Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Target-responsive liposome activated by catalytic hairpin assembly enables highly sensitive detection of tuberculosis-related cytokine

Haiyan Cui,[a] Bing Bo,[a] Jun Ma,[a] Yingying Tang,[b] Jing Zhao[b,*] and Heping Xiao[a,*]

[a] *Clinic and Research Center of Tuberculosis, Shanghai Key Lab of Tuberculosis, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200433, China*

[b] *Center for Molecular Recognition and Biosensing, School of Life Sciences, Shanghai University, Shanghai 200444, China*

Experimental section

Chemicals and materials

Oligonucleotides (HP1, HP2, HP1-S) were synthesized by Takara Biomedical Technology Co., Ltd (Beijing, China), and their sequences are listed in Table S1. Peptide probe was synthesized and purified by GL Biochem Ltd. (Shanghai, China) and its sequences are listed in Table S1. Recombinant IFN-γ (specific activity of 2.0×10,000,000 IU/mg), IFN-γ inducible protein (IP-10) and interleukin-2 (IL-2) was purchased from ProSpec Ltd. (Israel). Sodium citrate and bovine serum albumin (BSA) were purchased from Bio Basic Inc. (Shanghai, China). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), fluorescein (FAM), DOPG (1,2-Dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt), DOPC (1,2-Dioleoyl-sn-glycero-3-phosphocholine) and N-hydroxysuccinimide (NHS), were obtained from Sigma Aldrich. Other chemicals were of analytical grade and used as received. All buffer solutions were prepared with double-distilled water (18 MΩ cm) obtained from a Direct-8 Millipore purification system.

Preparation of FAM-encapsulated liposomes

FAM-encapsulated liposomes were prepared according to the procedure from the literature with slight modifications (*Electrochim. Acta.,* 2017, **252**, 362-367; *Chem. Sci.,* 2017, **8**, 3047-3053). Briefly, 8 mg DOPC and 8 mg DOPG were first dissolved in 2 mL $CHCl_3$ containing 10 mM FAM. After well vertexing, the organic solvent was removed by rotary evaporation under 40 °C, leaving a thin lipid film. Then, the dry residue was hydrated in 1 mL of 20 mM phosphate buffer solution (PBS) and the resulting dispersion was sonicated for 30 min. Finally, the desired FAM-encapsulated liposomes were obtained by extruding 15 cycles through polycarbonate membranes (pore diameter: 100 nm) with LiposoEasy LE-100 (Morgec, Shanghai). The characterization and optimization of FAM-encapsulated liposomes were performed by transmission electron microscopy (TEM) and fluorescence spectroscopy. As shown in Fig. S4, the prepared liposomes with spherical shapes are observed with the size of ~150 nm, while the utilized FAM concentration that is critical for self-quenching is optimized to be 10 mM.

Synthesis of magnetic nanoparticles (MNPs) and HP1-modified MNPs

MNPs were synthesized using a co-precipitation method (*New J. Chem.,* 2017, **41**, 14414-14419). In brief, 100 mL of 0.5 M NaOH solution was first purged with nitrogen to remove oxygen and then heated to 80 °C. After that, 10 mL of fresh-prepared solution containing 0.5 M $FeSO_4$, 1 M $FeCl_3$ and 0.4 M HCl was added drop wise into the NaOH solution under rapid stirring. 80 min later, a solution of sodium citrate (1.94 M) was added and continuously heated to 90 °C. After further stirring for 60 min, the precipitate was isolated by magnetic separation and washed with double-distilled water three times. Finally, the resulting MNPs were re-suspended with 50 mL double-distilled water and stored at 4 °C. For the preparation of HP1-modified MNPs (HP1/MNPs), 0.22 M EDC and 0.22 M NHS were first mixed with MNPs (1 mL) for 30 min under ultrasonication. After magnetic separation, 1 μM HP1 solution (prior to use, HP1 should be heated to 95 ºC for 5 min and gradually cooled to room temperature to form the stem-loop structure) was added to MNPs with continuous stirring for 120 min. The resulting mixture was isolated by magnetic separation and washed with double-distilled water three times, to obtain desired HP1/MNPs.

