Supporting Information

A Bifunctional DNA Probe for Sensing pH and microRNA using a Nanopore

Yun Zhang,[‡a,d] Peng Song,[‡b] Bingyuan Guo,*[a] Wenying Hao,[a] Lei Liu[c] and Hai-Chen Wu*[a,d]

[a]*Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China*
E-mail: haichenwu@iccas.ac.cn; Tel: +8610-82614948

[b]*Department of Geriatric Oncology, General Hospital of the Chinese People's Liberation Army, Beijing 100853, China*

[c]*Key Laboratory for Biomedical Effects of Nanomaterials & Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China.*

[d]*University of Chinese Academy of Sciences, Beijing 100049, China*

26th November 2021
Note added after first publication: This Supplementary Information file replaces that originally published on 10th August 2020. Figure S1 has been updated to correct the captions and labels for Fig. S1b-g and to remove Fig. S1h-j.

Materials and Methods

All the chemicals were purchased from Sigma-Aldrich, Alfa Aesar, and J&K, and used without further purification. 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPc) was purchased from Avanti Polar Lipids (Alabaster, AL). All DNA and RNA oligomers (Table S1) were purified by HPLC and purchased from Sangon Biotechnology Co. Ltd. (Shanghai). Dynabeads^{TM} MyOne^{TM} Streptavidin T1(~7-10×10^9 beads/mL) was obtained from Invitrogen (Shanghai). Micro Bio-Spin P6 gel columns (Tris buffer) were purchased from Bio-Rad (Hercules, CA). All the columns were pre-equilibrated three times with 80 μL deionized water prior to use. All miRNA samples were dissolved in DEPC water and stored at -80 ^{o}C. DNA and RNA concentration were determined on a NanoDrop 2000C pectrophotometer. Amicon Ultra-0.5 centrifugal filter devices were purchased from Millipore. DNA ESI mass spectrometry data were analyzed on Thermo-Finnigan LCQ Deca XP Plus. CD spectra were carried out with a JASCO J-815 spectrometer. Wild type αHL-D8H6 proteins were produced as described previously.[1]

Buffer preparation

In order to ensure the stability and accuracy of buffer pH, different buffer salts were chosen for different pH solutions. 22.365 g of KCl (99.999%, Sigma-Aldrich), buffer salt (pH 4.5-6.5: 0.213 g MES, 99.0%, Sigma-Aldrich; pH 7.0-7.5: 0.136 g KH_2PO_4, 99.0%, J&K; pH 8.0-9.0: 0.121 g Tris, 99.0%, Sigma-Aldrich) and 0.095 g of magnesium chloride (99.9%, Sigma-Aldrich) were dissolved in 80 mL of deionized water (Millipore, MA). 1 M HCl or 1 M NaOH was used to adjust the solution to desired pH. The solution was diluted with deionized water to 100 mL. The final buffer solution consists of 3 M KCl, 10 mM buffer salt and10 mM $MgCl_2$ at desired pH.

General procedure for the preparation of DNA-Fc⊂CB[7] probes

3.3 μL alkyne-containing DNA (100 μM), 1.2 μL deionized water, 2.0 μL azidomethylferrocene (dissolved in acetonitrile, 200 μM), 1.0 μL sodium ascorbate (20 mM), 0.5 μL copper nitrate (20 mM) were added to 2.0 μL HEPES (100 mM) buffer, with a final volume of 10 μL. The reaction was incubated for 2 h at room

temperature, and then 2.0 μL EDTA solution (100 mM) was added to terminate the reaction. The DNA product was purified with Micro Bio-spin P6 columns. The filtrate (DNA-Fc) was analyzed by ESI-MS to confirm the conjugation between DNA-Alkyne and ferrocene azide. Next, 10 μL CB[7] aqueous solution (5.0 mM) was added to the DNA solution and incubated for 2 h to afford the final DNA probes.

pH detection experiments

DNA 4 (0.8 μL, 125 μM), DNA 13 (0.7 μL, 143 μM) and DNA 14 (0.7 μL, 143 μM) were incubated with DNA3-Fc (3.1 μL, 31.7 μM) in 10.0 μL incubation buffer (10 mM PB, 20 mM NaCl, 2.5 mM $MgCl_2$, pH 5.0) overnight at 4°C, respectively, to form the triplex molecular beacon probes tMB1-3. Then 10 μL CB[7] (5 mM) was added to the sample and incubated for 2 h. The sample after incubation was ready for single channel recording experiments.

Procedure for the circular dichroism measurements of triplex probes

DNA3 (4.1 μL, 122 μM) and DNA4 (3.7 μL, 135 μM) were added to 15.0 μL incubation buffer (10 mM PB, 20 mM NaCl, 2.5 mM $MgCl_2$, pH 5.0) and incubated overnight at 4°C to form the triplex probe tMB1. Circular dichroism (CD) spectra were recorded in a 1 cm path length cuvette at 20^{o}C using a Jasco J-810 spectropolarimeter (Jasco, Easton, MD) with a final concentration of 1.25 μM tMB1 in different pH buffers. The typical instrumental parameters to record the CD spectra were: 200-400 nm measurement range, 1 nm data pitch, 1 nm band width, 0.5 sec response, standard sensitivity, 500 nm/min of scanning speed. The CD spectra of tMB1 were recorded with the spectral contribution from the buffer subtracted.

