Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

A target-induced logically reversible logic gate for intelligent and rapid detection of pathogenic bacterial genes

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EXPERIMENTAL PROCEDURES

Materials and Chemicals

The used oligonucleotides were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The DNAs were dissolved in distilled water and quantified by measuring their UV-visible absorption at 260 nm. The concentration was calculated according to the extinction coefficients, $\varepsilon_{260\ nm}$ ($M^{-1}\cdot cm^{-1}$): A=15400, G=11500, C=7400, T=8700. Before using, the DNA solutions were heated at 88°C for 10 min and then gradually cooled to room temperature. A stock solution of N-methylmesoporphyrin IX (NMM) (100 μM) was prepared in dimethyl sulfoxide and stored in darkness at -20°C, which was diluted to the desired concentration with buffer solution before use. $AgNO_3$ (99.8%) was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China), and $NaBH_4$ was obtained from Sigma/Aldrich (USA). Graphene oxide (GO) was purchased from XFNano Material Tech Co., Ltd. (Nanjing, China). The water used throughout all experiments was purified by a Millipore system (Millipore Corporation, Billerica, MA, USA). Bacteria were bought from American Type Culture Collection (ATCC, Rockville, MD). Bacteria genome DNA extraction kit was bought from Hangzhou Bioser Technology Co., Ltd., Zhejiang, China.

Synthesis of DNA-AgNCs

The DNA-AgNCs were synthesized according to a previous report.[30] Briefly, a mixture of DNA solution (10 μM, 100 μL) and $AgNO_3$ (120 μM, 133.3 μL) was shaken for 1 min. After incubation for 20 min, freshly prepared $NaBH_4$ solution (120 μM, 66.7 μL) was added into the solution, followed by vigorous shaking for another 1 min. Then, 1× phosphate buffer (700 μL, 20 mM phosphate, 10 mM KNO_3, 1 mM $Mg(NO_3)_2$, pH 7.4) was added to the mixture to reach a final

volume of 1000 μL. The solution was kept in dark at 4°C for over 18 h.

Spectrofluorimetry measurements

The fluorescence spectra were recorded with a Fluoromax-4 Spectrofluorometer (HORIBA JobinYvon, Inc., France) at room temperature. The slit widths for both the excitation and emission of NMM and silver nanocluster were set at 20 nm. The excitation wavelength was set at 399 nm and 680 nm for the fluorescence measurements of NMM and DNA-AgNCs, respectively.

Feynman logic gate operation

For the Feynman logic gate, a mixture of HP_1, HP_2, DNA-AgNCs, GO and NMM was used as the platform. Then, input DNA solutions (IN1, IN2, and IN1/IN2) with the desired concentration were added and then incubated for 50 min at room temperature before the fluorescence measurement. The final volume of the sample was 500 μL with a final concentration of 40 nM AgNCs, 40 nM NMM, 40 μg mL^{-1} GO, 400 nM IN1, 400 nM IN2, 400 nM HP_1 and 400 nM HP_2.

Native polyacrylamide gel electrophoresis (PAGE)

A native polyacrylamide gel electrophoresis gel (20%) was prepared by mixing 6.66 mL 30% acrylamide/bis-acrylamide gel solution (29:1), 2 mL 5×TBE buffer (89 mM Tris, 89 mM boric acid, and 2 mM EDTA, pH 8.3), 70 μL 10% ammonium persulfate, 10 μL 8 N,N,N',N'-tetramethylethylenediamine and 1.27 mL of deionized water. Each sample was prepared with PB buffer (20 mM PB, 200 mM KCl, and 10 mM $MgCl_2$, pH 7.4), and the concentration of each DNA strand was 200 nM. Each sample was subjected to heat at 95°C for 5 min and cooled down to room temperature over 2 h. A total of 9 μL from each sample was mixed with loading buffer (1 μL) before loading onto the gel. The gel was run under a constant voltage of 80 V over a period of approximately 3 h. Next, the gel was immersed in diluted SYBR Green I solution for 30 min and

imaged on a Bio-Rad ChemDoc XRS (Bio-Rad, USA). The gel electrophoresis was performed on a DYY-6C electrophoresis analyser (Liuyi Instrument Company, China)

Circular dichroism (CD) measurements

The CD spectra of the used DNAs in 1× phosphate buffer (20 mM phosphate, 100 mM KNO_3, 5.0 mM $Mg(NO_3)_2$, pH 7.4) were collected using a Bio-Logic MOS450 (France) in a 1-cm path length quartz. The concentration of each DNA strand was 5.0 μM. During the experiments, the lamp was kept under a stable stream of dry and highly purified nitrogen. The data were collected from 220 nm to 300 nm at 0.1 nm intervals with a bandwidth of 1 nm at 25°C. The background of the buffer solution was subtracted from the CD data.

