Supporting Information

Plasmonic multi-logic gate platform based on sequence-specific binding of estrogen receptors and gold nanorods

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1. Material and Methods

1.1. Materials

In 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%), lysozyme from chicken egg white (90 %) and the oligos containing the wild-type ERE consensus sequence (wtERE, 5'-AGTAAGCTCCAGGTCATTATGACCTGGAGCTTACT-3') were purchased from Sigma-Aldrich. Hexadecyltrimethylammonium bromide (CTAB, >98%) was purchased from Tokyo Chemical Industry. Human recombinant estrogen receptor α and β (ERα and ERβ) were purchased from Life Technologies, Thermofisher Scientific. To form dsDNA, the sense and antisense strands were annealed at 95 ºC for 5 min and cooled down for 3 h in 10 mM Tris-HCl buffer (pH 7.0). All the water employed in the experiments was obtained with a Mili-Q Integral 5 system.

1.2. Characterization

Transmission electron microscopy (TEM) images were acquired with a FEI Titan TEMoperating at 200 kV. The optical extinction spectra were recorded using a Spectramax M2/M2^e UV/Vis/NIR spectrophotometer. The dynamic light scattering (DLS) and zeta potential measurements were recorded with a Zetasizer Nano Z from Malvern Instruments. pH was measured with an 827 pH lab from Metrohm.

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 (0.1 M) and $HAuCl_4$ (0.25 mM) while vigorously stirred. The solution was stirred for 30 s and left undisturbed for 60 min.

AuNRs 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 s and 60 μL of the seed solution were added. Finally, the growth solution was vigorously stirred for 30 s and left undisturbed for 12 h. The AuNRs were isolated by centrifugation twice at 8500 rpm for 15 min followed by removal of the supernatant.

1.4. Determination of metallic Au concentration

The concentration of metallic Au (Au^0) in solution was determined by the Edgar *et al.* method.[1] Briefly, a calibration curve at 400 nm was built by well-aging different CTAB stabilized AuNP solutions of known Au^0 concentration for a month. This ensured that the reduction of gold salts was fully reached and no size effect would interfere with the measures. Metallic gold presents inter-band transitions in the range from 350 to 450 nm.[2,3] Therefore, the absorbance within this range is relatively independent from the shape and it has been widely used in the past to quantify metallic gold.[1,4,5] A comparison between the results of this method and ICP-MS was performed by Edgar *et al.*,[1] presenting discrepancies below 20%.

1.5. AuNRs aggregation by dsDNA

dsDNA solutions with different concentrations were prepared in 10 mM Tris-HCL buffer (pH 7.0). 1 μL of those solutions was added into a solution made of 25 μL of AuNR (final Au^0 concentration of 257 μM) and 74 μL of DI water. The final dsDNA concentration in solution ranged from 0 to 100 nM. The mixtures were incubated at room temperature for 10 min and the UV-Visible spectra were registered.

1.6. Protein-dsDNA binding assays

Binding assay at low concentration regime. The binding assay was performed by incubating 25 μL of AuNRs (final Au^0 concentration of 257 μM) with 1 μL of dsDNA (final concentration 10 nM) in 70 μL of DI water at room temperature for 10 min. The resulting solutions were mixed with 4 μL of different diluted solutions of ERα, ERβ or lysozyme in 12.5 mM Tris-HCl buffer (pH 8.0, 9.0 and 8.0 respectively). The final protein concentrations ranged from 0 to 35 nM for ERα and lysozyme and from 0 to 70 nM for ERβ. The mixtures were incubated for another 40 min at room temperature before their characterization.

Binding assay at high concentration regime. The binding assay was performed by incubating 25 μL of AuNRs (final Au^0 concentration of 257 μM) with 1 μL of dsDNA (final concentration 75 nM) in 67.5 μL of DI water at room temperature for 10 min. The resulting solutions were mixed with 6.5 μL of different concentrated solutions of ERα or lysozyme in 50 mM Tris-HCl buffer (pH 8.0). The final concentrations of proteins ranged from 0 to 262.5 nM. The mixtures were incubated for another 40 min at room temperature before their characterization.

