Supporting Information

Oligonucleotide Sensor Based on Selective Capture of Upconversion Nanoparticles Triggered by Target Induced DNA Inter-Strand Ligand Reaction

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1. Synthesis of the $NaYF_4$:Yb,Er@SiO_2-ssDNA-N_3 Nanoparticle

1.*a Synthesis of $NaYF_4$:Yb,Er Nanoparticles*

Upconverting nanoparticles (UCNPs) were synthesized by a modification of the Ostwald ripening method.[1] First, 0.78 mmol of YCl_3, 0.2 mmol of $YbCl_3$, and 0.02 mmol of $ErCl_3$ were dissolved with 6 mL of oleic acid and 15 mL of 1-octadecene in a three necked round bottom flask by heating the mixture to 160 ºC for 90 minutes under nitrogen atmosphere. After that, the mixture was cooled to room temperature and 10 mL

of a methanol solution containing 100 mg of NaOH (2.5 mmol) and 148.16 mg of NH_4F (4 mmol) were added drop-wise under vigorous stirring. The mixture was stirred for 30 min and finally the methanol was evaporated by heating at 100 ºC. Traces of methanol were extracted with a vacuum pump. The flask was placed on a heating mantle and heated up to 315 ºC at reflux for 60 minutes. After this time, the solution was transferred to a decantation funnel and cleaned 4 times with deionized water. Then, the oil phase was centrifuged with ethanol at 8500 rpm, 10 min. The pellets were redispersed with 1 mL of hexane, and centrifuged with ethanol again. Finally, the pellets were rinsed with ethanol, redispersed in 18 mL of hexane and stored for further use.

1.b Synthesis of $NaYF_4$:Yb,Er@SiO_2 Nanoparticles

A SiO_2 shell was grown on the surface of the UCNPs via the reverse microemulsion method.[2,3,4] Briefly, 240 mg of Igepal™ CO-520, 1.2 mL of UCNPs and 3.8 mL of hexane were mixed and dispersed using an ultrasonic bath. Next, 40 µL of Ammonia (30%) was added and the mixture was sonicated again until obtaining a homogeneous solution. Finally, 30 µL of TEOS (0.14 mmol) was added under stirring to start the reaction. After 8 h, the reaction was stopped and the microemulsion was destabilized using methanol. The resulting solution was centrifuged and the process was repeated three times using ethanol as solvent (9500 rpm, 10 min). Finally, the nanoparticles were collected and stored in 5 mL of ethanol. After this reaction, the z-potential of the particles was -37 mV.

1.c Synthesis of $NaYF_4$:Yb,Er@SiO_2-COOH Nanoparticles

$NaYF_4$:Yb,Er@SiO_2 nanoparticles were modified with amine (-NH_2) groups by adding 100 µL (0.45 mmol) of APTES to the UCNPs dispersed in 5 mL of ethanol and stirred overnight. After this time, the resulting product was centrifuged three times with ethanol at 9500 rpm for 10 min and once with anhydrous N,N-Dimethylformamide (DMF) at 10500 rpm during 12 min. After the surface modification with amine groups, the z-potential of the nanoparticles was +27 mV. The resulting pellet was dispersed with 3 mL of dry DMF and transferred to a vial, where it was kept under stirring. Then, a solution of 150 mg of succinic anhydride (1.5 mmol) in 2 mL of DMF was added dropwise to the $NaYF_4$:Yb,Er@SiO_2 dispersion and the reaction was allowed to progress overnight. Finally, the nanoparticles were recovered by centrifugation (11000 rpm, 12 min),

centrifuged twice with deionized water and stored in deionized water at a concentration of 2.2 mg mL^{-1}. After this reaction the z-potential of the nanoparticles change to -32 mV.

1.d. Immobilization of ssDNA-N_3 on the surface of UCNPS@SiO_2-COOH

Amine functionalized DNA strands (H_2N-C_6-TTTTTTTGTATATTTATA-N_3) were grafted on the surface of the upconversion nanoparticles using the EDC and sulfo-NHS coupling reaction. Hence, 4 mg of nanoparticles were transferred to an eppendorf tube and centrifuged. The resulting pellet was redispersed in 4 mL of Bionic (1X) and kept under stirring in a vial. Then, 60 µL of EDC·HCl 0.2 M and 120 µL of Sulfo-NHS 0.2 M were added, and the nanoparticles were let to activate for 1.5 h. Amine functionalized ssDNA (40 µL, 49µM) was added and the coupling reaction was allowed to progress for 3h. After this time, the nanoparticles were recovered by centrifugation, washed by centrifugation twice with Bionic (1x), and finally stored in DMSO at a concentration of 8 mg mL^{-1}. Thermogravimetric analysis indicated that the amount of DNA anchored on the surface of each nanoparticle is roughly 40 chains.

