Supporting Information

The Construction of CRISPR/Cas9-Mediated FRET 16S rDNA Sensor for Detection of *Mycobacterium tuberculosis*

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Material and Methods

Sequence. The detailed sequences of the 16S rDNA fragment of *M. tuberculosis*, Forward primer for preparing sgRNA, P1 probe, P2 probe and single base mismatch sequences could be seen in Table S1.

Table S1

The sequences of oligonucleotide used in this work.

Oligonucleotide Type	Sequences
P1	5'-TTTAGGTGC-3'
P2	5'- TAGGTGAGGTCTGCTACCCACAGCCGGTGCACCTAA-3'
16S rDNA fragment	5'-ACCGGCTGTGGGTAGCAGACCTCACCTA-3'
1 position mismatch	5'- ACCGGATGTGGGTAGCAGACCTCACCTA-3'
5 position mismatch	5'- ACCGGCTGTAGGTAGCAGACCTCACCTA-3'
10 position mismatch	5'- ACCGGCTGTGGGTAACAGACCTCACCTA-3'
15 position mismatch	5'- ACCGGCTGTGGGTAGCAGATCTCACCTA-3'
Forward primer for preparing sgRNA	5'-TTAATACGACTCACTATAGGCTGTGGGTAGCAGACCTCACTTTTAGAGCTAGAAATAGCA-3'

Preparation of simulated sputum samples. The centrifuge tube containing 1 mL of the sputum samples and 4.0 mL of 4% sodium hydroxide solution was centrifuged at 4 K rpm for 10 min and the supernatant was discarded. The sediment was washing using 5 mL of sterile PBS buffer and M7H9 medium, this detection sample solution was prepared for use.

M. tuberculosis with the concentration of 10^4 CFU/mL was added to sterile detection samples to prepare the simulated positive sputum samples, and sterile detection sample were used as negative sputum samples.

Acquisition of target fragments. The DNA of strains was obtaining by Chelex-100 strategy. 1 μL of AvaII restriction enzyme and 1 μL of MslI restriction enzyme were

added to the supernatant after centrifugation in order, and the incubation was performed individually at 37 °C for 10 min.

Preparation of UCNPs nanoparticle. Briefly, $Er(NO_3)_3 \cdot 6H_2O$ (55.4 mg), $Yb(NO_3)_3 \cdot 5H_2O$ (305.4 mg), and $Y(NO_3)_3 \cdot 6H_2O$ (1225.6 mg) were added in 20 mL deionized water and incubated with EDTA (0.4 M, 20 mL). Then the mixed solution was quickly poured into NaF solution (2.1 g, 100 mL) under magnetic stirring and reacted in ice bath for 20 min and at 30 °C for 10 min, respectively. After centrifuged at 8K rpm for 10 min, the precipitate was dried at 500 °C for 1 h under protection of nitrogen. Finally, 5 mg/mL of upconversion nanoparticles with a diameter of 20 nm was synthesized by adding 0.1 M PBS in tube.

Synthesis of UCNPs@SiO_2 nanoparticle. The prepared step of the functional nanoparticle could be showed in Figure S1. 0.2 mL IGEPAL CO-520, 6 mL hexane solution as well as the UCNPs (8 mg) was incubated under ultrasonic oscillations for 5 min. Then, 40 μL of 30% aqueous ammonia was added and shook slowly until mixture became transparent to form microemulsion. 30 μL of 0.14 mM TEOS was subsequently added and mixed at 35 °C for 60 min. Reaction was completed by adding 4 mL of methanol to microemulsion. The functional nanoparticle was finally obtained after the centrifugation at 8000 rpm for 10 min using ethanol.

Preparation of UCNPs@SiO_2 -COOH nanoparticle. 150 μL of 0.68 mM APTES was added to 5 mL ethanol contained the prepared UCNPs@SiO_2 nanoparticle (25 mg). The mixture was stirred at 35 °C overnight, then centrifugation was performed at 8000 rpm for 5 min. Sediment was dispersed in 4 mL of anhydrous DMF, followed by adding 3 mL of DMF solution containing succinic anhydride (200 mg, 1.5 mM). The mixed solution was stirred at 35 °C overnight. The UCNPs@SiO_2-COOH nanoparticle was

thus obtained after the centrifugation at 8000 rpm for 10 min using ethanol. Finally, the nanoparticles were dispersed in borate-buffered solution.

