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Supporting Information

Independent Control over Size, Valence, and Elemental Composition in the Synthesis of Nanoparticle-DNA Conjugates

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[†]These authors contributed equally.

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Materials

All reagents were purchased from Acros Organics, Fisher Scientific, AK Scientific, TCI America, or Sigma-Aldrich, and used without further purification unless otherwise noted. For synthesis performed in the Argon-filled glovebox, all the solvents were taken from an MBraun solvent purification system. Water was purified by a Milli-Q water purification system. Dichloromethane (DCM), pyridine, tetrahydrofuran (THF), benzene, toluene, nitrobenzene, diisopropylethylamine (DIPEA), DMSO and DMF were stored over activated 4 Å molecular sieves. Triethylamine (TEA) was dried over KOH pallets. Other solvents were used as received. Pyridine-modified Grubbs 2nd Generation catalyst for ROMP polymerization,[1] *N*-glycine *cis*-5-norbornene-*exo*-2,3-dicarboximide NHS ester (**M_1**),[2] FAM monomer (**M_2**),[2] *N*-(2,3-dihydroxypropyl) *cis*-5-norbornene-*exo*-2,3-dicarboximide acetonide (**M_3**),[2] *N*-(Boc-aminoethyl) *cis*-5-norbornene-*exo*-2,3-dicarboximide (**M_4**),[3] *cis*-1,4-(*p*-azidoethylphenoxy)-2-butene (**CTA**),[4] and tris(allyloxy-methyl)aminomethane (Triallyl-Tris)[5] were synthesized according to procedures in literature. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl), Azadibenzo-cyclooctyne-maleimide (ADIBO-maleimide) and sulfosuccinimidyl 4-(*N*-maleimido-methyl)cyclohexane-1-carboxylate (sulfo-SMCC) was purchased from Alfa Aesar. Oligonucleotides used in this study were either purchased from Integrated DNA Technologies Inc. (Coralville, IA) or synthesized on ABI 394 DNA/RNA synthesizer followed by HPLC purification. The sequences of oligonucleotides used are listed below:

Alexa594-$M_{18}bT_{20}$-SH: 5'-Alexa594-TTG CTG AGT ATA ATT GTT-T_{20}-SH-3'

$M_{18}a$-BHQ2: 5'-AAC AAT TAT ACT CAG CAA-BHQ2-3'

ERRed-$M_{18}bT_{20}$-SH: 5'-ERRed-TTG CTG AGT ATA ATT GTT-T_{20}-SH-3'

$M_{18}bT_{20}$-SH: 5'- TTG CTG AGT ATA ATT GTT-T_{20}-SH-3'

HS-$M_{18}aT_{20}$: 5'-HS-T_{20}-AAC AAT TAT ACT CAG CAA-3'

Adenosine Aptamer Strand:

5'-ACT CAT CTG TGA AGA GAA CCT GGG GGA GTA TTG CGG AGG AAG GT-3'

Adenosine Fluorescence Strand:

5'-$Alexa_{594}$-TCA CAG ATG AGT AAA AAA AAA A-SH-3'

Adenosine Quencher Strand: 5'-CCC AGG TTC TCT-BHQ2-3'

AS1411 Aptamer Strand: 5'-GGT GGT GGT GGT TGT GGT GGT GGT GG-T_{10}-SH-3'

Scramble Control Strand: 5'-GAG AAC CTG AGT CAG TAT TGC GGA GA-T_{10}-SH-3'

Methods

Nuclear Magnetic Resonance (NMR). NMR spectra were recorded using a Varian U400, UI400, U500 or VXR500 spectrometer in the NMR Laboratory, School of Chemical Science, University of Illinois. The data was processed in MestReNova 6.1 and aligned/annotated in Adobe Illustrator CS4 or Microsoft Paint of Windows 10.

Mass Spectrometry. Mass spectral analyses were provided by the Mass Spectrometry Laboratory, School of Chemical Science, University of Illinois, using ESI on a Waters Micromass Q-TOF spectrometer, MALDI-TOF on a Bruker Daltonics UltrafleXtreme MALDI-TOF instrument.

Analytical Gel Permeation Chromatography (GPC). GPC experiments were performed on a Waters system equipped with a Waters 1515 isocratic pump, a Waters 2414 refractive index detector, and a Waters 2998 photodiode array detector. Separations were performed at 50 °C using DMF containing 0.1 M LiBr as the mobile phase. Absolute molecular weights were collected on the above GPC system equipped with an additional miniDAWN TREOS 3-angle laser light scattering detector (MALLS, Wyatt Technology, CA). The detection wavelength of TREOS was set at 658 nm. The MALLS detector was calibrated using pure toluene and used for the determination of absolute molecular weights. The molecular weight of all linear polymers was determined based on the *d*n/*d*c value of each sample calculated offline by using the internal calibration system processed by the ASTRA 6 software (version 6.1.1, Wyatt Technology, CA). The obtained data points were imported into OriginPro (version 8.1), plotted, and saved as vector image files (*.ai) for coloring and annotation in Adobe Illustrator CS6.

