Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information for

Cooperative dynamics of DNA grafted magnetic nanoparticles optimize magnetic biosensing and coupling to DNA origami

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Chemicals

Iron (III) acetylacetonate (99.9% trace metal basis), cobalt (II) acetylacetonate (≥99.0%), dibenzyl ether (DBE, ≥98%), oleic acid (OA, 90%), 1-octadecene (ODE, 90%), poly(maleic anhydride-alt-1-octadecene) (Mn=30.000-50.000), NH_2-PEG(3)-Azide, Tris-Base, EDTA, $MgCl_2$, NaCl were purchased from Sigma-Aldrich (Germany). All ssDNA oligonucleotides were obtained from Biomers GmbH (Germany). Sodium oleate (>97%) was purchased from TCI (USA). All organic solvents with ACS reagent-grading were purchased from Carl Roth (Germany) and used without further purification.

Magnetic nanoparticle synthesis

Cobalt-doped iron oxide nanocubes were synthesized via thermal decomposition of iron (III) acetylacetonate and cobalt (II) acetylacetonate in a mixture of dibenzyl ether, 1-octadecene, oleic acid, and sodium oleate at 290 °C for 30 min as previously described.[1]

Modification of poly(maleic anhydride-alt-1-octadecene) with NH_2-PEG(3)-azide linker

Poly(maleic anhydride-alt-1-octadecene) (PMAO) was modified with NH_2-PEG(3)-azide via anhydride ring opening reaction following the procedure published by Jin et al.[2] with some modifications. In a typical reaction, 145 mg (0.482 mmol monomer units) of PMAO were added into 7.5 mL dimethylformamide (DMF) in a round flask and heated up to 65 °C. Next, 100 mg (0.482 mmol) of NH_2-PEG(3)-Azide dissolved in 1 mL DMF was pipetted into the reaction flask and left to react for 24 h at 65 °C. After cooling the flask to room temperature, DMF was removed using a rotary evaporator operating at 5 mbar and 36 °C. The crude yellowish oily product was dissolved in 3 mL of tetrahydrofuran (THF) and dialyzed against THF using regenerated cellulose dialysis tubes (6 kDa cutoff size) to remove unreacted PEG and byproducts. Next, THF was removed thoroughly in a rotary evaporator and the resulting product was dissolved in 9 ml of chloroform and stored at 4 °C prior to usage.

Polymer coating procedure

The polymer coating of oleic acid coated NPs was performed following the protocol by Pellegrino et al.[3] In a typical procedure, 5.7 ml (containing ~ 35 mg/ml of PMAO-azide polymer) was poured into a round glass flask and sonicated for 10 min. Next, 2 ml of particle suspensions in chloroform (3 g(MNP)/l) were first further diluted with 5.5 ml of chloroform and then added dropwise into the polymer solution and sonicated again for 30 min to homogenize the mixture. The ratio of polymer to MNPs corresponds to 500 polymer units per nm^2 of a particle. Afterwards, chloroform was removed very slowly within 5-6 h through stepwise reduction of pressure to a final value of ~350 mbar and increase of temperature to 34 °C. After a complete removal of chloroform, particles were resuspended in 20 ml of sodium borate buffer (pH 8.7) by sonication for 1 h at ~45 °C. The particle suspension was then concentrated to 2 ml using spin filtration (Amicon regenerated cellulose spin filter, 15 mL, 30 kDa cutoff size) at 3200 rpm at 20 °C for 30 min. These particles offer click-chemistry conjugation and are named clickable magnetic nanoparticles (CMPs).

Purification and particle fractionation

To remove polymeric micelles that are formed during the polymer coating process, we fractioned CMPs on non-continuous sucrose gradient (10%:40%:60%, from top to bottom,

each fraction 4 ml) centrifuge columns. Typically, 500 µL of PMAO-coated particle suspensions in borate buffer were loaded into the sucrose centrifuge tube and centrifuged for 2 h at 4500 rpm at 4 °C. Next, singly polymer coated nanoparticles were collected in the 10% band using a long needle. The sucrose was then removed by 5 rounds of Amicon filtration (Amicon spin filter, 15 mL, 30 kDa cutoff size) at 3200 rpm and 20 °C for 12 min. After each round of spin filtration the particles were thoroughly resuspended in borate buffer by vigorous pipetting. Finally, the particles were concentrated to 2 mL of Tris-EDTA (5 mM Tris, 1 mM EDTA, 5 mM NaCl, pH 7.3) buffer by spin filtration.

