Supporting Information

Precise Detection of NSE and ProGRP with Nanoprobe for Early Diagnosis of Small Cell Lung Cancer

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Experimental Section

Materials

Non-thiol ssDNA (Table S1) was purchased from Sangon Biotech (Shanghai) Co., Ltd. ProGRP protein was obtained from Tedbio (Wuxi), and NSE protein was obtained from MedChemExpress. Chloroauric acid ($HAuCl_4$), sodium citrate, N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and selenocysteine were acquired from Sinopharm Group (Shanghai). Sodium ascorbate was purchased from Sigma-Aldrich. Sodium dodecyl sulfate (SDS) was obtained from Shanghai Saen. Glutathione (GSH) was obtained from Shanghai Macklin. The patient serum was collected from the Second Hospital of Shandong University. All chemicals used in the experiments were of analytical grade and unpurified. All experimental water was ultrapure (18.2 MΩ•cm).

Instruments

A Hitachi HT7700 transmission electron microscope was used to obtain TEM images of the nanoparticles. The fluorescence absorption spectra of the probes were measured using a Hitachi F-4600 fluorescence spectrometer and a Shimadzu UV2600 UV-visible spectrophotometer. All glassware used in the experiments was soaked in aqua regia (mixed with concentrated hydrochloric acid and concentrated nitric acid in a ratio of 3:1) for 4 h, then rinsed three times with ultrapure water and oven-dried for later use.

Synthesis of Au Nanoparticles and Their Probes

Au nanoparticles (Au NPs) with a diameter of 13 nm were synthesized using the

classical sodium citrate reduction method. In a 100 mL three-neck round-bottom flask, 70 mL of ultrapure water was added, and 700 μL of chloroauric acid ($HAuCl_4$) solution (20 mg/mL) was added under stirring while heating until boiling. Then, 3.5 mL of sodium citrate solution (1% w/w) was rapidly added under stirring. The solution changed from pale yellow to colorless and finally turned wine red. It was stirred and boiled for 20 min. After that, the heat was turned off, and the solution was stirred and cooled to room temperature before being transferred to a refrigerator at 4°C for storage for future use.

Following previously reported methods, 3,3'-diselenodipropionic acid was synthesized. The synthesis of selenium-containing ssDNA was modified from a reported protocol. A 62.6 μL solution of 3,3′-diselenodipropionic acid (0.0011 g/10 mL) was mixed with 275 μL NHS (0.0010 g/10 mL) and 275 μL EDC (0.0016 g/10 mL). After stirring at room temperature for 30 min, 360 μM of non-selenium ssDNA was added and stirred at room temperature for 24 h (300 rpm). The reaction mixture was then ultrafiltered to remove by-products, and the synthesized selenium-containing ssDNA was stored at 4°C. To obtain hybridized double-stranded DNA, equal concentrations of fluorescently labeled ssDNA and selenium-containing ssDNA were added to PBS buffer (10 mM, pH 7.4) and heated to 85°C for 5 min before cooling to room temperature.

Four milliliters of the synthesized Au NPs were placed in a glass vial. A 10% w/w sodium dodecyl sulfate (SDS) solution was added to reach a final concentration of 0.1%, and the solution was stirred at room temperature for 30 min. The ProGRP-

specific DNA-SeH hybrid duplex (400 nM) and NSE-specific DNA-SeH hybrid duplex (400 nM) were then sequentially added and irradiated for 3 h before being reacted in the dark for 24 h. After the reaction was completed, the final product was centrifuged at high speed (12,000 rpm, 20 min), resuspended in ultrapure water, and stored at 4°C. The concentration of the probe was calculated by measuring the ultraviolet absorbance of the Au NPs and the probe, and subsequent experiments diluted the probe to the desired concentration using PBS buffer (10 mM, pH 7.4).

To calculate the DNA loading on the probe, we determined the optimal excitation and emission wavelengths of FITC-labeled ssDNA and 5-TAMRA-labeled ssDNA as 494 nm and 520 nm, and 562 nm and 585 nm, respectively. Standard linear calibration curves were drawn by measuring the fluorescence intensity of ssDNA fluorophores at different concentrations (5-400 nM). The probe was diluted to 1 nM in PBS buffer (10 mM, pH 7.4) and an excess of selenocysteine (10 mM) was added. The mixture was stirred in the dark for 12 h to ensure complete release of the hybridized double-stranded DNA. After centrifugation, the fluorescence intensity of the supernatant was measured with a fluorescence spectrometer. The obtained fluorescence intensity values were compared with the standard equation to determine the number of DNA strands loaded on the probe.

Response of Probe under Simulated Physiological Conditions

The probe was incubated with varying concentrations of ProGRP (0-150 pg/mL) and NSE (0-200 ng/mL) for one h. Fluorescence signals of FITC and 5-TAMRA were collected at 0, 5, 10, 15, 20, 25, 30, 40, 50, and 60 min. The kinetic response of the

probe was then studied. FITC and 5-TAMRA fluorescence were immediately collected after adding ProGRP (150 pg/mL) or NSE (200 ng/mL) to a 1 nM probe solution respectively.

The probe was incubated separately with ProGRP (150 pg/mL), NSE (200 ng/mL), and various interfering substances (KCl, $MgCl_2$, glucose, BSA, and HSA, each at 100 μM) for one h. Fluorescence signals of FITC and 5-TAMRA were then collected for each sample.

The probe was dispersed in PBS buffer (pH values ranging from 6.0 to 8.0) at a final concentration of 1 nM. Two experimental groups were set up: one without target addition and another with ProGRP (150 pg/mL) and NSE (200 ng/mL) as targets. Both groups were incubated at 37°C for 60 min. After incubation, FITC and 5-TAMRA fluorescence were measured using a fluorescence spectrometer to observe the fluorescence variation with pH.

