Electronic Supplementary Material (ESI) for RSC Advances
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Electronic Supplementary Information

S1. Materials and methods

All reagents and solvents were obtained from commercial suppliers and used without further purification.

High Pressure High Temperature (HPHT) ND (mean diameter: 20 nm) were purchased from Van Moppes (Syndia SYP 0-0.02). Peptide Nucleic Acid (NH_2-TTTTTTTTTT-Gly-COOH and NH_2-A18-resin/Bhoc) were purchased from Panagene. Labeled DNA sequences polyadenine- Cyanine3 with 10 bases and polythymine- fluorescein with 10 bases) were obtained from Sigma-Aldrich.

FT-IR spectra were measured in a transmission mode using a Thermo Nicolet 8700 spectrometer. 150 mg KBr pellets were prepared with 3 mg of NDs. For 24 hours before the measurement and during the acquisition, samples are maintained under vacuum at 310 K to eliminate adsorbed water.

The thermogravimetric analyses were performed using a TGA Q50 TA instrument with a ramp of 5ºC/min under N2.

Fluorescent images were acquired by using a Zeiss Axiovision 4 microscope with a Canon camera. 2 fluorescent set were used (Zeiss set filter 17 et 20 HE)

Fluorescence measurements were carried out with a Horiba Jobin-Yvon fluorospectrometer (Fluoromax 4, 150W xenon lamp).

NDs conjugation with peptide nucleic acid

This general procedure was applied for the grafting of PolyT and PolyA. NDs (10 mg) were suspended in DMF and neutralized with diisopropylethylamine (DIEA). N-Hydroxysuccinimide (NHS) (17 mg, 148 µmol) and ethyl(dimethylaminopropyl) carbodiimide (EDC) (30 mg, 156 µmol) were added to the solution. The reaction was stirred during overnight. The crude NDs were extensively washed with DMF and ether to eliminate the excess of the coupling agents. Then, NDs were suspended in DMF and neutralized with DIEA. PNA (100 nmol) (4) was dissolved in DMF and added to the solution with NDs. The reaction was stirred during 24 hour. The product was purified by subsequent reprecipitation with DMF and ether. The black solid was dried. The purified nanodiamonds conjugated with peptide nucleic acid were characterized by Kaiser Test, TGA and FT-IR. Only for PolyA coupling, this procedure was followed by a last reaction in TFA/DCM in order to deprotect the lateral amino group (Boc protection) and release ND-PNA from the resin.

Several parameters such as solvent type (dimethylformamide, dioxane, water), temperature (0°C to RT), reactive order during the reaction were tested in order to optimize the reaction.

Synthesis of ND-NH_2

NDs (10 mg) were suspended in DMF and neutralized with diisopropylethylamine (DIEA). N-Hydroxysuccinimide (NHS) (17 mg, 148 µmol), ethyl(dimethylaminopropyl) carbodiimide (EDC) (30 mg, 156 µmol), N-Boc-ethylenediamine (21 mg, 131 µmol) were added to the solution. The reaction was stirred overnight. Then, Boc-protected NDs was extensively washed with DMF and ether, and dried. Then Boc was removed using a mixture of TFA/DCM 1:1 during 1 hour. ND-NH_2 were recovered, extensively washed by subsequent reprecipitation with water and ethanol and dried.

Hybridization of ND-PNA

1 mg of ND-PNA was dispersed in Phosphate Buffer Solution (PBS). The solution were prepared with 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride .7 µL of a 100 µM water solution of DNA-Cy3 were added and the

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solution was stirred during 1 hour. Functionalized NDs were extensively washed with PBS buffer and examined under a fluorescent microscopy

Same hybridization conditions were used to study the recognition of ND-PNA to labeled DNA mixture.

Nanoparticles PS-NH_2

Orange fluorescent 52 nm aminated polystyrene nanobeads (λex: 488 nm, λem: 550-90 nm) (PS-NH2 Ref. AMOF050NM) were from Magsphere Inc. PS-NH_2 nanobeads were suspended at a concentration of 1 mg/mL in DMEM medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin (Invitrogen).

