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Versatile fluorescence detection of microRNA based on novel DNA hydrogel-amplified signal probes coupled with DNA walker amplification

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Experimental Section

1.1. Apparatus

Transmission electron microscopy (TEM) images were recorded using a JEM-2000EXinstrument (Hitachi). Field-emission scanning electron microscopy (FE-SEM) was carried out on a JEOL JSM-6700F instrument. Photoluminescence (PL) spectra were obtained on a F-4500 spectrophotometer (Shimadzu). Absorption measurements were carried out using a Varian Cary 300 UV-vis spectrophotometer. All optical measurements were carried out at room temperature under ambient conditions.

Table S1. Sequences of the DNA

Name	Sequence（5'---3'）
H_1	COOH-CTGGACGATATGCACCTCCGGCGATTGAGAAGGTGCATATC
H_2	Acrydite-TGGTAGGTCAAGGTGCATATGGATGTTAGAGATATGCACCTTCTCAATCG
H_3	TCTAACATCCATATGCACGCGATTGAGAAGGTGCATATGGAGTCTAATCGGCGGGTAAA
S_1	Acrydite- TTTACCCGCCGATTAGAC
Walker	NH_2-TTTTTTGTACGCTAGACTTGACCCTCCGGCGAGACGGTAAAGATGGCTTTTTT
Protecting	AAAAAAGCCATCTTTACCAGACAGTGTTA
Support	NH_2-TTTTTTATTCATTTTACCGTCTCGCCGGAGGTGCATATCGTCCAG
miRNA-141	UAACACUGUCUGGUAAAGAUGG
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
miRNA-155	UUAAUGCUAAUCGUGAUAGGGGU
miRNA-182	UUUGGCAAUGGUAGAACUCACACU

1.2. Reagents

Tellurium powder and $NaBH_4$ (98.0%), trisodium citrate, $CdCl_2{\cdot}2.5H_2O$ (98%), 3-triethoxysilylpropylamine (APTES, 98%), ammonium hydroxide ($NH_3{\cdot}H_2O$), tetraethyl orthosilicate (TEOS) and tris (hydroxymethyl) aminomethane (Tris) were obtained from Aladdin (Shanghai, China). Acrylamide/bis-acrylamide 29:1 40% gel stock solution, ammonium persulfate (APS), N, N, N′, N′-tetramethylethylenediamine (TEMED) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). SYBR Green I was purchased from Qingdao Ke Er Co. Magnetic beads were provided by Tianjin BaseLine ChromTech Research Centre (Tianjin, China). The DNA probes in our study (Table-1) were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China).

1.3. Preparation of amino functionalized SiO_2 microsphere

The amino functionalized SiO_2 microspheres was produced using the modified Stober method.[S1] 30 mL of ethanol, 50 mL of ultrapure water, and 10 mL of $NH_3 \cdot H_2O$ were mixed to form a uniform system under magnetic stirring. A mixed solution of 5 mL of TEOS and 20 mL of ethanol were added dropwise into this solution under stirring, and magnetic stirring was last for 6 h. After that, 5 mL of APTES was put into the above solution, the mixture was stirred at room temperature for more than 12 h. Finally, the SiO_2 microsphere was isolated by centrifugation and washed with ethanol, and then dispersed in 50 mL of ultrapure water for further use.