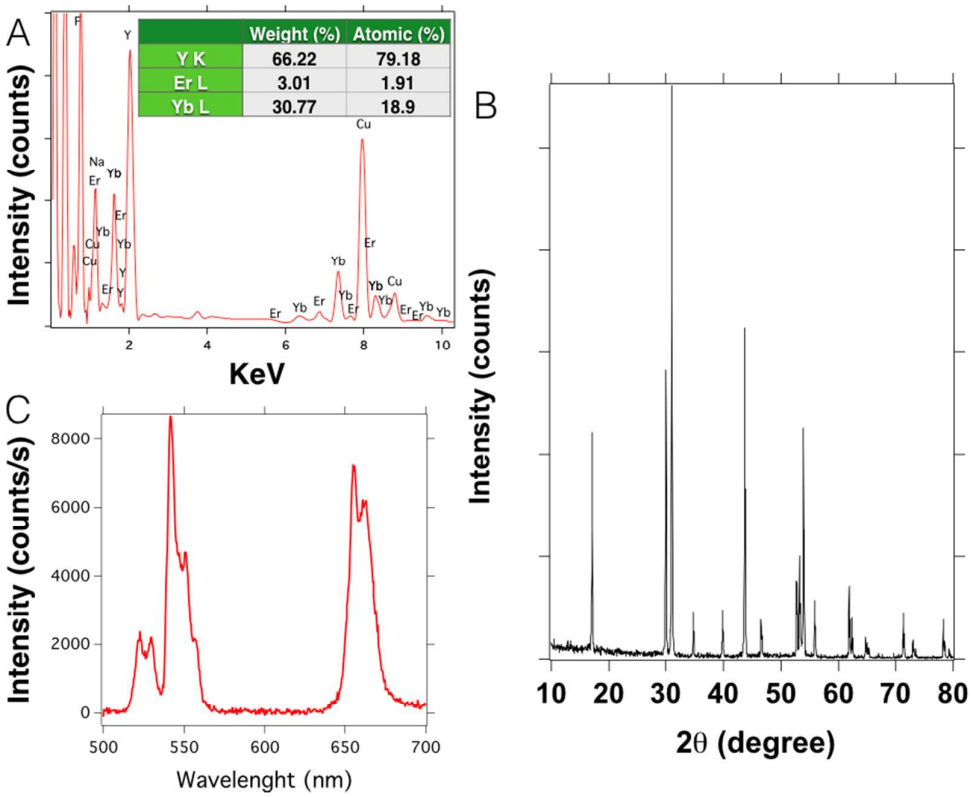

Figure S1. EDS spectrum of the bare $NaYF_4$;Yb,Er nanoparticles (A), X-Ray diffraction pattern of the as synthesized $NaYF_4$;Yb,Er nanoparticles (B) and emission spectrum of the silica coated upconversion nanoparticles in water after being excited with a CW laser at 980 nm. (C)

2. RNA Extraction and Target Detection

2.a Total RNA Extraction

The total RNA was extracted from healthy mosquitoes (Aedes albopictus) using the miRNeasy Mini Kit from Quiagen following the manufacturer's protocol**.**

2.b Target Detection

2.b.1. The standard process that was used for the detection assays was the following: First, 200 μL eppendorf tubes were filled with 90 μL of hybridization buffer (Hepes, 150 mM NaCl) containing a concentration of target sequence between 10^{-12} and 10^{-18} moles. In this work we have evaluated the response of the sensor against DNA and RNA targets obtaining similar results independently of the nature of the complementary strand. 1 μg of UCNPs-ssDNA-N_3 and $2x10^{-12}$ moles of biotinylated-oligo were added to the eppendorf giving a final volume of 100 μL. The resulting mixture was let to hybridize for 1 hour at room temperature. After this time, the content of the eppendorf was transferred to streptavidin-coated wells, and incubated at room temperature for 2 hours. Finally, the wells were washed six times with assay buffer and dried upside-down at room temperature, before reading. To analyze the reproducibility of the results, we run at least, three independent experiments measuring the luminescence intensity at 10 different positions for each well. All the experiments were compared with the background signal obtained from blank samples, which were produced as indicated above but without the addition of the target sequence.

2.b.2 The target detection in the presence of total RNA was performed follows; First, 200 μL eppendorf tubes were filled with 90 μL of hybridization buffer (HEPES, 150 mM NaCl) containing 100 ng of total RNA and from 10^{-12} to 10^{-18} moles of the target sequence. After that, 1 μg of UCNPs-ssDNA-N_3 and $2x10^{-12}$ moles of biotinylated-oligo were added to the eppendorf giving a final volume of 100 μL. The next steps that include the hybridization time and the incubation were the same as indicated above.

