Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

In-situ Synthesis of Photoluminescence-quenching Nanopaper for Rapid and Robust Detection of Pathogen and Protein

Yucheng Liu,[a,c] Guobin Mao,[a] Wang Wang,[a] Songbai Tian,[a] Xinghu Ji,*[a] Meng Liu,[b] Zhike He*[a]

[a] Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China

[b] Key Laboratory of Industrial Ecology and Engineering (Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Dalin 116085, China;

[c] Division of Engineering in Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

*Corresponding author Tel: +86 27-68756557; E-mail: xhu@whu.edu.cn (X. Ji)

Tel: +86 27-68756557; E-mail: zhkhe@whu.edu.cn (Z. He)

EXPERIMENTAL SECTION

Reagents and materials

All reagents and apparatus are provided in the Supporting Information Materials and apparatus: Sodium phosphate monobasic dihydrate(($NaH_2PO_4 \cdot 2H_2O$), sodium phosphate dibasic (Na_2HPO_4), calcium chloride ($CaCl_2$), cytochrome c and MUC1 Prestige Antigens were purchased from Sigma–Aldrich (St. Louis, MO, USA). The aptamer of MUC1 and the aptamer of *E. coil* O157:H7 were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China), and the sequences of the oligonucleotide for synthesizing Apt-QDs are listed as followed:

MUC-1 Apt:

GCAGTTGATCCTTTGGATACCCTGGAAAAAAAG*G*G*G*G*G*G*G*

E.coil O157:H7 Apt:

TCGCGCGAGTCGTCTGGGGACAGGGAGTGCGCTGCTCCCCCCGCATCGTC

CTCCCAAAAAAAAAG*G*G*G*G*G*G*G*

The preparation of DNA functionalized QDs

The procedure for synthesizing DNA functionalized CdTe: Zn^{2+} QDs was provided in our previous work in detail.[8]

The fabrication of paper-based analytical devices

Photolithography was chosen to pattern the paper in this article, and the detailed step descriptions were listed in our previous work.[14]

In-situ preparation of cyt.c embedded nanopaper

The nanopaper was prepared by sequentially adding 2 μL of cyt.c solution containing phosphate solution and 2 μL of aqueous calcium chloride solution ($CaCl_2$) onto the cellulose paper. To be specific, the 1mg of cyt.c was first dissolved in 1mL of PBS buffer solution (0.1M, 10 mM NaCl, pH 7.4), forming 1mg/mL cyt.c solution. The in-situ synthesis of cyt.c embedded nanopaper begun with adding 2 μL cyt.c solution

(1mg/mL) into the detection zone of the paper chip, followed by adding 2 μL $CaCl_2$ (200 mM) into the same place. Then the paper was quickly dried for 10 min at 37℃, forming nanostructured morphology. Finally, the nanopaper was stored at room temperature for further usage. For comparison, cyt.c was also added on the cellulose paper using the same condition without addition of $CaCl_2$. To compare with cyt.c embedded nanopaper, the morphology and performance test of cyt.c absorbed cellulose paper were also evaluated.

Evaluation of the effect of inorganic component on the fluorescence of QDs

In addition, the fluorescence of QDs in the cellulose paper containing $CaCl_2$ and PBS was also tested to evaluate the effect of inorganic component on the fluorescence of QDs. Specifically, 2 μL of $CaCl_2$ solution (200 mM) and 2 μL of PBS buffer solution (0.1M, 10 mM NaCl, pH 7.4) was added in the the detection zone of the paper chip. Then the paper was quickly dried for 10 min at 37℃. Red, yellow, green QDs were added into the as-prepared cellulose paper containing inorganic calcium phosphate component and pure cellulose paper, respectively. The paper after reaction was placed under the Ultraviolet lamp, and the fluorescence of each array zone of was captured by smartphone-camera. The photo was analyzed by ImageJ software, and the pixel intensity was retrieved.

Storage stability of cyt.c embedded nanopaper

The activity of cyt.c was evaluated by the quenching efficiency to the fluorescence of QDs. To demonstrate the storage stability of nanopaper, the cyt.c embedded nanopaper and the cyt.c absorbed cellulose paper containing same concentration of cyt.c were placed at room temperature to quench the fluorescence of the QDs every ten days for two months, respectively.

