

Electronic Supporting Information

Au nanoparticles deposited on ultrathin two-dimensional covalent organic framework nanosheets for in vitro and intracellular sensing

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Table S1. DNA sequences used in this work.

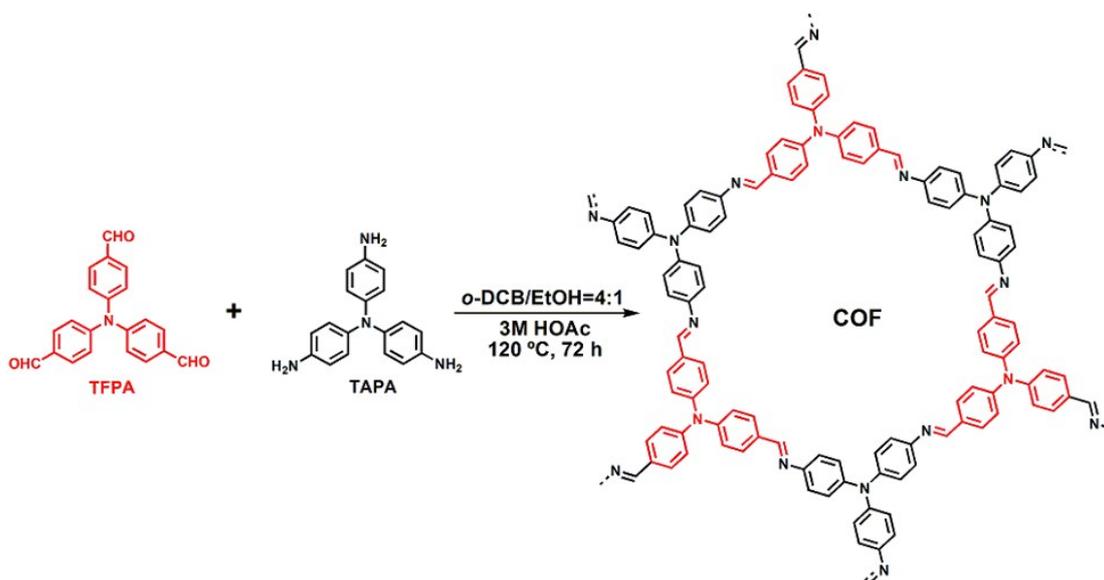
| Name | Sequence (5'–3') |
|--------------------------------|---|
| Probe1 | CCC TTT AAC CCC TTT TCT TTG CTA AAA CTG GAT CCT CAA TTG |
| Probe2 | CCT CCT TCC TCC TT TAC CAC ATC ATC CAT ATA ACT GAA AGC CAA |
| HAV(T1) | CAA TTG AGG ATC CAG TTT TAG CAA AGA A |
| HBV(T2) | TTG GCT TTC AGT TAT ATG GAT GAT GTG GTA |
| Single-base mismatch DNA (SM1) | CAA TTG AGG ATC <u>T</u> AG TTT TAG CAA AGA A |
| Single-base mismatch DNA (SM2) | TTG GCT TTC AGT TAT ATG GAT GAT <u>G</u> A GTA |
| Random DNA | TAG CTT ATC AGA CAG ATG TTG ACT ATC AGA |
| Probe (P3) | CCT CCT TCC TCC TTT GGG TTA GGG TTA GGG TTA GGG TTT |

Experimental Section

Materials. Tris(4-aminophenyl)amine (TAPA, 98%), tris(4-formylphenyl)amine (TFPA, 96%), 1,2-dichlorobenzene (o-DCB, 99%), and acetic acid (AcOH, 99.5%) were purchased from Tokyo Chemical Industry Co., Ltd. Gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, $\geq 99.9\%$), polyvinyl pyrrolidone (PVP) (average $\bar{M}_w = 10000$) and sodium citrate were purchased from Sigma-Aldrich. Ethanol (EtOH), acetone, dichloromethane, and methanol with analytical purity were purchased from Merck. All DNA strands were purchased from Sangon Biotech CO., LTD. (Shanghai, China). The human bone marrow mesenchymal stem cells (MSCs) were obtained from Cyagen Biosciences Inc (Gongzhou, China). Deionized water was obtained from the Milli-Q System (Millipore, Billerica, MA). All the chemical reagents were used as received without further purification.

