Supplementary Material

Preparation of AuPt@ZIF-67 nanomaterials and their application in flow injection chemiluminescence immunoassay

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Apparatus

The flow injection chemiluminescence measurement was carried out using an IFFM-E luminescence analyzer (China Xi'an Remax Analytical Instrument Co., Ltd.) equipped with an IFFS-A multifunctional chemiluminescence detector. Teflon tubing (0.8 mm inner diameter) is used to connect all components in the flow system.

The Scanning Electronic Microscopy (SEM) was supplied by Hitachi SU8010 SEM (Hitachi Co., Japan). Transmission Electron Microscope (TEM) images were obtained from Tecnai G2 F20S-TWIN 200KV (FEG, FEI Co., Ltd., USA). The infrared spectrum was obtained by Fourier transform infrared (FTIR) spectrometer Tensor 27 (BRUKER OPTICS, Germany). The ultrasonic treatment is carried out on the DS-3510DTH ultrasonic cleaner (40 kHz, 180 W, Shanghai Bioanalysis Ultrasonic Instrument Co., Ltd.). The XPS spectrum was obtained by X ray photoelectron spectroscopy (EXCALAB 250 XI,Thermo Scientifc Co., Ltd., USA). The Mapping images was obtained by Field emission transmission electron microscope (Talos F200X G2, Thermo Scientifc Co., Ltd., USA).

Materials and Reagents

Polyclonal antibodies to AFB1 and their coating antigens were provided by our laboratory and assessed by ELISA. Carboxyl resin beads (diameter: about 150 μm; sphericity: >99%; crosslinking degree: 7%; content of water: 30%~40%) were purchased from Nanjing Microsphere High Efficiency Isolation Carrier Co., Ltd. (Nanjing, China).

Casein and luminol (98%) were purchased from Sigma Co., Ltd (St Louis, USA). 2-Morpholineethanesulfonic acid (MES) was purchased from TCI Shanghai Chemical Industry Development Co., Ltd. Sodium hydroxide (NaOH), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, 99%), 2-methylimidazole (2-MIM, 98%) and Chloroauric acid (HAuCl4·4H2O, 47.8%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Chloroplatinic acid hexahydrate (H2PtCl6·6H2O, 98%) was provided by Jiangsu Aikon. Monopotassium phosphate (KH_2PO_4, 99%), tris(hydroxymethyl) aminomethane (Tris, 99%), hydrogen peroxide (H_2O_2, 30%), Tween-20, N-hydroxysuccinimide (NHS, 97%) and ethyl-3-(dimethylaminopropyl) carbodiimide (EDC, 98%) were provided by J & K Scientific (Beijing, China). Bovine serum albumin (BSA, 98%) and Hemin were provided by Shanghai Aladdin Biochemical Technology Co.. All other reagents and materials were commercially available and of analytical reagent grade.

Solutions and Buffers

Tris-HCl buffer solution was prepared by dissolving 12.113 g Tris in 1 L deionized water and using 4 M HCl to adjust its pH to 8.5. Assay buffer of 0.01 M phosphate-buffered saline (PBS, pH 7.4) was prepared by NaCl, $Na_2HPO_4 \cdot 12H_2O$, KCl, and KH_2PO_4. In order to facilitate preservation, we first prepared high-concentration PBS, which was diluted 10 times before use. The washing buffer (PBST) was 0.05% (v/v) Tween-20 in PBS, and the blocking solution was 2% casein in PBS, and stored in a refrigerator at 4 ℃ for later use.

The stock solution of luminol (0.01 M) was used by dissolving 0.0885g luminol in 50 mL of 0.1 M NaOH and then placed in the dark for more than 24 hours. Before use, the luminol and H_2O_2 solutions were diluted to optimal concentrations with 0.1 M Tris-HCl buffer solution (pH 8.5). The CL substrate solution consisted of 0.5 mM luminol and 10 mM H_2O_2. Obtain the coating antigen stock by diluting the 2 mg mL^{-1} stock to 1 mg mL^{-1} with 0.01 M PBS and store in a refrigerator at 4 ℃ for future use. All reagents and chemicals are of analytical grade. All aqueous solutions were prepared with sub-boiling distilled deionized water.