Cyt.c absorbed paper based aptasensor platform for E. coli detection

2μL of cyt.c solution (5.0 mg/mL) was added to the in the the detection zone of the paper. Followed by quickly dry for 10 min at 37℃ to get the Cyt.c absorbed paper. 90

μL of dilution of *E. Coil* O157:H7 (10^5 CFU/mL) was incubated with 10 μL of *E. Coil* O157:H7 aptamer functionalized green CdTe: Zn^{2+} QDs (25 μM) for 45 min under agitation at room temperature. As control, the background zone was placed with standard buffer free from *E. Coil*. Then 2 μL of the reaction solution above was dropped into the nanopaper for 5 min. For each sample with different concentration of *E. Coil* O157:H7, three parallel experiments were conducted to ensure repeatability. Finally, the nanopaper after reaction was placed under the Ultraviolet lamp, and the fluorescence of each array zone of the nanopaper was captured by smartphone-camera. The photo was analyzed by ImageJ software, and the pixel intensity was retrieved.

Nanopaper based aptasensor platform for E.coli detection

90 μL of dilution of *E. Coil* O157:H7 (10^2-10^6 CFU/mL) was incubated with 10 μL of *E. Coil* O157:H7 aptamer functionalized green CdTe: Zn^{2+} QDs (25 μM) for 45 min under agitation at room temperature. As control, the background zone was placed with standard buffer free from *E. Coil*. Then 2 μL of the reaction solution above was dropped into the nanopaper for 5 min. For each sample with different concentration of *E. Coil* O157:H7, three parallel experiments were conducted to ensure repeatability. Finally, the nanopaper after reaction was placed under the Ultraviolet lamp, and the fluorescence of each array zone of the nanopaper was captured by smartphone-camera. The photo was analyzed by ImageJ software, and the pixel intensity was retrieved. The fluorescence changes induced by the *E. Coil* O157:H7 was calculated as followed:

$(F_I/F_0)/(F_Q/F_0) = F_I/F_Q$

Real sample analysis for E. Coli

The performance of the nanopaper based platform for E. Coli analysis in real matrices was evaluated by river water as model sample. The same procedure in standard buffer was conducted for various concentration of *E. Coli* (10^3, 10^4, and 10^5 CFU/mL) in East Lake water. And the recovery of *E. Coli* from the lake water was calculated by fluorescence intensity in real matrices with that in standard buffer.

Cyt.c absorbed paper based aptasensor platform for MUC1 detection

2μL of cyt.c solution (1.0 mg/mL) was added to the in the the detection zone of the paper. Followed by quickly dry for 10 min at 37℃ to get the Cyt.c absorbed paper. 90 μL of diluted MUC1 solution in Tris-HCl buffer (10^4 nM) was incubated with 10 μL of MUC1 aptamer functionalized red CdTe: Zn^{2+} QDs for 60 min at room temperature. As control, the background zone was placed with Tris-HCl buffer free from MUC1. Then 2 μL of the reaction solution above was dropped into the nanopaper for 5 min. For each sample with different concentration of MUC1, three parallel experiments were conducted to ensure repeatability. Finally, the nanopaper after reaction was placed under the Ultraviolet lamp, and the fluorescence of each array zone of the nanopaper was captured by smartphone-camera. The photo was analyzed by ImageJ software, and the pixel intensity was retrieved.

Nanopaper based aptasensor platform for protein MUC1 detection

90 μL of diluted MUC1 solution in Tris-HCl buffer (10-10^5 nM) was incubated with 10 μL of MUC1 aptamer functionalized red CdTe: Zn^{2+} QDs (25 μM) for 60 min at room temperature. As control, the background zone was placed with Tris-HCl buffer free from MUC1. Then 2 μL of the reaction solution above was dropped into the nanopaper for 5 min. For each sample with different concentration of MUC1, three parallel experiments were conducted to ensure repeatability. Finally, the nanopaper after reaction was placed under the Ultraviolet lamp, and the fluorescence of each array zone of the nanopaper was captured by smartphone-camera. The photo was analyzed by ImageJ software, and the pixel intensity was retrieved. The fluorescence changes caused by the MUC1 was calculated as followed:

$(F_I/F_0)/(F_Q/F_0) = F_I/F_Q$

Real sample analysis for MUC1

The performance of the nanopaper based platform for MUC1 analysis in real matrices was evaluated by human serum. The same procedure in standard buffer was conducted for various concentration of MUC1 (10^2, 10^3, and 10^4 nM) in 20-fold diluted human

serum, respectively. And the recovery of MUC1 from human serum was calculated by comparing fluorescence intensity in real matrices with that in standard buffer.

