Supporting Information
The Development of a Silica Nanoparticle-based Label-free DNA biosensor

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Chemicals. Alexa Fluor 546 carboxylic acid, succinimidyl ester, neutravidin, Alexafluor 546-modified streptavidin (3 dyes per molecule of streptavidin) and PBS buffer were purchased from Invitrogen Canada. Tetraethoxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTES, 99+%) were obtained from Gelest. Succinic anhydride, sodium chloride, tween 20 and morpholinoethanesulfonate (MES) buffer were purchased from Aldrich. Ammonium hydroxide (NH_4OH, 28-30 wt %) was obtained from EMD Chemicals. N,N-dimethylformamide (DMF), and ethanol were purchased from EM Science, and Commercial Alcohols, respectively. All chemicals were used as purchased. Oligonucleotides were all purchased from Integrated DNA technologies, Inc. (Coralville, IA) in a standard desalting purification grade. Throughout the preparation of nanoparticles, if water was used purified water (18 MΩ cm) was used exclusively. Water was purified with use of a Millipore Q-guard 2 purification system (Millipore Corporation). Dynamic light scattering measurements were carried out using a Nanosight nanoparticle size analysis system in water solution. Transmission electron microscopy

was carried out with a JEM-2100F FETEM (JEOL) microscope operating at 200 kV. The samples were prepared by dipping the TEM grind into a solution of the nanoparticle in water or ethanol. Scanning electron micrographs were obtained using a Hitachi S-4800 FE microscope, operating at 1.2 kV. Samples were prepared by pipetting a few drops of a nanoparticle-bead conjugate in water onto an aluminum SEM sample stub with a pipette.

Preparation of Alexafluor 546 surface modified ss-oligonucleotide-modified nanoparticles (A_{probe})

The silica nanoparticle scaffold employed in this investigation was prepared via the Stöber method and measured ~ 200 nm in diameter (measured by dynamic light scattering, Nanosight, UK) and ~ 180 nm by TEM. They were prepared in ethanol solution (28 mL) through the addition of 3.14 mL ammonium hydroxide and 1.22 mL of tetraethoxyorthosilane. Following mixing on an orbital shaker overnight, 15 mL of the nanoparticle solution was removed and placed in a 20 mL glass vial and 3-aminopropyltriethoxysilane (300μL, to make a 2% solution) was added to the solution and the mixture was heated and stirred at 70 ºC for 2 hours. The resulting amine-modified nanoparticles were then purified via repeated centrifugation cycles in ethanol, water and the nanoparticles were finally redispersed in 10 mL ethanol.

To add different concentrations of Alexafluor 546 and neutravidin to the various amine-modified nanoparticles (architecture A), three separate 25μL solutions of the amine-modified nanoparticles were mixed with

1) 50µL (0.1mg/100µL) solution of AF546-NHS and 1µL (100 mg/100µL) succinic anhydride solution

2) 10uL (0.1mg/100µL) solution of AF546-NHS and 1µL (100 mg/100µL) succinic anhydride solution

3) 5µL (0.1mg/100µL) solution of AF546-NHS and 5µL (100mg/100µL) succinic anhydride solution.

Each nanoparticle solution was mixed for 2 hours and purified with repeated washes with MES buffer (25 mM MES, pH 6). The nanoparticles were then dispersed in 20 µL MES buffer solution and to each solution was added 15 µL of neutravidin (1 mg/mL) and 25 µL of a 0.1M solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in MES buffer. The solutions were mixed for 2 hours and purified with MES buffer, and PBS buffer with 1M NaCl (150 mM phosphate buffer) one time to remove any physisorbed neutravidin. The nanoparticles were finally redispersed in 100 µL 1X PBS/0.1% tween solution.

Preparation of Streptavidin Alexafluor 546 surface modified ss-oligonucleotide-modified nanoparticles (B_{probe})

The same amine-modified nanoparticles described above were used for this modification. A 5 mL solution of the amine-modified nanoparticle was centrifuged and redispersed in DMF. The nanoparticles were then mixed with a 5% solution of succinic anhydride in DMF to convert all accessible amine groups to carboxylate groups. The resulting carboxylate-modified nanoparticles were washed with DMF 4 times and eventually redispersed in ethanol (5 mL). A solution of the COOH-modified

nanoparticles (25 μL) was then isolated and dispersed in 25 μL MES buffer. Streptavidin-Alexafluor 546 (15 μL of a 1mg/mL solution) and 25 μL of a 0.1M solution of EDC were added and the solution was mixed for 2 hours. The resulting Streptavidin-Alexafluor 546-modified nanoparticles were then washed with MES buffer, 1X PBS/0.1% tween and 1M NaCl, and 1X PBS/0.1% tween and the nanoparticles were eventually dispersed in 100μL of 1X PBS/0.1% tween.

