Electronic Supplementary Information

Fluorescence proximity assay based on a metal–organic framework platform

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Table of Contents

Section 1. Materials and Instrumentation

Zirconium chloride ($ZrCl_4$), 2-aminoterephthalic acid (NH_2-H_2BDC), *N,N*-dimethylformamide (DMF) were purchased from Sigma-Aldrich Chemical Co., Ltd. (Shanghai, China). Carboxylic-functionalized single-walled carbon nanotubes (SWCNTs) were purchased from Sigma–Aldrich (USA). Graphene oxide (GO) was made from natural graphite flake. All other reagents were of analytical grade and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used as the water source throughout the work. Prostate specific antigen (PSA), mouse monoclonal anti-PSA antibodies (clone no. P27B1 as Ab1 and P27A10 as Ab2), human serum albumin (HSA), and immunoglobulin (IgG) were purchased from Shuangliu Zhenglong Biochem Lab (Chengdu, China). Carcinoembryonic antigen (CEA) and anti-CEA antibodies (mouse monoclonal antibodies, clone no. bsm-1623M as Ab1 and bsm-1624M as Ab2) were purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China). Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) was supplied by Heowns Biochem LLC (China). Dithiothreitol (DTT) was from Shanghai Sangon Biotechnology Co. Ltd. (China). TE buffer (10 mM Tris-HCl, containing 1 mM EDTA and 0.3 M NaCl, pH 7.9) was used for the preparation of oligonucleotide stock solutions. PBS1 (55 mM phosphate, containing 150 mM NaCl, 20 mM EDTA, pH 7.4) and PBS2 (55 mM phosphate, containing 150 mM NaCl, 5 mM EDTA, pH 7.4) were used to prepare the DNA-Ab probe. Hybridization buffer (HB, pH 7.4) contained 10 mM Tris-HCl, 50 mM NaCl,

and 10 mM $MgCl_2$. Oligonucleotides were obtained from Sangon Biotechnology Inc. (Shanghai, China), and the oligonucleotide sequences were as follows:

DNA1: 5′-FAM-GCG**GATCTATGTATC**ACATATTTTTTTTTTTTTTTTT*AGATCAGCTCTAGCTCTTTG*-SH-3′

Thiolated DNA2 (L12): 5′-SH-*TACTCACTTTACCAACCAGC*TTTTTTTTTTTTTTTTTA**GATACATAGATC**-3′

DNA2 (L10): 5′-*TACTCACTTTACCAACCAGC*TTTTTTTTTTTTTTTTTACT**TACATAGATC**-3′

DNA2 (L12): 5′-*TACTCACTTTACCAACCAGC*TTTTTTTTTTTTTTTTTA**GATACATAGATC**-3′

DNA2 (L14): 5′-*TACTCACTTTACCAACCAGC*TTTTTTTTTTTTTTTTTA**GTGATACATAGATC**-3′

Ref DNA: 5′-*GCTGGTTGGTAAAGTGAGTA*TTTTCTTTAATTTCTTATTTCTCTTAATTTTAATTTGTTT*CAAAGAGCTAGAGCTGATCT*-3′

The morphology of the as-synthesized nanomaterials was investigated with a XL-30E scanning electron microscope (SEM). UV−vis absorption spectra were obtained using a UV-3600 UV−vis−NIR spectrophotometer (Shimadzu Co. Kyoto, Japan). Fluorescence (FL) spectra were recorded at room temperature in a quartz cuvette on a FLSP920 fluorescence system. Powder X-ray diffraction (PXRD) was recorded on a Bruker D8-Focus Bragg−Brentano X-ray Powder diffractometer equipped with a Cu

sealed tube (λ= 1.54178 Å) at 40 kV and 40 mA.

Section 2. Experimental Section

Synthesis of UiO-66-NH_2. The UiO-66-NH_2 was synthesized according to the previous report with minor modifications.[1] Typically, $ZrCl_4$ (75 mg), and NH_2-H_2BDC (31 mg) in DMF (10 mL) were ultrasonically dissolved in a Teflon liner (20 mL) at room temperature, and then 28 μL of water was added and placed in an oven at 120 °C for 24 h. After cooling down to room temperature, the solid MOFs were harvested by centrifugation, then dried under vacuum at ambient temperature.

