

## Supporting Information

### Hybridization chain reaction engineered dsDNA for Cu metallization:

### An enzyme-free platform for amplified detection of cancer cells and microRNAs

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### Materials and Instrumentation

3-(N-morpholino)-propane sulfonic acid (MOPS) and sodium ascorbate were purchased from Alfa Aesar. Copper sulfate anhydrous ( $\text{CuSO}_4$ ) was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Sodium chloride (NaCl) was purchased from Sigma-Aldrich (USA). PBS buffer solution (10 mM) and MOPS buffer solution (10 mM MOPS, 300mMNaCl, 1mM  $\text{MgCl}_2$ , pH 7.5) were used in this study. All synthetic oligonucleotides were ordered from Shanghai Sangon Biological Engineering Technology (China) and the sequences were listed in Table 1. MCF-7 cells, A549 cells, K562 cells HepG2 cells and NIH-3T3 cells were purchased from Cell Bank of Chinese Academy of Sciences & Services (Shanghai, China). All other reagents were of analytical reagent grade and used as received. Nanopure water (18.2 M $\Omega$ ; Millipore Co., USA) was used throughout the experiment.

Fluorescence measurements were carried out using a JASCO FP-6500 spectrofluorometer (Jasco International Co., Japan). Transmission electron microscopy (TEM) images were recorded using a FEITECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. CD spectra were determined using a Jasco 810 (Jasco International Co., Ltd., Tokyo, Japan).

### Cell cultures

The MCF-7 cells, A549 cells, K562 cells, HepG2 cells and NIH-3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% (v/v)  $\text{CO}_2$ .

The cell density was determined by using a hemocytometer. A certain amount of cells

dispersed in cell media buffer were centrifuged at 3500 rpm for 5 min and re-dispersed in PBS buffer solution. Then, the cells were washed with PBS for three times. Finally, the cells were dispersed in PBS buffer solution.

## **Gel electrophoresis**

Nucleic acid samples were loaded on a 15% polyacrylamide gel electrophoresis and electrophoresed at room temperature at 100 V for 2 hour. The experiment was carried out in  $1 \times$  TBE buffer.

## **UV absorbance and melting studies**

UV melting experiments were carried out on a Cary 300 UV/Vis spectrophotometer equipped with a Peltier temperature control accessory. Absorbance changes at 260 nm versus temperature were collected at a heating rate of  $1.3\text{ }^{\circ}\text{C min}^{-1}$ .

## **CD spectroscopy**

Circular dichroism spectra were recorded with a JASCO J-810 spectropolarimeter equipped with a temperature controlled water bath. CD spectra were recorded from 320 to 210 nm in 50nm increments with an average time of 1 minute and three scans were accumulated and automatically averaged.

## **Synthesis of dsDNA-CuNPs**

**For cancer cells detection**, DNAa (3  $\mu\text{M}$ ) and DNAb (3  $\mu\text{M}$ ) in PBS buffer were heated to  $95^{\circ}\text{C}$  for 5 min and then allowed to cool to room temperature for at least 60 min before use. The partially complementary duplexes of DNAa and DNAb were formed and 20  $\mu\text{L}$  of the DNAa-DNAb were incubated with cancer cells in PBS buffer solution with a total volume of 120  $\mu\text{L}$  for 2 h at  $25\text{ }^{\circ}\text{C}$ . Then, the solution was centrifuged at 3500 rpm for 5 min. After that, 40  $\mu\text{L}$  suspensions were added into PBS buffer solution which contained 500 nM  $\text{H}_1$  and  $\text{H}_2$ .  $\text{H}_1$  and  $\text{H}_2$  were heated to  $95^{\circ}\text{C}$  for 5 min separately and then allowed to cool to room temperature for at least 60 min before use. Then, before sodium ascorbate (2 mM) was added, the solution was incubated at  $25\text{ }^{\circ}\text{C}$  for 2 h. And after 30 seconds shaking,  $\text{CuSO}_4$  (100  $\mu\text{M}$ ) was introduced into the solution with the finally volume of 60  $\mu\text{L}$ . Finally, the synthesis of

fluorescent CuNPs was completed within the 10 min incubation time at room temperature.

