Supporting Information (SI)

"A Broadly-Applicable Assay for Rapidly and Accurately Quantifying DNA Surface Coverage on Diverse Particles" by Haixiang Yu,[1†] Xiaowen Xu,[1†] Pingping Liang,[1] Kang Yong Loh,[2] Bhargav Guntupalli,[1] Daniel Roncancio[1] and Yi Xiao[1*]

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1. Materials

Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate dihydrate, tris(hydroxymethyl) aminomethane (Tris), Trizma pre-set crystals, sodium chloride, sodium hydroxide, magnesium chloride, ammonia fluoride, yttrium (III) acetate hydrate, ytterbium (III) acetate hydrate, 2-(N-morpholino)ethanesulfonic acid (MES), sodium dodecyl sulfate (SDS), oleic acid (OA), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), 1-octadecene, tetraethyl orthosilicate, 3-mercaptopropyl trimethoxysilane, 4-maleimidobutyric acid N-hydroxysuccinimide ester (GMBS), streptavidin (SA) from *Streptomyces avidinii* and polyvinylpyrrolidone were purchased from Sigma-Aldrich. Erbium (III) acetate hydrate was purchased from Strem Chemicals. Ethanol was purchased from Decon Laboratories. 10× phosphate buffered saline (10×PBS) was purchased from Lonza. Phosphate buffer (PB) (1 M, pH 7.4), calcium chloride dehydrate, ethylenediaminetetraacetic acid (EDTA) disodium salt, formamide, Tween 20, ammonia hydroxide, dimethyl sulfoxide (DMSO), cyclohexane, acrylamide, ammonium persulfate and N, N, N', N'-tetramethylethylenediamine were bought from Fisher. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was purchased from Thermo Scientific. DL-dithiothreitol (DTT) was obtained from Promega. SYBR Gold was obtained from Invitrogen. Acetic acid was purchased from Acros Organics. Exonuclease III (*E. coli*) and DNase I (RNase-free) were purchased from New England Biolabs. SA- (1-μm diameter, Dynabeads MyOne Streptavidin T1) and carboxylic acid-functionalized magnetic beads (1-μm diameter, Dynabeads MyOne Carboxylic Acid) were bought from Life Technologies. Carboxylic acid-functionalized silica beads (1-μm diameter) were obtained from Polysciences. Qdot 705 SA-conjugated quantum dots (18-nm diameter) were obtained from ThermoFisher Scientific. SA-modified upconversion nanoparticles (UCNPs) and OA-coated UCNPs were synthesized and characterized by our collaborator at the University of Illinois at Urbana−Champaign.

2. Instrumentation

UV-vis absorption spectra, fluorescence spectra and time-dependent fluorescence changes were collected on an Infinite M1000 Pro microplate reader (Tecan). Modification of DNA onto magnetic and silica beads was performed on a Labquake tube rotator (Thermo Scientific). Magnetic beads were separated with a Dynabeads magnetic particle concentrator (Life Technologies). Sonication was done with a FS60D ultrasonic cleaner (Fisher Scientific). DNA concentrations were measured on a Nanodrop 2000 (Thermo Scientific). Transmission electron microscopy (TEM) images of UCNPs were obtained by a JEOL 2100 Cryo TEM with an accelerating voltage of 200kV. UCNP fluorescence was characterized by a FluoroMax-P fluorimeter (HORIBA Jobin Yvon) modified with a commercial continuous-wave infrared laser (ThorLabs) that emits at 980 nm. Weight measurements were

taken using an XS 105 Dual range weighing balance (Mettler Toledo). The gel electrophoresis picture was recorded using ChemiDoc MP imaging system (Bio-Rad).

3. Methods

3.1 Synthesis and DNA modification of gold nanoparticles (AuNPs). AuNPs (13 ± 1 nm diameter) were synthesized by a modification of Turkevich's method.[1] AuNP concentration was determined by using the absorbance of five-fold diluted colloids and the extinction coefficient at 520 nm ($\varepsilon = 2.7 \times 10^8$ M^{-1} cm^{-1}),[2] according to Beer's law. Prior to modification, SH-2X, SH-1X or SH probe DNAs were mixed with 100 mM freshly-prepared TCEP in deionized water, and the mixture was incubated at room temperature in the dark for 2 hrs to reduce the disulfide bond and activate thiolated DNA. Different amounts of reduced DNA probes were added into separate aliquots of 3 mL of freshly-prepared 10 nM AuNPs under stirring to achieve a DNA:AuNP ratio of 40, 60, 80, 120, 150, 200, or 300. After standing at room temperature for 12 hrs, salt aging was employed using a previously reported method with modifications.[3] Briefly, concentrations of PB and SDS were first brought to 10 mM and 0.01%, respectively. After a 20-min incubation, a solution of 2 M NaCl prepared with 0.01 M PB and 0.01% SDS was added to the modified AuNP solution to obtain 0.05 M NaCl. The solution was sonicated for 10 s and then incubated at room temperature for 20 min. For DNA:AuNP ratios of 40, 60, 80, 120, 150, or 200, the same salt aging process was repeated until the concentration of NaCl reached 0.3 M. For the DNA:AuNP ratio of 300, the final salt concentration was increased to 1.0 M to achieve maximum DNA loading. The DNA-modified AuNPs were then incubated for another 12 hrs and separated from unconjugated DNA using a Millipore 100K molecular weight cut-off (MWCO) spin filter and washed six times with 10 mM Tris-HCl buffer (pH 7.4). Finally, the DNA-modified AuNPs were resuspended in the same buffer to obtain a particle concentration of 100 nM.

3.2 Synthesis and characterization of OA-capped upconversion nanoparticles (UCNP-OA). UCNP-OA particles were synthesized using a previously reported method with modifications.[4] Briefly, 2 mL of $Ln(CH_3COO)_3$ (0.2 M, Ln^{3+} = Y, Yb, Er) in water was mixed with 3 mL of OA and 7 mL of 1-octadecene in a 50 mL round bottom flask. The mixture was heated to 135 ^oC to remove water for 1.5 hr before cooling to 50 ^oC. At 50 ^oC, a mixture containing 4 mL of 0.4 M NH_4F and 2 mL of 0.5 M NaOH was added and stirred for 30 min. Subsequently, the mixture was heated to 100 ^oC for 15 min to remove methanol and all gaseous byproducts. The mixture was heated to 290 ^oC under nitrogen gas for 2 hrs, and then washed with cyclohexane and ethanol three times before redispersing the particles in 4 mL of cyclohexane. Upconversion properties of the UCNP-OA were characterized by fluorescence spectrometry (SI, Figure S20). The size and shape of the UCNP-OA were characterized by TEM (25 ± 1 nm) (SI, Figure S21). The molarity of the UCNPs (2.65 μM)

was calculated from the mass concentration of the UCNPs in the stock solution, by subtracting the combined mass of solvent and UCNP per 100 μL from the mass of solvent in 100 μL. The mass of a single particle was calculated from the diameter of the spherical UCNP and the density of a host $NaYF_4$ lattice, as previously reported.[5]

3.3 DNA modification on UCNP-OA. 5'-unmodified 2X probe DNAs were conjugated onto UCNP-OA following a modified version of our previously published method.[6] Briefly, 1 mL of previously synthesized UCNP-OA particles (2.65 μM) were dispersed temporarily in ethanol. A solution of 0.25 M HCl was added to obtain ligand-free UCNPs. These were subsequently washed with ethanol three times before redispersing in water. We mixed 75 nmole of 5'-unmodified-2X probe DNA with 25 pmole of ligand-free UCNPs in 250 μL PCR-grade water, followed by a 24-hr incubation in a thermomixer at 20 °C. The DNA-modified UCNPs were then separated from excess DNA using a Millipore 30K MWCO spin filter, and washed six times with 20 mM Tris-Ac buffer (pH 7.9). Finally, the DNA-modified UCNPs were suspended in the same buffer to obtain a particle concentration of 100 nM.

3.4 Functionalization and characterization of SA-functionalized UCNPs (UCNP-SA). 1 mL of previously synthesized UCNP-OA particles (2.65 μM) were dispersed temporarily in ethanol, and a solution of 0.25 M HCl was added to obtain ligand-free UCNPs. The UCNPs were subsequently washed with ethanol three times before redispersing in water. 200 mg polyvinylpyrrolidone K-30 was mixed with the ligand-free UCNPs in water and the resultant mixture was incubated and stirred for 2 hrs at room temperature. The UCNPs were then sonicated for 20 min before adding 20 mL of ethanol. The mixture was sonicated for another 20 min before adding 800 μL of ammonium hydroxide solution. The mixture was further sonicated for another 20 min before finally adding 200 μL of tetraethyl orthosilicate. The final mixture was kept at room temperature under stirring overnight. The resultant UCNP-SiO_2 was washed with ethanol three times before resuspending in 10 mL of ethanol. This stock solution of UCNP-SiO_2 was further diluted 4-fold in ethanol, and 1 mL of the diluted UCNP-SiO_2 was mixed with 1880 μL of ethanol and 120 μL of 3-mercaptopropyl trimethoxysilane in 4% ethanol for 45 min. The mixture was washed with ethanol three times before finally dispersing in 1 mL DMSO. 1 mL of this SiO_2-coated UCNP solution was combined with 1 mL of 0.5 mM GMBS in DMSO and stirred for 30 min. The reaction solution was washed once with DMSO and three times with 1× PBS. The GMBS-modified UCNP-SiO_2 particles were resuspended in 1 mL of 1× PBS. The size and shape of the resulting UCNP-SiO_2 particles were characterized by TEM (76±5 nm) (SI, Figure S22). 1 mL of GMBS-modified UCNP-SiO_2 was added to 1 mL of 20 μg/mL SA in 1× PBS.[7] The upconversion properties of the UCNP-SAs were characterized by fluorescence spectrometry (SI, Figure S23).

