Supporting Information

A microchip electrophoresis-based fluorescence signal amplification strategy for highly sensitive detection of biomolecules

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Experimental section

Reagents and solutions: Recombinant human IFN-γ was purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. T7 Exo and 10×NEB buffer 4 were purchased from New England Biolabs (Ipswich, MA, USA). Hairpin probe and a fluorescein amidite-labeled DNA (FAM-DNA) signal probe were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd, and their sequences were listed in Table S1. Sodium borate and sodium dodecyl sulfate (SDS) were obtained from Shanghai Chemical Reagent (Shanghai, China). All other chemicals were of analytical reagent grade. Water was purified with a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA). The IFN-γ was dissolved in water and stored at -20 °C. All solutions were filtered through 0.45 μm membrane filter before use. Human plasma samples were kindly provided by the No. 5 Hospital (Guilin, China).

Table S1. DNA oligonucleotides sequence used in this work

name	sequences (5′ to 3′) description
hairpin probe[a]	***GGGGTTGGTTGTGTTGGGTGTTGTG***TTTT TTTACACAACACTCGC
FAM-DNA signal probe	FAM-GCGAGTGTTGTGTAAA

[a]The italic bold letters in the hairpin probe are the aptamer sequences of IFN-γ, the underlined letters are completely complementary to the FAM-DNA signal probe, and contain the recognition sequences for T7 Exo.

Apparatus and microfluidic chip: MCE separation and detection was implemented by a laboratory-built MCE-LIF system described previously.[1] Sample loading and electrophoresis separation was achieved by using a multi-terminal high voltage power supply which ranges from 0 V to 5000 V. A self-made glass/PDMS microfluidic chip was used in this work. The size and structure of the chip were shown in Fig. S0, the depth and width of the channels were 25 μm and 45 μm, and the diameter and depth of reservoirs were all 4 mm and 2 mm. The effective separation length was 34 mm. The sampling volume was calculated to be about 110 pL.

References

[1] Y. Qin, J. Zhao, Y. Huang, S. Li and S. Zhao, *Anal. Methods,* 2016, **8**, 1852-1857.

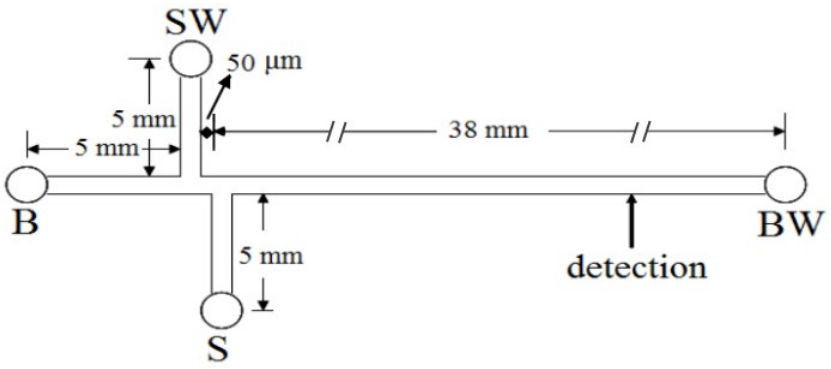

Figure S0. The layout and dimensions of the glass/PDMS microchip used in this work. S: sample reservoir; SW: sample waste reservoir; B: buffer reservoir; BW: buffer waste reservoir.

Amplified reaction: Hairpin probe was heated to 95 °C for 10 min, and slowly cooled down to room temperature before use. Then, a volume of 2.0 μL of the hairpin probe (0.5 μM) solution was mixed with 2.5 μL FAM-DNA signal probe (2.5 μM), 2.5 μL 10×NEB buffer 4, 2.0 μL T7 Exo and varying concentrations of IFN-γ solution, the mixed solution was diluted with water to 25 μL, and the final concentration of FAM-DNA signal probe was 0.25 μM. The reaction mixture was incubated at 37 °C for 1 h. After that, MCE-LIF measurements were performed.

