

Supplementary Material
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**RecJf exonuclease-assisted signal amplification for sensitive detection
of di(2-ethylhexyl) phthalate by Pd@Au NBs labeled electrochemical
aptasensor based on Au@Ni-CoHNBs/PEI-g-C₃N₄**

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S1 Materials, reagents and apparatus

Diethylhexyl phthalate (DEHP), dipropyl phthalate (DPrP, >99.7%), and tetrachloroauric acid trihydrate (III) ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) were purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). dimethyl phthalate (DMP, $\geq 99\%$), Dibutyl phthalate (DBP, >99.5%), sodium tetrachloropalladate (II) (Na_2PdCl_4), Hexadecyltrimethylammonium bromide (CTAB), tris (2-carboxyethyl) phosphine hydrochloride solution (TCEP), 6-Mercapto-1-hexanol (MCH), Thionin (Thi) and urea were supplied by Shanghai Yuan Ye Biological Technology Co., Ltd. (Shanghai, China). Sodium borohydride (NaBH_4) was bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cobaltous nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was bought from Alfa Aesar Chemical Co., Ltd. (Shanghai, China). Nickelous nitrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) and L-ascorbic acid (AA) were purchased from Xilong Chemical Co., Ltd. (Guangdong, China). RecJf exonuclease and $10 \times \text{NE Buffer}$ were brought in by New England Biolabs Ltd. (MA, USA). All the oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). the sequences were listed in the Supporting Information. The following were the DNA oligonucleotide sequences used in this experiment^[1]:

Apt: 5'-ACGAACGGAAGCGCGAAGCTTGCGC-SH-3'

cDNA: 5'-CTTCCGTTCGT-SH-3'

Tris-HCl buffer was prepared by 10 mM Tris, 1 mM EDTA and 100 mM NaCl, which was adjusted the pH 7.4, and used it after sterilizing the high-pressure cooker. All other chemicals were of analytical reagent grade and deionized water was used throughout experiments.

Electrochemical experiments, including cyclic voltammetry (CV) electrochemical impedance spectroscopy (EIS), differential pulse voltammograms (DPV) were carried out with CHI660D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd, China). The morphologies, crystal structure and element composition analysis of different materials were characterized by transmission electron microscope (TEM, Hitachi 7700, Japan), energy dispersive spectroscopy (EDS, JEOL JSM-2100 F, Japan),

X-ray diffraction (XRD, CuK α radiation, D/max 2550VB X-ray diffractometer, Rigaku), X-ray photoelectron spectra (XPS, Escalab 250xi, China), UV–vis absorption spectra were collected using a UV-6100 s double beam spectrophotometer (Mapada, China) and Fourier transform infrared spectroscopy (FTIR) was carried out on Nicolet 6700 (Thermo Fisher Scientific, america).

S2 Synthesis of g-C₃N₄, Ni-Co hollow nanoboxes and AuNRs

The g-C₃N₄ nanosheets were prepared according to the reported literature^[2] with some modifications, 20 g urea was firstly dissolved into 20 mL deionized water to form a transparent solution under 50°C. Then, the resulting solution was transferred into a covered ceramic crucible (100 mL) and calcined at 550°C for 4 h after sealing, using the heating program of 10°C/min. Finally, a light-yellow product of ultrathin g-C₃N₄ nanosheets was obtained.

The synthesis of the Ni-Co hollow nanoboxes refers to the previous literature with minor modifications^[3]. As a precursor for the preparation of Ni-Co hollow nanoboxes, ZIF-67 nanocubes were first prepared. Typically, 290 mg of Co(NO₃)₂·6H₂O was dissolved in 10 mL of deionized water containing 7.5 mg of CTAB. Then the solution was quickly injected into 70 ml of an ultrapure aqueous solution containing 4.540 g of 2-methylimidazole (the color immediately changed from colorless to purple) and stirred for 20 min, the product was collected by centrifugation and washed with ethanol for six times. After preparing ZIF-67 nanocubes, the synthesis of Ni-CoHNBs was performed. First, dispersed 30 mg of the prepared ZIF-67 nanocubes in 20 mL of ethanol, then added 5 mL of ethanol solution containing 50 mg of Ni(NO₃)₂, the solution was stirred for 1 min and the product vessel was put into an ultrasonic bath (at low power) for 60 min. Finally, the product was washed with ethanol several times and dried at 70°C.

