Electronic Supplementary Material (ESI) for Analyst.

Supplementary data

Highly labeled methylene blue-ds DNA silica nanoparticles for signal enhancement of immunoassays: application to the sensitive detection of bacteria in human platelet concentrates

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Experimental Section

MB-DNA NP assembly synthesis

Oligonucleotides were synthesized using standard phosphoramidite chemistry with ultra-mild nucleobase protecting groups on an Applied Biosystems 394 RNA/DNA synthesizer at 1 μmol scale. All automated synthesis reagents including 0.05M potassium carbonate in methanol, 9-O-Dimethoxytrityl-triethylene glycol 1-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Spacer Phosphoramidite 9) and 3-dibutylamino-7-[(3-(4,4'-dimethoxytrityloxy)propyl)(3-[(2-cyanoethyl)(N,N-diisopropyl)phosphoramidityl]propyl)amino]phenothiazin-5-ium chloride (methylene blue phosphoramidite) were provided by Glen Research (Sterling, Virginia, USA). Biotin phosphoramidite was ordered from Sigma-Aldrich.

Absorbance measurements and thermal analyses were performed on a Varian Cary 100 Bio UV-Visible spectrophotometer (Agilent technologies, Santa Clara, CA) equipped with a Peltier temperature controller. Quantification of oligonucleotides was performed according the equation in Fig. S8, with absorbance measured at 260 nm and molar extinction coefficient estimated by the nearest neighbor model method ($\varepsilon_{260\ nm}$ = 295 200 $M^{-1}.cm^{-1}$ for sequence T_5CAT CAA CAG ACG AAC CAT_{10}, $\varepsilon_{260\ nm}$ = 335 700 $M^{-1}.cm^{-1}$ for sequence T_5CAT CAA CAG ACG AAC CAT_{15}, $\varepsilon_{260\ nm}$ = 376 200 $M^{-1}.cm^{-1}$ for sequence T_5CAT CAA CAG ACG AAC CAT_{20}). The molar extinction coefficient of the complementary sequence TGG TTC GTC TGT TGA TG was $\varepsilon_{260\ nm}$ = 156 200 $M^{-1}.cm^{-1}$.

RP-HPLC analyses and purifications were performed with an Agilent 1200 series high performance liquid chromatography (HPLC) using a Lichrospher RP18, 5 μm, WP300 column with 1 $mL.min^{-1}$ flow rate of mobile phase. Analyses were monitored at 260 nm and 677 nm using a linear gradient in 30 min from 100% solvent A (0.05 M triethylammonium acetate buffer (TEAAc), pH 7) to 100% solvent B (water/acetonitrile 1/1, 0.05 M triethylammonium acetate buffer, pH7).

Electrochemical probe synthesis

Methylene blue labeled oligonucleotide synthesis

DNA electrochemical probe (MB-PEG-MB-PEG-MB-PEG-TGG TTC GTC TGT TGA TG) was obtained using automated DNA synthesis at 1 µmol scale incorporating commercially available spacer-9 phosphoramidite (PEG) and methylene blue phosphoramidite (MB). The coupling times were respectively 60s and 180s for each modifier. After synthesis, the oligonucleotide was cleaved from the support with 16h treatment in 0.05M K_2CO_3/methanol. After concentration on 4K Amicon ultra filters, the oligonucleotide was purified by RP-HPLC. The fractions were collected at the retention time of 27.1 min, concentrated to dryness by evaporation under vacuum. Quantification was performed by UV-Visible spectrophotometry at 260nm (89nmol, MALDI-TOF m/z $[M]^-$: found 7429.5, calcd: 7426.7).

Nanoparticle functionalization

Hydroxyl functionalization of fluorescent nanoparticles

Rhodamine-labeled silica nanoparticles were functionalized according to previously reported procedures. Briefly 3-(triethoxysilyl)propyl isocyanate (2.3mL, 9.26mmol) was slowly added to a solution of 6-Aminohexan-1-ol (9.26mmol, 1.08mg) in isopropanol (2.5mL) under argon atmosphere in order to obtain a freshly prepared solution of 3-(triethoxysilyl)propyl-hydroxyhexyl urea (TESPHU). After 30 min stirring at room temperature, the solution was then added dropwise to the silica nanoparticle solution (NP, 190mg) in DMF (20mL, $8.3.10^{13}$ part.mL^{-1}) with triethylamine (1.3mL, 9.26mmol). This solution was finally stirred at 120°C. After 16h, the nanoparticle suspension was then centrifuged (10,000 g, 45 min), the supernatant was eliminated and the nanoparticles redispersed in DMF. This washing procedure was repeated three times. Nanoparticle concentration was then determined by measuring absorbance at 560nm ([NP] = $4.41.10^{13}$ part.mL^{-1}).