Microchip electrophoresis: Before repetitive runs, 0.1 M NaOH solution, water, and electrophoretic buffer solution (25 mM borate buffer solution at pH 9.0 containing 30 mM SDS) were used to rinse the electrophoresis channels for 6 min each. Electrophoretic buffer solutions were then added into all reservoirs, and filled into all channels by using vacuum in reservoir BW. After that, the electrophoretic buffer in reservoir S was removed and the sample solution was added. Then, a group of electrical potentials (600 V for reservoir S, 250 V for reservoir B, 350 V for reservoir BW, and reservoir SW at grounded) were set at the mode of loading sample. The sample solution could be moved from reservoir S to reservoir SW in pinched mode. After 20 s, potentials at reservoir B, S and SW were set to be 2300, 1400 and 1400 V, and reservoir BW at grounded for sample separation and fluorescence detection.

The viability of proposed strategy:

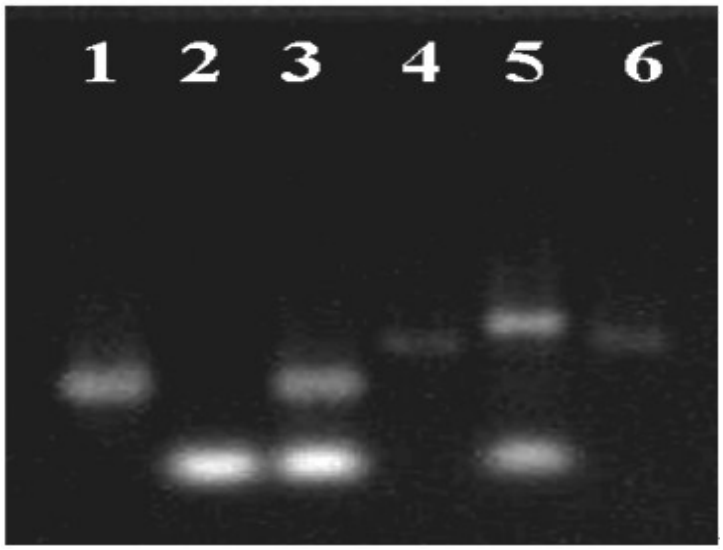

Figure S1. Agarose gel electrophoresis analysis. (1) Hairpin probe; (2) FAM-DNA; (3) hairpin probe+FAM-DNA; (4) hairpin probe+IFN-γ; (5) hairpin probe +FAM-DNA+IFN-γ; (6) hairpin probe+FAM-DNA+IFN-γ+T7 Exo. The concentrations of hairpin probe, FAM-DNA and IFN-γ were 1.2 μM, 4.0 μM and 1.5 μM, respectively.

Optimization of the conditions for signal amplification and electrophoresis separation:

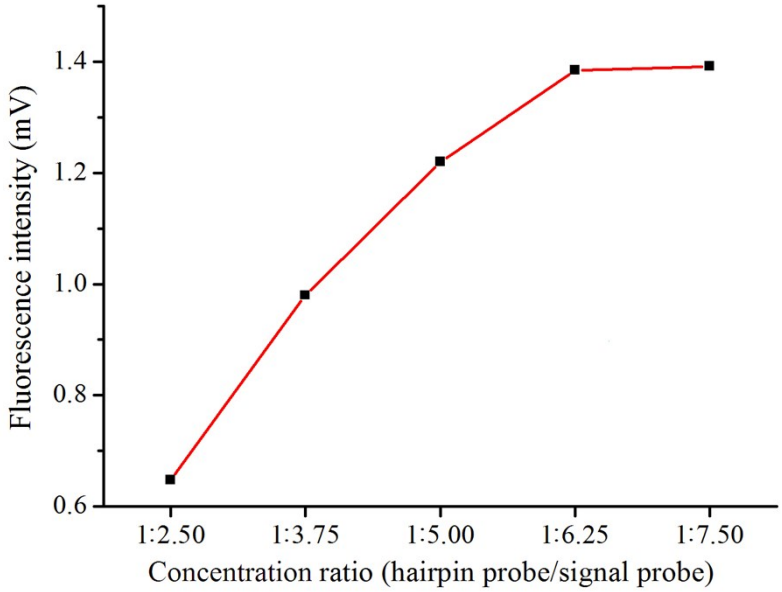

Figure S2: Optimization of the concentrations ratio of hairpin probe and signal probe. Electrophoresis buffer was 25 mM borate solution (pH 9.0) containing 30 mM SDS. The concentrations of T7 Exo and IFN-γ were 20 U and 1.5 nM, respectively.