AuNRs were synthesized by seed-mediated growth approach in aqueous solutions^[4]. Firstly, the seed solution was prepared as follows: 250 μ L of 0.01 M HAuCl₄·3H₂O was mixed with 9.75 mL of 0.1 M CTAB. Then, 600 μ L of ice-cold NaBH₄ (0.01 M) was added in the resultant mixture to form a yellow-brown solution under magnetic stirring. Finally, the seed solution was kept in a 25 °C water bath for 2 h without disturbance. The growth solution was prepared as follows: 0.912 g CTAB

and 0.11 g bromosalicylic acid were dissolved in 49 mL of distilled water. Then, 240 μL of 0.02 M AgNO_3 was added in the solution and left undisturbed at 30°C for 15 min. Subsequently, 500 μL of 0.05 M $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was added in the mixture and stirred for 15 min. Finally, 130 μL of 0.1 M AA was quickly injected and stirred vigorously for 30 s. To grow AuNRs, 80 μL of seed solution was injected in the freshly prepared growth solution and stirred for 30 s, and allowed to stand at 30°C for 12 h. After centrifugation at 12,000 rpm and washing twice, AuNRs were suspended in 5 mL distilled water.

S3 Characterization of nanomaterials

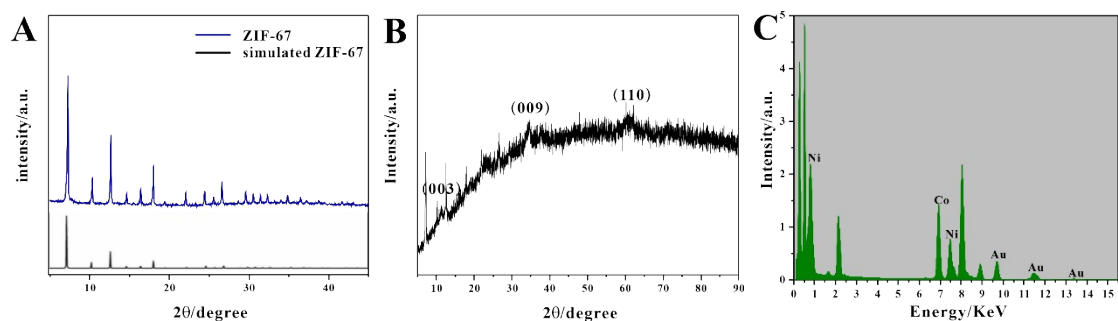


Figure S1. XRD patterns of (A) ZIF-67 and (B) Ni-CoHNBs; EDS of (C) Au@Ni-CoHNBs

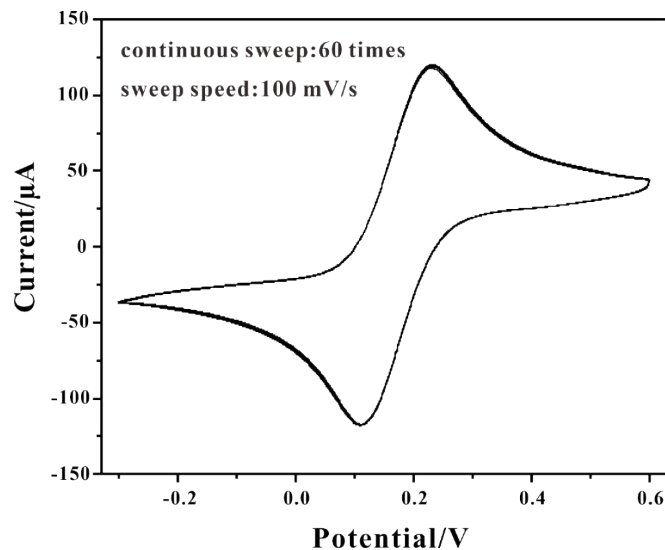


Figure S2. CV of Au@Ni-CoHNBs/PEI/g- C_3N_4 modified bare AuE continuous 60 times.