Amino functionalization of CPG support

The activation of silanols was performed by heating under reflux a suspension of controlled pore glass (CPG, 1.1g) in concentrated nitric acid (5mL) in a round-bottom flask. Then the CPG was washed with distilled water in a fritted-glass filter until the neutral pH of the washing fractions.

A freshly prepared solution of 10% (v/v) aqueous APTES (pH 2 adjusted with concentrated HCl, 8.5mL) was added on CPG in a round-bottom flask and the suspension was stirred at round temperature for 2.5

hours at 70°C. Supernatant was then removed and a siloxane stabilization process was accomplished by heating the CPG overnight at 120°C in an oven. Then the CPG were washed and dried in a fritted-glass filter by acetonitrile (3x7mL) followed by dichloromethane (3x7mL) for a quick air drying.

Hydroquinone linker[1] functionalization of CPG was carried out by a solution of HQDA (99.2mg, 0.41mmol), with DMAP (51mg, 0.42mmol), HBTU (158mg, 0.42mmol) and DIEA (154µL, 0.88mmol) in dry DMF (11mL). After 2 hour stirring at room temperature, the CPG was washed with DMF (3x7mL) and dichloromethane (3x7mL). To cap residual amine functions, CPG were stirred overnight under nitrogen in an equimolar solution of TMSCl (3.3mL, 26mmol) and HMDS (5.4mL, 26mmol). Then, the hydroquinone linker-CPG was washed with acetonitrile (3x7mL), water (3x7mL), acetonitrile (3x7mL) and dichloromethane (3x7mL).

Nanoparticle Coupling on CPG support

CPG were suspended in anhydrous acetonitrile (20mL) in a round-bottom flask with HBTU (207mg 0.54mmol), DIEA (280µL 1.60mmol) and DMAP (95.4mg, 0.78mmol) and stirred at room temperature for 2.5 hours. Then, supernatant was removed and CPG was washed with anhydrous DMF (2x5mL). The suspension of TESPHU-nanoparticles was then added (10.3mL, $4.41.10^{13}$ part.mL^{-1}) and the mixture was stirred for 16 hours at room temperature. Supernatant was removed and NP-CPG assembly was washed with DMF (2x5mL). Supernatant and washing solution were combined in a 20mL volumetric flask in order to determine the nanoparticle grafting yield at 560nm with a UV-visible spectrophotometer (69%, $2.7.10^{13}$ NP.g^{-1}).

Then, NP-CPG assembly was capped with a 1% (v/v) solution of piperidine in DMF (10mL) for 1 hour at room temperature in order to block residual activated ester functions. Extensive washing with acetonitrile and dichloromethane was then performed before drying the support with a mild air flush. Efficiency and homogeneity of the grafting was analyzed by MEB.

Nanoparticle supported functionalization

The fluorescent Nanoparticles grafted on CPG support were functionalized by automated synthesis. 1µmol scale synthesis coupling program was performed for 40 to 50 mg of NP-CPG support. To reach

a 10% yield coupling with biotin, diluted solutions of biotin phosphoramidite (10mM) and tetrazole in acetonitrile (45mM) were used and the coupling time step was reduced to 20 sec. The DNA syntheses were monitored at 498nm using dimethoxytrityl quantification.

Nanoparticle release from the support

The ultramild nucleobase protecting groups of oligonucleotides were removed by 16 hour treatment with a 0.05M K_2CO_3/methanol solution. Then, the supernatant was eliminated and the solution was washed with methanol (3x500µL) and water/acetronitrile 1/1 (v/v) solution. The nanoparticles were released from CPG by incubating the support in 1mL of 0.1% (m/v) solution of DBU in water-acetonitrile 1/1 (v/v) solution. DBU solution was stirred in a thermomixer at 30°C during 30 min before recovering. A fresh DBU solution was added to the CPG suspension every 30 min. The release kinetics were followed by quantifying nanoparticle concentration in each DBU solution with UV-visible measurement at 560nm. Released nanoparticles were washed with water (1x4mL then 3x2mL) and concentrated on 30K Amicon Ultra filter (5000g, 10 min).