The probe was divided into two groups. First, the probe was diluted to 1 nM with PBS buffer. The first group was not treated, while 2 U/L of DNase I was added to the second group. Both were incubated at 37°C for 1 h, and the fluorescence intensity of FITC and 5-TAMRA was measured at intervals of 10, 20, 30, 40, 50, and 60 min. Next, the effect of nuclease on the probe's response to targets was tested. The probe was divided into two experimental groups: one was treated with DNase I only, and the other was treated with DNase I and the targets ProGRP and NSE. Both groups were incubated at 37°C for 60 min, and then the fluorescence signals of FITC and 5-TAMRA were collected.

The probe at 1 nM was added to a solution containing 5 mM GSH and incubated at 37°C for 0-12 h. Fluorescence intensity of the supernatant was measured at 0, 1, 2, 4, 8, and 12-h intervals after centrifugation. To further investigate the effect of GSH within 12 h, the probe at 1 nM was added to solutions of varying GSH concentrations (0, 10, 50, 100, 200, 500, 1,000, and 5,000 μM) and incubated at 37°C for 12 h. After centrifugation, the fluorescence intensity of the supernatant was measured.

Thermal Stability Testing

The probe was diluted to 1 nM in PBS buffer. The diluted solution was then incubated for 5 min at temperatures of 30, 40, 50, 60, 70, and 80°C. Fluorescence changes of FITC and 5-TAMRA were measured using a fluorescence spectrometer.

Clinical Patient Testing

First, blood samples from five SCLC patients collected from the Second Hospital of Shandong University were processed. The blood was centrifuged at 4°C for 5 min (4,000 rpm), and the serum layers were collected and stored at -80°C. The serum was diluted tenfold, and the experimental group received a final concentration of 1 nM of the probe, while the control group consisted of serum samples of the same concentration without the probe. Both groups were incubated at 37°C for 40 min. To determine if the serum from other cancer patients affected the detection results, we also collected blood samples from five patients with different cancers, including stomach cancer, prostate cancer, liver cancer, breast cancer, and ovarian cancer, and numbered them from 1 to 5. The serum was processed similarly to before, and tenfold-diluted serum was added to the probe at 1 nM, with the same concentration of serum samples set as controls.

Both groups were incubated at 37°C for 40 min, after which the fluorescence intensities of FITC and 5-TAMRA were measured.

Supplementary Figures

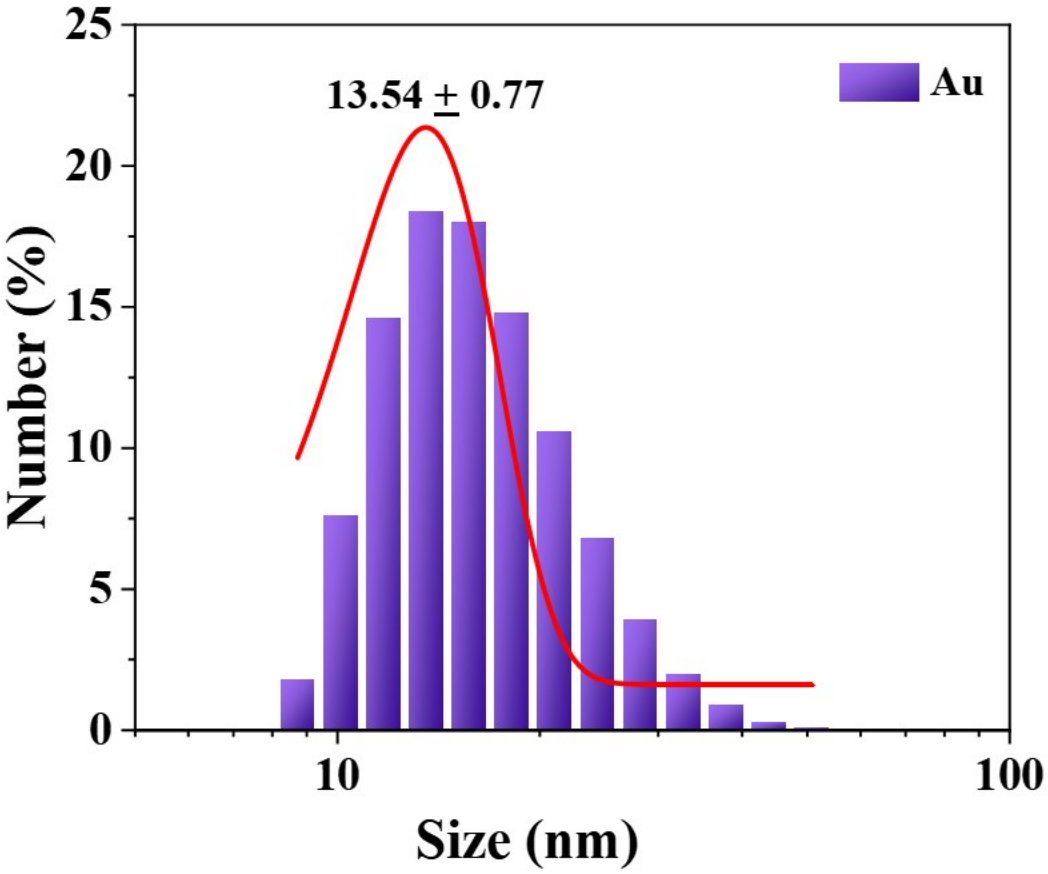

Figure S1. Dynamic light scattering (DLS) data of the Au NPs.