Calculation of detection limit

We calculate the limit of detection (LOD) using the formula LOD=3N/S. In this equation, N represents noise, and S represents slope. For the calculation of noise (N), we analyzed the signal values of 7 sets of blank samples and computed the standard deviation, considering this standard deviation as the magnitude of the noise. Regarding the slope (S), our approach differs from typical chemical sensors. For typical chemical sensors, S is usually equivalent to the slope of the standard curve because concentration and signal response exhibit a positive correlation. However, in our study, the standard curve takes a logarithmic form with a negative correlation, and its slope does not represent the sensitivity of the immune sensor. Therefore, we calculate the signal response in the low-concentration region (after subtracting the blank signal). Specifically, we measure the signal values of seven samples with a concentration of 0.002 ng mL^{-1}, calculate the ratio of the signal change to concentration, and use this ratio as the slope (S). Finally, we apply the formula LOD=3N/S to calculate the limit of detection. The specific formula is illustrated below. LOD = 3N/S; S = $(I_{black} - I_{0.002})/C_{0.002}$.

Figure S1

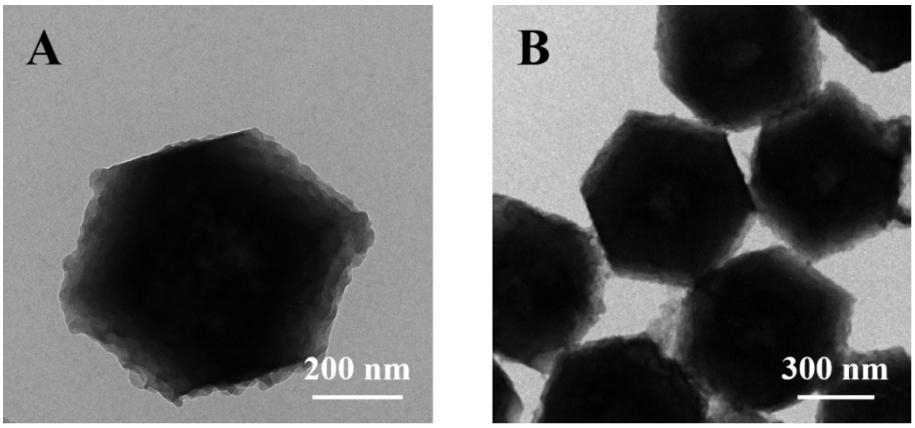


Fig. S1. (A, B) TEM of AuPt@ZIF-67.

Figure S2

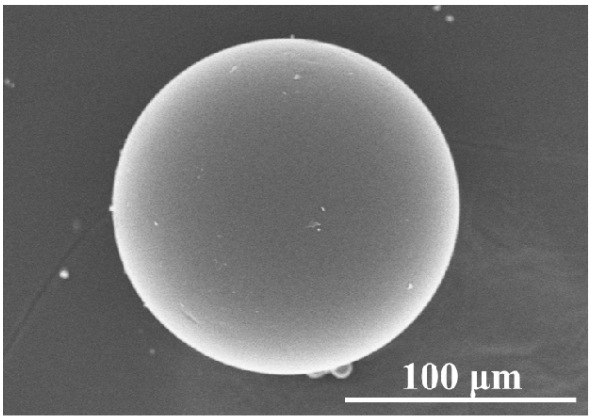


Fig. S2. SEM image of the carboxyl resin beads.

Figure S3

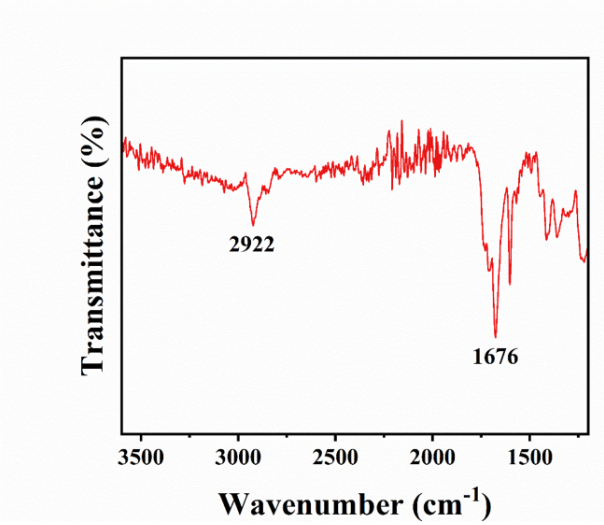


Fig. S3. IR image of the carboxyl resin beads.

