Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

A robust CRISPR-Cas12a biosensor coated with metal-organic framework

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Experimental Section

Chemicals and Materials.

Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) and 2-methylimidazole (2-MIM) were purchased from Alfa Aesar Co., Ltd. (Shanghai, China). Engen® Lba Cas12a (cpf1) proteins were ordered from NEW ENGLAND BioLabs Inc. (Beijing, China). All the chemicals were of analytical grade without any further treatment. Deionized water was obtained from a Millipore water purification system (Milli-Q, ≥18.2 MΩ) for the experiments. RNA was purchased from Invitrogen Inc. (Shanghai, China). Reporter DNA was synthesized by Takara Bio Inc. (Beijing, China). All of the other DNA sequences were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). All oligonucleotides were purified by high-performance liquid chromatography (HPLC). The sequences of the oligonucleotides are listed in Table S2.

Apparatus and Characterization.

Scanning electron microscope (SEM). For SEM images, the MOFs were immobilized on mica substrate, followed by the step of metal spraying. Then, the results were recorded on Nova NanoSEM450 (FEI, US).

Transmission electron microscopy (TEM). For TEM images, the MOFs were resuspended in water and drop-cast onto a TEM grid, then the results were accorded on JEM 2100F (JEM, Japan) at 200 kv.

Spectrophotometry. Fluorescence emission measurements were performed using F-7000 spectrometer (Hitachi, Japan). UV-vis absorption spectra were examined by a UV-2450 spectrometer (Shimadzu, Japan).

Powder X-ray Diffraction (PXRD). PXRD data was measured on a D8 Advance diffractometer (Bruker, Germany). Flat plate diffraction data was collected from the range 2θ = 2-50° at 40 kV/40 mA with Cu Kα line.

Zeta potential. The Cas12a, Cas12a@crRNA, and Cas12a/crRNA@ZIF-8 were dissolved or resuspended in water with a concentration of 1 μM at 25 °C. Then, the sample cell was placed on a NanoBrook 90Plus instrument (Brookhaven, USA) for the analysis.

BET analysis. The nitrogen isotherms of MOFs were obtained on a Micropore &Chemisorption Analyzer (Micromeritics ASAP2460, USA) at 77 K.

FT-IR analysis. FT-IR spectroscopy was measured by a NEXUS640 infrared spectrometer system (NICOLET).

Preparation of Cas12a/crRNA@ZIF-8.

Cas12a protein and crRNA were mixed at a molar ratio of 1:1 and incubated at room temperature for 10 minutes with a final concentration of 10 μM (40 μL). Then 450 μL of 2-MIM solution (2.5M, pH 7.4) was added to the Cas12a/crRNA complex solution, followed by 50 μL of $Zn(NO_3)_2$ (0.5 M) solution was slowly added to the above mixture under stirring conditions. After 20 minutes of continuous stirring, the resulting products were obtained by centrifugal separation and washed with deionized water three times to remove excess residues. Meanwhile, the supernatant solution was collected. Finally, the solids were then dried at 40 °C under reduced pressure overnight for later use.

To calculate the encapsulation efficiency of Cas12a/crRNA in ZIF-8 nanoparticles, we first constructed a standard curve based on the UV absorption of the free

Cas12a/crRNA solution at 260 nm. The efficiency can be calculated by measuring the UV absorption of supernatant solution.

Stability experiment.

Free Cas12a/crRNA complex and the Cas12a/ CrRNA@ZIF-8 nanocomposites were treated under three environmental conditions (heat treatment, organic reagent, and proteolysis). Heat treatment: 300 μL sample solution (1 μM) was taken and treated at 50 °C, 60 °C, and 70 °C for 30 minutes. Organic reagent: to conduct the experiments, free Cas12a/crRNA and Cas12a/crRNA@ZIF-8 were soaked in methanol and acetone for 1 hour, followed by the steps of centrifugation and resuspension using MWCO device (Millipore, 100 kDa). Proteolysis: two kinds of samples were soaked in solution containing 4 mg/mL proteolytic enzyme (protease K) for 1 hour. After the treatments, the detection performance of Cas12a/crRNA complex released from MOFs and free Cas12a/crRNA was compared.

Release and recovery of Cas12a/crRNA.

To release the encapsulated Cas12a/crRNA (MW: ~138 kDa), Cas12a/crRNA@ZIF-8 (1 mg/mL) was dispersed in PBS buffer (20 mM) with different pH (5.5, 6, and 7.4). When all the nanocomposites were dissolved, the process of release was completed. The Cas12a/crRNA complex was recovered using ultrafiltration with 100 kDa MWCO devices to remove the residues, followed by the washing step for two times. The centrifugal parameter is 11000 × g for 15 minutes.