Apparatus. Transmission electron microscopy (TEM) characterization was performed with JEOL 2100F (Japan) operated at 200 kV. Atomic force microscopy (AFM) images were recorded with a Dimension 3100 AFM with a Nanoscope IIIa controller (Veeco, Fremont, CA, USA). Fourier transform infrared spectroscopy (FTIR) spectra were collected on a PerkinElmer FTIR Spectrum GX. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed with a Dual-view Optima 5300 DV ICP-OES system. X-ray photoelectron spectroscopy (XPS) measurement was carried out on a VG ESCALAB 220I-XL system. The X-ray diffraction (XRD) patterns were recorded by Shimadzu XRD-6000 with a $\text{Cu K}\alpha$ ($\lambda = 1.54178 \text{ \AA}$) source. Fluorescence spectra were collected using a Shimadzu RF-5301PC fluorophotometer.

Synthesis of the COF nanosheets (NSs).



As reported by Zhang's group, COF nanosheets (NSs) were synthesized by the following method.¹ TAPA (5 mg, 0.017 mmol), TFPA (5 mg, 0.017 mmol) and *o*-DCB/ethanol (4:1 v/v, 1.5 mL) were mixed in a 10 mL Pyrex tube. A clear mixture was obtained by sonication for 5 min, and then acetic acid (0.06 mL, 3 M) was added. After that, the Pyrex tube was frozen in a liquid N₂ bath at 77 K, and degassed by three freeze-pump-thaw cycles. The tube was sealed by using vacuum and heated at 120 °C for 72 h. The product was collected by centrifugation, sonicated and washed with anhydrous ethanol, anhydrous acetone, and anhydrous dichloromethane, subsequently. The final sample was dried under vacuum for 12 h to form a yellow powder.

Synthesis of the Au nanoparticles (NPs)/COF NSs. As reported, for reproducibly narrow nanoparticle size distributions, the hybrid liquid-type reduction method superseded the initial reduction with only sodium citrate or polyvinyl pyrrolidone (PVP),²⁻⁴ and the combination of steric stabilization from the PVP and electrostatic stabilization from the citrate ions prevents agglomeration.⁵ The systemic control of size in synthesis of gold nanoparticle had been reported by Wheatley et al. using PVP and

sodium citrate, with mean particle sizes about 3-20 nm.⁶ In this regard, 50 µg COF power was dispersed into 50 mL of anhydrous ethanol and sonicated for 6 h to obtain the exfoliated COF nanosheets, and then moved into a 100 mL flask. The solution was stirred and heated to 80 °C equipped with a reflux condenser. PVP (12 mg) and sodium citrate (32mg, 0.11 mmol) were added into the COF NSs solution. After 5 min, 1 mL ethanol solution of H₂AuCl₄ (8 mg, 0.02 mmol) was rapidly added into the flask. The molar ratio of the repeat unit of PVP to H₂AuCl₄ was about 5:1 in this experiment. After 2 h, the product was collected by centrifugation at 5,500 rpm for 5 min, and washed three times with water to remove excess ethanol and PVP.

ICP-OES measurements. 1 mL HNO₃ (70%) was added into the double Au NPs/COF NSs obtained in the above synthesis method (containing COF NSs about 100 µg), and then the solution was diluted with 6mL Milli-Q water. After ICP–OES measurement, the concentration of Au was 2.85 ppm. The loading of Au was 19.95 µg on NPs/COF NSs, and the result of the mass ratio of COF to Au is about 5:1.

Synthesis of Fluorescent Ag nanoclusters (NCs). Because of the conformational flexibility and negative charge of phosphate backbone for single-stranded DNA bases, silver ions (Ag⁺) has a high affinity for DNA bases (affinities of >10⁵ M⁻¹) to facilitate the nucleation of neutral clusters (Figure 1).⁷ These loaded Ag⁺ can be reduced by the strong reducing agent (NaBH₄) to form oligonucleotide-encapsulated nanoclusters along the contour of the DNA template.^{8,9} Dickson and Petty had demonstrated that the ratio of BH₄⁻:Ag⁺:oligonucleotide at 6:6:1 is the most widely employed proportion reaching the maximum fluorescence intensities of Ag NCs.^{10, 11} In this work, the 30 µL

DNA (100 μM) solution was diluted in to 134 μL of citrate buffer (20 mM, pH 7.0) and heated at 95 $^{\circ}\text{C}$ for 5 min, and then the solution was kept in the ice bath for 1 h. After that, 3 μL of AgNO_3 (6 mM) was added into the DNA solution, followed by vigorous shaking for 3 min. After keeping the mixture in the ice bath for 15 min, the freshly prepared NaBH_4 (3 μL , 6 mM) was added, and the mixture was shaken vigorously for 5 min. The final mixture was kept in the dark for 24 h at room temperature before the measurement.