MicroRNA detection experiments

DNA14 (1.6 μL, 125 μM) and DNA3-Fc (6.3 μL, 31.7 μM) were added to 12.1 μL incubation buffer (10 mM PB, 20 mM NaCl, 2.5 mM $MgCl_2$, pH 5.0) and incubated overnight at 4°C to form the triplex probe. The total volume was 20 μL. Then the triplex probe was incubated with various concentrations of microRNA-10b (final concentration: 0.005, 0.05, 0.5, 1, 10, 50, 100 and 200 nM) or **U**-mismatch sequence (miRNA1, 200 nM), **G**-mismatch sequence (miRNA2, 200 nM), **C**-mismatch

sequence (miRNA3, 200 nM) and two-mismatch sequence (miRNA4, 200 nM) respectively for 0.5 h at room temperature. Meanwhile, magnetic beads suspension (100 μL, 10 mg/mL) was washed 3 times with 100 μL 1×BW buffer (1 M NaCl, 0.5 mM EDTA, 5 mM PB, pH 5.0). The resulting probe solution was mixed with magnetic beads in 200 μL buffer 1×BW buffer and vortexed gently for 15 min. The supernatant was collected with the aid of a magnetic separator, and magnetic beads were washed with 1×BW buffer. The solutions were combined and ultra-centrifuged with an Amicon Ultra-0.5 centrifugal filter (3 KD). 10 μL CB[7] (5.0 mM) was added to the concentrated sample and incubated for 2 h. The sample after incubation was ready for single channel recording experiments which were carried out in pH 8.0 buffer unless otherwise stated.

pH detection experiments in spiked serum samples

DNA 14-Fc (0.7 μL, 143 μM) were incubated with DNA3-Fc (3.1 μL, 31.7 μM) in 10.0 μL diluted fetal bovine serum (serum/incubation buffer = 1:20, v/v) overnight at 4°C, to form the tMB probe tMB3. Then 10 μL CB[7] (5.0 mM) was added to the sample and incubated for 2 h. The sample after incubation was ready for single channel recording experiments.

MicroRNA detection experiments in spiked serum samples

DNA14 (1.6 μL, 125 μM) and DNA3-Fc (6.3 μL, 31.7 μM) were added to 12.1 μL diluted fetal bovine serum (serum/incubation buffer = 1:20, v/v) overnight at 4°C, to form the tMB probe tMB3. The total volume was 20 μL. Then the triplex probe was incubated with various concentrations of microRNA-10b (final concentration: 0.05, 5, 10, 50, 100 and 200 nM, respectively, for 0.5 h at room temperature. The subsequent steps were the same as described above for microRNA detection in buffer solutions. 10 μL CB[7] (5.0 mM) was added to the concentrated sample and incubated for 2 h. The sample after incubation was ready for single channel recording experiments which were carried out in pH 8.0 buffer unless otherwise stated.

Single-channel current recording

DPhPc was used to form a synthetic lipid bilayer across an aperture 100-150 μm in diameter in a 25-μm-thick polytetrafluoroethylene film (Goodfellow, Malvern, PA) that divided a planar bilayer chamber into two compartments, *cis* and *trans*. Both compartments contained 1 mL of buffer solution. DNA samples were added to the *cis* compartment, which was connected to ground. The *trans* compartment was connected to the head-stage of the amplifier. For pH measurements, all experiments were carried out in 3 M KCl, 10 mM buffer salt, 10 mM $MgCl_2$, at 22.5±2 °C, under desired pH. For microRNA detection, all experiments were carried out in 3 M KCl, 10 mM Tris, 10 mM $MgCl_2$, pH 8.0, at 22.5±2 °C, unless otherwise stated. Ionic currents were measured by using Ag/AgCl electrodes with a patch-clamp amplifier (Axopatch 200B; Axon instruments, Foster City, CA), filtered with a low-pass Bessel filter with a corner frequency of 10 kHz and then digitized with a Digidata 1440A A/D converter (Axon Instruments) at a sampling frequency of 100 kHz.

Data analysis

Current traces were analyzed with Clampfit 10.2 software (Axon Instruments). Events were detected using the Event Detection feature, and used to construct amplitude and dwell time histograms. Current signature events were manually selected for statistical analysis. OriginPro 2016 (OriginLab, Northampton, MA) and Clampfit 10.2 were used for histogram construction, curve fitting and graph presentation. Adobe Illustrator was used for making figures.