Results and Discussion

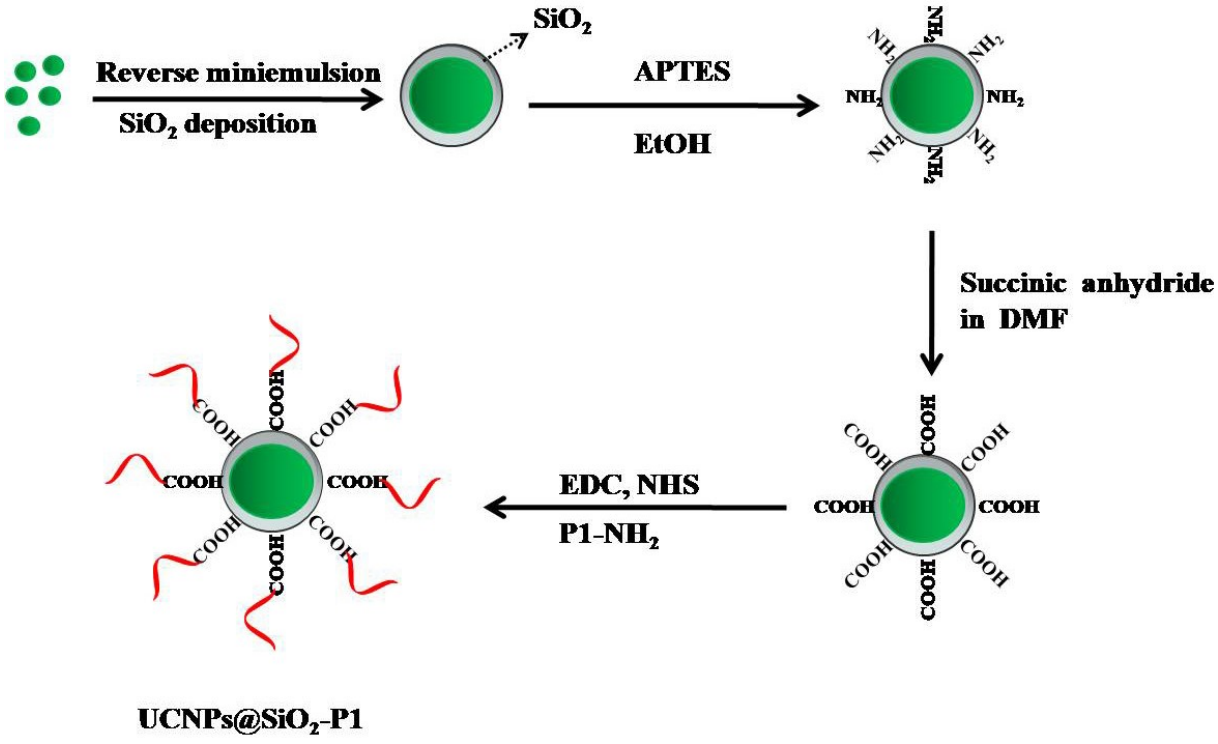

Figure S1. The functionalization of UCNPs@SiO_2-P1.

