Electronic Supplementary Material (ESI) for Chemical Communications

Reusable plasmonic aptasensor: Using a single nanoparticle to establish a calibration curve and to detect analytes

Longhua Guo and Dong-Hwan Kim*

School of Chemical and Biomedical Engineering, Nanyang Technological University, 637457, Singapore

Experimental section

Materials. Thrombin-binding aptamer (TBA, 5' - C6- S-S-$(T)_5$-GGT TGG TGT GGT TGG-3') was synthesized by Sigma (Singapore). Human α-thrombin and tris[2-carboxyethyl] phosphine (TCEP) were supplied by Merk Pte. Ltd. Human serum (from human male AB plasma, sterile-filtered), human serum albumin (HSA), 6-mercapto-1-hexanol, hexadecyltrimethylammonium bromide (CTAB), gold(III) chloride trihydrate (99.9%), sodium Borohydride, silver nitrate and ascorbic acid were purchased from Sigma-Aldrich Pte. Ltd. (Singapore). The 10× tris-Acetate-EDTA (TAE, pH 8.0) was provided by Vivantis Ltd. (UK). Glass cover slips (22 mm × 22 mm, no. 1.5) and microscope glass slides (26 mm × 76 mm, thickness 1.0-1.2 mm) were obtained from Thermo Fisher Scientific Inc. (USA).

Buffer solutions used in this study were as follows; 1× TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH 8.0) was used for the storage of the aptamer-modified AuNRs 2D chip, thrombin binding buffer consisted of 50 mM Tris-HCl (pH 7.4), 140 mM NaCl, 1 mM $MgCl_2$ and 0.1% BSA.

Darkfield microscope system. An Olympus IX71 inverted microscope employing an oil immersion ultra-darkfield condenser (IX-ADUCD U-DCW, NA 1.2-1.4, Olympus) and a 100× Plan Semi Apochromat objective (UPLFLN100×OI, NA 1.3-0.6, Olympus) were used for

plasmonic scattering detection. Illumination was provided by an integrated 100 W halogen source (IX2-ILL100, Olympus). A two-way TV adapter with a selectable output was connected to the microscope's camera port, which allowed the field of view to be imaged by either a color digital camera (DS-Fi1-U2, Nikon) or a line-imaging spectrometer (SpectraPro2150i, Princeton). In the detection plane of the spectrometer was a PIXIS 100F cooled CCD camera. A motorized stage with 50-nm step-size linear encoders (Ludl Flat-top Inverted Stage 96S106-N3-LE2, BioVision Technologies) was used as the sample stage to provide a programmable scanning platform. The detailed set-up of this optical system has been previously reported.[1]

Au nanorods synthesis. Gold nanorods were chemically synthesized by a seed-mediated growth procedure, as reported previously.[2, 3] Briefly, the seed solution was prepared by adding a freshly prepared, ice-cold aqueous NaBH4 solution (0.01 M, 600 µL) to an aqueous mixture solution of CTAB (0.1 M, 7.5 mL) and $HAuCl_4$ (0.01 M, 0.25 mL). Vigorous stirring was applied to the mixture solution for 2 min, and then the solution was kept at 25 °C for at least 10 min before it was used. The growth solution was made by first mixing aqueous solutions of CTAB (0.2 M 50 mL), silver nitrate (0.01 M 0.6 mL), $HAuCl_4$ (0.01 M, 5 mL) and distilled water (42.2 ml). Then, a freshly prepared aqueous ascorbic acid solution (0.01 M, 5.5 mL) was added. After the resulting solution was mixed by inversion, 50 µL of seed solution was added. The mixture was again vigorously stirred for 20 s and then allowed to sit overnight, resulting in a purple-colored suspension of AuNRs. The nanorods used in this study were 50.01 ± 5.27 nm in length and 18.15 ± 2.17 nm in diameter (n = 150), as measured by FE-SEM (JEOL JSM-6701F, see Figure S1).