To further purify the particles, we performed one round of magnetic washing. To do so, we used a 1.5 ml MACS column (Miltenyi Biotec) in combination with a MiniMACS separator (permanent magnet). First, the MACS column was placed in the separator and a collection vial was placed under the column to collect all effluent. At first, the column was rinsed with 500 µl of TE buffer and then the particle suspension was dropwise loaded on top of the column. After making sure that the whole buffer is passed through the column, the column was removed from the permanent magnet. Afterwards, the MNPs were collected in a glass vial by adding 3×565µl of TE buffer and pushing the plunger supplied with the column. The particle suspension was then stored at 4 °C prior to DNA functionalization. The particles after polymer coating are called clickable magnetic nanoparticles (CMPs).

DNA labeling of CMPs

The following equation was used to calculate the amount of ssDNA (100 µM) needed to label CMPs with ssDNA strands of 20-mer thymine (T) at different R(x) = (ssDNA/nm^2 of CMP) ratios:

$$V_{DNA} = \frac{R(x) \cdot V_{MNP} \cdot C_{MNP} \cdot S_{MNP}}{C_{DNA}} \qquad \text{Eq. 1}$$

with V_{DNA} (µl), V_{MNP} (µl), C_{MNP} (µM), and C_{DNA} (µM) the volume and concentration of the ssDNA solution and the CMP suspension, respectively. The surface area of a cubic particle is given by $S_{MNP} = 6 \cdot (D_h/\sqrt{3})^2$ in (nm^2), with D_h the volume weighted particle hydrodynamic diameter measured by DLS, since the DLS analysis assumes a spherical shape for particles.

In a typical experiment, a 180 µl of CMP suspension in TE buffer at a particle concentration of 112 nM was mixed with 112 µl, 224 µl, 447 µl l, 559 µl or 894 µl of 100 µM 5'-T(20)-DBCO-3' ssDNA (100 µM in TE) to obtain R(x) of 0.15, 0.3, 0.6, 0.48, and 1.2, respectively. After mixing, the sample was incubated at room temperature overnight. To maximize the binding efficiency of ssDNA to CMPs, we applied a so-called salt aging procedure using NaCl salt the next day.[4] We increased the concentration of NaCl in the mixture incrementally by 100 mM every hour up to 400 mM. After each salt adjustment step, the mixture was homogenized by vortexing and sonicating for ~20 s. At the end of the salt aging procedure, the mixture was again incubated at room temperature overnight. Next, the excess of ssDNA was washed out by 3 rounds of centrifugation at 14000 rpm at 5 °C for 13 min. Finally, the sample volume was adjusted again to 180 µl to obtain the original particle concentration. The samples were stored at 4 °C prior to further use.

DNA origami synthesis

DNA origami folding is performed in a one-pot reaction. First, all necessary ingredients were mixed together: the scaffold (p8064 for 24HB, p8634 for 6HB), staples and buffer (1x TE, 24mM $MgCl_2$ for 24HB and 18mM $MgCl_2$ for 6HB). Typically, we fold the scaffold with a 5x excess of the staples, so for a scaffold concentration of 20 nM, a staple concentration of 100 nM is used. The mixture was first heated up and then went through an annealing program (65 °C to 64 °C at 15 min/°C, 64 °C to 59 °C at 5 min/°C, 59 °C to 39 °C at 45 min/°C, 39 °C to 36 °C at 30 min/°C, and 36 °C to 20 °C at 5 min/°C). The first purification of the origami was performed by PEG precipitation[5] to increase the concentration before purifying it with an agarose gel (1%, 70 V, 80 min). The gel running buffer was 1xTAE with 11 mM $MgCl_2$.

Coupling of magnetic particles to origami

The CMP-DNA conjugates prepared at R(x) = 1.2 were mixed together with the purified DNA origami with an excess of 10:1 particles per binding site in 1xTAE (11 mM $MgCl_2$) using the gel buffer conditions. The mixture went through the following temperature program: (40 °C to 20 °C at 10 min/°C, repeat 3 times). After gel purification, the sample was imaged with negative-stain TEM imaging.

Characterization of magnetic nanoparticles

Transmission electron microscopy (TEM)

Transmission electron microscopy studies were carried out using a JEOL TEM microscope operating at 100 kV. The TEM samples were prepared by drop casting 5 µl particle suspensions in chloroform on a TEM grid (formvar-carbon coated copper grids with the mesh size of 300) and letting it completely dry in a fume hood. Negative-stain TEM samples were prepared by staining with uranyl formate (1%) for 10 s after 5 min incubation of particle suspension on TEM grid. The particle size D_c histogram was acquired by the analysis of a large field of view TEM image using the automatic particle analysis routine of the ImageJ software.