1.4. Synthesis of CdTe quantum dots in aqueous solution

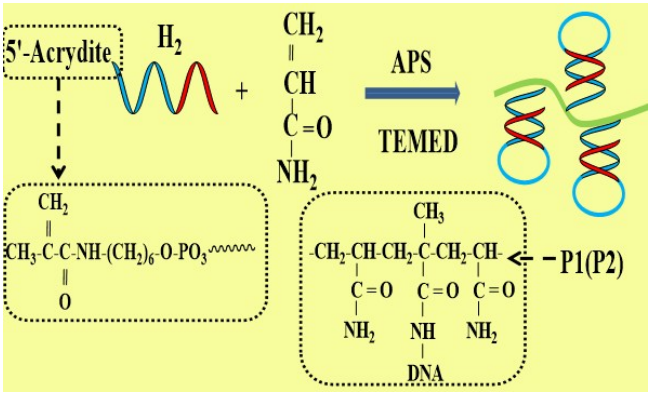
The CdTe QDs in aqueous solution were prepared by two steps. The sodiumhydrogen telluride (NaHTe) was prepared according to the method published by Gu et al. [S2] In nitrogen gas environment, 50.0 mg of $NaBH_4$ and 80.0 mg of Te powder were added to 2.0 mL of ultrapure water. After Te powder disappeared completely, the clear NaHTe solution was obtained. 63 mL of 2.5 mM $CdCl_2$ solution and 33 μL of 3-mercaptopropionic were placed in a 100 mL round flask, degassed with N_2 gas, and the pH was adjusted to 8.0 with 0.2 mol L^{-1} NaOH. Then the NaHTe solution was quickly injected into the reaction flask, heated to 100 ˚C, and refluxed under nitrogen environment for 8 h. After the reaction solution was cooled to room temperature, the orange red CdTe QDs solution was obtained. For purification, the obtained QDs were precipitated with ethanol, centrifugation, and redissolved in water (pH = 8.0).

1.5. DNA walker-based amplification process (Scheme 1A)

100 μL of carboxyl magnetic beads (MBs) was washed three times with 200 μL of PBS (0.1 M, pH 7.4), then magnetic beads were activated in 200 μL of PBS containing 0.4 M NHS and 0.6 M EDC for 2 h. The protecting DNA (1 μL, 1.5 μM) was firstly mixed with walker DNA (1 μL, 1.5 μM) at 37 ˚C for 2 h. Next, MBs (20 μL) and support DNA (20 μL, 1.5 μM) were added to the solution, stirring overnight and magnetically separated to obtain the DNA-MBs. After that, target miRNA-141 (1 μL, 1.5 μM) was added to hybridize with protecting DNA for 2 h to release the walker probe. Subsequently, the releasing walker DNA hybridized with support DNA. Nicking endonuclease (5 U Nt.BsmAI) was added to the above mixture and incubated at 37 ˚C for 2 h, releasing single stranded DNA (yellow S_3) and walker DNA (green DNA). After magnetic separation, a large number of single stranded DNA (yellow S_3) was obtained.

1.6. Synthesis of the acrylamide copolymer chain (Scheme 1B)

Acrydite-modified DNA was used to synthesize the copolymer chains (Scheme S1).[S3] 20 μL of H_2 (10 μM) and 20 μL of S_1 (10 μM) were separately added into 80 μL of 10 mM binding buffer (10 mM Tris-HCl buffer, pH 8.0, containing 5 mM KCl , 140 mM NaCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$, 2% (w/v) acrylamide). The mixture solutions were bubbled with nitrogen for 5 min, followed by addition of 1.4 μL of freshly prepared initiator solution (100 μL solution including 5 μL of TEMED and 10 mg of APS) with nitrogen bubbling for another 5 min, and the solutions were further polymerized at 4 ˚C for 12 h. The resulting copolymers were filtered through Microcon (Millipore) spin filter unit (MWCO: 30 kDa,10 kDa) to eliminate unreacted monomer units, salts, and the initiator, then the polymers were rinsed with water three times and dissolved in HCR buffer (pH 7.2, 25 mM HEPES and 25 mM $MgCl_2$) to form P1 and PS1 (Scheme 1B). Then, H_3 was added to PS_1 (S_1-modified polymer) for forming the polymer P2 (Scheme 1B). P1 and P2 were incubated at 95 ˚C for 5 min, immediately ice incubated for 30 minutes to form a hairpin structure. The resultant solution was stored at 4 ˚C for further use.



Scheme S1. The preparation process for P1and P2.

1.7. Assembly of substrate strands H_1 on SiO_2 microsphere (Scheme 1C)

50 μL of 1.0×10^{-7} M H_1 was activated with 25 μL of 0.1 M EDC and 25 μL of 0.025 M NHS at room temperature for 1 h, then 50 μL of amino-modified SiO_2 microspheres was added and incubated at 37 ˚C for over 6 h. The resulting solution was centrifuged to remove the excess sequence, and the precipitate was resuspended in 50 μL of deionized water and stored at 4 ˚C for use.