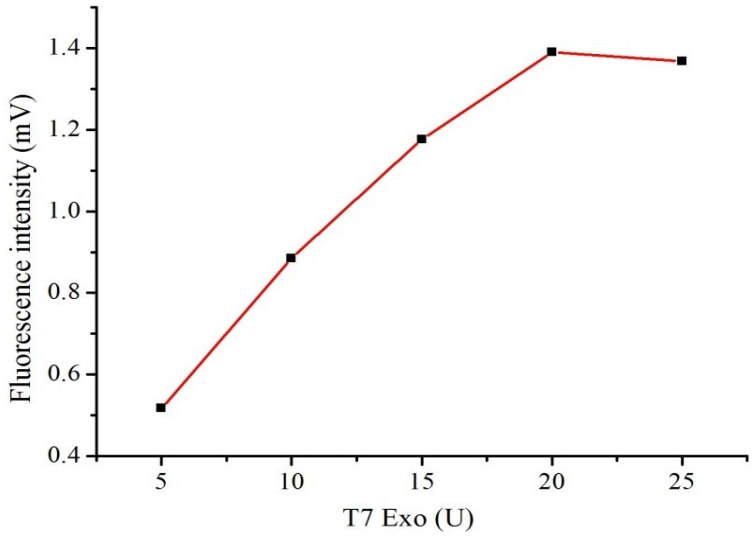

Figure S3: Optimization of the amount of T7 Exo. Electrophoresis buffer was 25 mM borate solution (pH 9.0) containing 30 mM SDS. The concentrations of hairpin probe, FAM-DNA and IFN-γ were 40 nM, 250 nM and 1.5 nM, respectively.

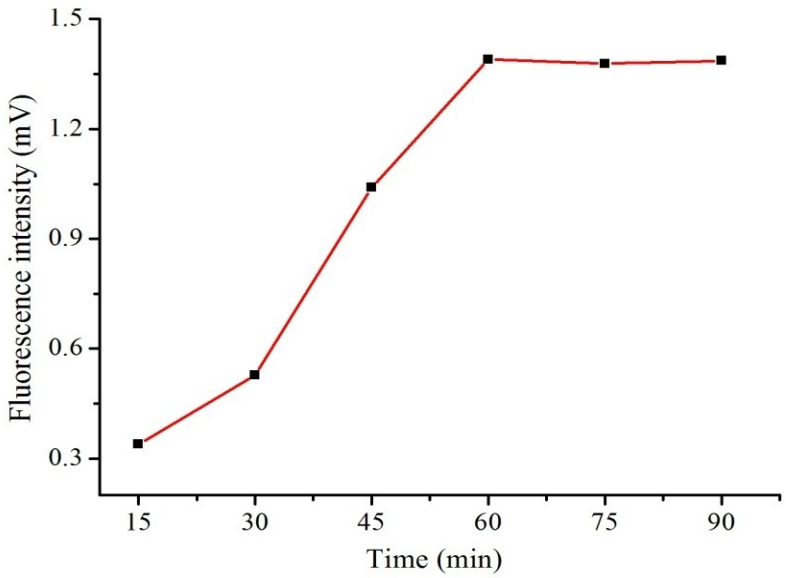

Figure S4: Optimization of the incubation time. Electrophoresis buffer was 25 mM borate solution (pH 9.0) containing 30 mM SDS. The concentrations of hairpin probe, FAM-DNA, T7 Exo and IFN-γ were 40 nM, 250 nM, 20 U and 1.5 nM, respectively.

