | Up to 500 | 0.5 |
| RGO ¹² | — | 4.7 |
| CuO nanowire ¹³ | 20–400 | 0.5 |
| N-CQDs ¹⁴ | 10-100 | 1.8 |
| GQDs/IL-SPCE ¹⁵ | 25–400 | 6.64 |

Table S2 Composition of the artificial saliva (SAGF® medium).

| Compound | Concentration (mg L⁻¹) |
|---|--|
| KCl | 964 |
| Na ₂ SO ₄ ·10H ₂ O | 763 |
| KH ₂ PO ₄ | 654 |
| NaHCO ₃ | 631 |
| CaCl ₂ ·2H ₂ O | 228 |
| Urea | 200 |
| KSCN | 190 |
| NH ₄ Cl | 178 |
| NaCl | 126 |

Table S3 Composition of the different oral formulations of nutritional products

| Number | Composition | VC content |
|---------------|--|-------------------|
| 1 | Sweeteners (Sorbitol, Sucralose), Vitamin C, Anti-Caking | 0.05 g |
| 2 | Agents (Stearic Acid, Silicon Dioxide, Magnesium Stearate), | 0.1 g |
| 3 | Orange Flavouring, Corn Starch, Bulking Agent (Hydroxypropyl Methylcellulose), etc. | 0.1 g |
| 4 | | 0.1 g |
| 5 | Vitamin C (as Ascorbic Acid), Bulking Agents (Hydroxypropyl Methylcellulose, Microcrystalline Cellulose), Anti-Caking | 0.1 g |
| 6 | Agents (Stearic Acid, Silicon Dioxide, Magnesium Stearate), etc. | 0.1 g |
| 7 | | 0.1 g |
| 8 | Citric Acid, Vitamin C, Acidity Regulator (Sodium Hydrogen Carbonate), Sweeteners (Sorbitol, Sucralose), Polyethylene Glycol, Orange Flavouring, Colour (Riboflavin), etc. | 1.0 g |
| 9 | | 1.0 g |

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