Supporting Information

Detection of Monoamine Oxidase B Using Dark-field Light Scattering Imaging and Colorimetry

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Experimental Section

Materials and Reagents. All reagents in this work were analytically pure and needed no further treatment. Gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), ammonium hydroxide ($NH_3 \cdot H_2O$) and potassium hydroxide (KOH) were obtained from Sinopharm Chemical Reagent (Shanghai, China). Silver nitrate ($AgNO_3$) and ascorbic acid (AA) were all purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Sodium dodecyl sulfate (SDS) and benzylamine were obtained from Macklin Reagent (Shanghai, China). Human monoamine oxidase B (MAO-B) was obtained from Sigma-aldrich Trading Co., Ltd (Shanghai, China). Ultrapure water with a resistivity of 18.2 MΩ·cm was produced using a Milli-Q apparatus (Thermo Scientific, USA) and used in the preparation of all the solutions.

Apparatus. Absorption spectra were collected by UV–vis spectrophotometer (Cary 100, Agilent, Singapore). Transmission electron microscopy (TEM) images were carried out with a JEM-2100 TEM (JEOL, Japan). The dark-field spectrum measurements were carried out with an inverted optical microscope (Eclipse Ti2-E, Nikon, Japan) equipped with a dark field condenser ($0.8 < NA < 0.95$) and a 60 × objective lens (NA = 0.7). White light source (100 W halogen lamp) through condenser was focused onto the sample and excited the nanoprobes to generate plasmon resonance scattering light. The dark-field color images were captured by using a true-color digital camera (Nikon DS-Fi3, Japan) and the scattering spectra was recorded by a spectrometer (FERGIE, Princeton Instruments, USA). In order to avoid interference and ensure accuracy, all scattering spectra of the nanoprobes were

corrected by subtracting the background spectrum generated by the instrument itself.

Preparation of AuNSs. AuNSs were prepared by a seed-mediated growth method.[1] Briefly, 15 mL of 1% citrate solution was added to 100 mL of boiling 1 mM $HAuCl_4$ solution under vigorous stirring. After boiling for 15 min, the solution was cooled to room temperature. The seed solution was obtained after filtration by a 0.22 μm nitrocellulose membrane and then stored in the dark at 4 °C. Then 500 μL of the above synthesized seed solution (13 nm) was added to 50 mL of 0.25 mM $HAuCl_4$ solution containing 50 μL of 1 M HCl in a 100 mL glass vial under stirring. After mixing, 1 mL of 0.6 mM $AgNO_3$ and 250 μL of 100 mM ascorbic acid (AA) were added simultaneously. The color of the solution turned from light red to blue rapidly after stirring for 30 s. Subsequently, 500 μL of 10 mM sodium dodecyl sulfate (SDS) solution was added, and the mixture was shaken for 5 min at 25 °C. The Au nanostars solution was given one centrifugal wash at 5000 g for 15 min to halt the nucleation, which was carried out in a 30 mL centrifuge tube. The resulting precipitate was redispersed in 50 mL of deionized (DI) water, and then kept at 4 °C for further use.

For obtaining uniform nanoparticles, all glassware used in the above experiments was thoroughly cleaned with aqua regia (volume ratio 3:1, HCl/HNO_3). Then all glassware was washed with DI water, and dried before use.

Preparation of Tollens' reagent. Tollens' reagent was prepared according to the literature.[2] Five hundred microliters of 100 mM $AgNO_3$ solution was mixed with 70 μL of 0.8 M diluted KOH until formation of the silver oxide, which precipitated from

the solution as a brown solid. Then, 0.6 M ammonia was added dropwise into the solution until it became clear. Finally, DI water was added to make the final volume of the solution to 1 mL. The resulting Tollens' reagent contained 50 mM $[Ag(NH_3)_2]^+$ complexes, and needed to be freshly prepared and stored in the dark.

Colorimetric detection of MAO-B. All enzymatic reactions were carried out in disposable plastic PE tubes. Three microliters of 0.1 M benzylamine was mixed with different concentrations of MAO-B and incubated at 37 °C for 2 h. After that, 150 μL AuNSs solution and 20 μL of Tollens' reagent were introduced to the reaction mixture. Then DI water was added to make the total volume reach 300 μL. After incubated at 37 °C for 1 h, the mixture was measured with UV−vis spectrometer. The change of the solution color could be recognized with the naked eyes. Bovine serum albumin (BSA), glucose oxidase (GOX), horseradish peroxidase (HRP) and alkaline phosphatase (ALP) were chosen as control substances to verify the selectivity of the method.

