Supplementary Information

SNP hydrodynamic diameter characterization by dynamic light scattering

SNP hydrodynamic diameter were characterized by using a dynamic light scattering (DLS) technique (Zetasizer Ultra, Malvern Panalytical). All samples were diluted in filtered deionised water to a concentration of 12 µg/mL to avoid multiple scattering effects and were equilibrated at 25 °C prior to analysis. In the case of Estapor 200 nm SNPs, analysis was performed in three conditions including bare non-functionalized SNP, streptavidin-functionalized SNP and streptavidin-functionalized SNP incubated with biotinylated DNA. Biotinylated DNA was incubated for 5 minutes with streptavidin-functionalized SNP, and the bound complexes were washed to remove unbound nucleic acids before resuspension in deionized water. Measurements were acquired in triplicate for each sample. Intensity-weighted hydrodynamic diameter was calculated using the instrument software analysis. Changes in hydrodynamic size were used to assess NPS size difference specified by suppliers and the impact of surface functionalization and biomolecular binding on NPS. Fig. S1 presents relative percentage of particles on size distribution based upon the intensity of scattered light.

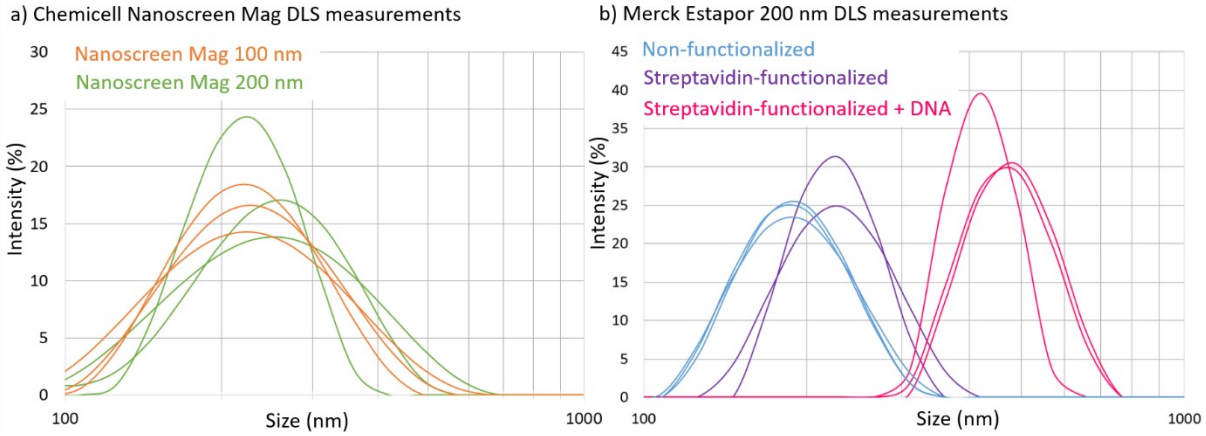

Fig. S1 NPS hydrodynamic diameters determined by dynamic light scattering size distributions. Measurements were performed in triplicates for each condition. a) Results for Chemicell Nanoscreen Mag 100 nm and 200 nm SNP. b) Results for Merck Estapor 200 nm SNP in three different conditions: (i) non-functionalized NPS, (ii) streptavidin-functionalized NPS, and (iii) streptavidin-functionalized NPS incubated with biotinylated DNA.

Measured hydrodynamic diameters of 213 ± 1 nm and 293 ± 21 nm were obtained for Nanoscreen Mag 100 nm and 200 nm SNP respectively. Hydrodynamic diameters of 186 ± 1 nm, 275 ± 8 nm, 602 ± 23 nm were obtained respectively for the bare, streptavidin-functionalized and DNA bound streptavidin-functionalized Estapor 200 nm SNP.