Transmission Electron Microscopy (TEM). TEM experiments were conducted with a JEOL 2100 Cryo transmission electron microscope. Negative staining of polymer nanoparticles with ammonium molybdate was applied when preparing TEM samples to achieve better contrast. For the negative staining method, samples were prepared by drop casting the polymer nanoparticle and ammonium molybdate mixture solutions onto a carbon-coated copper TEM grid (Ted Pella), followed by removing excess solution with

filter paper after 10 min. TEM grids were then left dried another 15 min before imaging. For the bright-field imaging, 3 nm ultrathin TEM grid (Ted Pella) was used.

Scanning Tunneling Electron Microscopy (STEM). STEM experiments were performed on a Hitachi 2300 instrument in z-contrast mode. EDX mapping was conducted on the same instrument with dual energy dispersive X-ray detectors. Signals were collected in the range of 0 to 20 keV.

Atomic Force Microscopy (AFM). AFM studies were performed using a Dimension Icon – Bruker instrument. ONP measurement was performed using PeakForce Tapping mode at ambient conditions. AFM images were analyzed by NanoScope Analysis v1.4 (Bruker).

DLS and Zeta-Potential Measurement. To estimate the size and charge of the ONPs, dynamic light scattering and zeta-potential experiments were performed using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). ONPs were dispersed in water for size and zeta-potential measurement. Errors represent standard deviation of three experiments.

Agarose Gel Chromatography. The agarose gel used to test ONP samples contained 1% agarose by weight/volume. Samples were run on an FB-SB-710 electrophoresis unit (Fisher Biotech) at room temperature (70 V, constant voltage) in TBE buffer. After running for 1 h, the gel was directly imaged by a digital camera or recorded by gel fluorescence reader.

UV-Vis Spectroscopy. Absorbance measurement of the gold nanoparticles were carried out by UV-Vis spectrophotometer (Hewlett–Packard 8453) during the kinetic study. The obtained data points were imported into OriginPro (version 8.1), plotted, and saved as vector image files (*.ai) for coloring and annotation in Adobe Illustrator CS6.

Fluorescence Spectroscopy. Organic nanoparticle and DNA strand fluorescence was measured by a Horiba FluoroMax-4 fluorospectrometer (Horiba, NJ, USA) with FluorEssence (v3.5) software from the instrument manufacturer. The obtained data points were imported into OriginPro (version 8.1), plotted, and saved as vector image files (*.ai) for coloring and annotation in Adobe Illustrator CS6.

Fluorescence Microscopy. Fluorescence microscope images and darkfield images were acquired using a Zeiss Axiovert 200M inverted microscope with an EC Epiplan 50X HD objective (NA = 0.7) and CCD camera. The digital camera was white-balanced using Zeiss Axiovision software before data acquisition.

Cell Culture and Measurements. MCF-7 cells were grown in chamber slides in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/ml aqueous penicillin G, 100 μg/mL streptomycin, and 10% FBS at concentrations to allow 70% confluence in 48 h.

On the day of experiments, chambers are rinsed once with fresh medium, before they are refilled with pre-warmed Opti-MEM medium (serum reduced) containing 2 μM ONPs in glass-bottom dish. After 3 h incubation, cells were washed with PBS 3 times and fresh DMEM was added. For flow cytometry study, after 3 h incubation with ONPs, MCF-7 cells were washed with PBS and detached by 0.05% trypsin. The suspended cell solution was collected by centrifugation at 2,000 g for 5 min and further washed with PBS three times. Flow cytometry was performed using a BD FACSCanto system under 488 nm excitation. Control cells without any treatment were used to set the gating. Each measurement set was performed using 10,000 cells.

Syntheses

Note: some of the synthetic protocols are already included in the main text. They are also included here to facilitate reading.

Synthesis of Monovalent, Mid-size ONP for DNA Conjugation. In a 20 mL vial was charged with $\mathbf{M_1}$ (31.8 mg, 45 eq.), $\mathbf{M_2}$ (4.5 mg, 3 eq.) and $\mathbf{M_3}$ (55.5 mg, 90 eq.). 4 mL of dry DCM was added and the mixture formed a homogeneous solution. Pyridine-modified 2nd generation Grubbs catalyst (0.025 M in DCM, 88.9 μL, 1 eq.) was added with vigorous stirring, and the green solution gradually turned yellow during 8 min of fast stirring. $\mathbf{M_3}$ (6.2 mg, 10 eq.) was added to construct the second block (spacer block), and the stirring was continued for another 2 min. **CTA** (0.125 M, 88.9 μL, 5 eq.) was added, and the mixture was stirred for 3 h to allow complete chain-end functionalization. Butyl vinyl ether (1 mL) was added to quench the catalyst. Solvent was removed under reduced pressure. The solid residue was re-dissolved in DCM (1.5 mL) and precipitated in ether (12 mL) in a 15 mL polypropylene centrifuge tube. The mixture was sonicated, centrifuged, and the supernatant was discarded. The solid was further triturated, sonicated, and centrifuged in ether (12 mL) another 2 times. The solid was thoroughly dried under reduced pressure to give the product polymer as an off-white solid. Yield: 78 mg.

