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A facile scanometric strategy for ultrasensitive detection of protein using aptamer-initiated rolling circle amplification

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Experimental details

Materials. Recombinant human vascular endothelial growth factor 165 (VEGF) was purchased from Bio Basic Inc. (Canada). Phi29 DNA polymerase, T4 DNA ligase, Exonuclease III, *E.coli* (ExoIII), Exonuclease I, *E.coli* (ExoI) and dNTP mixture were obtained from Fermentas (Lithuania). Salmon sperm DNA, bovine serum albumin (BSA), 3-glycidoxypropyltrimethoxysilane (GPTMS), bis(p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BSPP), and silver enhancer solutions A and B were purchased from Sigma-Aldrich (USA). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). Sodium borohydride ($NaBH_4$) was obtained from Sinopharm Chemical Reagent Co. Ltd (China). All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water (≥ 18 MΩ, Milli-Q, Millipore).

The oligonucleotides with the following sequences were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China):

Aptamer for VEGF: 5'-NH_2-$(CH_2)_6$-AAA AAA AAA CCG TCT TCC AGA CAA GAG TGC AGG G-3'

Circular template: 5'-p-TCT GGA AGA CGG ACA ACA TGA AGA TTG TAG GTC AGA ACT CAC CTG TTA GAA ACT GTG AAG ATC GCT TCC CTG CAC TCT TG-3'

thiolated DNA probe: 5'-ACT GTG AAG ATC GCT -$(CH_2)_3$-thiol-3'

Apparatus. The UV-vis absorption spectrum was recorded with an UV-3600 UV-vis-NIR spectrophotometer (Shimadzu, Japan). The TEM image was observed under a JEM-2100 transmission electron microscope (JEOL Ltd, Japan). Scanometric images were obtained with a flatbed scanner (HP scanjet 2400, Hewlett-Packard).

Preparation of Au nanoparticle probe. 3.5 nm Au nanoparticles (AuNPs) were synthesized according to the method reported previously.[1] Typically, 0.6 mL of ice-cold 0.1 M $NaBH_4$ was added into 20 mL aqueous solution containing 0.25 mM $HAuCl_4$ and 0.25 mM trisodium citrate with stirring. The orange-red solution was stirred for 1 h to obtain AuNPs solution, which was stored at 4 °C.

1 mg BSPP was added to the AuNPs solution (4 mL), and the mixture was stirred overnight. The AuNPs were then subjected to ultrafiltration using Vivaspin concentrator (Sartorius, 10,000 MW) at 10,000 g for 10 min to remove excessive BSPP. The upper phase was washed twice with pH 7.4 PBS, and dissolved in 500 μL PBS to obtain a solution of BSPP-protected AuNPs. 100 μL of 10 μM thiolated DNA probe was then added to the solution and left overnight under shaking. The resulting mixture was ultrafiltrated using Vivaspin concentrator (10,000 MW) at 10,000 g for 10 min at 4 °C to remove the non-conjugated DNA. The upper phase was washed thrice with PBS by ultrafiltration. The obtained AuNP probe was dissolved in 500 μL PBS and kept at 4 °C. Prior to use, the AuNP probe was blocked with 0.5% BSA, 50 μg mL^{-1} salmon sperm DNA and 0.025% Tween 20 at 4 °C for 1 h.

Preparation of circular DNA. 10 μM of circular template oligonucleotide and 10 μM of aptamer oligonucleotide were mixed in 100 μL of ligation buffer (50 mM pH 7.5 Tris-HCl buffer, 10 mM $MgCl_2$, 10 mM dithiothreitol and 0.5 mM ATP), and incubated at 37 °C for 30 min. Then 160 units of T4 DNA ligase was added and incubated at 22 °C for 1 h. After ligation, T4 DNA ligase was inactivated by heating the reaction mixture at 65 °C for 10 min. Then the exonucleases, 400 U ExoI and 2000 U ExoIII, were

applied to digest the residue linear DNA at 37 °C for 50 min.[2] The circular DNA was extracted with phenol/chloroform (1:1) and chloroform, respectively, and the product was precipitated with ethanol, which was finally dissolved in 100 μL double distilled H_2O and stored at -20 °C.[3]

Preparation of aptamer chip. A glass slide (25 mm × 75 mm × 1 mm) was firstly treated with piranha solution (30% hydrogen peroxide and 70% sulfuric acid) for 24 h, and silylanized by dipping it in toluene solution of 1% GPTMS for 24 h at room temperature to obtain epoxy-coated slide.[4]

5 μL of 50 nM aptamer solution was dropped on the epoxy-coated slide at a defined location, respectively, to form a droplet array of aptamer, which was then incubated at room temperature for 3 h in a humidified chamber. After the slide was thrice washed with ultrapure water and the remaining active epoxy groups were reduced with sodium borohydride (1 mg in 4 mL 25% ethanol), the resulting aptamer chip was twice rinsed with ultrapure water and used for following operation.