After DNA synthesis, the ratio of DNA per NP was estimated using UV-visible spectrophotometry comparing absorbance at 260nm and 560nm. The NP integrity was confirmed by TEM**.**

Quantification

The amount of DNA grafted onto nanoparticles was quantified by measuring absorbance in water (200µL) at 260nm and 560nm with a microplate reader. Nanoparticle concentration was estimated from calibration curves obtained at 560 nm (rhodamine absorbance). DNA concentration was recorded at 260 nm according to the nearest neighbour model (see supporting information). Two adjustments were made to obtain the exact concentration. Firstly, nanoparticle absorption at 260nm was subtracted using the calibration curves. Secondly, the molar extinction coefficient was corrected considering the overall coupling yield per cycle of DNA synthesis with complete and incomplete DNA strands. The proportion of truncated sequences was evaluated during solid phase synthesis using DMT quantification. Three main parts of the sequence were considered for quantification, i.e. 1) after spacer synthesis, 2) after coding sequence synthesis and finally 3) after complete strand synthesis. With the approximation that

all coupling steps had similar yields during supported synthesis, the final yield of DNA synthesis was around 50%. To fit with this estimation, we approximated a molar extinction coefficient that considers the epsilons of the different parts of the sequence corrected by their corresponding coupling yield. Finally, as only complete strands were quantified, the final yield of supported synthesis was used as correction.

Nanoparticle stability evaluation

Nanoparticle (batch of 7500 ODN/NP, 20nmol of DNA) stability was evaluated for 2 months in ethanol (200µL) at 4°C. At 0, 15, 30, 60 days, 50µL of the nanoparticle solution was extracted and diluted in 150µL water. After two centrifugations (7,500g, 8 min), nanoparticle functionalization with DNA was quantified to evaluate the stability of DNA grafting.

1. R. T. Pon, S. Yu and Y. S. Sanghvi, *Bioconjugate Chem.*, 1999, **10**, 1051-1057.

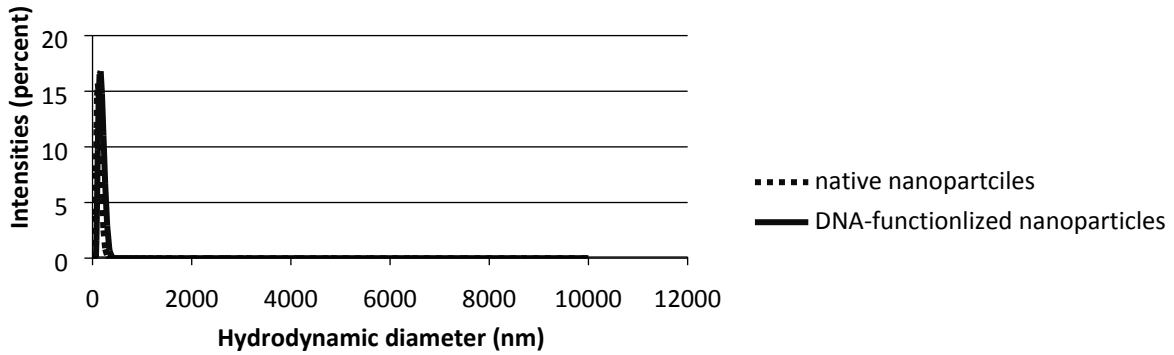


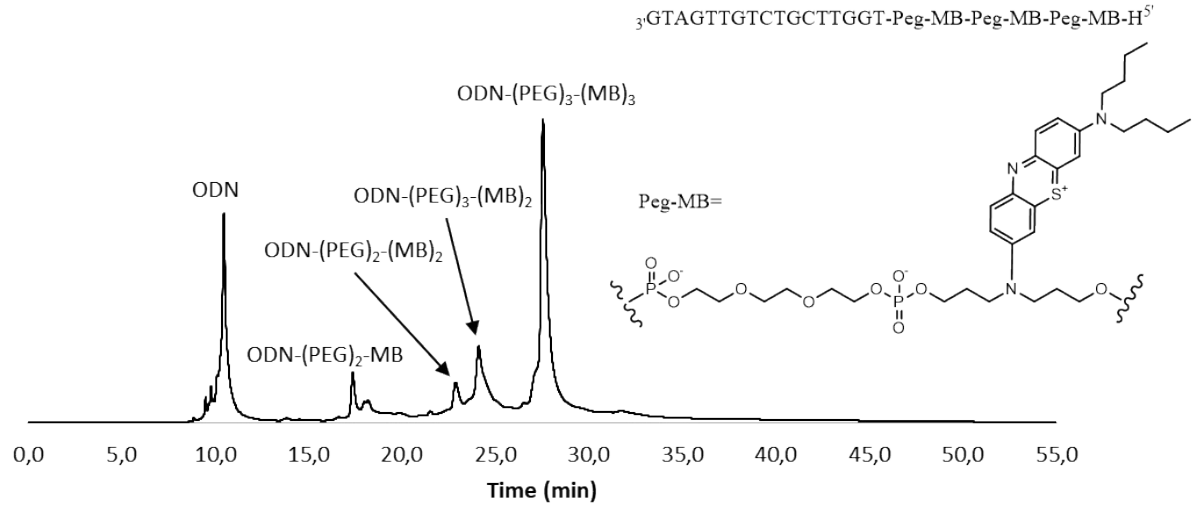
Figure S1. Size measurements by DLS of native silica nanoparticles and DNA functionalized nanoparticles with the $^{5'}$(Biot-T_5CAT CAA CAG ACG AAC $CAT_{20})_3$, sequence. Analyses were performed in water.