Modification of the A_{probe} and B_{probe} with ss-oligonucleotides.

Both streptavidin and neutravidin are tetrameric proteins well known to interact strongly with biotinylated substrates and were used to anchor biotinylated probe ss-oligonucleotides (5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-3') to generate $\mathbf{A_{probe}}$ and $\mathbf{B_{probe}}$. Specifically, the 100 μL $\mathbf{A_{probe}}$ and $\mathbf{B_{probe}}$ solutions were mixed with 10μL of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-3' in 1X PBS/0.1% tween. The solutions were mixed for 2 hours and washed with 1X PBS/tween, 1X PBS/0.1%tween/1M NaCl and 1X PBS/0.1%-tween and finally dispersed in 100 μL 1X PBS.

Quantification of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-3' on A_{probe} and B_{probe}.

For each $\mathbf{A_{probe}}$ and $\mathbf{B_{probe}}$, 10μL of the nanoparticle solution was centrifuged and redispersed in 60μL of hybridization buffer (hybridization buffer is comprised of 4 mL 20X SSPE buffer, 4 mL of formamide, 100μL of a 4% solution of polyvinylpyrrolidone and 1.9mL of water). A 20 μL aliquot of 10 μM Cy5-modified target oligomer in water

was then added, the solution was mixed for 2 hours and purified to remove unbound Cy5-modified target oligomer. The relative amount of hybridized DNA in each sample was then quantified using UV-visible spectroscopy, where the absorption peak at 654 nm could be used to quantify the amount of hybridized Cy5-labelled target oligomer (3'-TGTTTATGGACATTAATCGCAACGG-Cy5-5') in each sample. In addition, comparison of the peak intensity and relative extinction coefficient of the Alexa Fluor 546 component (λ_{max}=550 nm) and the Cy5 component (λ_{max}=654 nm) allows the comparison of the relative number of probe molecules (FRET donors when the duplex with the cationic polymer is added to the probe oligomer) and FRET acceptors (the AF546 molecules).

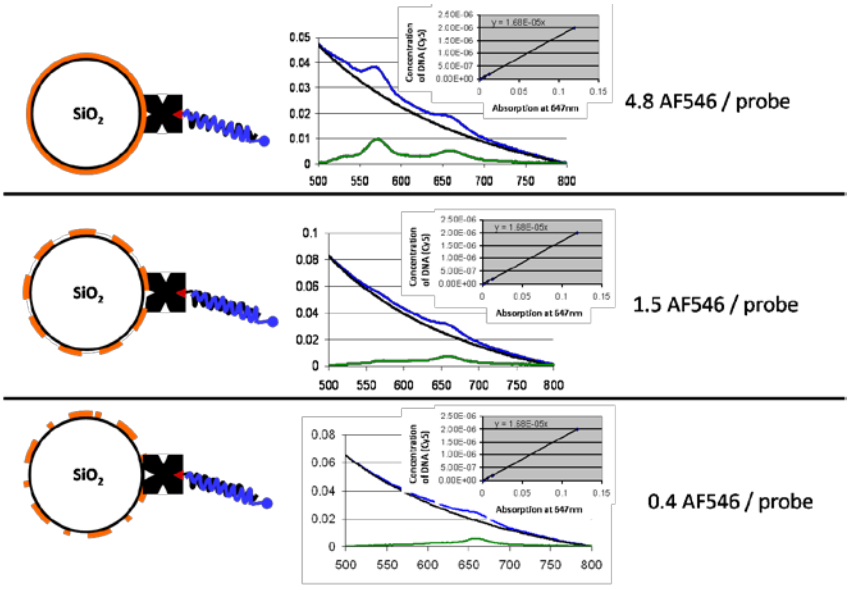


Figure S1. The UV-visible absorption spectra of the A_{probe} nanoparticles where the blue spectra are for the A_{probe} nanoparticles, the black spectra are unfunctionalized nanoparticle itself and the green spectra are the background subtracted spectra used to quantify the relative number of AF546 molecules (absorption at 567 nm) and the

hybridized Cy5-modified target oligomer (absorption at 654 nm). The number of AF546 molecules per probe (or target) oligomer is highlighted to the right of the spectra. Inset on each UV-visible absorption spectrum is the calibration curve used to quantify the Cy5-modified target oligomer.

