The interaction of an amorphous metal-organic cage-based solid (aMOC) with miRNA/DNA and its application on quartz crystal microbalance (QCM) sensor

Xia Jin, Yu-Ping Zhang, Dian-Mei Li, Ding Ma, Sheng-Run Zheng, Chu-Hong Wu, Jia-Yin Li, Wei-Guang Zhang**

School of Chemistry, South China Normal University, Guangzhou, 510006, P. R. China.

* Corresponding author: Dr. Sheng-Run, Zheng

E-mail address: zhengsr@scnu.edu.cn

Tel./Fax.: +86-20-39310187

Supporting Information

EXPERIMENTAL SECTION

Chemicals

HPLC-purified miRNA and DNA were obtained from Sangon Co. Ltd. (Shanghai, China; DEPC =diethylpyrocarbonate). The sequences are listed in Table 1. All other reagents were of analytical grade and were commercially available from Guangzhou Chemical Reagent Factory and were used without further purification.

Table S1 Synthetic oligonucleotides used in this study

Name	Sequence
Thiol--modified capture probe DNA (cpDNA)	5'-SH-CCTACTACCTCAGCCTA-3'
Signal Probe DNA (spDNA)	5'-AACTATACAACCTACTACCTCA-3'
let-7a	5'-UGAGGUAGUAGGUUGUAUAGUU-3'
let-7c	5'-UGAGGUAGUAGGUUGUAUGGUU-3'
let-7e	5'-UGAGGUAGGAGGUUGUAUAGUU-3'
let-7b	5'-UGAGGUAGUAGGUUGUGUGGUU-3'
Carboxyfluorescein-labelled probe DNA (FAM-pDNA)	5'-FAM-AACTATACAACCTACTACCTCA-3'

Apparatus and characterizations

The AT-cut quartz crystals (13.0 mm diameter, 5 MHz resonant frequency) covered with gold electrodes on both sides were used. All online QCM detections were implemented on a homemade aqueous media measuring the QCM system (Fig. S1). The system consisted of a QCM biosensor, a reaction cell, an oscillator detector, a frequency counter and a computer. Infrared (IR) spectra were measured on an IR Prestige-zl spectrophotometer (Shimadzu, Japan) with KBr pellets in the range from 4000 to 400 cm^{-1}. EDS spectra were recorded on a Phenom Pro X Desktop Scanning Electron Microscope-Energy Spectrum Integrative Machine. Zeta potentials were recorded by a Zetasizer Nano ZS90 system from Malvern Instruments with a liquid concentration of 0.75 mg/mL. Fluorescence experiments were performed on a fluorescence analysis instrument (Hitachi F4600, Japan).

Synthesis of aMOC-2

A mixture of L (61 mg, 0.2 mmol) and $Pd(NO_3)_2 \cdot 2H_2O$ (26.6 mg, 0.1 mmol) was dissolved in DMSO (6 mL) and heated at 50 °C for 8 h under the protection of argon. Then, the resulting yellow solution was added into dioxane (24 mL) to yield a pale-yellow precipitate. The

precipitate was obtained *via* centrifugation, washed three times with ethyl ether (24 mL) and then immersed in ethyl ether (8 mL) for another 3 days. The amorphous pale-yellow power was collected and dried. Yield: 70% (based on Pd).

Synthesis of signal probe DNA (spDNA) @MOC-2 complex

Before this experiment, particle size screening was performed for *a*MOC-2 powder. The *a*MOC-2 powder (10 mg) is dispersed in water (20 mL) and the suspension is formed by ultrasonic for 1 hours. Then, the suspension is centrifuged in 5000 r/min rotating speed for 20 minutes, and the upper suspension is taken to obtain *a*MOC-2 powder with specific particle size. The average size is determined and confirmed to be 240 ± 10 nm through the Zetasizer nano instrument before subsequent use. Then, the upper suspension is concentrated and freeze-dried, and the powder was used for the following experiment.