Analysis of Target DNAs and K^+ . In this experiment, all the DNA solutions were heated at 95 $^{\circ}\text{C}$ for 5 min and cooled down to room temperature. Probe DNA (50 nM) was mixed with Au NPs/COF NSs (12 $\mu\text{g}/\text{mL}$) in citrate buffer solution for 10 min. After that, the target molecules with different concentration were added. Fluorescence measurements were performed at room temperature.

Multiplexed Fluorescent DNAs Detection. Two types of probe DNA (25 nM for each) were added into Au NPs/COF NSs solution (12 $\mu\text{g}/\text{mL}$), and the mixture was incubated for 10 min. Then two target DNA sequences (50 nM for each) were added in the mixture at room temperature to hybridize with probe DNA. The fluorescence measurements were carried out at room temperature.

Cytotoxicity Assay. MSCs were dispersed in replicate 96-well microtiter plates with a total volume of 150 μL in each well. After overnight culture, the nanoprobe (12 $\mu\text{g}/\text{mL}$) was added and incubated for 24 h. Subsequently, 0.1 mL of MTT solution (0.5 mg mL^{-1}) was added to each well at 37 $^{\circ}\text{C}$ and incubated for 4 h. After the addition of DMSO (100 μL well $^{-1}$), the plate was shaken at room temperature for 5 min. The

absorbance was measured at 490 nm.

Cell Culture for imaging. MSCs were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (Sigma-Aldrich), and incubated at 37 °C in 5% CO₂ atmosphere. For the imaging experiment, cells were internalized with K⁺ sensor for 2 h. Then the medium containing the sensor was removed, and new medium was added. Hoechst33342 was used to stain the nucleus for 20 min, and then calcein-AM was used to stain the cytoplasm for 20 min. Hoechst33342 was excited at 405 nm and the blue emission was collected in the range of 420-460 nm. Calcein-AM was excited at 488 nm and the green emission was collected in the range of 500-560 nm. Sensor was excited at 488 nm and the red emission was collected in the range of 630-700 nm.

Monitoring the intracellular K⁺ efflux. Cells were incubated with K⁺ sensor at 37 °C for 2 h. Then the excess sensor was removed, and new medium was added. The stimuli materials were added for 1 h. The final concentrations of ATP, amphotericin B, nigericin, bumetanide, and ouabain, were 100 μM, 3.25 μM, 5 μM, 10 μM and 10 μM, respectively.

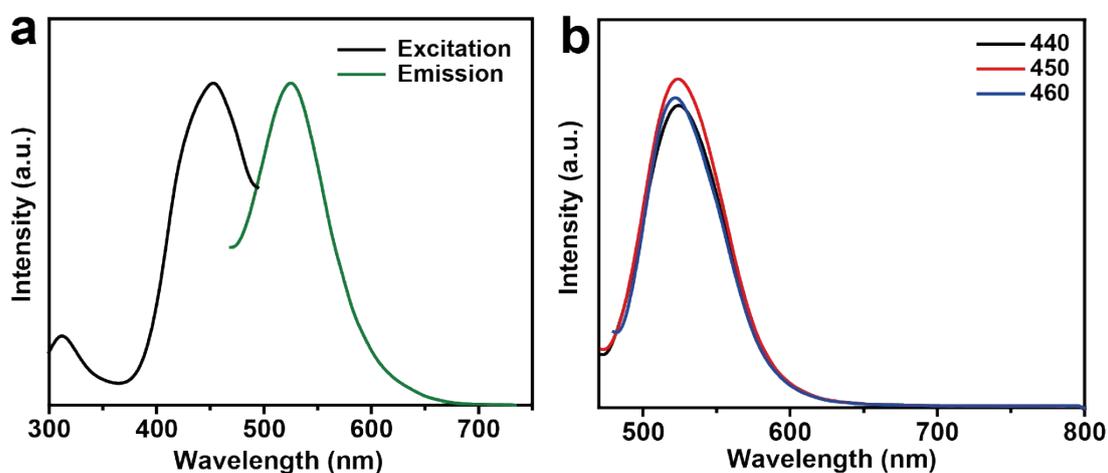


Fig. S1 (a) Excitation and emission spectra for the green-emitting fluorescent probe. (b) Emission spectra of the green-emitting fluorescent probe at different excitation wavelength.