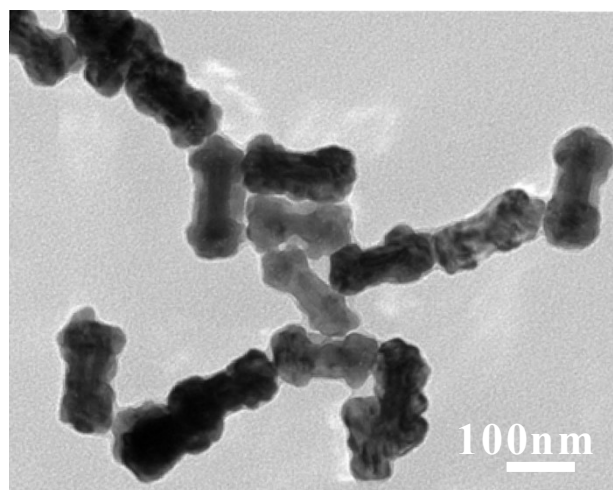


Figure S3. TEM images of Pd@Au NBs.

Table S1. BET surface area report of nanocomposites

Material	Sample weight (g)	Analysis free space (cm ³)	Analysis bath temp (K)	Analysis adsorptive	surface area (m ² /g)
ZIF-67	0.0667	47.5320	77.3	N ₂	22.4690
Ni-CoHNBs	0.1152	46.5961	77.3	N ₂	37.9959
Au@Ni-CoHNBs	0.1188	47.9760	77.3	N ₂	53.3606

S4 Feasibility analysis of amplification strategy

According to the Randles–Sevcik equation, the electroactive surface area of different electrodes was quantitated:

$$I = 2.69 \times 10^5 \times A \times D^{1/2} n^{3/2} \nu^{1/2} \times C$$

where A is the electroactive surface area (cm²), D represents the diffusion coefficient of [Fe(CN)₆]^{3-/4-} ($D = 7.6 \times 10^{-6}$ cm²/s), n is the number of electrons transferred ($n=1$), ν is the scan rate (V/s, $\nu=0.1$), and C is the concentration of the redox probe (mol/cm³, $C=5$).^[5]

S5 Optimization of loading content of Thi in Pd@Au NBs

As shown in Figure S3, the DPV current signal showed an increasing trend with increasing amounts of Thi. And the current response becomes stable after 1.0 as the volume ratio of Thi to Pd@Au NBs increases due to the Thi having reached its maximum loading content in Pd@Au NBs. Therefore, the volume ratio of Thi to

Pd@Au NBs was chosen to be 1.0.