Synthesis of the Parent Linear Polymer of Monovalent, Small-size ONP for DNA Conjugation. In a 20 mL vial was charged with $\mathbf{M_1}$ (31.8 mg, 15 eq.), $\mathbf{M_2}$ (13.5 mg, 3 eq.) and $\mathbf{M_3}$ (55.5 mg, 30 eq.). 4 mL of dry DCM was added and the mixture formed a homogeneous solution. Pyridine-modified 2nd generation Grubbs catalyst (0.025 M in DCM, 267 μL, 1 eq.) was added with vigorous stirring, and the green solution gradually turned yellow during 5 min of fast stirring. $\mathbf{M_3}$ (9.3 mg, 5 eq.) was added to construct the second block (spacer block), and the stirring was continued for another 1 min. **CTA** (0.125 M, 267 μL, 5 eq.) was added, and the mixture was stirred for 3 h to allow complete chain-end functionalization. Butyl vinyl ether (1 mL) was added to quench the catalyst. Solvent was removed under reduced pressure. The solid residue was re-dissolved in DCM (1.5 mL) and precipitated in ether (12 mL) in a 15 mL polypropylene centrifuge tube. The mixture was sonicated, centrifuged, and the supernatant was discarded. The solid was further triturated, sonicated, and centrifuged in ether (12 mL) another 2 times. The solid was thoroughly dried under reduced pressure to give an off-white solid. Yield: 71 mg.

Synthesis of the Parent Linear Polymer of Monovalent, Large-size ONP for DNA Conjugation. In a 20 mL vial was charged with $\mathbf{M_1}$ (31.8 mg, 90 eq.), $\mathbf{M_2}$ (2.3 mg, 3 eq.),

and **M_3** (55.5 mg, 180 eq.). 4 mL of dry DCM was added and the mixture formed a homogeneous solution. Pyridine-modified 2nd generation Grubbs catalyst (0.025 M in DCM, 44.5 µL, 1 eq.) was added with vigorous stirring, and the green solution gradually turned yellow during 12 min of fast stirring. **M_3** (6.2 mg, 20 eq.) was added to construct the second block (spacer block), and the stirring was continued for another 3 min. **CTA** (0.125 M, 44.5 µL, 5 eq.) was added, and the mixture was stirred for 3 h to allow complete chain-end functionalization. Butyl vinyl ether (1 mL) was added to quench the catalyst. Solvent was removed under reduced pressure. The solid residue was re-dissolved in DCM (1.5 mL) and precipitated in ether (12 mL) in a 15 mL polypropylene centrifuge tube. The mixture was sonicated, centrifuged, and the supernatant was discarded. The solid was further triturated, sonicated, and centrifuged in ether another 2 times. The solid was thoroughly dried under reduced pressure to give an off-white solid. Yield: 83 mg.

Synthesis of the Parent Linear Polymer Multivalent (25), Mid-size ONP for DNA Conjugation. In a 20 mL vial was charged with the activated ester monomer **M_1** (31.8 mg, 50 eq.), **M_2** (4.1 mg, 3 eq.), **M_3** (41.6 mg, 75 eq.), and **M_4** (15.3 mg, 25 eq.). 4 mL of dry DCM was added and the mixture formed a homogeneous solution. Pyridine-modified 2nd generation Grubbs catalyst (0.025 M in DCM, 80 µL, 1 eq.) was added with vigorous stirring, and the green solution gradually turned yellow during 7 min of stirring. Butyl vinyl ether (1 mL) was added to quench the catalyst for 10 min, and solvent was removed under reduced pressure. The solid residue was re-dissolved in DCM (1.5 mL) and precipitated in ether (12 mL) in a 15 mL polypropylene centrifuge tube. The mixture was sonicated, centrifuged, and the supernatant was discarded. The solid was further triturated, sonicated, and centrifuged in ether another 2 times. The solid was dried under reduced pressure to give the product polymer as an off-white solid. Yield: 70 mg.

Triallyl-Tris Functionalization of the Polymers. The product polymer from the above procedure (70 mg) was dissolved in 4 mL of DCM in a 20 mL vial. Nitrobenzene (50 µL) and TATris (100 µL, > 5 eq. of NHS ester, > 250 eq. of the polymer) was added. The vial was capped and sealed by parafilm, and was heated to 40 °C for 16 h with stirring. Most of the solvent was removed under reduced pressure, and the viscous residue was re-dissolved in DCM (1.5 mL) and precipitated in a 2:1 (v/v) mixture of cold ether-hexanes (-15 °C) in a 15 mL polypropylene centrifuge tube. The mixture was sonicated, centrifuged, and the supernatant was discarded. The solid was further triturated, sonicated, and

centrifuged in ether another 2 times. The solid was dried under reduced pressure to give the allylated polymer as an off-white solid. Yield: 58-67 mg.