The specificity of the nanopaper based aptasensor platform

The specificity was evaluated by replacing the target MUC1with BSA, SA, and AFP. 90 μL of BSA, SA, AFP and MUC1 at same concentration (10^3 nM) as well as blank buffer were incubated with 10 μL of MUC1 aptamer functionalized red CdTe: Zn^{2+} QDs for 60 min at room temperature. As control, the background zone was placed with Tris-HCl buffer free from MUC1. Then 2 μL of the reaction solution above was dropped into the nanopaper for 5 min. For each sample with different concentration of MUC1, three parallel experiments were conducted to ensure repeatability. Finally, the nanopaper after reaction was placed under the Ultraviolet lamp, and the fluorescence of each array zone of the nanopaper was captured by smartphone-camera. The photo was analyzed by Image J software, and the pixel intensity was retrieved.

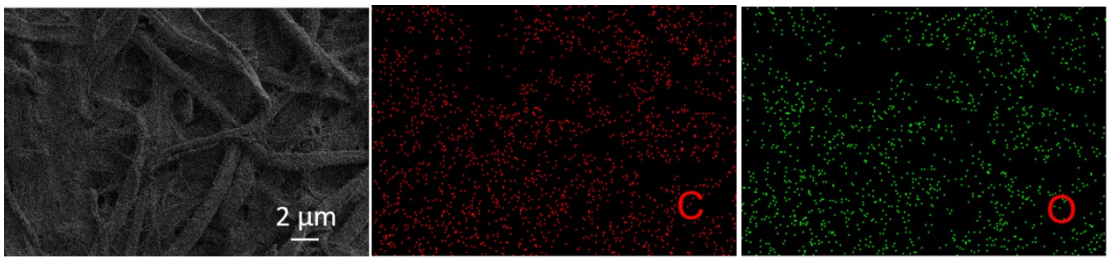

Figure S1. The EDS mapping of pure filter paper.

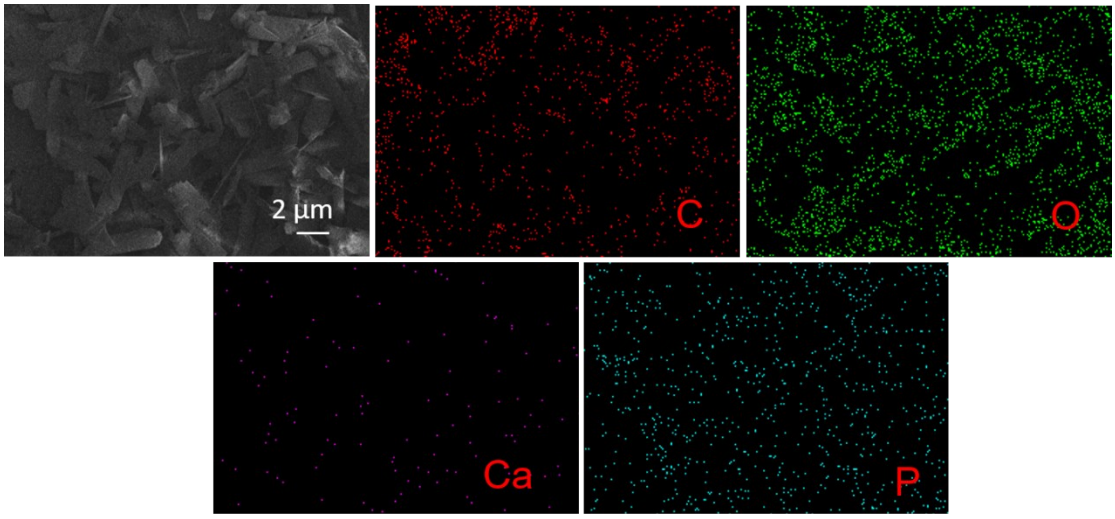

Figure S2. The EDS mapping of cellulose paper adding PBS.