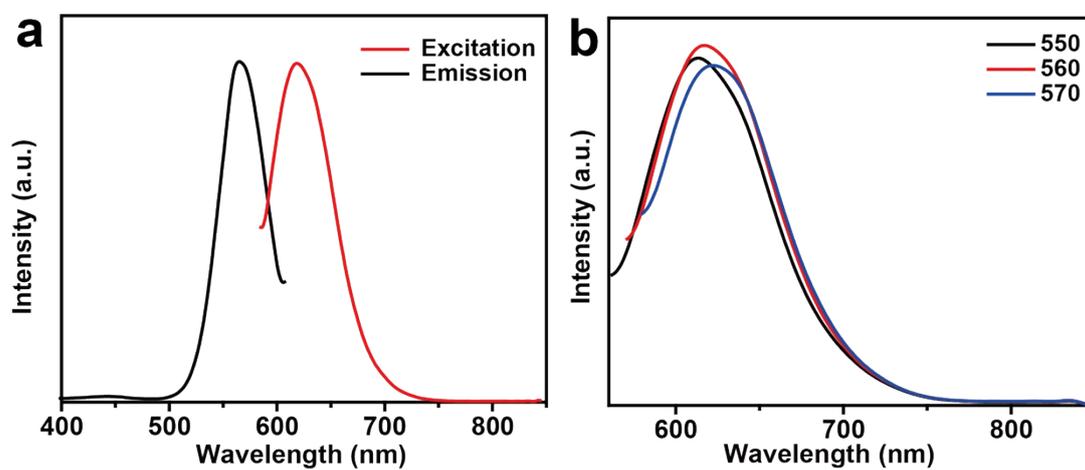


Fig. S2 (a) Excitation and emission spectra for the red-emitting fluorescent probe. (b) Emission spectra of the red-emitting fluorescent probe at different excitation wavelength.

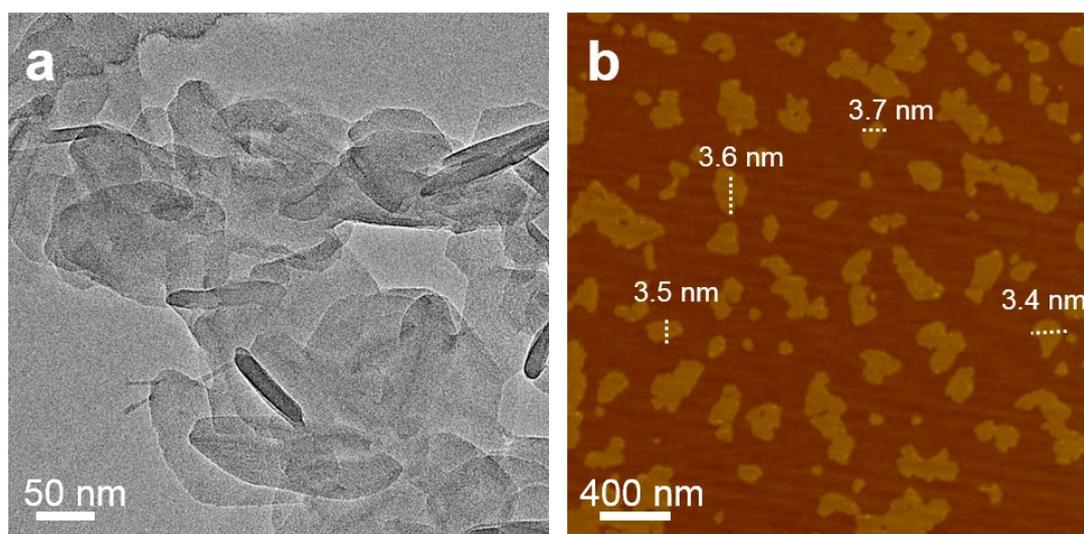


Fig. S3 (a) TEM image and (b) AFM image of COF NSs.

