Electronic Supplementary Information

Resonance Rayleigh scattering detection of epidermal growth factor receptor based on aptamer-functionalized gold-nanoparticle probe

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Experimental

Materials and reagents

Chloroauric acid (HAuCl₄) and sodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

The aptamers and oligonucleotide used in this study were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China) and have the following sequence:

The thiolated 51 mer EGFR aptamer: 5’ SH-(CH₂)₆-UGC CGC UAU AAU GCA CGG AUU UAA UCG CCG UAG AAA AGC AUG UCA AAG CCG-3’

Random RNA oligonucleotide (as negative control): UUG UAC UAC ACA AAA GUA CUG

Recombinant human EGFR protein (Fc Chimera) (catalog no. ab155726) was obtained from Abcam (Cambridge, UK). Bovine serum albumin (BSA), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Instrumentation

A Hitachi F-4600 fluorescence spectrophotometer (Hitachi Ltd. Tokyo, Japan) was used to record the RRS spectra and to measure the scattering intensities with the slits (ex/em) of 5.0/5.0 nm, the photomultiplier tube (PMT) voltage was 400 V. A TU-1901 UV-Vis spectrophotometer (Purkinje General Instrument Co. Ltd., Beijing, China) was used for recording the absorption spectra. A JEM-1011 transmission electron microscope (JEOL, Japan) was used to observe the morphology. Electrophoresis was performed in a Mini-PROTEAN electrophoresis cell (Bio-Rad, Richmond, CA, USA). Images were obtained using an Amersham™ Imager 600 (GE Healthcare Biosciences, Pittsburg PA).

Synthesis of AuNPs

AuNPs were synthesized by reduction of the HAuCl₄ solution with sodium citrate and
the synthesis method was described in the reference with a minor modification [1]. Briefly, 100 mL of 0.01% (w/v) of the HAuCl₄ solution was heated to boiling for 15 min, and then 5 mL of 1% (w/v) sodium citrate solution was added. The solution turned deep blue within 20 s, and then the final color changed to wine-red after 60 s. Heating continued for 20 min after the solution color remained unchanged. After cooling down to room temperature, the prepared AuNPs stock solution was stored at 4 °C for further use.

**Preparation of Apt-AuNP probe**

An aliquot of the aqueous AuNP solution (4.8 nM, 10 mL) was mixed with the thiol-modified 51-mer RNA aptamer (100 μM, 200 μL) at room temperature for 12 h. The mixtures were then centrifuged for 15 min at 16,000 rpm to remove the excess RNA. After two centrifuge/wash cycles, the precipitate was redispersed in 20 mM of N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES, pH 7.4, Sigma, St. Louis, MO).

**Cell culture and preparation of cell lysates**

Human esophageal cancer (Eca109) cells and normal human esophageal epithelial cells were purchased from the Cell Resource Center, Peking Union Medical College (which is the headquarters of National Infrastructure of Cell Line Resource, NSTI) (Beijing, China). The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Gibco Life Technologies, Carlsbad, CA, USA)), 100 μg/ml streptomycin and 100 U/ml penicillin (Gibco, USA) at 37°C with 5% CO₂ and saturated moisture.

Human breast cancer cells (MDA-MB-435 cells) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C in a humidified chamber containing 5% CO₂.

At 80% confluency, Eca109, HEEC and MDA-MB-435 cells were lysed using appropriate volumes of M-PER (Thermo Scientific, Rockford, IL), the mammalian protein extraction reagent and supernatant from cells was collected. Cell lysates and
supernatant were clarified by centrifugation at 10 000 rpm for 10 min and used for analyte detection by RRS.

**Experimental produce**

Suitable amounts of Apt-AuNP solution were placed in a 10mL calibrated flask, followed by different amounts of Recombinant human EGFR protein solution. After 10 min, the mixture was diluted with HEPES buffer solution (pH 7.4) to the mark and mixed thoroughly. The RRS spectra of the system was recorded with synchronous scanning at $\lambda_{em}=\lambda_{ex}$ ($\Delta\lambda=0$nm). The RRS intensity ($I_{RRS}$) for the complex and $I_0$ RRS for the reagent blank at their maximum wavelength ($\lambda_{max}$) was measured, $\Delta I_{RRS}=I_{RRS}-I_0$ RRS. The absorption spectra were also recorded. All measurements were performed at room temperature (15~25°C).