Inductively-coupled plasma optical emission spectroscopy (ICP-OES)

The particle concentration was determined by first measuring Fe and Co concentrations using ICP-OES on a Varian (715ES) instrument. The sample was prepared by adding 25 µl of CMPs and 1 ml of aqua regia (HCl : HNO_3 @ 3:1) into a 10 ml volumetric flask. The flask was then incubated for 1 h at ≈ 60 °C to facilitate the digestion process and then left overnight under fume hood. Next day, Milli-Q water was added into the flask up to the grading level. The calibration curves were prepared prior to every sample set. To convert Fe and Co concentrations to the particle concentration, numbers of oxygen, cobalt, and iron atoms in a FCC crystal structure with a lattice constant a = 8.40 Å were taken into calculation.

Agarose gel electrophoresis shift assays

To study electrophoretic migration behavior of particles prior and after ssDNA labeling, native agarose gel electrophoresis (AGE) without staining was applied. Typically, 0.5%

agarose gel in 1×TAE running buffer was made by dissolving 250 mg of agarose broad band gel in 50 ml of TAE buffer by 1 min microwave irradiation and 1 min magnetic stirring. The homogeneously dissolved gel solution was casted on a gel mold and left to solidify for 30 min. Afterwards, the casted gel was placed in electrophoresis chamber (Biorad) and samples were pipetted (containing 20% glycerol) into pockets. All AGE shift assays were run at 132 V (90 mA) for 30 min. The gels were imaged under white and UV light exposure.

Analysis of gel images

To quantify the migration of particles on the gel, we used the Image Lab software of the Gel Doc instrument. The relative front R_f parameter was derived from these analyses, showing how far the particle band is moving in the gel relative to the length of the area that was selected as a band length. The R_f values were obtained using analysis tools available in the Image Lab software.

Depletion assays

To determine the number of ssDNA per particle at different nominal grafting ratios R(x), we measured the UV absorption at 260 nm on the supernatants after DNA functionalization and centrifugation of CMPs (14000 rpm, 4 °C, 13 min). To eliminate the background caused by ever existing CMPs in the supernatant, we set the absorption at 310 nm as the background. The concentration of ssDNA in the supernatant c in µg/l is calculated using the Beer-Lambert law given by:

$$c = \frac{A}{\varepsilon \cdot L} \qquad \text{Eq. 2}$$

with A the absorption, L the optical path length in cm, ε the coefficient of excitation with $\varepsilon = 0.027 \cdot 10^{-3}$ $(\mu g/l)^{-1} \cdot cm^{-1}$ for ssDNA. By plugging c into the following equation the average number of ssDNA per CMPs can be estimated.

$$n = \left(C_{DNA} \cdot V_{DNA} - \frac{c \cdot V_s}{M(ssDNA)}\right) \cdot \frac{1}{C_{MNP} \cdot V_{MNP}} \qquad \text{Eq. 3}$$

with C_{DNA} the DNA concentration in µM and V_{DNA} the volume of the DNA sample in µl, V_s the volume of the supernatant in µl, M the molecular weight of ssDNA (6250 g/mol for 20T), C_{MNP} the particle concentration in µM and V_{MNP} the volume of the particle suspension in µl. The results presented in Fig. 1h are the average value of three independent UV absorption measurements.

Dynamic light scattering (DLS)

DLS measurements were performed using a Malvern Zetasizer instrument at 173° backscattered measurement mode. Measurements were performed on particle suspensions in TE buffer (pH 7.3) at a typical particle concentration of ~ 0.015-0.2 g/l at room temperature.

Sample preparation for atomic force microscopy (AFM)

For the AFM samples, we deposited 20 µl of CMPs or CMP-DNA conjugates at different grafting densities in TE buffer containing either 5 mM $MgCl_2$ or 300 mM NaCl on freshly cleaved bare muscovite mica. The sample was incubated 5 min before washing with 20 ml MilliQ water and drying with a gentle stream of filtered argon gas.[6]

AFM imaging

The dry AFM images were recorded in tapping mode at room temperature using a Nanowizard Ultraspeed 2 (JPK, Berlin, Germany) AFM with silicon tips (FASTSCAN-A, drive frequency 1400 kHz, tip radius 5 nm, Bruker, Billerica, Massachusetts, USA). Images were scanned over different fields of view, with a pixel size of 1 nm/pixel and with a scanning speed of 5 Hz. The free amplitude was set to 20 nm. The amplitude setpoint was set to 80% of the free amplitude and adjusted to maintain a good image resolution.