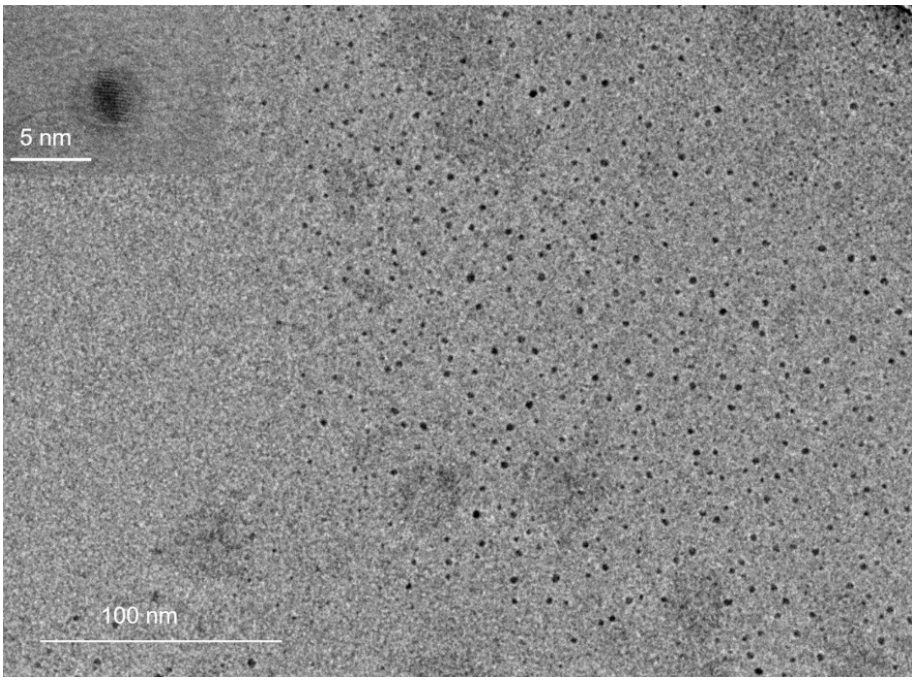

Figure S3. TEM image of aptamer functionalized QDs.

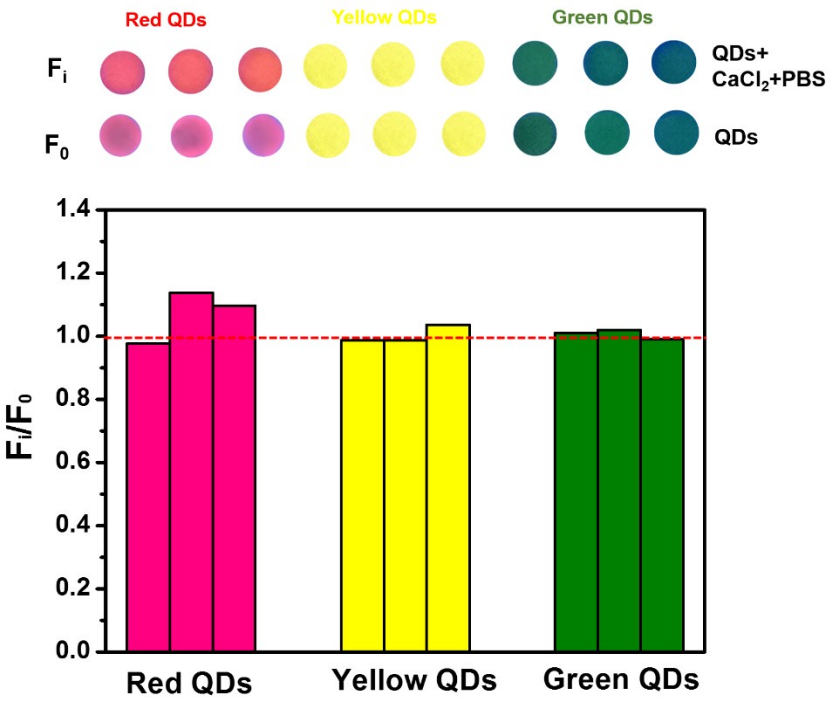

Figure S4. Fluorescence intensity ratio between the QDs incubated in cellulose paper containing calcium phosphate inorganic component (Fi) and the QDs incubated in pure cellulose paper (F_0).

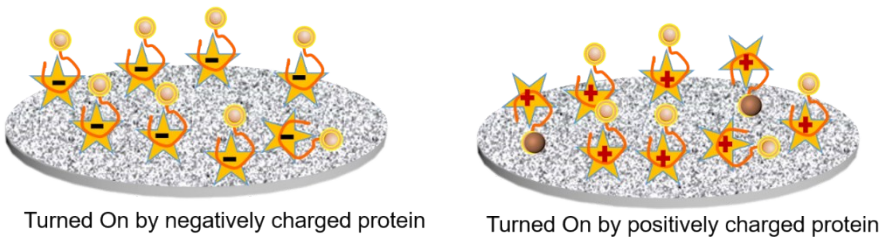

Figure S5. The conformation of protein-Apt-QDs complex in nanopaper platform with different charged target protein.