Typical Procedure for Intramolecular Crosslinking by RCM. A 1 L round-bottom flask was filled with 450 mL of anhydrous DCM under nitrogen atmosphere. TATris functionalized allyl polymer from previous step (50 mg) was dissolved in 1 mL of DCM in a 7 mL vial, and 10 mg of 1st generation Grubbs catalyst was dissolved in 1 mL of DCM in another vial. 0.5 mL of the polymer solution was added into the 1 L flask, stirred at room temperature for 5 min, and 0.5 mL of the catalyst solution was added. After 3 h, the remaining polymer and catalyst solutions (stored at -20 ^{o}C during this 3 h) were added. The solution was kept stirring at room temperature, and 5 mg catalyst added was added after 6 h, and another 5 mg after 18 h. The mixture was stirred for a total time of 40 h after the first catalyst addition, and 1 mL of butyl vinyl ether was added to quench the catalyst. The solution was stirred 15 min and evaporated under reduced pressure. The solid residue was dissolved in DCM, precipitated in diethyl ether to remove most of the catalyst, and dried on a rotary evaporator. The polymer was further purified by passing through silica gel eluted by DCM in a glass pipette. The collected fraction was evaporated, and the residue was re-dissolved in DCM (1.0 mL) and precipitated in ether (12 mL). The precipitates were collected after centrifuge, further washed with ether, and dried under reduced pressure to give a greyish-white powder. Yield: 46 mg.

Typical Procedure for Dihydroxylation of the Alkene ONPs. In a 20 mL glass vial, 40-50 mg of alkene ONP was suspended in a mixture of water (3 mL) and acetone (12 mL). *N*-Methylmorpholine *N*-oxide (50% wt. in H_2O, 0.5 mL) and K_2OsO_4 (1 mg) was added to the mixture. The vial was loosely capped and heated in a 40 ^{o}C aluminum heating block for 3 - 4 h, and the cap was removed. The vial was covered by a piece of chemical paper wipe so that acetone can freely and slowly evaporate. Heating and stirring was continued another 16 h and most of acetone was evaporated during this process (and the solution should be homogeneous at this time). The solution was decanted to remove insoluble residue (black, if there was any). The green solution was further purified by dialysis against water using a membrane (Spectra/Por 7 Membrane Tubing, MWCO = 1000 kDa) for 3 h and was directly used in the acid deprotection step.

Typical Procedure for Acid-deprotection of the Dihydroxylated ONPs. Briefly dialyzed ONP solution (containing 40-50 mg of ONP, approximately 5 mL of solution) was added trifluoroacetic acid (2 mL) to remove the ketal and Boc protecting groups. The homogeneous solution might become slightly cloudy after the acid addition. The reaction

mixture was warmed to 40 °C, and was stirred for 2.5 h and the solution became mostly clear. The solution was cooled to room temperature and was directly dialyzed against dilute $NaHCO_3$ (4.0 g in 4 L of water), then DI water, using a membrane (Spectra/Por 7 Membrane Tubing, MWCO = 1000 kDa). The solution was filtered and lyophilized to yield a yellow to yellowish-white solid. Yield: 27-40 mg. ***Note:*** *the azido group on the monovalent ONP is not stable. Conjugation to DNA should be performed in a few days after synthesis. The deactivated monovalent ONP-N_3 (presumably becomes ONP-NH_2) can have its activity partially recovered by treatment with imidazole-1-sulfonyl aizde hydrogen sulfide.*[6-7]

ADIBO Functionalization of DNAs. All the DNAs used for conjugation have a protected thiol group on their 3'-end (*e.g.*, a structure of 5'-DNA-3'-S-S-$CH_2CH_2CH_2OH$). To functionalize the DNA, the thiol group must be first deprotected using TCEP·HCl. Briefly, the DNA (1 mM, 5 μL, 5 nmol) was dissolved in 100 μL of acetate buffer (10 mM, pH = 5.2) in a 1 mL plastic tube. TCEP·HCl (freshly prepared solution, 100 mM, 10 μL, 1 μmol) was added. The reaction tube was incubated at room temperature for 2 h. The solution was loaded on a 0.5 mL Amicon tube (3 kDa MWCO), centrifuged for 10 min (13300 RPM), and the filtrate at the bottom was discarded. The concentrated DNA solution was further washed by pure water (0.4 mL) for 4 times to fully remove excess TCEP, leaving the pure DNA with activated thiol group on the filtering unit.

