Electronic Supporting Information

Construction of copper nanoclusters/MnO_2 nanosheets-based fluorescence method for butyrylcholinesterase activity detection and anti-Alzheimer's drugs screening

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CONTENTS

Experimental Section

1. Chemicals and Reagents

Cupric sulfate ($CuSO_4$), polyethyleneimine (MW = 10000), sodium hydroxide (NaOH), ethanol, methanol, sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), butyrylcholinestearse (BChE), potassium permanganate ($KMnO_4$), sodium dodecyl sulfate (SDS), galantamine, and butyrylthiocholine (BTCh) were bought from Aladdin Reagent Company (Shanghai, China). Glycine (Gly), arginine (Arg), cholesterol (Chol), α-glucosidase (α-Glu), tacrine, and acid phosphatase (ACP) were obtained from Sigma-Aldrich Company (USA). Trypsin (Try), bovine serum albumin (BSA), human serum albumin (HSA), cysteamine and ovalbumin (Ova) were bought from Sangon Biotech Co., Ltd. (China). All chemicals were of analytical grade and used without further purification and modification. Ultrapure water (18.2 MΩ·cm, Taihe, China) was employed for all the experiments.

2. Apparatus and Measurements

Transmission electron microscopic (TEM) measurements were performed by dropping the solution on a TEM grid and dried for 12 h at room temperature, then the samples were observed on a JEM-2100F field emission electron microscope (JEOL, Japan) under an accelerating voltage of 100 kV. Dynamic light scattering (DLS) and zeta potential measurements were conducted on a Zetasizer Nano ZS instrument (Malvern, England). Fourier transform infrared (FTIR) spectra were recorded as KBr pellets at room temperature using a Bruker IR spectrometer (Nicolet, WI, USA). X-ray photoelectron spectroscopy (XPS) results were acquired with an X-ray photoelectron spectrometer (Thermo Electron, USA). UV-vis spectra were collected from a UV-2450 spectrophotometer (Shimadzu, Japan). All fluorescence spectra were collected by an RF5301 fluorescence spectrophotometer (Shimadzu, Japan) equipped with a quartz cuvette of 1.0 cm path length with a xenon lamp as the excitation source.

3. Synthesis of Cu-Cy

Cu-Cy was synthesized according to the method in previous report.[1] Typically, 0.636 g cysteamine was added into an aqueous solution of $CuCl_2$. The solution pH was adjusted to pH 8, followed by being stirred for 2.5 h at room temperature and then heated to the boiling point of water for 0.5 h. The crude product was collected by centrifugation (7000 rpm, 10 min) and washed 3 times with a mixture of ethanol and water (v/v = 5 : 4), then dried in a vacuum oven at 30 °C for 12 h.

4. Fluorescence Quenching of PEI-CuNCs Induced by MnO_2

50 μL PEI-CuNCs solution was added into 1 mL PBS buffer solution (0.1 M, pH 7.4), then different amounts of MnO_2 nanosheets solutions were added. The solution was diluted to 2.5 mL with ultrapure water, followed by thorough mixing, and then the emission spectra were taken.

5. Selectivity and Sensitivity Assays

To evaluate the selectivity of the sensing assay for BChE activity, 0.1 mL BTCh (20mM), Na^+, K^+, Ca^{2+}, Mg^{2+}, Arg, Gly, Chol, (50 μL, 1 mM), BSA, HSA, Ova (50 μL, 10 mg/mL), Try, α-Glu, and ACP (50 μL, 1U/mL), and 0.1 mL PBS buffer solution (pH=7.4, 0.1 M) were added into a centrifuge tube in the absence and presence of BChE (13 U/L). After incubated at 37 ºC for 25 min, the solution was added into 225 μL MnO_2 nanosheets solution (2 mg/mL) and vigorously shaken for 2 min. Next, the above solution was added into 50 μL PEI-CuNCs solution in the presence of 500 μL PBS buffer solution (0.1 M, pH 7.4). The solution was diluted to 2.5 mL with ultrapure water, followed by a thorough mixing, and then the emission spectra were taken.

