Ligase-assisted signal-amplifiable DNA detection using upconversion nanoparticles

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Supporting Information

Chemicals and materials

$Y(NO_3)_3 \cdot 6H_2O$, $Yb(NO_3)_3 \cdot 5H_2O$, $Tm(NO_3)_3 \cdot 5H_2O$, polyacrylic acid (PAA, MW ~15000), NaOH, NH_4F, ethylenediaminetetraacetic acid (EDTA) and acetone were purchased from Sigma (St. Louis, MO). SYBR Green I was purchased from Life Technology (Carlsbad, CA). Ethylene glycol (EG), 1-Ethyl-3-[3-dimethylamonopropyl]carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Thermo Scientific (Rockford, IL).

Single-stranded DNA fragments were ordered from IDT DNA (Coralville, IA). The sequences were DNA_seg1 (5' amino-CAG TAA CGG CAG A-3') amine-modified at the 5'-end, DNA_seg2 (5'-CTT CTC CAC AGG AGC CGT TAC TG-3') phosphorylated at the 5'-end, DNA_tar (5'-TCC TGT GGA GAA GTC TGC CGT TAC TG-3'), and DNA_mut (5'-TCC TGA GGA GAA GTC TGC CGT TAC TG-3'). Thermostable DNA ligase (Ampligase®) and 10x Ampligase® reaction buffer were purchased from Epicentre (Madison, WI). The melting points of various DNA strands were calculated using the OligoAnalyzer 3.1 program available at the vendor website (www.idtdna.com).

Synthesis of $NaYF_4$:Yb^{3+},Tm^{3+} upconversion nanoparticles

PAA (0.3 g), NaCl (23.4 mg), YCl_3 (48.5 mg), $YbCl_3$ (13.9 mg), and $TmCl_3$ (1.1 mg) were added into 3 ml EG, using vortex and sonicator to make the mixture homogenous (Solution A). Separately, 0.03 g of

NH_4F was added to 2 mL of EG in a Teflon container (Solution B). Solution A was added drop wise into Solution B under stirring. The Teflon container was then placed in a sealed, stainless-steel capsule, and incubated for 2 hr at 200 °C. The resulting solution was clear with light yellow in color. The nanoparticles were collected after centrifuging the solution for 1 hr at 15,000 rpm to remove the supernatant. They were washed 3 times by ethanol and twice by DI water, before dispersed in 2 ml DI water for storage.

Conjugation of DNA_seg1 to UCNPs

Two mL of washed UCNP aqueous solution was pre-treated with 10 µL of EDC (0.2 M) and 10 µL of NHS (0.05 M) for 5 min under stirring at 8000 rpm. Then, 30 µL of 100 µM DNA_seg1 was added into the reaction mixture and stirred at 8000 rpm overnight. The resulting nanoparticles were washed 3 times by DI water, before dispersed in 500 µL DI water.

Ligation and hairpin loop amplification in thermal cycles

Fifty µL of DNA_seg1 conjugated UCNP solution was mixed with 10 µL of 10× Ampligase® reaction buffer, 20 µL of 1 µM DNA_seg2 and 1 µL of Ampligase with different amounts of 0.1 µM DNA_tar. The solution of a total volume of 100 µL was then treated in 90 °C for 30 second. Then the solution was cooled down to 72 °C and maintained for 1 min. At last the solution was cooled down to 45°C and maintained for 3 min. This thermal cycle was repeated up to 20 times.

TEM measurement

TEM samples were prepared by air-drying a drop of the sample solution on a 300-mesh Formvar-covered carbon-coated copper grid (Electron Microscopy Sciences, PA). TEM observations were performed using a Biotwin 12 transmission electron microscope (FEI, Netherlands). The images were analyzed by ImageJ software. The UCNPs were relatively mono-dispersed with the diameter of 143 ± 19 nm after analyzing 712 particles.

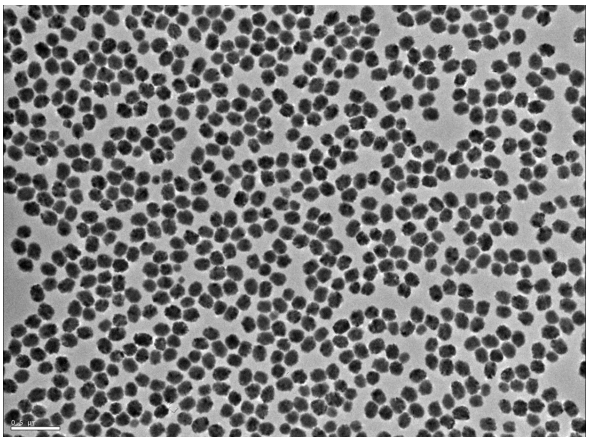

Figure S1. TEM picture of $NaYF_4$:Yb^{3+},Tm^{3+} upconversion nanoparticles (Scale bar: 500 nm).

FTIR measurement

Surface functional group characterization was performed on an ATR cell (Nicolet 6700 FT-IR, Fisher Thermo Scientific).