Detection of *mec A* and *Lac Z* in buffer solution

All used DNAs were annealed at 90°C for 10 min and then cooled down to room temperature slowly before conducting experiments. Different concentrations of *Lac Z* or *mec A* gene was added into mixture of 10 nM AgNCs, 10 nM NMM, 40 μg mL^{-1} GO, 40 nM HP_1 and 40 nM HP_2 and then and then incubated for 50 min at room temperature before the fluorescence measurement.The final volume of the sample was 500 μL in 1× phosphate buffer (700 μL, 20 mM phosphate, 10 mM KNO_3, 1 mM $Mg(NO_3)_2$, pH 7.4). The excitation wavelength was set at 399 nm and 680 nm for the fluorescence measurements of NMM and DNA-AgNCs, respectively. Slit widths for both excitation and emission of fluorescence spectra were set as 20nm.

Detection of *mec A* and *Lac Z* in bacterium lysate

The pure culture of E. coli O157:H7 was grown in 10 mL Luria-Bertani liquid medium at 37°C for 12 h under 200 rpm rotation. Then the resultant bacterial cells were separated and washed twice in ultrapure water by centrifugation (12000 rpm, 5 min). E.coli lysate was prepared by incubated bacterial cells at 100°C for 15 min. The *mec* A and *Lac* Z genes with different concentrations (0.3 nM, 0.4 nM, 0.6 nM) were spiked into the E. coli lysate, which were detected

according to the same procedure as that in buffer solution.

Table S1. DNA sequences used in this work.

Strand name	DNA sequences(from 5′-terminal to 3′-terminal)
DNA-AgNCs	GGGTCCACATAAGCTGATTAGGAT CACCCACCCACCCTCCC Input-recognition segment segment for AgNCs
mecA gene (IN1)	ATTGGGATCATAGCGTCATT
Lac Z gene (IN2)	TGCCGCTCATCCGCCACATATCCTG
HP_1	CCACATTACCTGATTGGGATCAAAAAAAAAATGACGCTATGATCCCAAT CAGGATATGTGG GGGTGGGTGGG segment S1 G-rich segment
HP_2	TGATCCAAATCAGGATATGTGGCGGATGAGCGGCAGGGTCCACATATCCTGATTGGGATCA TAGCG segment S2

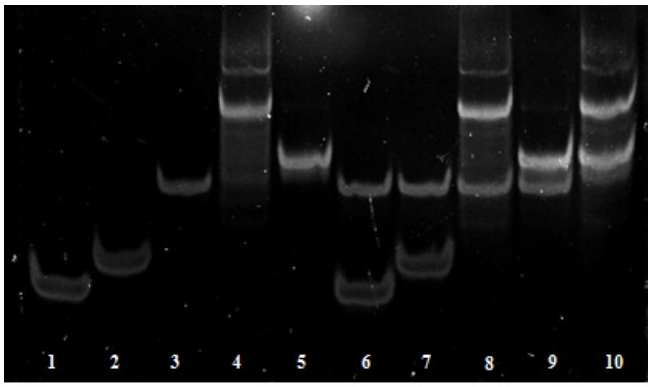

Figure S1. Polyacrylamide gel analysis (PAGE) of the DNA strands used in the Feynman logic gate-controlled detection of pathogenic genes. Lane 1: IN1; Lane 2: IN2; Lane 3: DNA-AgNCs; Lane 4: HP_1; Lane 5: HP_2; Lane 6: IN1+ DNA-AgNCs; Lane 7: IN2+ DNA-AgNCs; Lane 8: HP_1 +DNA-AgNCs; Lane 9: HP_2+DNA-AgNCs; Lane 10: HP_1+HP_2.

