Supporting Information

An All-Graphdiyne Electrochemiluminescence Biosensor for Ultrasensitive Detection of MicroRNA-21 Based on Target Recycling with DNA Cascade Reaction for Signal Amplification

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Materials and reagents

Graphdiyne (GDY) was purchased from Jiangsu Xianfeng Nanomaterials Technology Co., Ltd. (Nanjing, China). N-hydroxysuccinimide (NHS, 98%), 1-ethyl-(3-dimethylaminopropyl) carbon diimine hydrochloride (EDC, 98%), 6-mercapto-1-hexanol (MCH), Tris (2-carboxyethyl) phosphine (TCEP), diethyl pyrocarbate (DEPC), $HAuCl_4 \cdot 3H_2O$, $NaBH_4$ were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). The oligonucleotides were obtained from Sangon Biotechnology Inc. (Shanghai, China), as shown in Table S1. Phosphate buffered saline (PBS) at pH 7.4 was used to wash the electrode and prepare aptamer solutions. All the reagents were analytical grade unless otherwise stated. The serum samples provided by the Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine were used for real sample analysis.

Apparatus

ECL was measured on an MPI-EII multi-parameter electrochemiluminescence system (Xi'an Remax Electronic Science & Technology Co. Ltd, China). The Eelectrochemical workstation CHI440 (Shanghai Chenhua Instrument Co., Ltd., China) was used to perform the cyclic voltammetry (CV) detection. Electrochemical impedance spectroscopy (EIS) was performed by the PGSTAT128N Autolab potentiostat/galvanostat (The Netherlands). Scanning electron microscopy (SEM) was tested by SUPPRA 55 Sapphire (Carl Zeiss, Germany), transmission electron microscopy (TEM) was tested by Tecnai F30 (FEI, USA), which were used to characterize the morphology of the materials. D8 ADVANCE X-ray powder

diffractometer(AXS, Germany), Nicolet iS10 Fourier transform infrared spectrometer (Thermo Fisher Scientific, USA), UV-2100 UV-vis spectrophotometer (Beijing Beifen Ruili Co., LTD, China) and FL-4600 fluorescence spectrometer(Hitachi, Japan) were utilized to characterize the experimental materials, respectively. Deionized water was taken from Milli-Q water purification system (Millipore, USA).

ECL, CV and EIS detection were performed in the three-electrode system, the working electrode was modified glassy carbon electrode(GCE), the counter electrode was platinum pole, and the reference electrode was Ag/AgCl(sat. KCl) electrode or saturated calomel electrode (SCE). The scanning ranges of ECL potential detection and CV detection were -1.8~0 V and -0.2~0.6 V, respectively. The scan rates of ECL detection was 200 mV/s, and the scan rates of CV detection was 100 mV/s.

Preparation of AuNPs/GDY

The preparation of AuNPs/GDY was slightly adjusted according to the literature method[1-2]. Firstly, 2 mg of GDY was dispersed in 4 mL of ultrapure water and sonicated for 30 min. Secondly, 0.05 mol/L $HAuCl_4 \cdot 3H_2O$ solution was added to the GDY dispersion under stirring conditions. The mixture was sonicated for 10 min at room temperature and then stirred for 1 h, which was repeated 3 times. Subsequently, the newly prepared 0.05mol/L $NaBH_4$ solution was quickly added to the above solution and stirred for 20min. Then, 0.02 mol/L sodium citrate solution was added dropwise and stirred for 30 min. To remove unreacted substances, the reaction solution was centrifuged, washed three times with ultrapure water, and dried under vacuum at 60°C. Finally, the obtained precipitate was redispersed in 4 mL of

ultrapure water to obtain AuNPs/GDY, which was stored at 4 °C.