Supplementary Figures

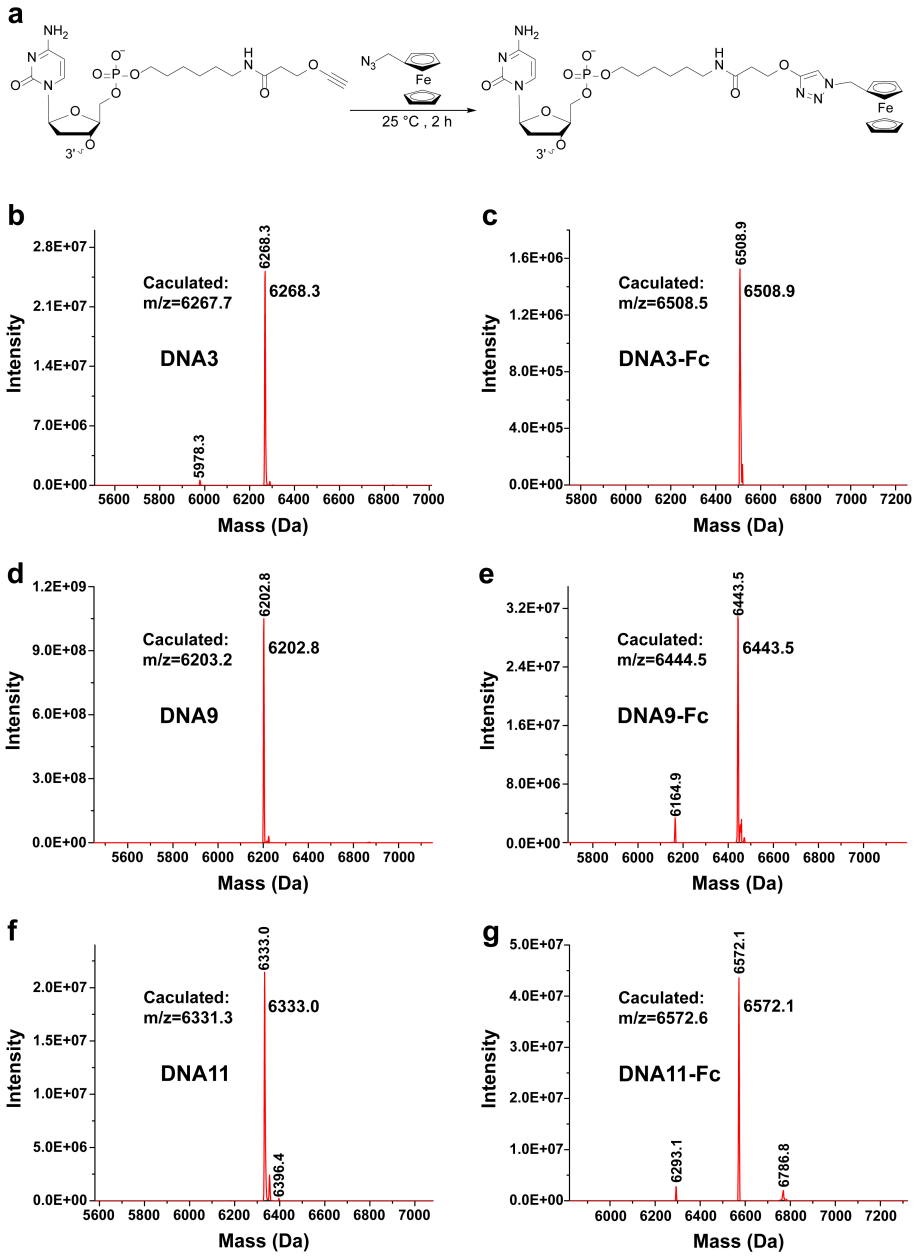


Fig. S1 Modification of DNA1, DNA3, DNA5 and mass spectroscopic characterization. (**a**) Chemical modification of DNA1, DNA3, DNA5 with azidomethylferrocene via "click" chemistry. Mass spectroscopic characterization of DNA3 (**b**); DNA3-Fc (**c**); DNA9 (**d**); DNA9-Fc (**e**); DNA11 (**f**); DNA11-Fc (**g**).

