Supporting Information

Plasmonic nanosensor with inverse sensitivity for circulating DNA quantification

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1. Material and Methods

1.1. Materials

In the series of experiments that are presented in this article, the following products were used as received. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$, 0.1 N), hydrogen chloride (HCl, 37% wt in water), L-ascorbic acid, sodium borohydride ($NaBH_4$, 98%) were purchased from Sigma-Aldrich. Hexadecyltrimethylammonium bromide (CTAB, >98%) was purchased from Tokyo Chemical Industry. The oligos used in this study were purchased from Integrated DNA Technologies. Table 1 shows their sequences. For the ssDNAs hybridization, the sense and antisense strands were annealed at 95 ºC for 5 min and cooled down for 4 h in 10 mM Tris-HCl buffer (pH 7.4), yielding their corresponding dsDNA. All the water employed in the experiments was obtained with a Mili-Q Integral 5 system.

Table S1. Oligonucleotides with estrogen receptor element sequences

Name	*Sequence 5' to 3'*
180-bp ssDNA	CTGGCACTGCCCCGCCCCACCCCTGACTTGCCAGTGAGTCCCAGACAGGCTGGCGG GATGACACAGGTCACTGTGACCACCTGAGTCACACGCCGTCACTGTGAGGCCGTGA GTGCCCCAGGCACCGGGACCTGGGGACTGTGCTCTGCGGCCTGTGTACCCCACAGA ACCGGTTCCTTG
180-bp ssDNA-rev	CAAGGAACCGGTTCTGTGGGGTACACAGGCCGCAGAGCACAGTCCCCAGGTCCCGG TGCCTGGGGCACTCACGGCCTCACAGTGACGGCGTGTGACTCAGGTGGTCACAGTG ACCTGTGTCATCCCGCCAGCCTGTCTGGGACTCACTGGCAAGTCAGGGGTGGGGCG GGGCAGTGCCAG

1.2. Characterization

Transmission electron microscopy (TEM) images were acquired with a Philips CM300 FEG TEM operating at 300 kV. The optical extinction spectra were recorded using a Synergy 2 Multi-Mode Reader spectrophotometer from BioTek Instruments, Inc. The fluorescence spectra were obtained by an InfiniteM200 from Tecan. The dynamic light scattering (DLS) measurements were performed with a DynaPro PlateReader-II from Wyatt Technology Corporation. The nanoparticle zeta-potentials were recorded with a Zetasizer Nano Z from Malvern Instruments.

1.3. Synthesis of AuNRs

The AuNRs were synthesized via seed-mediated method. Briefly, *Au seeds* were obtained by adding at once 0.6 mL ice-cold $NaBH_4$ (10mM) into a 10 mL solution of CTAB (0.1M) and $HAuCl_4$ (0.25 mM) while vigorously stirred. The solution was stirred for 30 s and left undisturbed for 30 min.

Au NRs were synthesized by adding 250 μL of $AgNO_3$ (4 mM) into 5.0 mL solution of CTAB (0.1 M). The solution was kept undisturbed for 15 min, after which 5 mL of $HAuCl_4$ (1 mM) and 12 μL of HCl (37%) were added. After slow stirring, ascorbic acid (75 μL, 79 mM) was introduced. The mixture was vigorously stirred for 30 sec and 60 μL of the seed solution were added. Finally, the growth solution was vigorously stirred for 30 sec and left undisturbed for 12 h. The Au NRs were isolated by centrifugation twice at 7000 rpm for 15 min followed by removal of the supernatant. The final precipitate was diluted in 20 mL of water before used.

1.4. Colorimetric detection of dsDNA

The assay was performed after preparing dsDNA solutions 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 Au NRs. The final concentrations of dsDNA ranged from 0 to 100 nM. The mixtures were incubated at room temperature for 10 min and the UV-Vis spectra were recorded afterwards.