3.5 DNA modification of UCNP-SA and SA-coated quantum dots (QD-SA). Biotin-2X probe DNAs were modified onto UCNP-SA and QD-SA following the manufacturer's recommendations. Briefly, 25 pmole of UCNP-SA or QD-SA were mixed with 5 nmole of biotin-2X probe DNA in 100 μL 1 × PBS containing 10× BSA, followed by a 2-hr incubation at room temperature. DNA-modified UCNPs and QDs were then separated from excess DNA using a Millipore 100K MWCO spin filter and washed six times with 20 mM Tris-Ac buffer (pH 7.9). Finally, the DNA-modified UCNPs and QDs were suspended in the same buffer to obtain a particle concentration of 100 nM.

3.6 DNA modification of SA-coated magnetic beads (MB-SA). Biotin-2X probe DNAs were modified onto MB-SA following the manufacturer's recommendations. Briefly, 50 μL of 10 mg/mL (equivalent to 14 pM) MB-SA particles were washed three times with 1× binding & washing (B&W) buffer (5 mM Tris-Ac, 0.5 mM EDTA, 1 M NaCl, pH 7.5) and resuspended in 100 μL of 2× B&W buffer. 100 μL of 5 μM biotin-2X probe (in deionized water) was then added to the beads, and the mixture was incubated on a tube rotator for 30 min at room temperature. The DNA-modified magnetic beads were washed three times with 1× B&W buffer to remove unbound oligonucleotides, washed three more times with 20 mM Tris-Ac buffer (pH 7.9), and finally resuspended in 50 μL of 20 mM Tris-Ac buffer (pH 7.9) to achieve a final concentration of 14 pM.

3.7 DNA modification of carboxylic acid-functionalized magnetic beads (MB-COOH). NH_2-2X probe DNAs were conjugated onto MB-COOH particles according to the manufacturer's recommendations. Briefly, 100 μL of 10 mg/mL (equivalent to 16 pM) MB-COOH beads were washed twice with 100 mM MES buffer (pH 4.8) and resuspended in 10 μL of MES. 12 μL of 417 μM NH_2-2X probe was mixed with 8 μL of 1.25 M EDC in 100 mM MES buffer (pH 4.8), and the above solution was added to 10 μL of MB-COOH beads. The suspension was incubated for 2 h at room temperature and then overnight at 4 °C under rotation. The DNA-modified beads were washed three times with TT buffer (250 mM Tris-Ac, pH 8.0, 0.01% Tween-20), further washed three times with 20 mM Tris-Ac buffer (pH 7.9), and finally resuspended in 100 μL of 20 mM Tris-Ac buffer (pH 7.9) to achieve a final concentration of 16 pM.

3.8 DNA modification of carboxylic acid-functionalized silica microspheres (SiO_2-COOH). Dry SiO_2-COOH microspheres were dissolved in purified water and bath sonicated for 15 min. 100 μL of 20 mg/mL dispersed microspheres (equivalent to 31 pM) were washed three times with 100 mM MES buffer (pH 4.8), and resuspended in 10 μL of the same buffer. 12 μL of 417 μM NH_2-2X probe was mixed with 8 μL of 1.25 M EDC in 100 mM MES buffer (pH 4.8), and the above solution was added to the 10 μL of SiO_2-COOH microspheres. The mixture was incubated for 2 hrs at room temperature and further incubated

overnight at 4 °C under rotation. The DNA-modified microspheres were washed three times with 20 mM Tris-Ac buffer (pH 7.9), and finally resuspended in 100 μL of 20 mM Tris-Ac buffer (pH 7.9) to achieve a final concentration of 31 pM.

3.9 Quantitation of DNA surface coverage on AuNPs via DTT displacement. 1.25 μL of SH-2X probe DNA-modified AuNPs (final concentration 3.1 nM) were mixed with 41.75 μL of 20 mM Tris-Ac (pH 7.9) buffer containing 0.51 M DTT. After incubation at room temperature for 12 hrs, the solution was centrifuged at 21,130 rcf × 10 min to remove the AuNP precipitate, after which 40 μL of supernatant was transferred into a 384-well microplate to record the fluorescence spectra from 505 nm to 850 nm (λ_{ex} = 495 nm). To establish the standard calibration curve, 1.25 μL of 20 mM Tris-Ac buffer (pH 7.9) containing known concentrations of SH-2X probe was mixed with 41.75 μL of 20 mM Tris-Ac (pH 7.9) buffer containing 0.51 M DTT. The calibration samples underwent the same incubation and centrifugation process as the DNA-modified AuNP samples. The number of oligonucleotides per particle (N) was calculated from the ratio between the DNA concentration as determined by the calibration curve and the particle concentration in the sample.

3.10 Characterization of the sequence effect of cDNA. To investigate the effect of the nucleotide at the site opposite the abasic site on Exo III AP endonuclease activity, we designed an unmodified 1X probe, and four cDNAs with 3' poly-$(T)_8$ overhang and different nucleotides (A, T, C and G) at the site opposite the abasic site of the 1X probe DNA. The poly-$(T)_8$ overhang was added to inhibit the enzyme's exonuclease activity. 200 nM of the cDNA was mixed with 1 μM 1X probe DNA in the reaction buffer and incubated for 30 min before addition of 0.004 U/μL of Exo III. The sample was analyzed by 15% denaturing polyacrylamide gel electrophoresis (PAGE) after 0, 30 min and 120 min of digestion. 3 μL of each sample in loading buffer was loaded into each well, and separation was carried out at 20 V/cm for 3 hours using 0.5× TBE running buffer. The gel was stained with 1X SYBR Gold solution for 25 min before imaging.

3.11 Characterization of the limit of quantitation of Exo III-based and DTT-based assays. The Exo III-based and DTT-displacement assays were performed as described above with different concentrations (final concentration 31 fM to 3.1 nM) of SH-2X probe DNA-modified AuNPs (79 strands/particle). We calculated the limit of quantitation by generating a calibration curve from fluorescence intensity data collected from standard samples consisting of different concentrations of DNA-modified AuNPs prepared via either method. The limit of quantitation was calculated as $10 \times S_a/b$, where S_a is the standard deviation of the blank sample and b is the slope of the established calibration curve.

3.12 Quantitation of DNA surface coverage on AuNPs via DNase I hydrolysis. 1.25 μL SH-2X probe DNA-modified AuNPs (final concentration 3.1 nM) were mixed with 38.75 μL of DNase I buffer (10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.6) and 3 μL of 1.67 U/μL DNase I. After incubation at room temperature for 16 h, the solution was centrifuged at 21,130 rcf × 10 min to remove the AuNP precipitates, and 40 μL of supernatant was transferred into a 384-well microplate to record fluorescence spectra from 504 nm to 850 nm (λ_{ex} = 495 nm). A standard calibration curve was established by mixing 1.25 μL of 20 mM Tris-Ac buffer (pH 7.9) containing known concentrations of SH-2X probe, 38.75 μL DNase I buffer and 3 μL of 1.67 U/μL DNase I. These samples underwent the same incubation and centrifugation process. For assays performed in the presence of cDNA, we mixed 1.25 μL of DNA-modified AuNPs with 2 μL of 4 μM cDNA, 36.75 μL DNase I buffer and 3 μL of 1.67 U/μL DNase I. We established a standard calibration curve by mixing 1.25 μL of 20 mM Tris-Ac buffer (pH 7.9) containing known concentrations of SH-2X probe, 2 μL of 4 μM cDNA, 36.75 μL DNase I buffer and 3 μL of 1.67 U/μL DNase I. The number of oligonucleotides per particle (N) was calculated from the ratio of the sample DNA concentration as determined by the calibration curve to the particle concentration in the sample.

3.13 Quantification of biotinylated DNA on QD-SA and UCNP-SA via heating-based elution. 1.25 μL of biotin-2X probe DNA-modified MB-SA or UCNP-SA stock solution was mixed with 41.75 μL of 10 mM EDTA, pH 8.2 in 95% formamide at 90 °C for 10 min. The solution was centrifuged at 21,130 rcf × 10 min to remove the particles, and the fluorescence spectra of the supernatant from 504 nm to 850 nm (λ_{ex} = 495 nm) was collected. A corresponding standard curve was established by incubating a mixture of 1.25 μL of 20 mM Tris-Ac buffer (pH 7.9) containing different concentrations of biotin-2X probe and 41.75 μL of 10 mM EDTA, pH 8.2 in 95% formamide at 90 °C for 10 min. The QD-SA cannot be totally removed by the centrifugation step, and since the QD-SA particles did not significantly quench FAM fluorescence, we collected the fluorescence spectra from 504 nm to 850 nm (λ_{ex} = 495 nm) in the presence of QD-SA particles. The same amount of unmodified QD-SA was added into the calibration samples before heat elution. The number of oligonucleotides per particle (N) was calculated from the ratio of the sample DNA concentration as determined by the calibration curve to the particle concentration in the sample.

