Supplementary Information

Bioinspired Single-Atom Nanozymes for Microplastic Degradation

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Materials and instruments

1. Materials

3-Amino-1,2,4-triazole (C₂H₄N₄, \geq 96.0%), Copper nitrate (Cu(NO3)₂·3H₂O, 99.9%), Copper nanoparticles (Cu NPs, 50 nm 99%), Copper oxide(CuO, 99.9%), Sodium borohydride (NaBH₄, 98%) · Sodium hydroxide (NaOH, 98%), ethylene glycol (98%), anhydrous ethanol (99.5%), 4-amino-antipyrine (4-AP, 98%), was purchased from Sinopharm Chemical Reagent Co., LTD. (China). 2, 4-Dichlorophenol (2,4-DP, 98%) and Low-density polystyrene (LDPS, 99.9%) were purchased from Shanghai Macklin Biochemical Co., LTD. (China). The ultrapure water used in all experiments met the following specifications: a resistivity of at least 18 M Ω cm⁻¹, an electrical conductivity of no more than 0.1 µs·cm⁻¹ and a total organic carbon (TOC) level of less than 10 ppb.

2. Characterization

The structures of the samples were characterized by high-resolution transmission electron microscopy (HRTEM, JEOL JEM-2010, Japan). The elemental distributions were investigated by the energy-dispersive X-ray spectrometry (EDS) mapping. Scanning electron microscope (SEM) was performed on an S-4800 instrument (Hitachi, Japan). High-resolution transmission electron microscopy (HRTEM) images of the typical samples were obtained on a Tecnai G2 F30 (Netherlands) apparatus at an accelerating voltage of 200 kV. Xray diffraction (XRD) patterns of the samples were recorded on a Bruker D8 Advance diffractometer (AXS-Bruker, German) with Cu a radiation and nickel filter ($\lambda = 0.15406$ nm), the operating voltage and current was 40 kV and 40 mA, respectively. X-ray photoelectron spectroscopy (XPS) analysis was performed on a Thermo ESCALAB 250XI multifunctional imaging electron spectrometer (USA), using monochromatic Al K radiation (1486.6 eV) operating at an accelerating power of 15 kW. The binding energy was calibrated using a C 1s peak at 284.8 eV as standard and quoted with an accuracy of ± 0.1 eV. Electron paramagnetic resonance (EPR) spectra were detected on a Bruker EMX EPR Spectrometer (JES FA200, Japan): A nanozyme dispersion solution of 1 mg mL⁻¹ was prepared with methanol as the dispersant, and then 200 μ L of the dispersion solution was added with 20 µL of 5,5-dimethyl-1pyrroline-noxide (DMPO) and stirred for 5 min to test at room temperature. The content of copper in the Cu SAs nanozyme was determined by inductively coupled plasma emission spectrometer (ICP-OES): Agilent ICP-OES730 (USA), and the final content of the measured elements in each sample was determined by spectrograms, and the test results were obtained.

3. Synthesis of Cu-C₃N₅ and Cu SAs nanozymes

 $g-C_3N_5$ nanosheets were prepared by one-step thermal polymerization.3-Amino-1,2,4-triazole powder (3.0g) were added into an alumina crucible. The crucible was then heated to 550 °C in a muffle oven at a rate of 2.5 °C min⁻¹, and kept at 550 °C for 3 h. After completion, the crucible was cooled to room temperature in the muffle oven, and the residues were ground into powders. The resultant powders were washed with ethanol and ultrapure water for 2-3 times, centrifuged at 12000 rpm for 5 minutes, and the supernatant was discarded. Afterward, the precipitate was dissolved in ultrapure water and frozen with liquid nitrogen for 1 h, and warmed naturally to room temperature. The resultant solution was ultrasonically broken by ultrasonic cell pulverizer (VCX750, Sonics&Materials, Inc., USA) in an ice bath for 24 h. C₃N₅ nanosheets (10 mg) were dispersed in ultrapure water. CuCl₂ solution (25 mM, 200 µL) was added dropwise into the above solution. After stirring for 1 h, pre-cooled NaBH₄ solution (0.1 M, 400 µL) was added dropwise. The mixed solution was then immediately washed several times with ultrapure water. The hybridized nanosheets were collected by centrifugation at 12000 rpm for 5 minutes and dispersed in ultrapure water.