First, 2 mg *a*MOC-2 powder was dispersed in 2 mL ultrapure water to form an *a*MOC-2 suspension though ultrasonication for 30 min. A certain volume (from 20 to 80 μL) of the suspension was added to a spDNA Tris-HCl solution (50 nM, 1 mL), and the mixture was reacted at 37 °C for 3 h.

Luminescent study

The fluorescence measurements were performed with an excitation slit width of 5.0 nm and an emission slit width of 5.0 nm. The emission spectra were collected from 500 to 600 nm under an excitation wavelength of 480 nm, and the fluorescence intensity at 530 nm was used for analysis. The concentration of miRNA was kept constant at 50 nM, and 80 μL nucleic acid was used in the FL test. The preparation method for aMOC@miRNA is consistent with the above experiment.

Assembly of capture probe DNA (cpDNA)-coated QCM chip

The QCM gold pieces were first immersed in a piranha solution (98% H_2SO_4:30% H_2O_2 (v/v) = 7:3) for 10 mins, then washed with deionized water several times and finally dried with

nitrogen. The pretreated gold piece was immersed in DNA Tris-HCl solution (1 mL, 50 nM) for 1 hour at room temperature. Then, the piece was washed with ultrapure water and dried with N_2. After drying, the piece was immersed in 6-mercapto-1-hexanol solution (4 μM) for 30 minutes and washed with ultrapure water and dried with Ar to form the DNA-coated QCM chip.

Detection of miRNA by QCM sensor

To prevent MOF deposition, QCM sensors were tested face down. Before the QCM test, the DNA-coated QCM chip was incubated with a let-7a solution at a certain concentration for 1 h and dried by Ar and used as a QCM biosensor. First, phosphate buffer was injected into the QCM cell, and the crystal frequency was measured as the background signal. After the signal change was less than 1 Hz of frequency drift in 10 min, the experiment could be continued. Then, the spDNA@MOC-2 complex tris-HCl solution/suspension was injected into the QCM liquid cell until it was filled, and the solution/suspension in the QCM cell was kept still until a stable signal with signal change less than 1 Hz was obtained. After 5000 seconds, the frequency change was recorded and were use for data analyzing.

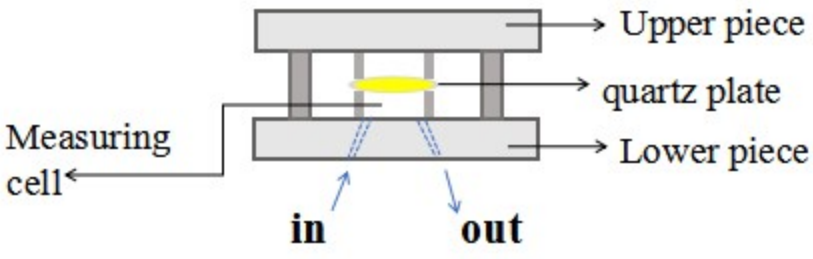


Fig. S1 Schematic diagram of Static detection quartz crystal microbalance sensor system.