Fluorescence spectroscopy characterization of DNA to SNP specific binding *via* streptavidin-biotin interaction

Fluorescence spectroscopy was conducted to characterize the specificity of streptavidin-functionalized SNP to biotin-coupled DNA. Two types of DNA were used, one type is DNA coupled to a fluorophore, and second type is DNA coupled to a fluorophore and a biotin, referred as non-biotinylated DNA and biotinylated DNA respectively in Fig. S2. SNP used were Merck Estapor 200 nm particles, that were functionalized with streptavidin as previsouly described [1] and incubated at a concentration of 0.29 mg/mL with 4 µg/mL of DNA in a solution containing Tris HCl 10 mM, Denhardt 1X and hybridization solution 0.5X, for 20 min on an orbital shaker at 600 rpm. This solution is referred as total solution in Fig. S2. As detailed in Fig. S2a, centrifugation was performed at 10 krpm during 10 min to collect supernatant, containing free unbound DNA, and pellet, containing SNP. The pellet was washed three times by suspension in Tris HCl 10 mM and centrifugation followed by supernatant removal, and finally resuspended in Tris HCl 10 mM. Fluorescence spectroscopy measurements were

conducted on 100 µL of each total solution, supernatant and pellet in triplicate for n=2 independent experiments. Spectrophotometer used for experiment is a Tecan Spark device, excitation and emission wavelengths were set at λ_{Ex} = 495 nm and λ_{Em} = 525 nm respectively, both with bandwidths set at 7.5 nm, gain was set at 100 and z position at 2 cm. The limit of detection was determined to be 18 a.u. Experimental results are presented in Fig. S2b for each non-biotinylated DNA and biotinylated DNA

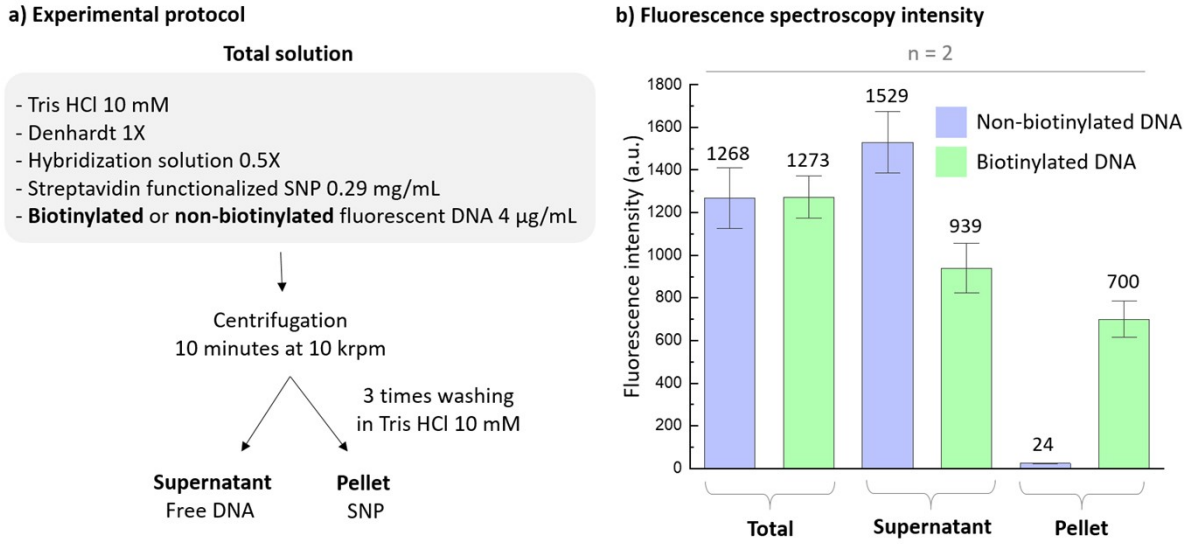

Fig. S2 Biotinylated DNA to streptavidin functionalized SNP specific binding characterization by fluorescence spectrophotometry. (a) Composition description of nine different solutions characterized in fluorescence spectroscopy. (b) Bar chart represents the fluorescence spectroscopy results for the three total, supernatant and pellet solutions, for each condition of non-biotinylated fluorescent DNA and biotinylated fluorescent DNA. Measurements were performed in triplicates, number n of independent experiments conducted is indicated on the graph in each of the nine solution conditions. The limit of detection is 18 a.u.

conditions.