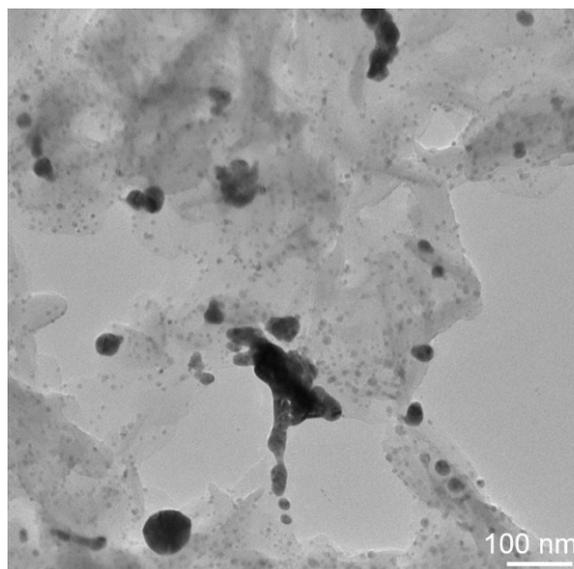


Fig. S4 TEM image of aggregated Au NPs.

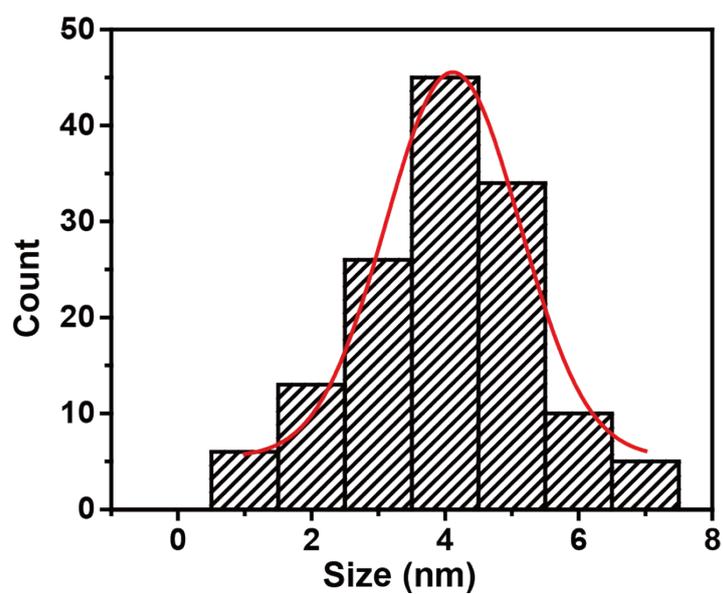


Fig. S5 The size distribution of Au NPs deposited on COF NSs.

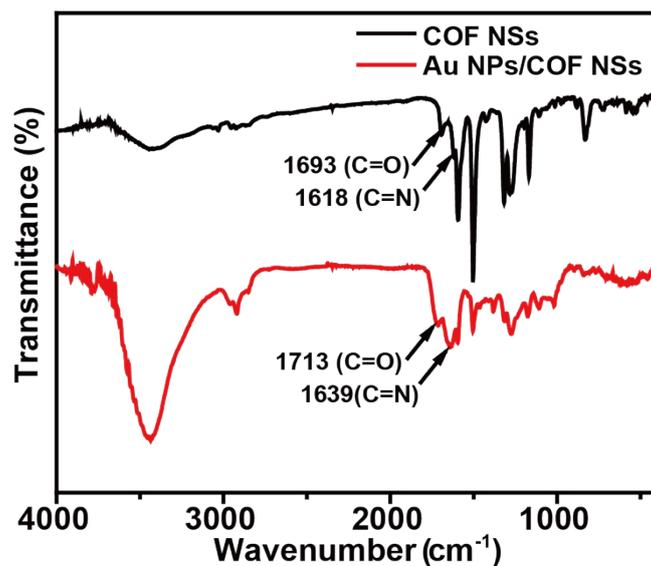


Fig. S6 FTIR spectra of COF NSs and Au NPs/COF NSs.

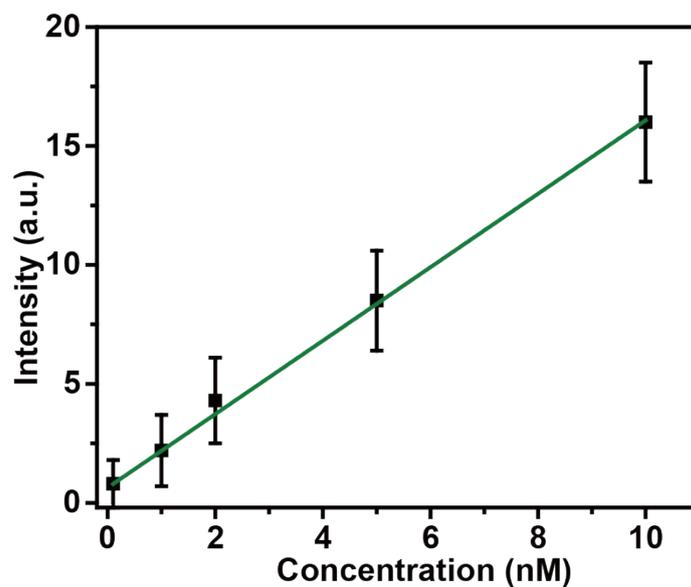


Fig. S7 Linear relationship between the fluorescence intensity and the concentration of T1 (0.1 to 10 nM) based on Au NPs/COF NSs biosensor.