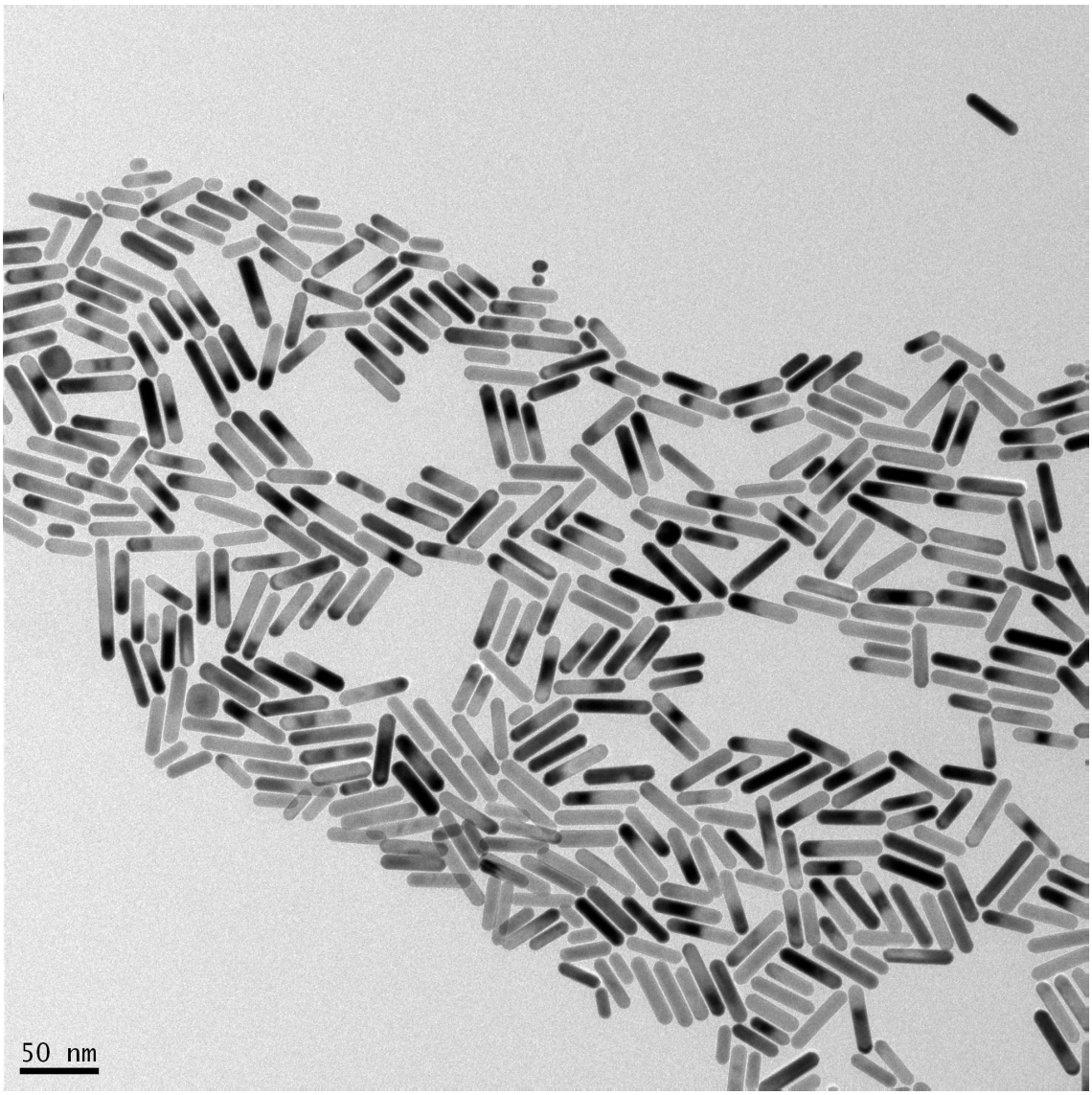

Fig. S1 TEM image of AuNRs used in the protein-DNA binding assays. AuNR dimensions are 42.6 (±10.0) × 10.4 (±1.2) nm with aspect ratio of 4.1 (±0.8).