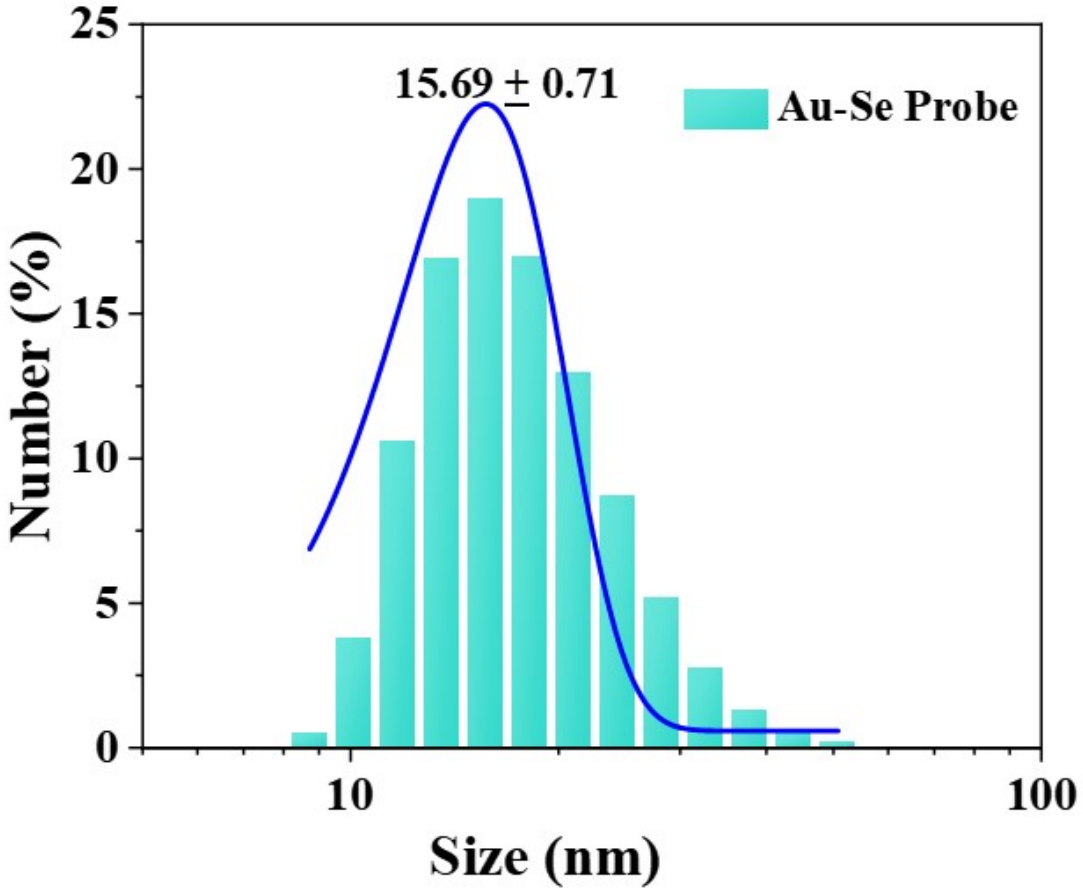

Figure S2. DLS data of the Probe.

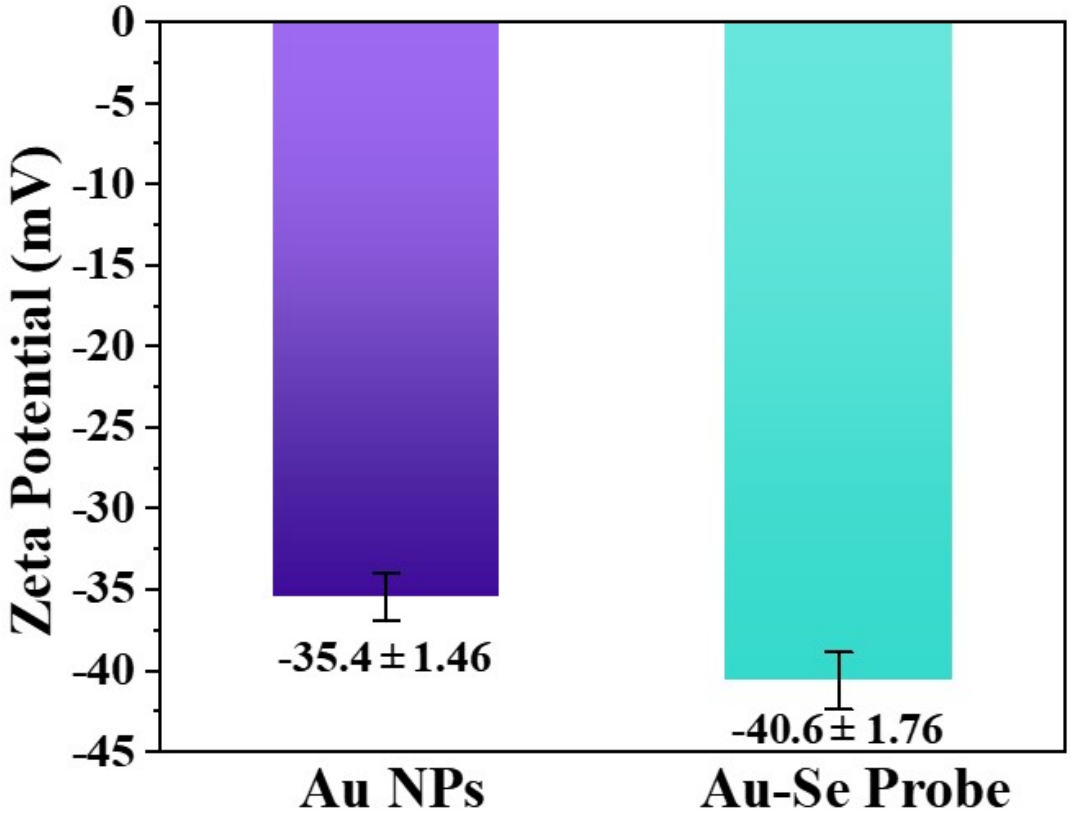

Figure S3. Zeta potential of the (purple) Au NPs and (green) the Probe.