AFM image analysis

Postprocessing of AFM data was performed in the software SPIP (v.6.4, Image Metrology, Hørsholm, Denmark) using the particle and pore analysis to determine the height of the nanoparticles. Contaminations (and for some parts of the analysis clusters) were excluded manually.

Complex ac-susceptibility (ACS) spectroscopy

The ACS spectra were recorded using our home-built AC spectrometer operating from 200 Hz to 1 MHz at magnetic field amplitudes of $\mu_0 H$ = 95 µT. The measurements were carried out at 295 K on 150 µl of particle suspensions at a particle concentration of 112 nM.

Magnetic particle spectroscopy (MPS)-based magnetic biosensing of target nucleic acids

Purification of CMPs with agarose gel electrophoresis

For magnetic particle spectroscopy (MPS)-based magnetic bioassays, we purified the CMPs before DNA labeling using agarose gel electrophoresis to remove aggregates and have only single-coated CMPs. The gel purification assays were run at 132 V for 40 min (Figure S4a). The piece of gel that was "cut & squeeze" is highlighted by a red rectangle. We then labeled the gel purified CMPs with probe DNA strands (5′-ACT GCT TAT GCT AAT AGT GTA AAA AAA AAA-DBCO-3′ (30 nucleotides, 5A nt spacer underlined, GC = 23.3%,) at five different ratios R(x) of 0.0375, 0.075, 0.135, 0.15, and 0.3 calculated using Eq. 1. These ratios correspond to 9.0 ± 0.9, 30.5 ± 3.3, 46.8 ± 2.1, 60.4 ± 2.2, and 106.9 ± 3.3 probe DNA/CMP, as estimated from the depletion assays, and are plotted in Figure 4 of the manuscript. The correlation between the R(x) ratio used for DNA labeling of gel unpurified and gel purified CMPs and the number of T(20) ssDNA and probe DNA per CMP, respectively, is shown in Figure S5b.

We performed magnetic biosensing assays on a different sequence than 20-mer T to avoid unspecific cross-linking between CMPs upon sensing complementary sequences. The target sequence is: 5′-TTT TTA CAC TAT TAG CAT AAG CAG T-3′ (25 nt).

In these assays, the ratio of target DNA to probe DNA on CMPs was set to 5 to ensure excess of the target. Typically, the mixture of CMP-DNA conjugates and target DNA in TE buffer with 500 mM NaCl was shaken at 300 rpm for 2 h. Next, the mixture was kept at 4 °C overnight. The MPS measurements were then performed directly on the mixture in a wash-free fashion. To determine the measurement uncertainty, three independent measurements were performed on each sample.

Magnetic particle spectroscopy (MPS)

The MPS measurements on particles in TE buffer were performed using a custom-built MPS setup (immunoMPS),[1] which is especially designed for sensitive magnetic immunoassays. The setup operates at an excitation frequency of 590 Hz and a magnetic field amplitude of $\mu_0 H$ = 15 mT. The measurements were performed on 60 µl particle suspensions at a typical particle concentration of 28 nM and at a temperature of 295 K.

MPS-based magnetic bioassays and analysis of MPS harmonics ratio

Our magnetic assays are based on changes in Brownian magnetic relaxation processes of MNPs that happen when the particle hydrodynamic size/volume increases upon hybridization of the DNA probes on the particles with the target DNA sequences in solution. A molecular binding event slows down the Brownian relaxation of the particles in ac magnetic fields and thereby reduces the amplitude of the MPS harmonics. The MPS higher harmonics are, however, more sensitive to changes in the particle relaxation dynamics than the fundamental excitation frequency. This means that the 5th harmonic drops more than the 3rd harmonic upon the binding event. Therefore, the ratio of the 5th to 3rd harmonics (HR53) drops after a successful binding to the target sequence compared to the HR53 of the particle sample without the target sequence. The MPS harmonics ratio is, therefore, a particle concentration-independent measure to judge success of MPS-based assays.

Cooperative dynamics: Hill equation

Relative gel front R_f, hydrodynamic size D_h, and the ACS peak frequency f_p vs. R(x) were fitted with a Hill equation given by:

$$R_f(R), D_h(R), f_c(R) = A_1 + A_2 \cdot \frac{1}{1 + \left(\frac{R_{half}}{R}\right)^n}$$

A_1, A_2, n, and R_{half} are fitting parameters. R_{half} is the nominal grafting density that produces a half-maximal change in the respective parameters and n is the Hill coefficient. For the fit to the R_f data the parameter A_1 is set to zero.