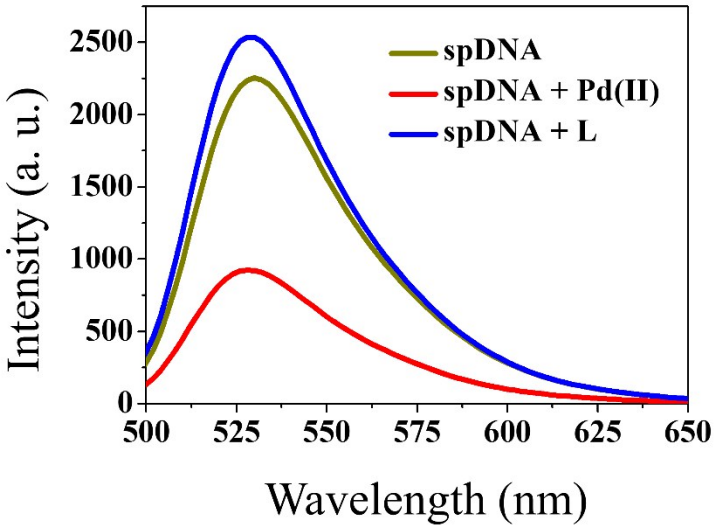
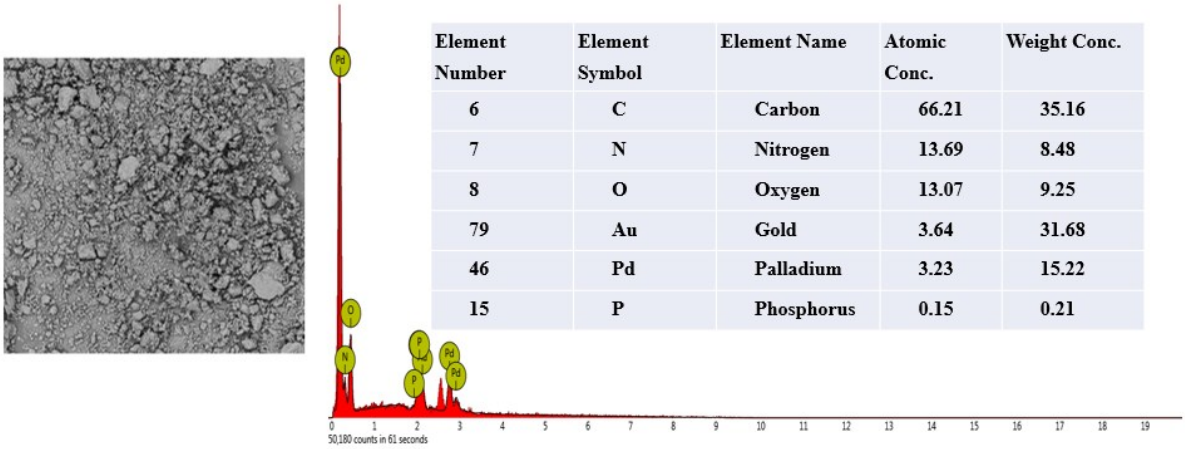


Fig. S2 Fluorescence spectra of FAM-spDNA without and with the addition of Pd(II) and L.



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	66.21	35.16
7	N	Nitrogen	13.69	8.48
8	O	Oxygen	13.07	9.25
79	Au	Gold	3.64	31.68
46	Pd	Palladium	3.23	15.22
15	P	Phosphorus	0.15	0.21

Fig. S3 The EDS spectrum of aMOC-2@spDNA complex.

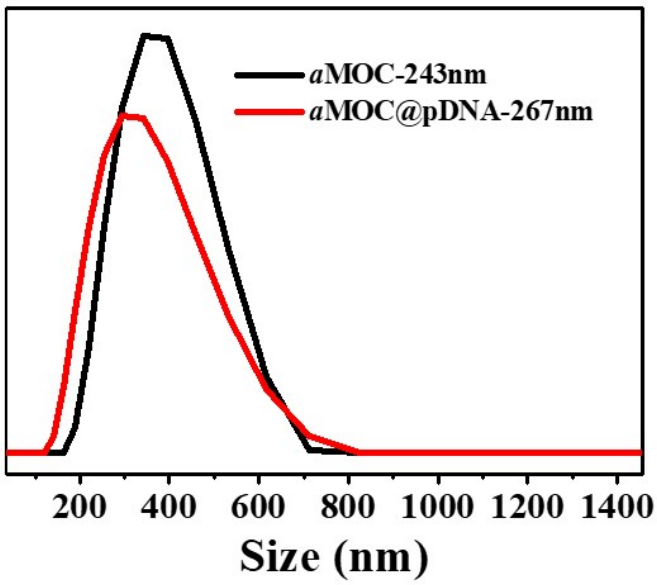


Fig. S4 The average size of aMOC-2 and aMOC-2@spDNA complex.