Figure S4

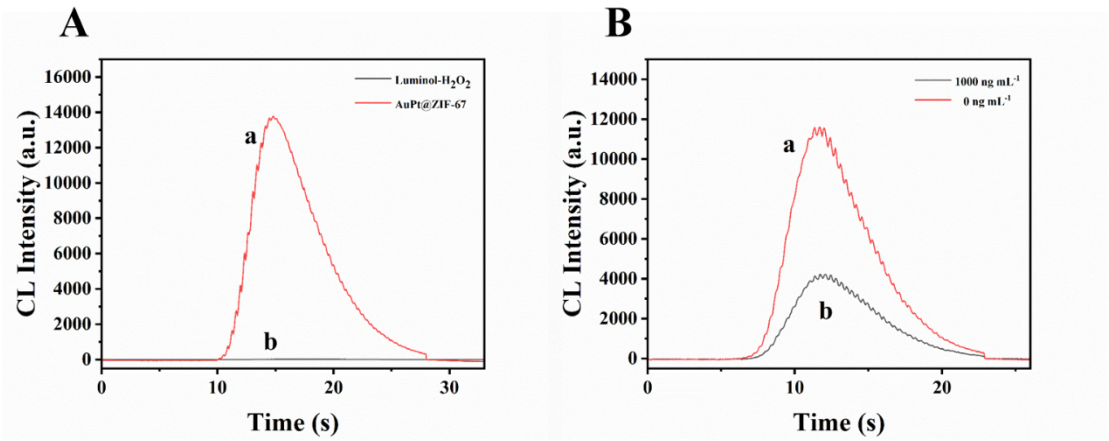


Fig. S4. (A) Relationship between chemiluminescence signal intensity and time: (a) AuPt@ZIF-67-Luminol-H_2O_2 and (b) Luminol-H_2O_2; (B) Relationship between chemiluminescence signal intensity and time: (a) Without AFB1 standard solution and (b) Containing 1000 ng mL^{-1} AFB1 solution.

Figure S5

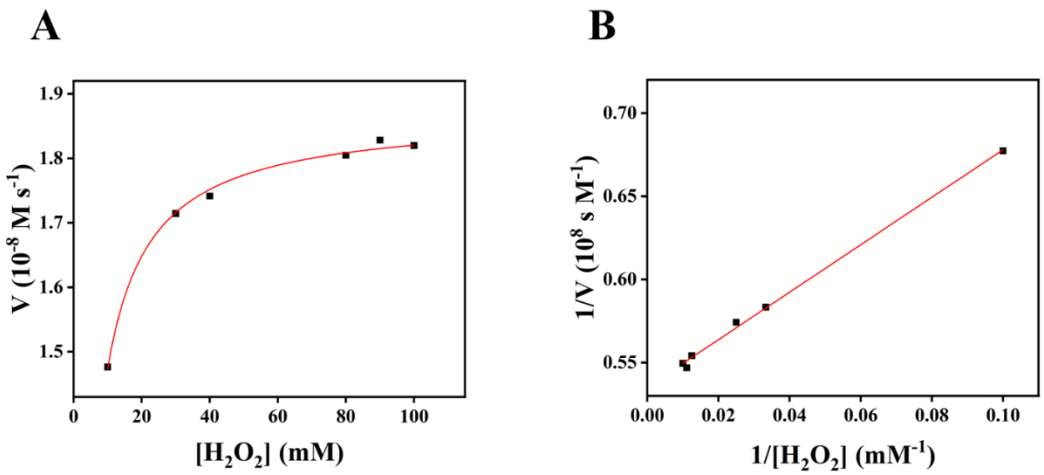


Fig. S5. Steady-state kinetic analysis: (A)Michaelis-Menten model; (B) Lineweaver-Burk plot.