The detection limit (DL) was calculated based on IUPAC recommendations ($DL = 3S/k$, in which S is the standard deviation of 10 blank samples, and k is the slope of the calibration curve).

6. Screening of BChE Inhibitors

0.1 mL inhibitors with various concentrations were added into the mixture of 0.1 mL BChE (5 U/mL) and 0.1 mL PBS buffer solution (0.1 M, pH 7.4), and the solutions were incubated for 15 min. Then, 0.1 mL BTCh (20 mM) was added into the mixture and incubated at 37 ºC for 25 min. Afterwards, the mixture was added into 225 μL MnO_2 nanosheets solution (2 mg/mL) and vigorously shaken for 2 min. Next, the above solution was added into 50 μL PEI-CuNCs solution in the presence of 500 μL PBS buffer solution (0.1 M, pH 7.4). The solution was diluted to 2.5 mL with ultrapure water, followed by a thorough mixing, and then the emission spectra were taken. The inhibition efficiency (*IE*) was calculated by the following equation.

$$IE = [I_{\text{no inhibitor}} - I_{\text{inhibitor}}] / [I_{\text{no inhibitor}} - I_{\text{no inhibitor and BChE}}]$$

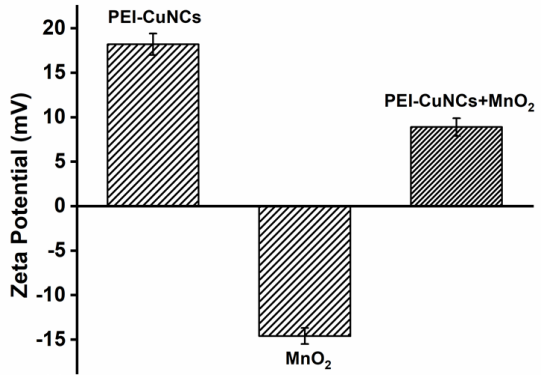

Fig. S1. Zeta potentials of PEI-CuNCs, MnO_2 nanosheets, and PEI-CuNCs+MnO_2 nanosheets.

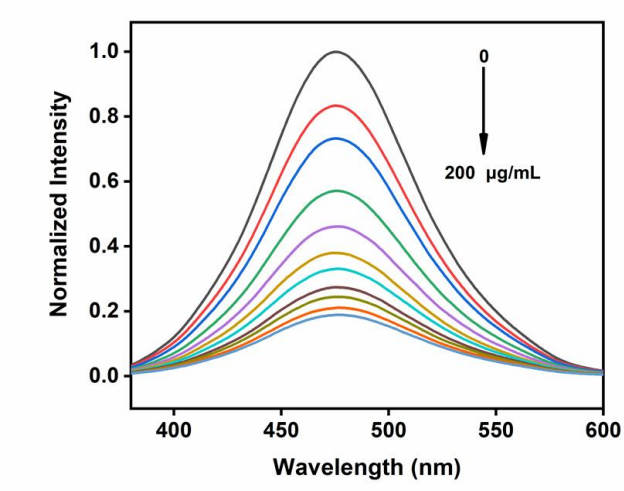

Fig. S2. Fluorescence spectra of PEI-CuNCs in the presence of MnO_2 with different concentrations.

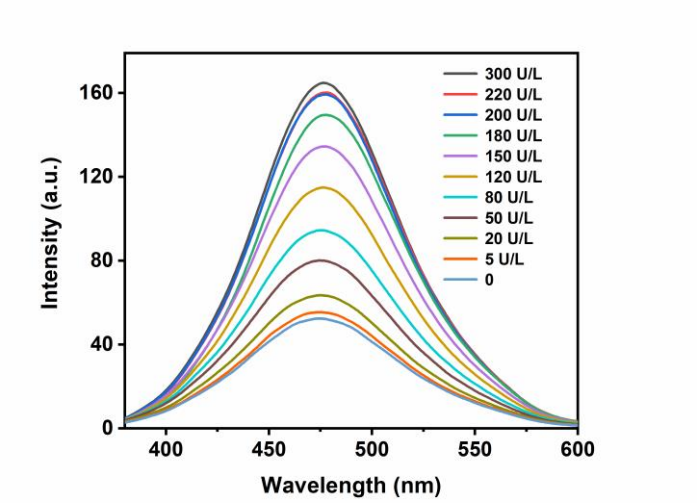

Fig. S3. Fluorescence spectra of PEI-CuNCs upon the introduction of BChE with various activities.