3.14 Calculation of DNA surface coverage on different particles. DNA surface coverage was calculated from the amount of DNA per particle (n) and the surface area (A) of the particle. *n* was calculated by the equation $\mathrm{n} = \frac{\mathrm{N}}{6.02 \times 10^{11}}$ pmole, where N is the number of

oligonucleotides per particle. A can be calculated as $\pi d^2 \times 10^{-14}$ cm^2, where d is the average diameter of the particle in nm. Thus:

$$\text{DNA surface coverage} = \frac{n}{A} = \frac{N}{6.02\times10^{11}\,\text{pmole}^{-1}\times\pi d^2\times10^{-14}\,\text{cm}^2} = 52.9\times\frac{N}{d^2}\ \text{pmole/cm}^2$$

4. List of Tables

DNA sequences. DNA oligonucleotides were synthesized by Integrated DNA Technologies with HPLC purification. DNA was dissolved in 1×TE buffer as a stock solution of 500 μM. The concentrations of DNA were determined according to UV absorbance at 260 nm. The sequences of the oligonucleotides are as follows:

Table S1: Sequence ID and DNA sequences used in this work.

Sequence ID	Sequence
SH probe	5'-HS-$(CH_2)_6$-TTTTTTACCACATCATCCA TATAACTGAAAGCCAAACAGTTTTTTTT-$(CH_2)_6$-FAM-3' [a]
SH-2X probe	5'-HS-$(CH_2)_6$- TTTTTTACCACATCATCC/iSpC3/TATAAC TGAAA/iSpC3/CCAAACAGTTTTTTTT-$(CH_2)_6$-FAM-3'
SH-1X probe	5'-HS-$(CH_2)_6$-TTTTTTACCACATCATCC/iSpC3/TATAAC TGAAAGCCAAACAGTTTTTTTT-$(CH_2)_6$-FAM-3'
1X probe	5'-ACCACATCATCC/iSpC3/TATAACTGAAAGCCAAACAGTTTTTTTT-3' [b]
Biotin-2X probe	5'-Biotin-$(CH_2)_6$-TTTTTTACCACATCATCC/iSpC3/TATAAC TGAAA/iSpC3/CCAAACAGTTTTTTTT-$(CH_2)_6$-FAM-3'
NH_2-2X probe	5'-NH_2-$(CH_2)_6$-TTTTTTACCACATCATCC/iSpC3/TATAAC TGAAA/iSpC3/CCAAACAGTTTTTTTT-$(CH_2)_6$-FAM-3'
5'-unmodified -2X probe	5'-TTTTTTACCACATCATCC/iSpC3/TATAAC TGAAA/iSpC3/CCAAACAGTTTTTTTT-$(CH_2)_6$-FAM-3'
cDNA	5'-CTGTTTGGCTTTCAGTTATATGGATGATGTGGT-3'
cDNA-A	5'-CTGTTTGGCTTTCAGTTATAAGGATGATGTGGTTTTTTTTT-3'
cDNA-T	5'-CTGTTTGGCTTTCAGTTATATGGATGATGTGGTTTTTTTTT-3'
cDNA-C	5'-CTGTTTGGCTTTCAGTTATACGGATGATGTGGTTTTTTTTT-3'
cDNA-G	5'-CTGTTTGGCTTTCAGTTATAGGGATGATGTGGTTTTTTTTT-3'
cDNA-8A	5'-AAAAAAAACTGTTTGGCTTTCAGTTATATGGATGATGTGGT-3'
28-nt DNA	5'-TATAACTGAAAGCCAAACAGTTTTTTTT-3'

a. FAM represents fluorescein label b. /iSpC3/ represents internal propanyl abasic site

Table S2: DNA surface coverage on SH-2X probe DNA-modified AuNPs as calculated by various methods.

DNA: AuNP ratio	Exo III digestion		DTT displacement		DNase I hydrolysis - cDNA		DNase I hydrolysis + cDNA	
	Strands /particle	Surface coverage (pmole/cm^2)	Strands /particle	Surface coverage (pmole/cm^2)	Strands /particle	Surface coverage (pmole/cm^2)	Strands /particle	Surface coverage (pmole/cm^2)
60	34±1	10.6±0.2	34±1	10.6±0.2	22±1	6.9±0.2	33±1	10.3±0.2
80	44±2	13.6±0.5	42±3	13.2±0.8	27±1	8.6±0.4	43±1	13.5±0.3
120	56±3	17.7±0.8	54±2	17.0±0.8	45±2	14.1±0.5	53±1	16.6±0.5
150	63±1	19.8±0.2	63±2	19.8±0.7	43±4	13.3±1.1	57±1	17.8±0.2
200	71±1	22.3±0.3	72±2	22.4±0.6	52±1	16.4±0.4	62±1	19.5±0.3
300	79±3	24.9±0.8	80±1	24.9±0.4	62±3	19.5±0.8	61±1	19.0±0.2

Table S3: Scope of application for various methods of quantifying DNA surface coverage

Quantification methods	**AuNPs**	**Inorganic nanocrystals (UCNPs)**		**Magnetic beads**		**Silica particles**
	Thiol-gold bond	**SA-biotin interaction**	**Electrostatic interaction**	**SA-biotin interaction**	**Covalent linkage**	**Covalent linkage**
DTT displacement[3]	√	×	×	×	×	×
Heating-based elution[8]	×	√	×	√	×	×
DNase I hydrolysis[9]	√	N/A	N/A	N/A	N/A	N/A
Radioisotope labeling[8, 10–12]	√	N/A	N/A	√	√	√
Exo III–based assay (This work)	√	√	√	√	√	√

5. List of Figures

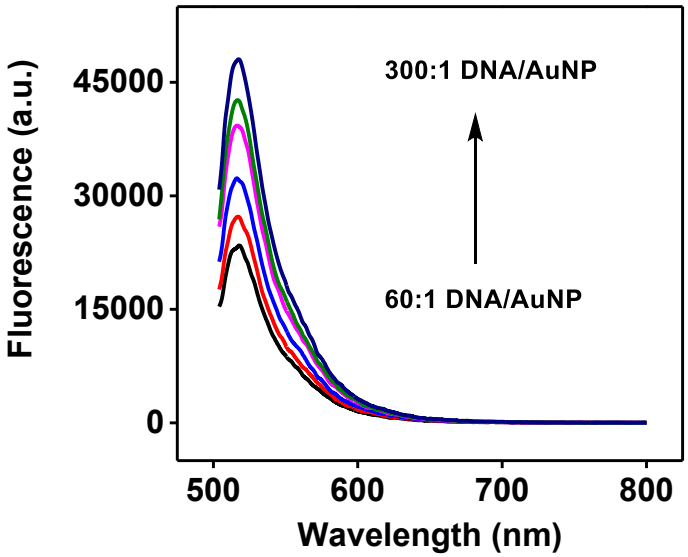

Figure S1: Determination of DNA surface coverage on SH probe DNA-modified AuNPs with our Exo III-based assay. Fluorescence spectra of the supernatant from AuNPs modified with a DNA:AuNP ratio of 60, 80, 120, 150, 200 or 300 after incubation with Exo III (0.2 U/μL) and 200 nM cDNA-8A for 255 min in reaction buffer. These spectral data are plotted in Figure 3A.

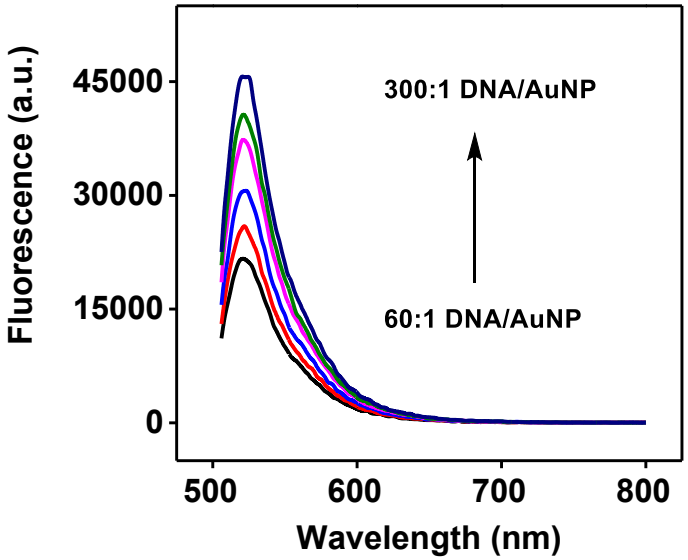

Figure S2: Determination of DNA surface coverage on SH probe DNA-modified AuNPs with the DTT displacement assay. Fluorescence spectra of supernatants from AuNPs modified with a DNA:AuNP ratio of 60, 80, 120, 150, 200 or 300 after treatment with DTT (0.5 M as final concentration) for 12 h in reaction buffer. These spectral data are plotted in Figure 3A.

