Electronic Supplementary Information

Development of a single-molecule biosensor with ultra-low background for simultaneous detection of multiple retroviral DNAs

Zi-yue Wang,[‡a] Ya-ru Meng,[‡a] Jinping Hu,[‡b] Jian-Ge Qiu,*[c] and Chun-yang Zhang*[a]

[a] College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, China.

[b] School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China.

[c] Academy of Medical Sciences, Zhengzhou University, Zhengzhou, Henan, 450000, China.

* Corresponding author. E-mail: cyzhang@sdnu.edu.cn; jiangeqiu@zzu.edu.cn.

[‡] These authors contributed equally.

1. Chemicals and materials

RNase H (Catalog: M0297S), 10× RNase H Reaction Buffer (Catalog: B0297S; 750 mM KCl, 500 mM Tris-HCl, 30 mM $MgCl_2$, 100 mM DTT, pH 8.3), RNase inhibitor (Catalog: M0314S) were obtained from New England Biolabs (Beverly, MA, USA). The superparamagnetic beads of 2.8 μm in diameter with a streptavidin monolayer covalently coupled to the surface (MBs; Catalog: 11206D) were obtained from Life Technologies (Carlsbad, CA, USA). Fetal bovine serum (FBS; Catalog: SA211) was obtained from CellMax (Beijing, China). QIAamp DNA Mini Kit (Catalog:51304) was obtained from Qiagen (Redwood, CA, USA). Human T lymphoblastic leukemia cells (HuT-78 cells) and human multiple myeloma cells (U266B1 cells) were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). All other regents were of analytical grade without further purification. Ultrapure water was prepared by a Millipore filtration system (Millipore, Milford, MA, U.S.A.).

2. Average loading density of signal probes modified on the MBs

In this assay, the average loading density of signal probes modified on the surface of MBs may directly affect the experimental results, and it should be investigated. The detailed operation procedures were as follows. Firstly, 5 μL of 10 mg/mL streptavidin-coated MBs was washed twice with 2× binding and washing (B&W) buffer (10 mM Tris-HCl, 1 mM EDTA, and 2 M NaCl, pH 7.5). After resuspension with 10 μL of 2× B&W buffer, 10 μL of 1 μM signal probe 1 and 10 μL of 1 μM signal probe 2 were added to the MBs suspensions and incubated in the dark for 15 min on a roller mixer at room temperature to form the signal probes-MBs conjugates through streptavidin-biotin interaction. Secondly, the resultant signal probes-MBs conjugates were washed

three times using 1× B&W buffer (5 mM Tris-HCl, 500 μM EDTA, and 1 M NaCl, pH 7.5) to remove the uncoupled signal probes, followed by resuspending in 20 μL of DEPC water. The signal probes were then eluted from MBs by incubation at 95 ° C for 60 s, followed by quickly placing the tubes on the DynaMag™-PCR magnetic rack to separate the supernatant. After magnetic separation, the supernatant was subjected to fluorescence emission spectra measurement and single-molecule detection.

The average loading density of signal probes modified on the MBs was calculated as follows. Firstly, we determined the Cy3 and Cy5 fluorescence intensities corresponding to different amounts of signal probe 1 and signal probe 2, respectively. As shown in Figs. S1A and S1B, the amounts of signal probes are proportional to the fluorescence intensities in the range from 0.125 pmol to 10 pmol. The regression equations are $F_1 = 478.11 + 251.48\ N_1$ ($R^2 = 0.9694$) for signal probe 1 and $F_2 = 429.90 + 463.40\ N_2$ ($R^2 = 0.9382$) for signal probe 2, where F_1 and F_2 represent the fluorescence intensities of Cy3 and Cy5, and N_1 and N_2 represent the amount of signal probe 1 (pmol) and signal probe 2 (pmol), respectively. The average amounts of signal probe 1 and signal probe 2 on the surface of MBs are calculated according to Eq. (1) and Eq. (2), respectively.

$$\overline{N_1} = \frac{N_1}{m} = \frac{(F_1 - 478.11)/251.48}{50}\ \text{pmol/μg} \qquad (1)$$

$$\overline{N_2} = \frac{N_2}{m} = \frac{(F_2 - 429.90)/463.40}{50}\ \text{pmol/μg} \qquad (2)$$

where $\overline{N_1}$ and $\overline{N_2}$ are the average number of signal probe 1 and signal probe 2 on the surface of MBs. Secondly, we measured the fluorescence intensities of the eluted signal probes following the procedures described above. Figs. S1C and S1D are the fluorescence emission spectra in response to the eluted signal probe 1 and signal probe 2, respectively. The average loading density of signal probes modified on the MBs is calculated to be 0.1704 pmol/μg for signal probe 1 and 0.1588

pmol/μg for signal probe 2.

