Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics
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Electronic Supplementary Information

Magnetic actuation of ss-DNA release from nanoparticle clusters

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Synthesis of $CoFe_2O_4$ nanoparticles

$CoFe_2O_4$ nanoparticles were prepared introducing minor modifications to the Massart method, as previously reported. In summary, 32 ml of a 1M $FeCl_3$ solution and 16 ml of 1M $Co(NO_3)_2$ were added to 1 ml of concentrated nitric acid. The mixture was heated to boiling and then quickly mixed under vigorous stirring with a boiling 1M NaOH solution (200 ml). The mixture was maintained at the boiling temperature under stirring for 90 minutes. The particles were separated by magnetic high decantation, washed with water and added to 2 M HNO_3 (20 ml). The precipitate was again separated by magnetic decantation, then dispersed in a boiling solution obtained by dissolving 0.5 M $FeCl_3$ (28 ml) and 0.5 M $Co(NO_3)_2$ (14 ml) and kept under vigorous agitation for 30 minutes. The precipitate obtained after this treatment was isolated, washed with HNO_3 1M (15 ml) and dispersed in water. Fe and Co contents of the dispersion were checked by Inductively Coupled Plasma Emission spectrometer (ICP-AES).

Fe=2.88 mg/ml; Co=1.08 mg/ml

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Synthesis of Au@$CoFe_2O_4$ nanoparticles

A gold shell was formed on the magnetic core, obtained as previously described, by reduction of $HAuCl_4$ with $NaBH_4$, according to a known procedure (Lyon, J. L.; Fleming, D. A.; Stone, M. B.; Schiffer, P.; Williams, M. E. *Nano Lett.* **2004**, *4*, 719–723). A 10mM $HAuCl_4$ solution (320 μl) was added under agitation to a 4.6 mM solution $CoFe_2O_4$ NPs (880 μl) diluted with 800 μl of water. The reduction was performed adding drop by drop a freshly prepared 10mM $NaBH_4$ solution (kept in a bath of cold water at 4°C) under vigourous stirring. The dark brown solution of Cobalt ferrite NPs turned immediately purple upon addition of the reducing agent and gradually changed to deep pink. The nanoparticles were magnetically separated and washed with water to remove the unreacted reagents or byproducts. The precipitate was then redispersed in 1500 μl of a buffer solution of sodium citrate 1.3 mM (pH 4.5) and sonicated for 10 min. Fe, Co and Au contents of Au@$CoFe_2O_4$ dispersion were checked by inductively coupled plasma emission spectrometer (ICP-AES).