The thiol-DNA (5 nmol) was diluted to 100 μL with 10 mM phosphate buffer (pH = 7.4) in a 2 mL plastic centrifuge tube. ADIBO-maleimide (10 mM in dioxane, 20 μL, 200 nmol) was added. This homogeneous solution mixed thorough using a vertex and was incubated at room temperature overnight. The solution was diluted to 0.8 mL with water. DCM (0.5 mL) was added, and the tube was sealed and vigorously vortexed. The tube was centrifuged (10000 RPM) for 1 min, and the lower layer (DCM) was removed using an autopipette. This extraction procedure was repeated 3 more times, and the aqueous solution was bubbled with N_2 to remove residual DCM. Finally, the solution was loaded on a 0.5 mL Amicon tube (3 kDa MWCO) and washed by water 5 times to give the pure DNA-ADIBO. MALDI indicated a peak with a +337 Da shift compared to the original DNA-S-S-$CH_2CH_2CH_2OH$.

Conjugation of Monovalent Azido-ONP and DNA-ADIBO. Azido-ONP and DNA-ADIBO were mixed in 100 μL of pH 7.4 phosphate buffer (10 mM) in a 1 mL plastic tube. For monovalent ONP, ONP/DNA = 10:1 to ensure complete reaction of DNA, which was the

more expensive reagent. The tube was incubated at 37 ºC for 4 d. The resulting solution was loaded on a column packed with DEAE Sepharose CL-6B gel (GE Healthcare, column was 10 cm long with 1 cm diameter). The mixture was first eluted with an acidic buffer (20 mM citrate buffer, 300 mM NaCl, pH = 3) until all the unreacted ONP was removed (monitored by fluorescence). The mixture was then eluted with 2 M NaCl and the product band (containing ONP-DNA and unreacted DNA) was collected (monitored by fluorescence or DNA's UV-vis absorption). The collected fractions were combined and loaded on a 0.5 mL Amicon tube (30 kDa MWCO, pre-treated, see notes), centrifuged (10000 RPM, 10 min) to remove the unreacted DNA. The filtrate was discarded and water (0.4 mL) was added to the Amicon tube again, centrifuged, to wash away the DNA. The washing was repeated for totally 8 times. The pure ONP-DNA conjugate is then collected from the filtering unit. ***Note:*** *a major yield loss of this reaction happened at the Amicon washing step, as the ONP tend to be absorbed and fixed on the Amicon membrane via non-specific binding. To alleviate this problem, before using the Amicon filter unit, it was soaked in bovine serum albumin (BSA) solution (6 mg/mL) for 1.5 h. The filtering unit full of BSA solution was then taken on a centrifuge (13300 RPM, 10 min), and washed thoroughly with water. In this way, some BSA proteins will be absorbed on the filter membrane and reduce the chance of an ONP-DNA conjugate getting absorbed. The method was shown to be effective without causing BSA contamination.*

Conjugation of Multivalent ONP-NH_2 and DNA-SH. The synthesized multivalent ONP-NH_2 (100 uM, 40 μL) was added to 260 μL of phosphate buffer (10 mM, pH 7.4). Sulfo-SMCC (1.0 mg, large excess) was added, followed by a small amount of dioxane (ca. 20 μL) to help the dissolution. The solution was incubated at room temperature for 2 h, and extracted by DCM (0.5 mL) for 3 times to remove dioxane. The aqueous solution was bubbled with N_2 to remove residual DCM, and purified by ultracentrifugation (5 times) to remove the excess sulfo-SMCC using Amicon tube (MWCO = 3 kDa). The obtained multivalent ONP-maleimide should be used immediately.

The thiol-DNA (protected by disulfide bond, 1 mM, 100 μL) was added to 300 μL of acetate buffer (10 mM, pH 5.2). TCEP•HCl (>40 eq.) was added. The solution was incubated at room temperature for 3 h. The product was purified by ultracentrifugation (5 times) using an Amicon tube (MWCO = 3 kDa) to give free-thiol-bearing DNA-SH.

The prepared multivalent ONP-maleimide and DNA-SH were mixed in 200 μL of phosphate buffer (10 mM, pH 7.4) and incubated at room temperature overnight. The

product was purified by ultracentrifugation (10 times) using an Amicon tube (MWCO = 30 kDa) to remove the excess DNA-SH.

Growth of Metal Nanoparticles on ONP Templates. Metal nanoparticles were prepared using different chlorometallates through sucrose or ascorbic acid reduction with a typical protocol as the following. For AuNP prepared by using sucrose as reductant, 10 μL polymer template (50 μM) was added in 940 μL citrate buffer (5 mM, pH = 3.00). Then, 25 μL $HAuCl_4$ (10 mM) was added. After mixing, incubate the solution at room temperature for 10 min. Then, add 25 μL 1 M sucrose to reduce chloroaurate. For control samples, the volume of polymer template solution was filled by buffer. All of the SEM and TEM samples reacted overnight. For AuNP synthesized using ascorbic acid as reductant, 5 μL polymer template (50 μM) was added in 445 μL citrate buffer (5 mM, pH =3.00). Then, 25 μL $HAuCl_4$ (10 mM) was added. After mixing, incubate the solution at room temperature for 10 min. Then, add 25 μL 100 mM ascorbic acid to reduce chloroaurate. PtNP and PdNP were prepared analogously using $PtCl_6^{2-}$ and $PdCl_4^{2-}$ as metal sources. Small variations of the concentrations of the citrate, metal source and reducing agent were applied to optimize the experimental conditions for different batches of ONP samples.