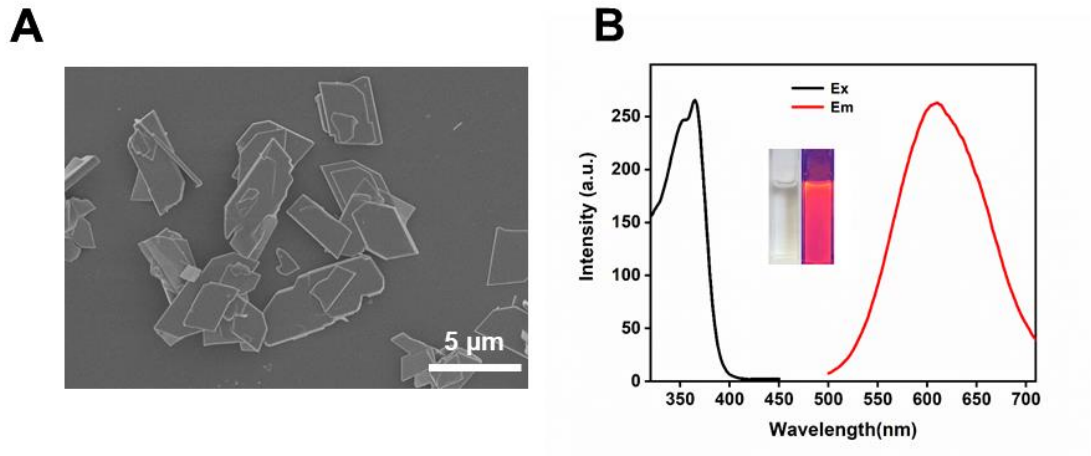

Fig. S4. (A) SEM image of Cu-Cy nanosheets. (B) Fluorescence excitation (black, λ_{em} = 609 nm) and emission (red, λ_{ex} = 365 nm) spectra of Cu-Cy. Inset: Cu-Cy dispersion under visible (left) and 365 nm UV (right) light.

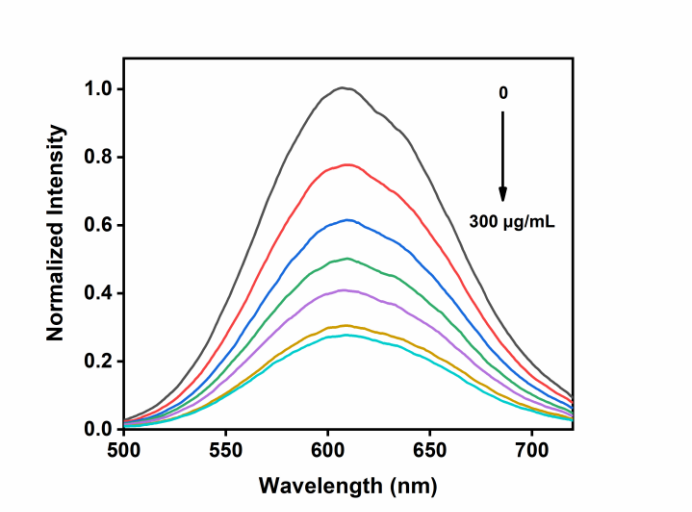

Fig. S5. Fluorescence spectra of Cu-Cy in the presence of MnO_2 with different concentrations.

