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Supporting Information

A ratiometric colorimetric detection of folate receptor based on terminal protection of small-molecule-linked DNA

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1. Experimental Section

1.1 Reagents and materials

HPLC-purified oligonuleotides (sequences are (5' to 3') S1: CAGCTGCTGGTA-HS-SH C6; S2: CAGCAGCTGCTACAGCAGCTG- C6 NH2) were obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris), trisodium citrate, Exonuclease (Exo I), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysulf-osuccinimide (Sulfo-NHS), bovine serum albumin (BSA), thrombin, and other reagents were obtained from the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). HAuCl4, FA and FR were purchased from Sigma Aldrich Chemical Co. NEB buffer I was purchased from New England Biolabs (Beijing, China). Ultrapure water obtained through PSDK2-10-C (Beijing, China) was used throughout the experiments.

1.2 Preparation of Au NPs

All glassware used in the following procedures were cleaned in a bath of freshly prepared solution of HNO3–HCl (1 : 3, v/v), rinsed thoroughly in ultrapure water and dried in air. The 13 nm AuNPs were prepared by the classical citrate reduction route with a slight modification[1]. Briefly, a sodium citrate solution (2 mL, 1 mg/mL) was rapidly added to a boiled HAuCl4 solution (50 mL, 1 mg/mL) under vigorous stirring. The mixed solution was boiled for 30 min and further stirred for 15 min to ensure complete reduction. The resulting wine-red solution was cooled to room temperature gradually and filtered by a filter film (The pore size: <0.45 μm) to remove the impurities, and the filtrate was stored at 4 oC. The average diameter of Au NPs was verified by the scanning electron microscope (SEM) (Hitachi S-4800, Japan). The concentration of the AuNPs was estimated by UV-vis spectroscopy based on an extinction coefficient of 2.7×108 M-1cm-1 at 516 nm for 13 nm particles.

1.3 Preparation of S1modified Au NPs (S1-AuNPs)

Thiolated DNA modified Au NPs were prepared according to the procedure described previously with minor modification [1]. In brief, 0.5 mL 40 nM S1 solution was gradually added to 5 mL of the concentrated Au NPs solution under stirring conditions and incubated for 16 h at room temperature; subsequently, 10 mM phosphate buffer solution (PBS, pH 7.4) was added drop-by-drop while stirring (called the 'salt aging process'); then, after incubation overnight (40 h), the unbound oligonucleotides were removed via centrifugation at 12 000 rpm for 25 min followed by resuspension of the sediment in 1 mL of 10 mM PBS (300 mM NaCl, pH 7.4). This step was repeated three times to sufficiently remove the excess oligonucleotides, and stored at 4 oC prior to the use for the detection of FR.

1.4 Preparation of FR protected FA-linked S2 (cross-linker: FR/FA-S2)

FA was conjugated to the amine moiety of the S2 at 3' end using the succinimide coupling (EDC-NHS) method. Briefly, 0.5 mL S2 (20 μM) was mixed with 0.5 mL Tris-HCl (100 mM, pH 7.4) containing 10 mM folate, 1 mM EDC, and 5 mM Sulfo-NHS, and incubated for 3 h at 37 °C in the dark. The final product (FA-S2) solution was then dialyzed against PBS buffer (pH 7.4) using a membrane with molecular weight cutoff of 1000 Da to remove excessive folate. Afterwards, a series of varying concentrations of FR were employed into the preceding solutions for sufficiently incubating at 37 °C for another 2 h in the dark. Finally, through acceding respectively to 400 U/mL Exo I-containing working buffer (50 units Exo I in 50 μL of 2× NEB buffer I), the aforementioned mixture solutions were sustained for about 30 min at 37 °C and terminated by an 80 °C water-bath for 20 min for hydrolyzing absolutely. After cooling to room temperature, the cross-linker that FA-S2 with different concentrations of FR (FR/FA-S2) was fabricated.

1.5 Terminal protection-based colorimetric assay for FR

Typically, 400 μL of the above prepared S1-AuNP solution was mixed with 200 μL of the prepared FR/FA-S2 solution and shaken for 5 min gently to get a stable solution. After 2 h duration at 37 °C in the dark, the solution was transferred into centrifuge tube. Finally, the color changes were detected by the naked eye and/or by recording the UV-vis absorption spectra on a UV-3010 spectrometer (Hitachi, Japan). The photographs were taken with a digital camera, and the absorption measurements were performed using a UV-vis spectrophotometer with 1 mm slit width cell between 400 and 800 nm. The sensor responses were calculated by dividing the extinction of the S1-AuNP suspension at 650 nm by the extinction at 520 nm (A650/A520).