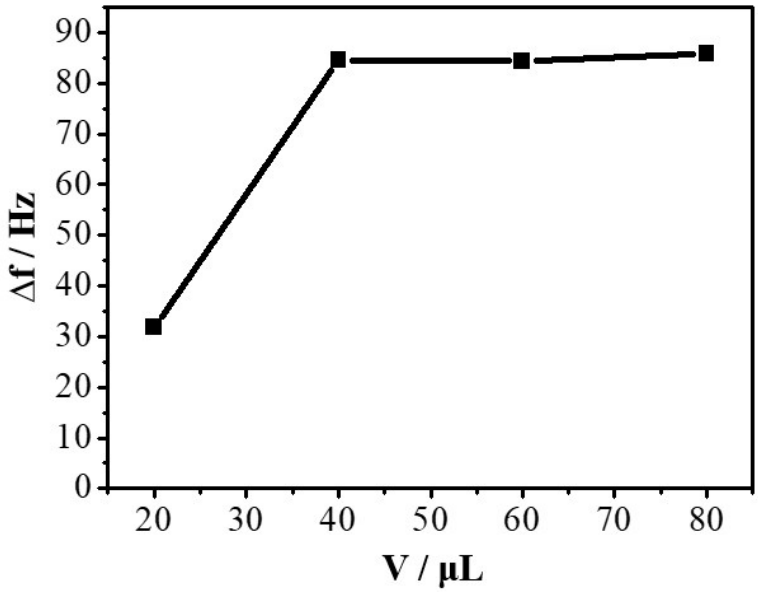


Fig. S5 The influence of the amounts of aMOC-2 on the frequency shift of QCM sensor.

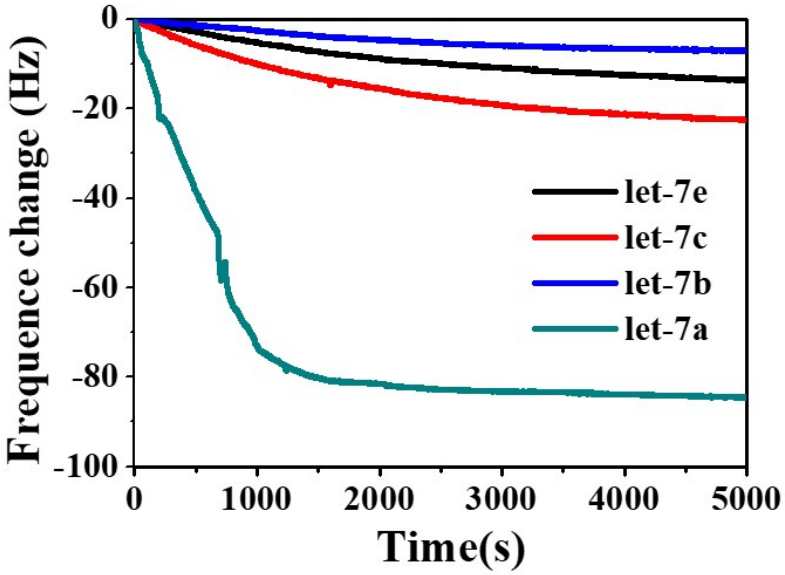


Fig. S6 Real-time frequency responses of aMOC-2@spDNA on QCM sensor incubated with different miRNAs.

Tab S2 Accuracy validation

Sample	Let7a	Calculated Δf（Hz）	Experimental Δf（Hz）	RSD (%)	recovery (%)
1			85.7		98.17%
2			86.6		99.2%
3	50nM	87.3	82.4	1.7%,	94.39%
4			85.6		98.05%
5			85.4		97.82%
1			48		98.97%
2			46.3		95.46%
3	10pM	48.5	46.2	1.4%	95.26%
4			46.5		95.88%
5			46.4		95.67%
1			6.6		101.5%
2			7		107.7%
3	1fM	6.5	6.7	4.5%	103.1%
4			6.9		106.2%
5			7.5		115.3%

Tab S3 Determination of let-7a added in 10% bovine serum (n=3) with the QCM sensor

Serum sample	Added concentration	Average found concentration	Recovery %	RSD
1	50 nM	51.23 nM	102%	1.6%
2	10 pM	10.90 pM	109%	1.1%
3	1 fM	1.01 fM	101%	2.6%