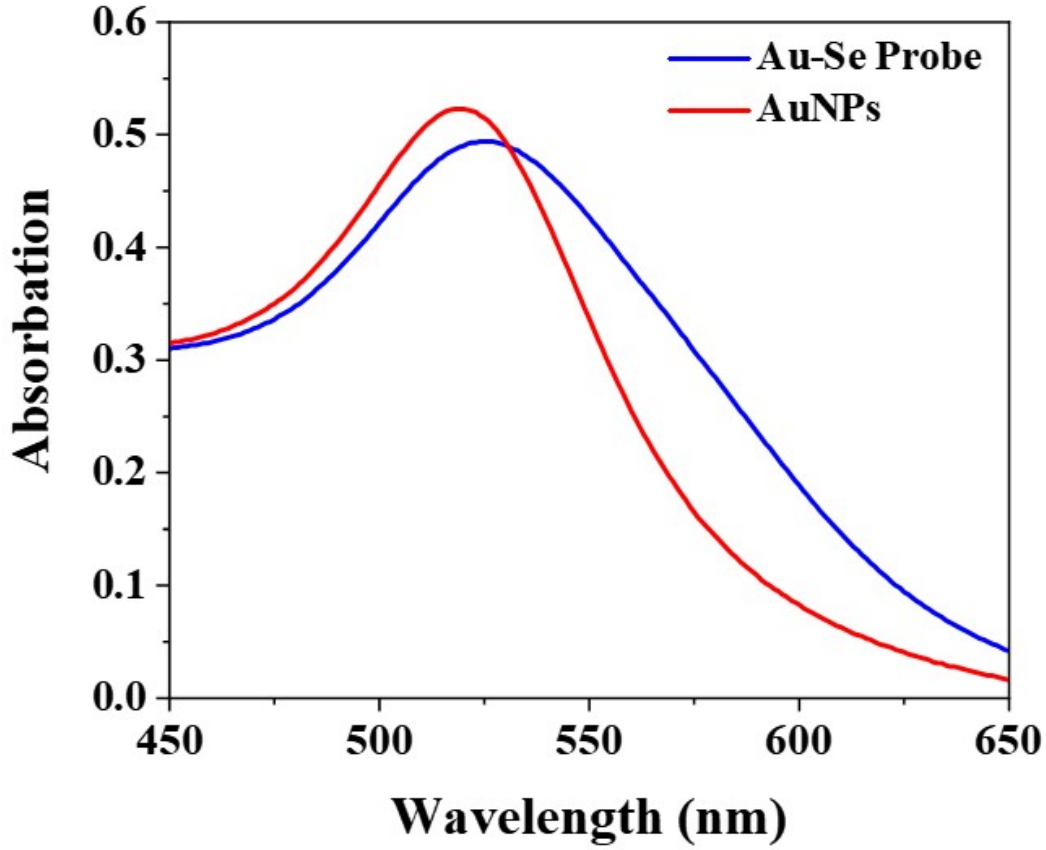

Figure S4. UV-Vis absorption spectra of the (red) Au NPs and (blue) Probe.

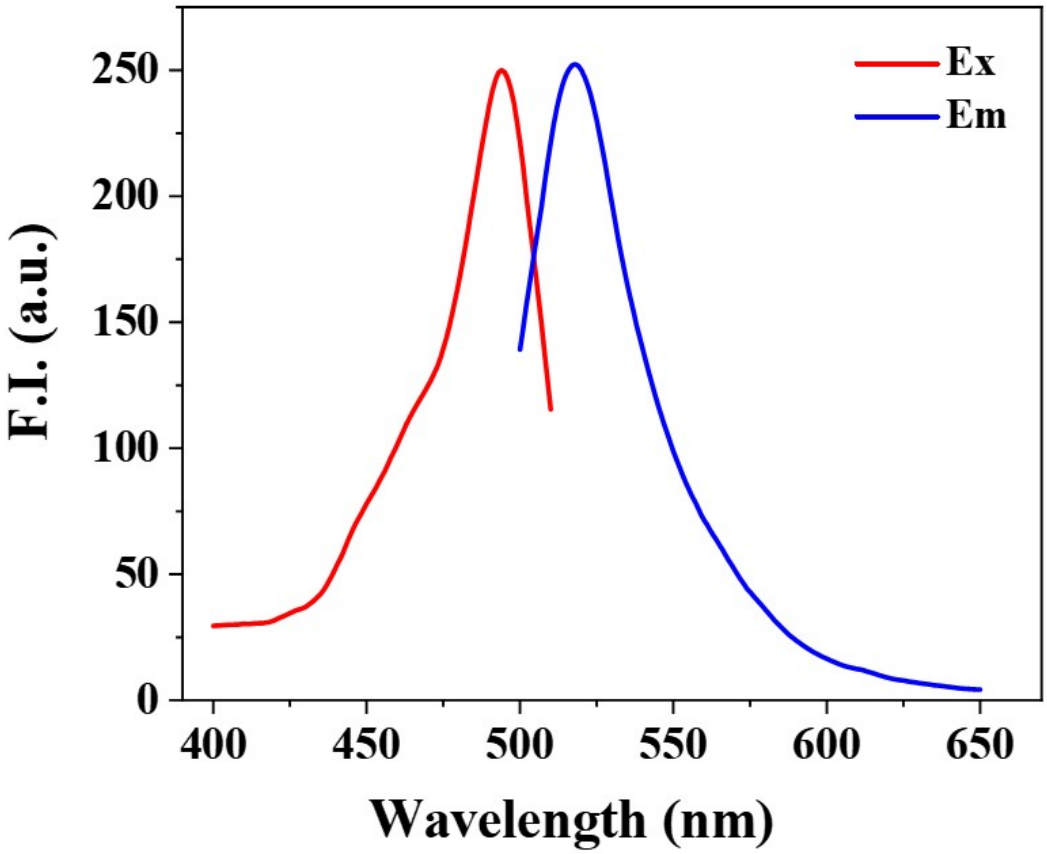

Figure S5. The optimal excitation and emission wavelengths for FITC-labeled ssDNA.