1.8. Preparation of quantum dot probe (P3 and P4) (Scheme 1D)

80 μL of CdTe quantum dots was activated with 10 μL of 0.1 M EDC and 10 μL of 0.025 M NHS at room temperature for 1 h, then 125 μL of P1 were added and incubated at 37 ˚C for 16 h. The solution was centrifuged to remove excess DNA. The precipitate was redispersed in 125 μL of ultrapure water, so the CdTe QDs/P1 (P3) probe was obtained. In

addition, CdTe QDs/P2 (P4) was assembled by using the same method, and stored at 4 ˚C for use.

1.9. Fabrication of the biosensing system for detecting targets

The process was shown in Scheme 1C and 1E, 25 μL of S_3 was added to the solution with 25 μL of SiO_2-H_1, the hybridization reaction was performed at 37 ˚C for 90 min. The solution was centrifuged to remove the uncombined S_3, then P1, P2 and SYBR Green I (or P3 and P4) were added and incubated at 37 ˚C for 120 min to perform the chain hybridization reaction.

After the chain hybridization reaction of P3 (CdTe QDs/P1) and P4 (CdTe QDs/P2) on the SiO_2-H_1-S_3 microsphere was performed at 37 ˚C in an oscillator, the mixture was centrifuged to remove the uncombined QDs probe (P3 and P4), then the QDs fluorescence was measured for target assay.

1.10. Total RNA Extraction

22Rvl cancer cells were cultured in six-well plates, followed by washing twice with ice-cold phosphate buffered saline solution (pH 7.4) and adding 1.0 mL of Trizol Reagent. After incubation at room temperature for 5 min, 200 μL of trichloromethane ($CHCl_3$) was added. The mixture was then centrifuged at 4 ˚C with 12000g for 10 min. Subsequently, total RNAs were purified by extraction with 2-propanol and precipitation with ethanol, respectively. Finally, the total RNA sediment was redissolved in RNase-free water and stored at -80 ˚C before use.

Results and Discussions

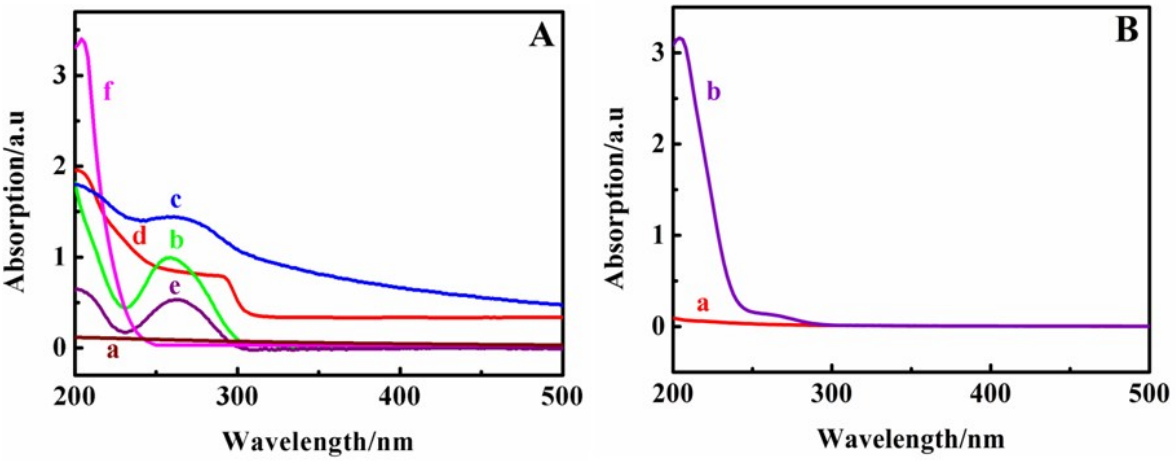


Figure S1. UV−vis absorption spectra of (A) (a) SiO_2 microspheres, (b) H_1, (c) SiO_2-H_1, (d) acrydite monomer, (e) acrydite–DNA (H_2), (f) DNA-modified acrydite polymer (P1). (B) (a) CdTe QDs, (b) P3.

