SUPPORTING INFORMATION

Tunable plasmonic colorimetric assay with inverse sensitivity for extracellular DNA quantification

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Methods

Chemicals. The following products were used as received. Cetyltrimethylammonium bromide (CTAB, > 98%) was purchased from Tokyo Chemical Industry, Singapore. Sodium borohydride ($NaBH_4$, 98%), L-ascorbic acid (crystalline), hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$, 0.1 N) and hydrogen chloride (HCl, 37% wt in water) and nitric acid (HNO_3) were purchased from Sigma-Aldrich, Singapore. The oligonucleotides used in this study were purchased from Integrated DNA Technologies, Singapore. The dsDNA molecules (sequence in Table S1) were obtained by the hybridization of the sense and antisense ssDNA, where the solutions containing both strands were annealed at 95 °C for 5 min and cooled down for 4 h in 10 mM Tris-HCl buffer (pH 7.4). All the water used in the experiments described in this article was obtained from a Mili-Q Integral 5 system.

Instrumentation. Transmission electron microscopy (TEM) images were acquired with a Philips CM300 FEG TEM operating at 300 kV. The UV-Vis spectra of the solutions were recorded with a Synergy 2 Multi-Mode Reader spectrophotometer from BioTek Instruments, Inc. Gold concentrations were quantified with inductively coupled mass spectrometry (Thermo Fisher Scientific™, iCAP™ Q). The AuNR solutions were digested prior quantification by mixing 10 μL of nanorods with 100 μL of HCl and 100 μL of HNO_3 for 1 h. The digested solutions were finally diluted with 4.79 mL of Mili-Q H_2O.

Synthesis of Gold Nanorods. The gold nanorods (AuNRs) were synthesized through a seed-mediated method. Briefly, gold seed solution was prepared by adding at once 0.6 mL of freshly prepared $NaBH_4$ (10 mM) to a 10.0 mL solution of CTAB (0.1 M) and $HAuCl_4$ (0.25 mM) while being vigorously stirred. The solution was stirred for an additional 30 s and left undisturbed for 45 min.

AuNRs with aspect ratio (AR) of 2.0, 2.7, 3.3 and 3.8 were synthesized by adding 50, 100, 150 and 200 μL of $AgNO_3$ (4 mM), respectively, into 5.0 mL solutions of CTAB (0.1 M). The resulting solutions were stirred for 30 s, and 5.0 mL of $HAuCl_4$ (1 mM) and 12 μL of HCl (37%) were added. After a gentle stirring, ascorbic acid (75 μL, 79 mM) was introduced. The mixtures were vigorously stirred for 30 s and 60 μL of the seed solution were added. Finally, the growth solutions were vigorously stirred for 30 s and left undisturbed for 12 h. The AuNRs were washed twice by centrifugation (7000 rpm for 15 min followed by removal of the supernatant). The final precipitates were diluted in 20 mL of water before used.

Colorimetric Assay for DNA Quantification. The colorimetric assay was performed after preparing oligonucleotide solutions (ssDNA or dsDNA) with different concentrations in 8 mM Tris-HCl buffer (pH 7.4). 25 μL of those solutions were added into 75 μL of as-prepared AuNRs. The final concentrations of oligonucleotides ranged from 0 to 100 nM. Rapid changes of color were observed immediately after the addition of DNA caused by the fast AuNR aggregation. The mixtures were left incubating at room temperature for 10 min before the UV-Vis spectra were recorded.

Table S1. ssDNA sequences used in the colorimetric assays. For dsDNA preparation the sense strands were hybridized with their complementary antisense.

DNA length	Sequence (5' to 3')
44 bases	GGG ATG ACA CAG GTC ACT GTG ACC T GCC CCA GGC ACC GGG ACC T
59 bases	GGG ATG ACA CAG GTC ACT GTG ACC ACC TGA GT CC GTG AGT GCCCCAGGC ACC GGG ACC T
74 bases	GGG ATG ACA CAG GTC ACT GTG ACC ACC TGA GTC ACA CGC C TGT GAG GCC GTG AGT GCCCCAGGC ACC GGG ACC T

Description of Analytical Concepts

1. *Limit of detection* (LOD) is defined as the smallest concentration whose intensity fulfils the following equation:

$$I_{LOD} = I_0 + 3 \cdot \sigma_0$$

where I_{LOD} and I_0 are the signal intensities at the LOD and blank, and σ_0 is the standard deviation of the blank.

2. *Inverse sensitive limit* (ISL) is the lowest concentration on the inverse sensitivity regime.

3. *Signal-to-noise ratio* at ISL (S/R_{ISL}) is defined as the signal intensity at ISL (I_{ISL}) divided by the standard deviation at ISL (σ_{ISL}):

$$S/R_{ISL} = \frac{I_{ISL}}{\sigma_{ISL}}$$

Table S2. Dynamic ranges and LOD of different AuNR solutions compared with literature values of eDNA from different waterbodies.