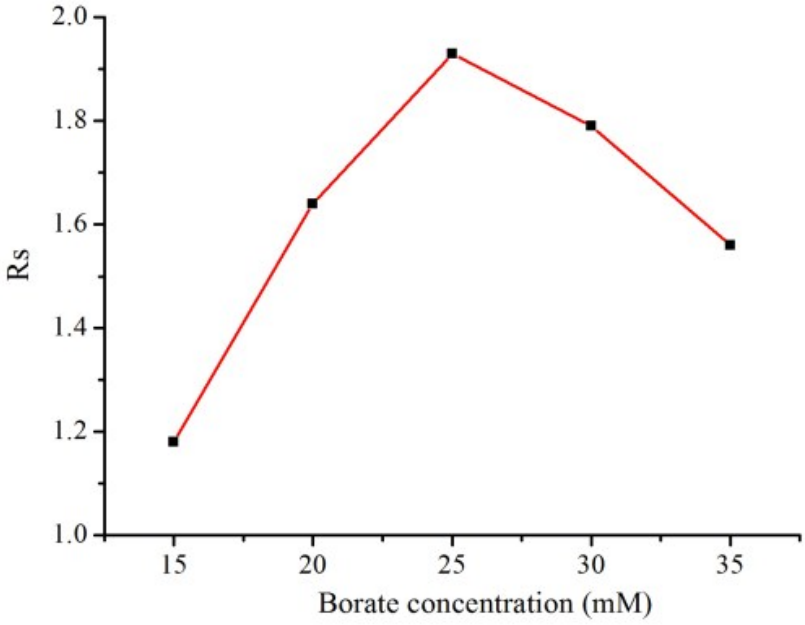

Figure S5: The effect of borate concentration. Electrophoresis buffer was borate solution (pH 9.0) containing 30 mM SDS. The concentrations of hairpin probe, FAM-DNA and IFN-γ were 40 nM, 250 nM and 1.5 nM, respectively.

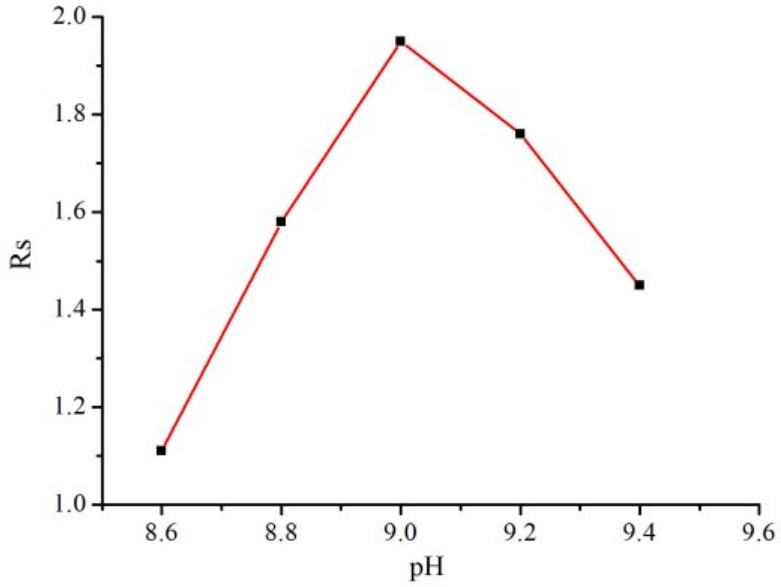

Figure S6: Effect of the electrophoresis buffer pH on Rs. Electrophoresis buffer was 25 mM borate solution containing 30 mM SDS. The concentrations of hairpin probe, FAM-DNA and IFN-γ were 40 nM, 250 nM and 1.5 nM, respectively.