Fe = 1.08 mg/ml; Co = 0.52 mg/ml; Au = 0.18 mg/ml

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EDX of Au@$CoFe_2O_4$ NPs

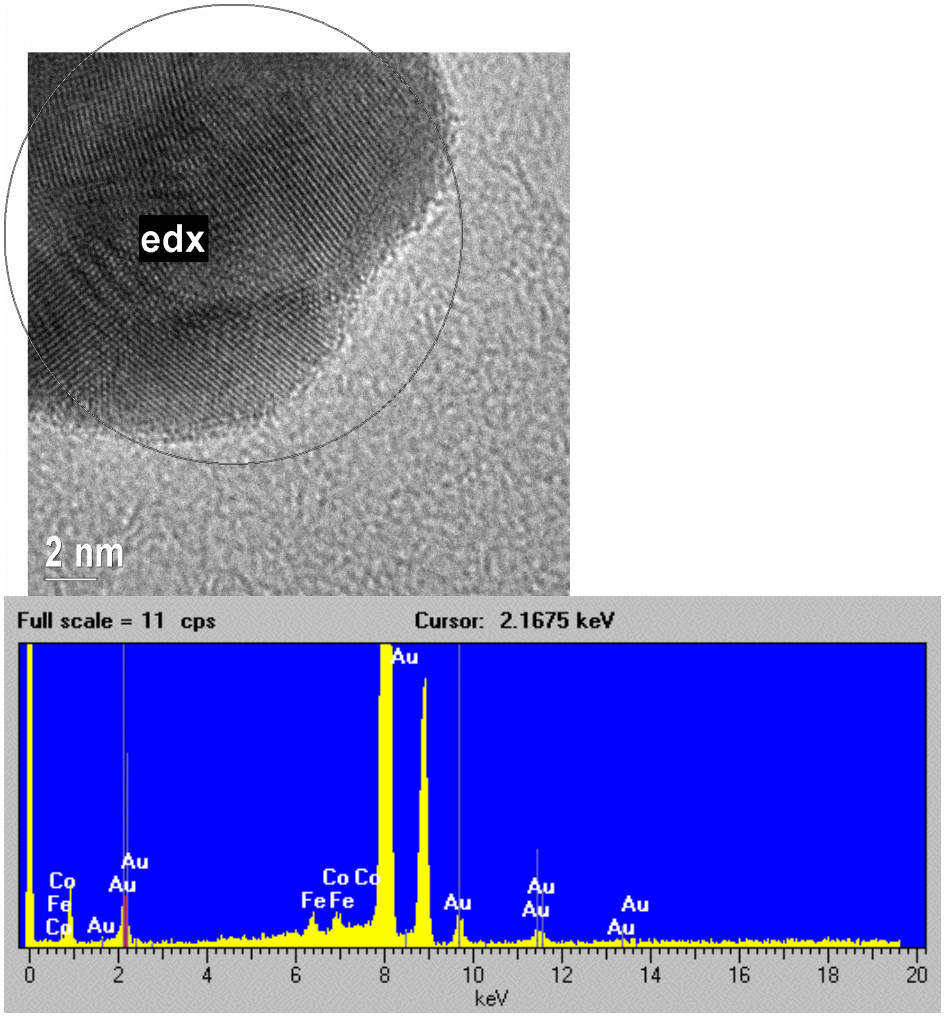

Fig ESI-1. HRTEM images and EDX analysis of Au-coated $CoFe_2O_4$ nanoparticles

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DLS results

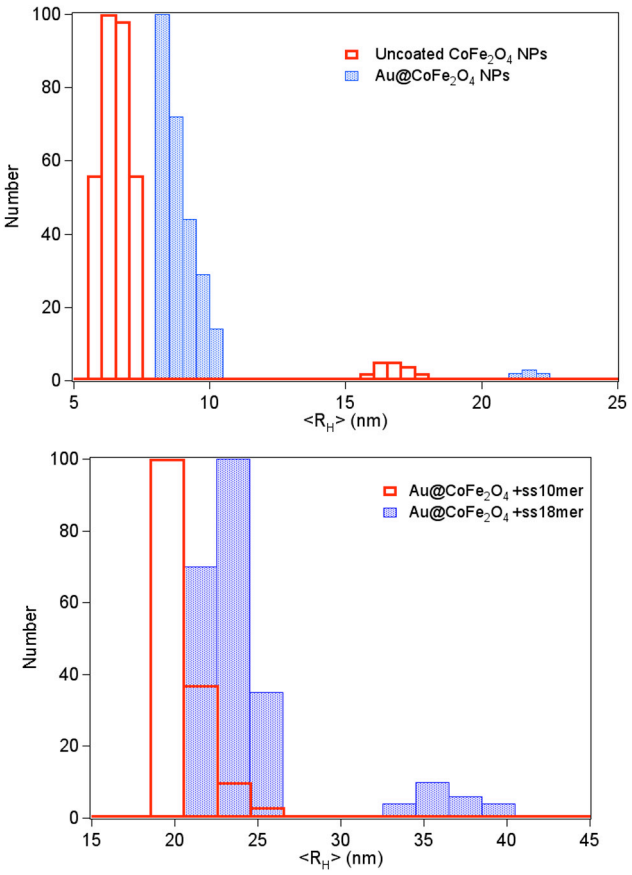

Figure ESI-2. Size distributions of (top) uncoated CoFe2O4 and Au@CoFe2O4 nanoparticles, and (bottom) ss10mer–Au@CoFe2O4 and ss18mer-Au@CoFe2O4 dispersions.

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Fluorescence of RhBITC

Rhodamine B isothiocyanate (mixed isomers) was purchased from Sigma Aldrich. All the solutions were prepared by using MilliQ water. The fluorescence of each sample was recorded using a spectrofluorometer ($\lambda_{excitation}$ ~ 561 nm).

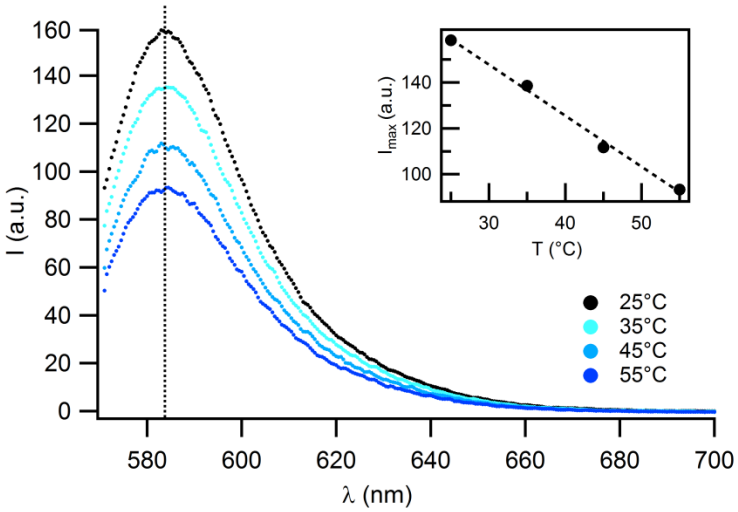

Figure ESI-3. Fluorescence emission spectra of RhBITC (Rhodamine B isothiocyanate) 20 µM at T=25°C, 35°C, 45°C, 55°C, excited at λex 561 nm. (Inset) Fluorescence intensity peak values ($\lambda_{emission}$ = 583 nm) trend vs temperature.