Performance tests of CRISPR-Cas12a sensor.

200 nM Cas12a protein, 200 nM crRNA, and 400 nM reporter DNA are incubated at 37 °C for 30 minutes to form Cas12a/crRNA complex with a final volume of 100 μL. For biosensing test, the compound was diluted to the final concentration of 50 nM Cas12a, 50 nM crRNA, and 100 nM reporter DNA, followed by the addition of different concentration of activator DNA with a final volume of 40 μL. After 20 minutes, the reaction system was treated at 95 °C for 2 minutes to stop reaction. The intensity of fluorescence signal was recorded using a fluorescence spectrophotometer with an excitation wavelength at 474 nm and emission wavelength at 520 nm.

Table S1. Wrapping efficiency of Cas12a/crRNA (10 μM) and crystallinity at different ZIF-8 ratio.

Ratio (2-MIM to Zn^{2+})	wrapping efficiency	crystallinity
1:1	53%	High
10:1	66%	High
20:1	71%	High
45:1	87%	High
100:1	56%	Low

Table S2. Oligonucleotide sequences used in this work

Name	Sequence
crRNA	5'-UAA UUU CUA CUA AGU GUA GAU AAG GUU UGU GUG UUU ACC UG-3'
Activator DNA	5'- CCC AGG TAA ACA CAC AAA CCT T-3'
Random DNA	5'- CCC ATT TAA ATT GAC AAA CCT T-3'
Reporter DNA	5'- FAM-TTTATT -3'-BHQ
crRNA-MUC1	5'-UAA UUU CUA CUA AGU GUA GAU ACCAGGGUAUCCAAA GGAUCAACUGC-3'
A-DNA	5'-GCA GTT GAT CCT TTG GAT ACC CTG GT-3'
B-DNA	5'-ACC AGG GTA TCC AAA GGA TCA ACT GC-3'

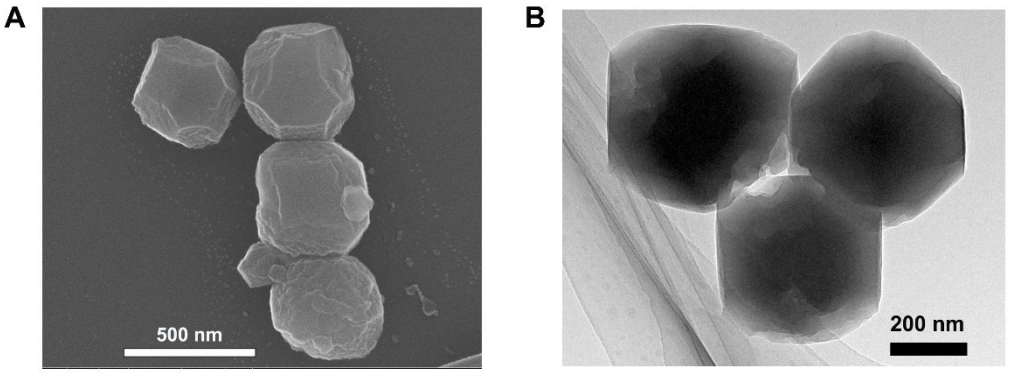

Fig. S1 (A) SEM and (B) TEM micrographs of Cas12a/crRNA@ZIF-8 with high magnification.

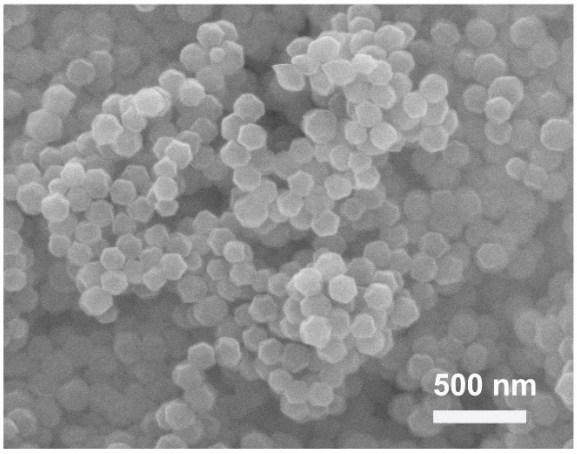

Fig. S2 SEM micrographs of ZIF-8 nanoparticles.

