Electronic Supplementary Information

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

Junbo Li[a,b], Yanqiang Yang [c], Jinghua Wang [d] , Bing Zhang[a], Honghong Chang [a] , Wenlong Wei [a, *]

[a] *Department of Chemistry and Chemical Engineering, Taiyuan University of Technology, Taiyuan, 030024, China. E-mail: weiwenlong5923@163.com*

[b] *Pharmaceutical Department, Changzhi Medical College, Changzhi, 046000, China.*

[c] *Changzhi Entry-exit Inspection and Quarantine Bureau, Changzhi, 046000, China.*

[d] *Department of Traditional Chinese Medicine, Heping Hospital Affiliated to Changzhi Medical College, Changzhi, 046000, China.*

*Correspondence address: Department of Chemistry and Chemical Engineering, Taiyuan University of Technology, Taiyuan, 030024, China. E-mail address: weiwenlong5923@163.com

Table of Contents

Experimental

Materials and reagents

Chloroauric acid ($HAuCl_4$) 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-$(CH_2)_6$-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 $HAuCl_4$ 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 $HAuCl_4$ 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% CO_2 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% CO_2.

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 10min, 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 $\lambda_{em}=\lambda_{ex}$ ($\Delta\lambda$=0nm). The RRS intensity (I_{RRS}) for the complex and $I0$ RRS for the reagent blank at their maximum wavelength (λ_{max}) was measured, $\Delta I_{RRS}=I_{RRS}-I0$ 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]:

$c=A_{450}/\varepsilon_{450}$ (1)

in which c is the concentration of AuNPs, A_{450} is the absorbance at λ=450nm, ε_{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 ,ε_{450} (d=13nm)=1.39×10^{8} $M^{-1}cm^{-1}$. 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 (2.81×10^{12} 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).

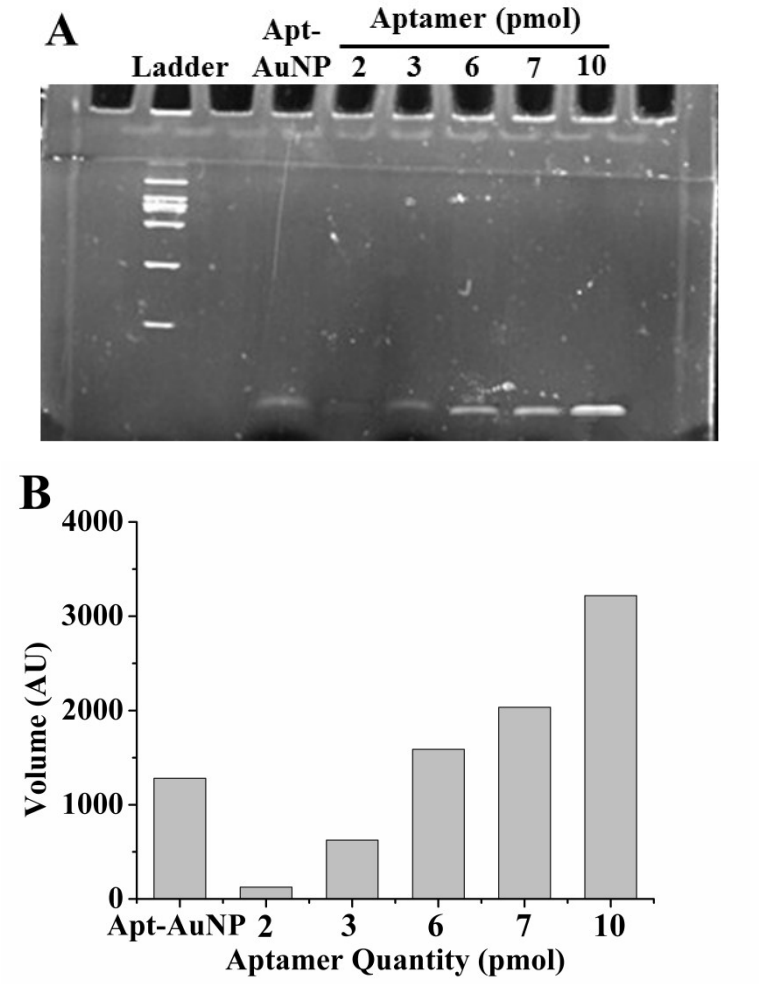

Fig.S1 (A) Photograph of the gel after GelRed staning. (B) Quantified results by the Image Quant TL