	Hydrodynamic Diameter (nm)	PdI	Zeta potential (mV)	Zeta potential standard deviation
Nanoparticles	103 ± 2 nm	0.121	-53.2	9.72
DNA functionalized nanoparticles	150 ± 3 nm	0.084	-54.8	7.32

PdI: Polydispersity Index

Table S1. Hydrodynamic diameters obtained by Dynamic Light Scattering (DLS) and zeta potential analyzed of native silica nanoparticles and DNA-functionalized nanoparticles with $^{5'}$(Biot-T_5CAT CAA CAG ACG AAC $CAT_{20})_3$, sequence. Analyses were performed in water.

A



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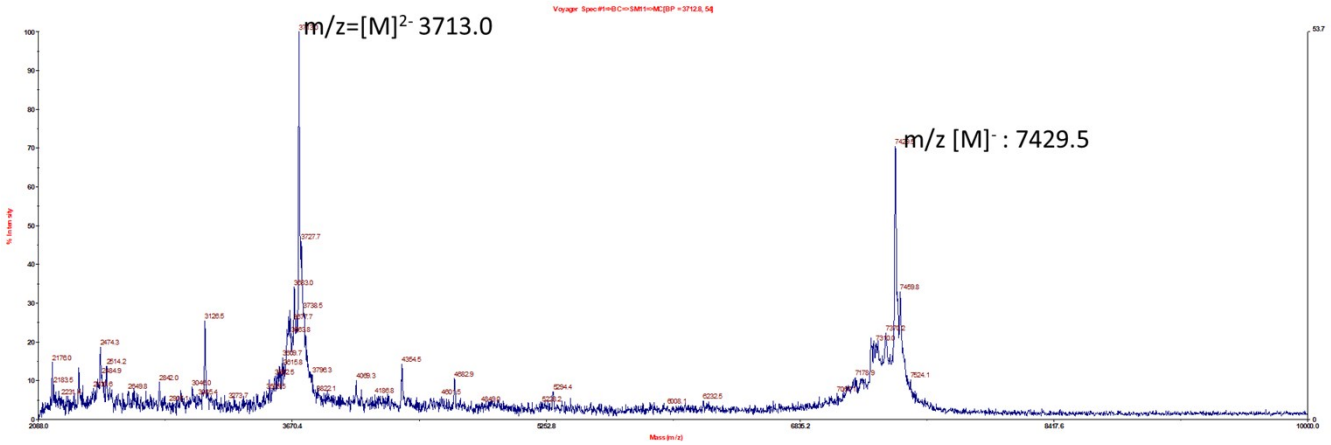

Figure S2.Characterization of the 3MB-DNA, A) HPLC analysis of the crude synthesis and B) MALDI-ToF mass spectrum