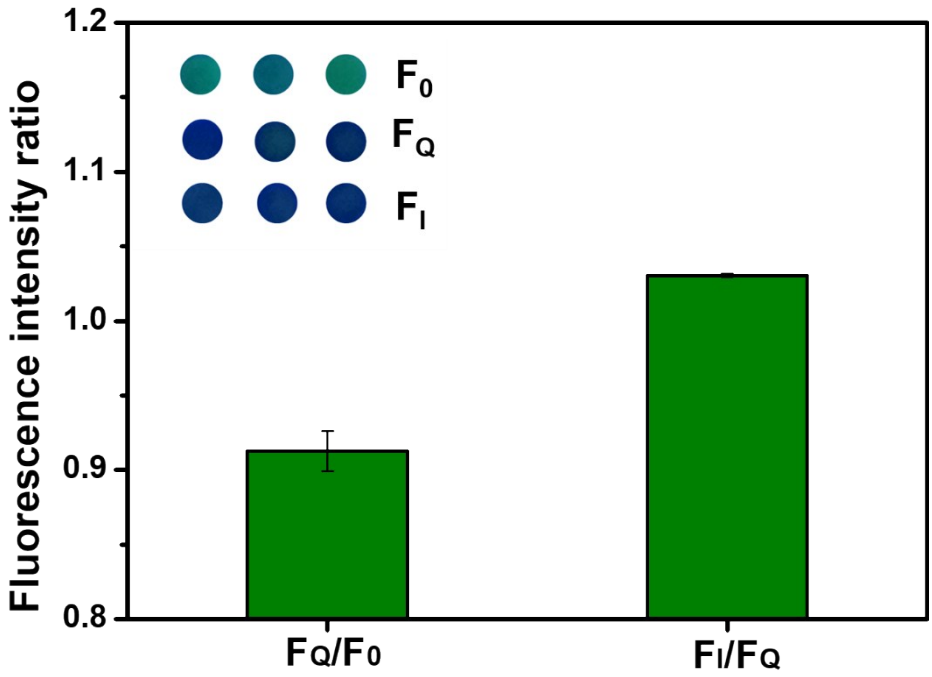

Figure S6. The performance of the cyt.c abosorbed paper for the E.coil O157:H7 analysis.

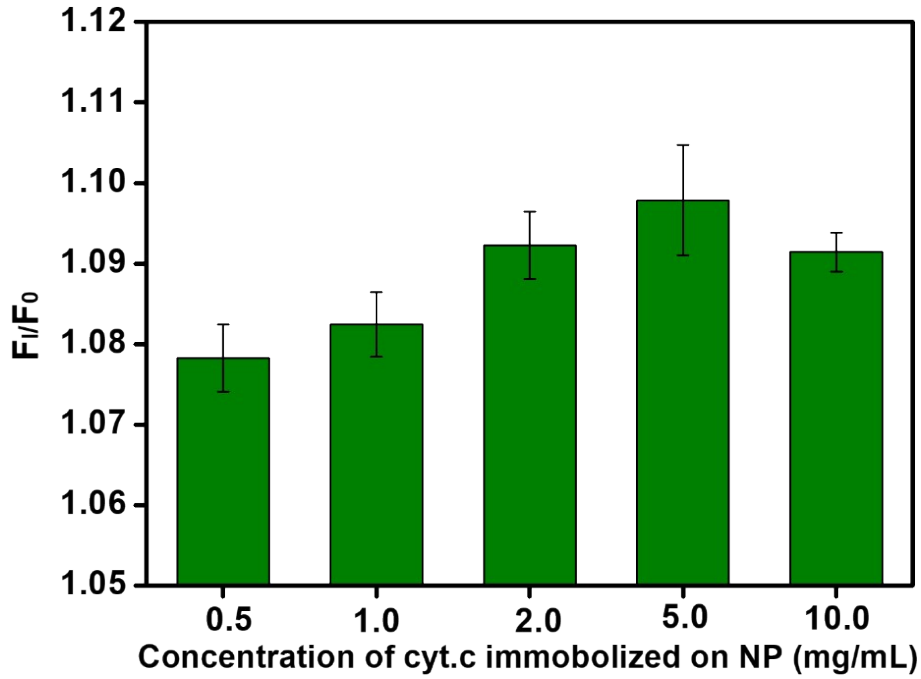

Figure S7. The optimization of the concentrations of cyt.c for pathogen detection.