UV-Vis Absorption

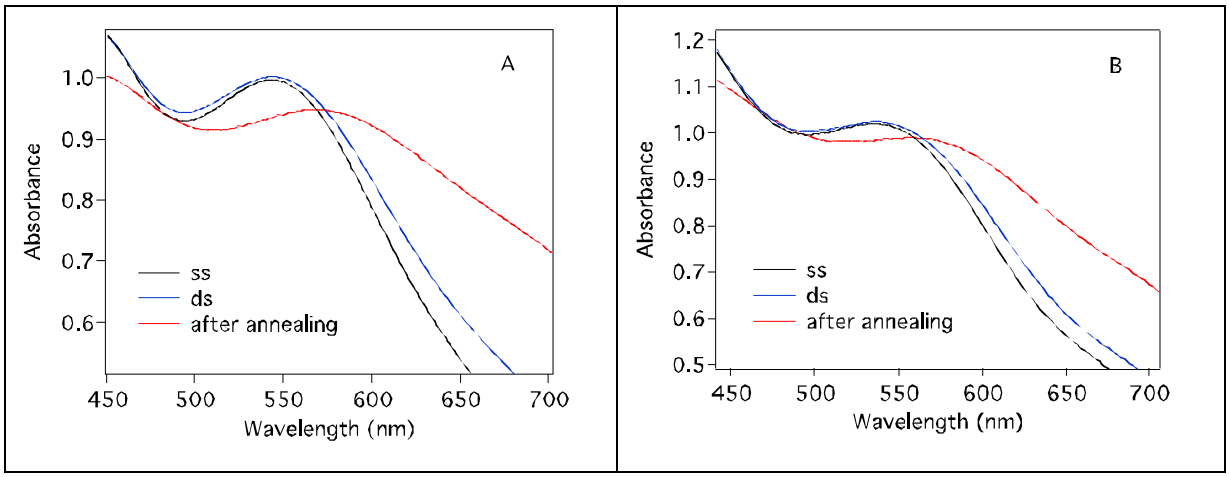

Figure ESI-4. Plasmon absorbance band shift for ssON-NPs, dsDNA-NPs before and after annealing, with (A) ss10mer (ss) and ss10mer+ss21mer (ds) and (B) ss18mer (ss) and ss18mer+ss37mer.

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EDX of ssDNA coated Au@$CoFe_2O_4$ NPs