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.

$$Number\ of\ Apt\ per\ particle = \frac{5\ pmol \times N_A}{5\mu L \times 2.81 \times 10^{12} particles/mL} = \frac{5 \times 10^{-12} mol \times 6.02 \times 10}{5 \times 10^{-3} mL \times 2.81 \times} = 214\ Apt\ molecules/particle$$

Comparison of absorption and resonance Rayleigh scattering spectra

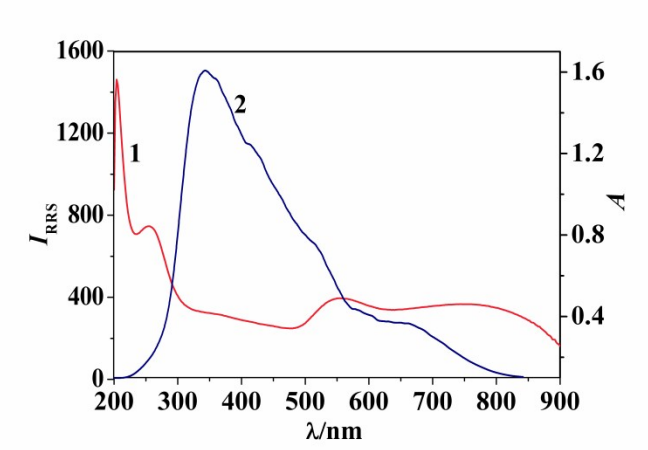

Fig. S2 Comparison of absorption (1) and resonance Rayleigh scattering (2) spectra

Optimization of Experimental Conditions

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

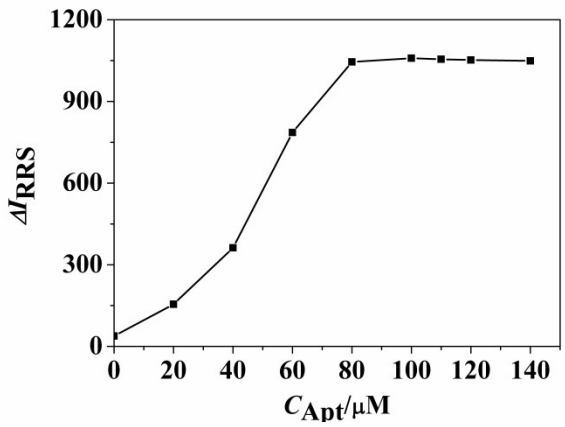

Fig. S3 Effect of Apt concentration

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 ΔI_{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.

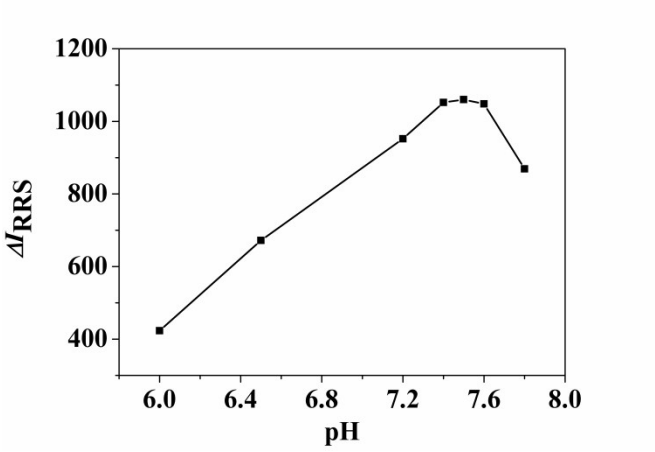

Fig. S4 Effect of pH

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, ΔI_{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 scatting intensity would be reduced, because the reagent blank would enlarge. Therefore, we choose 4.8 nM as a suitable probe concentration in the experiment.