Cell culture and flow cytometry

Human A549 cells (ATCC number: CCL-185) were routinely grown at 37°C in a humidified atmosphere of 5% CO2 and 9 % air, in Dulbecco's modified Eagle medium (DMEM) glutamax supplemented with 10% (v/v) inactivated fetal bovine serum and 1 mM antibiotic-antimycotic (Invitrogen). This medium is considered as complete DMEM. For nanoparticles exposure, $1.5x10^5$ A549 cells were seeded at least 24 h before exposure into 6 well micro plates. Then cell culture medium was removed and nanoparticles suspensions were added on each well for treatment. After 24 h of exposure, cells were washed and trypsinized for 5 min. Trypsin was inactivated by adding complete medium, cells were centrifuged for 5 min at 300xg and then resuspended in 500 µL of medium with serum in flow cytometry-compatible tubes (BD 352058). Multi-parametric analyses were performed on BD Facscalibur using FlowJo 7.5.5 software. A first analysis was done on size/granulometry parameters, to collect living and dead cells and to remove fragmented cells (Fig S2a). This first step allowed us to determine the gate where at least $2x10^4$ events per replica were recorded. Then upon these gated events, the To-Pro3 (Molecular Probes, Invitrogen) signal was collected on FL4 (λem: 661/16 nm) after He-Ne laser excitation at 636 nm and was used for the analysis of cell viability since this dye is compatible without interference with the equipment and with nanoparticle detection. The results were reported as the mean distribution of cell fluorescence, obtained for 3 replicas (with at least $2x10^4$ gated events per replica). Combining SSC and To-Pro3 measurements, a bi-parametric representation was then possible and distinguished three kinds of cell populations: living cells without nanoparticles (To-Pro3-negative/SSC-negative cells), living cells with nanoparticles (To-Pro3-negative/SSC-positive cells) and dead cells (To-Pro3-positive) (Fig S2a). It is not pertinent to make the distinction between dead cells with or without nanoparticles since, depending on the mechanisms of cell death and the stage of death, both alteration of membrane fluidity and membrane disruption could lead to loss of nanoparticles. To-Pro3-positive and SSC-positive cut-offs were determined for events recorded in the gate in control cells.

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S2 Cytotoxicity study of A549 cells exposed 24 h to ND-PNA.

Flow cytometry bi-parametric analysis (a) was performed to detect NDs (channel SSC) and to evaluate the mortality (channel FL4). Cut-offs were defined on the control condition (non-exposed cells). An example of one representative experiment (among 3 independent experiences) is reported on panel (b) for the detection of mortality after 24h of exposure to ND-PNA. Panel (c) represent one representative experiment (among 3 independent experiences) for the detection of mortality after 24 h of exposure to PS-NH_2 nanobeads (positive control). Finally panel (d) represent the overall results for the detection of ND-PNA and the associated mortality for A549 cells exposed 24 h to ND-PNA. Results are represented as the mean value of 3 independent experiences, the analysis of each experiment being performed on $2x10^4$ events.