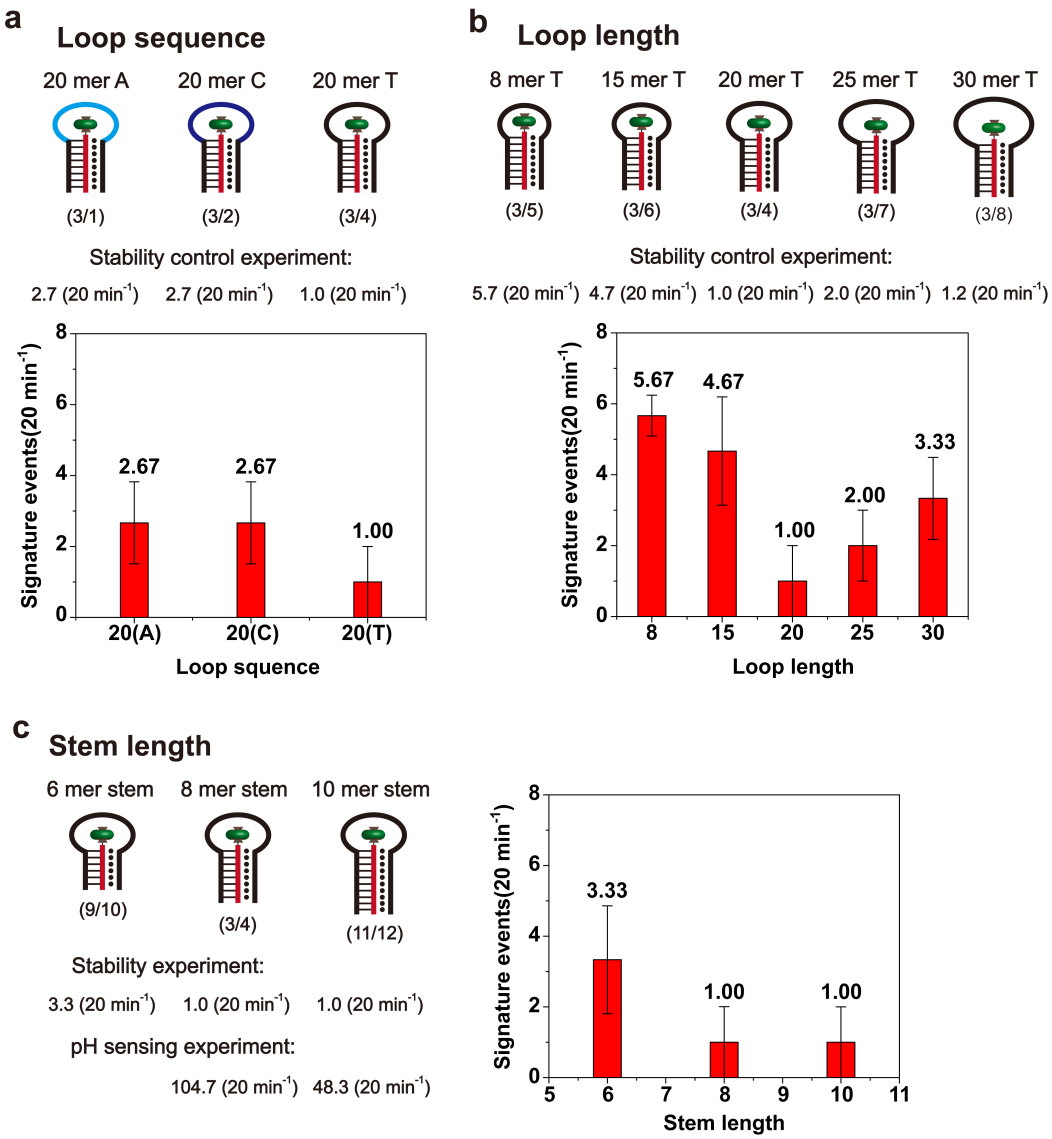


Fig. S2 Condition screening for measuring pH with tMB probe. (**a**) Determination of the loop sequence. Stability control experiments using three different loops: 20-mer polyA, 20-mer polyT and 20-mer polyC, respectively. The corresponding frequency of signature events (f_{sig}) for each probe is 2.67 ± 1.16, 2.67 ± 1.16, 1.0 ± 1.0 (20 min^{-1}), respectively. (**b**) Determination of the loop length. Stability control experiments using five different loop length: 8-mer polyT, 15-mer polyT, 20-mer polyT, 25-mer polyT and 30-mer polyT, respectively. The corresponding f_{sig} for each probe is 5.67 ± 0.58, 4.67 ± 1.53, 1.0 ± 1.0, 2.00 ± 1.00, 3.33 ± 1.16 (20 min^{-1}), respectively. (**c**) Determination of the stem length. The corresponding f_{sig} for each probe is 3.33 ± 1.53, 1.0 ± 1.0, 1.0 ± 1.0, respectively. The results showed that when the stem has only 6 base pairs, the beacon structure is not stable enough. However, if

the stem base pairings are increased to 10, the probe becomes so stable that the sfO cannot be released effectively. Therefore, 8-mer stem is the most appropriate for the tMB probe. The numbers in the parenthesis are DNA numberings. The data of stability experiment were acquired in the buffer of 3 M KCl, 10 mM MES and 10 mM $MgCl_2$, pH 5.0; the data of pH sensing experiment were acquired in the buffer of 3 M KCl, 10 mM MES and 10 mM $MgCl_2$, pH 6.5. All data were acquired with the transmembrane potential held at +200 mV. Number of individual experiments $n = 3$.

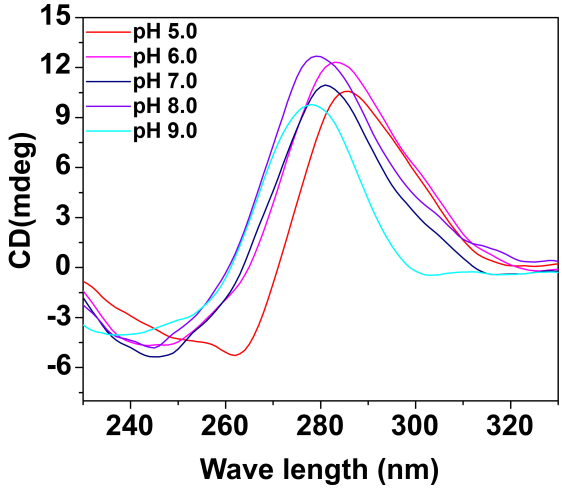


Fig. S3 Circular dichroism measurements of tMB1 probe at different pH. The final concentration of tMB1 probe is 1.25 μM. All data were acquired in the buffer of 3 M KCl, 10 mM buffer salt (pH 4.5-6.5: MES, pH 7.0-7.5: KH_2PO_4, pH 8.0-9.0: Tris) and 10 mM $MgCl_2$. Number of individual experiments n = 3.