Amino Functionalization of Glass Slides. The glass slide (25 mm × 75 mm × 1.2 mm) was functionalized with amino groups according to the literature.[3] Briefly, the glass slides were immersed in alkali water prepared by ethanol and sodium hydroxide for 2 h. Subsequently, the glass slides were rinsed with deionized (DI) water and ethanol, respectively. The cleaned glass slides were immersed in an ethanol solution containing 1 % (3-aminopropyl)-triethoxysilane (APTES) for 2 h and then washed with ethanol and dried under a stream of nitrogen. Finally, amino functionalized glass slides were successfully obtained.

Dark-field analysis of MAO-B. A home-built poly (dimethylsiloxane) (PDMS) has pores with a volume of about 100 μL, which was then combined with amino functionalized glass slides to prepare the sample cell for detecting MAO-B with dark-field scattering imaging. Three microliters of 0.1 M benzylamine and different concentrations of MAO-B were first mixed together and incubated at 37 °C for 2 h. After that, 150 μL of AuNSs solution and 20 μL of Tollens' reagent were added to the mixture and cultured at 37 °C for 1 h. The resulting solution was then added to the sample cell and incubated by electrostatic adsorption for 15 min. Excess particles were sucked away and washed with DI water. Subsequently, dark-field images were captured under the DFM, and the corresponding scattering spectra were collected. MAO-B activity could be determined according to the LSPR peak shift induced by silver deposited AuNS. In addition, the selectivity tests were carried out according to the above procedures. BSA, GOX, HRP and ALP were selected as control substances.

Determination of MAO-B activity in human serum samples

To monitor MAO-B level in human serum, a standard addition method was applied to analyze the human serum samples donated from Southeast University Hospital. Different concentrations of MAO-B were added to serum to obtain the spiked samples, which were analyzed by the established colorimetry and dark-field scattering method to calculate the recovery.

Experimental Data

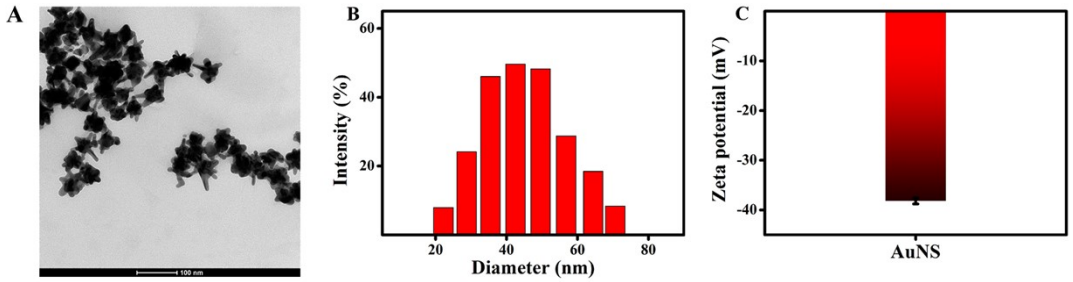

Figure S1 (A) TEM image of AuNSs. (B) Particle size distribution of AuNSs. (C) Zeta potential of dispersed AuNSs. The error bars represent standard deviations of three repetitive measurements.

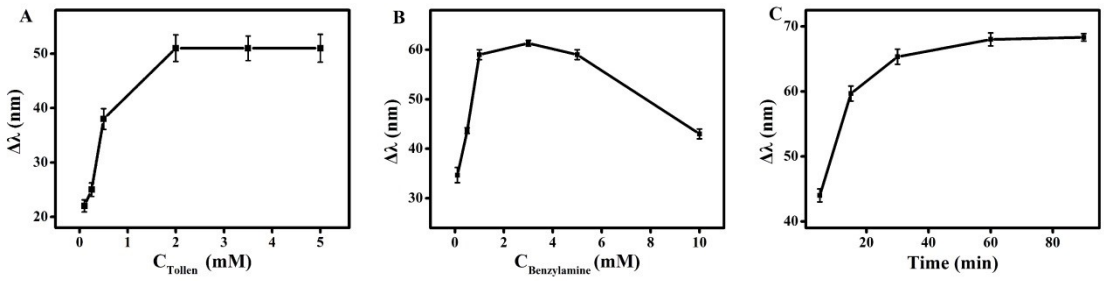

Figure S2 Optimization of the Tollens' reagent concentration (A), the substrate benzylamine concentration (B), and the deposition time of silver (C). The error bars represent standard deviations of three repetitive measurements.