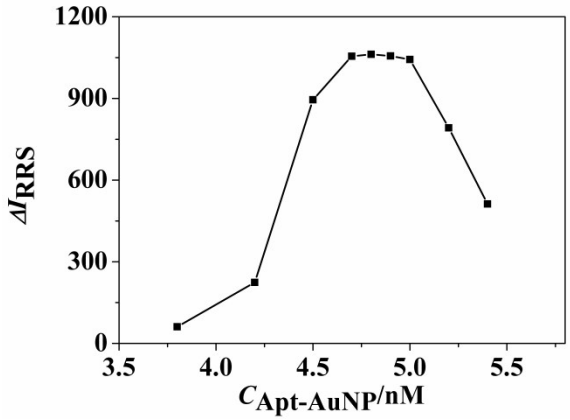

Fig. S5 Effect of Apt-AuNPs concentration

Concentration of EGFR: 80 ng·mL^{-1}; pH7.4; 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 ΔI_{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.

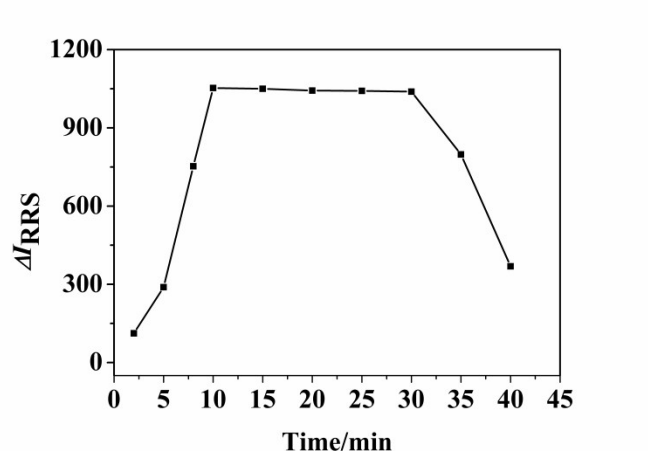

Fig. S6 Effects of reaction time

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

The effect of temperature on the recognition reaction is shown in Figure S7. The ΔI_{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.

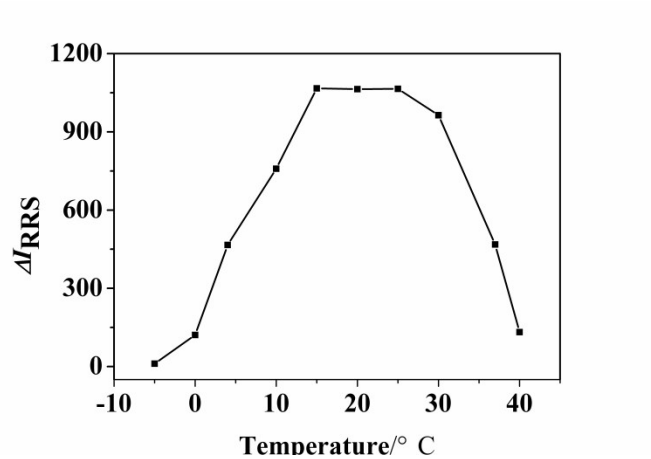

Fig. S7 Effect of reaction temperature

Concentration of Apt-AuNP: 4.8 nM; Concentration of EGFR: 80 $ng \cdot mL^{-1}$; pH7.4; reaction time: 10 min

Comparison of the method used for EGFR determination

Table S1 Comparison of the method used for EGFR determination

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

Reference

1 J. Gao, X. Huang, H. Liu, F. Zan and J. Ren, Langmuir, 2012, **28**, 4464-4471.

2 W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig, Analytical Chemistry, 2007, **79**, 4215-4221.

3 J. C. Chen, S. Sadhasivam and F. H. Lin, Process Biochem., 2011, **46**, 543-550.

4 H. Ilkhani, M. Sarparast, A. Noori, S. Z. Bathaie and M. F. Mousavi, Biosens. Bioelectron., 2015, **74**, 491-497.

5 D. Zhang, F. Ma, Q. Zhang and C. Y. Zhang, Chem. Comm., 2017, **53**, 11496-11499.

6 M. Regiart, M. A. Fernandez-Baldo, J. Villarroel-Rocha, G. A. Messina, F. A. Bertolino, K. Sapag, A. T. Timperman and J. Raba, Anal. Chim. Acta, 2017, **963**, 83-92.

7 P. I. Reyes, C. J. Ku, Z. Duan, Y. Lu, A. Solanki and K. B. Lee, Appl. Phys. Lett., 2011, **98**, 173702.

8 Z. Altintas, S. S. Kallempudi, and Y. Gurbuz, Talanta, 2014, **118**, 270-276.