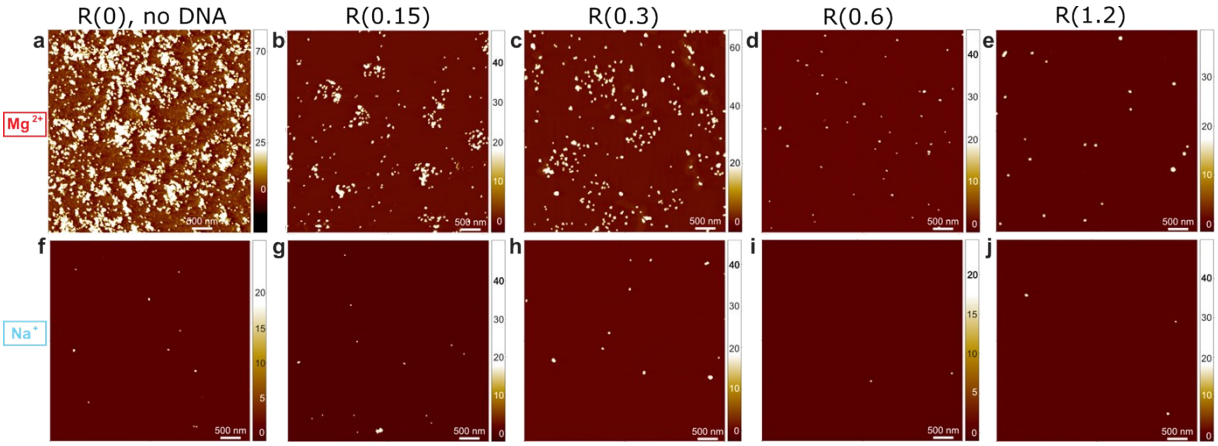

Figure S1. Atomic force microscopy images of CMPs with different grafting densities and ionic environments. Atomic force microscopy (AFM) height images of CMPs with no ssDNA grafting and of CMP-DNA conjugates at different grafting densities in the presence of 5 mM $MgCl_2$ divalent salt (top row) or 300 mM NaCl monovalent (bottom row). AFM images were recorded in tapping mode using a FASTSCAN-A cantilever on bare mica after drying. The z-ranges are indicated by the color map on the right side of each image (in nm).

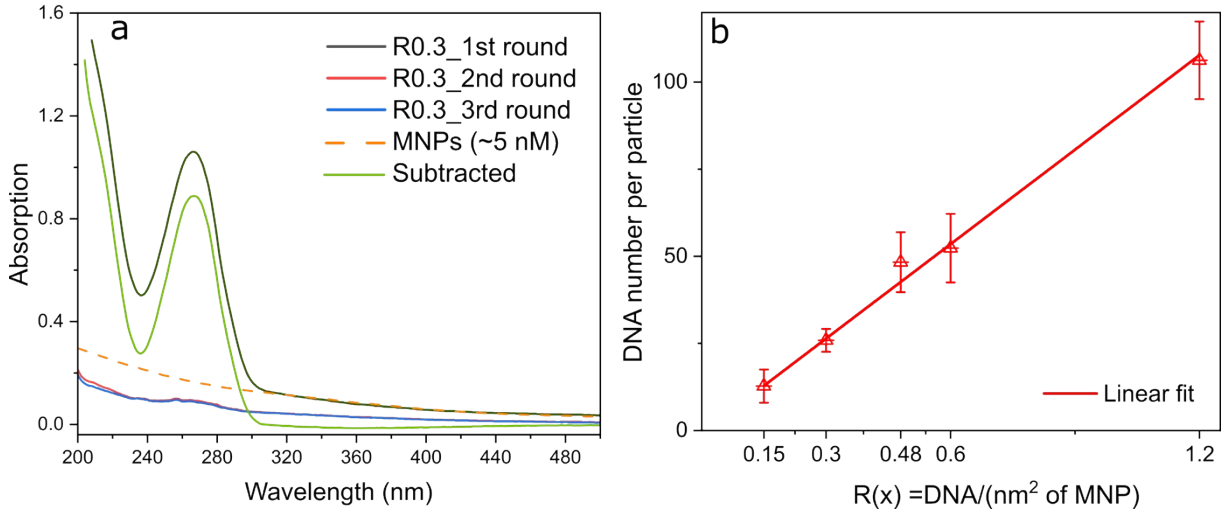

Figure S2. UV absorption spectra of supernatants and MNPs, background subtraction, and number of DNA/CMP. (a) UV absorption spectra of three sequential supernatants after particle centrifugation at 14000 rpm, here shown for CMP-DNA conjugates obtained at a nominal density of R(x) = 0.3. The absorption spectrum of MNPs at 5 nM concentration used as the background for data subtraction is shown, too. This is a typical concentration of MNPs that remains in the 1st supernatant after centrifugation. (b) Number of DNA per MNP as obtained from the depletion assays. This is an extended version of the data shown in Figure 1e of the main text.