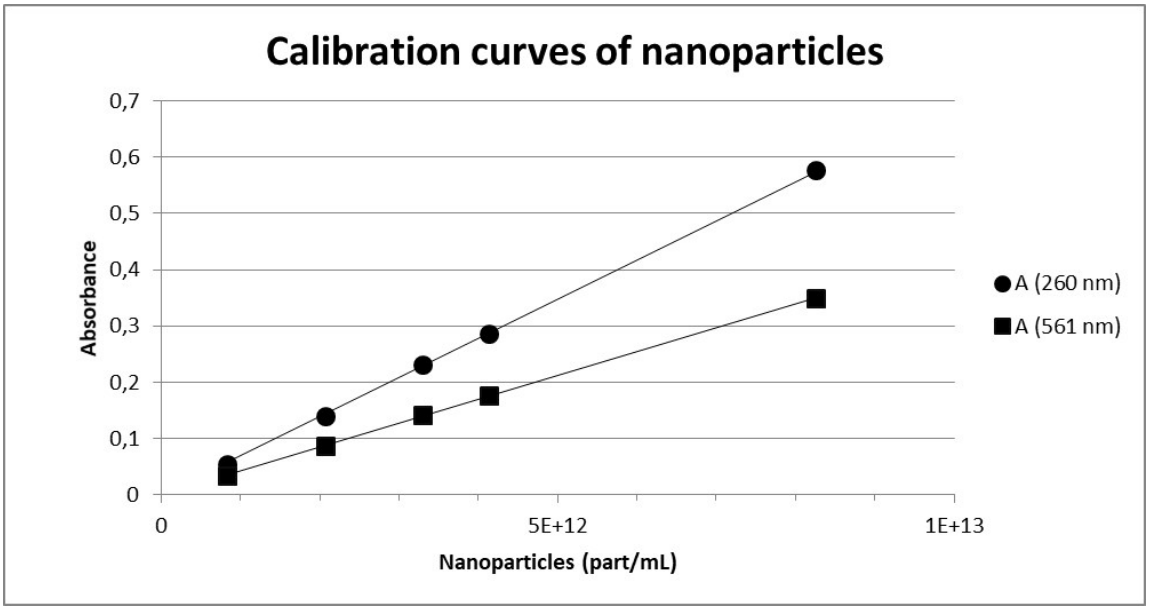

Figure S3. Calibration curves of rhodamine-silica nanoparticles at 260 and 561 nm. Measurements were performed in a microplate reader in a 200 µL analytical volume

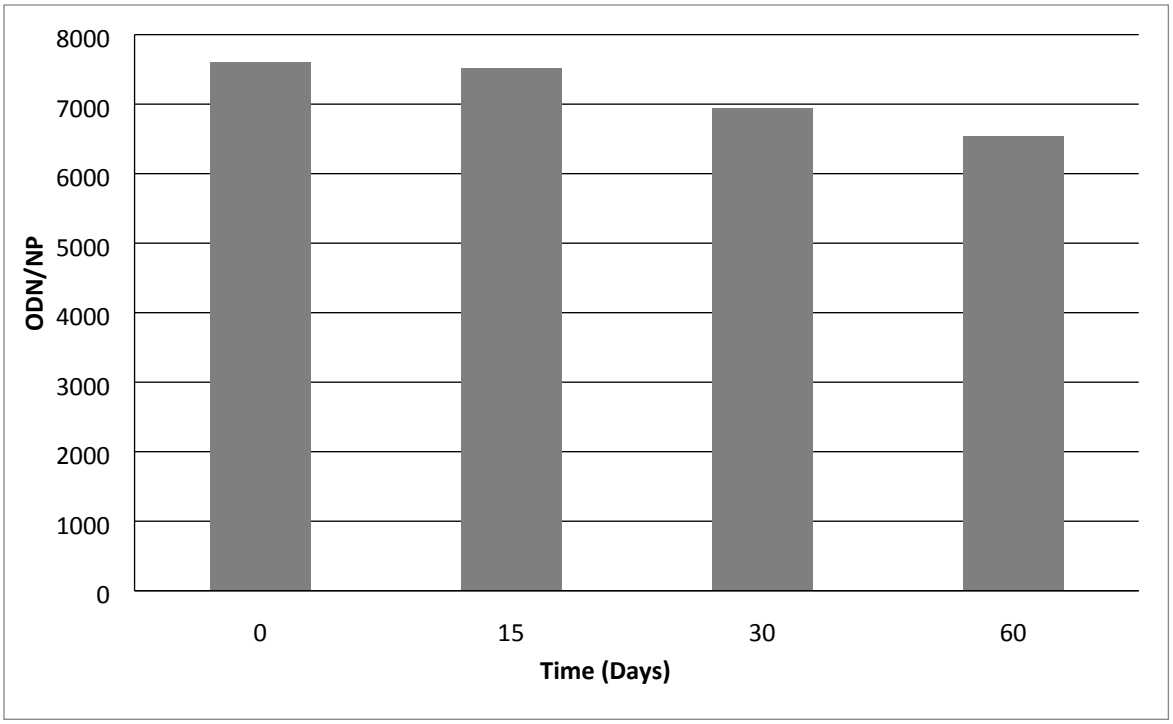

Figure S4. Stability study: quantification of the number of ODN strands grafted on the nanoparticles after storage in ethanol at 4°C. Quantification was performed in pure water after washing by two centrifugations at 7500g for 8 minutes in water and elimination of supernatant.