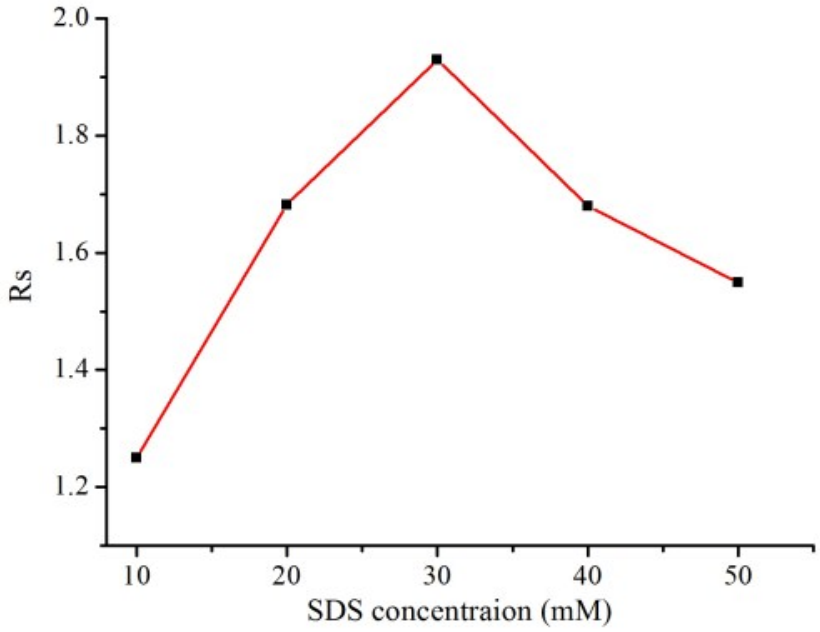

Figure S7. Optimization of the SDS concentration. Electrophoresis buffer was 25 mM borate solution (pH 9.0) containing different concentration of SDS. The

concentrations of hairpin probe, FAM-DNA and IFN-γ were 40 nM, 250 nM and 1.5 nM, respectively.

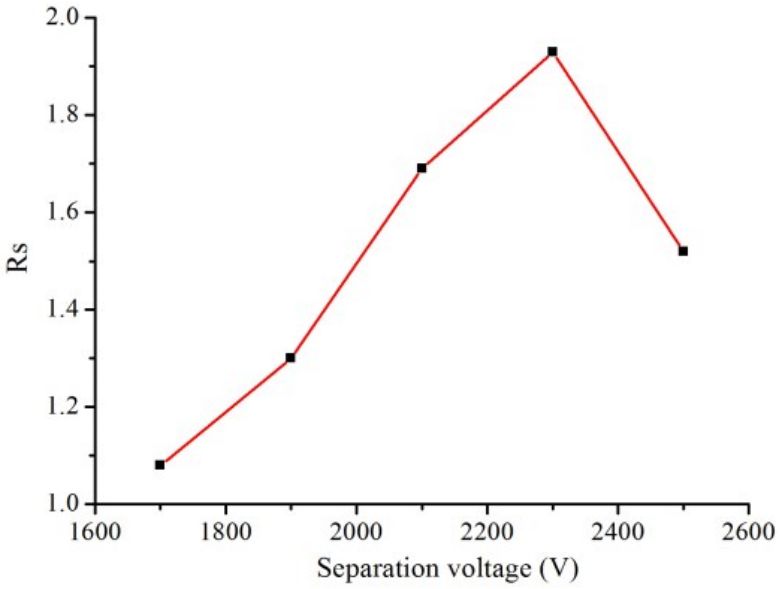

Figure S8. Effect of the separation voltage. Electrophoresis buffer was 25 mM borate solution (pH 9.0) containing 30 mM SDS. The concentrations of hairpin probe, FAM-DNA and IFN-γ were 40 nM, 250 nM and 1.5 nM, respectively.