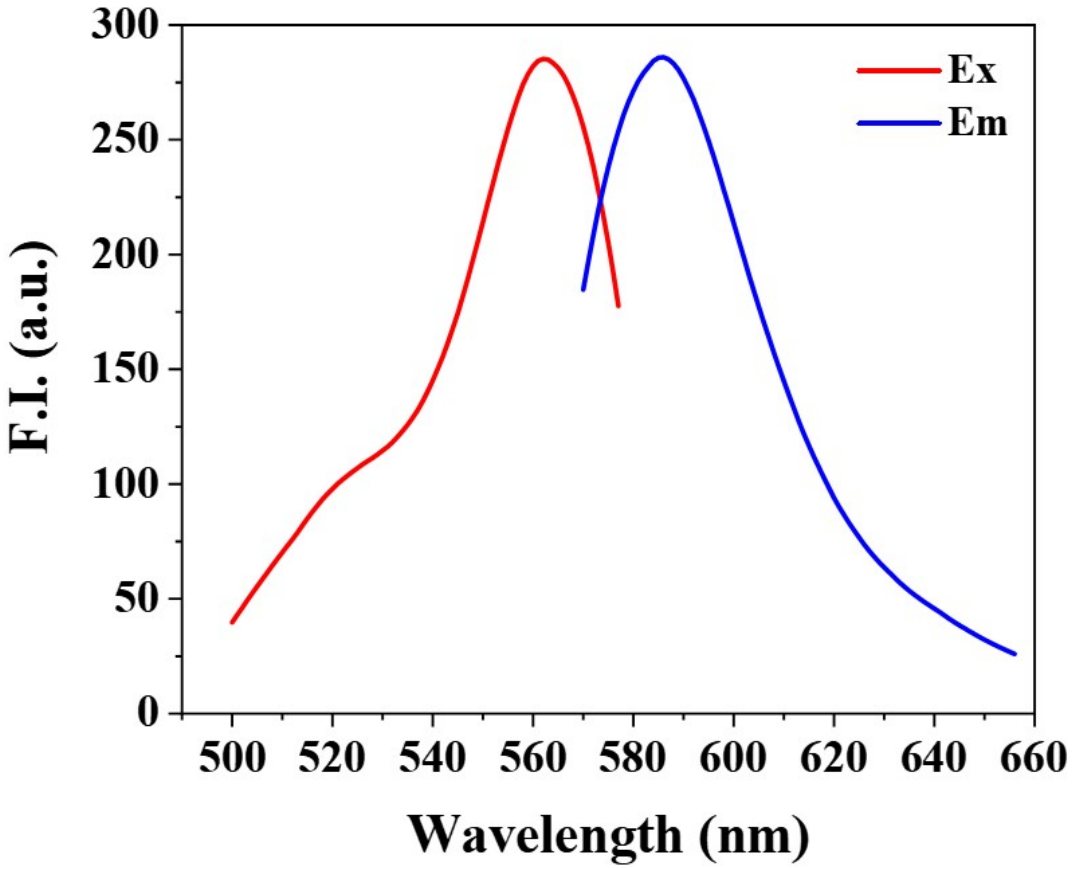

Figure S6. The optimal excitation and emission wavelengths for 5-TAMRA-labelled ssDNA.