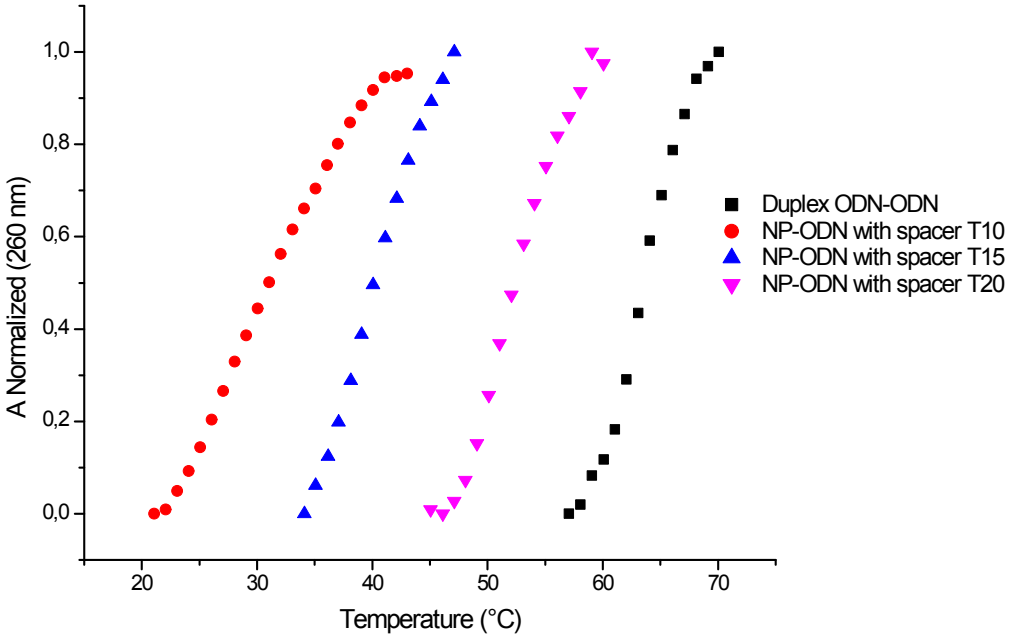

Figure S5. Normalized denaturation curves of hybridized duplex in solution or grafted on a nanoparticles. Measures were performed with 1µM DNA in Tris 50 mM NaCl 250 mM pH7 with the sequence $^{5'}$(Biot-T_5CAT CAA CAG ACG AAC $CAT_{10})_3$, grafted on the nanoparticles.