As illustrated in Figure S1, the belts represent the individual DNA strands of IN1, IN2, DNA-AgNCs, HP_1 and HP_2 from Lane 1 to Lane 5, respectively. In the mixture of IN1 and DNA-AgNCs (Lane 6), two individual belts appeared at the same position level of IN1 and DNA-AgNCs, indicating that no hybridization occurred between IN1 and DNA-AgNCs. No new belt could be found in the mixtures of (IN2 and DNA-AgNCs, Lane 7), (HP_1 and DNA-AgNCs, Lane 8), (HP_2 and DNA-AgNCs, Lane 9), and (HP_1 and HP_2, Lane 10),

suggesting that no hybridization happened between the two DNA strands as mentioned above.

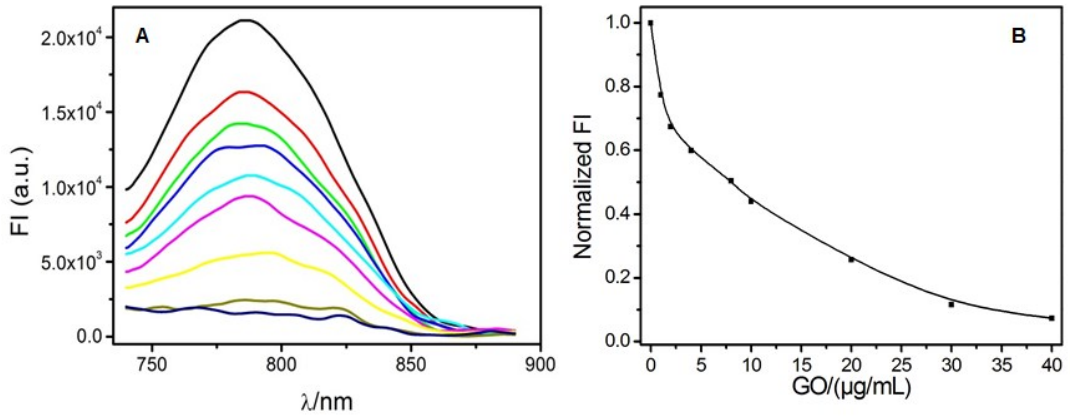

Figure S2. The fluorescent response (A) and the fluorescent intensity at 780 nm (B) of DNA-AgNCs (40 nM) in the presence of GO with different concentrations: 0, 1, 2, 4, 8, 10, 20, 30, and 40 μg/mL (from top to bottom).

The fluorescence of DNA-AgNCs was quenched once DNA-AgNCs were trapped on the GO via non-covalent π-π stacking interaction due to the fluorescence resonance energy transfer between GO and AgNCs. As shown in Figure S2, the fluorescence intensity of AgNCs decreased along with increasing concentration of GO and reached a minimum at 40 μg/ml GO. Here, 40 μg/ml of GO was selected for the following experiments.

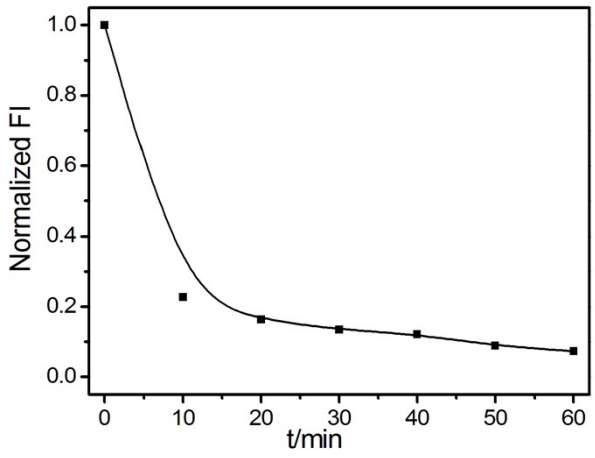

Figure S3. Fluorescence changes of the DNA-AgNCs against the incubation time after adding GO to the DNA-AgNCs.

As shown in Figure S3, the fluorescence intensity of AgNCs decreased quickly at the beginning and then reached a plateau after incubation for 20 min. Here, 20 min was selected as the incubation time for preparing the logic platform.