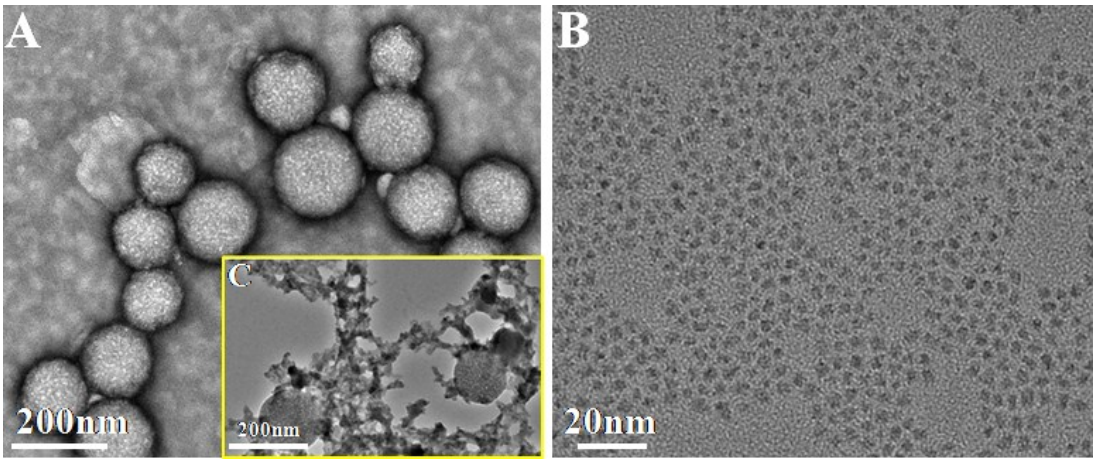


Figure S2. (A) TEM image of SiO_2-DNA hydrogel by HCR; (B) TEM image of CdTe QDs; (C) TEM image of DNA hydrogel-QDs signal probe (P3) on the SiO_2 microspheres.