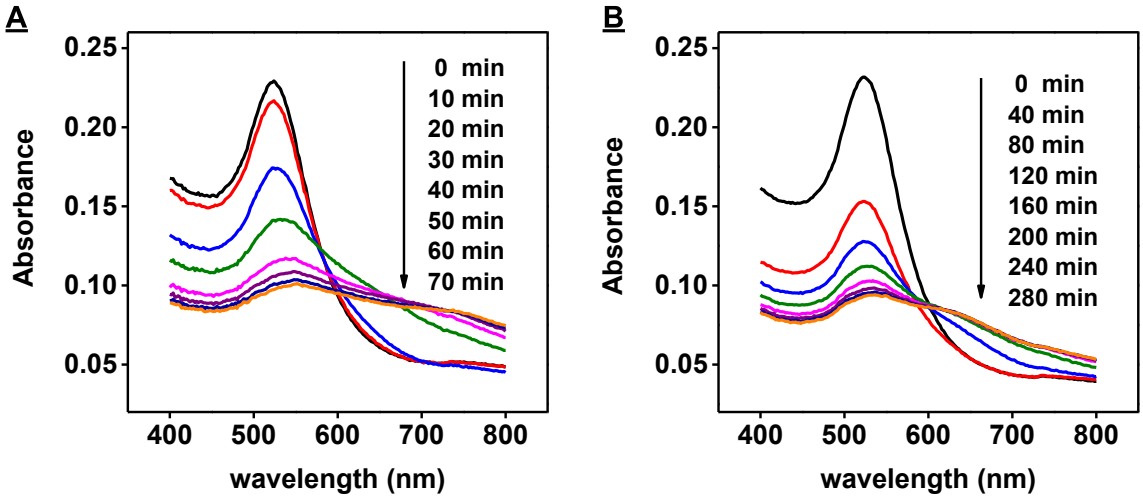

Figure S3: Time-dependent absorbance changes in Exo III-based assays with AuNPs modified with either (**A**) SH-2X probe DNA or (**B**) SH-1X probe DNA at a DNA:AuNP ratio of 300 (This refers to the initial ratio of DNA to AuNPs that was used for the particle modification). Surface coverage for 2X and 1X probe DNA-modified AuNPs were 79 and 77 strands/particle, respectively. The A_{650}/A_{520} of 2X and 1X probe DNA-modified AuNPs reached saturation after 60 min and 230 min, respectively. These spectral data are plotted in Figure 5B.

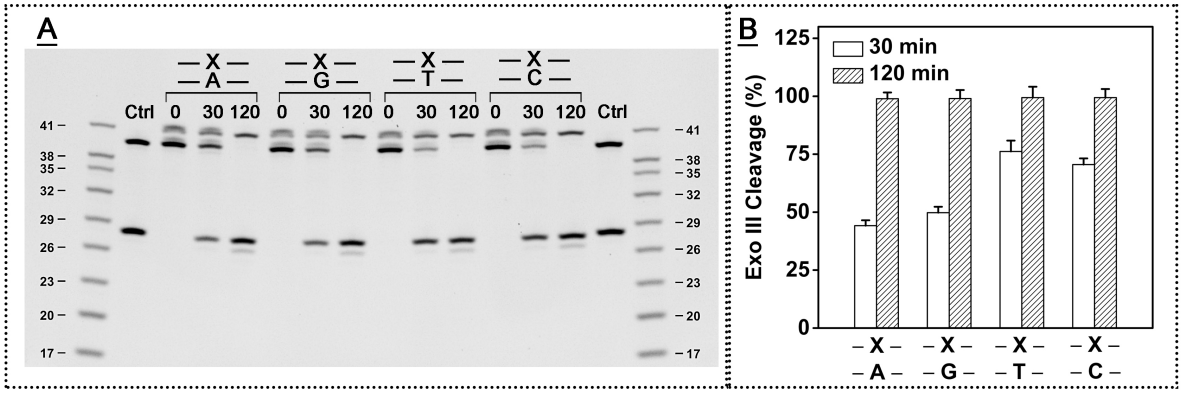

Figure S4: Sequence effect of cDNA on AP endonuclease activity of Exo III (0.004U/μL). (**A**) We incubated a mixture of 1 μM 1X probe DNA and 200 nM cDNA with different nucleotides at the site opposite the abasic site with 0.004 U/μL Exo III for 0, 30 or 120 min. 1 μM of 1X probe DNA and 1 μM of the synthesized 28-nt DNA was used as a control. (**B**) The cleavage of 1X probe DNA by Exo III was evaluated by $Int_{Exo\ III}/Int_0 \times 100\%$, where $Int_{Exo\ III}$ is the intensity of the 1X probe band after 30 or 120 min of digestion and the Int_0 is the intensity of the 1X probe band without Exo III digestion.

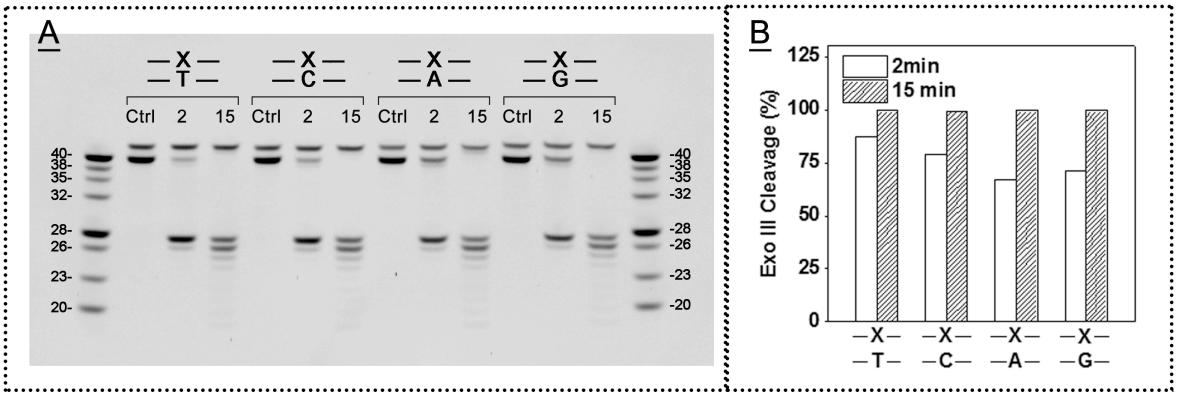

Figure S5: Sequence effect of cDNA on AP endonuclease activity of Exo III (0.2U/μL). (**A**) We incubated a mixture of 1 μM 1X probe DNA and 200 nM cDNA with different nucleotides at the site opposite the abasic site with 0.2 U/μL Exo III for 0, 2 or 15 min. 1 μM of 1X probe DNA and 1 μM of the synthesized 28-nt DNA was used as a control. (**B**) The cleavage of 1X probe DNA by Exo III was evaluated by $Int_{Exo\ III}/Int_0 \times 100\%$, where $Int_{Exo\ III}$ is the intensity of the 1X probe band after 2 or 15 min of digestion and the Int_0 is the intensity of the 1X probe band without Exo III digestion.

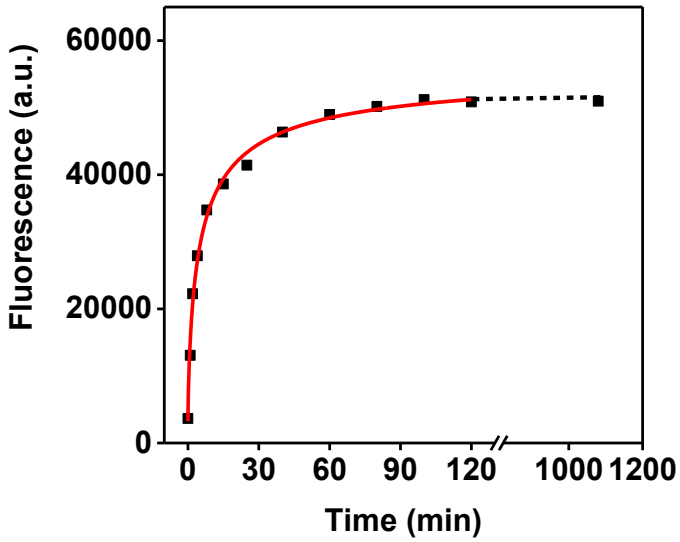

Figure S6: Time-dependent changes in FAM fluorescence intensity for maximally-loaded SH-2X probe DNA-modified AuNPs in our Exo III-based assay. AuNPs modified at a DNA:AuNP ratio of 300 (This refers to the initial ratio of DNA to AuNPs that was used for the particle modification) were incubated with Exo III (0.2 U/μL) and 200 nM cDNA in reaction buffer. The fluorescence intensity reached saturation after 60 min, with no further increase after 18 hrs.