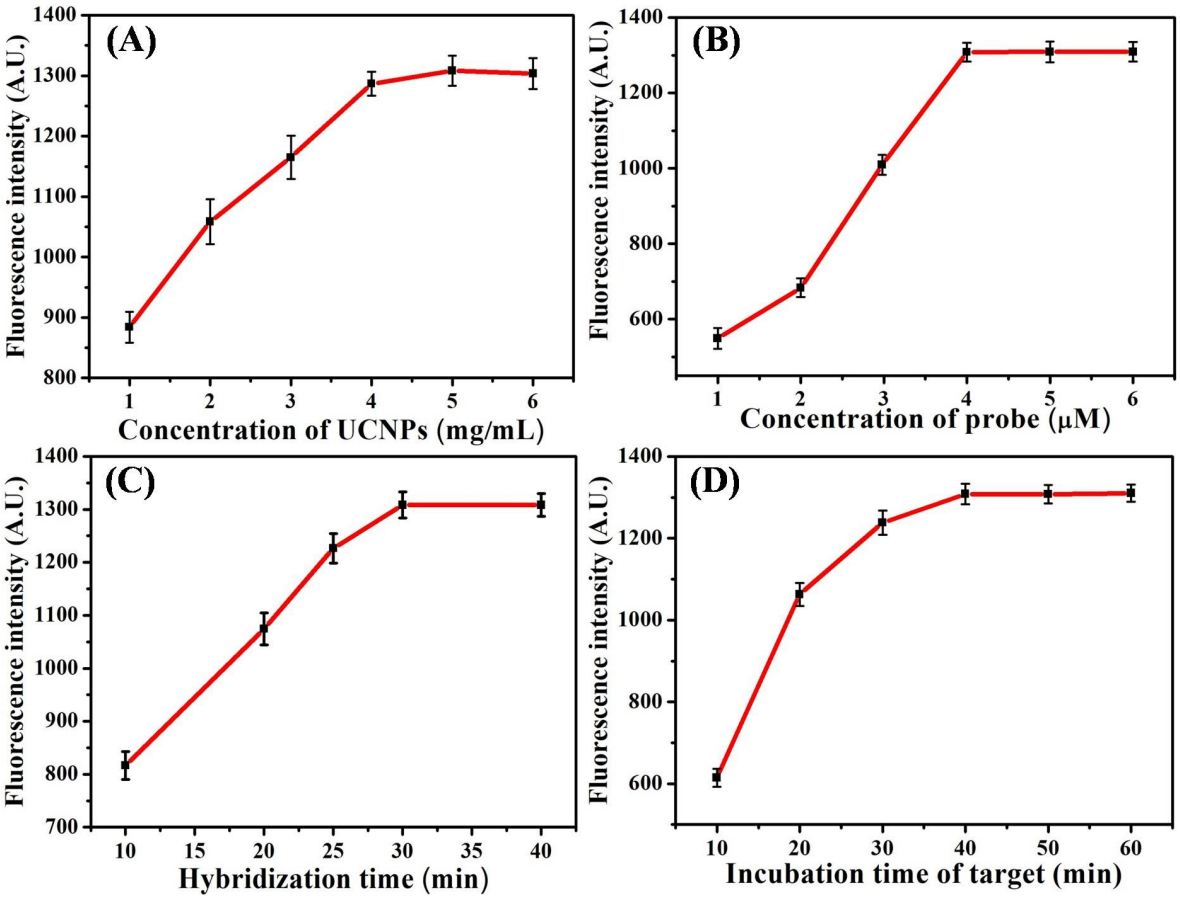

Figure S2. Optimization (A) the optimal concentration of UCNPs. (B) the optimal concentration of probes. (C) the optimal concentration hybridization time between UCNPs@SiO_2-P1 and Fe_3O_4@Au-P2. (D) the optimal the CRISPR/Cas9 system reaction time during target detection. The concentrations of target were at 10 pM. Error bars indicate standard deviation (n = 3).

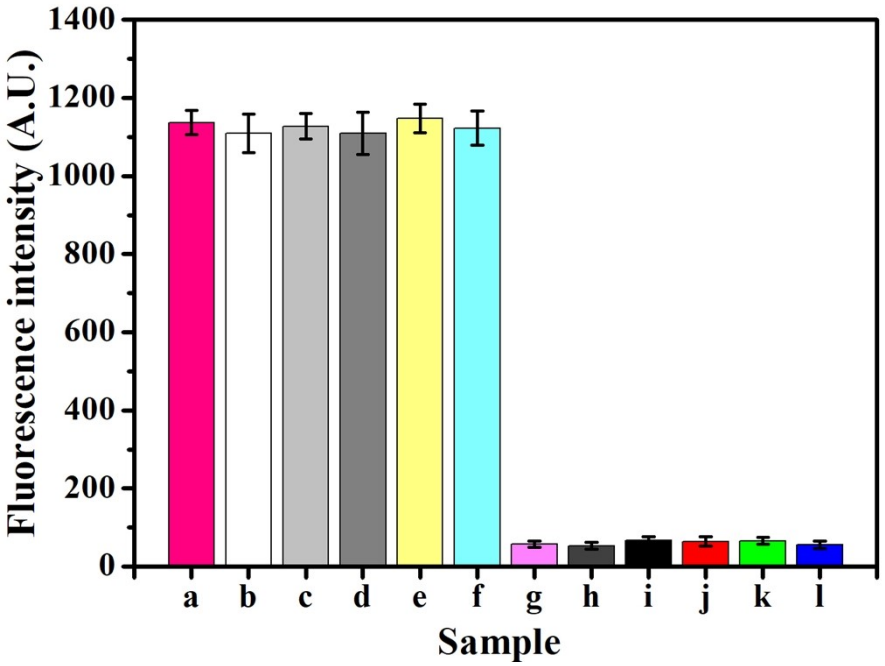

Figure S3. The fluorescent signal responses to different strains. (a) *M. tuberculosis* + *P. aeruginosa*, (b) *M. tuberculosis*+ *S. enteritidis*, (c) *M. tuberculosis* + *E. coli*, (d) *M. tuberculosis*+ *BCG vaccine*, (e) *M. tuberculosis* + *M. smegmatis*, (f) *M. tuberculosis,* (g) *P. aeruginosa,* (h) *S. enteritidis*, (i) *E. coli,* (j) *BCG vaccine*, (k) *M. smegmatis*, (l) blank sample. The concentrations of these strains were at 10^5 CFU/mL. Error bars indicate standard deviation (n = 3).