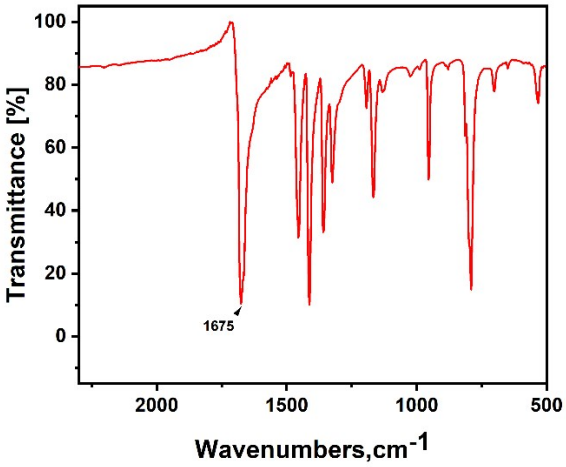

Fig. S3 FT-IR spectrum of Cas12a/crRNA@ZIF-8.

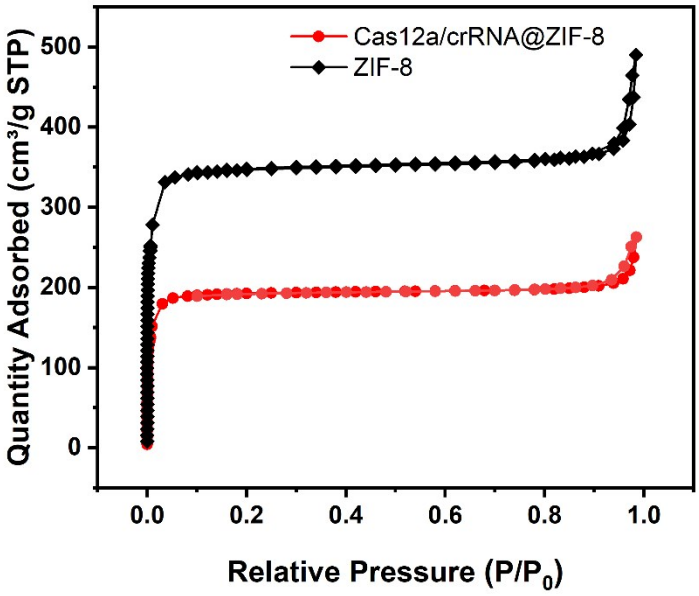

Fig. S4 Nitrogen adsorption-desorption isotherm of ZIF-8 and Cas12a/crRNA@ZIF-8. The BET area of ZIF-8 and Cas12a/crRNA @ZIF-8 are 1135.19 m^2/g and 630.39 m^2/g, respectively.

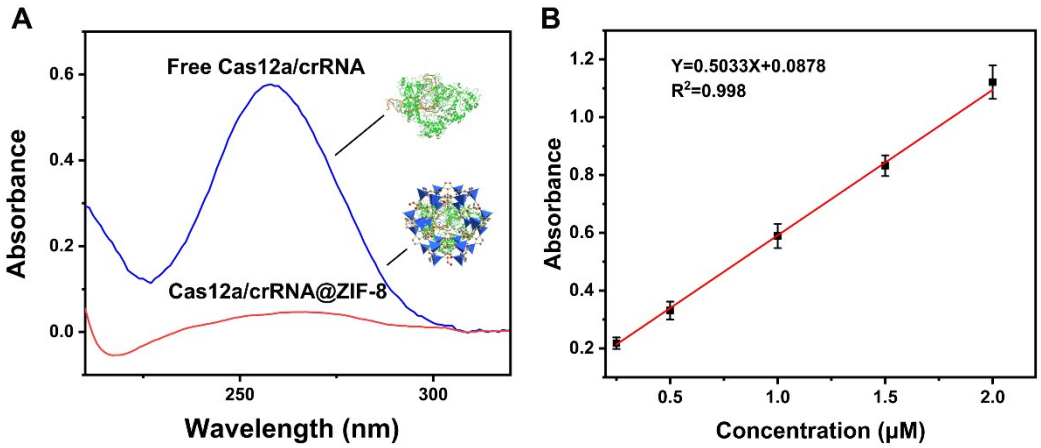

Fig. S5 (A) UV−vis spectra of Cas12a/crRNA@ZIF-8 compared to free Cas12a/crRNA complex. (B) Plot of absorbance at 260 nm versus varying concentrations of free Cas12a/crRNA complex from 0.1 to 20 μM.