Validation of CHA using gel electrophoresis analysis

8 μL of DNA samples (0.5 μM HP1, 0.5 μM HP2, 0.5 μM HP1 treated 50 nM IFN-γ, 0.5 μM HP1 and HP2 without or with treated with 50 nM IFN-γ) were first prepared (prior to use, HP1 and HP2 should be separately heated to 95 ºC for 5 min and gradually cooled to room temperature to form the stem-loop structure), followed by incubating with 2 μL of 5× loading buffer. Afterward, the samples were loaded onto a 10% non-denaturing polyacrylamide gel (PAGE) in 1× Tris-acetate-EDTA (TAE) buffer, and electrophoresis separation was then running at a constant voltage of 120 V. After being separated for 60 min, the resulting gel was stained with SYBR Green I for 30 min and photographed using a Gel Doc XR+ System (Bio-Rad, USA).

Target-responsive liposome-based fluorescence detection of IFN-γ

A typical experiment for fluorescence detection of IFN-γ using target-responsive liposome was performed by first mixing 25 μL of different concentrations of IFN-γ and 25 μL of DNA hybridization buffer (10 mM PBS containing 1 M NaCl, pH 7.4)

containing 0.6 μM HP2 (prior to use, HP2 should be heated to 95 ºC for 5 min and gradually cooled to room temperature to form the stem-loop structure). Thereafter, the mixture was treated with 400 μL of HP1/MNPs to sustain the target-catalyzed hairpin assembly for 2 h. After magnetic separation, the resulting MNPs was further treated with 100 μL of 10 mM PBS buffer containing 10 μM peptide probe to form Peptide/MNPs via click chemistry at room temperature. Finally, the MNPs were again magnetic separated and thoroughly rinsed, and used for incubating with 200 μL of FAM-encapsulated liposomes. The fluorescence emission spectrum of the resulting reaction mixture was collected 30 min later with the excitation wavelength at 460 nm.

Table S1. Sequences of oligonucleotides and peptide probe used in this work [a]

Name	Sequence
HP1	5'-NH_2-C_{12}-AAAAAAAATCCA*GGGGTTGGTTGTGT****TGGGTGTTGTGT****CCCCTGG****ATT****ACACAA**-3'
HP2	5'-DBCO-GTG**TTGTGT****AATCCAGGGG****ACACAACAC****CCA**-3'
HP1-S	5'-NH_2-C_{12}-AAAAAAAATCCA*GGGGTTGGTTGTGT****TGGGTGTTGTGT****CCCCTGG****ATT****ACACAA**-DBCO-3'
Peptide probe	FVQWFSKFLGRILGG-PEG-Azido

[a] The underlined letters indicate the stem sequences of the hairpins; the boldface letters indicate the complementary bases between the hairpins; the italic letters in HP1 represent the aptamer sequence of IFN-γ.

