Electronic Supplementary Information

Adenosine Triphosphate Responsive Metal–organic Frameworks Equipped with DNA Structure Lock for Construction of Ratiometric SERS Biosensor

Caijun Wu[1], ShufanWang[1], Xiliang Luo[2], Ruo Yuan[*1], Xia Yang[*1]

1 Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education; College of Chemistry and Chemical Engineering, Southwest University, Chongqing, PR China

2 Shandong Key Laboratory of Biochemical Analysis; College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, PR China

1 EXPERIMENTAL SECTION

1.1 Materials and Reagents

Chloroauric acid hydrate ($HAuCl_4 \cdot xH_2O$) from Sigma Chemical Co. (St. Louis, MO). Sodium citrate, silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), hexadecyl trimethyl ammonium bromide (CTAB), toluidine blue (TB), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), nickel (II) nitrate hexahydrate ($Ni(NO_3)_2 \cdot 6H_2O$), 2-amino terephthalic acid (2-NH_2-BDC), glutaraldehyde (GTD), N,N-dimethylformamide (DMF) and ethylene glycol were purchased from ChengDu Kelong Chemical Reagent Company (ChengDu, China). Hexanethiol (96 %, HT) were purchased from Sigma-Aldrich (USA). 10 mM Tri-HCl (pH = 7.0) buffer solution (1.0 mM EDTA, 10 mM TCEP, 100 mM NaCl) was prepared in advance. Interfering proteins used in this experiment were purchased from Zybio (Chongqing, China). Human serum sample acquired from Xinqiao Hospital of Third Military Medical University (Chongqing, China).

HPLC-purified DNA nucleotides provided by Sangon Biotech Company Ltd. (Shanghai, China), and their sequences were listed in Table S1. A hairpin structure can be formed by H1. The ternary Y-junction can be formed by hybridization of three single strands of DNA (S1, S2 and cDNA). And S2 as the ATP-aptamer can recognize ATP specifically with a specific affinity.

1.2 Apparatus

The morphologies of materials were identified by a Field-emission scanning electron microscope (FE-SEM, JSM-7800F, JEOL, Japan) and a scanning electron microscope (SEM, S-4800, Hitachi). Then we used a Tecnai F20 S-Twin microscope

(TEM, FEI, USA) measuring at 200 kV to record transmission electron micrograph images. The Brunauer-Emmett-Teller measurement (BET, ASAP 2020, USA) was used to measure the special surface area of MOFs. For SERS spectra measurements, we used a Raman spectrometer (Renishaw Raman spectrometer, Invia, UK) with the equipment of a 633 nm line from a He-Ne laser (17 mW of power on the 50 × objective). A clean silicon wafer was used to calibrate the Raman spectrometer with 520 cm^{-1} Raman shift before the SERS measurement.

Table S1. Sequence information of nucleic acid used in this experiment.

Name	Sequences (5'-3')
S1	NH_2-$(CH_2)_6$-AAA AAA AAT ACC CCC AGG GAA TCG A
S2	TCG ATT CCC TGG GGG AGT ATT GCG GAG GAA GG
Complementary DNA (cDNA)	CCT TCC TCC GCA ATA CTT ATT TTT TTT
Hairpin probe (H1)	SH-CCC CCC CCC AAA AAA AAT AAG TAT TGC GGA GGA AGG-Cy5

1.3 Synthesis of GNFs and GNFs@H1.

GNFs were synthesized by using the template method in the previous literature.[1] The H1 was heated to 90 °C for 5 minutes and then gradually cooled to room temperature to form the hairpin probe. The H1 (5'-SH) was dropped onto the GNFs for 2 hours at 4 °C to obtain GNFs@H1.

1.4 Synthesis of MOFs.

MOFs can be synthesized with slight modification of references.2, 3 0.03 g of 2-NH_2-BDC, 0.05 g of $Ni(NO_3)_2{\cdot}6H_2O$ and 0.05 g of $Zn(NO_3)_2{\cdot}6H_2O$ were completely dissolved in 13 mL mixed solution of DMF and ethylene glycol (V1:V2 = 8:5) with

stirring for 1 hour at room temperature. Then the mixture was put into a Teflon lined stainless steel reactor keeping 150 °C for 6 hours. After cooling naturally to room temperature, the collected green materials were washed twice with DMF and ethanol each. The final product was dried in a vacuum at 60 °C overnight.