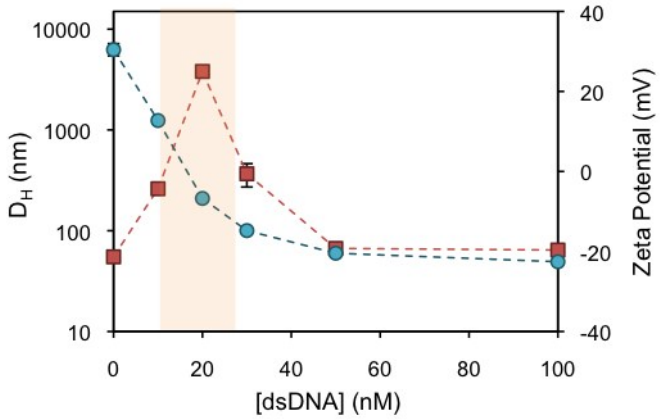

Fig. S2 (Red squares) hydrodynamic diameter (D_H) measured by DLS and (turquoise circles) zeta potential of AuNRs as a function of dsDNA concentration. The area with higher AuNR aggregation is highlighted in pale-orange.

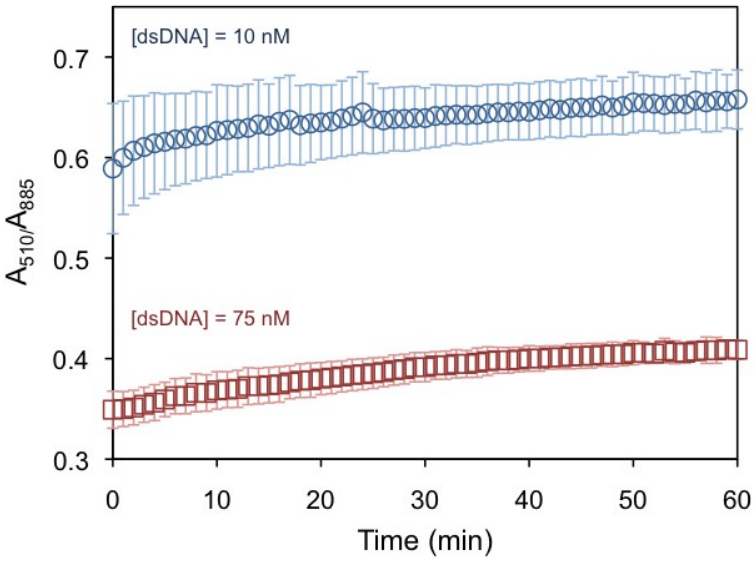

Fig. S3 Variation of dsDNA-AuNR absorbance ratio intensities at 510 and 885 nm after addition of Tris buffer over time. The volumes of Tris added into the system are the same as the ones added in the protein-dsDNA binding assays, *i.e.* 4 μL of 12.5 mM Tris and 6.5 μL of 50 mM Tris at 10 and 75 nM dsDNA, respectively.

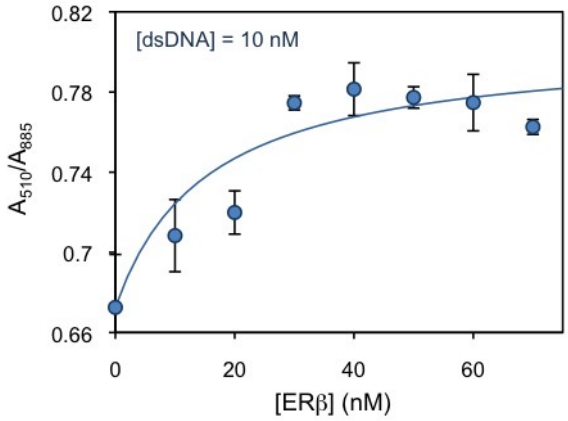

Fig. S4 AuNR absorbance ratio intensities at 510 and 885 nm as function of ERβ concentration at 10 nM dsDNA.