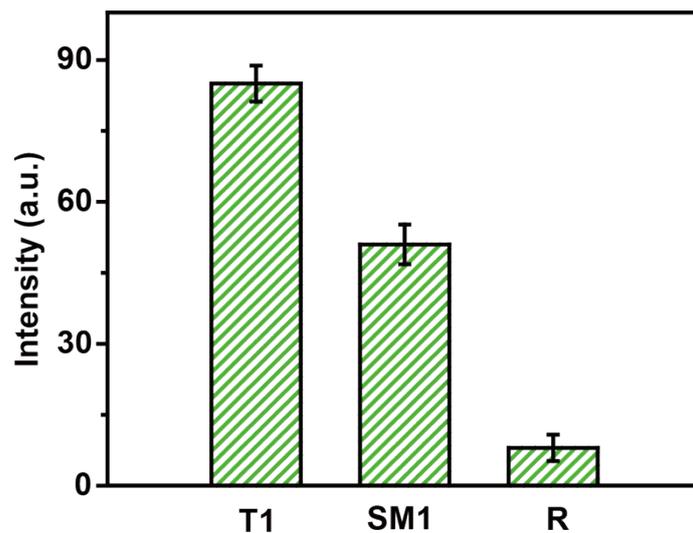


Fig. S8 Selectivity of Au NPs/COF NSs sensor toward T1, single-base mismatch DNA (SM1) and random DNA (RD).

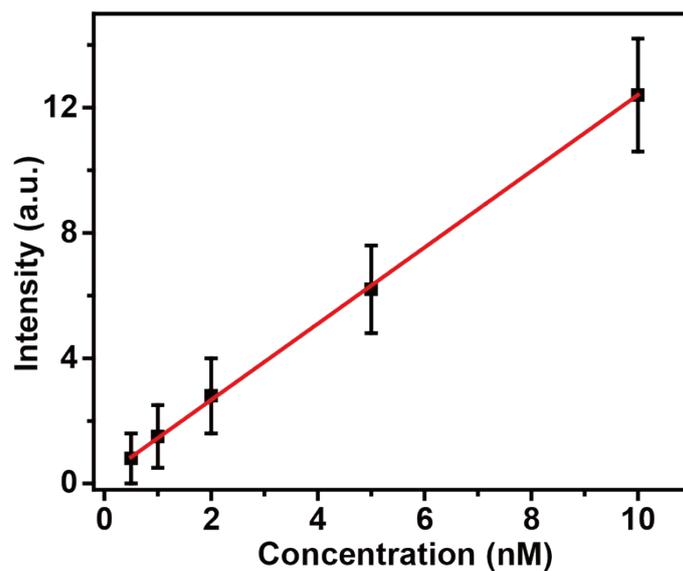


Fig. S9 Linear relationship between the fluorescence intensity and the concentration of T2 (0.5 to 10 nM).

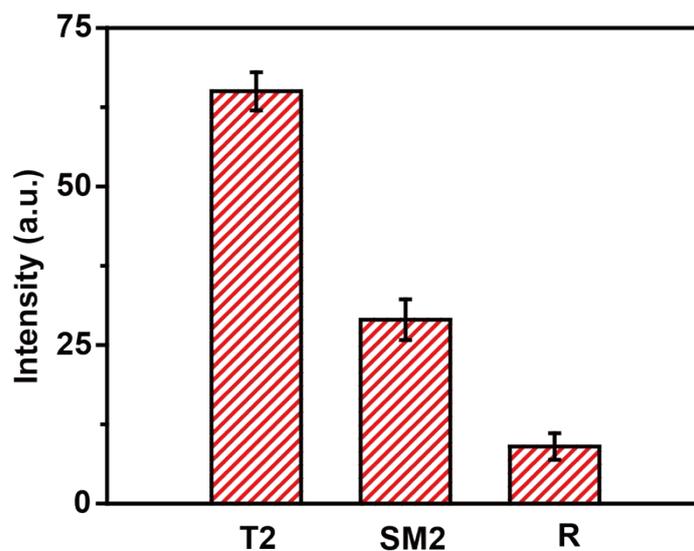


Fig. S10 Selectivity of Au NPs/COF NSs sensor toward T2, single-base mismatch DNA (SM2) and random DNA (RD).