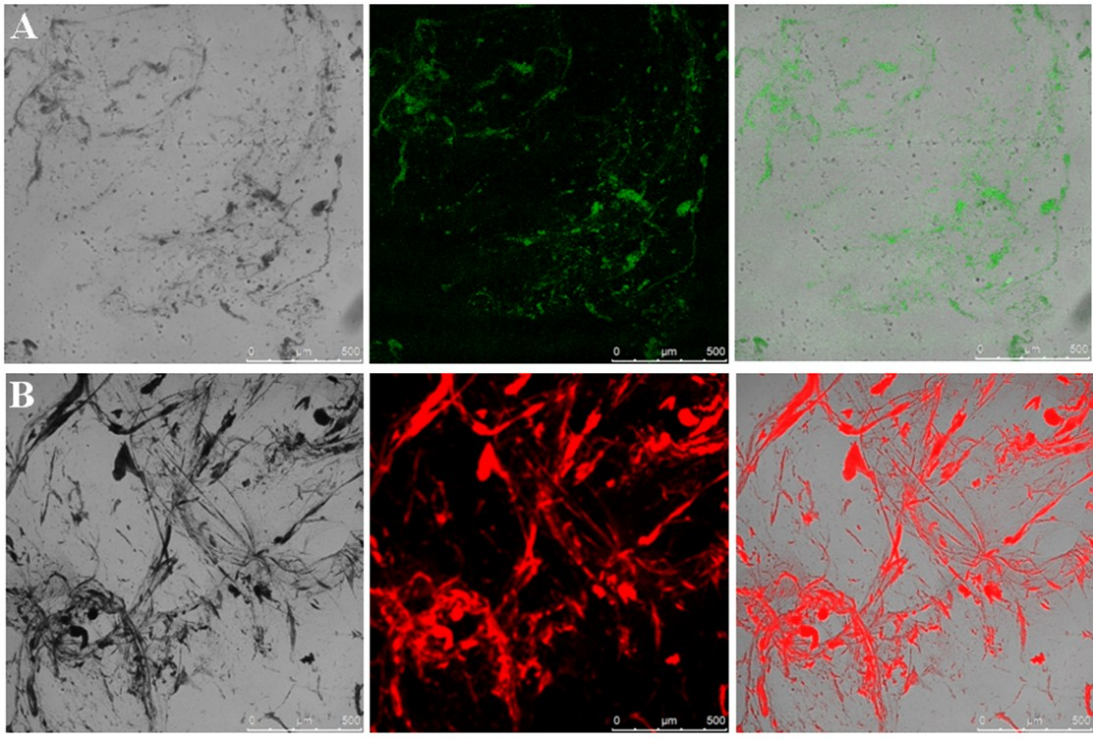


Figure S3. Confocal fluorescence microscopy images of (A) DNA hydrogel-SYBR Green fluorescence platform on SiO_2 microspheres, (B) DNA hydrogel-CdTe QDs fluorescence platform on SiO_2 microspheres.

The feasibility of the fluorescence assay based on SG I and QDs signal probes was further studied by confocal fluorescence microscopy. After HCR of P1 and P2 on the SiO_2-H_1-S_3 microsphere was performed and SG I dyes were added, forming the DNA hydrogel-SG I fluorescence platform. As shown in Figure S3A, the fluorescence confocal microscopy images showed bright green fluorescence on the long linear polymers, indicating that the SG I-based fluorescence method was successfully developed. Furthermore, when P3 and P4 with

CdTe QDs are assembled hydrogel by HCR on the SiO_2 microspheres, bright red fluorescence of CdTe QDs were observed (Figure S3B). The fluorescence imaging results prove that the DNA hydrogel-based versatile fluorescence platform using SG I and CdTe QDs can be used for target assay.

The feasibility of the fluorescence sensing system was verified by comparative experiments. As shown in Figure S4A, when only SG I is present in solution, there is almost no fluorescence signal (curve a). SG I is a widely used DNA intercalating dye, and displays dramatic increase in fluorescence upon interaction with dsDNA. [S4] In the absence of target, as short dsDNA in H_1 binds a little SG I, showing very low fluorescent signal (curve b). After P1 and P2 polymer were added, the fluorescence signal slightly increased for the nonspecific adsorption (curve c, blank). By comparison, when the target is present, DNA S_3 was produced by walking machine and then hybridized with H_1 on SiO_2, the fluorescence signal further increased due to more dsDNA (curve d). Subsequently, P1 and P2 was further added to perform HCR, forming the DNA hydrogel-amplified fluorescence platform, dramatically increased fluorescence signal was observed (curve e). The results demonstrate that the DNA hydrogel-SG I fluorescence sensor system combined with DNA walking amplification is feasible for target assay.