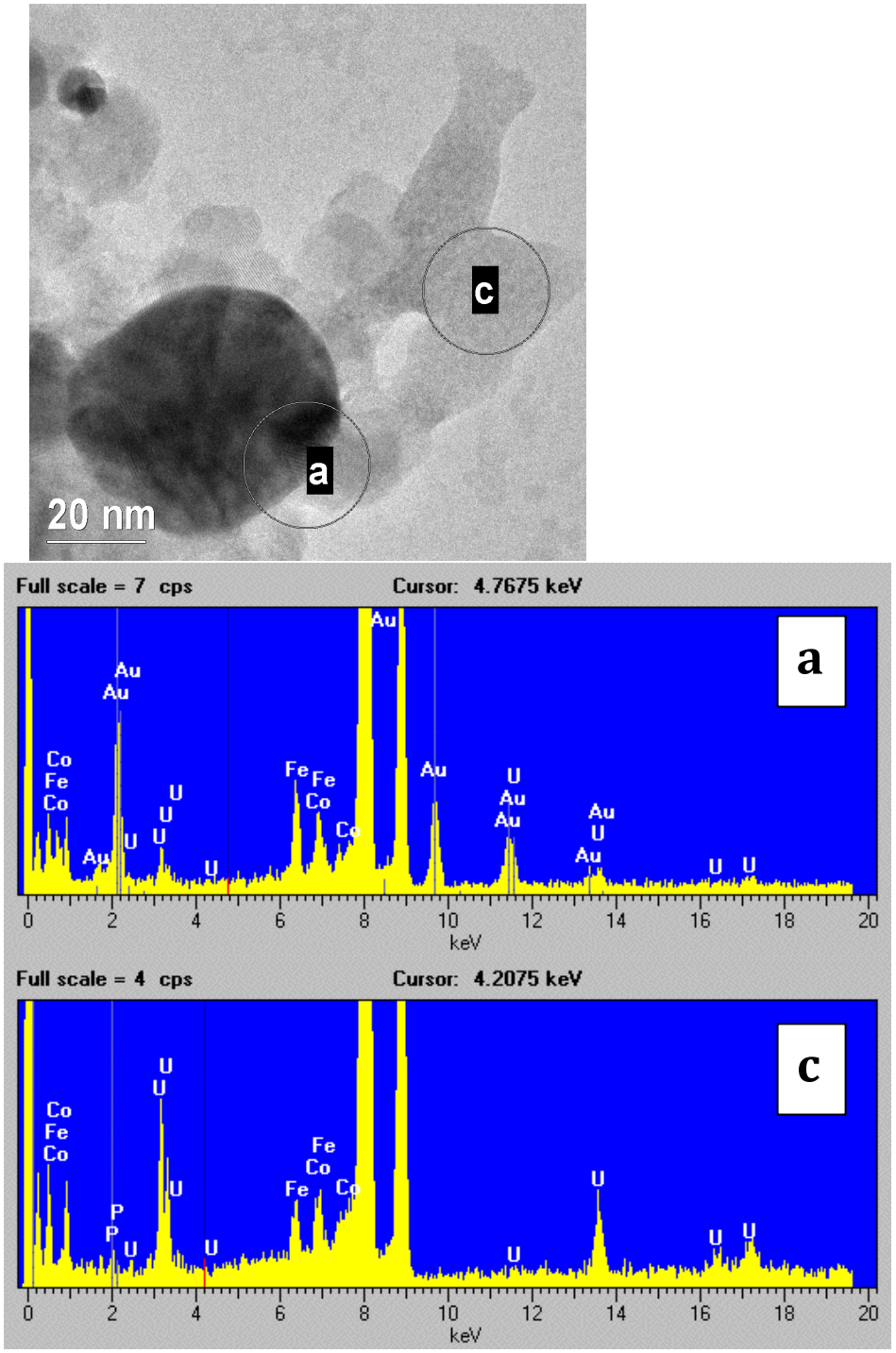

Fig. ESI-5. HRTEM images and EDX spectra of *ss*DNA coated nanoparticles

Thermal Cycling

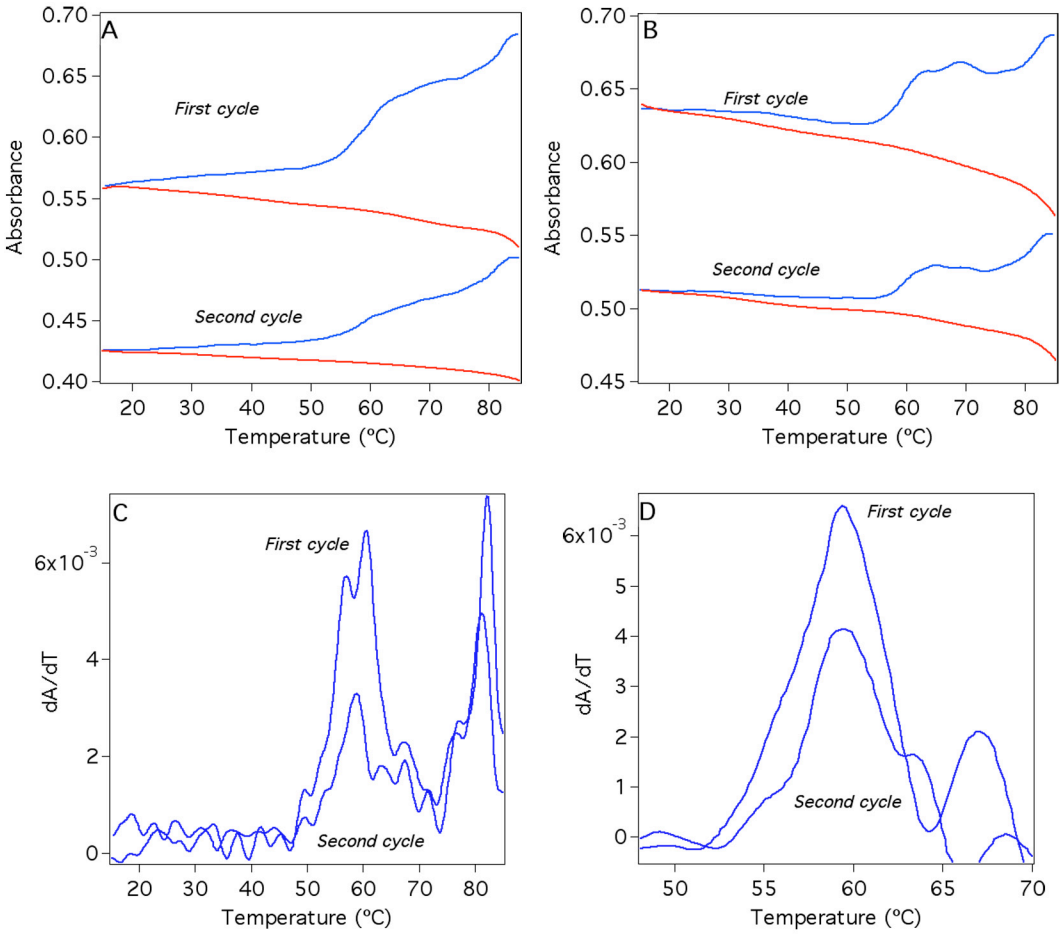

Figure ESI-6. Sequential temperature cycles (A and B, heating ramps in red, cooling ramps in blue) and the respective derivatives of the cooling ramps (C, D) for the clusters obtained with ss10mer+ss21mer (A, C) and 18mer+ss37mer (B, D).