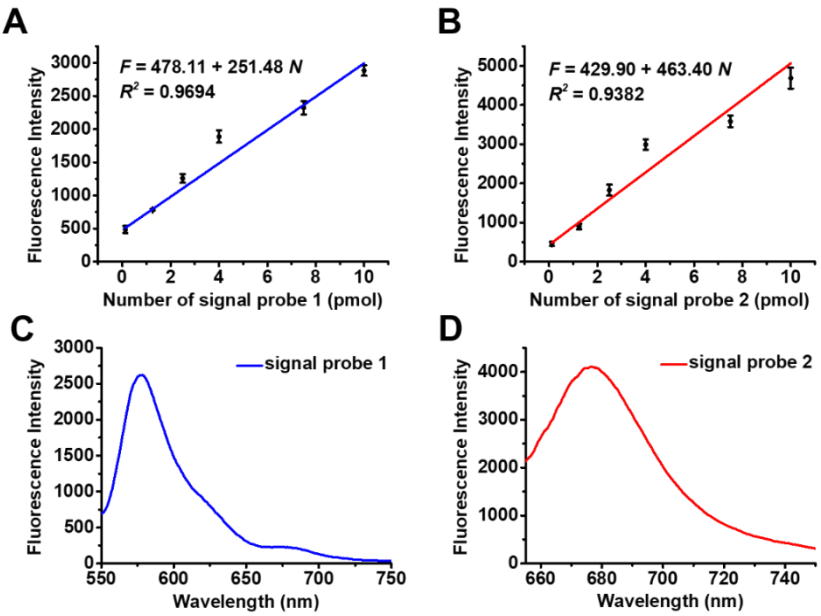

Fig. S1 (A) Variance of Cy3 fluorescence intensity with different amounts of signal probe 1. (B) Variance of Cy5 fluorescence intensity with different amounts of signal probe 2. (C) Cy3 fluorescence emission spectrum generated by the eluted signal probe 1. (D) Cy5 fluorescence emission spectrum generated by the eluted signal probe 2. The concentration of MBs conjugates is 2.5 mg/mL. Error bars represent the standard deviation of three experiments.

3. Time-dependent reaction dynamic for the RNase H-assisted cleavage of signal probes on MBs.

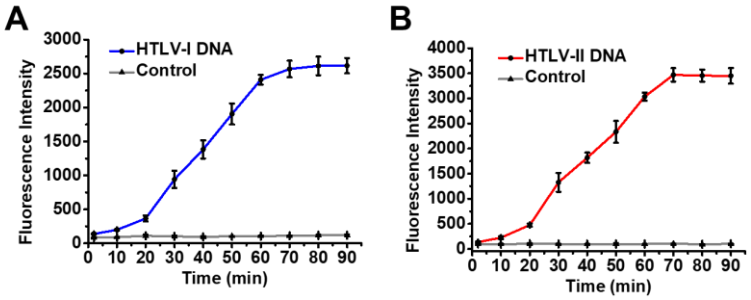

Fig. S2 (A) Time-dependent reaction dynamic for the RNase H-assisted cleavage of signal probe 1

on MBs. (B) Time-dependent reaction dynamic for the RNase H-assisted cleavage of signal probe 2 on MBs. The 1 μM HTLV-I DNA, 1 μM HTLV-II DNA, 1.25 U RNase H, and 2.5 mg/mL signal probes-MBs conjugates were used in the experiments. Error bars represent the standard deviation of three experiments.

4. Optimization of experimental conditions

4.1 Optimization of the amount of RNase H

RNase H is responsible for the cyclic cleavage of signal probes and recycling of targets, and it should be optimized carefully. As shown in Fig. S1, the Cy3 and Cy5 counts enhance with the increasing amount of RNase H from 0.5 to 1.25 U, and reach the maximum value at 1.25 U. Therefore, 1.25 U RNase H is used in the subsequent researches.