1.5. Fluorescence assays

dsDNA was incubated with thiazole orange (TO) in a proportion of 1:40 in Tris 10 mM buffer at room temperature for 30 min. Saturation of dsDNA with TO is achieved at ratio of 1 dye to 2 base pairs.[1] Because we wanted to make sure that all TO was bound to dsDNA, a ratio of 1 dsDNA to 40 TO was selected (1 dye to 4.5 base pairs). The fluorescence of several dilutions, ranging from 0 to 50 nM, was measured in the absence and presence of AuNRs (OD_{890} = 0.48).

2. Comparison between common and inverse sensitivity sensors

Fig. S1 shows the schemes of this inverse sensitivity concept (Fig. 1A) versus the traditional response concepts, i.e. inversely proportional (Fig. 1B) and directly proportional (Fig. 1C) responses. The inverse sensitivity sensors present the highest signal in their LOD. Therefore, the response intensity (I_1) at low concentrations (close to their LOD) is much higher than the background intensity (I_0), unlike in conventional sensors, where I_1 and I_0 have similar intensities. This analytical strategy enables a higher reliability for low concentration analyte detection by having a higher signal-to-noise ratio (SNR).

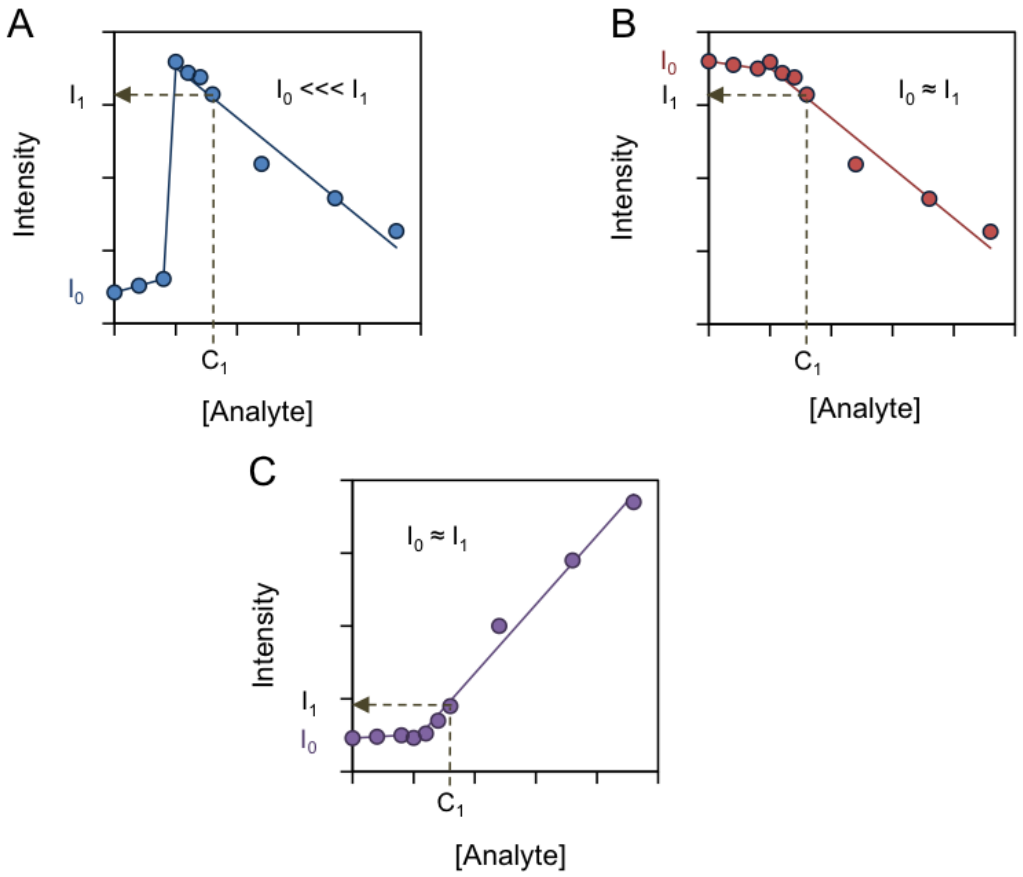

Fig. S1 Scheme of the response curves of sensors with (A) inverse sensitivity, (B) inversely proportional response and (C) directly proportional response. This scheme has been drawn for clarification purposes and it has not been made from real data.