1.5 Synthesis of MOFs@S1 and MOFs@Y-junction.

5.0 mg of MOFs were added into 2.5 % of GTD solution incubating for 3 hours at 30 °C, and washed twice with ultra-pure water. The GTD modified MOFs was incubated in 50 μL of 10 μM S1 for 2 hours at 37 °C. The precipitation was washed twice with Tri-HCl (pH = 7.0) buffer solution to remove the excess S1 and the MOFs@S1 was obtained. The functional MOFs were placed in 0.1 mg/mL of TB solution soaking for 24 hours to encapsulate TB in the pores. 50 μL of 10 μM S2 and 50 μL of 10 μM cDNA were pre-annealed for 5 minutes at 95 °C to form the nucleotide hybridization solution. Then the hybridization solution was mixed with functionalized MOFs, which was incubated for 2 hours at 37 °C and washed with ultra-pure water until colorless.

1.6 Process of SERS Strategy Assay.

Different concentrations of ATP was added to the DNA Y-junction modified MOFs. The mixture was incubated for 90 minutes at 37 °C and centrifuged with 8000 rpm/min for 3 minutes. The supernatant and HT were dropped onto the GNFS@H1. The Raman signal was detected after incubation at 37 °C for 2 hours. Scheme 1 showed the construction process of SERS biosensor.

1.7 Characterization of Material

As shown in Figure S1A and 1B, SEM image and TEM image of GNFs depicted that uniform flower nanoparticles were obtained with the diameter of about 200 nm. Moreover, the MOFs with 2 µm microspheres shape were exhibited in the SEM (Figure S1C). The UV-vis spectrum of GNFs showed that a absorption peak was around 620 nm (Figure S2A), which had a remarkable red-shift because the particle size and petal length of the GNFs increased.[1] The MOFs was measured by the N_2 physisorption measurements, and the BET surface area was 101.0 m^2/g with pore volume of 0.19 cc/g and average pore diameter of 7.8 nm (inset image of Figure S2B) respectively. This showed that the MOFs possessed the porous structure with large surface area. The XRD pattern of MOFs was shown in Figure S2C, and the peaks were in accordance with previous reports[4, 5], indicating that the MOFs were well crystallized.

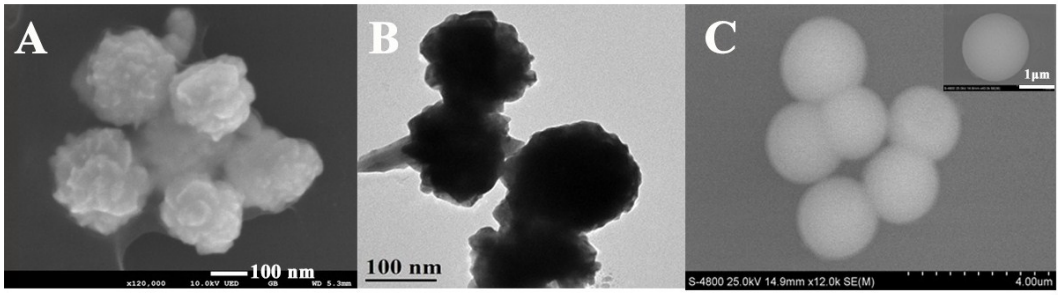

Figure S1. (A) SEM and (B) TEM of GNFs, SEM of (C) MOFs.