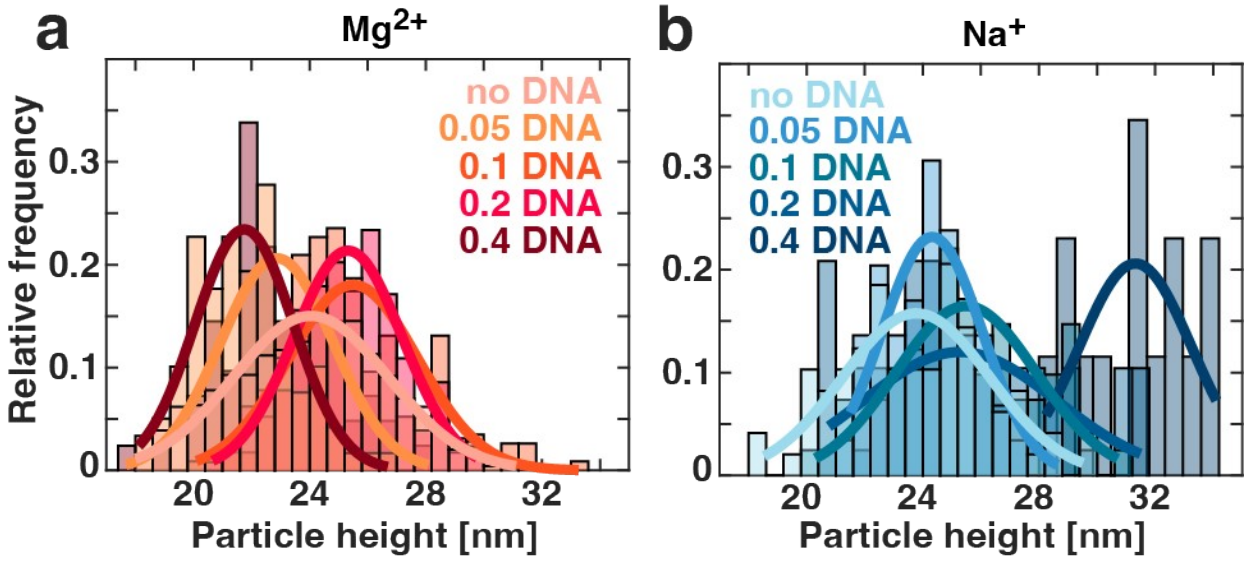

Figure S3. Particle height histograms from AFM imaging. Normalized particle height histograms of CMPs with no DNA grafting and of CMP-DNA conjugates at different grafting densities indicated in the legend. Data are in the presence of (a) 5 mM $MgCl_2$ and (b) 300 mM NaCl. The solid lines are Gaussian fits. Examples of the corresponding AFM images are shown in Figure S1. The mean ± standard deviation of the height distributions are shown in Figure 3e in the main text.