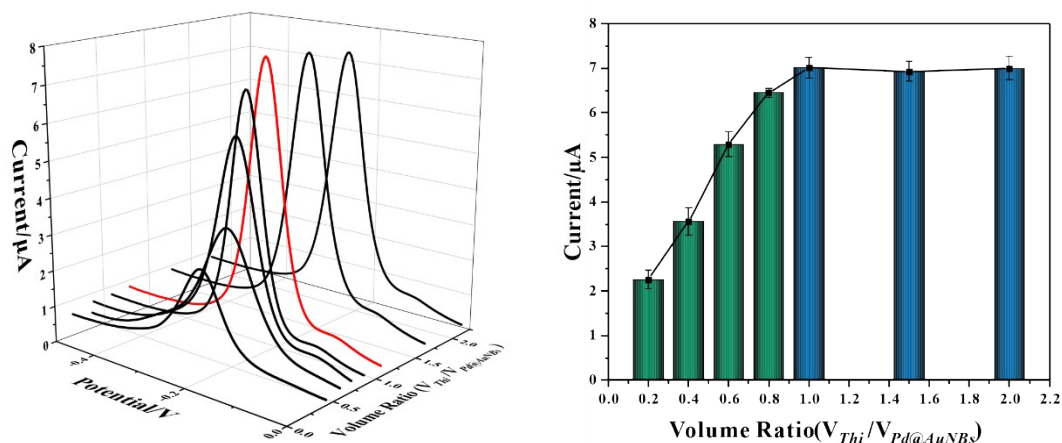


Figure S4. Optimization of volume ratio of Thi to Pd@Au NBs

S6 Analytical performance of the detection method for DEHP

The detection limit was calculated according to the following method:

According to IUPAC recommendation (IUPAC 1976), the analyte's signal at the detection limit (Sdl) is given by:

$$Sdl = S_{reag} + k * \sigma_{reag},$$

where S_{reag} is the electrochemical signal for a blank, σ_{reag} is the known standard deviation for the blank's electrochemical signal ($n=10$). As is well known, $k = \text{signal/noise (S/N)} = 3$. As suggested by Long and Winefordner (1983) (Long and Winefordner 1983), the use of $k=3$ allows a confidence level of 99.86 % for a normal distribution of the blank signals. The detection limit can be calculated by Sdl and calibration curves.

Table S2. Electrochemical response values of the blank solution for ten times

Number	I (μA)	Average	SD	RSD (%)
1	7.03			
2	6.89	6.99	0.16	2.29
3	6.97			

4	6.81
5	7.15
6	6.99
7	6.80
8	6.85
9	7.21
10	7.22

S7 Calculation of RSD

The calculation formula for the relative standard deviation (RSD) is the standard deviation (SD) divided by the average value (\bar{x}), as follows:

$$RSD = SD / \bar{x} \cdot 100$$

The following table shows the original data and the RSD obtained according to the formula.

Table S3. Original data for RSD Calculation

	\bar{X} (μA)	SD (μA)	RSD (%)
Reproducibility	1.82	0.04	2.22
	4.23	0.24	5.57
Liquor sample	3.32	0.18	5.51
	2.48	0.07	2.72

Reference

[1] Hyun Jeong Lima, A-Ru Kimb, Moon-Young Yoonb, Youngmin Youc, Beelee Chuad, Ahjeong Son. Development of quantum dot aptasensor and its portable analyzer for the detection of di-2-ethylhexyl phthalate[J]. *BIOSENSORS &*

BIOELECTRONICS, 2018, 121:1-9.

[2] Shuying Gao, Xuyu Wang, Changjian Song, Shijian Zhou, Fu Yang, Yan Kong. Engineering carbon-defects on ultrathin g-C₃N₄ allows one-pot output and dramatically boosts photoredox catalytic activity[J]. Applied Catalysis B: Environmental, 2021, 295.

[3] Peilei He, Xin-Yao Yu, and Xiong Wen (David) Lou. Carbon-Incorporated Nickel-Cobalt Mixed Metal Phosphide Nanoboxes with Enhanced Electrocatalytic Activity for Oxygen Evolution[J]. Communications, 2016.

[4] X. Ye, L. Jin, H. Caglayan, et al., Improved size-tunable synthesis of monodisperse gold nanorods through the use of aromatic additives, ACS Nano 6 (3) (2012), 2804-2817.

[5] Song, H.; Zhao, H.; Zhang, X.; Xu, Y.; Cheng, X.; Gao, S.; Huo, L. 3D hierarchical hollow hydrangea-like Fe³⁺@ ϵ -MnO₂ microspheres with excellent electrochemical performance for dopamine and hydrogen peroxide. Biosens. Bioelectron. 2019, 133, 250-257.