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SUPPLEMENTARY INFORMATION

Colorimetric nanosensor based on a selective target-responsive aptamer kissing complex

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Experimental section

Material

Adenosine, inosine, guanosine, xanthine, theophylline, Bis(p-sulfonatophenyl)-phenylphosphine (BSPP), chloroauric acid, sodium citrate and Tris(hydroxymethyl)aminomethane were obtained from Sigma-Aldrich (Saint-Quentin, France). Na_2HPO_4 was obtained from Fisher-Scientific (Illkirch, France). NaCl and $MgCl_2$ were obtained from Chimie-Plus Laboratoires (Bruyères de Pouilly, France) and Panreac Quimica (Barcelona, Spain), respectively. Water was obtained from a MilliQ system (Millipore, Saint-Quentin en Yveline, France). The oligonucleotides (Table S1) were synthesized and HPLC purified by Eurogentec (Angers, France). The identity of the oligonucleotides was confirmed by MALDI-TOF mass spectrometry.

Instrumentation

UV–visible spectra of colloidal solutions were recorded on a Shimadzu UV-1650pc spectrophotometer at wavelengths ranging from 400 to 800 nm in 10 mm quartz suprasil® cuvettes. Microscopy samples were prepared by deposition of a drop of colloidal solution onto a carbon-coated copper grid and observed on a JEOL1011 microscope for bright-field transmission electronic microscopy (TEM) working at 100 kV. The particle size was estimated manually using the ImageJ software.

Fluorescence anisotropy measurements

Tecan Infinite 1000 instrument was used to measure the fluorescence anisotropy. Oligonucleotides were structured prior to mixing. RNA was heated at 65°C for 3 min and chilled on ice before adding SE buffer (140 mM potassium acetate, 20 mM sodium acetate, 10 mM magnesium chloride, 20 mM HEPES pH 7.4 at room temperature). DNA samples were treated the same way except that they were heated up to 80°C. Samples were incubated at least for 5 min at room temperature. Adenosine, inosine, or buffer were added to the structured aptaswitches for 15 min at room temperature and mixed with the 3' end Texas-red labeled aptakiss at 10 nM in a final volume of 50 μL and then incubated for 4 hours at 4°C. Fluorescence anisotropy was measured in triplicate in 96 well greiner black microplates.

Excitation was set at 590 +/- 5 nm and emission was collected at 635 +/- 20 nm bandpass filters for the Texas Red probe. The fluorescence anisotropy (r) was calculated by the instrument software, as classically reported:

$$r = \frac{I_{vv} - GI_{vh}}{I_{vv} + 2GI_{vh}} \quad (1)$$

where Ivv and Ivh are the vertically and horizontally polarized components of the emission after excitation by vertically polarized light. The instrumental correction factor G was determined according to the manufacturer's instructions.

The relative r parameter (Δr) was calculated as follows:

$$\Delta r = r - r_0 \quad (2)$$

where r is the fluorescence anisotropy of the Texas Red labeled aptakiss in the presence of the aptaswitch and the target, r_0 is the fluorescence anisotropy in the absence of the target.

Gold nanoparticle synthesis and characterization

GNPs were prepared by citrate reduction as previously reported.[S1] Briefly, 3 mL of trisodium citrate (38.8 mM in water) were added to 30 mL of boiling $HAuCl_4$ (1 mM in water). The solution was maintained boiling under vigorous stirring for 10 min. The color of the suspension changed from pale yellow to dark red. The stirring was then continued until the mixture was cooled to room temperature (RT). The colloidal suspension was finally filtered on PVDF-membrane filters 0.22 µm (Roth) and left aging for 2 days at 4 °C before use. TEM analysis allowed the determination of the GNP size (13.2 +/- 1.4 nm). UV visible absorption measurements[S2] allowed to calculate a 12.5 +/- 6.5 nm size and a 12.5 +/-6.5 nM concentration. Each batch was used for 2 to 4 weeks following preparation.