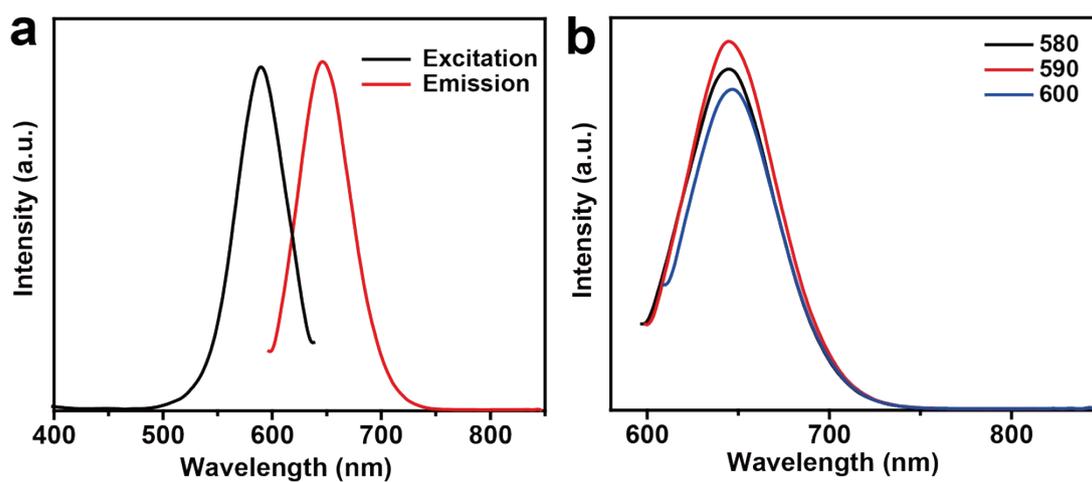


Fig. S11 (a) Excitation and emission spectra for the aptamer-based fluorescent probe. (b) Emission spectra of the aptamer-based fluorescent probe at different excitation wavelength.

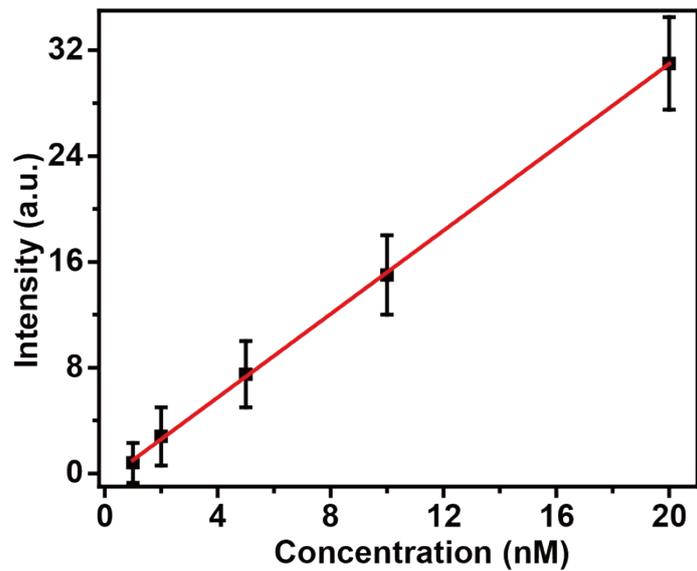


Fig. S12 Linear relationship between the fluorescence intensity and the concentration of K^+ from 1 to 20 nM.

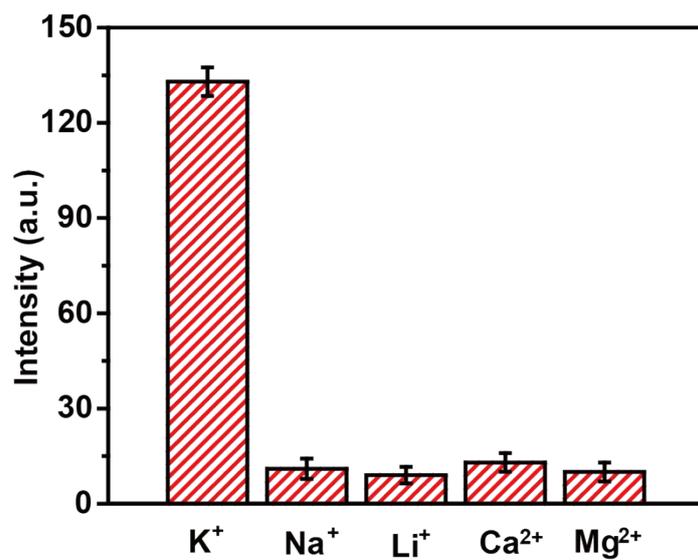


Fig. S13 Selectivity of aptamer-based sensor toward different cations.