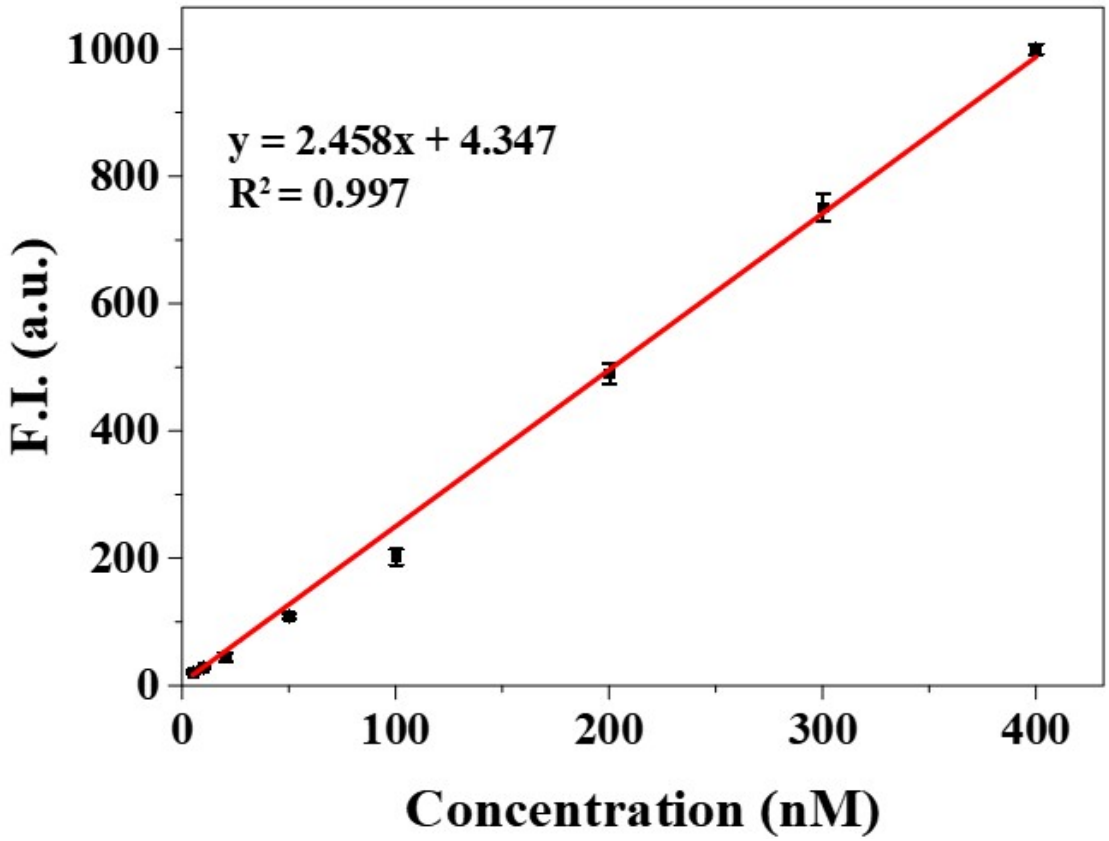

Figure S7. Standard linear calibration curve of FITC-labeled ssDNA at different concentrations.

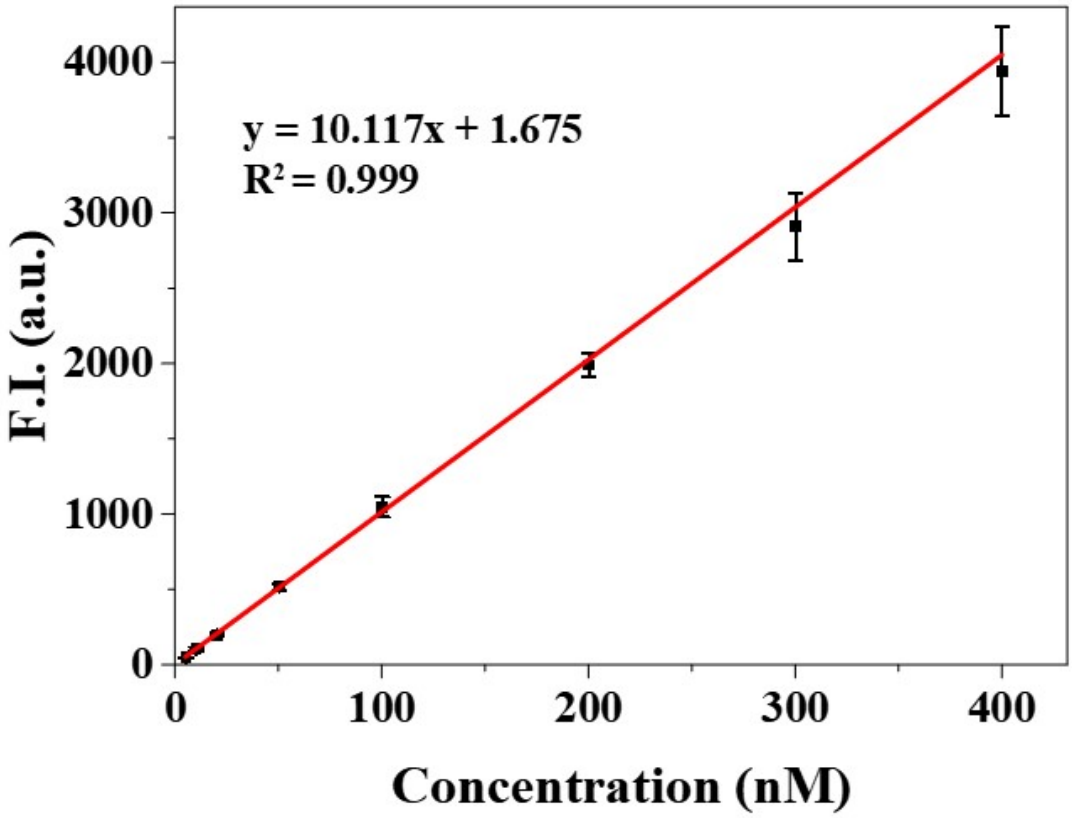

Figure S8. Standard linear calibration curve of 5-TAMRA-labeled ssDNA at different concentrations.

