Polynucleotide Functionalized Upconversion Nanoparticles for DNA Biosensoring

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SUPPORTING INFORMATION

The EDC coupling between the probe DNA and the UCNPs@SiO_2-COOH was confirmed with a control experiment in which we performed the same reaction using a 3´-modified DNA probe sequence. In this control experiment the DNA had a fluorescent dye (Cy3) which could be directly observed by fluorescence (λ_{exc} = 543 nm; λ_{em} = 562 nm) after several purification steps. This result suggested that the EDC coupling under our experimental conditions was successful in covalently attaching the probe DNA to the upconversion nanoparticles.

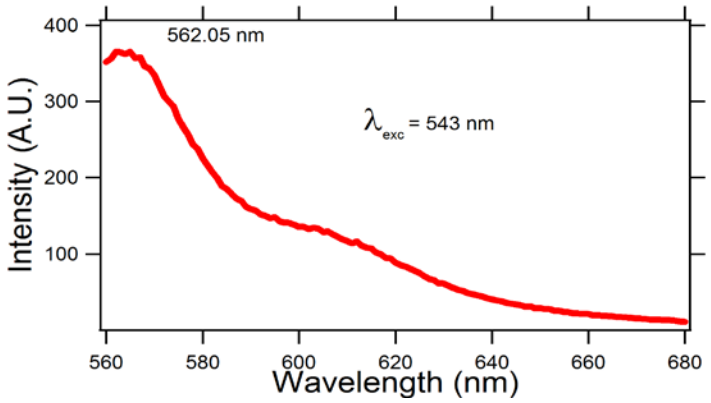

Figure S1: Fluorescence spectrum of the UCNPs@SiO_2-ssDNA-Cy3 nanoparticles collected with an excitation wavelength of 543 nm. The maximum fluorescence intensity at 562 nm corresponds to the fluorescence of the Cy3 dye.

The UV-Vis spectrum of graphene oxide reveals that the absorbance at 549 nm is higher than that at 654 nm. The upconversion fluorescence quenching in this work has followed the same trend for both wavelengths, which would indicate that this quenching is due to a FRET process rather than a simple absorption related process.

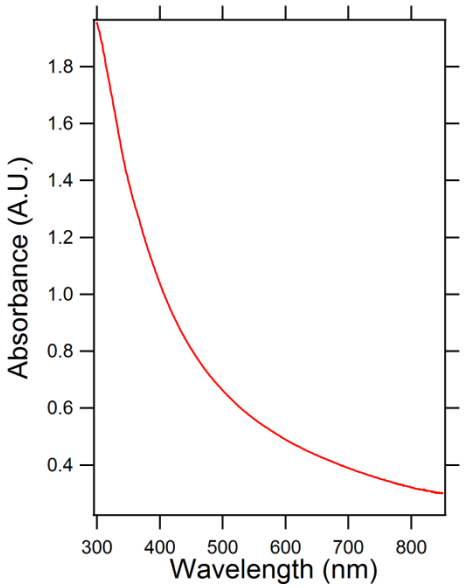

Figure S2: UV-Vis absorption spectrum of 0.3 mg/mL graphene oxide in PBS buffered aqueous solution.

The upconversion fluorescence intensity was represented as a function of the complementary DNA concentration, and we observed a double exponential increase in which at lower concentrations of cDNA the upconversion fluorescence intensity increased more than that at higher concentrations of cDNA. The double exponential equations obtained from the curve fitting for 549 nm and 654 nm, respectively, were the following:

$$y = (816.8 \pm 54) + (-428.09 \pm 50.8) \cdot e^{(-0.0067113 \pm 0.00214) \cdot cDNA} + (-182.74 \pm 32.7) \cdot e^{(-1.7031 \pm 1.11) \cdot cDNA}$$

$$y = (1984.5 \pm 40) + (-1136 \pm 37.7) \cdot e^{(-0.0069295 \pm 0.00062) \cdot cDNA} + (-298.59 \pm 36.8) \cdot e^{(-1.6515 \pm 0.567) \cdot cDNA}$$

In this work we have demonstrated that the hybridization conditions strongly influence the sensitivity of the DNA sensor. In Figure S3 we represented the upconversion fluorescence intensity as a function of the cDNA concentration at different hybridization conditions (90 ºC for 2 minutes and 1 hour at 40 ºC and 40 ºC for 2 hours). This figure shows graphically that the experimental detection limit was three orders of magnitude lower when the samples were hybridized at 90 ºC. We observed this result in both of the emission wavelength maxima of Erbium, i.e. 549 nm and 654 nm.