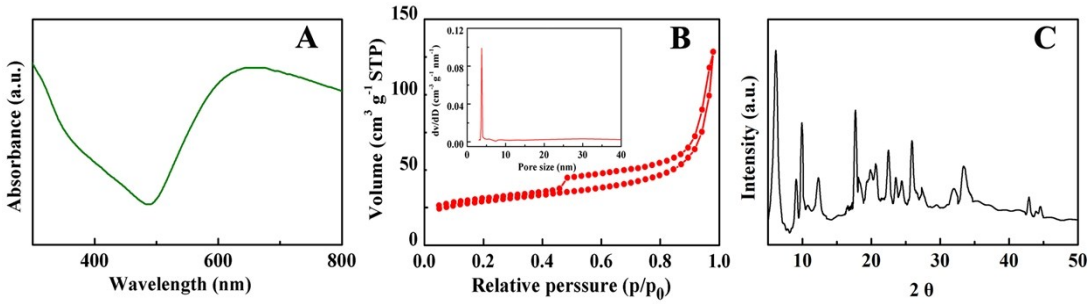

Figure S2. (A) The UV-vis spectrum of GNFs. (B) The N_2 adsorption-desorption isotherms of MOFs (Inset: pore diameter of MOFs). (C) The XRD pattern of MOFs.

1.8 UV-vis Absorption Characterization

The ultraviolet-visible (UV-vis) absorption spectrum was recorded by using UV-2450 ultraviolet-visible (UV-vis) absorption spectrum (Shimadzu, Tokyo, Japan). Firstly, different concentrations of toluidine blue (TB) standard solutions (0.0025，0.005，0.01，0.02 mg/mL) was prepared and the series of UV-vis absorption spectrums was measured, respectively (the solvent was ultra-pure water). Besides, it is known from literature[6] that the UV maximum absorption wavelength of TB is 630 nm in Figure S3A . As shown in Figure S3B, the standard curves for TB between the absorbance of TB at 630 nm and its concentrations from 0.0025 to 0.02 mg/mL was obtained. And the linear regression equation was $y = -5.395\times10^{-7} + 128.234c$ (R^2 = 0.999), where y represented the TB absorbance and c was the TB concentration. In addition, Figure S3C displayed that the absorbance intensity for MOF@TB and @TB blocked by Y junction (MOF@TB@Y junction) was 0.22 and 0.016. So it can be calculated according to the standard curves for TB to know the concentration of TB in different materials. When the TB was encapsulated in the MOFs, the TB was achieved equilibrium in and out of the pore of MOFs, the concentration was 1.716×10^{-3} mg/mL. And when the TB was blocked in the pore of MOFs by the Y junction, the concentration was 1.248×10^{-4} mg/mL, which may be caused by the adsorption effect on the surface of MOFs.

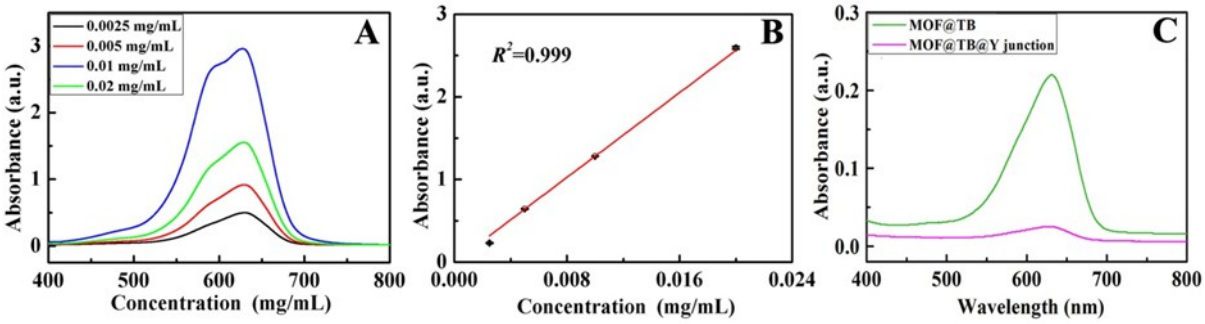

Figure S3. (A) The UV-vis absorption spectrums of TB standard solutions with different concentrations. (B) The standard curve of TB. (C) The UV-vis absorption spectrums of MOF@TB and MOF@TB@Y junction.