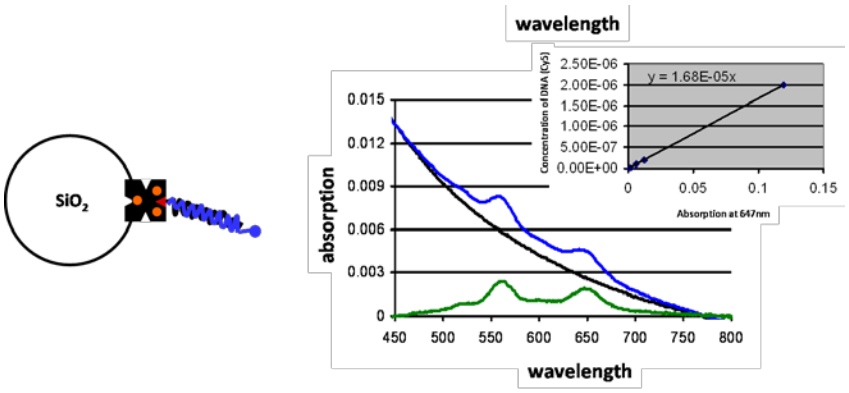


Figure S2. The UV-visible spectra of the B_{probe} nanoparticles where the blue spectrum is for the B_{probe} nanoparticles, the black spectrum is the unfunctionalized nanoparticle itself and the green spectrum is the background subtracted spectrum used to quantify the relative number of AF546 molecules (absorption at 567 nm) and the hybridized Cy5-modified target oligomer (absorption at 650 nm). The number of AF546 molecules per probe (or target) oligomer is three in this case. Inset on each UV-vis spectrum is the calibration curve used to quantify the Cy5-modified target oligomer.

Preparation of nanoparticle-modified magnetic beads (n-MB).

The same amine-modified nanoparticles described above were used for this modification. A 100 µL aliquot of the 20 µm beads (Kisker, product # PMC-18.0, 1% w/v) were magnetically confined and washed with 100 µL MES buffer three times. Following the third wash, the beads were redispersed in 50 µL MES buffer containing 0.1 M EDC. A 1 mL aliquot of the amine-modified nanoparticles ($1x10^{14}$ particles /mL) described above were centrifuged, redispered into 200 µL of MES and added to the 20 µm beads and mixed at room temperature for 6 hours. The resulting nanoparticle-modified beads were purified through repeated magnetic confinement s and redispersion

in fresh MES buffer (200 μL) until there was no evidence that free nanoparticle remained in solution (addition of fluorescamine did not yield a fluorescence signal).

Modification of the nanoparticle-modified beads with AF546 and succinic anhydride

A solution of succinic anhydride in DMF (100 mg/100μL) and a solution of AF546-NHS (0.1mg/100 μL) were prepared and added in a 1:10 ratio to a 50 μL aliquot of the nanoparticle-modified beads in ethanol. The solutions were mixed for 2 hours at room temperature and washed repeatedly with MES buffer (100 μL). The nanoparticles were finally dispersed in 200 μL MES.

Modification of the nanoparticle-modified beads (n-MB) with Neutravidin and 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-3'

The nanoparticle-modified beads were then magnetically confined and redispersed in 20 μL MES buffer solution and 15 μL of neutravidin (1 mg/mL) and 25 μL of a 0.1M EDC solution in MES buffer were added. The solution was mixed for 2 hours and purified with MES buffer and PBS buffer with 1M NaCl (150 mM phosphate buffer) one time to remove any physisorbed neutravidin. The neutravidin functionalized nanoparticle-modified beads were finally redispersed in 200 μL 1X PBS/0.1% tween solution. The nanoparticle-modified beads were then split into two 100 μL solutions. To the first 100 μL solution, 7.5 μL of a 1 μM solution of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-3' was added and mixed for 2 hours and washed with 1X PBS/tween, 1X PBS/0.1%tween/1M NaCl and 1X PBS/0.1%-tween and finally dispersed in 100 μL 1X PBS.