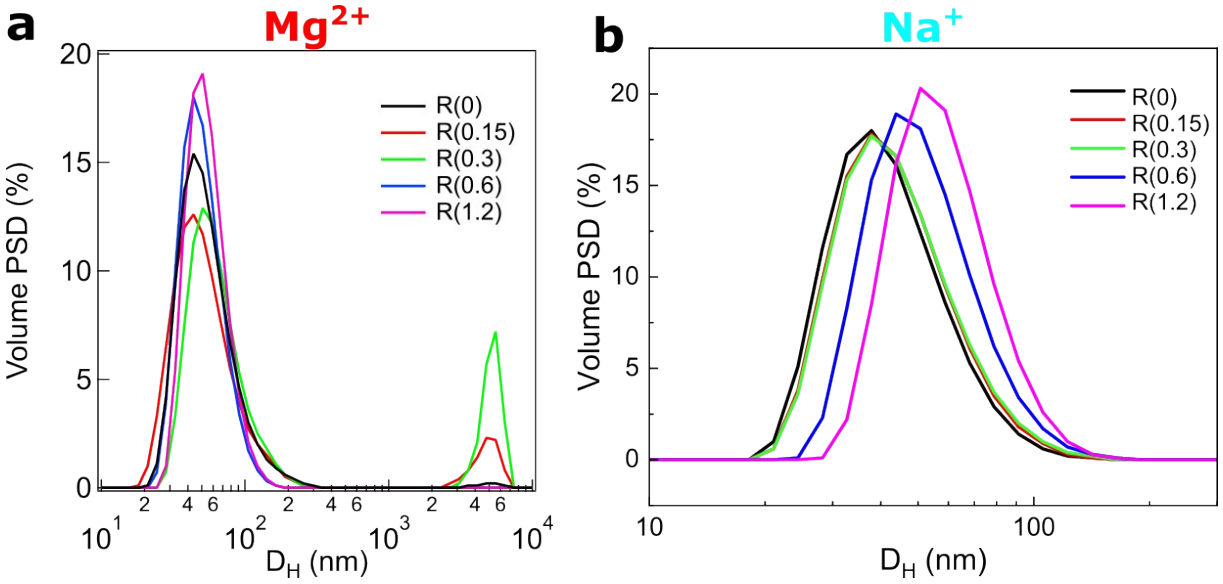

Figure S4. Particle size distributions from DLS measurements. Volume-weighted particle size distribution (PSD) of CMPs with no DNA grafting R(0) and of CMP-DNA conjugates at all grafting densities measured by DLS in the presence of (a) 5 mM $MgCl_2$ divalent ions and (b) 300 mM NaCl monovalent ions.