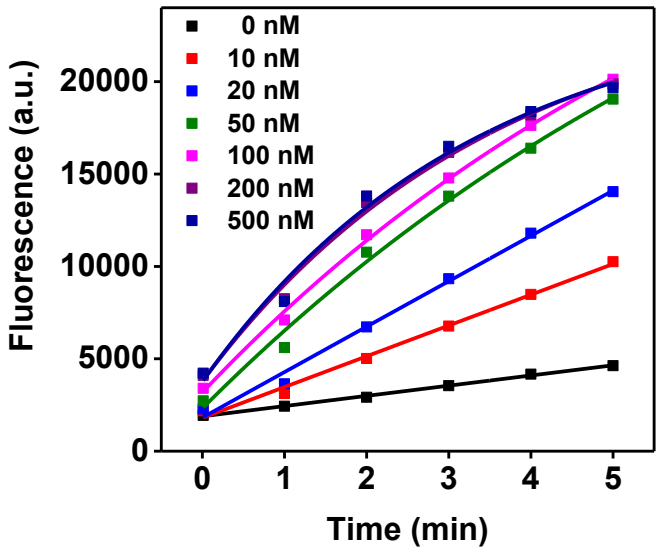

Figure S7: Effect of cDNA concentrations on the Exo III reaction rate. AuNPs modified at a DNA:AuNP ratio of 300 (This refers to the initial ratio of DNA to AuNPs that was used for the particle modification) were incubated with Exo III (0.01 U/μL) and different concentrations of cDNA in reaction buffer (20 mM Tris-Ac, 50 mM KAc, 25 mM NaCl, 20 mM $CaCl_2$, 3 mM $MgCl_2$ and 1 × BSA, pH 7.9). A low concentration of Exo III was used in this experiment to better present the dependence of cDNA concentration.

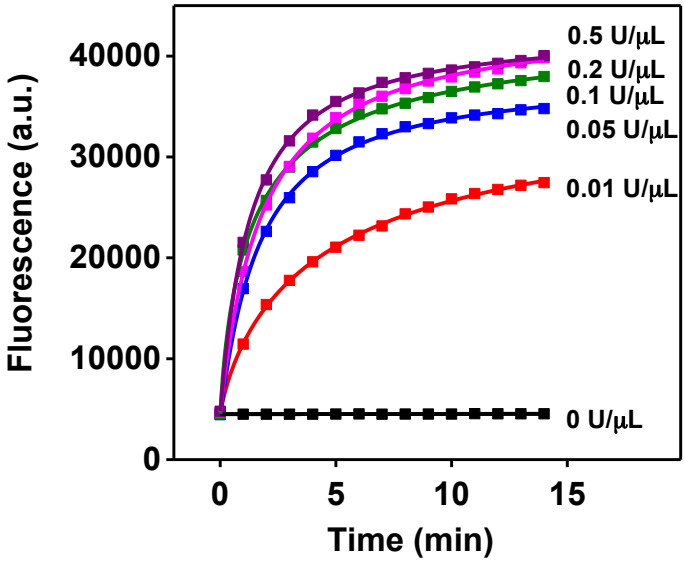

Figure S8: Effect of Exo III concentrations on the Exo III reaction rate. AuNPs modified at a DNA:AuNP ratio of 300 (This refers to the initial ratio of DNA to AuNPs that was used for the particle modification) were incubated with cDNA (200 nM) and different concentrations of Exo III in reaction buffer.

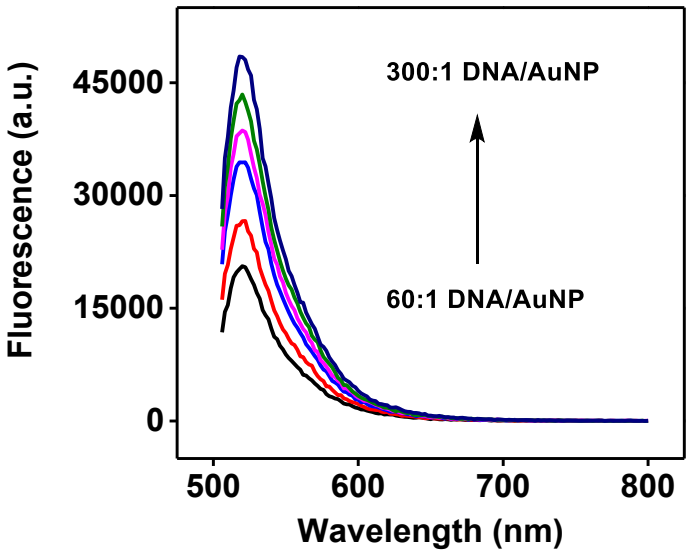

Figure S9: Determination of DNA surface coverage on SH-2X probe DNA-modified AuNPs with our Exo III-based assay. Fluorescence spectra of the supernatant from AuNPs modified with a DNA:AuNP ratio of 60, 80, 120, 150, 200 or 300 (This refers to the initial ratio of DNA to AuNPs that was used for the particle modification) after incubation with Exo III (0.2 U/μL) and 200 nM cDNA for 60 min in reaction buffer. These spectral data are plotted in Figure S11A.

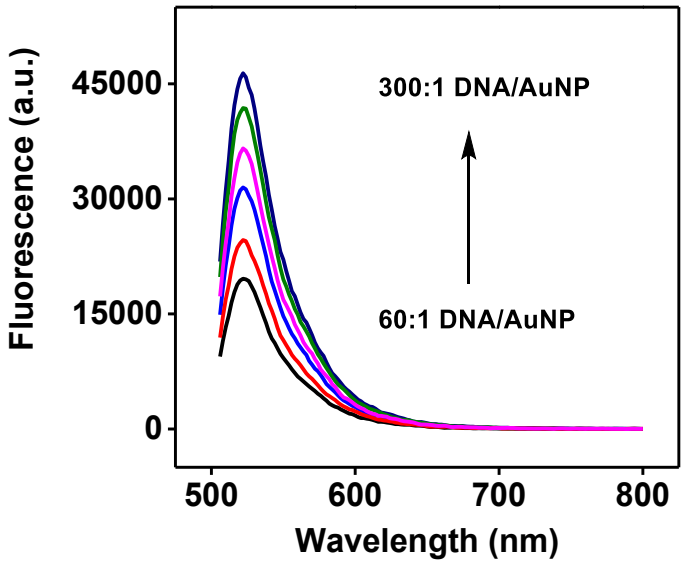

Figure S10: Determination of DNA surface coverage on SH-2X probe DNA-modified AuNPs with the DTT displacement assay. Fluorescence spectra of supernatants from AuNPs modified with a DNA:AuNP ratio of 60, 80, 120, 150, 200 or 300 (This refers to the initial ratio of DNA to AuNPs that was used for the particle modification) after treatment with DTT (0.5 M) for 12 h in reaction buffer. These spectral data are plotted in Figure S11A.

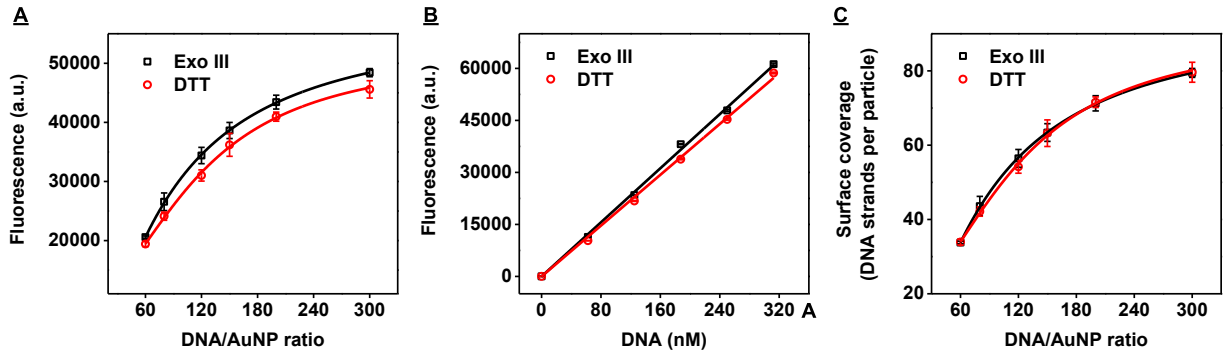

Figure S11. Comparison of our Exo III-based assay and the DTT displacement assay with six batches of SH-2X probe DNA-conjugated AuNPs prepared at different DNA:AuNP ratios. **(A)** Fluorescence intensities of supernatants after Exo III digestion (black) and DTT displacement (red). **(B)** Standard calibration curve obtained with known concentrations of FAM-labeled, SH-2X probe DNA after Exo III digestion (black) and DTT displacement (red). **(C)** Surface coverage of these DNA-modified AuNPs as characterized by Exo III digestion (black) and DTT displacement (red). Error bars show standard deviations obtained from three measurements.