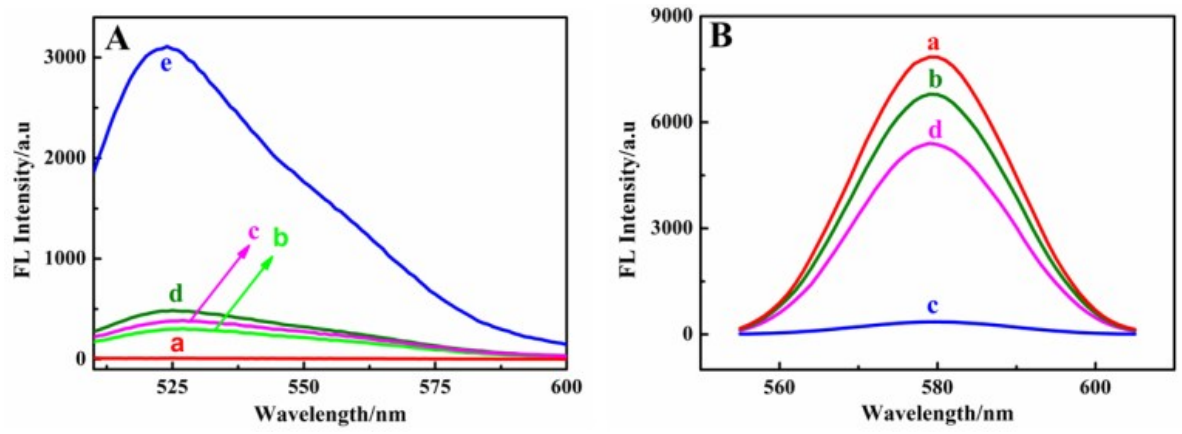


Figure S4. (A) Feasibility of the DNA hydrogel-SYBR Green I fluorescence sensing system. (a) SYBR Green I, (b) SiO_2+H_1, (c) SiO_2+H_1+P1+P2 (blank), (d) SiO_2+H_1+target, (e) SiO_2+H_1+target+P1+P2 (miRNA-141: 10^{-19} M). (B) Feasibility of the DNA hydrogel-CdTe QDs fluorescence sensing system. (a) CdTe QDs, (b) QDs-DNA probe (P3), (c) blank, (d) 10^{-10} M miRNA-141.

In addition, the fluorescence sensing system using CdTe QDs is investigated. As shown in Figure S4B, CdTe QDs displayed very high fluorescence signal (curve a). After the QDs-DNA probe (P3) was fabricated, the fluorescence signal slightly decreased due to the interaction of DNA with QDs (curve b). In the absence of target, there is still a low

fluorescence signal (curve c, blank) because a small number of QDs probes were adsorbed on the SiO_2 microsphere. When the target miRNAs were present, abundant QDs-DNA signal probes were introduced to the sensing system by HCR, therefore obviously higher fluorescence signal was observed (curve d), indicating that the DNA hydrogel-CdTe QDs fluorescence sensing system can be used for target assay.

2.8. Optimization of experimental conditions

In order to obtain the optimal fluorescence signal, experimental conditions were optimized. The SiO_2 microspheres solution with different dilution times (twice, five, seven, ten and twelve, respectively) in the sensing system was investigated. After the chain hybridization reaction on SiO_2 was performed, the fluorescence signal of the sensing system is shown in Figure S5A. As the dilution factor of SiO_2 solution increased, the fluorescence signal increased, and then reached a stable platform until the dilution time is 10. Therefore, SiO_2 microsphere solution was diluted 10 times for use in the experiment.

The hybridization time of HCR has important effect on DNA hydrogel formation. To achieve the best analytical performance of the detection system, the time of HCR was optimized. Figure S5B showed that the FL signal increased obviously with the prolongation of hybridization time. When the time exceeds 120 minutes, the fluorescence signal tends to be flat and no longer increases. Hence, 120 min was selected as the optimized time of HCR in the detection system.

The amount of SG-I has significant influence on fluorescent signal, the results were shown in Figure S5C. With increasing amount of SG-I, the fluorescence signal increased and became stable at 6 μL of SG-I. Therefore, 6 μL of SG-I was used in the experiment.

The effects of Nt.BsmAI amount on the sensing system was investigated. As shown in Figure S5D, the fluorescence signal increased with increasing amount of Nt.BsmAI, and no obvious change was observed after 5 U of Nt.BsmAI. Therefore, 5 U of Nt.BsmAI was used for detection.

The amount of quantum dots has significant effect on fluorescence signal, and the results were shown in Figure S5E. As the amount of CdTe QDs increases, the fluorescence signal increased and no obvious change was observed after 20 μL of QDs. Therefore, 20 μL of CdTe QDs was selected in the experiment.