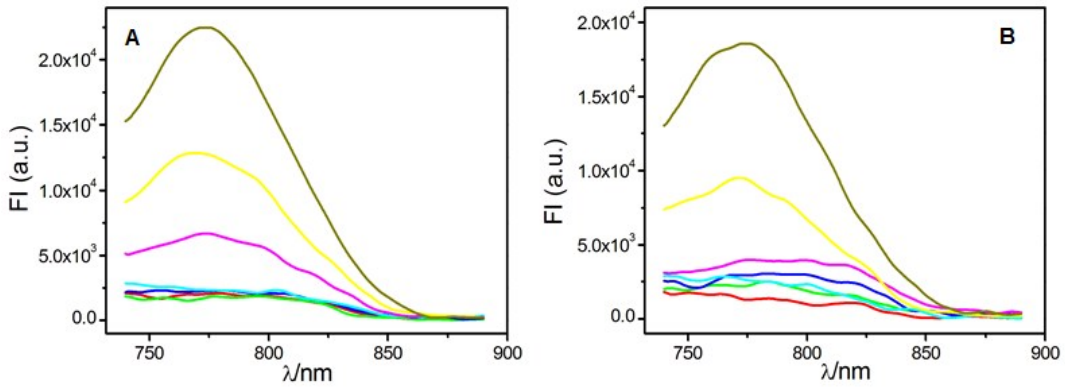

Figure S4. The restoration of the DNA-AgNCs fluorescence from the logic system by adding IN1 (A) or IN2 (B) at different concentrations: 0, 10, 20, 40, 100, 200 and 400 nM (from bottom to top).

To explore the optimized concentration of the input DNAs, the fluorescence response of AgNCs was monitored after adding IN1 or IN2 to the logic system. The fluorescence of AgNCs increased with increasing concentration of IN1 (Figure S4A) or IN2 (Figure S4B), which was restored over 80% at 400 nM of input concentration. Here, 400 nM was selected as the input concentration for IN1 and IN2 in the following experiments.

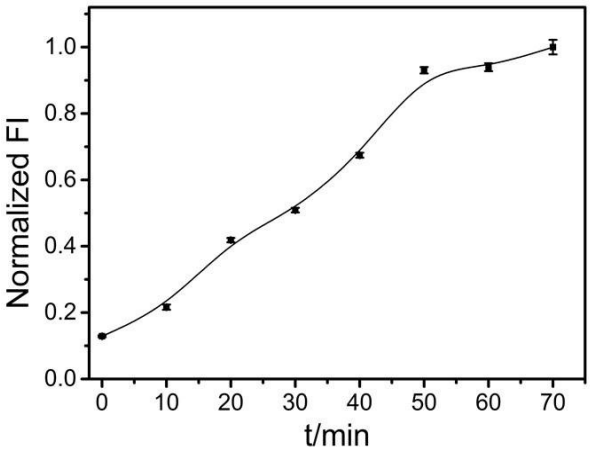

Figure S5. The fluorescence restoration of the DNA-AgNCs/GO against the reaction time after adding IN2 into the logic system. The error bars were obtained according to three times independent experiments.

The reaction time was explored by measuring the restored fluorescence of the DNA-AgNCs after adding the target DNA (IN2) into the sensing system, Figure S5. The fluorescence of the DNA-AgNCs increased with increasing reaction time and reached a plateau if the reaction time was over than 50 min. Thus, 50 min was selected as reaction time in the experiments.