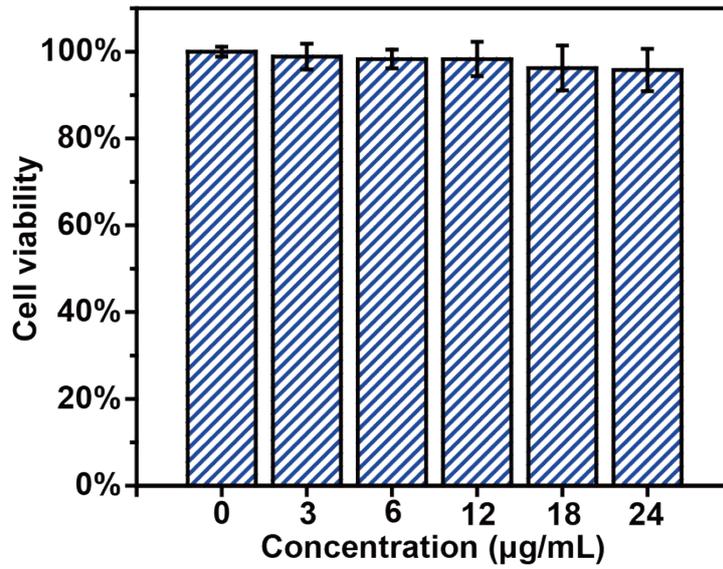


Fig. S14 Cytotoxicity tests by culturing MSCs with K^+ sensor for 24 h in the MTT assay.

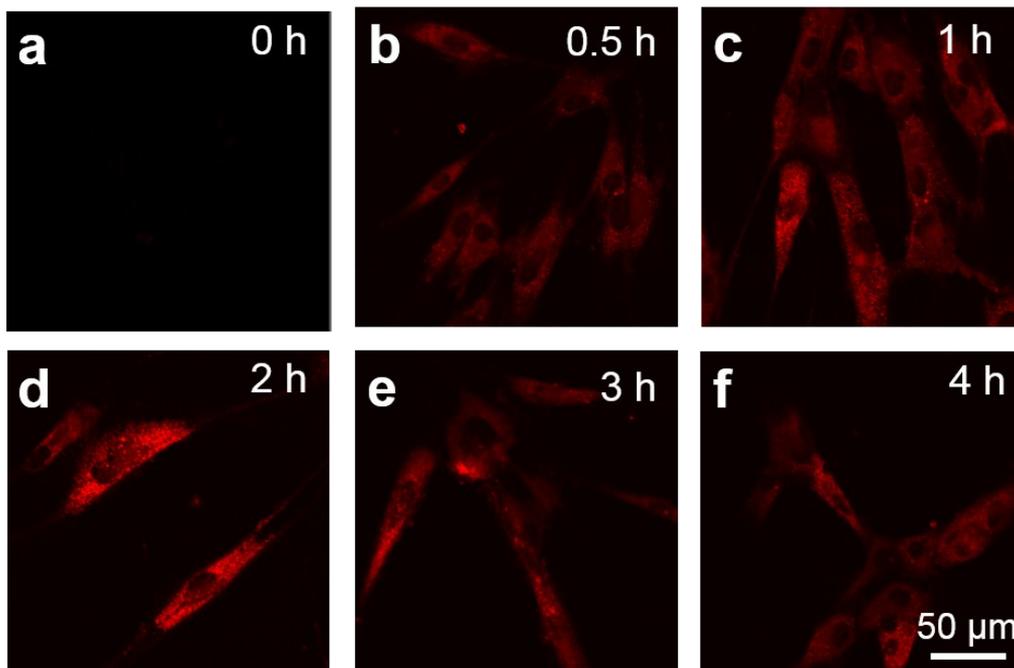


Fig. S15 Confocal fluorescence images of MCF-7 cells treated with the K^+ sensor for (a) 0, (b) 0.5, (c) 1, (d) 2, (e) 3, (f) 4 h.

References

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