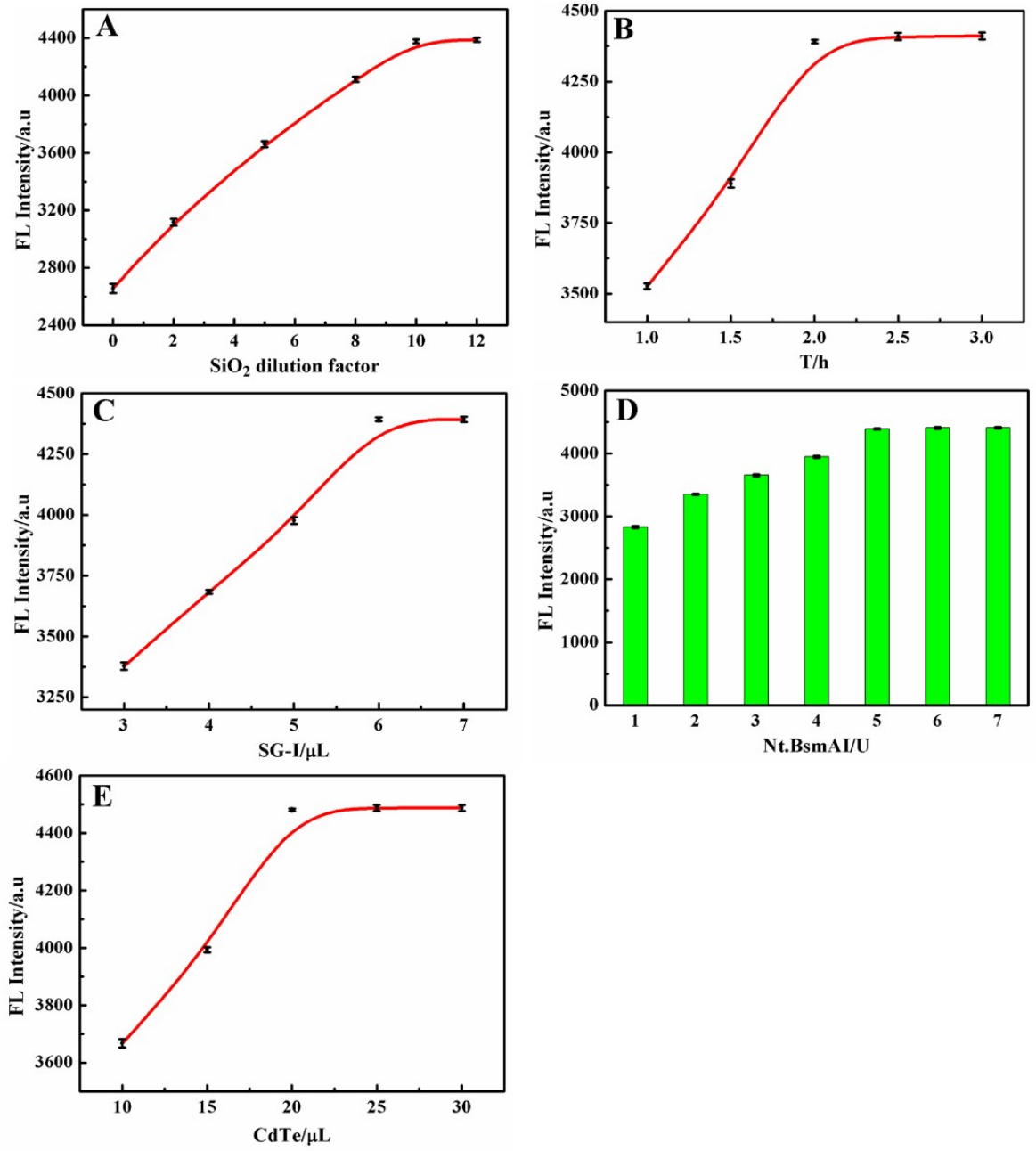


Figure S5. Effects of (A) SiO_2 microsphere dosage, (B) HCR time, (C) SG-I amount, (D) Nt.BsmAI amount, (E) CdTe QDs amount on FL signal for detection. (miRNA-141 concentration: 1.0 pM)