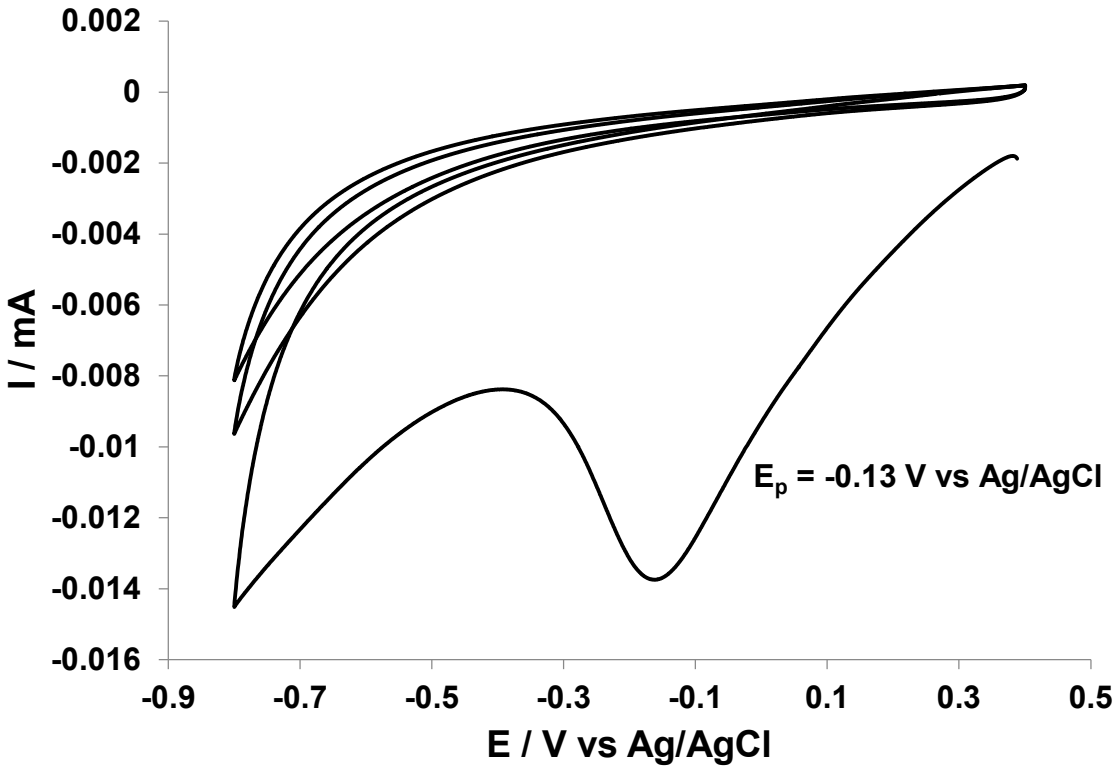

Figure S6. Cyclic voltammograms of 4-ABA electrografting. Measures were performed at 4°C in HCl 1M with 40 mM $NaNO_2$ and 1.5mM 4-ABA with a scan rate of 100mV.s^{-1}.

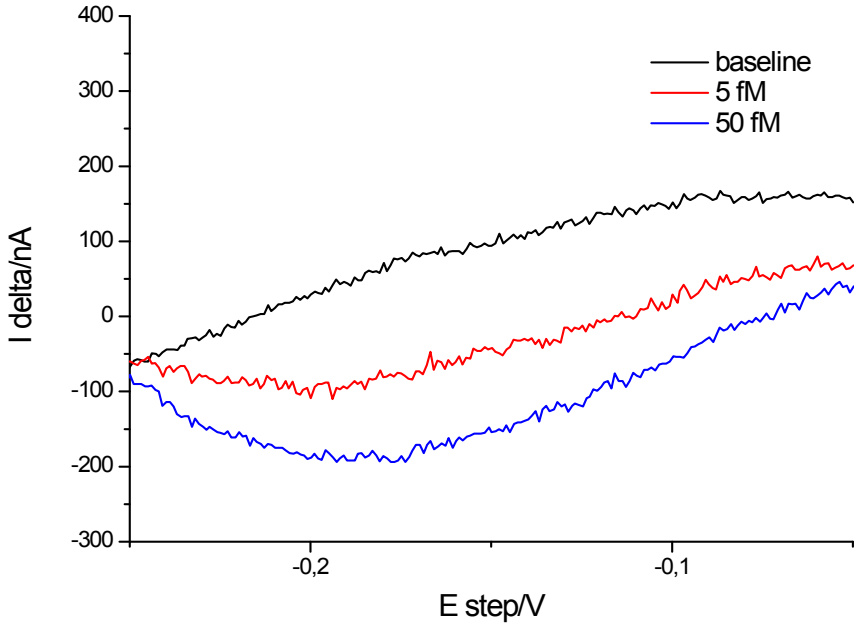

Figure S7. Square wave voltammograms of electrochemical probe at 5 fM (red) and 50 fM (blue). Measures were performed in 20 mM phosphate buffer with 250 mM KCl pH6 after 10 min of chronoamperometric accumulation at -0.35 V.

$$C_{ODN} = \frac{A_{260} - A_{260NP}}{\varepsilon_{final} \times \rho_{final} + \varepsilon_{coding} \times \rho_{coding} + \varepsilon_{spacer} \times \rho_{spacer}} \times \rho_{final}$$

Equation 1

C_{ODN} : DNA concentration in mol/L, A : absorbance at 260 nm, Ɛ : molar extinction coefficient of the sequence parts (spacer, coding, final), ρ : average coupling yield of the sequence.

Figure S8. Calculation of the grafted DNA on NPs (in mol/L) according to the nearest neighbour model