Preparation of GDYO QDs

GDYO was synthesized by acid-oxidation treatment according to the literature[3-4]. Firstly, 10 mg of GDY powder was mixed with 1 mL of concentrated HNO_3, 3 mL of concentrated H_2SO_4 and 10 mg of $KMnO_4$ powder. Then, the mixture was stirred in an oil bath at 80 °C for 24 h. Secondly, after the mixture was cooled to room temperature, the pH was adjusted to 8.0 with NaOH in an ice bath. Afterwards, The suspension was centrifuged at 8000 rpm for 10 minutes and washed several times. To obtain the pure GDYO, the centrifuged product was dialyzed (cutoff, 3500) in aqueous solution for 3 days. The GDYO aqueous suspension was sonicated for 24 hours and heated in an oil bath at 100°C for 6 hours. After the mixed product was centrifuged, the supernatant was taken to obtain 0.8 mg/mL GDYO QDs.

The full-scan spectra of X-ray photoelectron spectroscopy (XPS) in Fig. S1A was mainly a to probe the elemental composition of AuNPs/GDY. In the C 1s XPS spectrum (Fig. S1B), the peaks at 284.8, 285.6, 286.9, and 288.6 eV can be assigned to carbon in the form of C=C (sp^2), C≡C (sp), C-O, C=O, respectively. As is shown in the Fig. S1C, the O 1s can be respectively divided into two peaks: C-O at 531.8 eV and C=O at 532.9 eV, which may be due to the chemical reaction of oxygen with the terminal acetylene bonds or the adsorption of oxygen on the surface of GDY[13]. Fig. S1D shows two peaks at 84.1 and 87.9 ev are attributed to $4f_{7/2}$ and $4f_{5/2}$, respectively. The results above confirm the successful synthesis of GDYO QDs and Au/GDY.

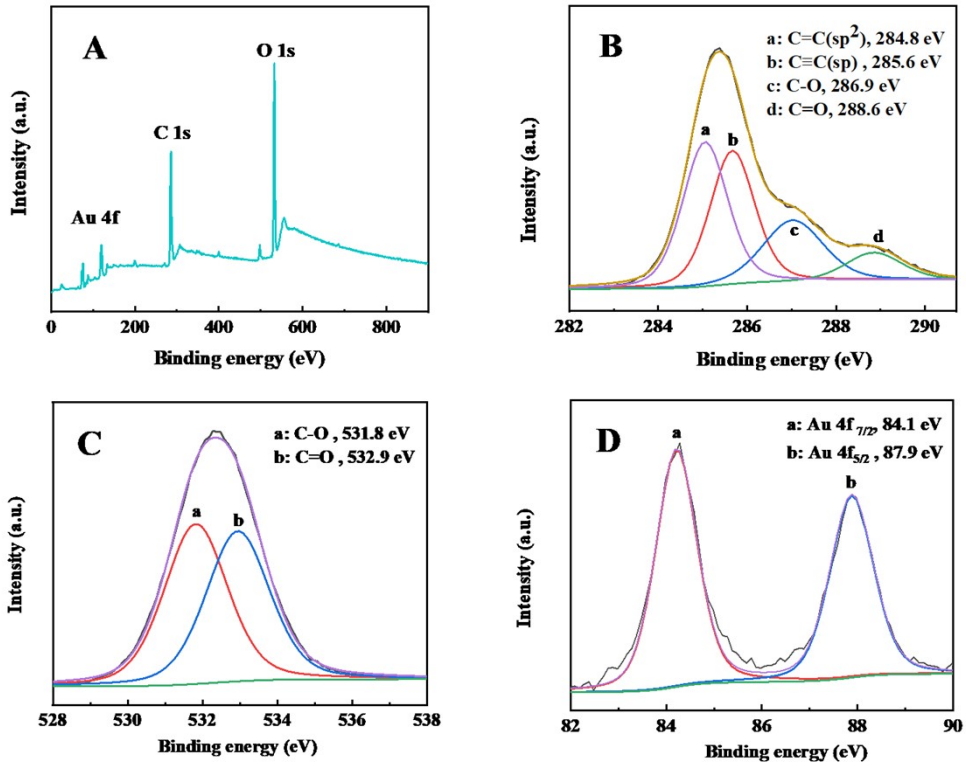

Fig. S1. X-ray photoelectron spectroscopy (XPS) analysis of AuNPs/GDY: (A)survey scans, (B) C 1s, (C) O 1s, (D) Au 4f .