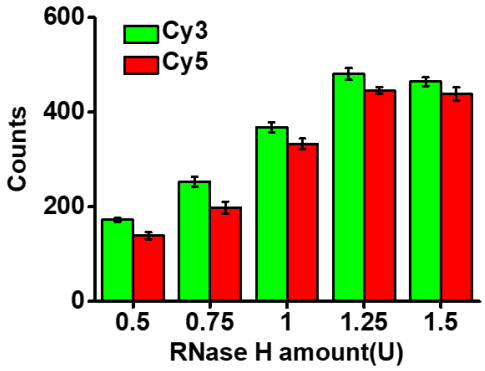

Fig. S3 Variance of Cy3 and Cy5 counts in response to different amounts of RNase H in the presence of 1 μM HTLV-I DNA (green column) and 1 μM HTLV-II DNA (red column), respectively. The concentration of signal probes-MBs conjugates is 2.5 mg/mL. Error bars represent the standard deviation of three experiments.

4.2 Optimization of the reaction time of RNase H

As shown in Fig. S2, the Cy3 and Cy5 counts enhance with the reaction time of RNase H from 10 to 70 min, followed by the decrease beyond 70 min due to the complete depletion of signal probes by RNase H. Thus, 70 min is used as the optimal reaction time of RNase H.

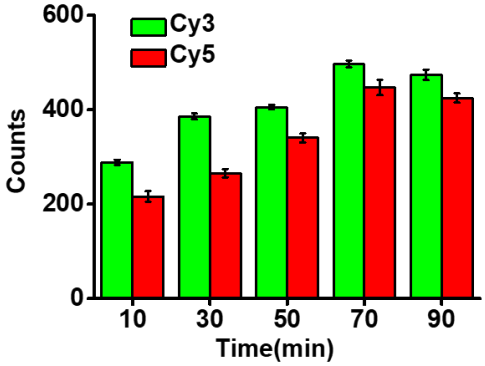

Fig. S4 Variance of Cy3 and Cy5 counts with reaction time of RNase H in the presence of 1 μM HTLV-I DNA (green column) and 1 μM HTLV-II DNA (red column), respectively. The amount of RNase H is 1.25 U, and the concentration of signal probes-MBs conjugates is 2.5 mg/mL. Error bars represent the standard deviation of three experiments.

4.3 Optimization of the reaction temperature of RNase H

The rection temperature of RNase H directly influences the activity of RNase H, and eventually affects the release of Cy3 and Cy5 fluorophores from the MBs. Thus, the reaction temperature of RNase H should be optimized. As shown in Fig. S3, the Cy3 and Cy5 counts enhances with the increasing reaction temperature of RNase H from 25 ℃ to 30 ℃, followed by the decrease beyond 30 ℃. Thus, 30 ℃ is used as the optimal reaction temperature of RNase H in the subsequent researches.

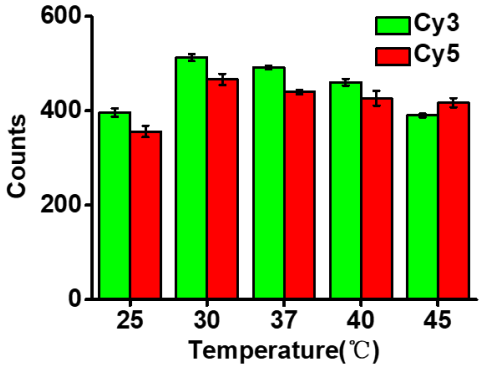

Fig. S5 Variance of Cy3 and Cy5 counts with different reaction temperatures of RNase H in the presence of 1 μM HTLV-I DNA (green column) and 1 μM HTLV-II DNA (red column), respectively. The amount of RNase H is 1.25 U, and the concentration of signal probes-MBs conjugates is 2.5 mg/mL. Error bars represent the standard deviation of three experiments.

5. Recovery assay

Table S1. Recovery studies by spiking HTLV-I DNA and HTLV-II DNA into 10% fetal bovine serum samples.

		Add (nM)	Measured (nM)	Recovery (%)	RSD (%)
Sample 1	HTLV-I DNA	1.00	0.98	98.00	1.43
	HTLV-II DNA	1.00	0.99	99.00	1.00
Sample 2	HTLV-I DNA	10.00	9.93	99.30	1.49
	HTLV-II DNA	10.00	10.01	100.10	1.05
Sample 3	HTLV-I DNA	100.00	100.40	100.40	1.04
	HTLV-II DNA	100.00	101.50	101.50	1.28