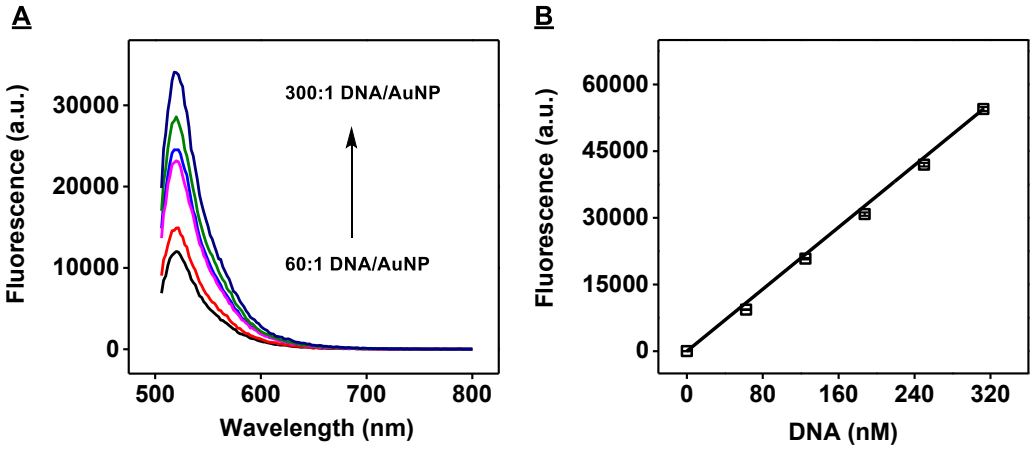

Figure S12: Determination of DNA surface coverage on SH-2X probe DNA-modified AuNPs with the DNase I-based method in the absence of cDNA. **(A)** Fluorescence spectra of supernatants from AuNPs modified at a DNA:AuNP ratio of 60, 80, 120, 150, 200 or 300 (This refers to the initial ratio of DNA to AuNPs that was used for the particle modification) and incubated with 0.125 U/μL DNase I for 12 hrs in DNase I buffer (10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.6). These spectral data are plotted in Figure 7B. **(B)** Standard calibration curve obtained by mixing known concentrations of SH-2X probe DNA with 0.125 U/μL DNase I in DNase I buffer. Error bars show the standard deviation of three measurements for each probe DNA concentration.

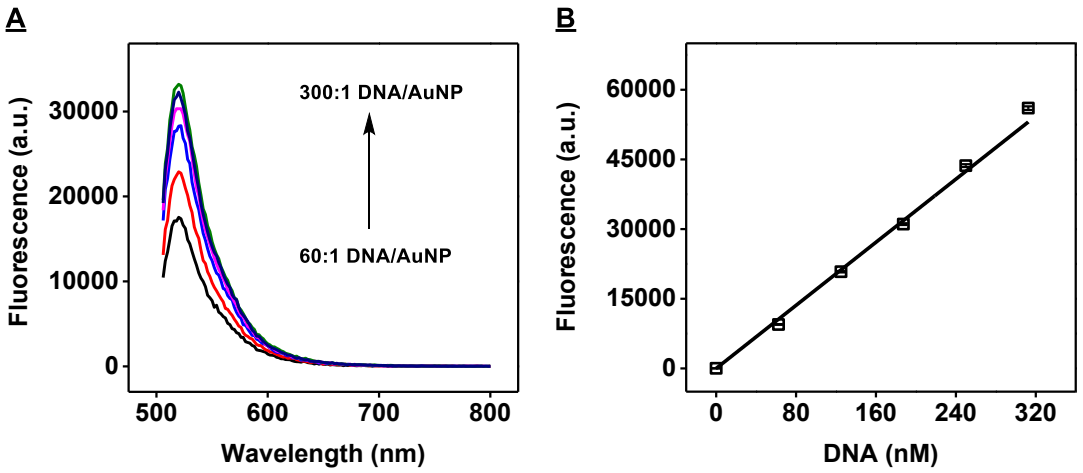

Figure S13: Determination of DNA surface coverage on SH-2X probe DNA-modified AuNPs with the DNase I-based assay in the presence of cDNA. **(A)** Fluorescence spectra were obtained as described in Supplementary Figure S11, except assay was also performed in the presence of 200 nM cDNA. These spectral data are plotted in Figure 7B. **(B)** Standard calibration curve obtained by mixing known concentrations of SH-2X probe DNA with 0.125 U/μL DNase I and 200 nM cDNA in DNase I buffer. Error bars show the standard deviation of three measurements for each probe DNA concentration.

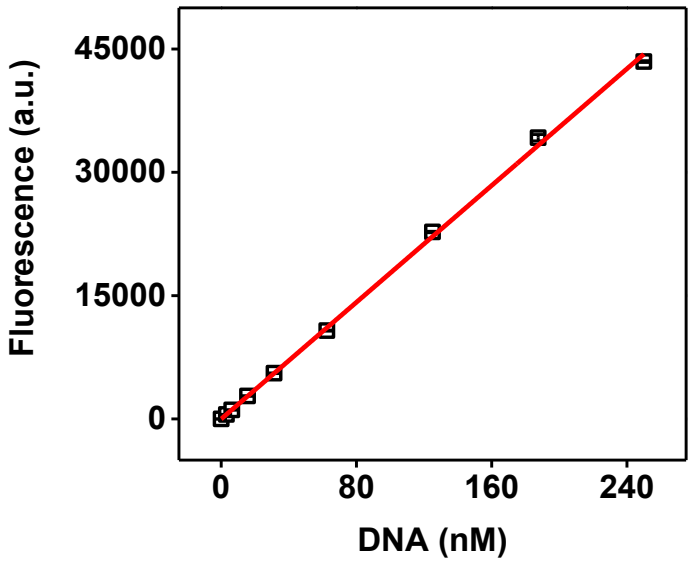

Figure S14: Standard calibration curve of our Exo III-based assay for SA-coated magnetic beads (MB-SA) and UCNPs (UCNP-SA), obtained by incubating known concentrations of biotin-2X probe DNA with 0.2 U/μL Exo III and 200 nM cDNA in reaction buffer for 60 min. Error bars show the standard deviation of three measurements for each probe DNA concentration.

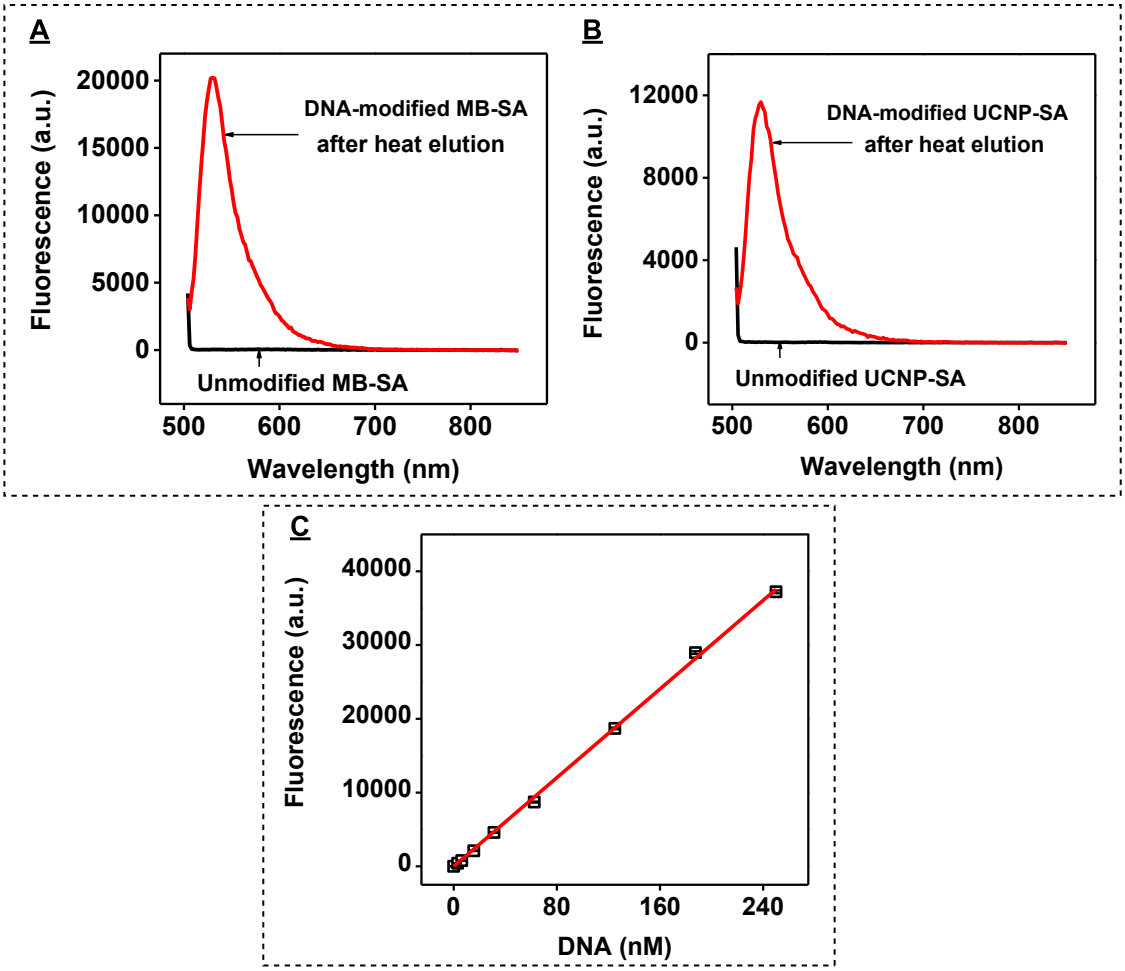

Figure S15: Quantification of DNA surface coverage on MB-SA and UCNP-SA particles via heating-based elution. Fluorescence spectra of unmodified particles in 95% formamide and 10 mM EDTA (black) and supernatants (red) of biotin-2X-modified **(A)** MB-SA and **(B)** UCNP-SA after heat-elution. **(C)** Standard calibration curve obtained by incubating known concentrations of biotin-2X probe DNA in 95% formamide and 10 mM EDTA and heating at 90 °C for 10 min. Error bars show the standard deviation of three measurements for each probe DNA concentration.