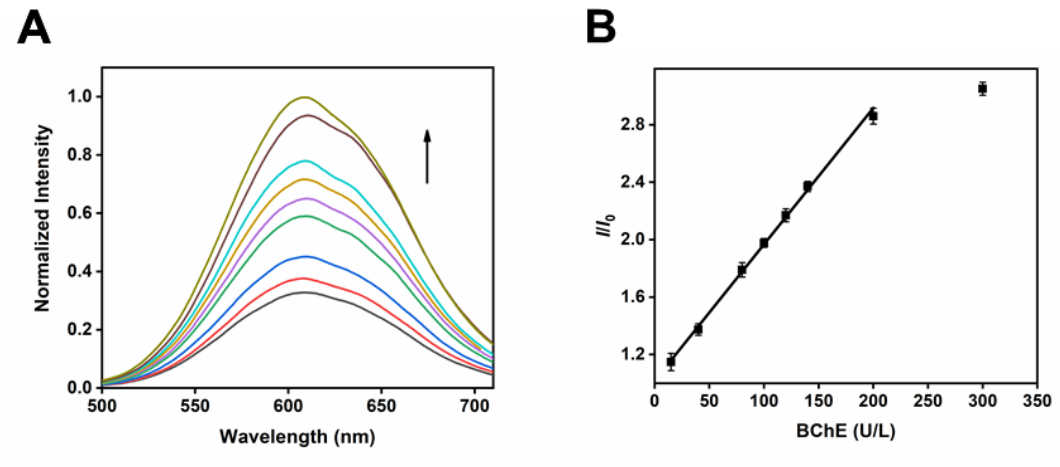

Fig. S6. (A) Fluorescence spectra of Cu-Cy/MnO_2 upon the introduction of BChE with various activities (λ_{ex} = 365 nm). (B) I/I_0 versus BChE activities. I and I_0 are emission intensities of PEI-CuNCs/MnO_2 with and without the addition of BChE, respectively.

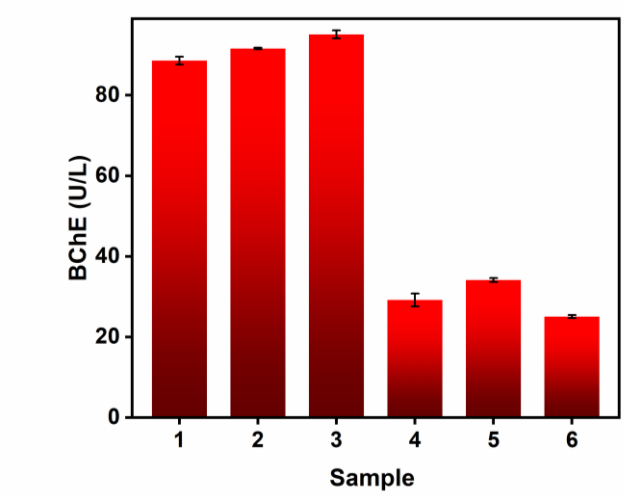

Fig. S7. Determination of BChE activity in 100-fold dilution of human serum samples. Samples 1-3 are from healthy persons and 4-6 are from cirrhotic patients.

Table S1 Comparison of different assays for detecting BChE activity.

Probe	Method	Linear range (U/L)	DL (U/L)	Ref.
AuNPs	Colorimetry	50−250	6.8	2
Polymeric membrane electrode	Potentiometry	7.5–150	6	3
CQDs	Fluorescence	60−220	2.7	4
CdTe QDs	Fluorescence	10–2000	10	5
S-dots	Fluorescence	0.05–10, 10–500	0.035	6
CyClCP	Fluorescence	0–150	3.75	7
DCPDA	Fluorescence	0.2–9.0	0.06	8
Fluorophore	Fluorescence	0–2000	1.2	9
Thiamine	Fluorescence	0.125–15	0.036	10
PEI-CuNCs/MnO_2	Fluorescence	5–200	2.26	This work
Cu-Cy	Fluorescence	15-200	4.45	This work

Table S2 Results of the standard addition method for the detection of BChE activity in healthy human serum samples.

Sample	Measured (U/L)	Added (U/L)	Detected (U/L)	Recovery (%)	RSD (n = 3, %)
1	92.2	20.0	113.2	105.0	2.2
2	94.7	30.0	124.6	99.7	5.0
3	96.5	50.0	149.8	106.6	4.7

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