Oligonucleotide immobilization

Firstly, citrate was substituted by BSPP at the gold nanoparticle surface. 3 mg of BSPP was added to 1 mL of gold nanoparticle solution (specific surface = 0.488 m^2/L) and gently stirred for 7 hours at room temperature (RT). The solution was then centrifuged at 12,000 g for 25 min. 940 µL of supernatant were eliminated and 440 µL of a BSPP solution (1 mg/mL) were added. It was stirred by vortex. NaCl (about 10 mg) was added to the solution until it turned blue. It was again centrifuged at 12,000 g during 25 min. The supernatant was eliminated and the particles were resuspended in 70 µL of a BSPP solution (1 mg/mL).

10 µL of oligonucleotide solution (1 µM) in buffer B1 (10 mM Tris buffer, pH 7.5; 150 mM NaCl) were denatured at 80 °C for 5 min, cooled down and left at 4 °C for 30 min. 20 µL of a BSPP solution (25 mg/mL) in B1 was then mixed with the oligonucleotide solution. Finally, 70 µL of the previously prepared gold nanoparticle solution was added to it. The resulting solution

was then left for 16 h at 4 °C. 7 µL of a 2-(2-mercaptoethoxy)ethanol (200 µM in B1) were mixed with the solution that was left for 1h30 at RT. It was then centrifuged at 12,000 g for 25 min and 97 µL of supernatant were removed. GNPs were resuspended by addition of 97 µL of 1.23-fold concentrated buffer B2 (50 mM phosphate buffer, pH 7.5; 10 mM $MgCl_2$) and the resulting solution was stirred by vortex. To note, special care was taken to prevent RNase contamination during the preparation of RNA kiss-GNPs by maintaining as much as possible a RNase-free environment (use of nuclease-free water, sterile pipette tips and tubes, autoclaved laboratory glasswares and GNPs as well as wear gloves when handling the reagents under a dedicated hood).

Aggregation ability of Apt-GNPs and CS-GNPs

A 1:1 mixture of 45 µL of aptamer modified gold nanoparticle solution mixed with 45 µL of CS modified gold nanoparticle solution and 10 µL of water was prepared. The mixture was incubated at 4°C for 30 min. UV–visible spectrum of the colloidal solution was recorded and the absorbance ratio at 650 and 520 nm (R = A650/A520) was determined.

Colorimetric adenosine detection through AKC principle

The same protocol was used for the nanosensing platform based on the aptaswitch and aptakiss modified GNPs, except that 60 min incubation at 4°C was needed. The final target concentration ranged from 0 to 500 µM. Control samples were also performed by mixing only one type of modified nanoparticles with water or target (500 µM). For each experiment, ΔR ($\Delta R = R - R_{blk}$) was calculated where R_{blk} was the mean value of the blank measurements. The titration curves were constructed by plotting ΔR *versus* the ligand concentration. A high ΔR value was associated to purple-colored GNP aggregates while a low ΔR value corresponded to red-colored dispersed nanoparticles. Relative ΔR was calculated as follows:

$$\textbf{relative } \Delta\mathbf{R} = \frac{\Delta\mathbf{R}\times\mathbf{100}}{\Delta\mathbf{R_{500}}} \quad (3)$$

where ΔR_{500} is the ΔR value for 500 μM adenosine concentration. The sensing platform reproducibility was assessed from triplicate measurements.

Complex medium analysis

Human serum from human male AB plasma (Sigma-Aldrich from Saint-Quentin, France) was employed to test the kissing based colorimetric assay under biological conditions. Serum was firstly filtered on a Nanosep 10K centrifugal device (Pall) spin column at 12 000 g for 1 h in a MIKRO 220 R centrifuge. A second filtration was performed on a Nanosep 3K centrifugal device

spin column at 12 000 g for 1 h. It was then 1/2 diluted in water then spiked with the target to obtain final 1/20 serum dilution and a final target concentration of 200 µM. The protocol for the colorimetric detection of adenosine was identical to that used with the buffer. We prepared the samples in triplicate to determine the recoveries obtained with serum media.