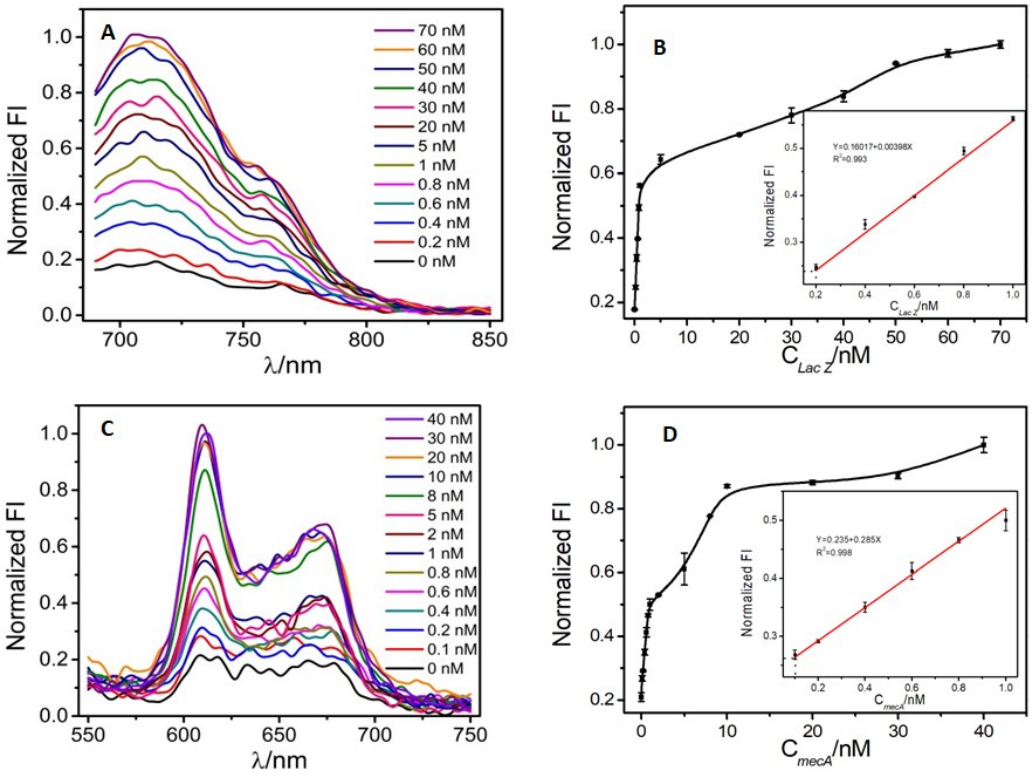

Figure S6. The fluorescent response of AgNCs (A) and NMM (C) against the *mec A* gene and *Lac Z* with different concentrations, respectively. The normalized fluorescent intensity of AgNCs at 699 nm (B) and NMM at 609 nm

(D) against the concentrations of *mec A* gene and *Lac Z*, respectively. The inset shows the linear relationship between the fluorescent signal and the target concentrations. Error bars were obtained from three independent experiments.

The detection sensitivities of *Lac Z* and *mec A* were investigated by plotting the fluorescent intensity of AgNCs at 699 nm against concentration of *Lac Z* and the fluorescent intensity of NMM at 609 nm against concentration of *mec A*, respectively. The fluorescent intensity of AgNCs linearly depends on *Lac Z* within concentration range from 200 pM to 1000 pM (R^2= 0.993), Figure S6A and Figure S6B. The limit of detection (LOD) of 13 pM was obtained according to the calculation of three times the standard deviation of ten blank measurements. The fluorescent intensity of NMM linearly depends on *mec A* within concentration range from 100 pM to 1000 pM (R^2= 0.998), Figure S6C and Figure S6D. The LOD of 60 pM was obtained according to the calculation of three times the standard deviation of ten blank measurements. The lowest detection concentrations in practice measurements were comparable with the recent report about pathogen gen detection.[1]

Table S2. Recovery test in bacterium lysate

Sample	*Lac Z*(nM)		Recovery(%)
	Spiked	Found mean±SD	
0	--	--	--
1	0.3	0.326±0.0120	108.6±3.6
2	0.4	0.449±0.0064	112.5±0.64
3	0.6	0.628±0.0349	104.7±3.5

Sample	*mec A*(nM)		Recovery(%)
	Spiked	Found mean±SD	
0	--	--	--
1	0.3	0.286±0.00623	95.3±2.1
2	0.4	0.318±0.0121	79.4±0.03

3	0.6	0.610±0.0525	101.7±8.7

The detection of *mec A* and *Lac Z* was implemented in E. coli (10 CFU/mL) lysate according to the same detection procedure. Before adding the target DNAs, no obvious signal change was detected for E. coli (10 CFU) lysate. *Lac Z* and *mec A* with different concentrations (0.3, 0.4, and 0.6 nM) were spiked into the E. coli lysate and then quantified following the same procedure of the developed assay. The detection results were listed in Table 1. Recoveries were found distributed within range from 104% to 110% for *Lac Z* detection and from 95% to 102% for *mec A* detection. The results validated the reliability of the developed strategy for pathogenic bacterial gene detection in a biologically relevant sample.

References:

1. Y. Sun, C. Zhao, Z. Yan, J. Ren, X. Qu, *Chem. Commun.* 2016, **52**, 7505-7508.