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Quant-iT™ OliGreen® ssDNA Reagent and Kit

Quant-iT™ OliGreen® ssDNA reagent was purchased from Invitrogen. Quant-iT™ OliGreen® ssDNA reagent is an ultra-sensitive fluorescent nucleic acid stain to detect low concentrations (as low as 100 pg/ml) of oligonucleotides and ssDNA in solution. The assay was used to determine the ss18-mer and ss10-mer bound to NPs and to quantify the staple released after LF-AMF exposure.

A calibration curve from 10 ng/ml to 1 μg/ml of ss-DNA was generated for each oligonucleotide (ss18-mer, ss10-mer and staples) using the experimental protocol. Briefly, different aliquots of 2 μg/ml of ss-DNA stock solutions were diluted with TE buffer (10 mM Tris, adjusted to pH 8.0 with HCl, 1 mM EDTA) and 1 ml of OliGreen® reagent was added to each cuvette. After 3 minutes of incubation, the fluorescence of each sample was read using a spectrofluorometer ($\lambda_{excitation}$~480nm; $\lambda_{emission}$~520nm). Each value was corrected by subtraction of the fluorescence of the blank (sample without oligonucleotides). The fluorescence of each sample, diluted with TE buffer, was measured after 3 minutes of incubation with 1 ml of OliGreen® reagent and the corresponding concentrations were derived from the calibration curve.

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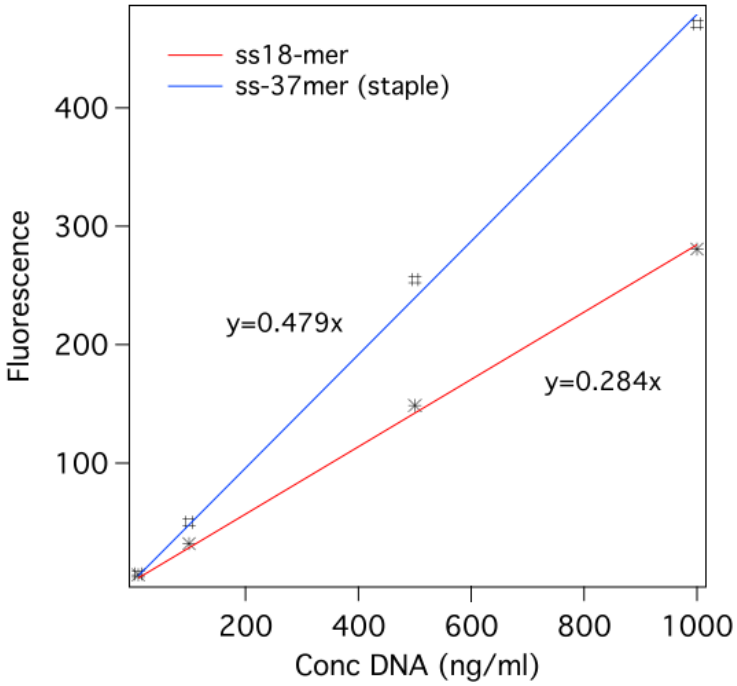
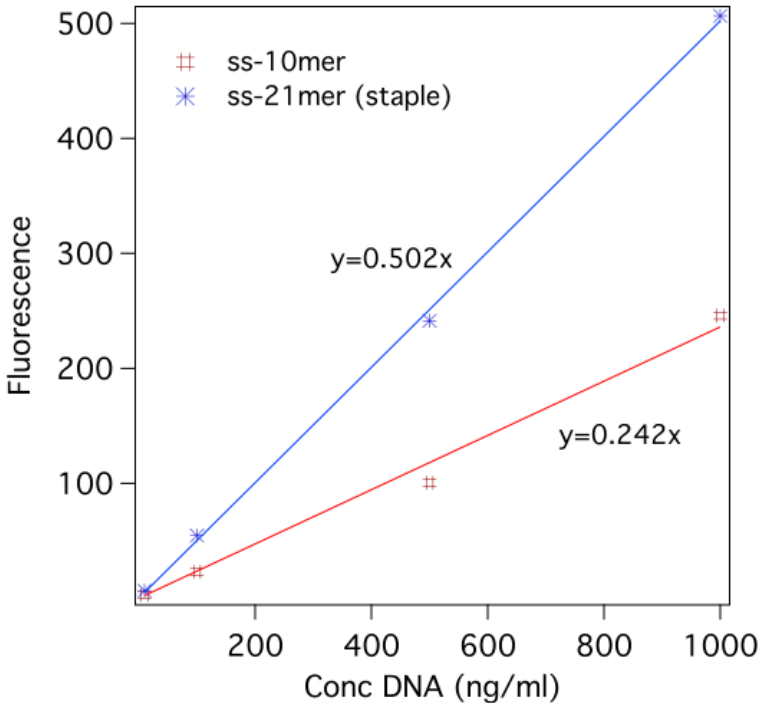
Calibration Curves of ss18-mer and staple (OliGreen)



Calibration Curves of ss10-mer and staple (OliGreen)



The quantity of of ss-18mer and ss-10mer bound to the MNPs were determined by

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comparison of the values detected in supernatants with the initial known concentrations.