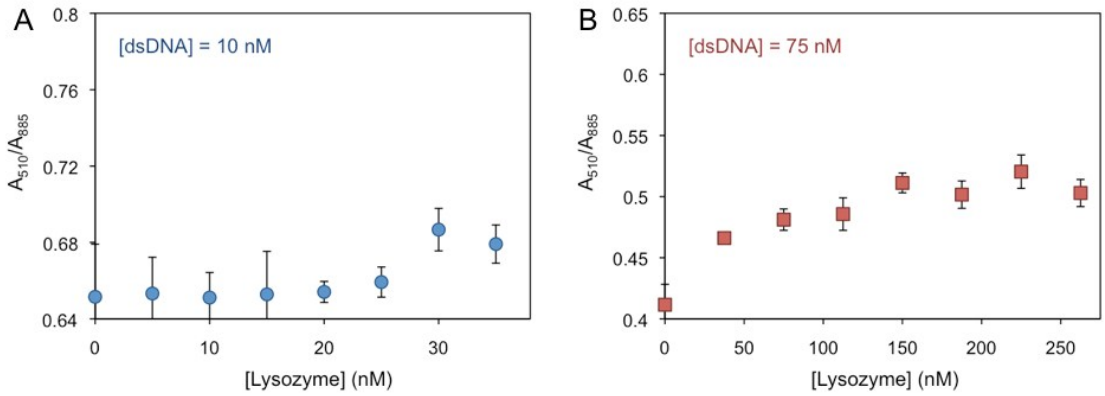

Fig. S5 AuNR absorbance ratio intensities at 510 and 885 nm as function of Lysozyme concentration in the presence of dsDNA of (A) 10 nM and (B) 75 nM.

Table S1. *p*-values obtained by one-sample Student's t-test, to study if the differences between the A_{510}/A_{885} ratios from the logic gate experiments and the reference values are statistical significant.

Logic Gate	*Input*	A_{510}/A_{885}	*St Dev*	*Reference value*	*p*
OR	(0/0)	0.651	0.026	0.650	0.896
	(1/0)	0.759	0.013	0.750	0.260
	(0/1)	0.763	0.022	0.750	0.414
	(1/1)	0.772	0.022	0.750	0.225
NOT	(0)	0.741	0.011	0.750	0.292
	(1)	0.669	0.010	0.650	0.081
A IMPLY B	(0,0)	0.741	0.011	0.750	0.292
	(1,0)	0.669	0.010	0.650	0.081
	(0,1)	0.754	0.031	0.750	0.844
	(1,1)	0.755	0.012	0.750	0.546
BUFFER	(0)	0.651	0.026	0.650	0.896
	(1)	0.759	0.013	0.750	0.260
TRUE	(0,0)	0.741	0.011	0.750	0.292
	(1,0)	0.746	0.011	0.750	0.593
	(0,1)	0.754	0.031	0.750	0.844
	(1,1)	0.764	0.010	0.750	0.136
FALSE	(0,0)	0.651	0.026	0.650	0.896
	(1,0)	0.654	0.006	0.650	0.368
	(0,1)	0.655	0.013	0.650	0.574
	(1,1)	0.665	0.017	0.650	0.083

Table S2. *p*-values obtained by Welch t-test (unequal variances t-test), to study if the differences between the A_{510}/A_{885} ratios from the logic 0 and 1 values are statistical significant.

Logic Gate	*Input 1*	A_{510}/A_{885}	*St Dev*	*Input 2*	A_{510}/A_{885}	*St Dev*	*p*
OR	(0,0)	0.651	0.026	(1,0)	0.759	0.013	< 0.001
	(0,0)	0.651	0.026	(0,1)	0.763	0.022	0.005
	(0,0)	0.651	0.026	(1,1)	0.772	0.022	0.004
NOT	(0)	0.741	0.011	(1)	0.669	0.010	0.004
A IMPLY B	(0,0)	0.741	0.011	(1,0)	0.669	0.010	0.004
	(0,1)	0.754	0.031	(1,0)	0.669	0.010	0.046
	(1,1)	0.755	0.012	(1,0)	0.669	0.010	0.002
BUFFER	(0)	0.651	0.026	(1)	0.759	0.013	< 0.001

Table S3. Cohen's *d*-values calculated to study the standardised differences between the A_{510}/A_{885} ratios from the logic 0 and 1 values.

Logic Gate	*Input 1*	A_{510}/A_{885}	*St Dev*	*Input 2*	A_{510}/A_{885}	*St Dev*	*d*
OR	(0,0)	0.651	0.026	(1,0)	0.759	0.013	4.53
	(0,0)	0.651	0.026	(0,1)	0.763	0.022	4.41
	(0,0)	0.651	0.026	(1,1)	0.772	0.022	4.76
NOT	(0)	0.741	0.011	(1)	0.669	0.010	6.85
A IMPLY B	(0,0)	0.741	0.011	(1,0)	0.669	0.010	6.85
	(0,1)	0.754	0.031	(1,0)	0.669	0.010	3.69
	(1,1)	0.755	0.012	(1,0)	0.669	0.010	7.79
BUFFER	(0)	0.651	0.026	(1)	0.759	0.013	4.53

References

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