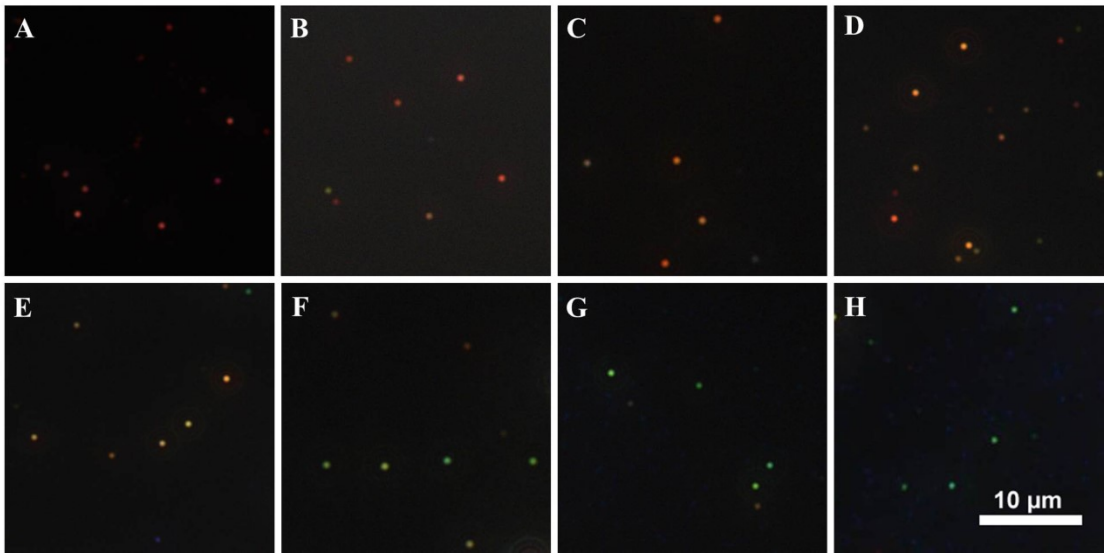

Figure S3 Dark-field images of AuNSs incubated with benzylamine, Tollens' reagent and different concentrations of MAO-B. (A-H): 0, 0.5, 1.0, 3.0, 5.0, 7.0, 10.0, and 20.0 ng/mL.

Table S1 Comparison of the analysis performance of various previously reported methods.

Method	System	Detection range	LOD	Reference
HPLC-DAD	H_2O_2-HRP/TMB	Qualitative	-	4
Surface-enhanced Raman scattering	Amine-aldehyde click reaction	Qualitative	-	5
Spectrophotometric	2,4-dinitrophenylhydrazine	0.0234−0.469 μg/mL	-	6
Electrochemical	H_2O_2-amperometric	10^{-5}−1.4×10^{-4} U	-	7
Fluorescence	MitoHCy-NH_2	1−10 μg/mL	-	8
Fluorescence	BiPhAA	0.25−2 μg/mL	0.02 μg/mL	9
Fluorescence	DEAN-MA/DEAB-MA	1−10 μg/mL	0.6 ng/mL	10
UV absorption spectrum	AuNS	0.01−1.0 μg/mL	8.0 ng/mL	This work
Dark-field scattering spectrum	AuNS	0.5−20.0 ng/mL	0.4 ng/mL	This work

Table S2 Detection of MAO-B in human serum samples by the proposed methods.

Colorimetry	**Added (μg/mL)**	**Found (μg/mL)**	**Recovery (%)**	**RSD (%, n = 3)**
1	0.050	0.043	96.11	1.5
2	0.500	0.459	91.74	4.2
3	1.000	0.945	94.74	2.5
Dark-field scattering	**Added (ng/mL)**	**Found (ng/mL)**	**Recovery (%)**	**RSD (%, n = 3)**
1	0.500	0.474	94.74	2.7
2	5.000	4.730	94.60	3.1
3	10.000	9.337	93.37	3.4

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