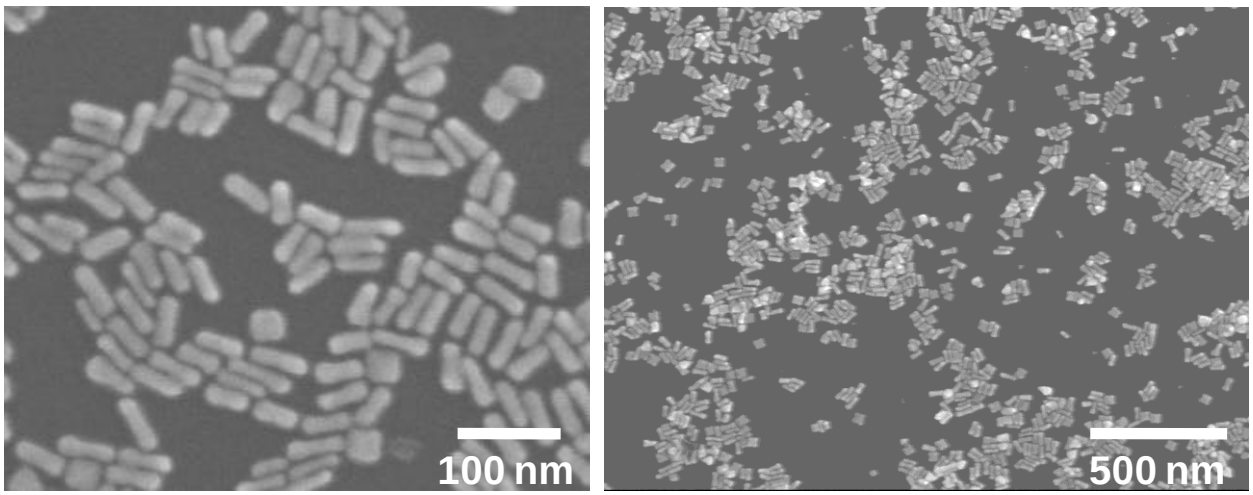

Figure S1 SEM images of AuNRs used in this work

AuNRs immobilization. Au nanorods were immobilized on 22 mm × 22 mm square microscope cover slips (no. 1.5, Thermo Fisher Scientific Inc., US) via a simple two-step procedure previously developed.[4] Briefly, the cover slips were first cleaned in a so-called 'piranha' bath (i.e., 30% H_2O_2 mixed in a 1:3 ratio with concentrated H_2SO_4) at 60 °C for 10 min (***Warning:*** *The piranha solution is very corrosive and must be handled with extreme caution; it reacts violently with organic materials.*) and then thoroughly rinsed with water. The cleaned cover slips were then incubated in a 30-fold diluted Au nanorod suspension with 0.00625 M CTAB for 5 min. The cover slips were finally sonicated for 2 min in water and ethanol. AuNR chips fabricated according to this approach have an average inter-particle distance of approximately 3 μm, and the robustness of AuNR immobilization was shown to be stable enough for bioanalysis. For chemical modification and target molecule detection, the AuNR 2D chip was mounted on a homemade, disposable flow cell, as previously reported.[4]

Preparation of aptamer-based protein sensing interface. Before the chemical modification of the AuNRs with thrombin binding aptamer (TBA), thiol groups in the aptamer were activated, as previously reported.[5] Briefly, the 5'-thiol aptamer (0.1 mM) was deprotected

by 0.l mM tris(2-carboxyethyl)phosphine (TCEP) in 50 mM acetate buffer (pH 5.2) for 1 h at room temperature. The deprotected aptamer was then diluted to 1 μM with deionized water and injected into the flow cell containing the AuNRs-modified 2D chip. The flow cell was kept in a dark drawer at room temperature overnight. After aptamer modification, the chip was rinsed with water, and then 100 μM 6-mercaptohexanol was injected into the flow cell and incubated for another 3 h to ensure full surface coverage of AuNRs so that nonspecific protein adsorption would be suppressed. The aptamer-modified glass chip was rinsed thoroughly with deionized water and incubated in 1× TEA buffer at 4 °C before use.

Binding measurement. Protein stock solutions were diluted with thrombin binding buffer to the appropriate concentration. Before protein detection, binding buffer was injected into the flow cell and incubated for 15 min. Then, protein solutions at specific concentrations were injected into the flow cell and incubated for 30 min. LSPR scattering spectra both before and after protein binding were recorded. Peak-fitting based on a Lorentzian algorithm was used for data processing by using OriginPro 8.0 for the accurate and efficient determination of λ_{max}, and the corresponding wavelength shift ($\Delta\lambda_{max}$) was calculated.

In order to test the performance of the sensor in complex matrices, standard solutions of thrombin were added to male human serum (diluted 1:1 with binding buffer). These spiked samples were tested and compared with results from samples in pure binding buffer.