Figure S6

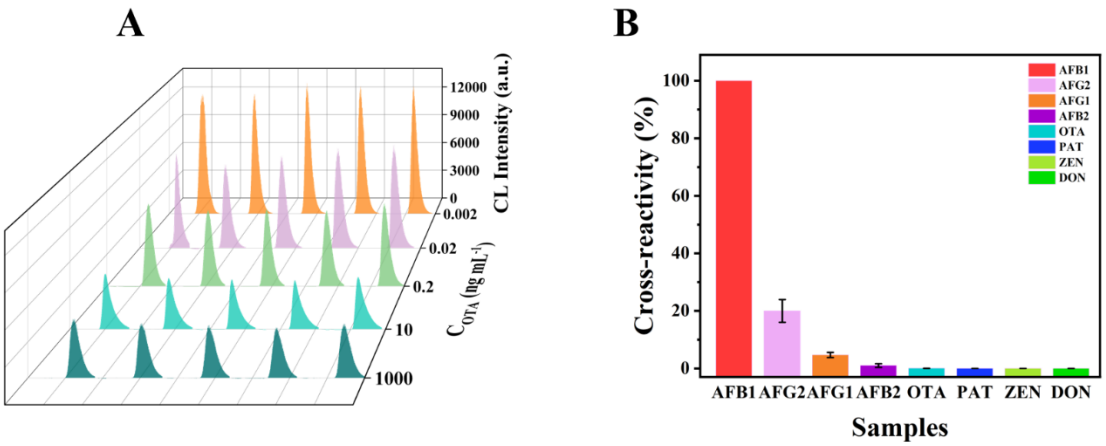


Fig. S6. (A) Five consecutive injections of AFB1 detection at concentrations of 1000, 10, 0.2, 0.02, and 0.002 ng mL^{-1}; (B) Selectivity of the CL immunosensor in the presence of seven different interfering substances at 100 ng mL^{-1}.

Table S1

Table S1. Comparison of kinetic parameters of AuPt@ZIF-67 with HRP and other nanozymes.

Catalyst	Substrate	K_m (mM)	V_{max} (10^{-8} M s^{-1})	References
Au_2Pt	H_2O_2	5.045	14.11	1
CoFe-LDH/CeO_2	H_2O_2	10.82	-	2
$Au_{21}Pd_{79}$	H_2O_2	5.89	8.19	3
HRP	H_2O_2	3.7	8.71	4
AuPt@ZIF-67	H_2O_2	2.68	1.87	This work

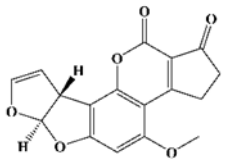
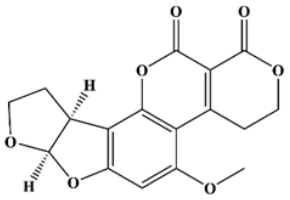
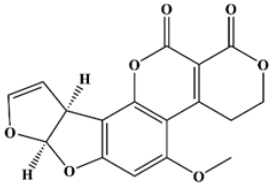
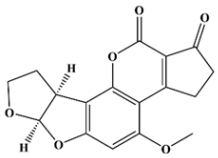
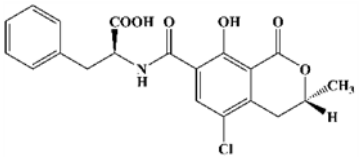
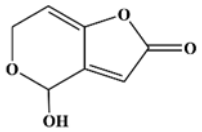
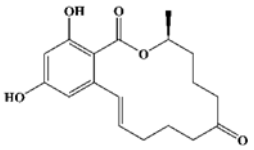
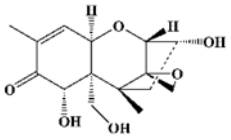
Table S2

Table S2. Properties of comparable methods for the determination of AFB1.

Methods	LODs (pg mL^{-1})	Analytical ranges (ng mL^{-1})	References
ECL	1	0.005~500	5
SERS	30	0.1~5	6
Fluorescence	70	1~100	7
HPLC-MS-MS	20	0.05~0.2	8
ECLSA	18	0.005~1	9
ICA	25	0.025~25	10
FI-CLIA	0.68	0.002~1000	This work

Table S3

Table S3. The molecular formula, CAS number and structural formula of aflatoxin B1 and 7 structural-similar interfering substances.

Substance	Molecular formula	CAS No.	Structural formula
Aflatoxin B1	$C_{17}H_{12}O_6$	1162-65-8	
Aflatoxin G2	$C_{17}H_{14}O_7$	7241-98-7	
Aflatoxin G1	$C_{17}H_{12}O_7$	1165-39-5	
Aflatoxin B2	$C_{17}H_{14}O_6$	7220-81-7	
Ochratoxin A	$C_{20}H_{18}ClNO_6$	303-47-9	
Patulin	$C_7H_6O_4$	149-29-1	
Zearalenone	$C_{18}H_{22}O_5$	17924-92-4	
Deoxynivalenol	$C_{15}H_{20}O_6$	51481-10-8	

Supplementary References

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