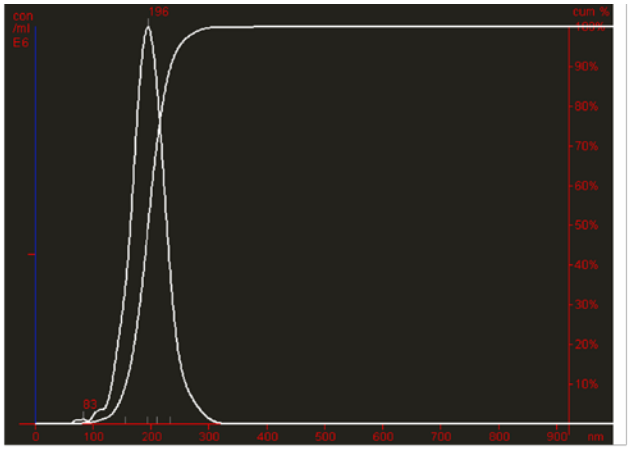
In order to test the concentration of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-3' anchored to the nanoparticle surface, the remaining 100 μL solution isolated above was mixed with 7.5 μL of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-AF546-3' (a fluorophore-modified probe) rather than the unlabelled probe. Following incubation and mixing for 2 hours, the n-MB was isolated and a UV-visible spectrum of the supernatent was recorded and compared to a control sample diluted to the same volume. This analysis suggests that there is 3.6×10^{-11} moles of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-3' per 100 μL in the sample above.

General Label-free Biosensing of DNA

A solution of the cationic polythiophene (7.4×10^{-5}M or 7.4×10^{-6}M *monomer* concentration) was mixed with the oligomer-modified DNA modified nanoparticles in water such that there is a charge balance between the ss-oligomer present on the nanoparticle (2 nM) and the polythiophene. Following the addition of the polymer the mixture was incubated on a thermo-mixer at 65ºC for 15 minutes. A fluorescence emission spectrum was then run on the sample in order to record a "fluorescence off" spectrum. After the emission spectrum was recorded 3 μL of a 0.1M solution of NaCl in 0.1% tween was added to the nanoparticle solution followed by the addition of the appropriate concentration of target ss-oligomer, the mixture was incubated on a thermo-mixer for 15 minutes at 65ºC and an emission spectrum was run on the nanoparticle.

Competiton assay for Label-free Biosensing of DNA and determination of the limit of detection.

A solution of the cationic polythiophene ($7.4x10^{-5}$M or $7.4x10^{-6}$M *monomer* concentration) was mixed with the oligomer-modified DNA modified nanoparticles in water such that there is a charge balance between the ss-oligomer present on the nanoparticle (2 nM) and the polythiophene. Following the addition of the polymer the mixture was incubated on a thermo-mixer at 65ºC for 15 minutes. A fluorescence emission spectrum was then run on the sample in order to record a "fluorescence off" spectrum. After the emission spectrum was recorded 3 μL of a 0.1M solution of NaCl in 0.1% tween was added to the nanoparticle solution followed by the addition of the noncomplementary ss-oligomer (either 3'-CTATCTGATTGTTGAAGAAGGATT-5' or 3'-TTGATTATTGTTATCCTGTTATGCC-5', 10nM), the mixture was incubated on a thermo-mixer for 15 minutes at 65ºC and an emission spectrum was run on the nanoparticle. After the emission spectrum was recorded the appropriate concentration of complementary target ss-oligomer (3'-TGTTTATGGACATTAATCGCAACGG-5') was added to the nanoparticle, the mixture was incubated on a thermo-mixer for 15 minutes at 65ºC and an emission spectrum was recorded. This procedure was followed for the acquisition of the limit of detection curves.



Results
Measurement Conditions
Analysis Conditions
Mean: 196 nm
Mode:196 nm
SD: 32 nm
D10: 156 nm
D50: 195 nm
D90: 234 nm

Results
User Lines: 0, 0 nm
Concentration: 1.72 x 10^8 particles/ml
Temperature: 23.20 °C
Viscosity: 0.93 cP
Frames Per Second: 34.76
Measurement Time: 40 of 40 seconds
Drift Velocity: 593 nm/s

Figure S3. A representative plot of the dynamic light scattering data used to characterize the nanoparticles.