For non-biotinylated DNA, measured fluorescence intensity are 1268 a.u., 1529 a.u. and 24 a.u. for total solution, supernatant, and pellet respectively. Pellet measurement results shows that minimal DNA is bound to SNP. For the biotinylated condition, measurements are 1273 a.u., 939 a.u., and 700 a.u. for total solution, supernatant, and pellet respectively. Pellet measurement shows significant DNA binding to SNP. This way we can assume specific binding of biotinylated-DNA to streptavidin functionalized SNP.

We notice that fluorescence intensity of total solution does not corresponds to the addition of supernatant and pellet fluorescence intensities. This is probably due to a masking effect of SNP in suspension on the fluorescence in the total solution. We can consider that the initial amount of fluorescent DNA corresponds to the supernatant for the non-biotinylated DNA condition, as the amount of fluorescence on the pellet is close to the limit of detection, suggesting that negligible amount of DNA was bound to SNP. The addition of supernatant and pellet fluorescence intensities in the case of biotinylated DNA are indeed close to the supernatant of non-biotinylated condition. We can therefore estimate that ~40% of biotinylated DNA and ~0% of non-biotinylated DNA were bound streptavidin-functionalized SNP.

Same experimental protocol was conducted in the absence of 0.5X hybridization solution in the initial total solution. Results of n=1 independent experiment with triplicate measurements for each condition are presented in Fig. S3.

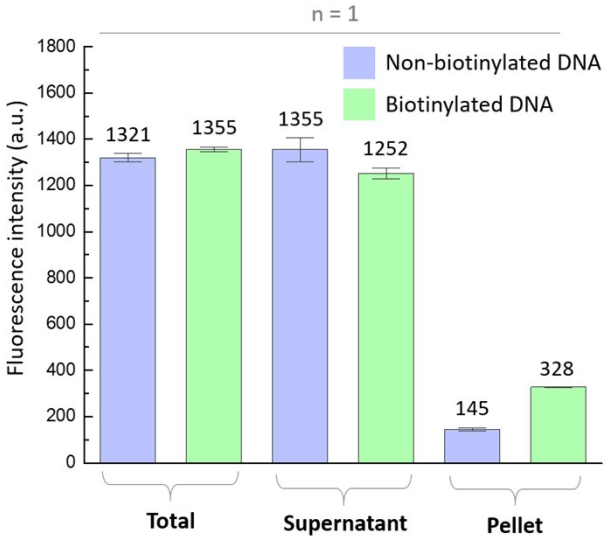

Fig. S3 Biotinylated DNA to streptavidin functionalized SNP specific binding in the absence of 0.5X hybridization solution characterization by fluorescence spectrophotometry. Bar chart represents the fluorescence spectroscopy results for the three total, supernatant and pellet solutions, for each condition of non-biotinylated fluorescent DNA and biotinylated fluorescent DNA. Measurements were performed in triplicates, number n of independent experiments conducted is indicated on the graph in each of the nine solution conditions. The limit of detection is 18 a.u.

In the absence of 0.5X hybridization solution, fluorescence intensity measurements of total solution of 1321 a.u. and 1355 a.u. for non-biotinylated fluorescent DNA and biotinylated fluorescent DNA respectively, are comparable with results obtained with its presence (Fig. S1b). We observe significant fluorescence intensity decresease in the supernatant of non-biotinylated condition (1355 a.u.) and increase for the biotinylated condition (1252 a.u.), in concordance with an increase in the fluorescence intensity of the pellet for the non-biotinylated condition (145 a.u.) and a decrease in for biotinylated condition (328 a.u.). This result suggests that presence of 0.5X hybridization solution prevents non-specific fluorescent DNA binding to streptavidin-functionnalized SNP, and enhance specific fluorescent DNA binding via biotin-streptavidin interaction. This effect may be due to the presence of denaturated salmon DNA fragments in the hybridization solution, which efficiently passivate surfaces without interfering to the biotin-streptavidin interaction.