Preparation of DNA-Ab. The DNA-labeled antibodies (DNA-Ab) were prepared by a modified procedure.[2] Briefly, anti-PSA antibody or anti-CEA antibody (2 mg mL^{-1}) was first reacted with a 20-fold molar excess of SMCC in PBS1 for 2 h at room temprature. The obtained Ab2-SMCC was purified by ultrafiltration using a 100 KD Millipore (10 000 rpm, 10 min). In parallel, 12 μL of 100 μM thiolated DNA was reduced with 16 μL of 100 mM DTT in PBS1 at 37 °C for 1 h. The reduced DNA was purified by ultrafiltration using a 10 KD Millipore (10 000 rpm, 10 min). Then, the resulting Ab-SMCC and DNA were mixed in 200 μL of PBS2, incubated overnight at 4 °C, and purified by ultrafiltration using a 100 KD Millipore (10 000 rpm, 10 min) for several times to remove the unreacted DNA. The obtained DNA-Ab was collected at a concentration of 6.0 μM in PBS2, which was diluted with PBS2 at 100-fold prior to use.

Mesurement Procedure. Firstly, 2 mg UiO-66-NH_2 were sonicated in 1 mL buffer solution and stored for use. Then, 1900 μL buffer, 20 μL fluorescent oligonucleotide labeled antibody (FAM-DNA1-Ab1) and 30 μL UiO-66-NH_2 were mixed together and

shaken for 60 min at room temperature. For PSA assays, the composite was mixed with 50 μL Ab2-DNA2 and different concentrations of PSA, being incubated for 3 h at 37 ^{o}C. After reaction, the resulting solution was subjected to flurescence measurements. The fluorescence spectra were recorded at room temperature in a quartz cuvette on a FLSP920 fluorescence system. All fluorescent measurements were carried out at room temperature. The emission spectra were collected from 500 to 660 nm under the excitation wavelength of 480 nm, and the fluorescence intensity at 518 nm is used for analysis.

Section 3. Characterizations for UiO-66-NH_2

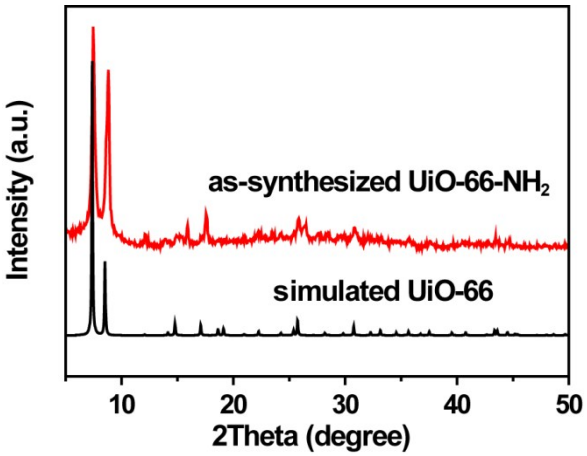


Fig. S1 Powder XRD patterns for experimental UiO-66-NH_2 and simulated UiO-66.

Section 4.

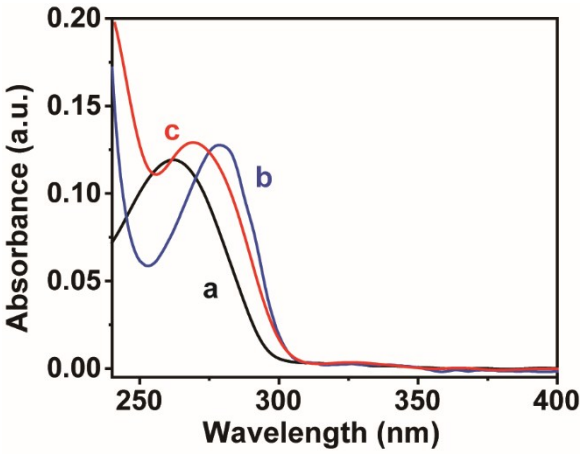


Fig. S2 UV-visible spectra of DNA (a), Ab (b), and DNA-Ab (c).