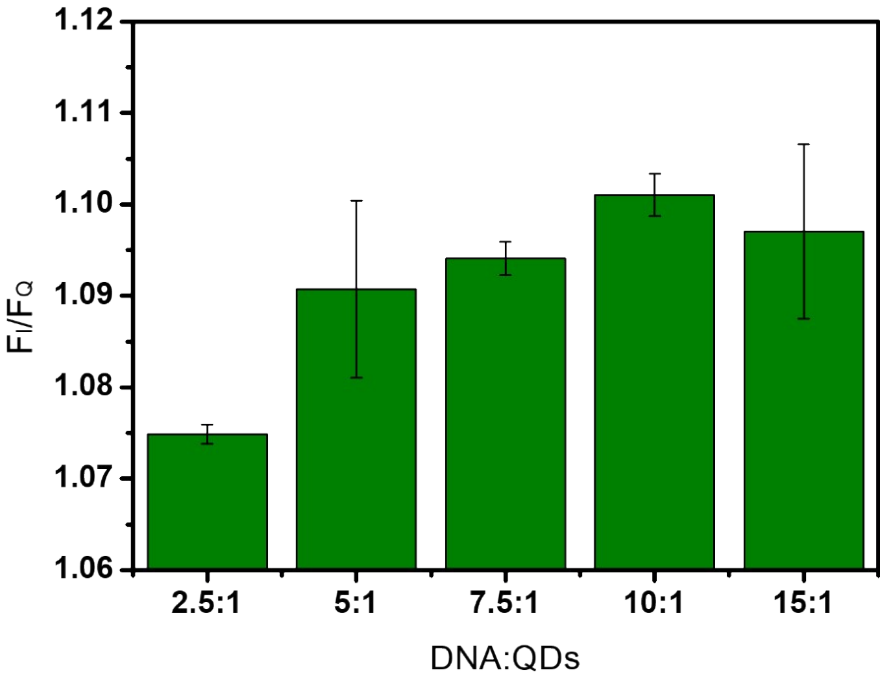

Figure S8. The optimization of the ratio of DNA to QDs for pathogen detection. (The final concentration of green QDs: 25 μM, and the primary added *E. coil*. Aptamer was set according to the ratio above.)

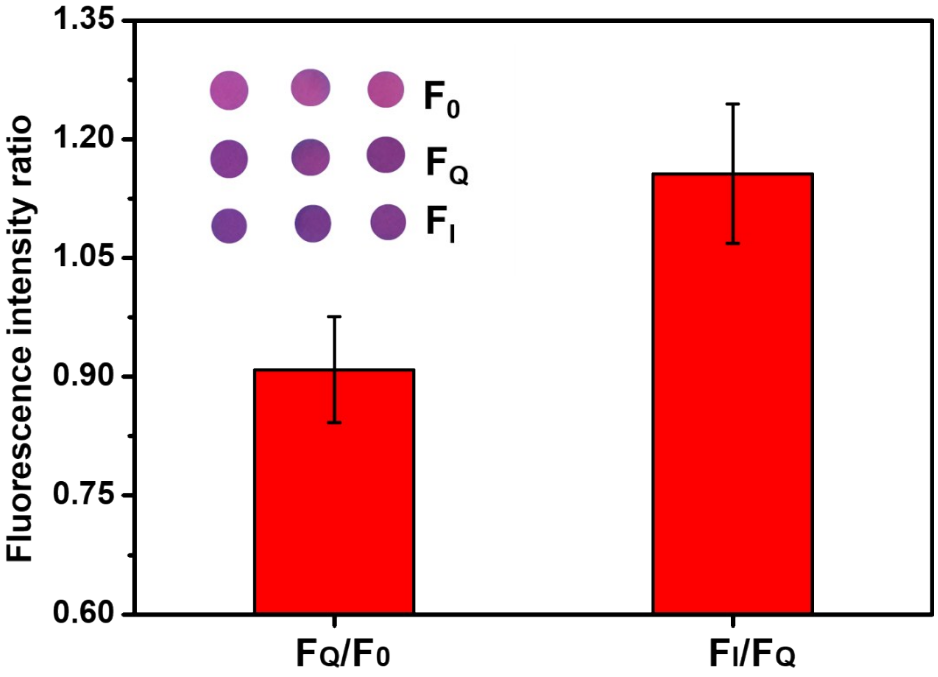

Figure S9. The performance of the cyt.c abosorbed paper for the MUC1 analysis.