1.9 Sensitivity of the Ratiometric SERS Biosensor based on one signal

The individual TB or Cy5 signal of the biosensor with different ATP concentrations were shown in Figure S4A and 4B. The linear relationship between the individual I_{Cy5} and the ATP concentrations is in the range from 1 to 200 nM, and the LOD is 11 nM (Figure S4A). While the linear relationship between the individual I_{TB} and the ATP concentrations is in the range from 1 to 200 nM, and the LOD is 12 nM (Figure S4B). These results showed that the ratiometric SERS biosensor strategy offers lower LOD and more excellent stability than the typical SERS methods based on single signal response.

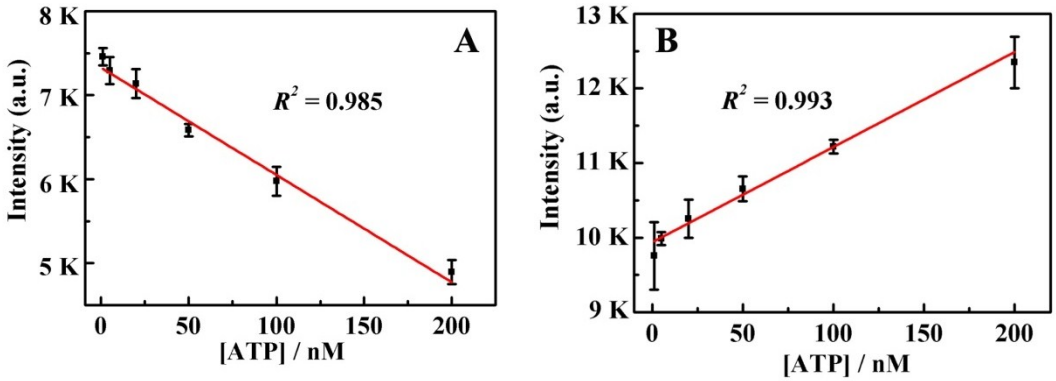

Figure S4. The calibration curve of individual (A) Cy5 or (B) TB Raman intensity and ATP concentration (error bars = RSD, *n* = 3). And the elevated concentration of ATP was 1, 5, 20, 50, 100, 200 nM, respectively.

If there are not H1, the biosensor has only one signal with TB. The variation trend of Raman intensity of TB on GNFs without hairpin DNA (H1) with different ATP concentration from 1 to 200 nM was shown in Figure S5A. And the linear relationship between the I_{TB} and the ATP concentrations is in the range from 1 to 200 nM, and the LOD is 2.3 nM (Figure S5B). So the detection limit and stability of this typical SERS methods based on single signal response mechanism was inferior compared with our ratiometric SERS biosensor strategy.

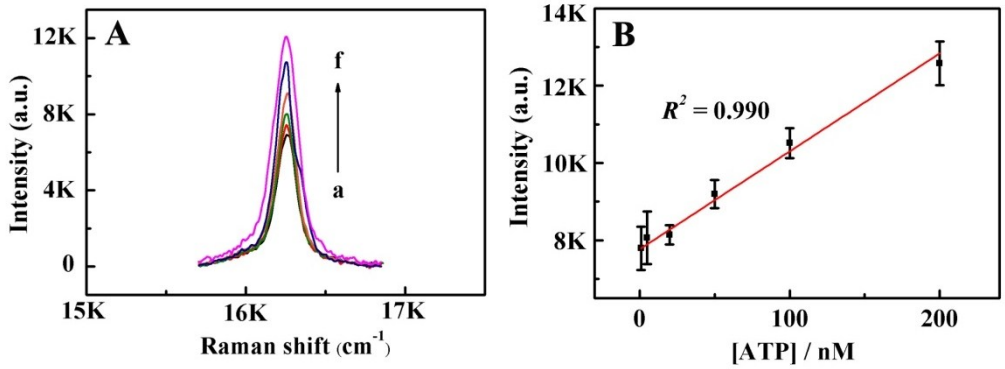

Figure S5. (A) The variation trend of Raman intensity of TB on GNFs without hairpin DNA (H1)with the increasing trend of the elevated concentration of ATP (nM) from a to f: (a) 1, (b) 5, (c) 20, (d) 50, (e) 100, (f) 200. (B) The calibration curve of TB Raman intensity on GNFs without hairpin DNA (H1) and ATP concentration (error bars = RSD, *n* = 3).