Cu SAs were synthesized by a one-step thermal polymerization process. 3-Amino-1,2,4-triazole (3.0 g) and copper nitrate (40 mg) were added to an alumina crucible. The crucible was then heated to 550 °C in a muffle furnace at 2.5 °C min⁻¹ and held at this temperature for 4 hours. The resulting material is ground to a powder. Any undoped copper material is removed by acid washing. The powder is then washed 2-3 times with ethanol and centrifuged at 12,000 rpm for 5 minutes and the supernatant discarded. The precipitate is then dissolved in ultrapure water, frozen in liquid nitrogen for 1 hour, and allowed to warm naturally to room temperature. An ultrasonic cell pulverizer (VCX750, Sonics & Materials, Inc.) was used in an ice bath.

4. Analysis of catalytic activity

The chromogenic reaction of phenolic compounds with 4-AP was used to assess the catalytic performance. Specifically, 20 μ L of Cu-C₃N₅ or Cu SAs dispersion (1 mg

mL⁻¹) was mixed with the reaction mixture, which was composed of 140 μ L of morphine ethanesulfonate monohydrate buffer (pH 6.8), 20 μ L of 4-AP (1 mg mL⁻¹), and 20 μ L of 2,4-DP (1 mg mL⁻¹). After a period of reaction, the supernatant was carefully aspirated after centrifugation at 9000 rpm for 5 min using a high-speed centrifuge and its absorbance was monitored at 510 nm using a UV-visible spectrometer (Cary 5000, Agilent Technologies, Inc., USA).

5. Michaelis-Menten kinetics

The kinetics of nanozymes were investigated through a chromogenic reaction with 4-AP, in which 2,4-DP was used as substrates. The absorbance at 510 nm was recorded in real time. MES buffer, 30 μ L Cu SAs (1 μ g mL⁻¹) or Cu-C₃N₅ nanozymes (1 μ g mL⁻¹), 50 μ L 4-AP (10 mM in H₂O), and 2.4-DP solution (10, 20, 40, 60, 80, 100 μ g mL⁻¹) were added to the reaction vessel in turn with a total volume of 1 mL, followed by rapidly shaking for 3 seconds before being put into test equipment. The K_m was assumed to be a fixed value and the selected concentrations of nanozymes were only to achieve satisfactory test signal values. Steady state containing varying concentrations of 2,4-DP was monitored at 510 nm ($\epsilon_{510 nm} = 136,000 \text{ M}^{-1} \text{ cm}^{-1}$) in time scan mode at 20 seconds intervals over 10 min. The kinetic parameters were obtained by the Michaelis-Menten equation:

$$v = \frac{V_{MAX}[S]}{K_m + [S]} \tag{S1}$$

v: the initial velocity; V_{max} : the maximal reaction velocity; [S]: the substrate concentration; K_M : The Michaelis constant.

6. Specific activity

Various concentrations of Cu SAs, Cu-C₃N₅, and laccase solutions (0.1, 0.2, 0.4, 0.6, 0.8, 1, and 2 mg mL⁻¹) were prepared and reacted respectively with the mixed solution, which contained 140 μ L of MES buffer (pH 6.0), 20 μ L of 4-AP (1 mg mL⁻¹), and 20 μ L of 2,4-DP (1 mg mL⁻¹). The absorbance time curves of the above assays were recorded at 510 nm in time scan mode using a UV-Vis spectrometer (Cary 5000, Agilent Technologies Inc, USA) The specific activity (U mg⁻¹) was obtained via the following formula:¹

$$SA = \frac{V/(\varepsilon \times l) \times (\Delta A/\Delta t)}{m}$$
(S2)

V is the total volume of the reaction solution (μ L); ϵ is the molar absorption coefficient of the colorimetric substrate, which is typically maximized at 136,000 M⁻¹cm⁻¹ at 510 nm for 2,4-DP; l is the path length of light traveling in the cuvette (cm); A is the absorbance after subtraction of the blank value, and $\Delta A/\Delta t$ is the initial rate of change in absorbance at 510 nm min⁻¹; m is the SAzyme weight (mg) of each assay.

7. Density functional theory (DFT) calculations

All the first-principles calculations were performed by the Vienna ab initio simulation package (VASP) using the framework of DFT. The projector augmented wave (PAW) method was used to treat the effective interaction of the core electrons and nucleus with the valence electrons and the generalized gradient approximation (GGA) in the form of Perdew-Burke-Ernzerhof (PBE) was adopted for the exchange-correlation functional. The Kohn-Sham electron wave functions were expanded using the plane-wave functions with an energy cutoff of 500 eV. The optimization was considered convergence when the spring force between adjacent images was less than 0.02 eV Å⁻¹, the total energy change upon two steps for the electronic self-consistent field iteration was less than 1E⁻⁵ eV, which has been confirmed to be precise enough under the convergence condition tests.