Supporting Figures

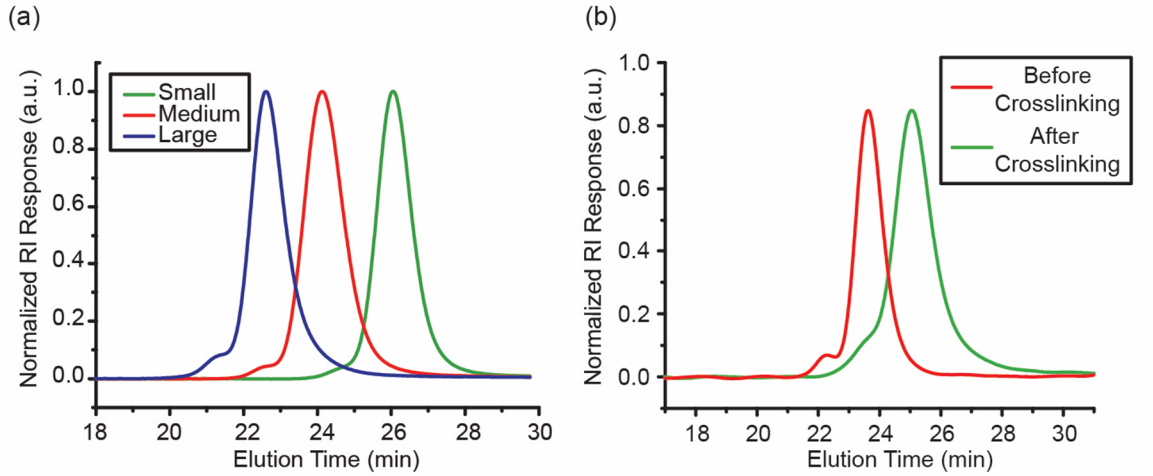

Figure S1. (a) GPC elution curve overlay showing linear polymers of different sizes. Longer retention time indicates larger size and higher molecular weight. (b) GPC elution curve overlay of multivalent linear polymer (before crosslinking) and ONP (after crosslinking). A reduction in size after crosslinking is clearly observed.