Table S2. The comparison between the proposed ratiometric SERS sensor and other biosensors for ATP detection.

Detection Method	Detection Target	Detection Limit	Linear Range	Refs
SERS	ATP	0.4 nM	1 - 2×10^2 nM	This work
Near Infrared Fluorescence	ATP	0.6 nM	–	[7]
Electrochemistry	ATP	0.5 nM	1 - 1×10^5 nM	[8]
Electrochemistry	ATP	0.6 nM	1 - 2×10^2 nM	[9]
Colorimetry	ATP	50 nM	50 - 1×10^3 nM	[10]
SERS	ATP	0.02 nM	0.1 - 1×10^2 nM	[11]

1.10 Selectivity of SERS Sensor.

In order to prove the selectivity of the SERS biosensor, four interfering proteins, including alpha fetoprotein (AFP), bovine serum protein (BSA), carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA), were measured under the same conditions. As shown in Figure S6A, almost same I_{TB}/I_{Cy5} values were obtained for the four interfering proteins and the blank experiment (no ATP). When ATP (100 nM) coexisted with the mixture of four interfering proteins, the I_{TB}/I_{Cy5} value was similar to that with only ATP (100 nM). Therefore, the above data indicated that this sensor had excellent specificity for detecting ATP.

1.11 Reproducibility of SERS Sensor.

The reproducibility of the Raman signal was a crucial indicator for the SERS sensing system. Therefore, the SERS spectra of 15 parallel samples were randomly

collected with the same concentration of ATP in Figure S6B, which had slight difference. Figure S6C shows the I_{TB}/I_{Cy5} values variation trend of these 15 points, and the relative standard deviation (RSD) was 5.24 % as a statistically meaningful result. As a result, the sensor had an excellent reproducibility.

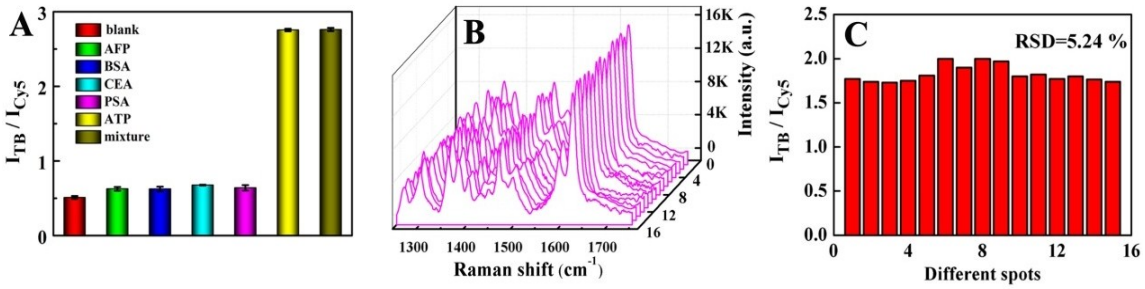

Figure S6. (A) I_{TB}/I_{Cy5} values of the SERS sensor with series of interfering proteins, and the concentration of AFP, BSA, CEA and PSA are 10 ng/mL, ATP was 100 nM. (B) The SERS spectra and (C) the I_{TB}/I_{Cy5} values of 15 different points after incubated with 100 nM ATP.

1.12 Practical Application.

We put a series of concentrations of ATP into serum samples which were diluted to 100-fold in healthy people serum to study the reliability of the SERS sensor. Table S3 displayed that the recovery rate of the sensor was 98.0~105.4 %, and the RSD was 0.10~0.62 %. Therefore, it proved that the SERS biosensor designed in this study was reliable and feasible for the clinical detection of ATP.

Table S3. Determination of different concentrations of ATP by the SERS platform in Human Blood Serum (*n* = 3).

Sample	Concentration of ATP added (nM)	Concentration obtained with sensor (nM)	Recovery (%)	RSD (%)
1	1	0.98	98.0	0.32
2	5	5.27	105.4	0.62
3	20	19.76	98.8	0.12
4	50	50.31	100.6	0.56
5	200	198.08	99.0	0.10

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