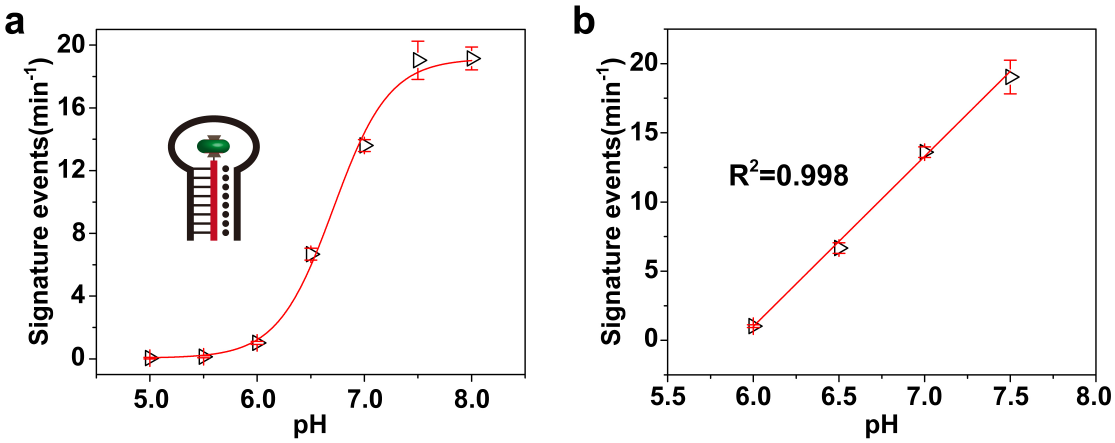


Fig. S4 Detection of solution pH with the tMB2 probe. (**a**) Correlation of the frequency of signature events (f_{sig}) of tMB2 probe with solution pH in the range of 5.0-8.0. (**b**) Correlation of f_{sig} of tMB2 probe with solution pH in the range of 6.0–7.5. The data could be fitted with a linear equation: y = 12.3x – 72.8. The final concentration of tMB2 probe is 100 nM. All data were acquired in the buffer of 3 M KCl, 10 mM buffer salt (pH 5.0-6.5: MES, pH 7.0-7.5 KH_2PO_4, pH 8.0: Tris) and 10 mM $MgCl_2$ at various pH, with the transmembrane potential held at +200 mV. Number of individual experiments n = 3.