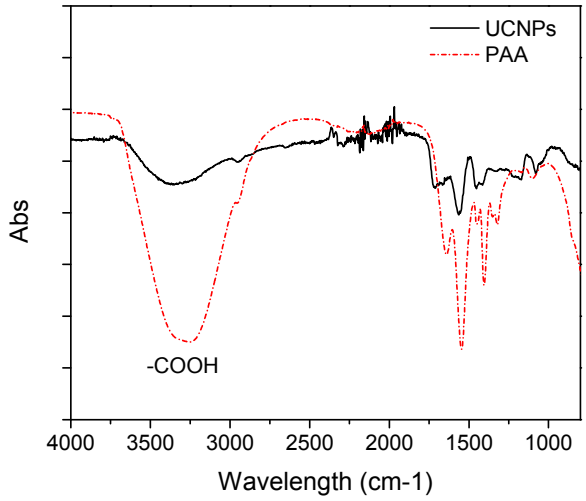

Figure S2. FT-IR spectra of $NaYF_4$: Yb^{3+},Tm^{3+} upconversion nanoparticles and PAA.

Determination of the amount of UCNP-conjugated DNA_seg1

First, a calibration curve of DNA_seg1 in 5 µM SG1 solution was obtained with different concentrations (0, 50, 100, 200, 400 nM) of DNA_seg1 (Figure S3B). The calibration curve showed a linear relationship between the fluorescence intensity of SG1 and the concentration of DNA_seg1 (in nM). Then 25 µL of UCNP-conjugated DNA_seg1 working solution was diluted by 20 times, and then mixed with 5 µM SG1 solution. The fluorescence intensity of SG1 at 530 nm was again measured, and compared to the calibration curve, yielding 220 nM of DNA_seg1 for the 500 µL of diluted solution. For these measurements (Figure S3A), SG1 was excited at 480 nm using a Xenon lamp with the slit width of 1 mm.

Based on these results, we were also able to calculate the yield of the EDC/NHS conjugation between UCNPs and DNA_seg1. The initial amount of DNA_seg1 added during the conjugation was 3 nmole. The conjugation yield was thus ~73%.

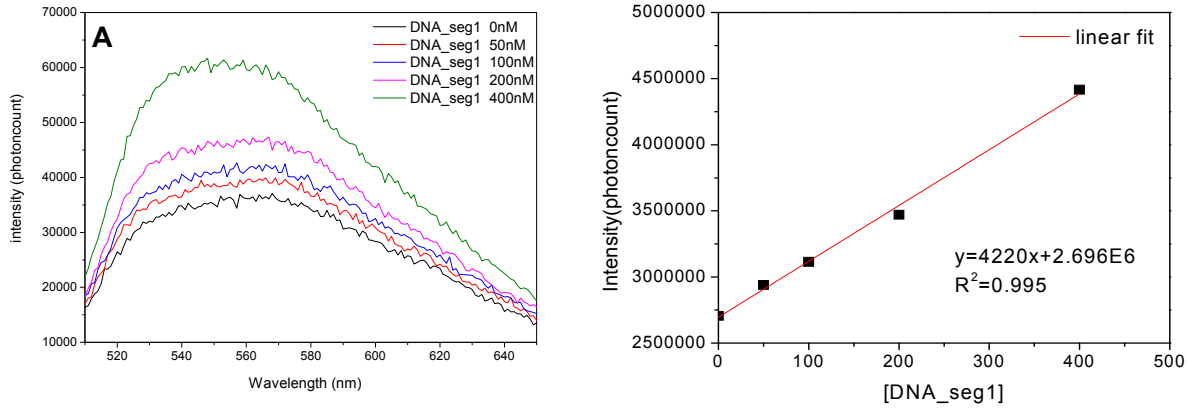

Figure S3. SG1 fluorescence emission excited at 490 nm with different amounts of DNA_seg1 (A), and a calibration curve (B).

Luminescence measurement at 980 nm excitation

One hundred µL of UCNP-DNA_seg1 mixture after respective thermal cycles was mixed with 50 µL of 0.1 M EDTA solution for 30 min, and washed twice by DI water before dispersed in 200 µL DI water.

The solution was then mixed with 200 µL of 37 µM SG1 solution in 100 µM sodium phosphate buffer solution (pH 7.8). The solution was placed in a quartz cuvette and put in the cuvette holder of the spectrofluorometer (Photon Technology International, NJ), which is equipped with a customized excitation source of a 980-nm laser (Laserglow Technology, Toronto). Emission signals were measured with a slit width of 10 mm.

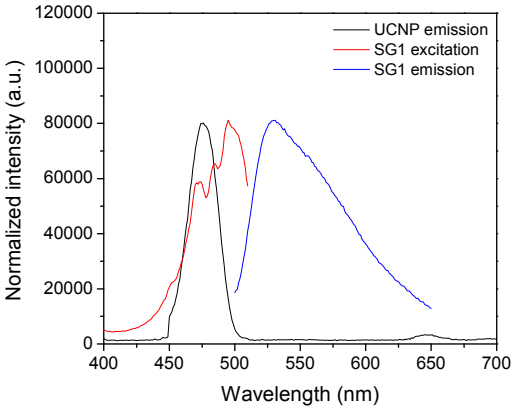

Figure S4. Normalized excitation and emission spectra of SG1 and emission spectrum of $NaYF_4$:Yb^{3+}, Tm^{3+} upconversion nanoparticles excited at 980 nm.