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2. Optimization of the experimental conditions

Based on the proposed strategy, a series of experimental conditions were conducted to establish the optimum analytical conditions for the detection of FR. Considering the potential false-positive results caused by the residual folate-linked S2 which are not hydrolyzed by Exo I, in the absence of FR we have studied the requied minimum concentration of Exo I and digestion time by measuring the absorbance. As shown in Fig. S1A, the absorbance ration of A_{650}/A_{520} reduced with the increase of Exo I concentration from 0 to 800 U/mL. At the same time, we detected the signals at different digestion times in the presence of 400 U/mL Exo I. Experimental results showed increased progressively until 30 min and then reached a plateau (Fig. S1B). Hence, 400 U/mL of Exo I and 30 min digestion time were chosen as the optimized conditions. As shown in Fig. S1C, a longer hybridization time between S1 and S2 would by better for the detection sensitivity. Nevertheless, considering that a short-time analysis is highly desired and 2 h is enough for the sensitive determination, we have chosen the 2 h hybridization time for the following detection.

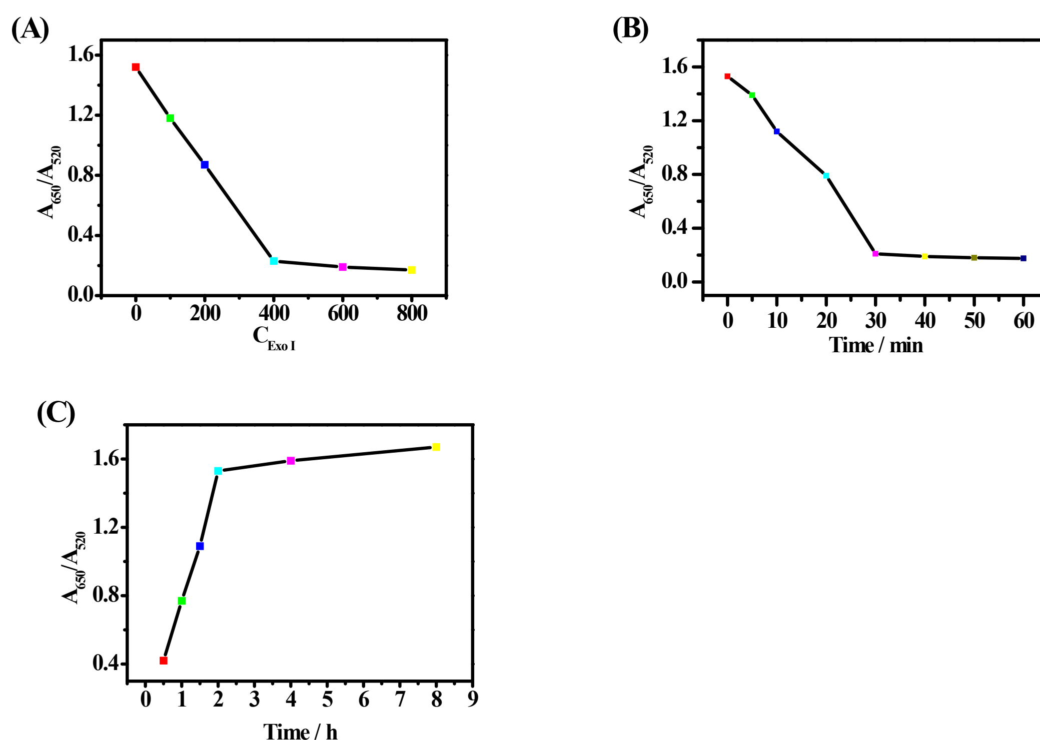

Fig. S1 (A) Effect of the concentration of Exo I on the detection performed in the absence of FR. (B) Effect of Exo I digestion time on the detection performed in the absence of FR. (C) Effect of the hybridization time between S1 and FR/FA-S2 on the detection performed in the presence of FR

3. Comparison of detection limit between the proposed method with other reported detection methods for FR

Method/System	Linear range	Detection limit	Features	Ref.
Fluorescence, RCA*	1 pM-2.5 nM	0.8 pM (about 0.03 ng/mL)	Low detection limit, but cumbersome	1
Fluorescence, Exo III-assisted G-quadruplex releasing	1 nM-100 nM	30 nM (about)	Label free, but low sensitivity	2
Fluorescence, PEI-coated CdS-ZnS QDs	0.2-1.2 μg mL^{-1}	10 ng/mL	Highly complex process, and low sensitivity	3
Electrochemistry, Exo III-assisted recycling*	0.3−15 ng/mL	0.19 ng/mL	high sensitivity	4
Electrochemistry, selective CNTs assembly	10 pM-1.0 nM	3 pM (about 0.114 ng/mL)	high sensitivity	5
Colorimetry, DNA-functionalized Au NPs, Exo III-assisted recycling*	0.1 - 10 pM	50 fM in UV-vis spectrometry; 10 pM (about 0.38 ng/mL) for direct visualization	High sensitivity and visualization, but cumbersome	6
Colorimetry, DNA-functionalized Au NPs	0.5-50 ng/mL	0.33 ng/mL for direct visualization	Low detection limit, and visualization	This work

***: amplified detection**

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4. Colorimetric detection of the standard FR concentrations in serum samples

samples	Standard FR concentration (ng/mL)	FR concentration detected by proposed method (ng/mL)	Relative error (%)
1	1	0.94	6
2	5	4.76	4.8
3	10	9.72	2.8
4	15	14.47	3.5

Table S2 Colorimetric detection of the standard FR concentrations in serum samples