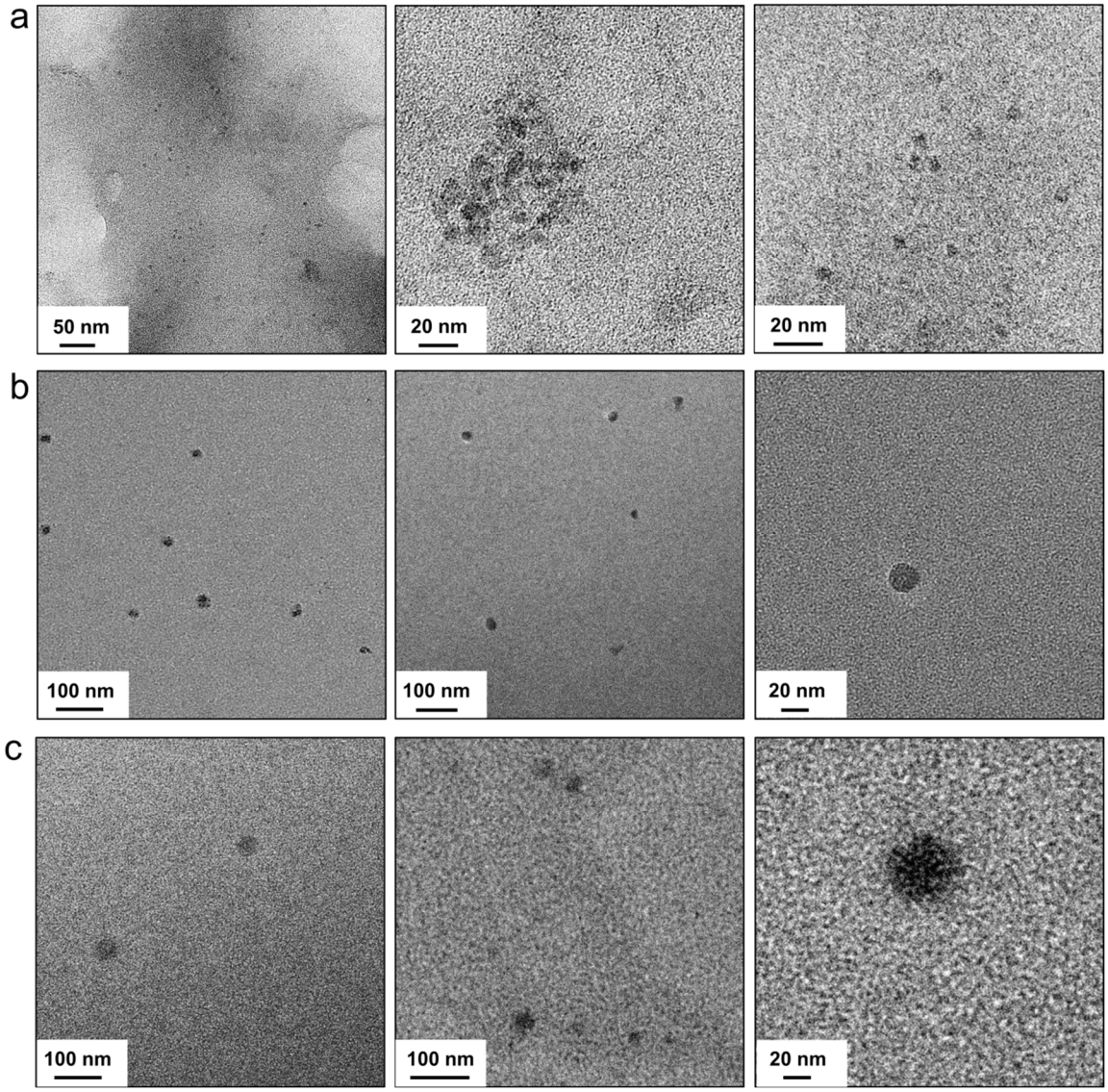

Figure S2. Additional bright-field TEM images of different sized monovalent azido-ONPs. (a) ONP^{S}, (b) ONP^{M}, and (c) ONP^{L} using ultrathin TEM grid with 3 nm of carbon layer.

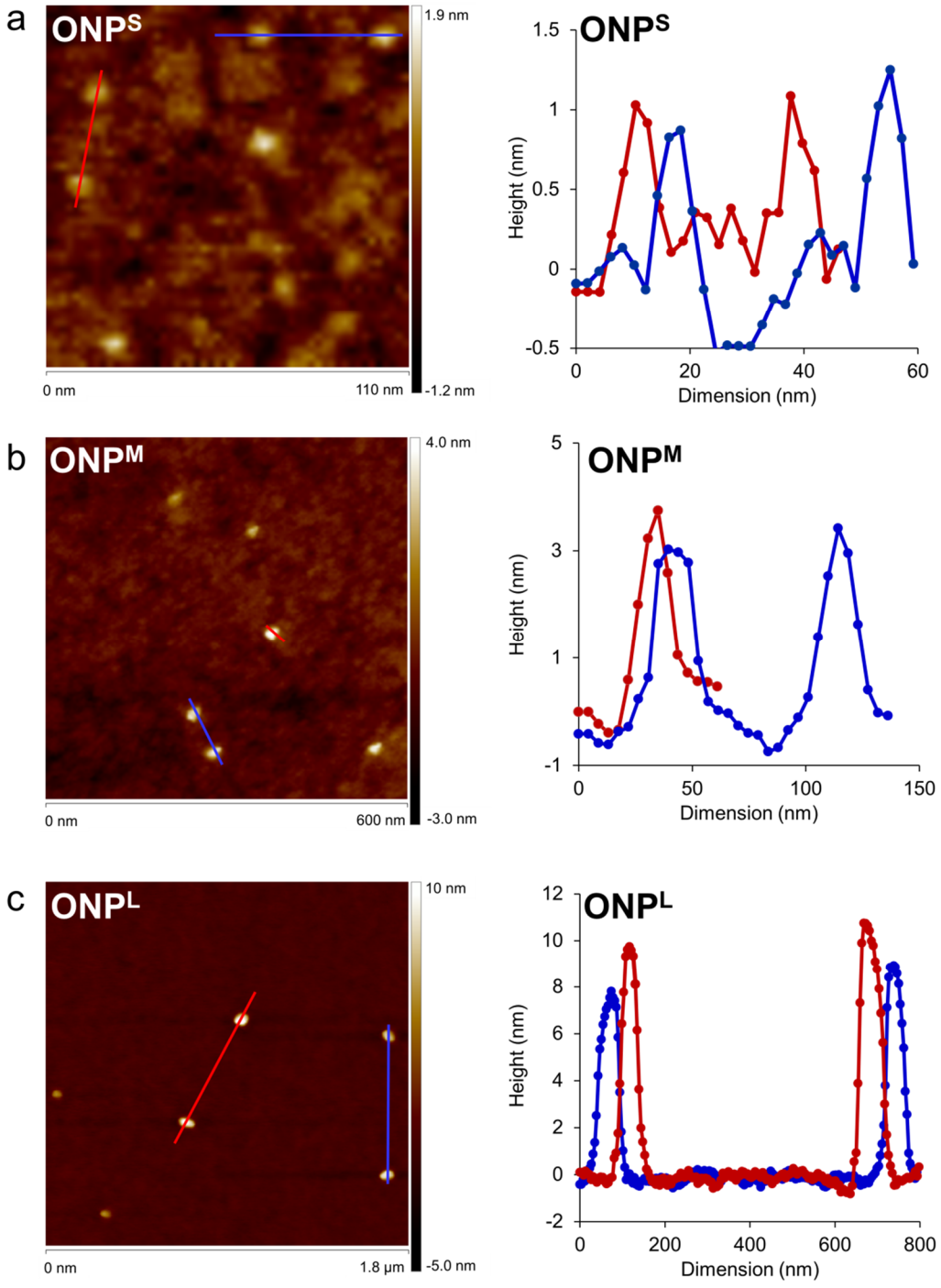

Figure S3. AFM images (left) and the corresponding cross-sectional analysis (right) of (a) ONP^S, (b) ONP^M, and (c) ONP^L.