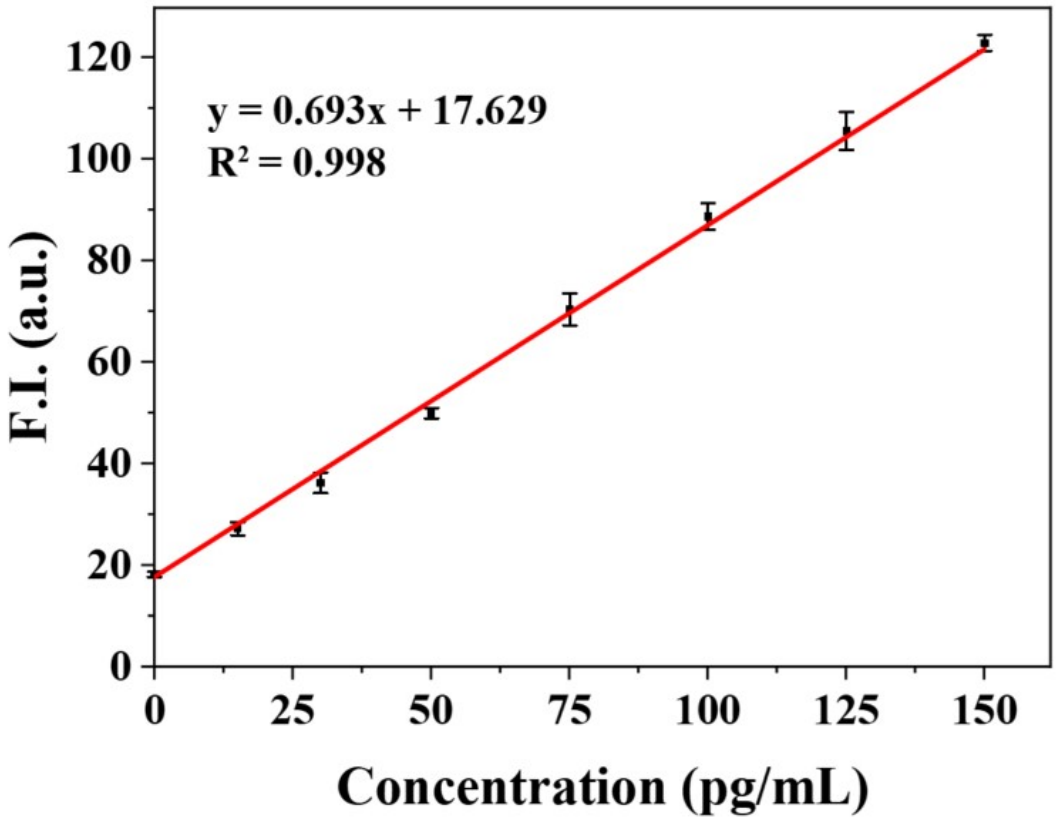

Figure S9. The fluorescence standard curve corresponding to the ProGRP

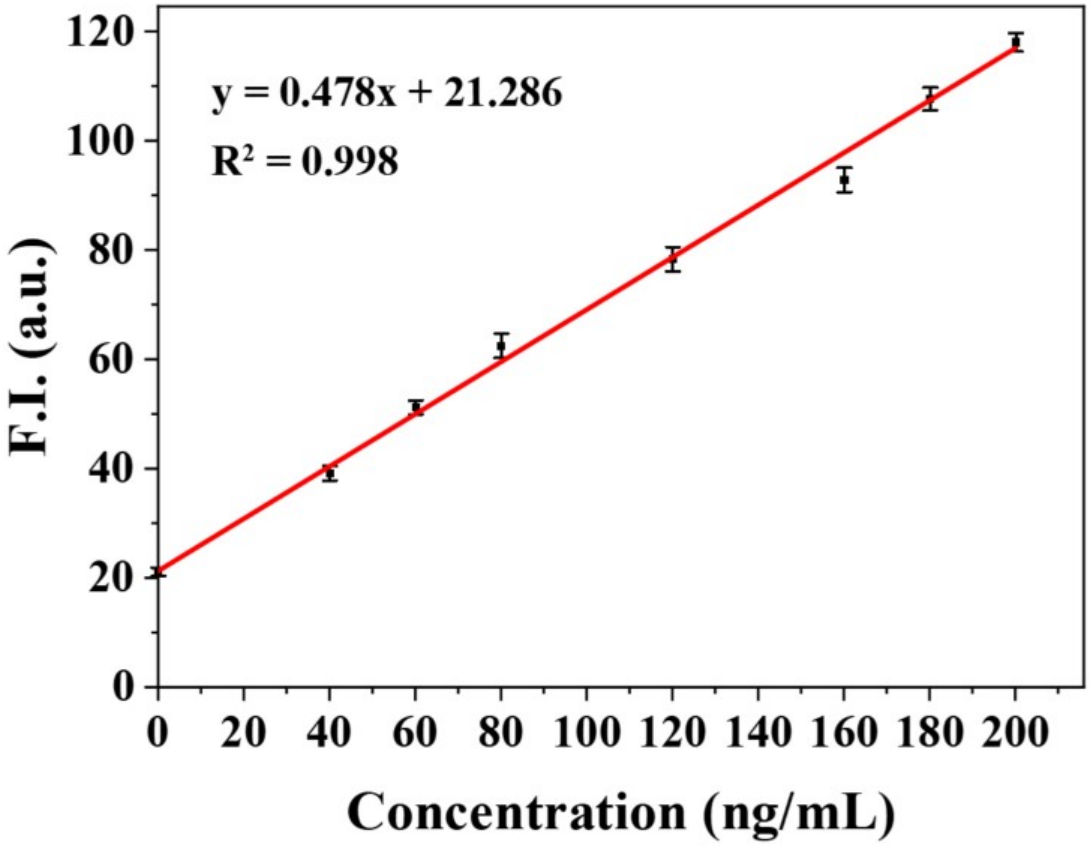

Figure S10. The fluorescence standard curve corresponding to the NSE.