2.b.3. The target detection in spiked-in serum samples was performed as follows. First, 200 μL eppendorf tubes were filled with 50 μL of hybridization buffer (Hepes, 150 mM NaCl) and mixed with 40 μL of human serum containing a target sequence concentration from 10^{-12} to 10^{-18} moles. After spiking the serum samples, they were immediately measured in order to reduce degradation effects. The fraction of serum inside the whole sample was 50% v/v. After that, 1 μg of UCNPs-ssDNA-N_3 and $2x10^{-12}$ moles of biotinylated-oligo were added to the eppendorf giving a final volume of 100 μL. The next steps that include the hybridization time and the incubation were the same as indicated above.

The amount of target sequences presented in the calibration curves is referred to the moles of target strands spiked in the samples.

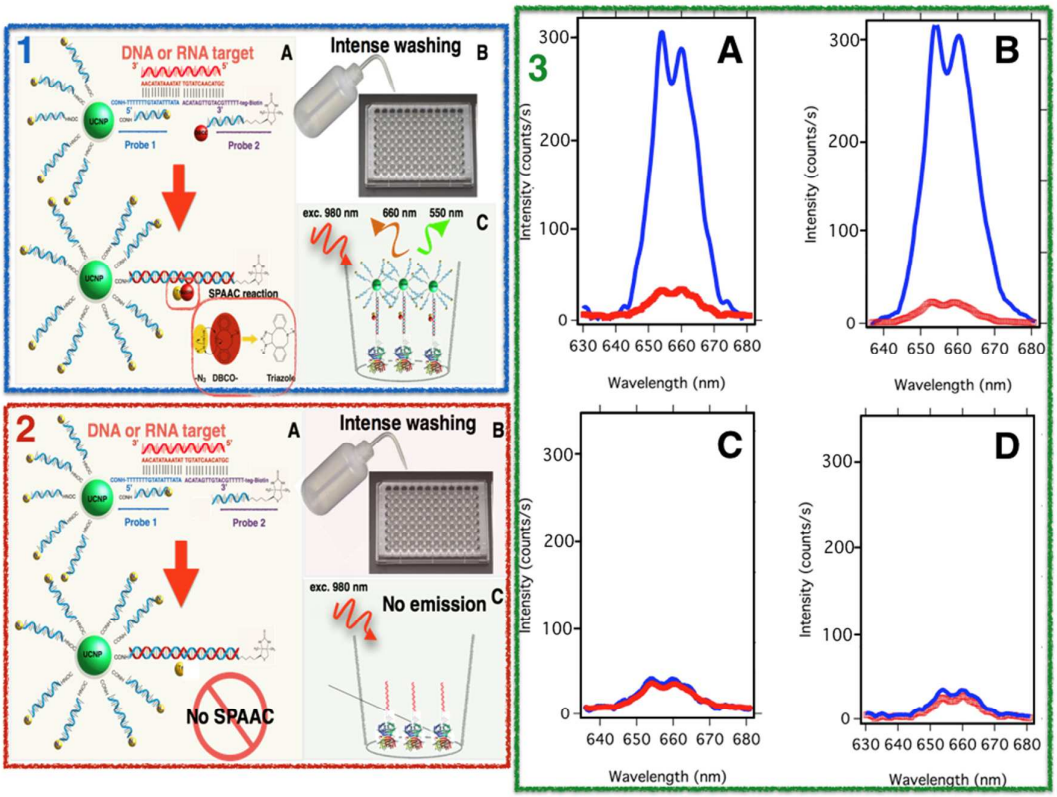

Figure S2. Panel 1 illustrates the measuring process where inter-strand ligand reaction is involved. Panel 2 depicts the same process in the absence of inter-strand ligand reaction. Panel 3 shows the photoluminescent spectra obtained from $NaYF_4$;Yb,Er@SiO_2-ssDNA-N_3 and DBCO-ssDNA-biotin able to produce inter strand ligation (blue) and $NaYF_4$;Yb,Er@SiO_2-ssDNA-N_3 and ssDNA-biotin, which are unable to produce inter-strand ligation (red) in the presence of 10^{-13} moles of target sequence and after washing the solid support with 10 mM HEPES buffer and different concentrations of NaCl at 50ºC, 150 mM in A) and 50 mM in B). C and D are the same experiments but in the absence of target sequence (control experiments).