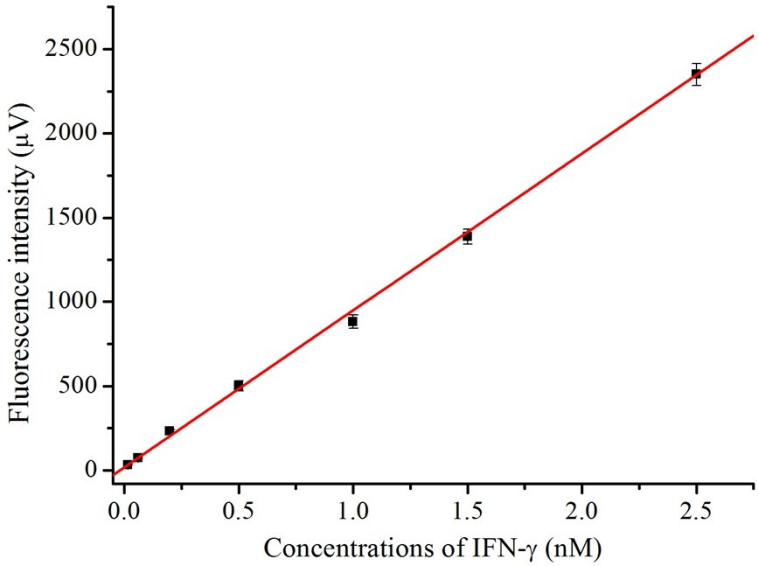

Figure S9. The calibration curve of IFN-γ concentrations for H=928.6C+18.08. Electrophoresis buffer was 25 mM borate solution (pH 9.0) containing 30 mM SDS.

The concentrations of hairpin probe, FAM-DNA and T7 Exo were 40 nM, 250 nM and 20 U, respectively.

Table S1 Comparison of detection limit between the proposed method with reported methods for IFN-γ detection.

Methods	Dynamic range (M)	Sensitivity (M)	Reference
Hybridization chain reaction-based electrochemical aptasensor.	5.0×10^{-10}～3.0×10^{-7}	3.0×10^{-10}	[1]
Hairpin aptamer and catalytic DNAzyme -based electrochemical detection.	0～1.2×10^{-7}	1.0×10^{-10}	[2]
Aptamer beacon -based FRET detection.	5.0×10^{-9}～1.0×10^{-7}	1.0×10^{-7}	[3]
Graphene and nuclease cleavage amplification-based electrochemical aptasensor.	1.0×10^{-13}～7.0×10^{-13}	6.5×10^{-14}	[4]
Structure-switching aptamer-based fluorescence detection.	3.0×10^{-9}～1.2×10^{-7}	2.0×10^{-9}	[5]
Aptamer-based electrochemical biosensor.	6.0×10^{-11}～1.0×10^{-8}	6.0×10^{-11}	[6]
Hairpin aptamer DNAzyme probes amplification-based visual detection.	0～1.0×10^{-7}	1.5×10^{-12}	[7]
T7 Exonuclease amplification-based MCE- LIF detection.	1.5×10^{-11}～2.5×10^{-9}	6.5×10^{-12}	This work

Table S2 Detection results and recovery of IFN-γ in human plasma samples.

Sample number	Found (pM)	RSD (%, n=5)	Added (pM)	Total found (pM)	RSD (%, n=5)	Recovery (%, n=5)
1	360.2	2.8	200.0	555.0	3.1	97.4
			400.0	774.2	3.6	103.5
2	230.4	3.3	200.0	442.0	2.9	105.8
			400.0	616.0	3.4	96.4
3	163.2	3.7	200.0	359.0	3.8	97.9
			400.0	546.0	3.3	95.7
4	252.6	2.9	200.0	461.6	2.8	104.5
			400.0	637.4	4.0	96.2

References

[1] J. Zhao,C. Chen, L. Zhang, J. Jiang and R. Yu, An electrochemical aptasensor based on hybridization chain reaction with enzyme-signal amplification for interferon-gamma detection. *Biosens. Bioelectron.,* 2012, **36**, 129-134.

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[3] N. Tuleuova, C. N. Jones, J. Yan, E. Ramanculov, Y. Yokobayashi and A. Revzin, Development of an aptamer beacon for detection of interferon-gamma. *Anal. Chem.,* 2010, **82**, 1851-1857.

[4] G. Yan, Y. Wang, X. He, K. Wang, J. Liu and Y. Du, A highly sensitive label-free electrochemical aptasensor for interferon-gamma detection based on graphene controlled assembly and nuclease cleavage-assisted target recycling amplification. *Biosens. Bioelectron.,* 2013, **44**, 57-63.

[5] L. Pan, Y. Huang, C. Wen and S. Zhao, Label-free fluorescence probe based on structure- switching aptamer for the detection of interferon gamma. *Analyst,* 2013, **138**, 6811-6816.

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