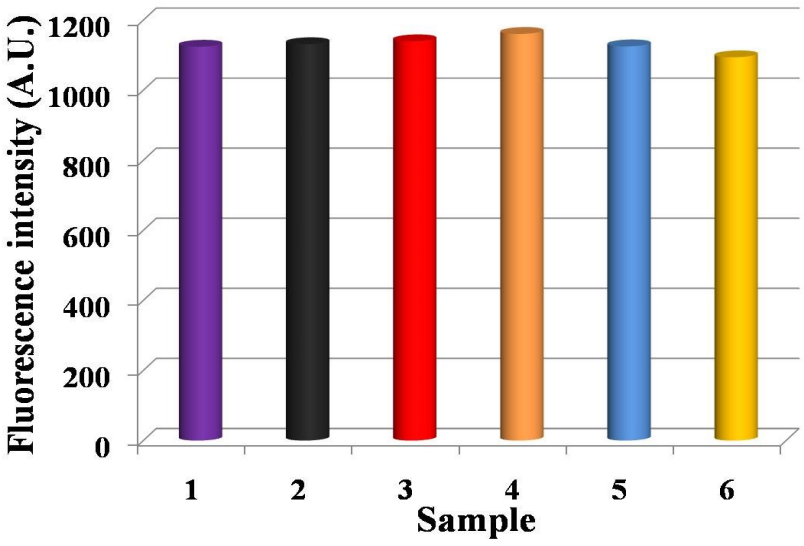

Figure S4. The repeatability study for detection of *M. tuberculosis* in M7H9 medium. The concentrations of *M. tuberculosis* were 10^5 CFU/mL.

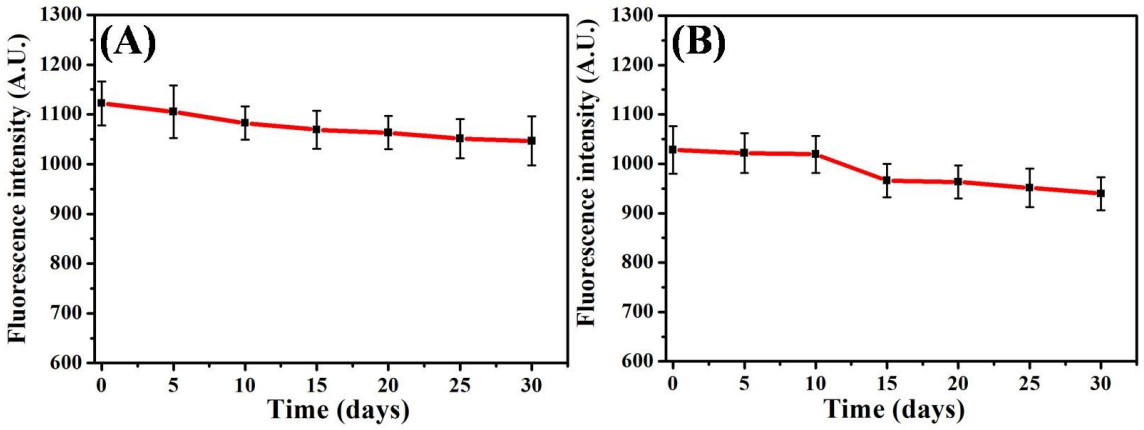

Figure S5. The stability study for detection of *M. tuberculosis* in M7H9 medium (A) and in simulated sputum sample (B). The concentrations of *M. tuberculosis* were 10^5 CFU/mL.

Table S2

Recovery assay of *M. tuberculosis* in the simulated sputum samples.

Samples	Added (CFU/mL)	Culture method (CFU/mL)	Constructed sensor (CFU/mL)	Recovery (%)	RSD (%, n=3)
Sputum 1	30	29.79	31.88	106.27	0.95
Sputum 2	3×10^2	3.15×10^2	2.83×10^2	94.33	1.56
Sputum 3	3×10^4	2.86×10^4	2.99×10^4	99.67	2.05
Sputum 4	3×10^6	3.06×10^6	2.88×10^6	96.00	1.38
Sputum 5	3×10^8	2.96×10^8	3.08×10^8	102.67	2.42
Sputum 6	3×10^9	3.26×10^8	3.12×10^8	104.00	1.94