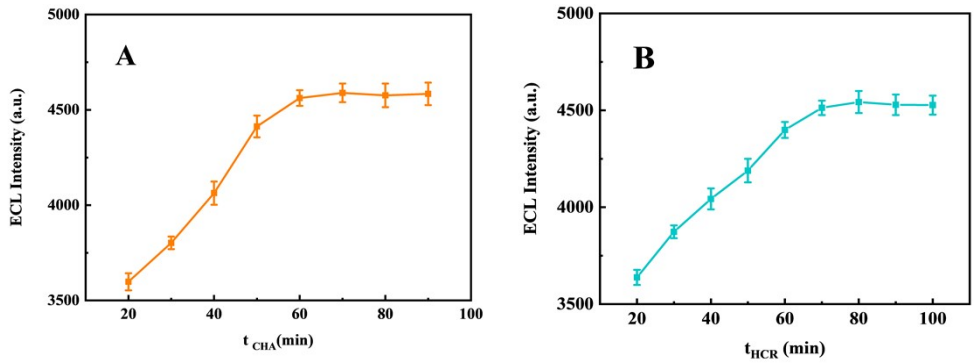

Fig. S2. Effects of (C) reaction time in 3D DNA walker and (D) HCR process on the ECL responses. The concentrations of SH-CP, H1/H2, and H3/H4 were 1 μM, miRNA-21 was 1 nM. Error bars: SD, n=3.

Table S1. Sequences of the oligonucleotides

Name	Sequences (from 5′ to 3′)
SH-CP	5’-SH-$(CH2)_6$-TTT TTT GTC TGA GGT-3’
miRNA-21	5’-UAG CUU AUC AGA CUG AUG UUG A-3’
H1	5’-TCA ACA TCA GTC TGA TAA GCT ACG ACA ACT GTA GAT GTA ACC TCA GAC-3’
H2	5’-TAC ATC TAC AGT TGT CGT AGC TTA TCA GAC CGC TGC CAA ACA ACT-3’
H3	5’-AGT TGT TTG GCA GCG GAA GTC CGC TGC CAA -3’
H4	5’-NH_2-$(CH_2)_6$-CGC TGC CAA ACA ACT TTG GCA GCG GAC TTC-3’
miRNA-141	5’-UAA CAC UGU CUG GUA AAG AUG G-3’
miRNA-155	5’-UUA AUG CUA AUC GUG AUA GGG GU-3’
miRNA-199a	5’-ACA GUA GUC UGC ACA UUG GUU A-3’
single-base mismatch RNA（smRNA）	5’-UAG CUU AUC AGA AUG AUG UUG A-3’
three-base mismatch RNA（tmRNA）	5’-UAG CUU GUC AGA AUG AUG AUG A-3’
Non-complementary（NC）	5’-UUG UAC UAC ACA AAA GUA CUG-3’

Table S2. Comparison of different sensors for miRNA detection

Method	Linear range	Detection limit	Reference
Phosphorescence	8-80 nM	1.60 nM	[5]
Field effect transistor	1 fM-1 nM	1 fM	[6]
Electrochemistry	10 fM-5 nM	2.49 fM	[7]
Stochastic Collision Electrochemistry (SCE)	0.001-0.1 nM	1 pM	[8]
Electrochemistry	5 fM-2 nM	1.5 fM	[9]
Photoelectrochemistry （PEC）	0.001-100 pM	0.31 fM	[10]
Electrochemiluminescene (ECL)	0.001-10 nM	0.00003 nM	[11]
Fluorescence	1-50 nM	0.5 nM	[12]
Electrochemiluminescene (ECL)	0.1 fM-1 n M	0.023 fM	This work

Table S3. Determination of miRNA-21 in human serum samples（*n*=3）

Sample	Detected (fM)	Added (fM)	Found (fM)	Recovery (%)	RSD (%)
1	Not detected	1.00	0.9874	98.74	4.62
		10.00	10.03	103.0	3.79
2	Not detected	1.00	1.053	105.3	4.84
		10.00	9.77	97.70	5.12
3	Not Detected	1.00	1.038	103.8	2.56
		10.00	10.19	101.9	4.33

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