Fluorescence imaging characterization of DNA to SNP specific binding *via* streptavidin-biotin interaction

Bright field and fluorescence imaging of SNP capture on the surface of the magneto-fluidic chamber was performed with SNP incubated with the two types of non-biotinylated fluorescent and biotinylated fluorescent DNA. SNP used were Merck Estapor 200 nm particles, that were incubated at a concentration of 0.29 mg/mL with 4 µg/mL of DNA in a solution containing Tris HCl 10 mM, Denhardt 1X, for 10 min on an orbital shaker at 600 rpm. Solutions of the two conditions were injected in 8 µL magneto-microfluidic chamber. Bright field and fluorescence images were acquired after 5 min capture, results for each condition are presented in Fig. S4. Imaging was performed using an Olympus IX70 microscope, a white LED lamp, a GFP fluorescence cube and a DP30BW 12 bit camera (1360 pixels x 1024 pixels, gain 8). Images were acquired using a 10x objective and exposition parameters were 100 % lamp power and 15 ms exposure time for the GF fluorescence and 1 % lamp power and 0.5 exposure time for bright field.

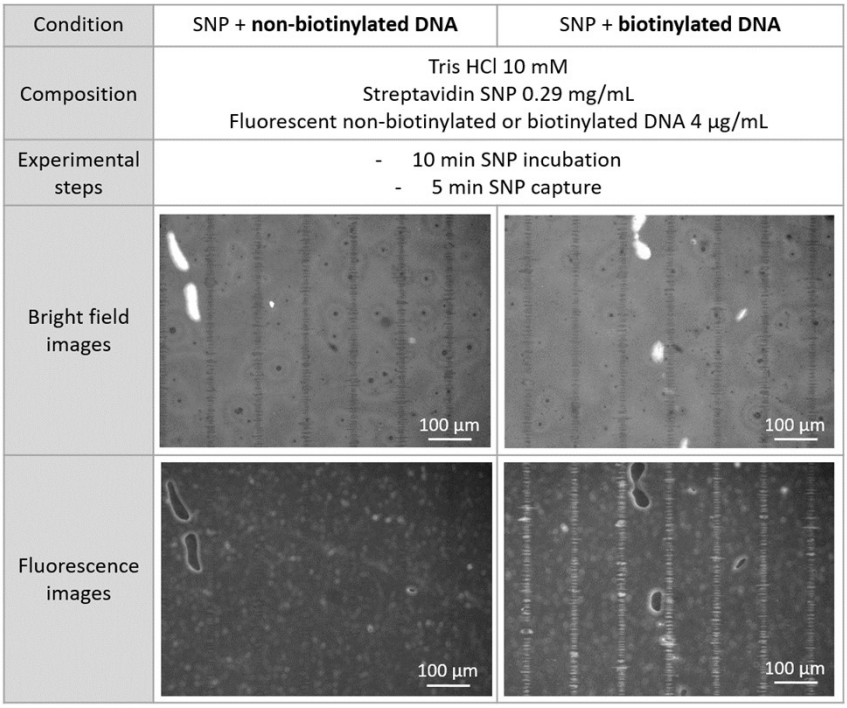

Fig. S4 Experimental details of SNP incubated with non-biotinylated DNA and biotinylated DNA prior to bright field and fluorescence imaging with corresponding imaging results.

We observe for both conditions SNP capture on line pattern in bright field. However, in fluorescence, SNP capture on line pattern is only visible for the fluorescent biotinylated DNA condition, showing that negligible amount of fluorescent DNA was bound to SNP for the non-biotinylated condition.

References

1. Orsini, E. et al. Magnetically Localized Detection of Amplified DNA Using Biotinylated and Fluorescent Primers and Magnetic Nanoparticles. Biosensors 15, 195 (2025).