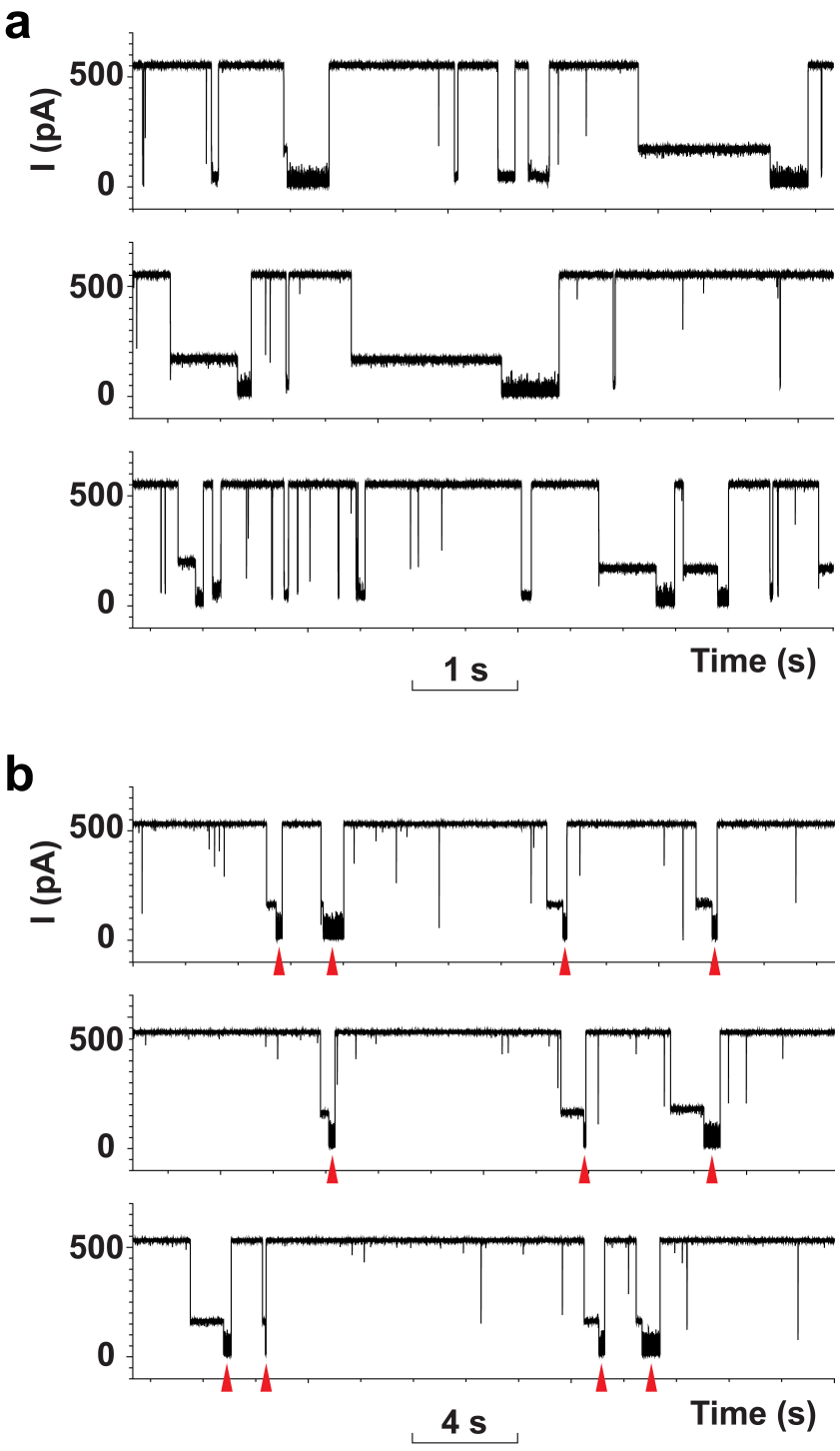


Figure S5. Comparison of the current traces before and after removal of duplex DNA in microRNA sensing with tMB probe. (**a**) There were many unwanted current events in the trace before the duplexes were removed. (**b**) After using magnetic beads, the trace became very clean. The red arrows indicate current signatures. All data were acquired in the buffer of 3 M KCl, 10 mM MES and 10 mM $MgCl_2$, pH 8.0, with the transmembrane potential held at +200 mV.

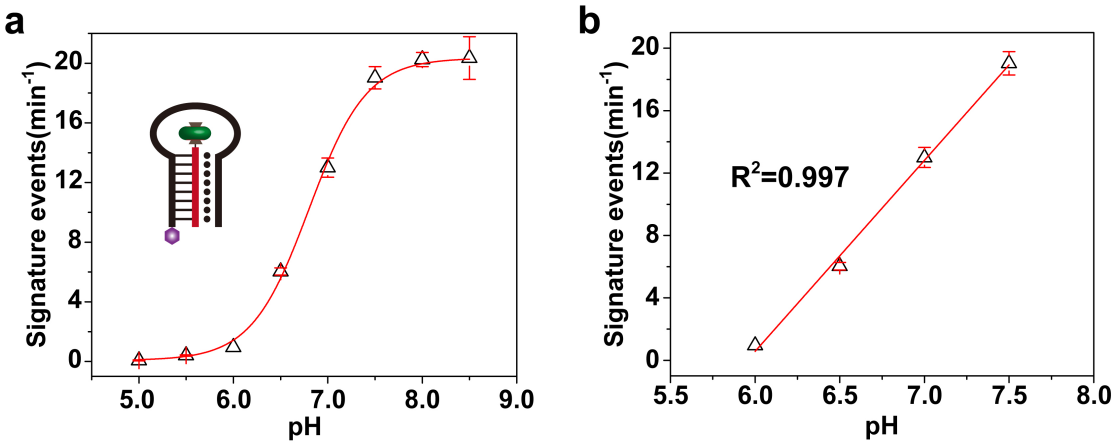


Fig. S6 Detection of solution pH with the tMB3 probe. (**a**) Correlation of f_{sig} of tMB3 probe with solution pH in the range of 5.0 to 8.5. (**b**) Correlation of f_{sig} of tMB3 probe with solution pH in the range of 6.0 to 7.5. At pH 6.0-7.5, the data could be fitted with a linear equation: y = 12.323x–72.951. The final concentration of tMB3 probe is 100 nM. All data were acquired in the buffer of 3 M KCl, 10 mM buffer salt (pH 5.0-6.5: MES, pH 7.0-7.5 KH_2PO_4, pH 8.0-8.5: Tris) and 10 mM $MgCl_2$ at various pH, with the transmembrane potential held at +200 mV. Number of individual experiments n = 3.

Supplementary Tables

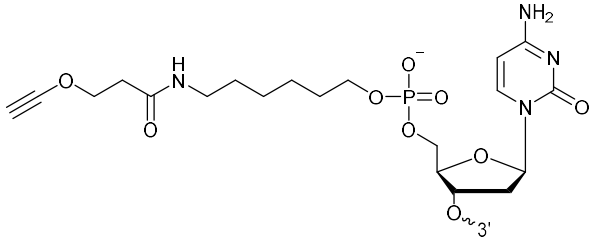
Table S1. Sequences of miRNA targets and DNA probes in this study.

Name	Sequence	Modification
DNA1	5'-TCTCTCTC**AAAAAAAAAAAAAAAAAA**ACTCTCTCT-3'	none
DNA2	5'-TCTCTCT**CCCCCCCCCCCCCCCCCCCC**CCTCTCTCT-3'	none
DNA3	**5'**-CCCCCCCCCCCCGAGAGAGA-3'	5'-alkyne modification
DNA4	5'-TCTCTCTC**TTTTTTTTTTTTTTTTTTTT**CTCTCTCT-3'	none
DNA5	5'-TCTCTCTC**TTTTTTTT**CTCTCTCT-3'	none
DNA6	5'-TCTCTCTC**TTTTTTTTTTTTTTTT**CTCTCTCT-3'	none
DNA7	5'-TCTCTCTC**TTTTTTTTTTTTTTTTTTTTTTTT**CTCTCTCT-3'	none
DNA8	5'-TCTCTCTC**TTTTTTTTTTTTTTTTTTTTTTTTTTTT**CTCTCTCT-3'	none
DNA9	**5'**-CCCCCCCCCCCCCCGAGAGA-3'	5'-alkyne modification
DNA10	5'-TCTCTC**TTTTTTTTTTTTTTTTTTTT**CTCTCT-3'	none
DNA11	**5'**-CCCCCCCCCCGAGAGAGAGA-3'	5'-alkyne modification
DNA12	5'-TCTCTCTCTC**TTTTTTTTTTTTTTTTTTTT**TCTCTCTCTCT-3'	none