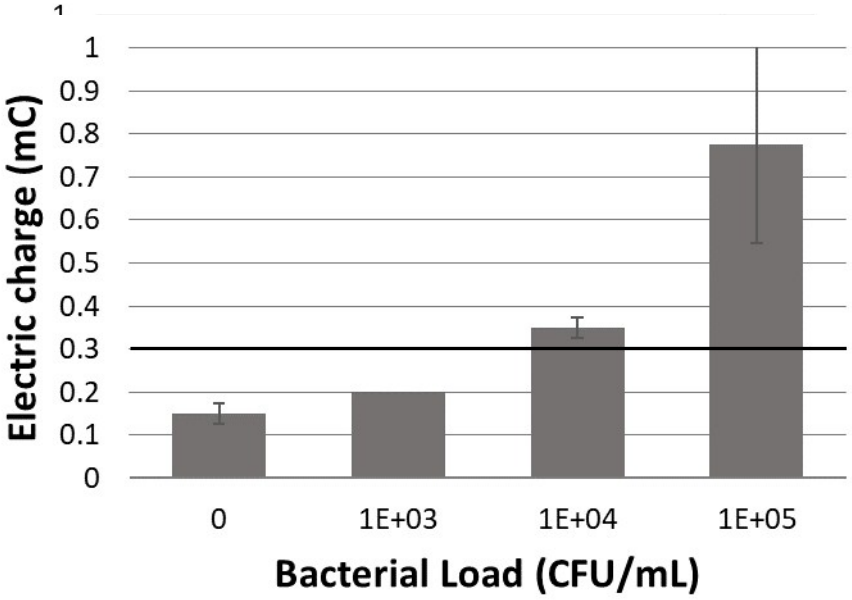

Figure S9. *E coli* quantification in platelet samples by the nanoparticle-based electrochemical assay. Analyses were performed with 9C12 antibody-functionalized magnetic beads and streptavidin-modified 16D12 antibody. The electrochemical signal was provided by MB-DNA-NP at the detection step. The electric charge was determined by integrating the reduction peak surface area of the square wave voltammograms recorded on aminobenzyl functionalized BDD electrodes (mean values of three experiments except for the experiment with 10^3 CFU/mL).

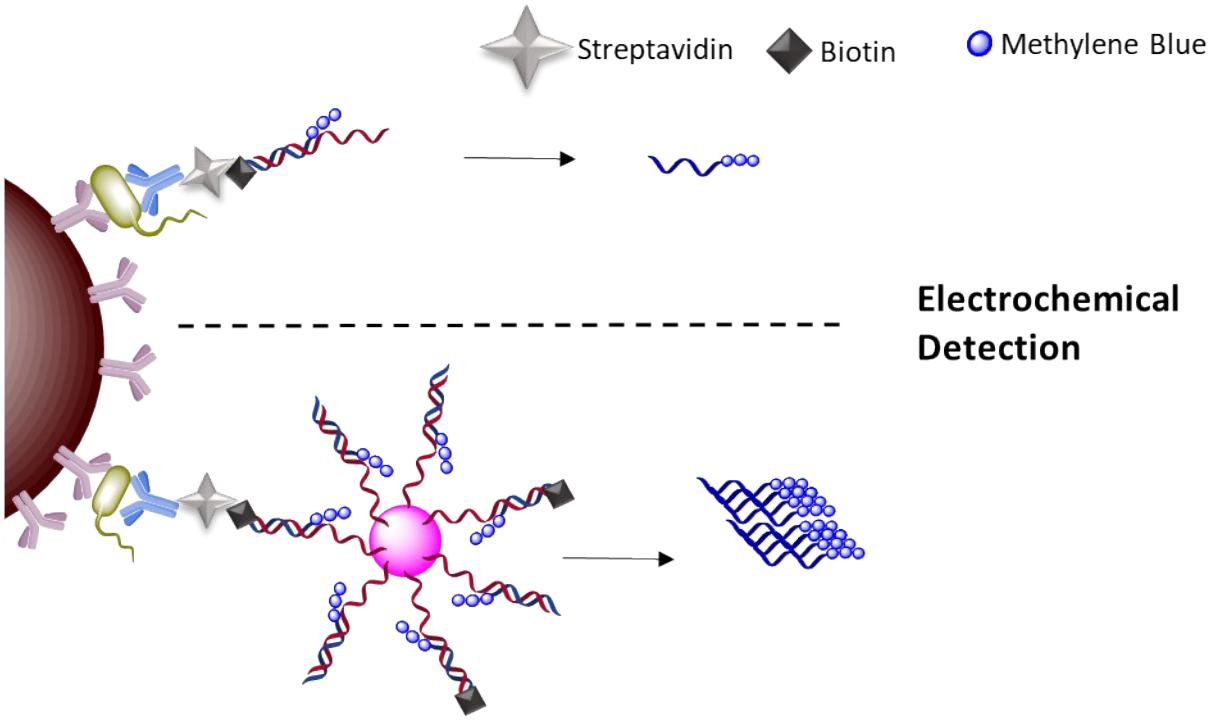

Figure S10. Presentation of the immuno-assay without and with the nanoparticle-based amplification of the electrochemical signal prior to detection

1. R. T. Pon, S. Yu and Y. S. Sanghvi, *Bioconjugate Chem.*, 1999, **10**, 1051-1057.