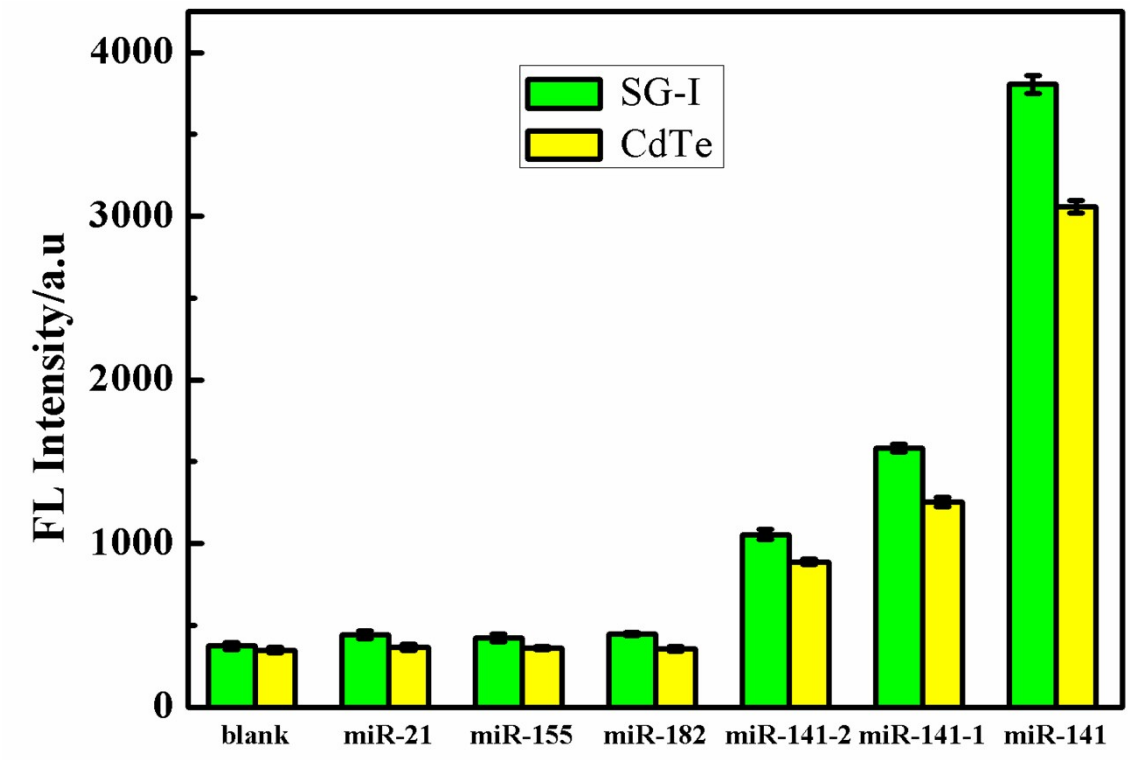


Figure S6. Selectivity of the fluorescence strategy for detecting different miRNAs (concentrations of miRNAs: 1.0 fM; miRNA-141-1: single-base mismatched miRNA-141; miRNA-141-2: double-base-mismatched miRNA-141).

Table S2. Comparison of Different Methods for Assay of miRNA

Methods	Linear range (ng/mL)	LOD (ng/mL)	refs
Fluorescence (SYBR Green I)	10^{-4} fM- 10 pM	10^{-4} fM	this work
Fluorescence (CdTe QDs)	10^{-3} fM -10 pM	1.1×10^{-4} fM	this work
Fluorescence	2.0 fM- 2.0 nM	0.8 fM	S5
ECL	10 fM - 100 pM	3.3 fM	S6
ECL	0.1 fM - 1 pM	0.03 fM	S7
DPV	0.01fM - 10 nM	25.1 aM	S8
SWV	0 fM - 50 fM	4.2 fM	S9
SERS	1.0 fM - 100 nM	0.17 fM	S10

Table S3. Determination of miRNA 141 Added in 22 Rvl human prostate cancer cells (n = 3) with the Proposed Strategy

sample	added/fM	obtain/fM	recovery/%	RSD/%
1	1	0.958	99.57	3.359
2	10	9.922	97.19	4.4251
3	100	102.95	102.57	5.4298
4	1000	997.86	99.31	3.0539
5	1	1.043	104.7	5.7402
6	10	10.017	93.45	6.2276
7	100	96.32	95.02	3.2151
8	1000	976.3	98.31	3.6804

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