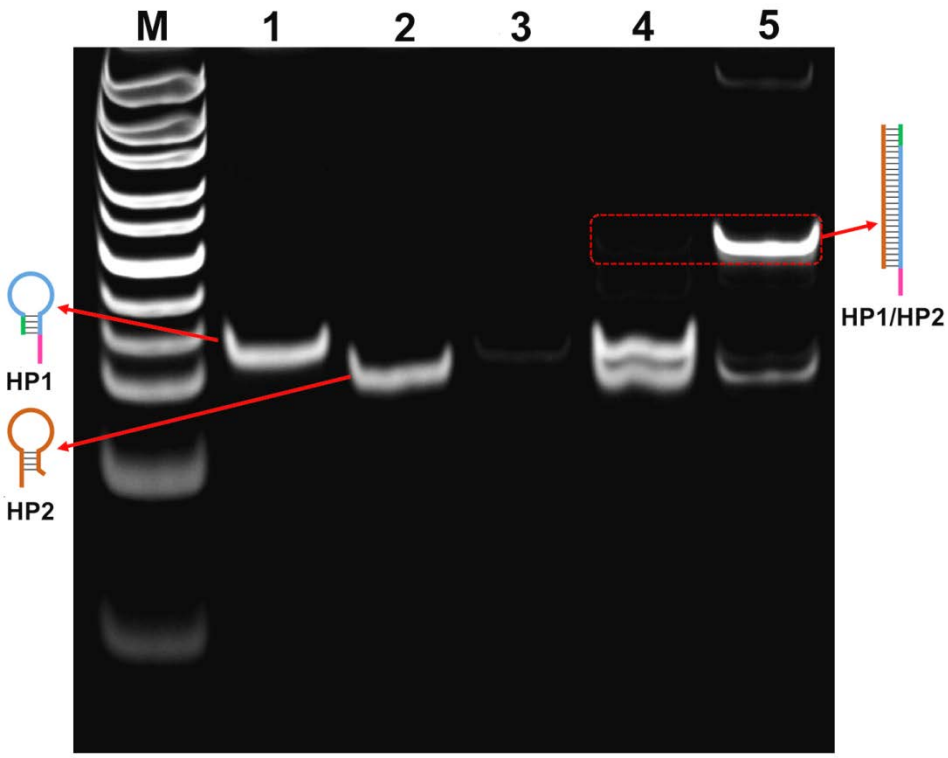

Fig. S1 Target-catalyzed hairpin assembly verified by gel electrophoresis. Lane M: DNA ladder; Lane 1: 0.5 μM HP1; Lane 2: 0.5 μM HP2; Lane 3: 0.5 μM HP1 treated with 50 nM IFN-γ; Lane 4: 0.5 μM HP1 and HP2; Lane 5: 0.5 μM HP1 and HP2 treated with 50 nM IFN-γ.

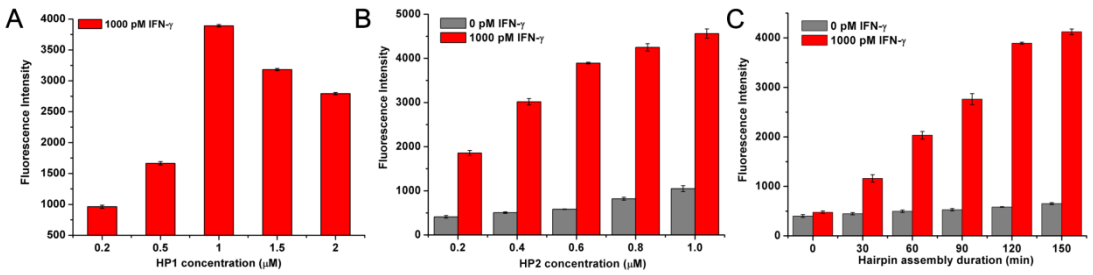

Fig. S2 (A) Effects of HP1 concentration on the fluorescence responses obtained upon detecting 1000 pM IFN-γ. (B) and (C) correspond to effects of HP2 concentration and reaction time for target-initiated CHA on the fluorescence responses obtained upon detecting 0 and 1000 pM IFN-γ.

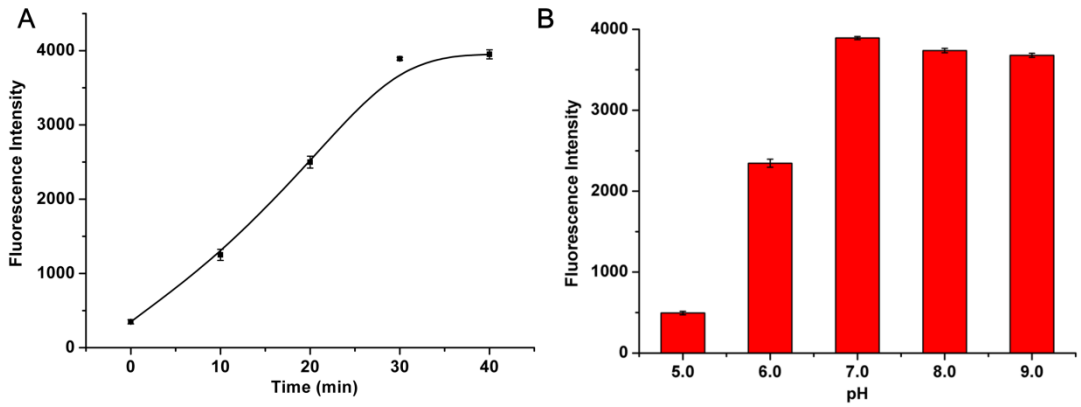

Fig. S3 (A) Effects of interaction time of peptide probe and liposome on the fluorescence responses obtained upon detecting 1000 pM IFN-γ. (B) Effects of pH of the buffer used for interaction of peptide probe and liposome on the fluorescence responses obtained upon detecting 1000 pM IFN-γ.