This indirect procedure was adopted because the fluorescence of OliGreen® ss-DNA reagent can not be measured in the presence of $CoFe_2O_4$@Au NPs, due to bleaching effects from the gold shell.

The amount of staple released after the magnetic field treatment was evaluated by testing the supernatant solution.

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Quant-iT™ PicoGreen® dsDNA Reagent and Kit

Quant-iT™ PicoGreen® dsDNA reagent was purchased from Invitrogen. Quant-iT™ PicoGreen® dsDNA reagent is an ultra-sensitive fluorescent nucleic acid stain to detect low concentrations (as low as as 25 pg/13l) of dsDNA in solution. This assay was used to determine the concentrations of dsDNA (ss-18mer and ss-10mer paired with the corresponding staples) released after LF-AMF exposure.

Similarly to the OliGreen assay, a five-point calibration curve from 1 ng/mL to 1 μg/ml of ds-DNA was generated for each sample (ss18-mer/staple 2:1, ss10-mer/staple 2:1) using the experimental protocol. The fluorescence of each sample was monitored with a spectrofluorometer ($\lambda_{excitation}$~480 nm; $\lambda_{emission}$~520 nm) and corrected for the reference value (sample without oligonucleotides). A standard curve of fluorescence versus ds-DNA concentration was built and used for the determination of ds-DNA concentration in the supernatant after magnetic treatment.

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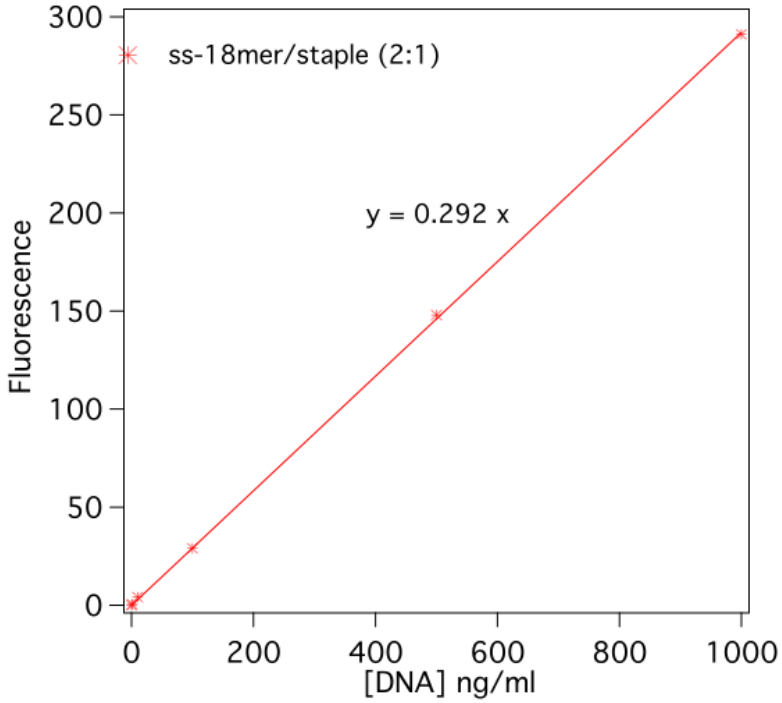
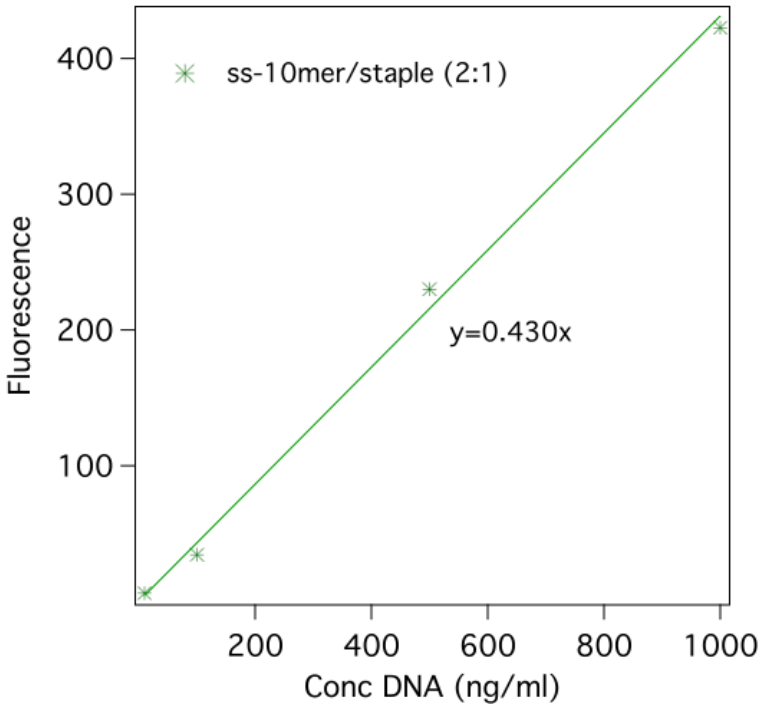
Calibration Curve ss-18mer/staple (2:1) (PicoGreen)