AuNR dilution ratio	Dynamic range of dsDNA concentrations detected ng/ml (nM)	eDNA concentrations reported in literature ng/ml	Sample origin
1/1	383.5 – 3835 (1.0 – 100)	568 – 3163	Sea water particulates[1]
1/2	95.9 – 3835 (2.5 – 100)		
1/4	38.4 – 958.8 (1 – 25)	88	Hypereutrophic water[2]
1/5	19.2 – 95.9 (0.5 – 2.5)	13.4 – 80.6	Sea water[3]

Table S3. Dimensions and optical properties of AuNRs used in the colorimetric assays.

Aspect ratio (AR)	**Length (nm)**	**Width (nm)**	**LSP_{max} (nm)**
2.0 ± 0.6	32.1 ± 6.7	16.6 ± 3.1	605
2.7 ± 0.5	38.4 ± 7.1	14.3 ± 1.9	705
3.3 ± 0.6	40.3 ± 9.1	12.3 ± 1.3	805
3.8 ± 0.6	49.8 ± 7.9	13.2 ± 1.6	840

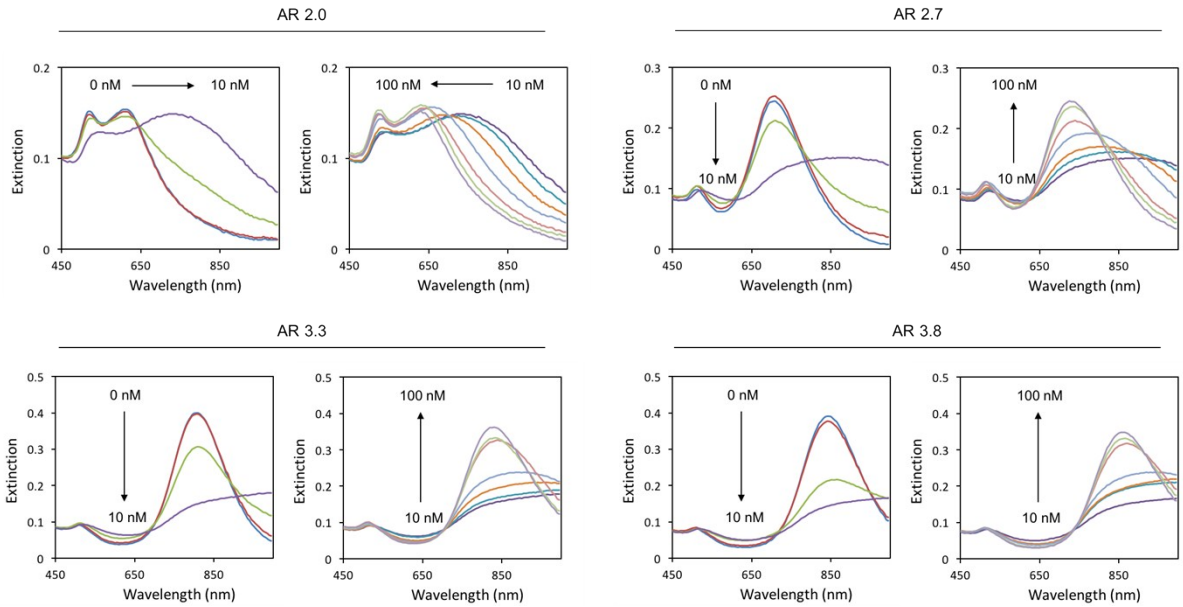

Figure S1. UV-Vis spectra of AuNRs with AR of 2.0, 2.7, 3.3 or 3.8 in the presence of 0, 1, 5, 10, 15, 20, 25, 50, 75 and 100 nM 59 base pair dsDNA.

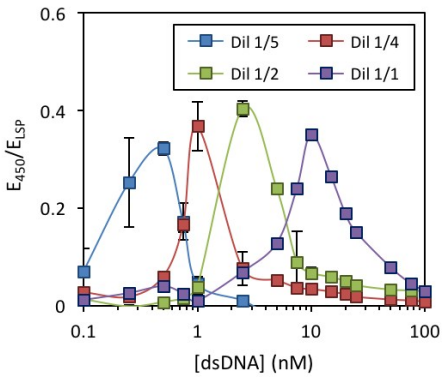

Figure S2. Response curve of four different AuNR solutions as function of dsDNA concentration. The original AuNR solution (purple) used in Figure 3 is noted as dilution ratio 1/1. As the dilution ratio increases the dynamic range and LOD of the assay shift to lower dsDNA concentrations.