Table S1 List of oligonucleotides used in the study (kissing sequences in blue, intramolecular base-pairing are underlined, complementary sequences in green)

Name	DNA/RNA	linker	Sequence (5'→3')
k3-3'TR	RNA	-	CGAGCCUGGGAGCUCG
k2-3'TR	RNA	-	UGCUCGGCCGCGCGAGCA
dADOsw3'A	DNA	-	GTTGGGGGAGCACACTCCCATTCTGCGGAGGAAAC
dADOsw3'B	DNA	-	GTTGGGGGACACACTCCCATTCTGGGAGGAAAC
dADOsw3'C	DNA	-	GTTGGGGGAACACTCCCATTCTGGAGGAAAC
5'SH-**Apt**	DNA	C6	CCTGGGGGAGTATTGCGGAGGAAGG
5'SH-**CS**	DNA	C6	CTTCCTCCGCAA
5'SH-**dADOsw3'B**	DNA	C6	GTTGGGGGACACACTCCCATTCTGGGAGGAAAC
5'SH-**k3**	RNA	C6	CGAGCCUGGGAGCUCG

Table S2 Limit of detection (LOD) for adenosine and analogues using functionalized GNP-based colorimetric aptasensor strategies.

Strategy	LOD	Ref
Strand displacement disassembly	300 µM	10
Folding disassembly	20-30 µM	11
Split aptamer assembly	250 µM	12
Improved split aptamer assembly	25 µM	25
AKC assembly	13 µM	This work

Table S3 Some representative examples of highly sensitive aptasensors (for adenosine and analogues) based on different transduction methods (FA: fluorescence anisotropy; SPR: surface plasmon resonance, NP: nanoparticle, QD: quantum dot).

Strategy	LOD	Ref
Graphene-based mass amplifier FA assay	2 pM	S3
NP-based mass amplifier FA assay	20 pM	S4
Electrogenerated chemiluminescence assay	7 fM	S5
Amplification-based SPR assay	0.2 pM	S6
QDs-based amplification electrochemical assay	45 pM	S7

Fig. S1 Specificity of the aptakiss-aptaswitch kissing interaction monitored by fluorescence anisotropy. Increasing concentration of **dADOsw3'B** (blue) or **C** (red) were tested for their ability to bind the aptakiss **k3** (solid lines) or **k2** (dotted lines) in the presence of 8 mM of adenosine.