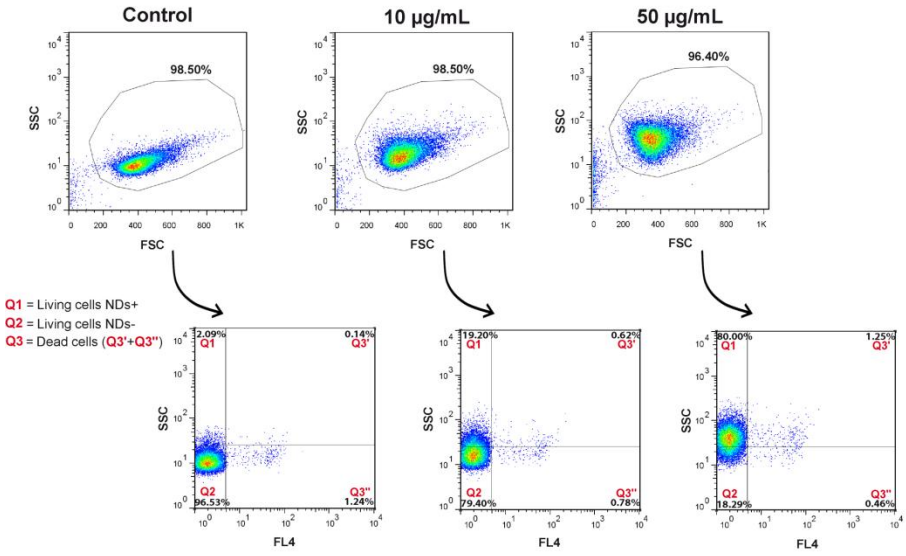

Fig S2 a: Bi-parametric analysis (ND-PNA detection/mortality)

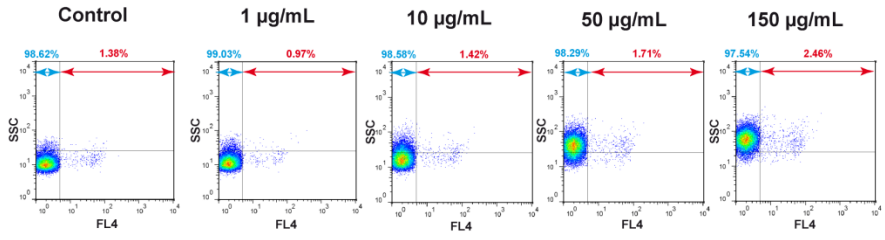

Fig S2 b: Detection of ND-PNA mortality at 24 h

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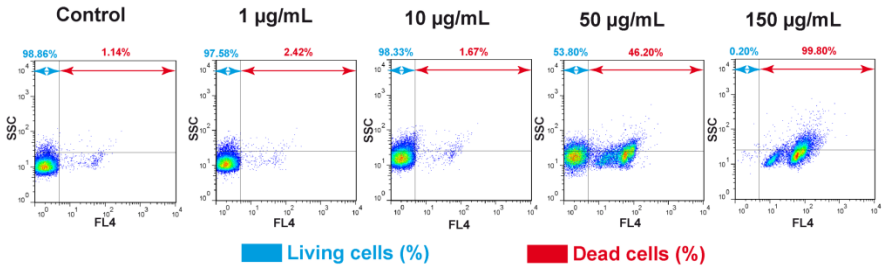
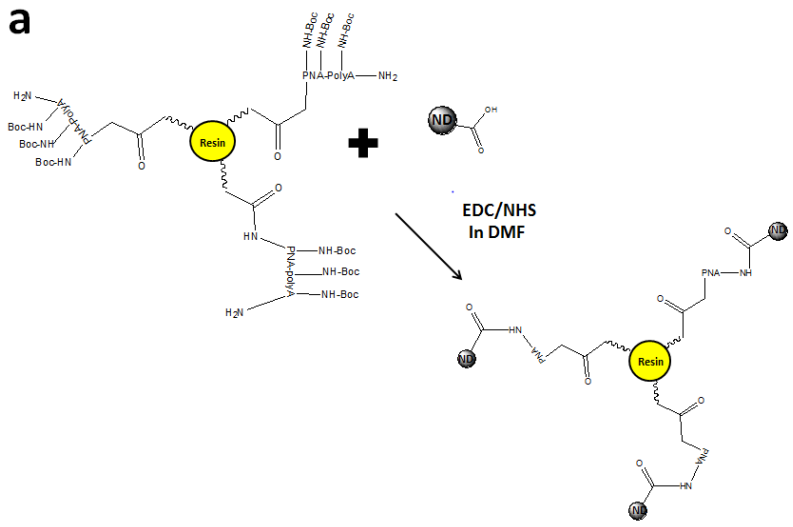