DNA13	5’-TCTCTCTC**CACAAATTCGGTTCTACAG GGTA**CTCTCTCT-3’	none
DNA14	**5’-Biotin**-AAAAAAAAAATCTCTCTC**CACAA ATTCGGTTCTACAGGGTA**CTCTCTCT-3’	5’-biotin modification
microRNA-10b	5’-UACCCUGUAGAACCGAAUUUGUG-3’	none
miRNA1	5’-UACCCUGUAGA**U**CCGAAUUUGUG-3’	**U** mismatch
miRNA2	5’-UACCCUGUAGA**G**CCGAAUUUGUG-3’	**G** mismatch
miRNA3	5’-UACCCUGUAGA**C**CCGAAUUUGUG-3’	**C** mismatch
miRNA4	5’-UACCCUGUA**U**AAC**U**GAAUUUGUG-3’	**two** mismatch

Note: all the loop sequences are in bold; all the stem sequences are underlined; the modified site on DNA is marked in red.

5’-alkyne modification



5’-biotin modification

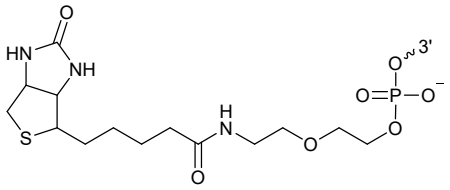


Table S2. Comparison of the different methods for pH detection.

Methods	pH responsive range	Linear range	Ref.
Colorimetry	~	6.0-7.2	[2]
Colorimetry	2.0-5.5	~	[3]
Fluorescence	~	6.0-7.5	[4]
Fluorescence	~	4.0-10.0	[5]
Fluorescence	~	4.0-6.0	[6]
Fluorescence	5.2-8.2	6.0-8.0	[7]
Fluorescence	~	5.59-8.09[a], 4.98-6.40[b]	[8]
Electrochemistry	~	5.8-8.0	[9]
MRI	~	5.09-8.01	[10]
MRI	~	6.0-7.6	[11]
SERS	4.0-9.0	~	[12]
SERS	5.01-9.10	~	[13]
FET	1.0-7.5	~	[14]
FET	~	4.0-10.0	[15]
Photoelectrochemistry	~	2.0-12.0	[16]
Nanopore	5.5-8.0	6.0-7.5	This work

(a) Bromothymol blue as pH sensitive dye. (b) Rhodamine B as pH sensitive dye.

Table S3. Comparison of Different Methods for miRNA Detection.

Methods	Target microRNA	Linear ragne	LOD	Ref.
Colorimetry	miR-21	0.1-1 μM	4.5 nM	[17]
Colorimetry	*let*-7a	0.05-1 μM	10 nM	[18]
Fluorescence	miR-122	0.5-50 nM	72 pM	[19]
Fluorescence/FCM	miR-21, miR-141	0.01-1 pM	3.39 fM[a]	[20]
Fluorescence	miR-141	0.75 pM-20 pM 0.4 nM-40 nM	32 fM	[21]
Fluorescence	miR-155	0.1-1.0 pM	33.4 fM	[22]
Electrochemistry	*let*-7d	1.0×10^{-3}-10 nM	0.17 pM	[23]
Electrochemistry	miR-125a	1-2.0×10^{3} μM	10 pM	[24]
Electrochemistry	miR-21	1.0×10^{-3}-10.0 nM	0.26 pM	[25]
Photoelectrochemistry	miR-319a	5–3.0×10^{3} fM	2.26 fM	[26]
Bioluminescence	*let*-7a	~	7.6 fM	[27]
SERS	miR-17	1-1.0×10^{3} pM	0.26 pM[b]	[28]
SPR	miR-155	0.02-10 nM	45 pM	[29]
SPR	miR-200b	~	500 pM	[30]
MS	miR-21, miR-125a, miR-200c	1-1.0×10^{3} pM	1 pM	[31]
ICPMS	miR-21	0.1–500 fM	41 aM	[32]
Nanopore	Multiple microRNAs	~	5 pM[c]	[33]
Nanopore	Multiple microRNAs	~	10 pM	[34]
Nanopore	microR-10b	~	5 pM	This work

(a) LOD for miRNA-21. (b) SERS peak at 1618 cm^{-1}. (c) LOD for *let*-7a.

Supplementary References

1 S. Wen, T. Zeng, L. Liu, K. Zhao, Y. Zhao, X. Liu and H.-C. Wu, *J. Am. Chem. Soc.*, 2011, **133**, 18312-18317.

2 C. Chen, G. Song, J. Ren and X. Qu, *Chem. Commun*., 2008, **46,** 6149-6151.

3 S. Saha, K. Chakraborty, Y. Krishnan, *Chem. Commun*., 2012, **48,** 2513-2515.

4 A. Orte, J. M. Alvarez-Pez and M. J. Ruedas-Rama, *ACS Nano*, 2013, **7,** 6387-6395.

5 B. Chu, H. Wang, B. Song, F. Peng, Y. Su and Y. He, *Anal. Chem*., 2016, **88,** 9235.

6 X. Liu, Y. Su, H. Tian, L. Yang, H. Zhang, X. Song and J. W. Foley, *Anal. Chem*., 2017, **89,** 7038-7045.

7 W. Shi, X. Li and H. Ma, *Angew. Chem., Int. Ed*., 2012, **51,** 6432-6435.

8 X. Liu, S.-Q. Zhang, X. Wei, T. Yang, M.-L. Chen and J.-H. Wang, *Biosens. Bioelectron*., 2018, **109,** 150-155.

9 J. Zhou, L. Zhang and Y. Tian, *Anal. Chem*., 2016, **88,** 2113-2118.

10 S. Düwel, C. Hundshammer, M. Gersch, B. Feuerecker, K. Steiger, A. Buck, A. Walch, A. Haase, S. J. Glaser, M. Schwaiger and F. Schilling, *Nat. Commun.* 2017, **8,** 15126.