**SDS-PAGE**

Analysis of the amount of aptamers conjugated on the surface of the AuNPs. AuNPs conjugates along with control RNA samples ranging from 2 to 10 pmols were loaded into a 12% SDS-PAGE gel, electrophoresed, and stained with GelRed. The gel was scanned with an Amersham Imager 600, and densitometry was analyzed using Image Quant TL 8.1 software.

**Ethics statement**

The study was approved by the ethics committee of the Changzhi Medical College, P. R. China. The collection and use of the volunteers' samples for test method development purposes was approved by the ethics committee of the Heping Hospital affiliated to Changzhi Medical College, P. R. China. The participants were informed that the samples were going to be stored in a repository. None of the authors have access to any identifying patient information.
Concentration of AuNPs

The concentration of AuNPs was calculated according to the method described in reference [2]:

\[ c = \frac{A_{450}}{\varepsilon_{450}} \]  

(1)

in which \( c \) is the concentration of AuNPs, \( A_{450} \) is the absorbance at \( \lambda = 450 \text{nm} \), \( \varepsilon_{450} \) is the molar decadic extinction coefficient. The TEM image showed the diameter of AuNPs we synthesized is about 13 nm, so, according to the literature, \( \varepsilon_{450} \) (\( d = 13 \text{nm} \)) = \( 1.39 \times 10^8 \text{ M}^{-1} \text{cm}^{-1} \). The \( A_{450} \) obtained from the absorption spectra of as-prepared AuNPs is 0.672. By using the equation 1, the concentration of as-prepared AuNPs was calculated as 4.8 nM (\( 2.81 \times 10^{12} \text{ particles/mL} \)).

Analysis of the amount of aptamers conjugated on the surface of the AuNPs.

AuNPs conjugates along with control RNA samples ranging from 2 to 10 pmols were loaded into a 12% SDS-PAGE gel, electrophoresed, and stained with GelRed (Fig.S1 A). The gel was scanned with an Amersham Imager 600, and free RNA in the AuNPs sample was quantified in Image Quant TL 8.1 software by taking the average intensity value within the band (after background subtraction). The control sample was used as reference and taken to represent the total loaded RNA (Fig.S1 B).
By calculating the signal intensity, we found that there was 5 pmol of RNA per 5 μL of AuNPs, which is equivalent to ~214 molecules of Apt/particle.

\[
\frac{5 \text{ pmol} \times N_A}{5 \mu L \times 2.81 \times 10^{12} \text{ particles/mL}} = \frac{5 	imes 10^{-12} \text{ mol} \times 6.02 	imes 10^{23} \text{ Apt molecules/mol}}{5 \times 10^{-3} \text{ mL} \times 2.81 	imes 10^{12} \text{ particles/mL}} = 214 \text{ Apt molecules/particle}
\]

Comparison of absorption and resonance Rayleigh scattering spectra
Fig. S2 Comparison of absorption (1) and resonance Rayleigh scattering (2) spectra

**Optimization of Experimental Conditions**

We firstly optimize the Apt concentration. As shown in Fig. S3, enhanced intensities ($\Delta I_{RRS}$) improve with the increasing concentration of Apt from 0 $\mu$M to 100 $\mu$M and then remains unchanged beyond the concentration of 100 $\mu$M probably due to the consumption of all available AuNPs. Therefore, 100 $\mu$M Apt is used in the following experiments, and the addition amount is 200 $\mu$L.

![Graph showing the effect of Apt concentration on ΔI_{RRS}](image)

**Fig. S3 Effect of Apt concentration**

Concentration of AuNP: 4.8 nM; Concentration of EGFR: 80 ng·mL$^{-1}$

Since the specific recognition reaction of Apt-AuNP and EGFR protein involves charging species, it is expected that the pH in would affect the reaction. The effect of the pH is shown in Figure S4. The $\Delta I_{RRS}$ of the system reached a maximum and remained relatively constant in the pH range 7.2 ~ 7.5, which may be attributed to the instability of Apt and EGFR protein at lower or higher pH. Therefore, HEPES buffer
solution of pH 7.4 was taken as the reaction medium in the following experiments.