Regeneration of aptamer. After each cycle of protein detection, the flow cell was rinsed with 2 M NaCl for 5 min and then rinsed with distilled water. It has been reported that aptamer would readily unfold its three-dimensional structure without damaging the oligomer structure at high ionic strength.[6, 7] Therefore, after this treatment, the bound protein is released from the

aptamer. Refilling the flow cell with binding buffer could easily regenerate the three-dimensional-structured aptamer.

Table S1

Table S1. Dynamic ranges and limits of detection for eight particles with different LSPR wavelengths

Particle number	Central wavelength (nm)	Dynamic range (g/ml)	LOD (ng/ml)*
1	682	3.0×10^{-9} to 1.0×10^{-5}	1.50
2	700	3.0×10^{-9} to 1.0×10^{-5}	1.20
3	722	3.0×10^{-9} to 1.0×10^{-5}	1.00
4	736	1.0×10^{-9} to 3.0×10^{-6}	0.80
5	745	1.0×10^{-9} to 3.0×10^{-6}	0.75
6	758	1.0×10^{-9} to 3.0×10^{-6}	0.63
7	779	1.0×10^{-9} to 3.0×10^{-6}	0.25
8	795	1.0×10^{-9} to 3.0×10^{-6}	0.22

*LODs were experimentally obtained based on S/N of 3.

Table S2

Table S2. Repeatability within a particle and among different particles for the detection of a standard solution containing 50.0 nM thrombin [a)]

Particle	Concentration found (nM)								RSD (%) [b)]
1	53.7	51.9	48.2	53.8	49.7	49.6	48.1	51.8	3.95
2	51.6	51.3	47.6	53.7	48	49.3	50.2	47.8	4.35
3	49.5	52.6	52.1	48.3	49.6	50.7	50.2	49.1	2.94
4	50.3	50.6	48.3	47.2	47.7	49.2	51.2	50.9	3.13
5	49.7	46.2	48.2	53.1	49.7	48.9	47.8	49.3	4.05
6	53.4	51.7	48.6	49.5	48.7	47.2	50.2	49.6	3.88
7	50.1	52.3	48.8	47.9	53.1	52.9	47.6	49.1	4.47
8	50.2	50.6	51.3	49.8	47.8	49.6	53.7	51.3	3.36
RSD (%) [c)]	3.25	3.99	3.32	4.22	3.57	3.28	4.10	3.24	

[a] Binding buffer: 50 mM Tris-HCl (pH 7.4), 140 mM NaCl, 1 mM $MgCl_2$ and 0.1% BSA; testing sample was prepared by spiking thrombin standard solution in binding buffer to a final concentration of 50 nM. [b] Relative standard deviation (RSD) within a same particle. [c] RSD among different particles.

Table S3

Table S3 Recovery of a standard thrombin added to human serum *

Added (ng/mL)	Found (ng/mL)	Recovery (%)
5	5.4±0.38	108.0
50	49.6±2.3	99.2
500	466.2±33	93.2

*Data were obtained from the same AuNR aptasensor with five replications for each concentration.

References

1. Guo, L., Ferhan, A. R., LEE, K. & Kim, D. H. Nanoarray-based Biomolecular Detection Using Individual Au-Nanoparticles with Minimized Localized Surface Plasmon Resonance Variations. Anal. Chem. 83, 2605-2612 (2011).
2. Jana, N. R., Gearheart, L. & Murphy, C. J. Seed-mediated growth approach for shape-controlled synthesis of spheroidal and rod-like gold nanoparticles using a surfactant template. Adv. Mater. 13, 1389-1393 (2001).
3. Nikoobakht, B. & El-Sayed, M. A. Preparation and growth mechanism of gold nanorods (NRs) using seed-mediated growth method. Chem. Mater. 15, 1957-1962 (2003).
4. Guo, L., Zhou, X. & Kim, D. H. Facile fabrication of distance-tunable Au-nanorod chips for single-nanoparticle plasmonic biosensors. Biosens. Bioelectron. 26, 2246-2251 (2011).
5. Liu, J. & Lu, Y. Preparation of aptamer-linked gold nanoparticle purple aggregates for colorimetric sensing of analytes. Nat. Protoc 1, 246–252 (2006).
6. Bini, A., Minunni, M., Tombelli, S., Centi, S. & Mascini, M. Analytical performances of aptamer-based sensing for thrombin detection. Anal. Chem 79, 3016-3019 (2007).
7. Baldrich, E., Restrepo, A. & O'Sullivan, C. K. Aptasensor development: elucidation of critical parameters for optimal aptamer performance. Anal. Chem 76, 7053-7063 (2004).