11 Y. Wu, T. C. Soesbe, G. E. Kiefer, P. Zhao and A. D. Sherry, *J. Am. Chem. Soc.,* 2010, **132,** 14002-14003.

12 H. Wei, M. R. Willner, L. C. Marr and P. J. Vikesland, *Analyst*, 2016, **141,** 5159-5169.

13 J. Wang, Y. Geng, Y. Shen, W. Shi, W. Xu and S. Xu, *Sens. Actuators, B*, 2019, **290,** 527-534.

14 Y. Zhang, J. Clausmeyer, B. Babakinejad, L. Córdoba, T. Ali, A. Shevchuk, Y. Takahashi, P. Novak, C. Edwards, M. Lab, S. Gopal, C. Chiappini, U. Anand, L. Magnani, R. C. Coombes, J. Gorelik, T. Matsue, W. Schuhmann, D. Klenerman, E. V. Sviderskaya and Y. Korchev, *ACS Nano*, 2016, **10,** 3214-3221.

15 N. C. S. Vieira, A. Figueiredo, A. D. Faceto, A. A. A. Queiroz, V. Zucolotto and F.

E. G. Guimarães, *Sens. Actuators, B*, 2012, **169,** 397-400.

16 X.- M. Shi, L.-P. Mei, N. Zhang, W.-W. Zhao, J.-J. Xu, H.-Y. Chen, *Anal*. *Chem*., 2018, **90,** 8300-8303.

17 L. Lan, R.-L. Wang, L. Liu and L. Cheng, *Anal. Chim. Acta.* 2019, **1079,** 207-211.

18 Y. Zhang, Z. Li, Y. Cheng and X. Lv, *Chem. Commun*., 2009, **22,** 3172-3174.

19 Y. Liu, T. Shen, J. Li, H. Gong, C. Chen, X. Chen and C. Cai, *ACS Sens.,* 2017, **2,** 1430-1434.

20 W. Peng, Q. Zhao, M. Chen, J. Piao, W. Gao, X. Gong J. Chang, *Theranostics*, 2019, **9,** 279-289.

21 S. Li, F. Zhang, X. Chen and C. Cai, *Sens. Actuators, B*, 2018, **263,** 87-93.

22 X. Miao, Z. Cheng, H. Ma, Z. Li, N. Xue and P. Wang, *Anal*. *Chem*., 2018, **90,** 1098-1103.

23 Y. Tao, D. Yin, M. Jin, J. Fang, T. Dai, Y. Li, Y. Li, Q. Pu and G. Xie, *Biosens. Bioelectron*., 2017, **96,** 99-105.

24 G. Yammouri, J. Mandli, H. Mohammadi and A. Amine, *J. Electroanal. Chem.* 2017, **806,** 75-81.

25 D. Zhu, W. Liu, D. Zhao, Q. Hao, J. Li, J. Huang, J. Shi, J. Chao, S. Su and L. Wang, *ACS Appl. Mater. Interfaces*, 2017, **9,** 35597-35603.

26 H. Yin, Y. Zhou, B. Li, X. Li, Z. Yang, S. Ai and X. Zhang, *Sens. Actuators, B*, 2016, **222,** 1119-1126.

27 Q. Xu, F. Ma, S.-Q. Huang, B. Tang and C.-Y. Zhang, *Anal*. *Chem*., 2017, **89,** 7077-7083.

28 M. Schechinger, H. Marks, S. Mabbott, M. Choudhury and G. Cote, *Analyst*, 2019, **144,** 4033-4044.

29 K. Zeng, H. Li and Y. Peng, *Microchim. Acta*, 2017, **184,** 2637-2644.

30 L. Hong, M. Lu, M.-P. Dinel, P. Blain, W. Peng, H. Gu and J.-F. Masson, *Biosens. Bioelectron.* 2018, **109,** 230-236.

31 Y. Kuang, L. Liu, Z. Wang and Y. Chen, *Talanta*, 2020, 120726.

32 X. Liu, S.-Q.Zhang, Z.-H. Cheng, X. Wei, T. Yang, Y.-L. Yu, M.-L. Chen and J.-H.

Wang, *Anal*. *Chem*., 2018, **90,** 12116-12122.

33 X. Wu, B. Guo, Y. Sheng, Y. Zhang, J. Wang, S. Peng, L. Liu and H.-C. Wu, *Chem. Commun*., **2018**, *54*, 7673-7676.

34 X. Zhang, Y. Wang, B. L. Fricke and L.-Q. Gu, *ACS Nano*, 2014, **8,** 3444-3450.