Section 5. Selection of nanoquenchers (MOF, SWCNTs, and GO)

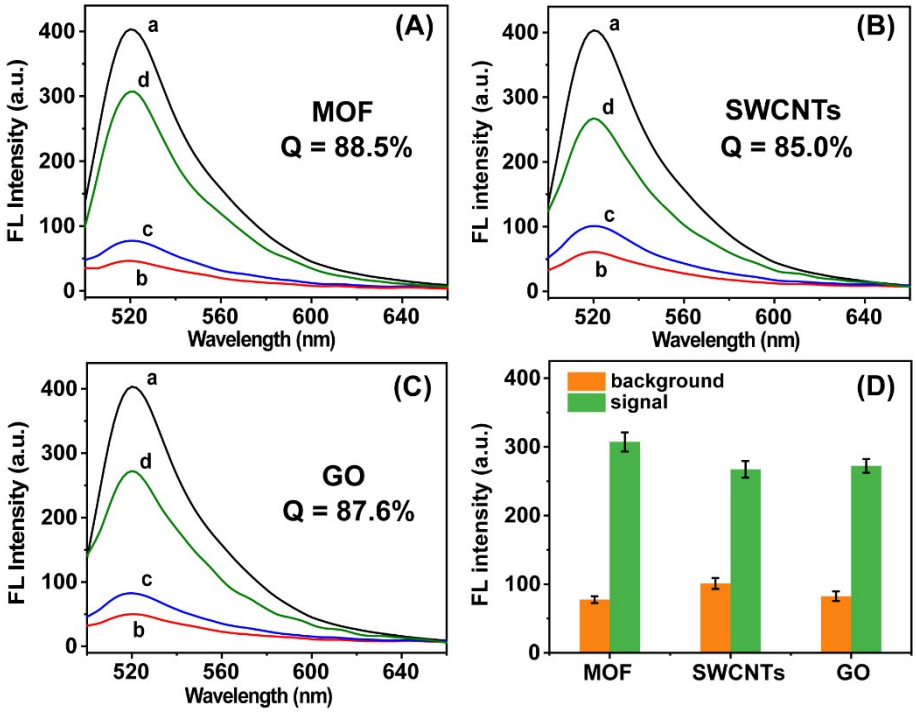
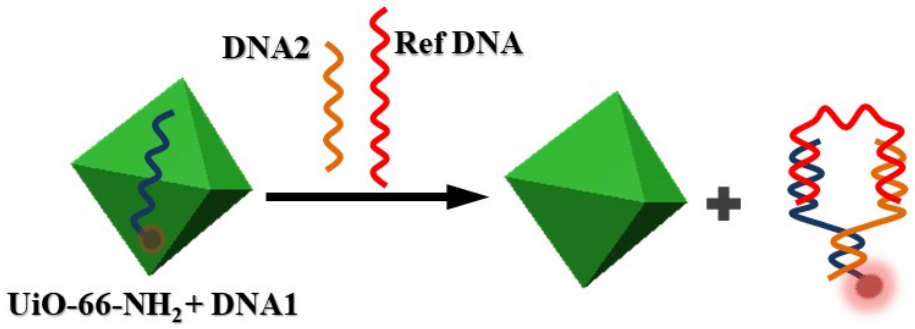


Fig. S3 Fluorescence spectra of DNA1-Ab1 in different systems with different nanoquenchers ((A) MOF, (B) SWCNTs, and (C) GO) (λ_{ex} = 480 nm): (a) DNA1-Ab1; (b) DNA1-Ab1 + nanoquencher; (c) DNA1-Ab1 + nanoquencher + DNA2-Ab2; (d) DNA1-Ab1 + nanoquencher + DNA2-Ab2 + PSA. (D) Effects of different nanoquenchers (MOF, SWCNTs, and GO) on fluorescence intensity. Error bars, SD, n = 3.

Section 6.



Scheme S1 Illustration of UiO-66-NH_2-based FPA by using Ref DNA instead of target protein.

The detection mechanism of FPA relied on simultaneous recognition of target protein by a pair of Ab-DNA affinity probes which brought DNA1 and DNA2 in proximity to process the Ab-DNA displacement from UiO-66-NH_2. Thus, the number of complementary bases between DNA1 and DNA2 should be optimized. In order to simplify the optimization procedure, Ref DNA containing 80 bases (bp) was used to mimic the sandwich immunocomplex.[3,4]

Section 7. The analytical performance of the proposed UiO-66-NH_2-based FPA for CEA detection