Protein detection protocol. Prior to use, the aptamer chip was washed in boiling water for 30 s. 5 μL of 100 nM circular DNA in pH 7.4 PBS containing 1% BSA and 100 μg mL^{-1} salmon sperm DNA was carefully added onto each aptamer spot and incubated at 37 °C for 1 h in a humidified chamber. The resulting slide was washed thrice with washing buffer I (50 mM pH 7.5 Tris-HCl buffer and 0.05% Tween 20), and twice rinsed with washing buffer II (50 mM pH 7.5 Tris-HCl buffer). Then 5 μL of VEGF solutions at different concentrations were added on the defined spots and incubated for 1.5 h at 37 °C in a humidified chamber to displace the circular DNA. After the slide was washed thrice with washing buffer I, and twice rinsed with washing buffer II, rolling circle amplification was initiated by dropping 5 μL reaction buffer containing 0.5 units of phi29 DNA polymerase, 50 mM pH 7.5 Tris-HCl buffer, 10 mM magnesium acetate, 33 mM potassium acetate, 1 mM dithiothreitol and 10 mM dNTP on each defined spot, and continued for 1 h at 37 °C in a humidified chamber. After the washing steps, 5 μL of AuNP probe was added to each spot and hybridized at 37 °C for 30 min in a humidified chamber. The slide was carefully washed thrice with washing buffer I, twice rinsed with washing buffer II to remove the non-tagged AuNP probe, and dried under a stream of nitrogen. Finally, the sliver enhancement was preformed on each spot by reaction with 5 μL 1:1 mixture of silver enhancer solutions A and B for 4 min.

After rinsed with ultrapure water and dried under a stream of nitrogen, the resulting slide was scanned with a flatbed scanner.

Absorption spectrum of AuNP probe

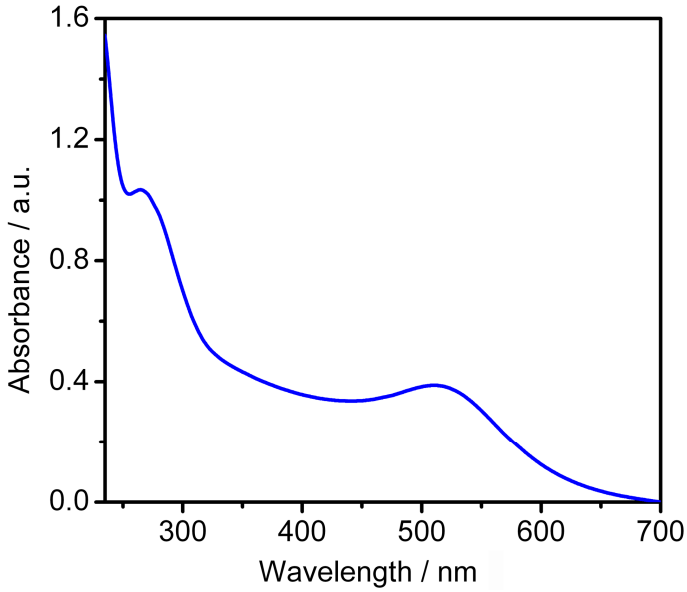

Fig. S1. *UV-vis absorption spectrum of AuNP probes*

TEM image of AuNPs labeled on RCA products

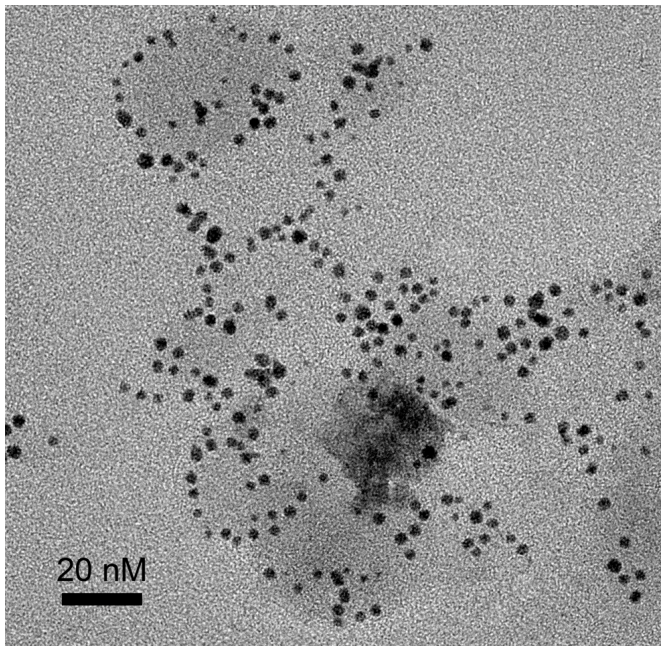

Fig. S2*. TEM image of AuNPs labeled on RCA products.*

References

1. A. Gole and C. J. Murphy, *Chem. Mater*., 2004, **16**, 3633-3640.

2. N. Li, C. Jablonowski, H. Jin and W. Zhong, *Anal. Chem*., 2009, **81**, 4906-4913.

3. Y. Y. Huang, H. Y. Hsu and C. C. Huang, *Biosens. Bioelectron*., 2007, **22**, 980-985.

4. Z. J. Yang, H. Liu, C. Zong, F. Yan and H. X. Ju, *Anal. Chem.*, 2009, **81**, 5484-5489.