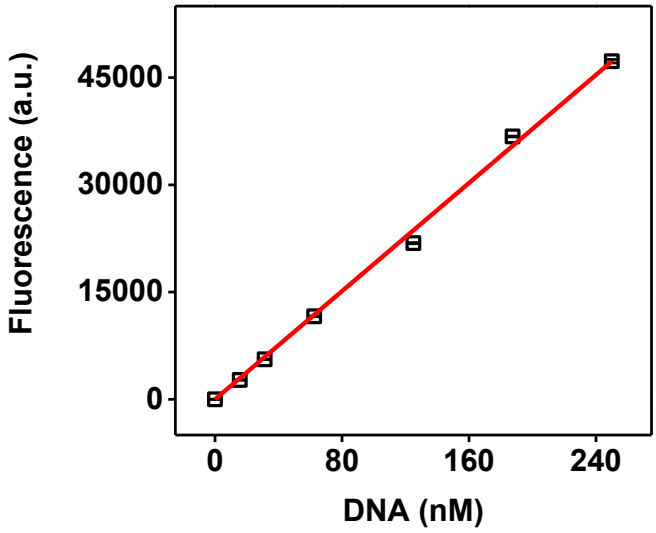

Figure S16: Standard calibration curve of our Exo III-based assay for SA-coated QDs (QD-SA). We incubated known concentrations of biotin-2X probe DNA with 0.2 U/μL Exo III, 200 nM cDNA and 3.1 nM unmodified QD-SA in reaction buffer for 60 min. Error bars show the standard deviation of three measurements for each probe DNA concentration.

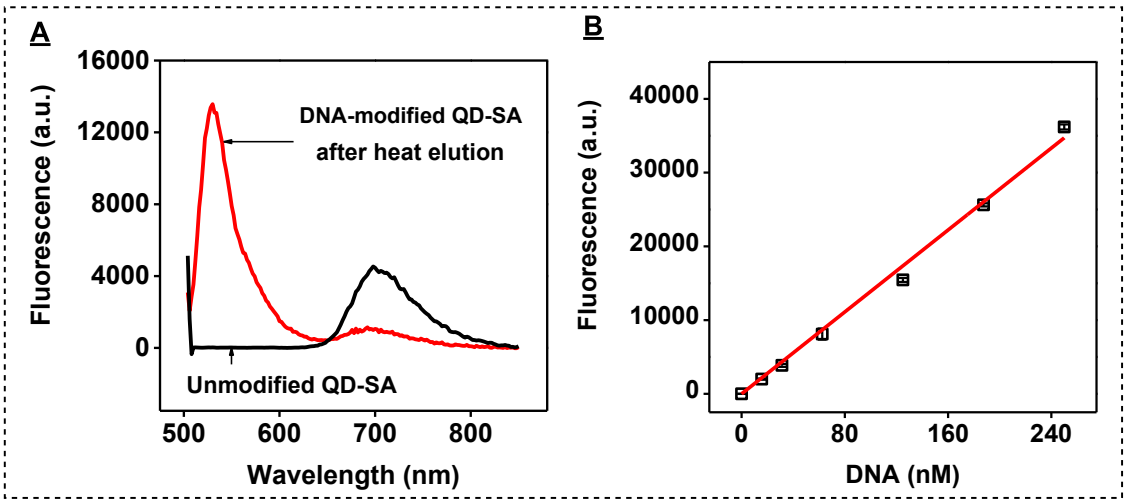

Figure S17: Quantification of surface coverage on biotin-2X probe DNA-modified QD-SA via heating-based elution. **(A)** Fluorescence spectra of unmodified QD-SA in 95% formamide and 10 mM EDTA (black) and supernatants (red) of biotin-2X probe DNA-modified QD-SA after heat-elution. **(B)** Standard calibration curve obtained by incubating known concentrations of biotin-2X probe DNA in 95% formamide containing 10 mM EDTA and 3.1 nM QD-SA and heating at 90 °C for 10 min. Error bars show the standard deviation of three measurements for each probe DNA concentration.

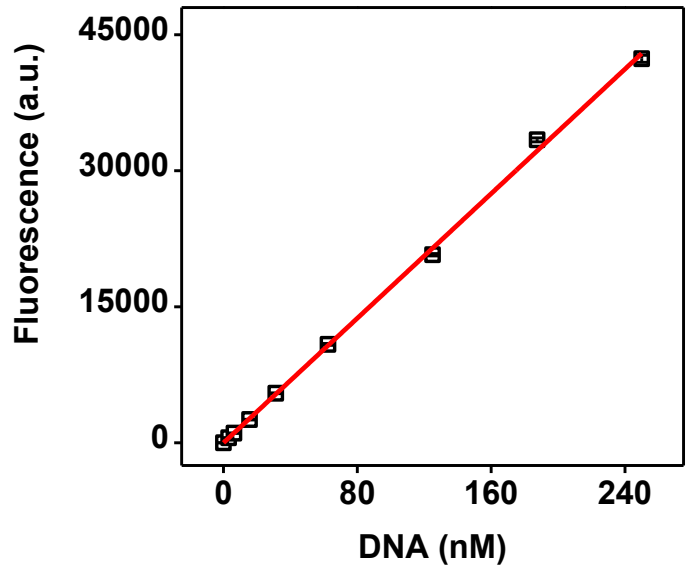

Figure S18: Standard calibration curve of our Exo III-based assay for carboxylated magnetic (MB-COOH) and silica (SiO_2-COOH) particles. Known concentrations of NH_2-2X probe DNA were incubated with 0.2 U/µL Exo III and 200 nM cDNA in reaction buffer for 16 hrs. Error bars show the standard deviation of three measurements for each probe DNA concentration.

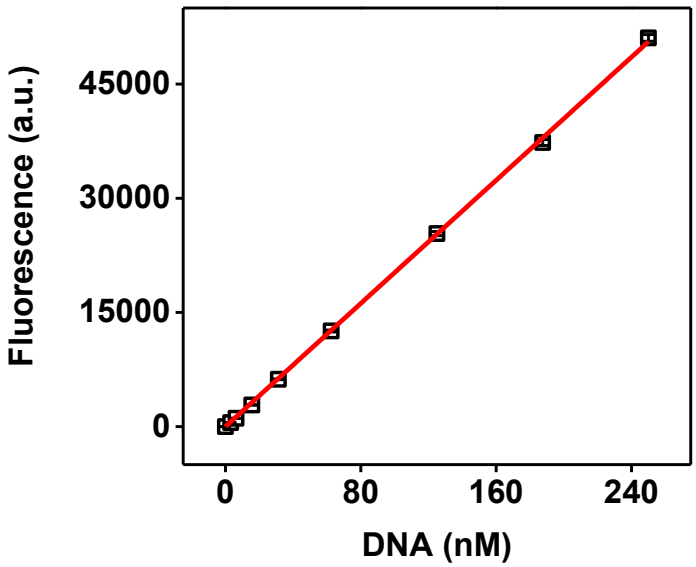

Figure S19: Standard calibration curve of our Exo III-based assay for oleic acid-capped UCNPs (UCNP-OA). Known concentrations of fluorophore-labeled, 5'-unmodified 2X probe DNA were incubated with 0.2 U/µL Exo III and 200 nM cDNA in reaction buffer for 60 min. Error bars show the standard deviation of three measurements for each probe DNA concentration.

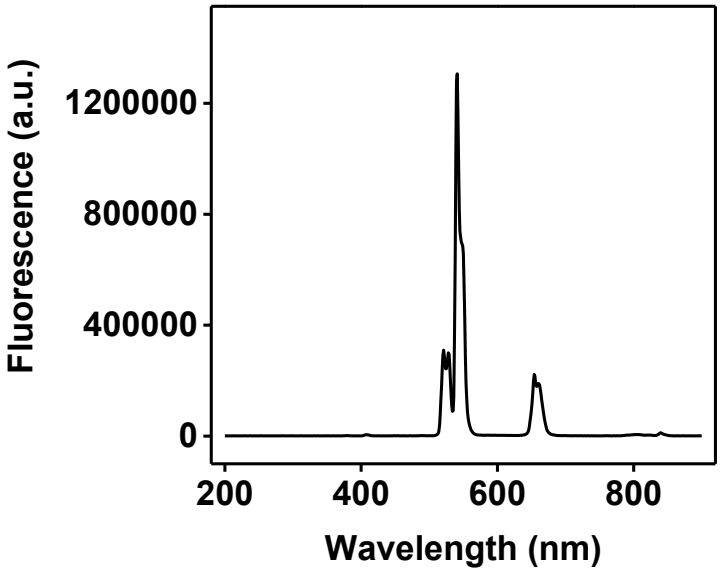

Figure S20: Fluorescence spectra of synthesized UCNP-OA in cyclohexane (7.8 µM) with 980-nm laser irradiation.