Calibration Curve ss-10mer/staple (2:1) (PicoGreen)



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Preparation of GUVs and experimental details

Giant Unilamellar vesicles (GUVs) were prepared using the electroformation method, originally developed by Angelova and Dimitrov [Angelova, M. and Dimitrov D. S. Biophysics of Membrane Transport, **1988**: 305-305. Angelova, M.I; Soleau, S.; Meleard, P.; Faucon, J. F.; Bothorel, P. Trends in Colloid and Interface Science, **1992**. **89**: 127-131]. A home-built chamber was set up sandwiching two Indium Tin Oxide (ITO)-coated microscope slides, separated by an O-ring spacer [Nappini, S.; Al Kayal, T.; Berti, D.; Nordén, B.; Baglioni, P. *J. Phys. Chem. Lett.* **2011**, 2, 713-718.]. The electrical connection with both sides of the capacitor was obtained by directly attaching two Cu tapes on the conducting faces. The lipid (POPC, 3 mg/ml) was dissolved in chloroform with 0.1% mol of the fluorescent probe Rhodamine DHPE, purchesed from Avanti Polar Lipids (excitation and emission wavelengths 560 and 580 nm, respectively). 10 μl of POPC solution was spread on each conducting face of ITO-coated microscope slides and dried under vacuum for at least 2h to remove the solvent. The O-ring was positioned around the film, and the two slides were sandwiched to form a chamber filled 350 μl of solution containing the Au@$CoFe_2O_4$-DNA clusters (Au final concentration ≈ 0.35 mM) and a PicoGreen solution diluted 30 times with sucrose 0.23M. Finally, the chamber was connected to a function generator and a low-frequency AC electric field (sinusoidal wave with a frequency of 10 Hz and amplitude of 2 V) was applied for 3 h. GUVs growth was monitored by optical microscopy and, when complete, the solution was gently removed from the electro-formation chamber. To reduce the fluorescence from PicoGreen-dsDNA not confined within the GUVs, the sample was diluted 1:50 with an iso-osmolar solution of glucose 0.23 M. The density difference between sucrose (inside the vesicles) and glucose (outside) lead to GUV deposition, helping in microscopy observation. The diameter of the vesicles obtained with this method ranged from 5 to 50 μm. The samples were placed in home made cells: the bottom plate was a microscopy coverglass (LAB-TEC, 8 Well) and the cell wall was a cylinder of glass (diameter 8 mm, height 1.5 cm). The release of the staple following the application of LF-AMF, was monitored by measuring the fluorescence intensity decrease of the PicoGreen probe in the aqueous pool of the GUVs.

To evaluate the effect of the laser irradiation on the bleaching of the fluorescent probe, the laser beam was alternatively switched on and off, as shown in Figure SI-8.

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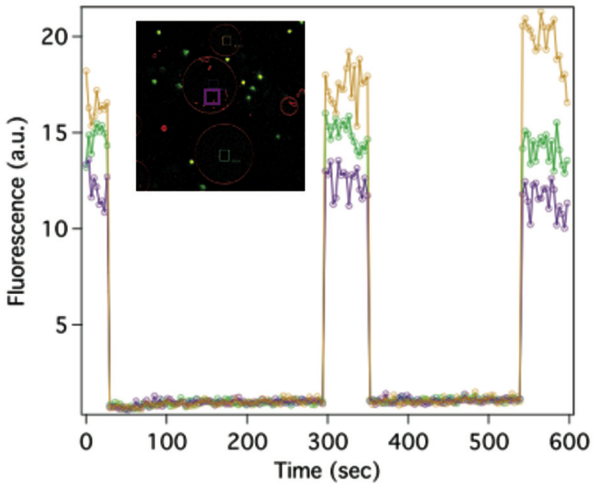

Figure ESI-7. Effect of laser irradiation on the fluorescence intensity inside three different GUVs as the beam is alternatively switched on and off.