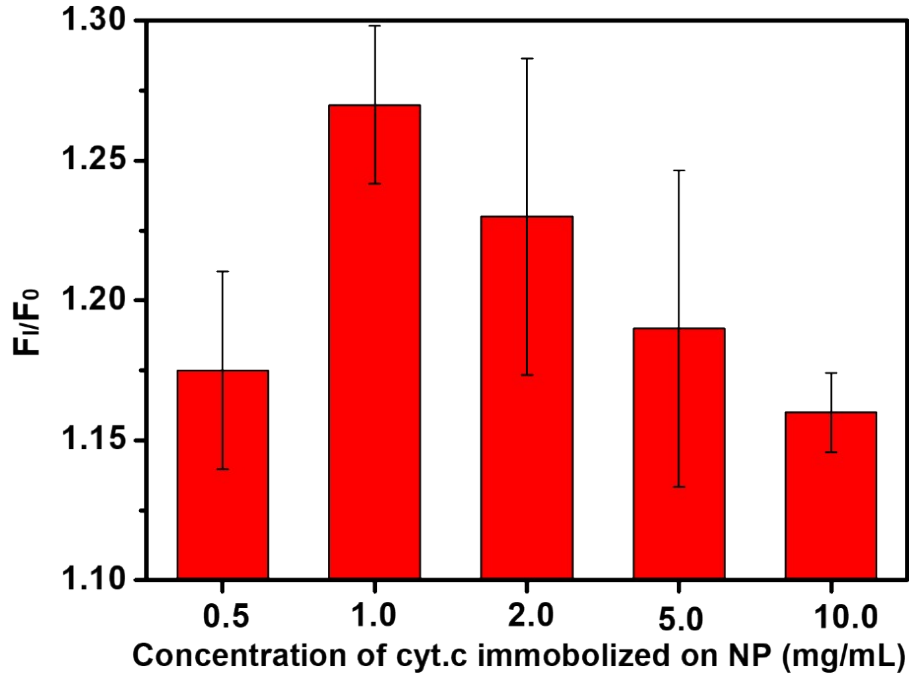

Figure S10. The optimization of the concentrations of cyt.c for protein MUC1 detection.

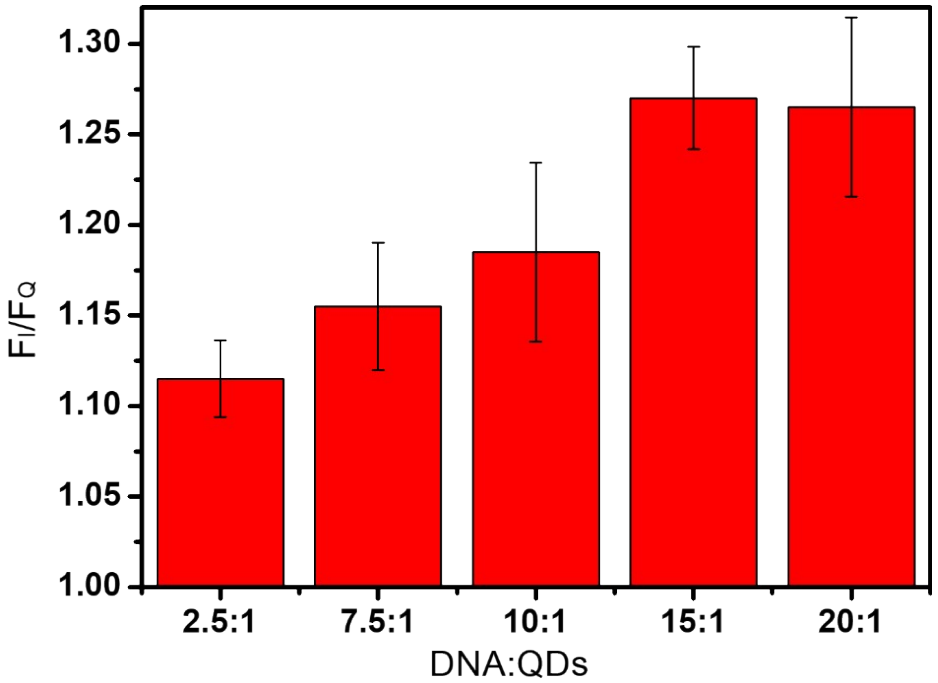

Figure S11. The optimization of the ratio of DNA to QDs for protein MUC1 detection. (The finial concentration of red QDs is 25 μM, and the primary added MUC1 Aptamer was set according to the ratio above.)