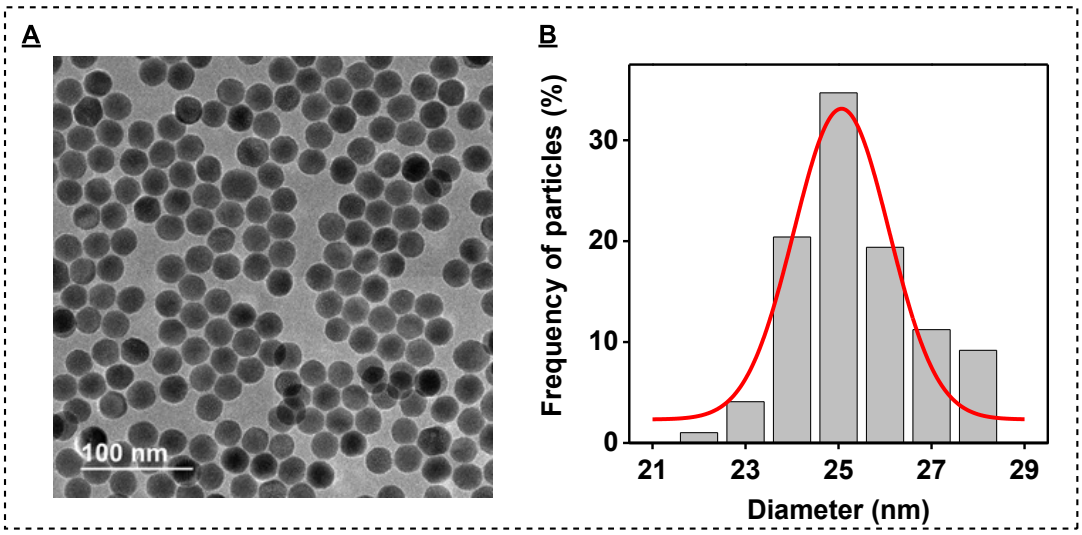

Figure S21: Characterization of synthesized UCNP-OA. **(A)** TEM image of 25±1 nm UCNP-OA. **(B)** Size distribution of synthesized UCNP-OA measured by ImageJ software.

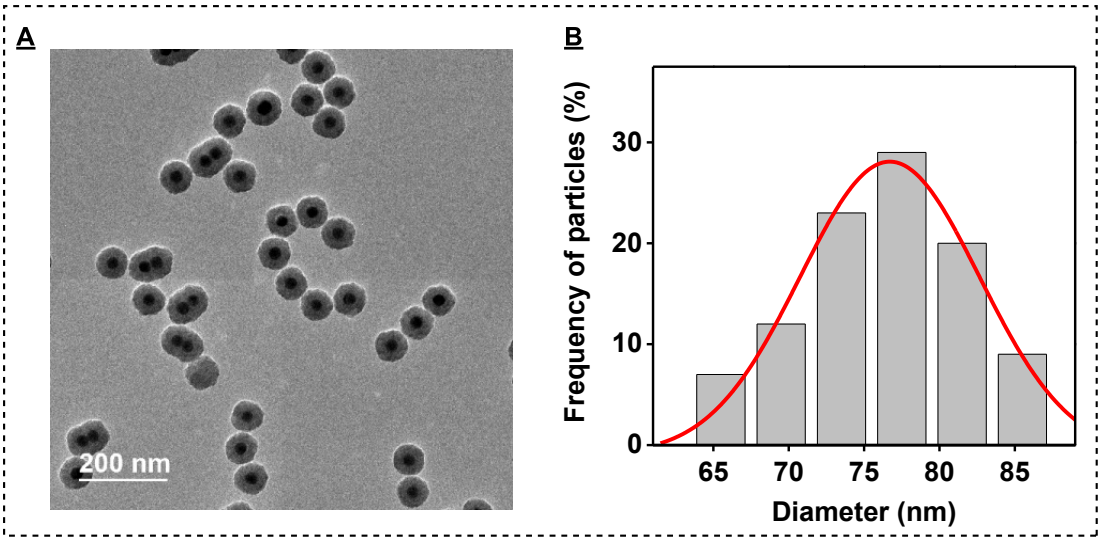

Figure S22: Characterization of silica-coated UCNPs. **(A)** TEM image of 76±5 nm silica-coated UCNPs. **(B)** Size distribution of silica-coated UCNPs measured by ImageJ software.

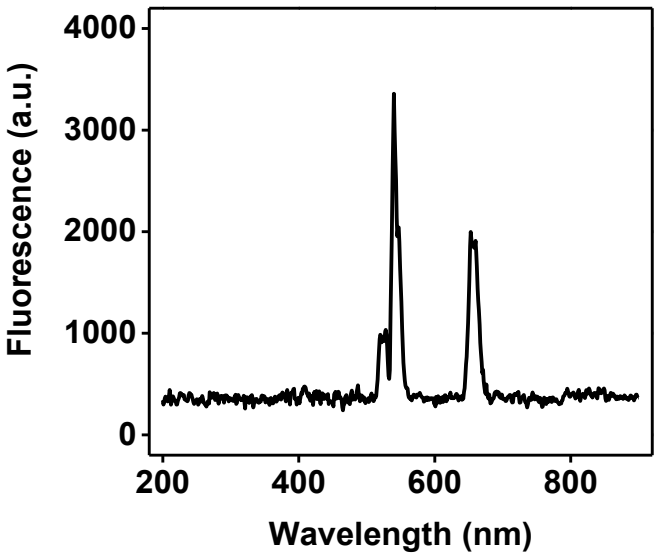

Figure S23: Fluorescence spectra of synthesized UCNP-SA in 1× PBS (33 nM) with 980-nm laser irradiation.

6. ESI references

(1) Turkevich, J., Stevenson, P. C., and Hillier, J. (1951) A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc. 11*, 55–75.

(2) Hill, H. D., and Mirkin, C. A. (2006) The bio-barcode assay for the detection of protein and nucleic acid targets using DTT-induced ligand exchange. *Nat. Protoc. 1*, 324–336.

(3) Hurst, S. J., Lytton-Jean, A. K. R., and Mirkin, C. A. (2006) Maximizing DNA loading on a range of gold nanoparticle sizes. *Anal. Chem. 78*, 8313–8318.

(4) Wang, F., Deng, R. R., Wang, J., Wang, Q. X., Han, Y., Zhu, H. M., Chen, X. Y., and Liu, X. G. (2011) Tuning upconversion through energy migration in core–shell nanoparticles. *Nat. Mater. 10*, 968–973.

(5) Carling, C. J., Nourmohammadian, F., Boyer, J. C., and Branda, N. R. (2010) Remote-control photorelease of caged compounds using near-infrared light and upconverting nanoparticles. *Angew. Chem., Int. Ed. 49*, 3782–3785.

(6) Li, L. L., Wu, P. W., Hwang, K., and Lu, Y. (2013) An exceptionally simple strategy for DNA-functionalized up-conversion nanoparticles as biocompatible agents for nanoassembly, DNA delivery, and imaging. *J. Am. Chem. Soc. 135*, 2411–2414.

(7) Wang, S. T., Wang, H., Jiao, J., Chen, K. J., Owens, G. E., Kamei, K. I., Sun, J., Sherman, D. J., Behrenbruch, C. P., Wu, H., and Tseng, H. R. (2009) Three-dimensional nanostructured substrates toward efficient capture of circulating tumor cells. *Angew. Chem., Int. Ed. 48*, 8970–8973.

(8) Tong, X., and Smith, L. M. (1992) Solid-phase method for the purification of DNA sequencing reactions. *Anal. Chem. 64*, 2672–2677.

(9) McKenzie, F., Steven, V., Ingram, A., and Graham, D. (2009) Quantitation of biomolecules conjugated to nanoparticles by enzyme hydrolysis. *Chem. Commun. 1*, 2872–2874.

(10) Álvarez, M., Carrascosa, L. G., Moreno, M., Calle, A., Zaballos, Á., Lechuga, L. M., Martínez-A, C., and Tamayo, J. (2004) Nanomechanics of the formation of DNA self-assembled monolayers and hybridization on microcantilevers. *Langmuir 20*, 9663–9668.

(11) Strömberg, M., Gunnarsson, K., Johansson, H., Nilsson, M., Svedlindh, P., and Strømme, M. (2007) Interbead interactions within oligonucleotide functionalized ferrofluids suitable for magnetic biosensor applications. *J. Phys. D: Appl. Phys. 40*, 1320–1330.

(12) Dugas, V., Depret, G., Chevalier, Y., Nesme, X., and Souteyrand, É. (2004) Immobilization of single-stranded DNA fragments to solid surfaces and their repeatable specific hybridization: covalent binding or adsorption? *Sens. Actuators, B 101*, 112–121.