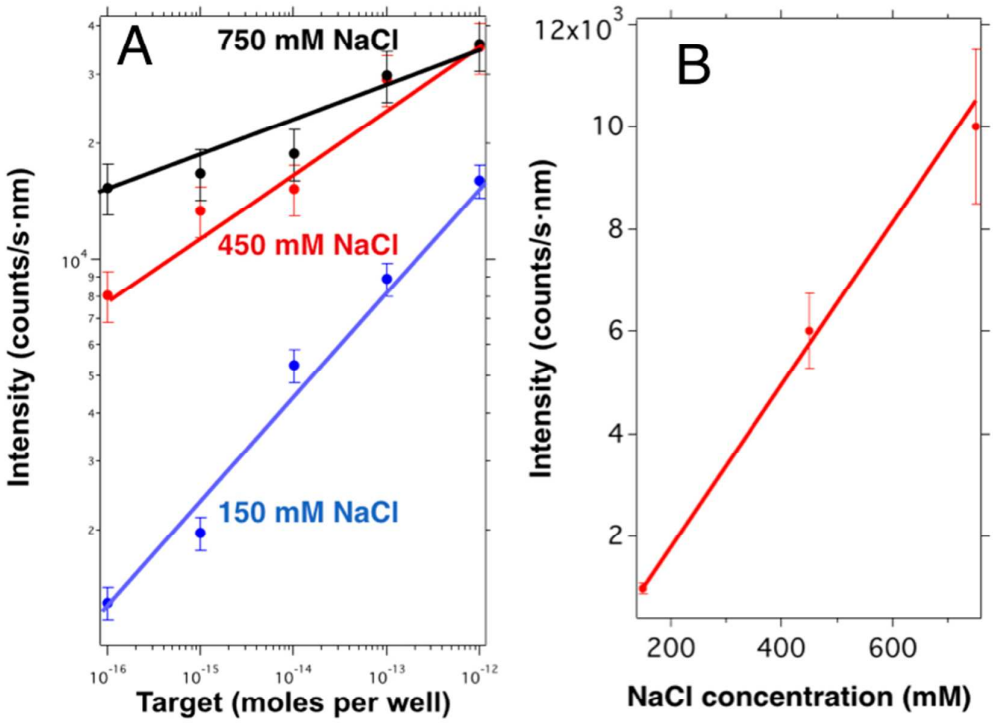

Figure S3. A) Calibration curve of the sensor using different ionic strength, B) signal intensity of the backgrounds as a function of the ionic strength. The results obtained from Figure S3 indicated that the best ionic strength was 150 mM of NaCl.

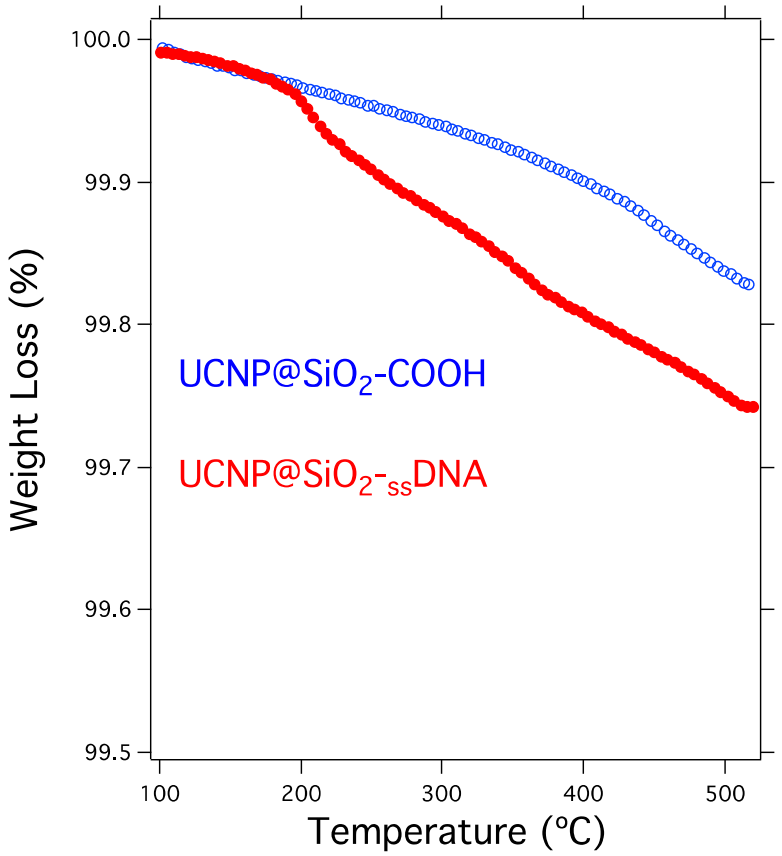

Figure S4. TGA experiment for UCNP@SiO_2-COOH and UCNP@SiO_2-ssDNA nanoparticles

References

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