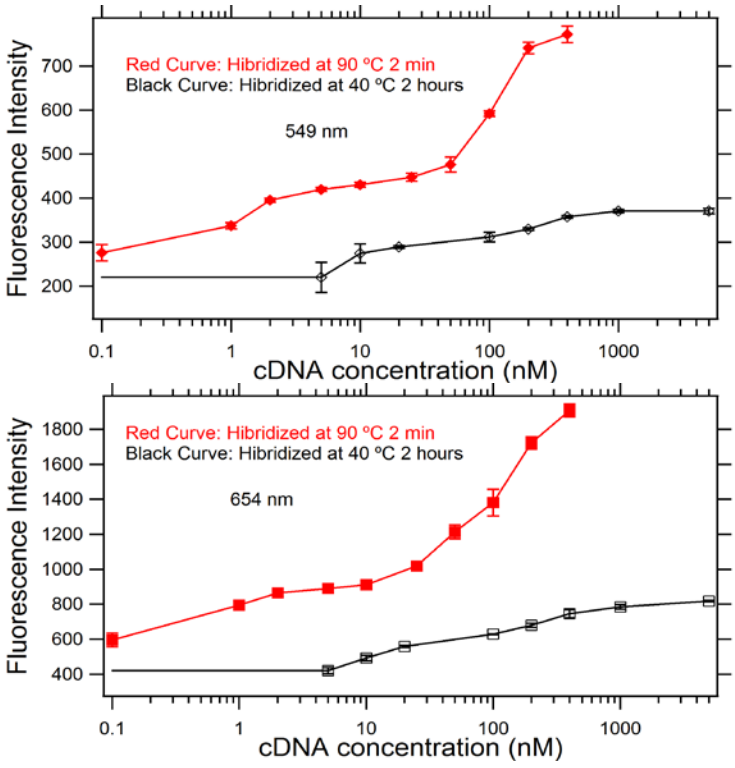

Figure S3: Upconversion fluorescence intensity as a function of the cDNA concentration at 549 nm (upper figure) and at 654 nm (lower figure). The red curves represent the results obtained when the hybridization was performed at 90 ºC for two minutes and 1 hour at 40 ºC. The black curves represent the results obtained when the hybridization was performed at 40 ºC for two hours.

When the hybridization was performed with a random non-complementary DNA sequence, the upconversion fluorescence intensity did not increase significantly. Thus, the data analysis revealed that the upconversion fluorescence at 549 nm and 654 nm remained quenched independently of the non-complementary DNA concentration. Figure S4 shows the curve fitting of these results.

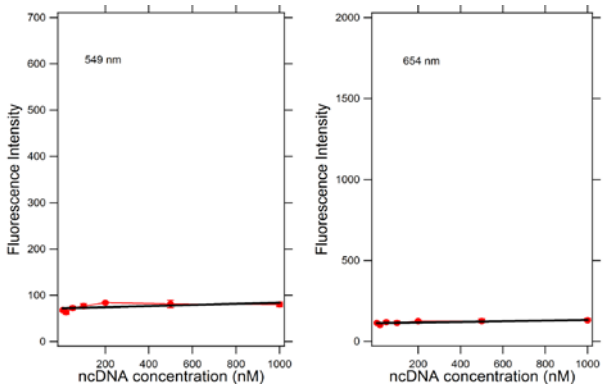

Figure S4: Curve fitting of the upconversion fluorescence intensity against the non-complementary DNA concentration for the emission at 549 nm (left) and 654 nm (right).

In summary, the upconversion fluorescence intensities obtained with the DNA concentration (cDNA and non cDNA) constant at 400 nM were significantly different. Figure S5 depicts the upconversion fluorescence intensity obtained at this concentration with cDNA with the different hybridization conditions and the upconversion fluorescence intensity obtained at this concentration of cDNA "hybridized" at 90 ºC.

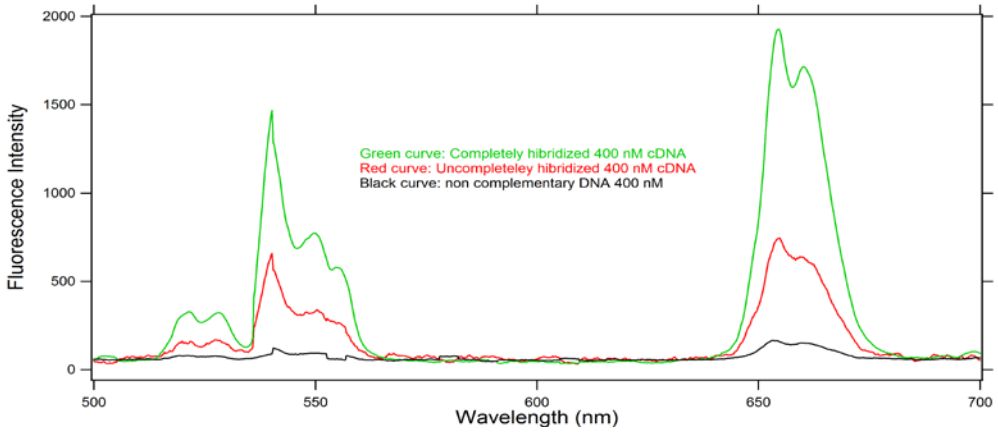

Figure S5: Upconversion fluorescence curves when the UCNPs@SiO_2-ssDNA were hybridized with: 400 nM of cDNA at 90 ºC for 2 minutes and 1 hour at 40 ºC (green); 400 nM of cDNA at 40 ºC for 2 hours (red); and 400 nM of non cDNA at 90 ºC for 2 minutes and 1 hour at 40 ºC (black).

TABLE OF CONTENTS