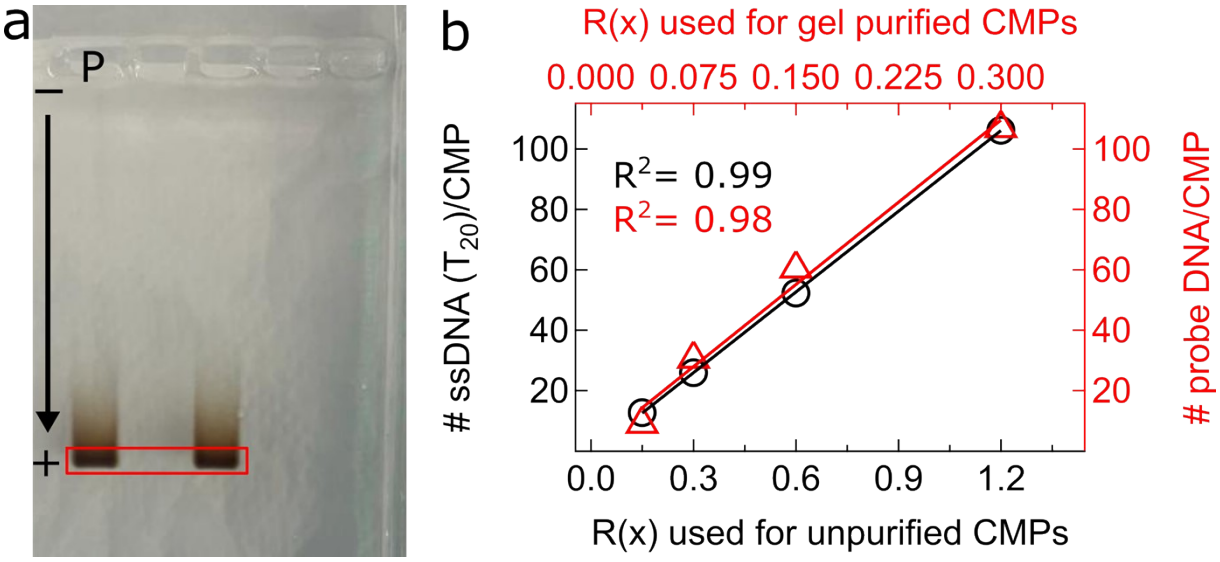

Figure S5. Agarose gel electrophoresis-based purification of particles and correlation between the number of ssDNA/CMP and the R(x) ratio for particles prior and after the gel purification. (a) A typical agarose gel electrophoresis procedure for removal of small aggregates, seen as a smeared tail of the narrow brownish band, to obtain singly-coated CMPs for magnetic biosensing assays. CMP samples were loaded in 1st and 3rd pocket. The gel was run at 132 V for 40 min. The red rectangle marks the piece of gel that was "cut & squeeze" to recover single CMPs. The squeezing of the gel was done between two glass sides covered with parafilm. P stands for pocket. (b) Numbers of T(20) ssDNA and the probe DNA per CMP estimated from the depletion assays as a function of the experimental R(x) ratios given in Eq. 1. These results show that DNA grafting densities very similar to the ones obtained on the unpurified CMPs can be achieved for the gel purified CMPs, yet by feeding four times less initial DNA into the DNA labeling reaction. The fact that the gel purified CMPs contain no aggregates (e.g. dimers, trimers etc.) may explain why smaller R(x) ratios are needed to yet obtain the similar grafting densities on these sample set. The black data points in panel (b) are exactly the same as the ones shown in Figure 1d in the main manuscript.

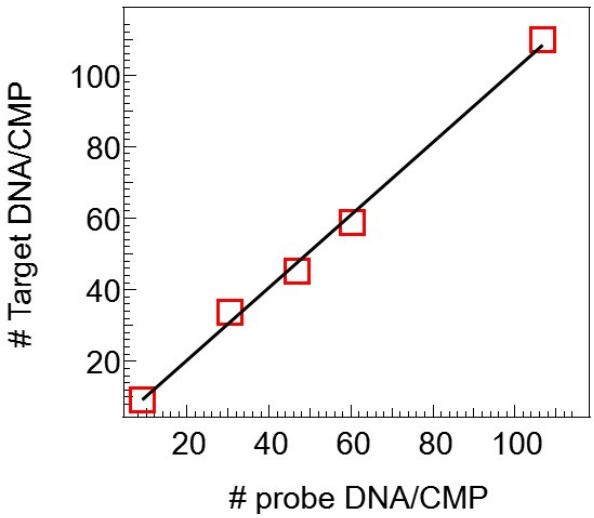

Figure S6. Hybridization efficiency on CMPs for the MPS-based magnetic assays. The number of the target DNA hybridized to the probe DNA on CMPs versus the probe DNA on CMPs as determined from the depletion assays. The black line is a linear function with slope ~ 1 and offset ~ 0. The results reveal that, in our assay design, the hybridization is quantitative, meaning that each probe DNA on CMPs gets one target DNA available in solution.

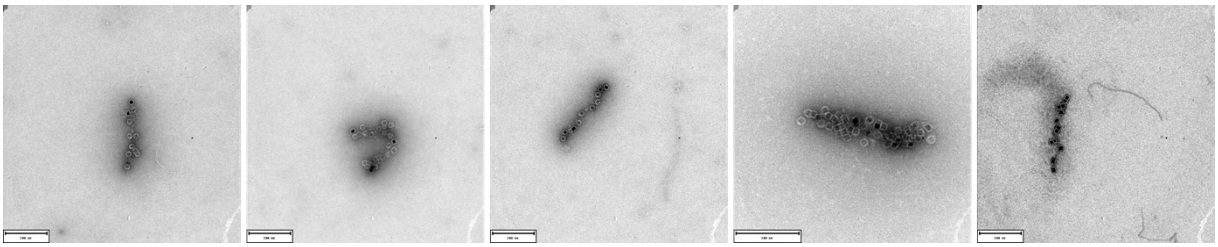

Figure S7. Collection of TEM images of 6HB DNA origami patterned with CMP-DNA conjugates at R(x) = 1.2. All images are negatively stained as discussed earlier.

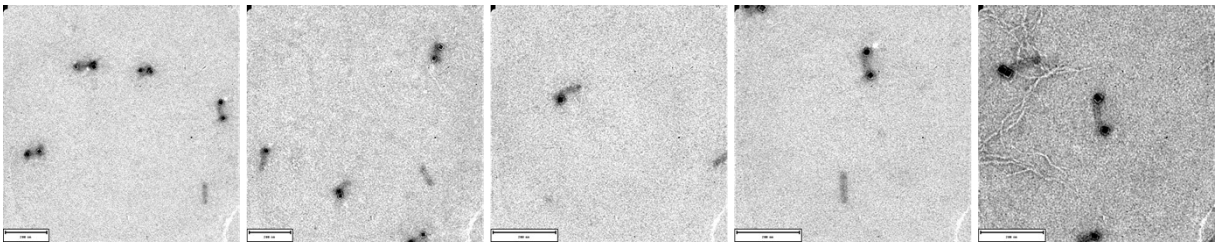

Figure S8. Collection of TEM images of 24HB DNA origami patterned with CMP-DNA conjugates at R(x) = 1.2. All images are negatively stained as discussed earlier.

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