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Circular Dichroism

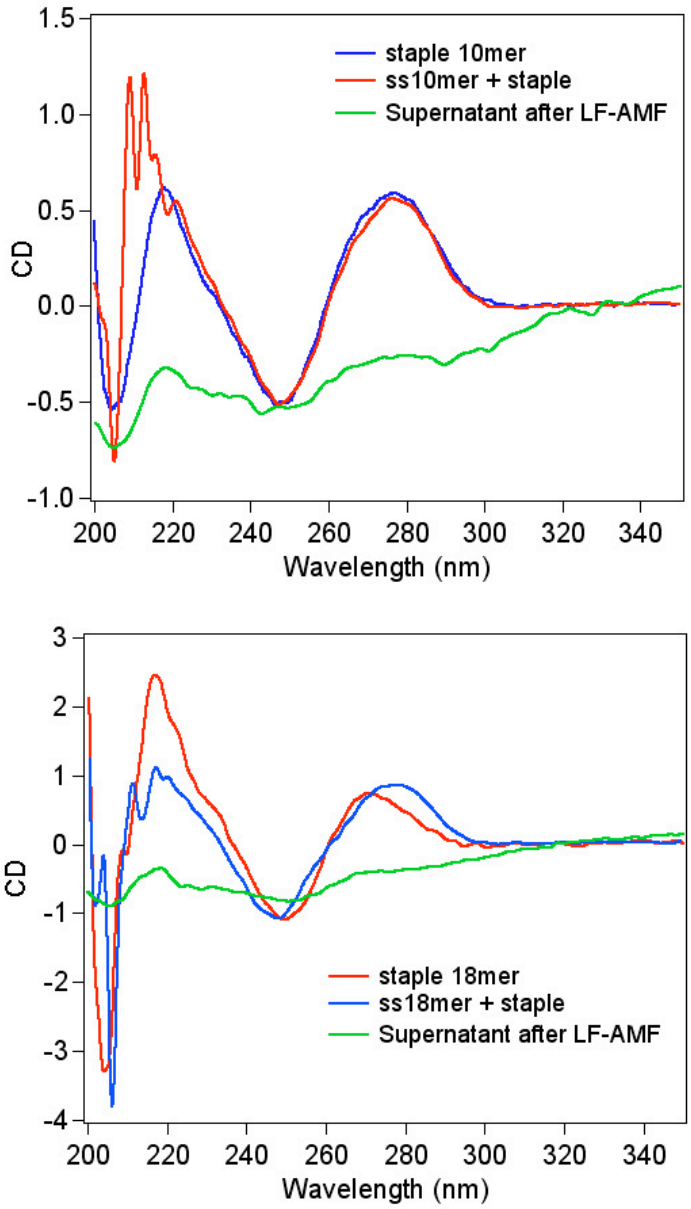

Figure ESI-8. Circular Dichroism of staple solutions (red), staple solutions mixed with their respective target *ss*DNAs (blue) and supernatants (green) after the application of the magnetic field.

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Alternating Magnetic Field Setup

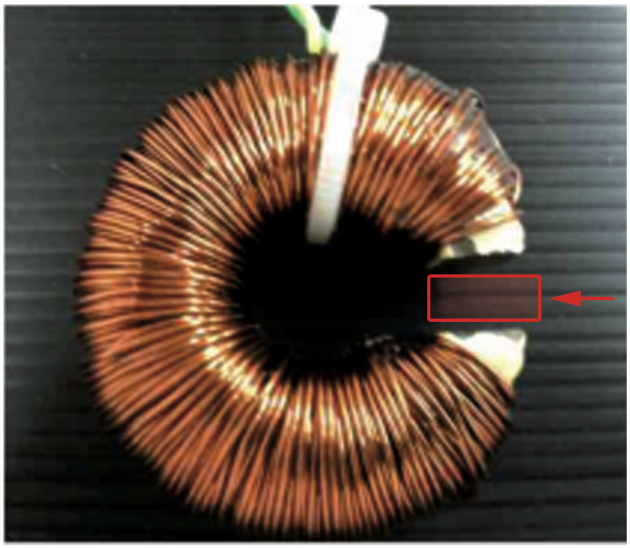

Figure ESI-9. Picture of the broken toroidal magnet used to apply the LF-AMF. The position of the sample during the LF-AMF treatment is indicated by the red arrow.

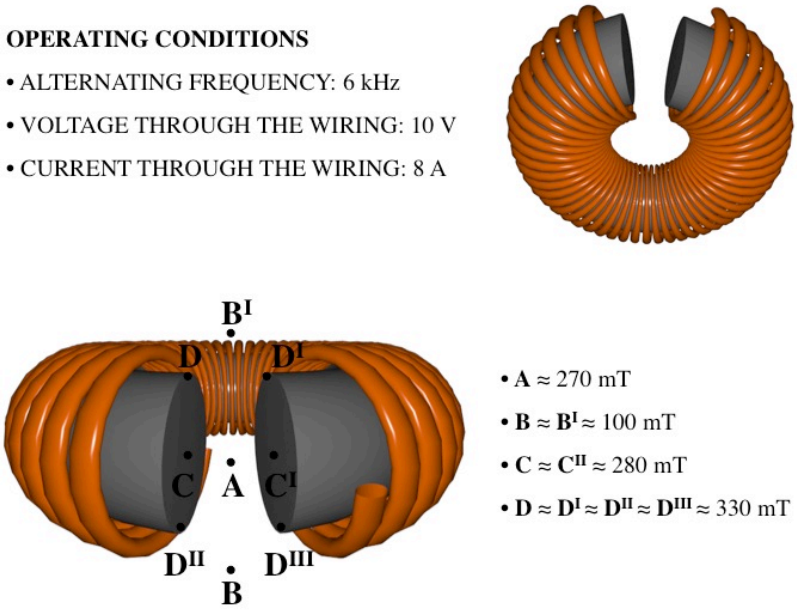

Figure ESI-10. Operating conditions and magnetic field values at different positions of the broken toroidal magnet used to apply the LF-AMF as measured by means of a GM-07 Gaussmeter (HIRST Magnetic Instruments Ltd, UK).