![Fig. S4 Effect of pH](image)

Concentration of Apt-AuNP: 4.8 nM; Concentration of EGFR: 80 ng·mL⁻¹; reaction temperature: 15~25 °C; reaction time: 10 min

The effect of Apt-AuNP probe concentration (calculated by AuNPs) on the intensity was studied. According to the experimental results, $\Delta I_{RRS}$ reached the maximum and keep stability in the range of 4.7~5.0 nM (See Fig.S5). Without enough probe, the reaction was incomplete, when the probe was excessive, the scattering intensity would be reduced, because the reagent blank would enlarge. Therefore, we choose 4.8 nM as a suitable probe concentration in the experiment.

![Fig. S5 Effect of Apt-AuNPs concentration](image)

Concentration of EGFR: 80 ng·mL⁻¹; pH 7.4; reaction temperature: 15~25 °C; reaction time: 10 min

Additionally, we optimized the time Apt-AuNP probe incubated with EGFR
protein. As shown in Fig. S6, the $\Delta I_{RRS}$ improves dramatically and reaches a plateau within 10 min due to the higher binging of the probe to EGFR. The decrease in readout signal with further increasing in incubation time over 30 min is due to the nonspecific adsorption of the probe. Thus, 10 min is selected as the optimal reaction time.

![Fig. S6 Effects of reaction time](image)

Concentration of Apt-AuNP: 4.8 nM; Concentration of EGFR: 80 ng·mL$^{-1}$; pH7.4; reaction temperature: 15~25 °C

The effect of temperature on the recognition reaction is shown in Figure S7. The $\Delta I_{RRS}$ increases with decreasing temperature. The intensity at 15~25 °C is higher than that at 37 °C. Although the rate of chemical reactions usually increases with temperature, the recognition reaction involves a negative change in entropy $\Delta S < 0$. Therefore, the increase of the temperature results in an increase in the free energy, and a decrease in the stability of the binding products. It is also found that decreasing the temperature to 10 °C increases the rate of nonspecific aggregation.
Fig. S7 Effect of reaction temperature

Concentration of Apt-AuNP: 4.8 nM; Concentration of EGFR: 80 ng·mL⁻¹; pH 7.4; reaction time: 10 min
## Comparison of the method used for EGFR determination

<table>
<thead>
<tr>
<th>Methods</th>
<th>Assay protocol</th>
<th>Analytical ranges/LODs</th>
<th>Comments</th>
<th>Reference</th>
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<tr>
<td>Quartz crystal microbalance (QCM)</td>
<td>Anti EGFR immobilized QCM</td>
<td>0.01-10 μg·mL⁻¹/100 ng·mL⁻¹</td>
<td>Small size, minimal electrode, complex, limited dynamic range, not low LOD</td>
<td>[3]</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Apt/Ab-based sandwich immunoassay</td>
<td>1-40 ng·mL⁻¹/50 pg·mL</td>
<td>Low LOD, narrow dynamic range</td>
<td>[4]</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>Aptamer-based target-/probe-mediated</td>
<td>1 fM-1 nM/0.16 fM</td>
<td>Low LOD, large dynamic range, operationally complex, long assay time</td>
<td>[5]</td>
</tr>
<tr>
<td>Microfluidic immunosensor</td>
<td>CMK-3/poly-(AC-co-MDHLA) anti-EGFR/AMS</td>
<td>0.01-50 ng·mL⁻¹/3.03 pg·mL⁻¹</td>
<td>Low LOD, Complex structure</td>
<td>[6]</td>
</tr>
<tr>
<td>ZnO thin film transistor immunosensor</td>
<td>ZnO-bioTFT</td>
<td>—/10 fM</td>
<td>Low LOD, highly sensitive, operationally complex</td>
<td>[7]</td>
</tr>
<tr>
<td>Capacitive</td>
<td>Using AuNPs for signal enhancement</td>
<td>20-1000 pg·mL⁻¹/20 pg·mL⁻¹</td>
<td>Low LOD, special equipment</td>
<td>[8]</td>
</tr>
<tr>
<td>RRS</td>
<td>Apt-AuNP probe</td>
<td>30-110 ng·mL⁻¹/0.7 ng·mL⁻¹</td>
<td>Highly selective and sensitive, short assay time, easy to operate</td>
<td>This work</td>
</tr>
</tbody>
</table>
Reference