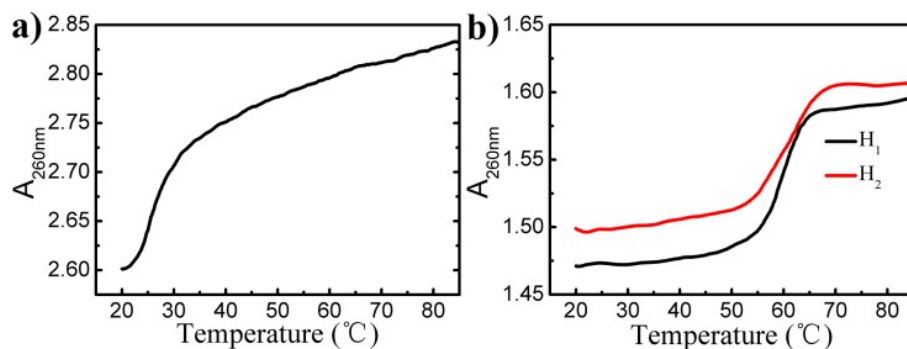
**For miRNAs detection**, different amount of miRNAs were added into MOPS buffer solution which contained 500 nM H<sub>1</sub> and H<sub>2</sub>. H<sub>1</sub> and H<sub>2</sub> were heated to 95°C for 5 min separately and then allowed to cool to room temperature for at least 60 min before use. Then, before sodium ascorbate (2 mM) was added, the solution was incubated at room temperature for 2 h. And after 30 seconds shaking, CuSO<sub>4</sub> (100 μM) was introduced into the solution with the finally volume of 60 μL. Finally, the synthesis of fluorescent CuNPs was completed within the 10 min incubation time at room temperature.

The fluorescence intensities of CuNPs were detected ten minutes later after sodium ascorbate and Cu<sup>2+</sup> were added into the DNA solution.

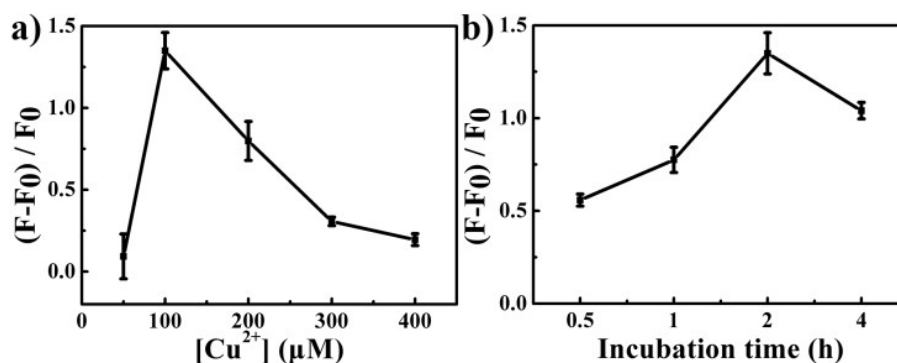
Table S1. DNA sequences used in the work

Name	Sequence
DNAa	5'-TCAACATCAGGCTATAGCACATGGGTAAAACGAC-3'
DNAb	5'-TAGCTTATCAGACTGATGTTGA-3'
H <sub>1</sub>	5'- <u>TCAACATCAGTCTGATAAGCTATGAACG</u> TAGCTTATCAGACTGA-3'
H <sub>2</sub>	5'-TAGCTTATCAGACTGATGTTGATCAGTCTGATAAGCTACGTTCA-3'
miR-21	5'-UAG CUU AUC AGA CUG AUG UUGA-3'
RNA1	5'-UAG CUU AUC ACA CUG AUG UUGA-3'
RNA2	5'-UAG CUU AUC ACA CUG AUGAUGA-3'
RNAr	5'-UUG UAC UAC ACA AAA GUA CUG -3'