Fig S2 c: Detection of PS-NH_2 mortality at 24 h

S3 PolyA coupling onto NDs



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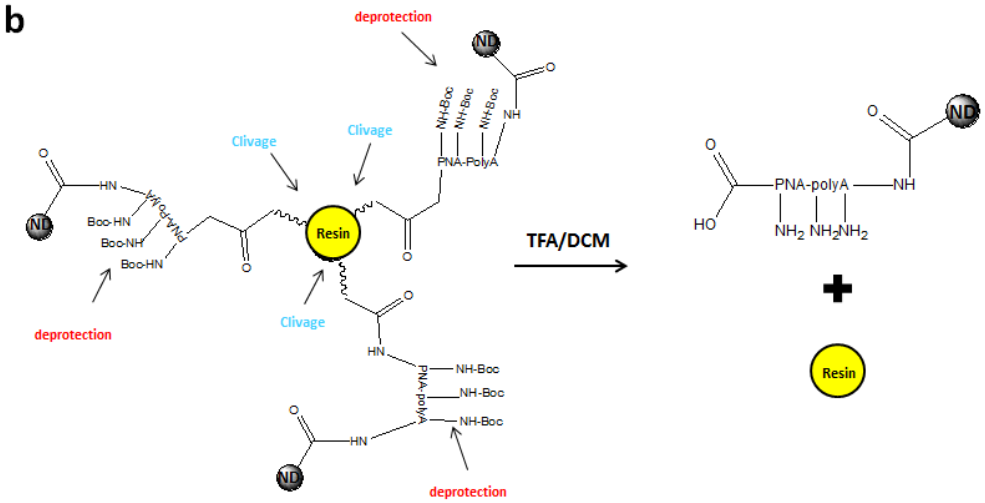

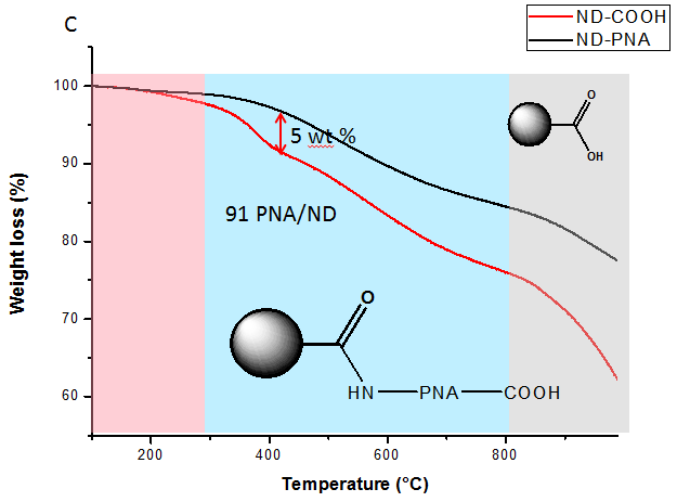

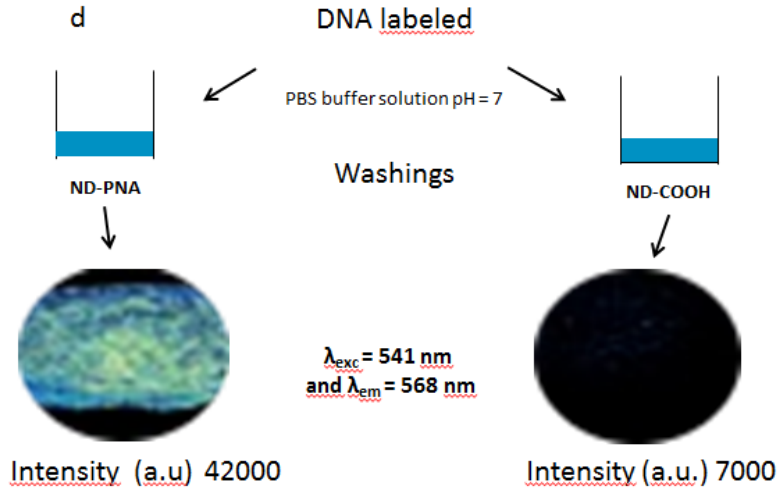

Additional figures :

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a & b : synthesis schemes of ND-Peptide Nucleic Acid polyA conjugates

c : Thermogravimetric Analysis (TGA) of ND as received and ND-polyA

d: Fluorescent microscopy pictures of ND-COOH and ND-PNA after hybridization with a fluorescent polyT sequence