3. Excitation and Emission spectra of dsDNA-TO_{40}

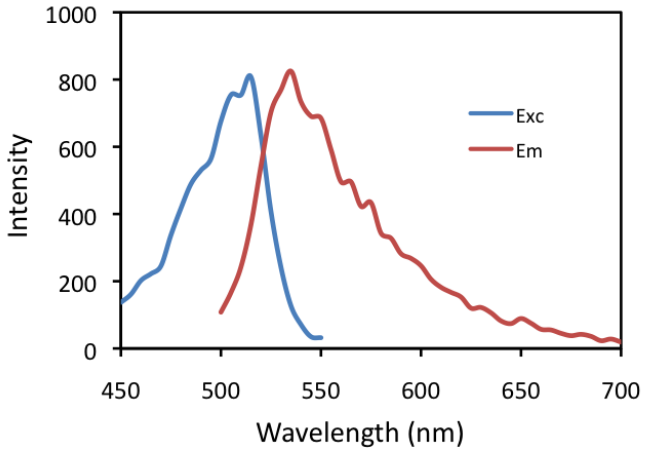

Fig. S2 Excitation and emission spectra of dsDNA-TO_{40} (5 nM). Excitation wavelength: 450 nM. Emission wavelength: 600 nm.

4. Performance summary of different AuNR solution sensors and reference cancer values

When the AuNR solution is added into the sample, the DNA concentration is diluted. Thus, the concentration values have to be corrected depending on the AuNR solution (75 µl) and the initial the sample volume. In Table S2 we present the results for an original sample volume of 25 µl.

Table S2 Dynamic ranges of different AuNR solutions and their equivalence for an original 25 µL sample. The dynamic ranges from an original 25 µL sample are compared with several reference values from cancer patients.

	Range of dsDNA Concentrations Detected in the Sensor Solution		*Range of dsDNA Concentrations Detected in 25 µl Sample*		*Reference Cancer Patient Values*		
[AuNR] (OD)	Min [dsDNA] ng/ml (*nM*)	Max [dsDNA] ng/ml (*nM*)	Min [dsDNA] ng/ml	Max [dsDNA] ng/ml	Cancer Type	Value ng/ml	Sample
0.93	1111.3 (*10*)	11112.6 (*100*)	4445.1	44450.6			
0.48	277.8 (*2.5*)	5556.3 (*50*)	1111.3	22225.3	Colorectal (stage I – II)[2]	1630 ± 430	Serum
					Colorectal (stage III – IV)[2]	1730 ± 450	Serum
0.22	111.1 (*1*)	555.6 (*5*)	444.5	2222.5	Melanoma (Metastasis)[3]	1056* (411 – 2021)	Serum
					Colorectal[4]	868* (22 – 3922)	Serum
					Lymphoma[5]	899* (171 – 2660)	Plasma
					Brest (stage III)[6]	589 ± 87	Serum
					Brest (stage IV)[6]	776 ± 271	Serum
0.08	55.6 (*0.5*)	222.3 (*2*)	222.3	889	Stomach[5]	593* (232 – 1111)	Plasma
					Lung[7]	318**	Plasma
					Gastrointestinal[8]	412 ± 63	Serum
0.05	22.2 (*0.2*)	222.3 (*2*)	88.9	889	Gastrointestinal (Benign)[8]	118 ± 14	Serum
					Melanoma (Metastasis)[3]	259* (83 – 604)	Plasma
					Mix[9]	180 ± 38	Serum

* Median

** Standard deviations or ranges are not provided.

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