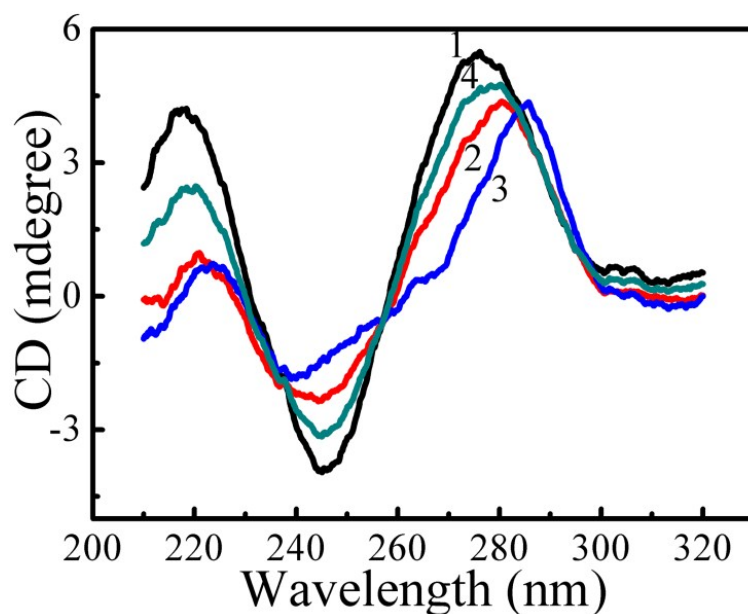
\*The DNAa was designed partially complementary to DNAb. The bold letters in the DNA are presented the aptamer bases for MUC1. In the hairpin sequences, loops were underlined and sticky ends were over lined. In the mismatch RNA, the italic letter represented the mismatched base.



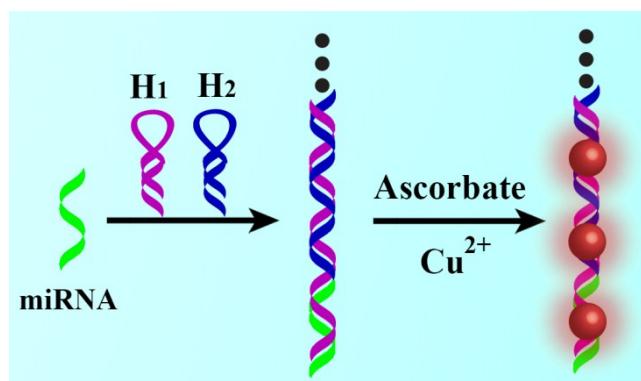
**Fig. S1** (a) UV melting curve of DNAa-DNAb (3 μM) in PBS buffer. (b) UV melting curves of H<sub>1</sub> (1 μM) and H<sub>2</sub> (1 μM) in PBS buffer, respectively.



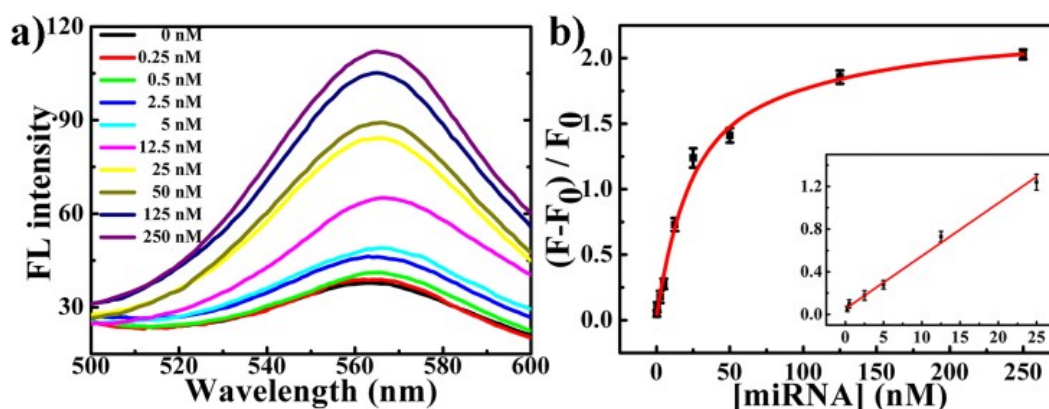
**Fig. S2** (a) Impact of concentration of  $Cu^{2+}$  on the fluorescence of the DNA-templated Cu NPs. 500 nM H<sub>1</sub>, 500 nM H<sub>2</sub>, 50 nM DNAb, incubation time of HCR process is 2 h, 2 mM sodium ascorbate. (b) Impact of incubation time of HCR process on the fluorescence. 500 nM H<sub>1</sub>, 500 nM H<sub>2</sub>, 50 nM DNAb, 2 mM sodium ascorbate, 100 μM  $Cu^{2+}$ . Error bars represent the standard deviation of three repeated experiments. The fluorescence intensity of CuNPs increased with the increasing of  $Cu^{2+}$  concentration firstly and then decreased. The final decrease could be attributed to the damage of DNA template by hydroxyl radicals that were generated at high concentrations of  $Cu^{2+}$  and ascorbate.<sup>1</sup>