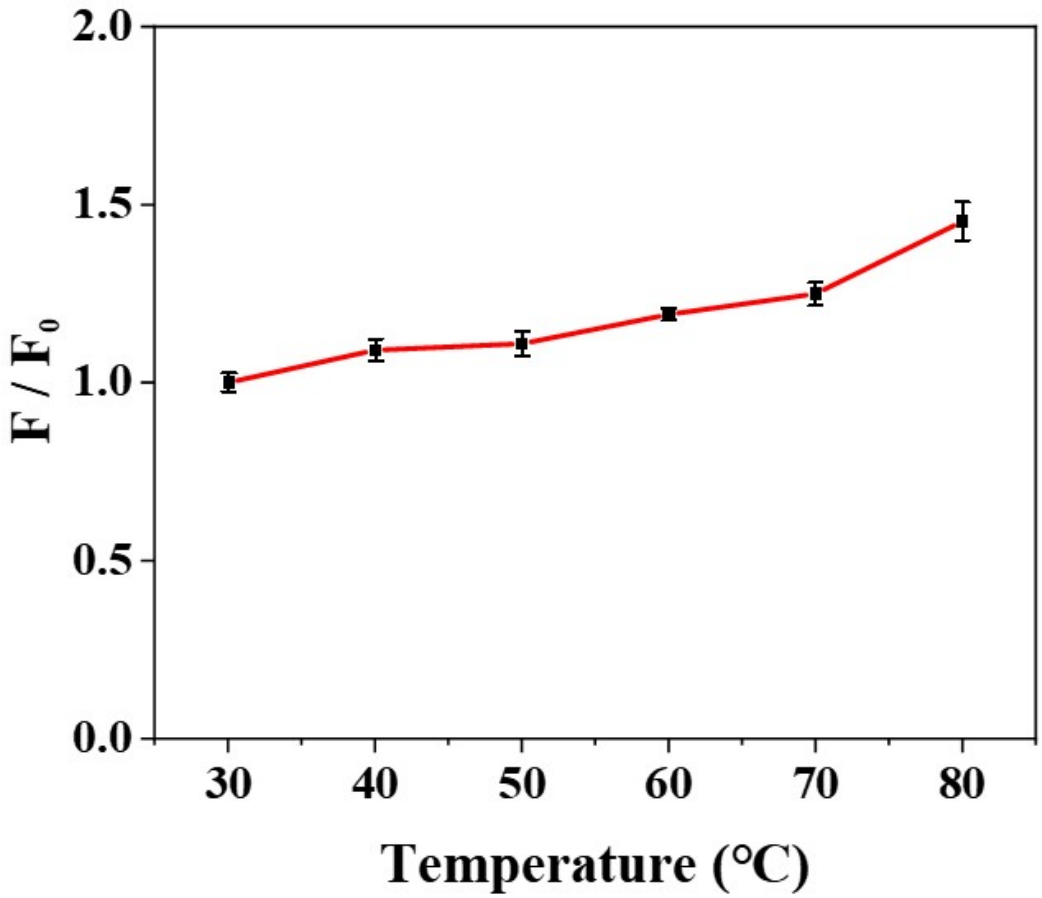

Figure S11. Fluorescence of Au-Se probe as a function of temperature for FITC

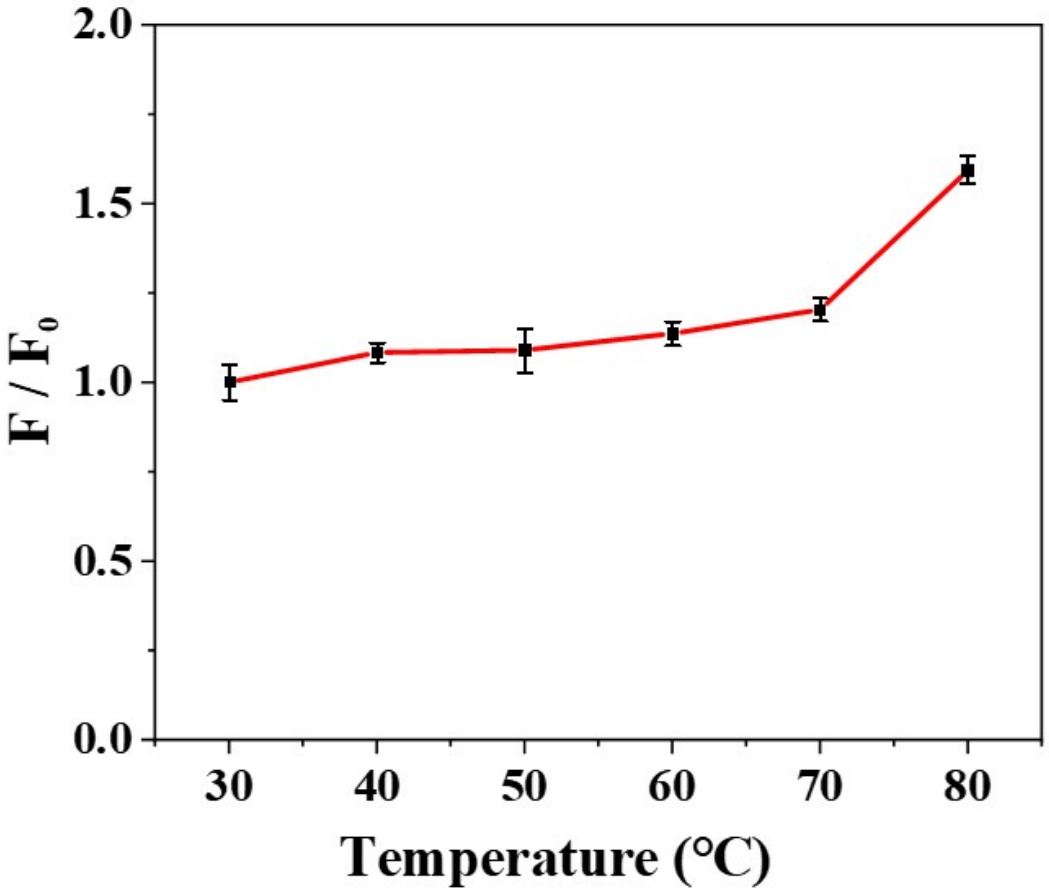

Figure S12. Fluorescence of Au-Se probe as a function of temperature for 5-TAMRA

Table S1. DNA sequences used in this work.

名称	序列(5'-3')
DNA-NH_2	NH_2-AAAAAAAAACCTAAGGAAATA
Aptamer of ProGRP	CATGCGGAGTAGATTCGAGCCCAGATAGTCCCT GGTTATTTCCTTAGG-FITC
DNA-NH_2	NH_2-AAAAAAAAACCCCTGATTCTG
Aptamer of NSE	CGGTAATACGGTTATCCACAGAATCAGG GG-5-TAMRA