Charge transfers were calculated and analyzed using the Bader charge method. As for the adsorption case of small molecules (H₂O and O₂), the adsorption energy (E_{ad}) was defined as

$$E_{ads} = E_{absorbate/support} - (E_{absorbate} + E_{support})$$
(S3)

and for the co-adsorption case, similar to the single molecular adsorption, the binding energy is defined as

$$E_{ads} = E_{absorbate/support} - (E_{absorbate1} + E_{absorbate2} + E_{support})$$
(S4)

where $E_{absorbate}$, $E_{support}$, and $E_{absorbate/support}$ are the total energies of the free adsorbate, the corresponding support, and the support with the adsorbate in the same slab, respectively. All three types of energies were derived from the scf calculations using the same calculated setting parameters. With this definition, a negative value indicates an exothermic adsorption and the more negative this value is, the large interaction between molecules and the substrate has been proved. The spin value of the triplet state of oxygen was set to 1. The infrared intensity of the radical intermediates was calculated using linear response within the Density Functional Perturbation Theory (DFPT). The obtained Born files were then analyzed using the Phonopy-Spectroscopy software to derive the infrared intensity of the oxygen-containing intermediates.

8. Degradation of microplastics

Cu-C₃N₅ (200 μ g mL⁻¹) or Cu SAs (200 μ g mL⁻¹) was mixed with LDPS (10 mg) in a volume of 10 mL. The samples were heated at various temperatures in a Teflon-lined autoclave for various durations. After the reaction, the samples were cooled down to the room temperature. The remaining microplastics were washed with ultrapure water and weighed after vacuum drying. The degradation was quantified based on the mass loss of microplastics after the reaction. Control experiments were performed in the same condition but in the absence of the Cu-C₃N₅ or Cu SAs. The relative molecular mass of the polymer was determined by a gel chromatograph (PL-GPC50, Agilent, USA). The total organic carbon (TOC) was measured by a TOC analyzer (Multi N/C 2100, Jena, Germany) with the detection limit 500 μ g L⁻¹. The experiment was repeated three times to take the average value.



Fig. S1. SEM image of (a) $Cu-C_3N_5$ and (b) Cu SAs.



Fig. S2. Standard PDF cards for Cu and CuO.



Fig. S3. (a) C 1s and (b) N 1s spectra of Cu SAs.



Fig. S4. Effect of (a) pH, (b) temperature, (c) time, (d) solution ion concentration, (e) number of cycles and (f) ethanol content on laccase-like activity.



Fig. S5. (a) Effect of different concentrations of Cu NPs on laccase-like activity. (b) Effect of different concentrations of $g-C_3N_5$ on laccase-like activity.



Fig. S6. (a) Michaelis-Menten kinetic curve of laccase. (b) Lineweaver-Burk plot for laccase.



Fig. S7. Gel permeation chromatography (GPC) of polystyrene degradation.

Material	Туре	Time	Concentration	Efficacy	Reference
F- J- microspheres	PET	48 h	1 mg mL^{-1}	61.8%	[2]
CrL_UiO-66	BHET	48 h	1 mg mL^{-1}	44.1%	[3]
CrL_UiO-66- NH ₂	BHET	48 h	1 mg mL^{-1}	37.4%	[3]
DMD-NiO	LDPE	240 h	1.5 mg mL^{-1}	38.16%	[4]
Pt@N-TiO ₂	PET- FMP	12 h	$200 \ \mu g \ m L^{-1}$	29%	[5]
Cu SAs	PS	20 h	$100 \ \mu g \ mL^{-1}$	90%	This work

Tab. S1. Comparison with reported microplastic degradation properties of nanomaterials.

Tab. S2 Comparison of kinetics of Cu SAs with other nanozymes.

Nanozyme	K _M (mM)	V _{max} (10 ⁻⁴ mM s ⁻¹)	Reference
CA-Cu	0.23	1.87	[6]
I-Cu	0.17	4.1	[7]
Cu-MIM	0.04	4.92	[8]
Tris-Cu	0.18	2.60	[9]
Cu-Mn	0.20	3.56	[10]
CH-Cu	0.42	1.22	[11]
Cu-C ₃ N ₅	2.755	2.57	This article
Cu SAs	0.129	5.64	This article

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