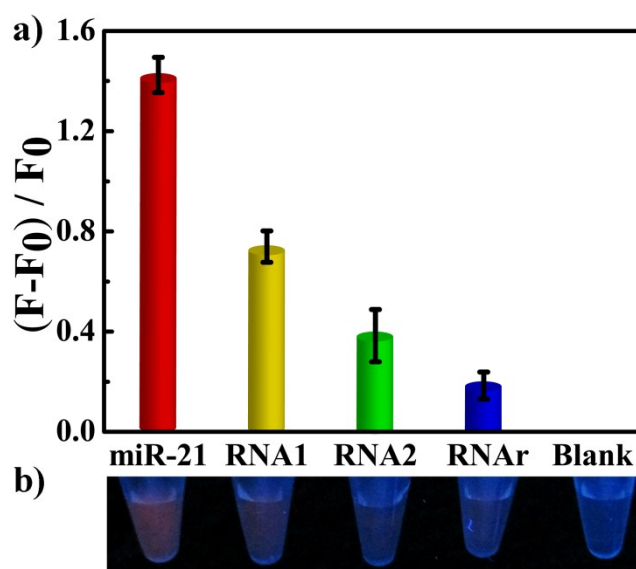
**Fig. S3** CD spectra of solutions containing 1)  $H_1$ , 2)  $H_2$ , 3)  $H_1 + H_2 + 50$  nM DNAb ( $[H_1] = 2 \mu\text{M}$ ,  $[H_2] = 2 \mu\text{M}$ ) and 4) the sum of CD spectra of  $H_1$  and  $H_2$  (spectra of  $H_1$  + spectra of  $H_2$ )/2). The positive peak of 3) was different from 1), 2) and 4), indicating that DNA polymers were formed in the presence of DNAb.



**Scheme S1.** Schematic illustration of miRNA detection based on the target-triggered hybridization chain reaction (HCR) and in situ formation of fluorescent CuNPs.



**Fig. S4** (a) Fluorescence emission spectra corresponds to the analysis of different concentrations of miR-21 from 0 nM to 250 nM. (b) Plot of the fluorescence intensity measured at 568 nm as a function of the miR-21 concentration. Inset: the linear plot. Error bars represent the standard deviation of three repeated experiments.  $F$  is the resulting fluorescence at the respective concentration of miR-21 and  $F_0$  is the fluorescence of the system without miR-21.



**Fig. S5** The specificity for miR-21 detection. (a) The fluorescence intensity of solutions containing miR-21, single-base mismatched RNA (RNA1), two-base mismatched RNA (RNA2) and random RNA (RNAr), respectively. (b) The corresponding photograph taken under a UV transilluminator. Error bars were estimated from at least three independent measurements.  $F$  is the resulting fluorescence at the respective concentration of miR-21 and  $F_0$  is the fluorescence of the system without miR-21.

## Reference for supporting information:

1. E. Stadtman, *Am. J. Clin. Nutr.*, 1991, **54**, 1125S.