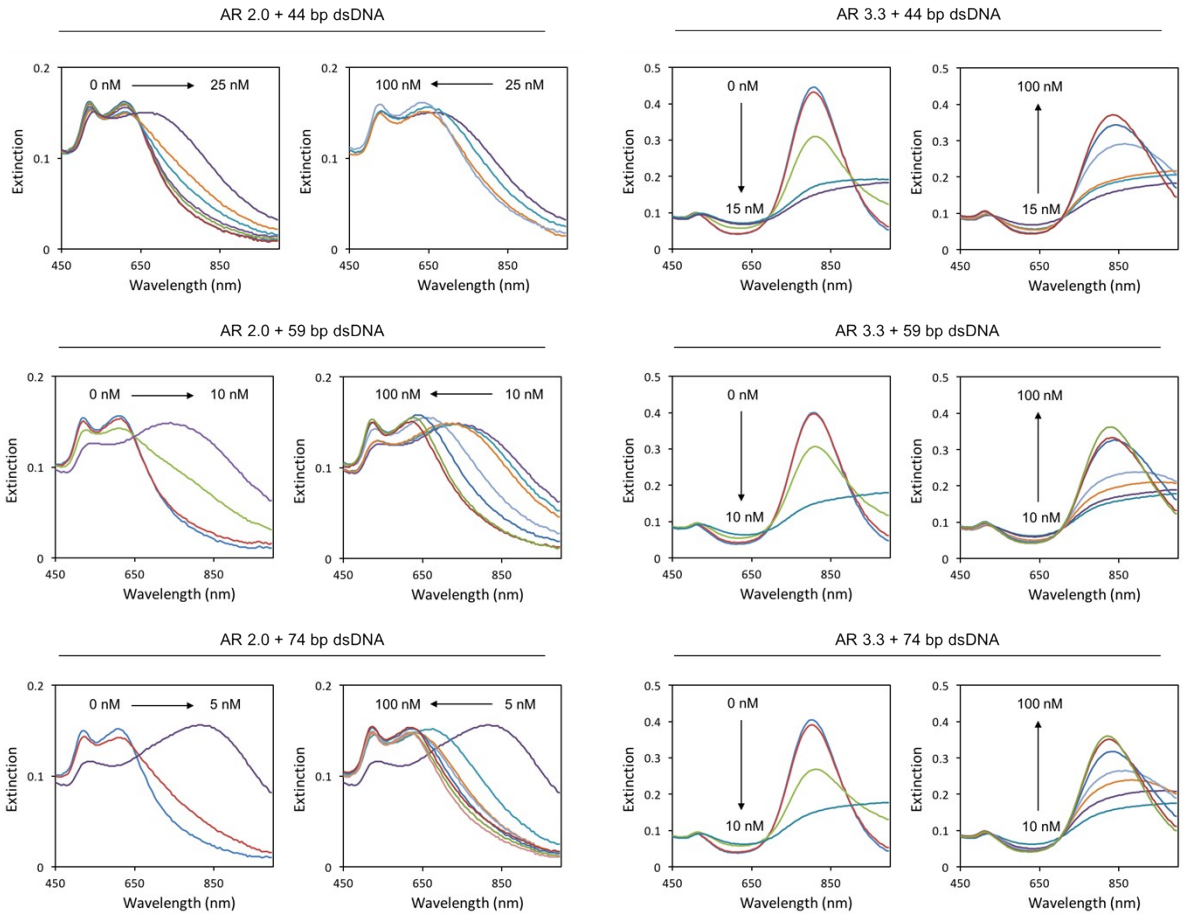

Figure S3. UV-Vis spectra of AuNRs with AR of 2.0 or 3.3 in the presence of 0, 1, 5, 10, 15, 20, 25, 50, 75 and 100 nM 44, 59 or 74 base pair dsDNA.

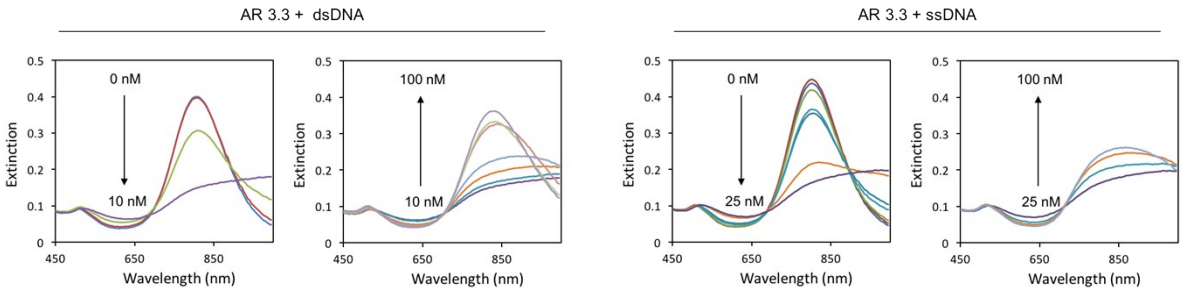

Figure S4. UV-Vis spectra of AuNRs with AR of 3.3 in the presence of 0, 1, 5, 10, 15, 20, 25, 50, 75 and 100 nM 59 base pair dsDNA or 59 base ssDNA.

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