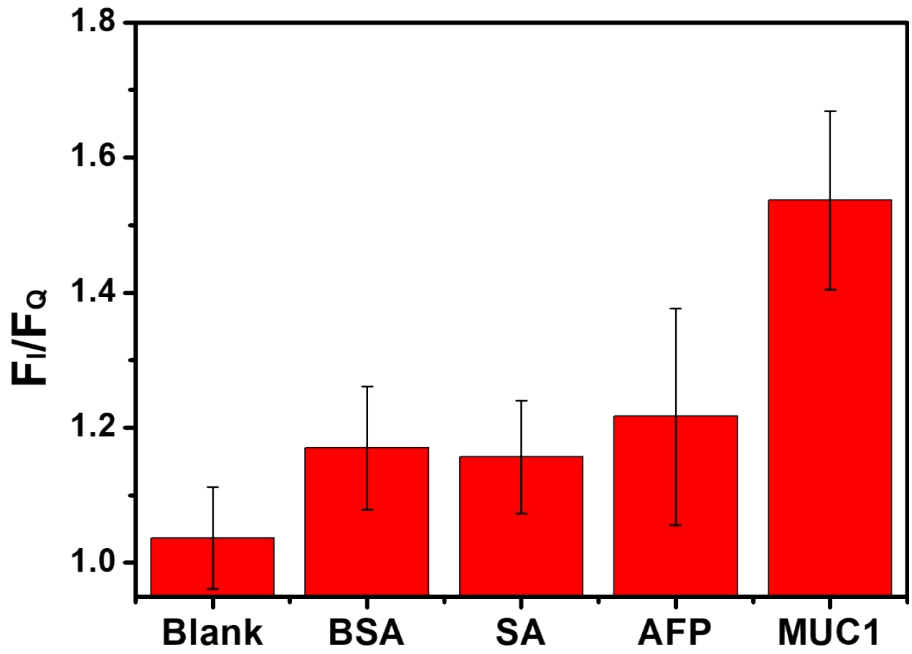

Figure S12. Specificity of the assay for MUC1 detection with different nontarget protein. Experimental conditions: 1.0 mg/mL cyt.c, 1 μg/mL different protein, 25 μM red QDs.

Table S1. Recovery results of *E. coil* O157:H7 in lake river.

Spiked bacteria [CFU mL^{-1}]	F_I/F_Q in standard buffer	F_I/F_Q in lake river	Recovery [%]
10^3	1.0994	1.0907	99.21
10^4	1.1304	1.1125	98.42
10^5	1.2506	1.2368	98.89

Table S2. Recovery results of MUC1 in human serum.

Spiked protein (nM)	F_I/F_Q in standard buffer	F_I/F_Q in human serum	Recovery [%]
10^2	1.2328	1.1488	93.19
10^3	1.8868	1.6991	90.05
10^4	2.0456	1.8874	92.27

Table S3 Comparison of the reported sensor for *E. coli* and MUC1 detection.

Target	Method	Range of detection	LOD	Reference
E. coli	Electrochemical Impedance Spectroscopy	10^3-10^7 CFU/mL	10^3 CFU/mL	1
	ELISA	10^3-10^7 CFU/mL	10^3 CFU/mL	2
	Gold nanoparticle-based Immunoassay	10^2-10^5 CFU/mL	148 CFU/mL	3
	Chemiluminescence Immunoassay	4.3×10^3–4.3×10^5 CFU/mL	1.2×10^3 CFU/mL	4
	Nanopaper-based Aptasensor	10^2-10^6 CFU/mL	10^3 CFU/mL	This work
MUC1	GO-based aptasensor	0.04−10 μM	28 nM	5
	QDs-based aptasensor	0.8−39.7 μM	250 nM	6
	Electrochemical Assay	0.05−1.5 μM	50 nM	7
	Carbon nanospheres based Aptasensor	0.1−2.12 μM	7.05 nM	8
	Nanopaper-based Aptasensor	10-10^5 nM	10 nM	This work

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