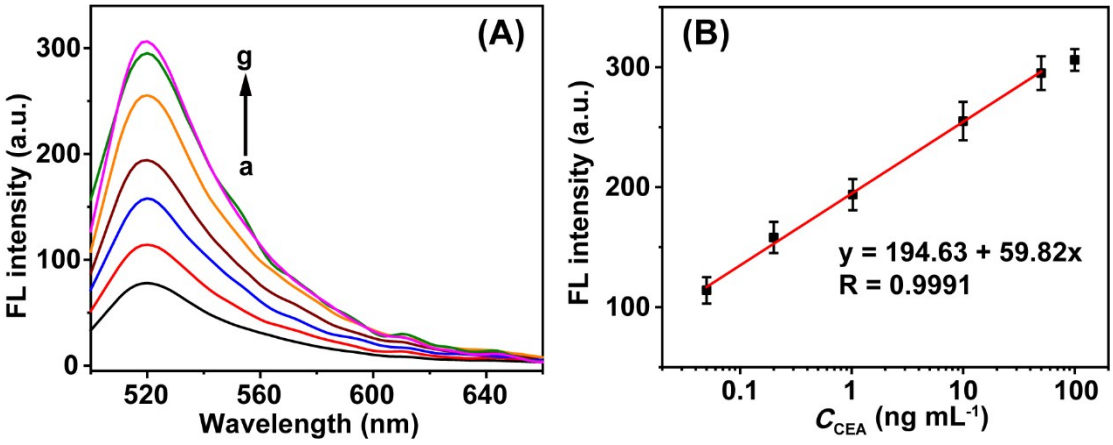


Fig. S4 (A) Fluorescence spectra under λ_{ex} = 480 nm of FPA to 0, 0.05, 0.2, 1, 10, 50, and 100 ng mL^{-1} (from a to g) CEA; (B) plot of fluorescence intensity vs. logarithm of concentrations of CEA. Error bars, SD, n = 3.

The FPA strategy based on a MOF platform was extended to detection of other biomarker protein, CEA. With the increasing concentrations of CEA, the fluorescence intensity increased correspondingly (Fig. S4A). The resulting calibration curve was illustrated in Fig. S4B. The fluorescence intensity was proportional to the logarithm value of the CEA concentration in a range of 0.05 to 50 ng mL^{-1}. The regression equation could be represented as I (a.u.) = 194.63 + 59.82 log C (ng mL^{-1}) with a correlation coefficient of 0.9991. The limit of detection (LOD) for CEA was estimated to be 2.4 pg mL^{-1} (signal-to-noise ratio of 3).

Section 8. The selectivity of proposed immunosensor

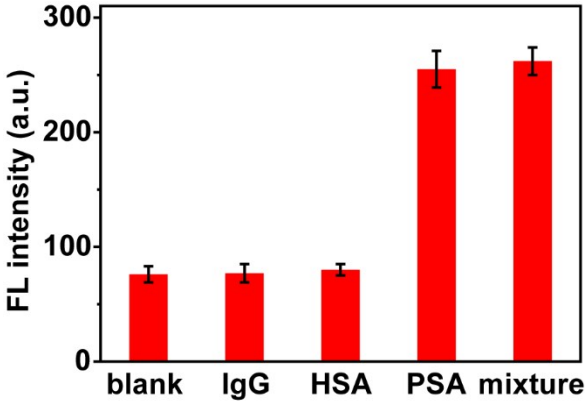


Fig. S5 Selectivity of FPA sensor for PSA detection (20 ng mL^{-1} IgG, 20 ng mL^{-1} HSA, and 10 ng mL^{-1} PSA). Error bars, SD, n = 3.

Section 9.

Table S1 Comparison of the performance of our proposed FPA with other published PSA immunosensors.

Analytical method	Linear range	Detection limit	Ref
LRET[a]	0–500 pM	1.0 pM	5
multicolor ECL[b]	1.0–20 ng mL^{-1}	–	6
electrochemistry	1.0–1000 ng mL^{-1}	10 pg mL^{-1}	7
SERS[c]	1.0 pg mL^{-1} to 10.0 ng mL^{-1}	0.65 pg mL^{-1}	8
metal-enhanced fluorescence	0.1–100 ng mL^{-1}	27 pg mL^{-1}	9
colorimetric immunoassay	20–2000 pg mL^{-1}	0.80 pg mL^{-1}	10
FPA	0.05–50 ng mL^{-1}	2.8 pg mL^{-1}	**This work**

[a]LRET: luminescence resonance energy transfer; [b]ECL: electrochemiluminescence; [c]SERS: surface-enhanced Raman scattering.

Section 10.

Table S2 Detection of PSA and CEA in serum samples (n = 4).

Tumor Markers	Added (ng mL^{-1})	Found (ng mL^{-1})	CV (%)	Recovery (%)
PSA	0.1	0.097	2.7	95.6
	1	1.056	3.2	102.8
	10	9.872	1.3	97.2
CEA	0.1	0.103	2.4	104.5
	1	0.985	1.6	96.7
	10	10.263	3.7	103.2

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