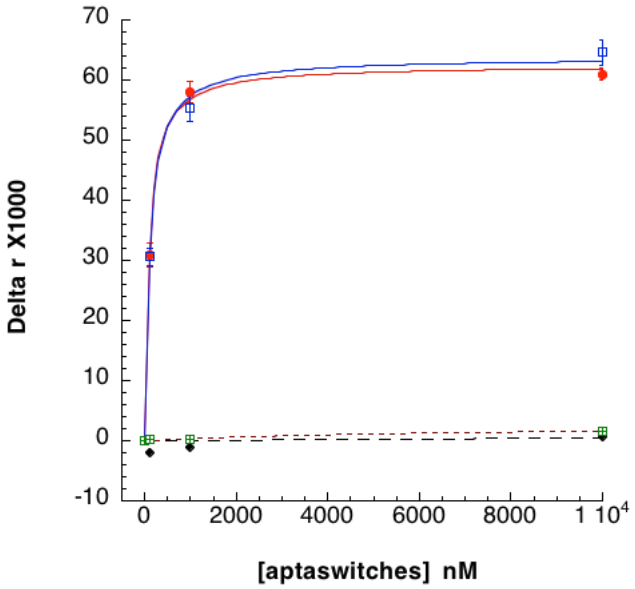


Fig. S2 Characterization of the synthesized gold nanoparticles using TEM

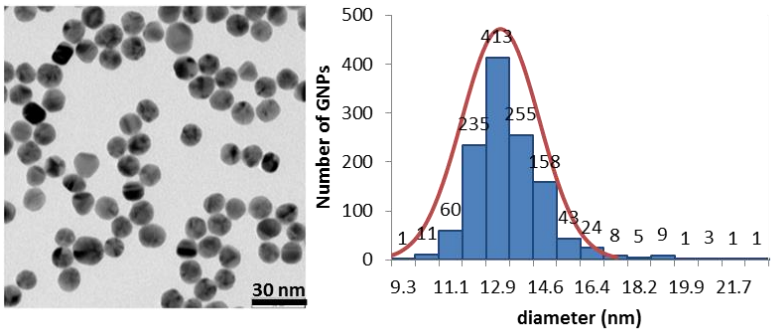


Fig. S3 Experimental protocol to graft DNA oligonucleotides onto GNPs

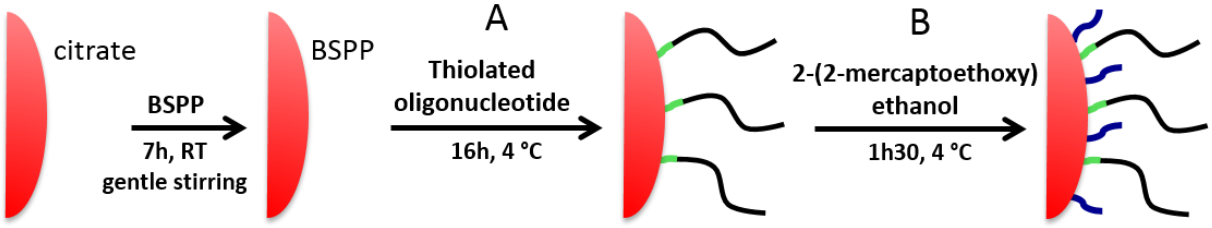
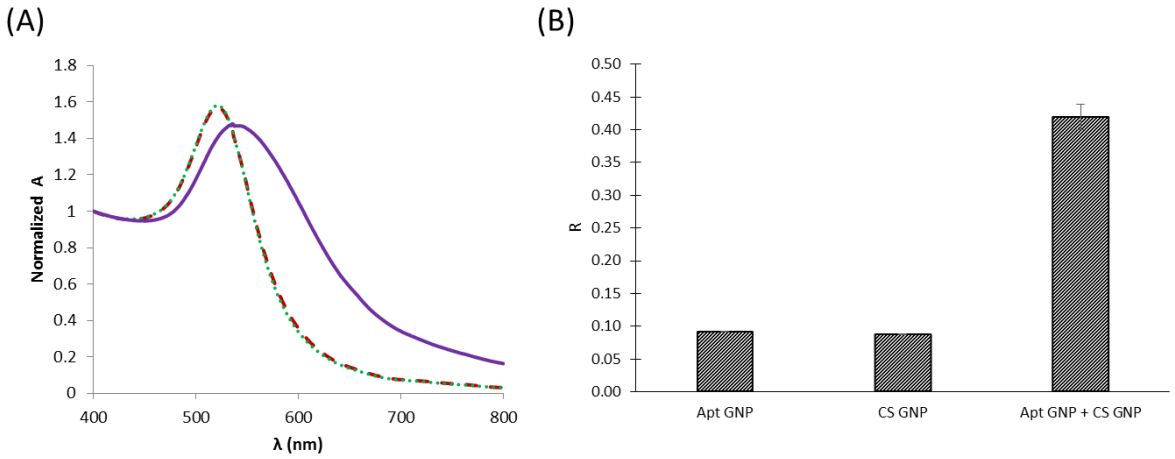


Fig. S4 (A) Normalized UV visible absorption spectra considering **Apt**-GNPs (green), **CS**-GNPs (red) and a mixture of them (purple) and (B) the corresponding signal. Operating conditions: phosphate buffer 50 mM, pH 7.5, $MgCl_2$ 10 mM, 30 min incubation at 4°C before measurement.



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