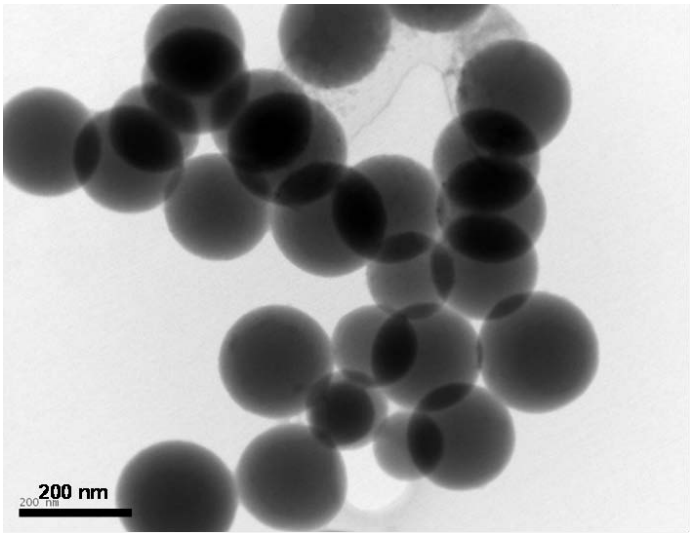


Figure S4. A representative transmission electron microscopy image of the nanoparticles.

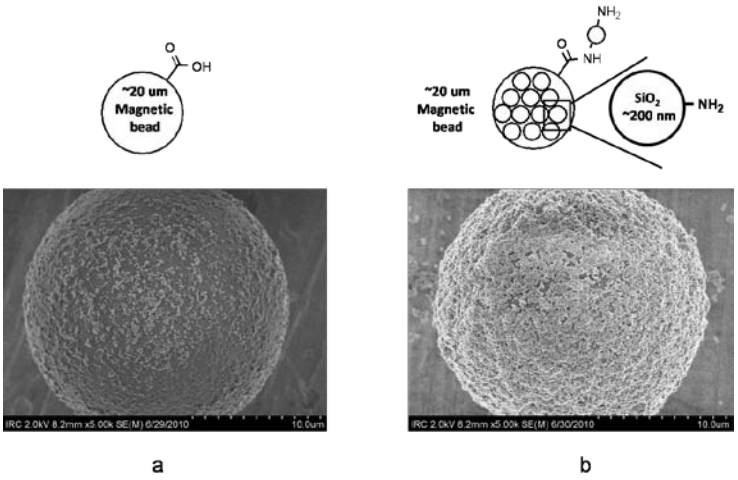


Figure S5. A representative scanning electron microscopy image of the 20μm beads (a) and the nanoparticle-modified 20μm beads (b).

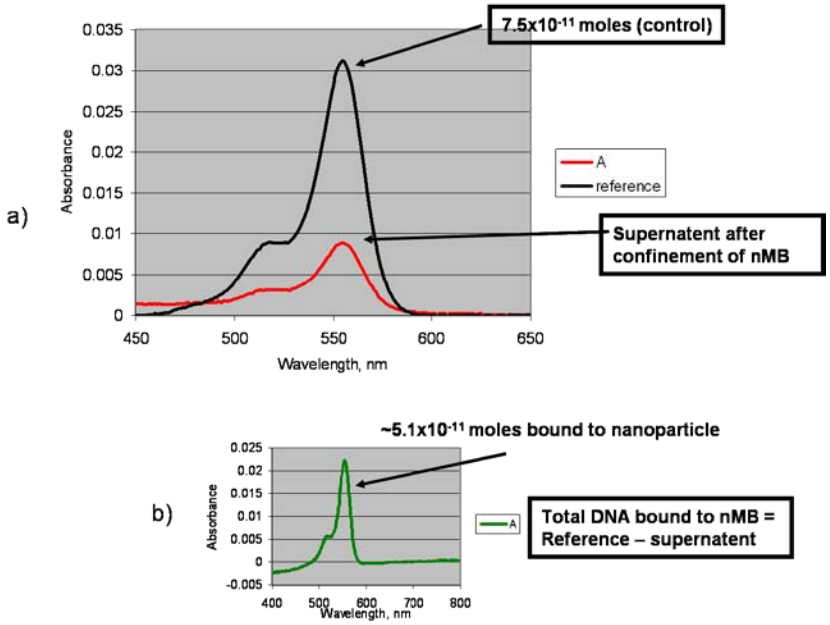


Figure S2. The UV-visible spectra of the ss-DNA (5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-AF546-3') used to quantify the concentration of oligomer on the n-MB particles, where the black spectrum is the total concentration of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-AF546-3' used in the immobilization reaction, and the red curve is the concentration of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-AF546-3' left over after the n-MB_{probe} is isolated (a). Below the green spectrum in (b) represents the concentration of 5'-biotin-C6 spacer-ACAAATACCTGTAATTAGCGTTGCC-AF546-3' bound to n-MB_{probe} based on a subtraction of the red spectrum from the black spectrum in (a).