Table S2 Comparison of different aptamer-based methods for IFN-γ detection.

Detection technique	Signal amplification	Detection limit	Reference
Electrochemical	None	11.56 pM	S1
Electrochemical	Hybridization chain reaction	2 pM	S2
Electrochemical	Self-assembly of stacking DNA	0.57 pM	S3
Colorimetric	Two independent exonuclease III assisted recycling cycles	0.1 pM	S4
Fluorescent	T7 exonuclease-assisted recycling	6.5 pM	S5
Fluorescent	Target-responsive liposome facilitated by catalyzed hairpin assembly	0.047 pM	This work

References

S1. S. Ding, C. Mosher, X. Y. Lee, S. R. Das, A. A. Cargill, X. Tang, B. Chen, E. S. McLamore, C. Gomes, J. M. Hostetter and J. C. Claussen, *ACS Sens.,* 2017, **2,** 210-217.

S2. Y. Yin, L. Shi, Z. Y. Chu and W. Q. Jin, *RSC Adv.,* 2017, **7,** 45053-45060.

S3. Y. Cao, W. W. Chen, P. Han, Z. X. Wang and G. X. Li, *Anal. Chim. Acta.,* 2015, **890,** 1-6.

S4. K. Zhang, T. L. Ren, K. Wang, X. Zhu, H. Wu and M. H. Xie, *Chem. Commun.,* 2014, **50,** 13342-13345.

S5. Y. Qin, L. Zhang, S. Li, J. Zhao, Y. Huang, S. Zhao and Y. M. Liu, *Chem. Commun.,* 2017, **53,** 455-458.

Table S3 Assay results for IFN-γ detection in serum samples

Samples	Added (pM)	Detected (pM)	Recovery (%)	Relative standard deviations (%)
1	10.00	9.32	93.2	3.17
2	100.0	104.6	104.6	4.26
3	1000	1039	103.9	3.78

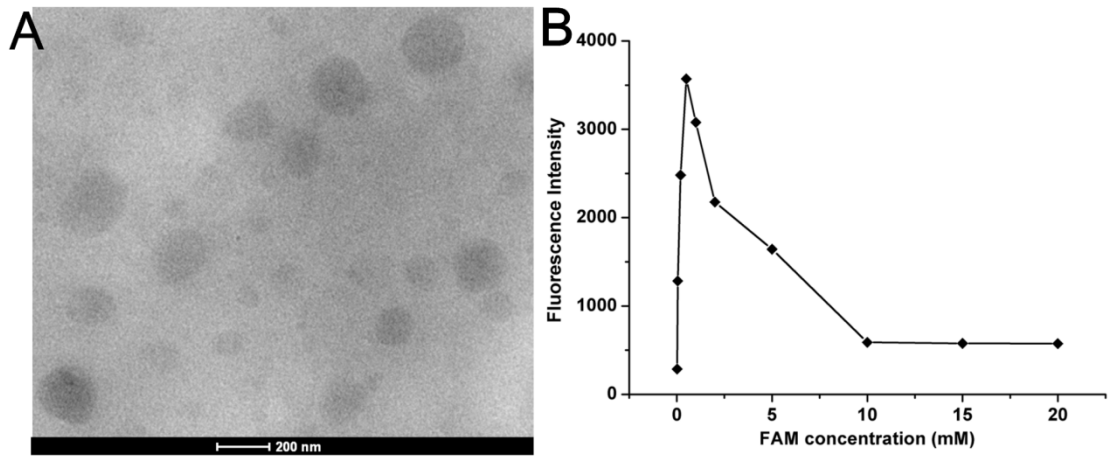

Fig. S4 (A) Transmission electron microscopy (TEM) characterization of FAM-encapsulated liposomes. (B) Effects of FAM concentration on the fluorescence of FAM-encapsulated liposomes.