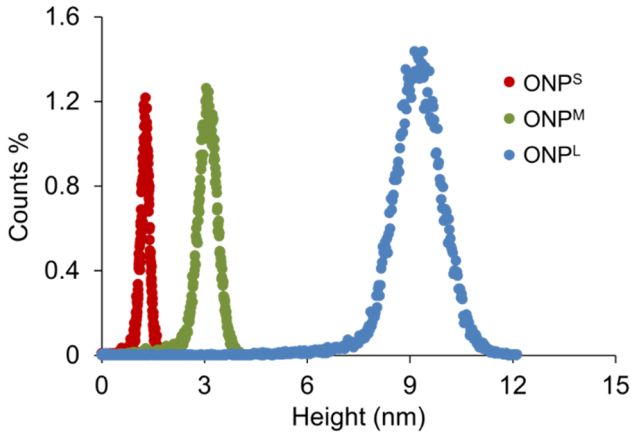

Figure S4. Height distribution analysis for ONP^S (red), ONP^M (green), and ONP^L (blue) from AFM images. The data was plotted by fitting with a Gaussian distribution model.

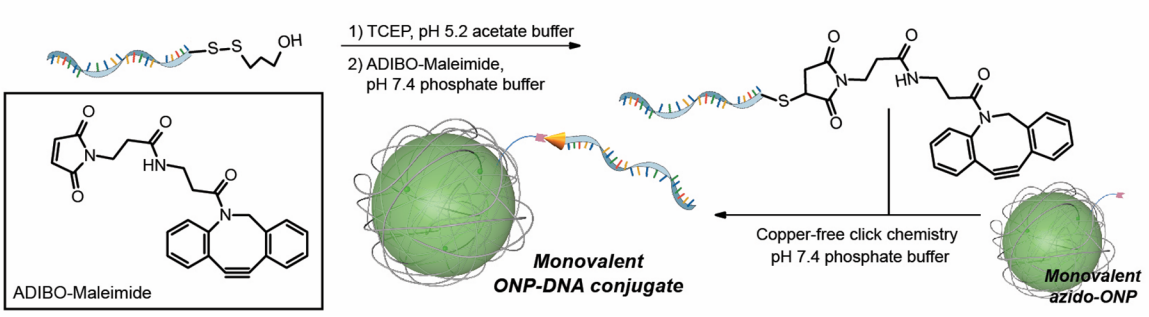

Figure S5. Chemical scheme of the synthesis of monovalent DNA-ONP conjugate via copper-free click chemistry.

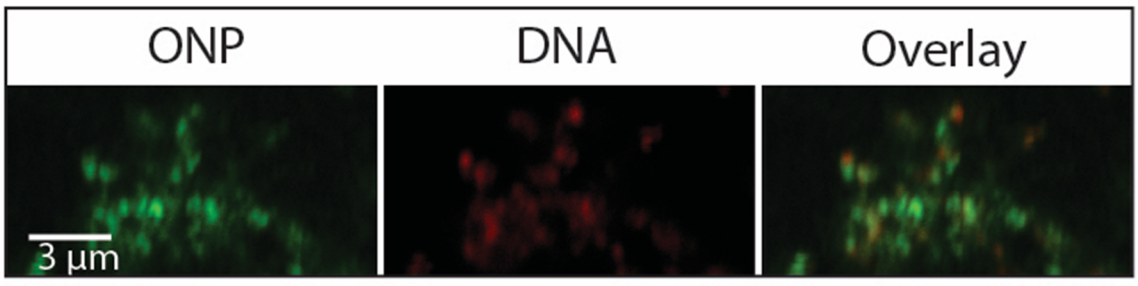

Figure S6. Fluorescence microscope images of the DNA-ONP conjugates. Co-localization of the green (from ONP) and red (from DNA) fluorescence indicates the covalent linkage between DNA and ONP.

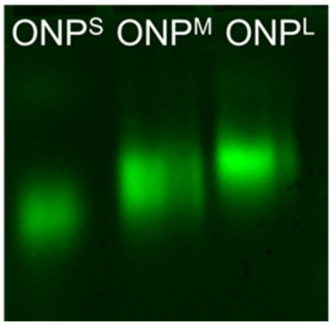

Figure S7. 1% agarose gel images of three different sized monovalent ONP-DNA conjugates. DNA-ONPs showed clear narrow band on agarose gel with the ONP^S showed the largest mobility.

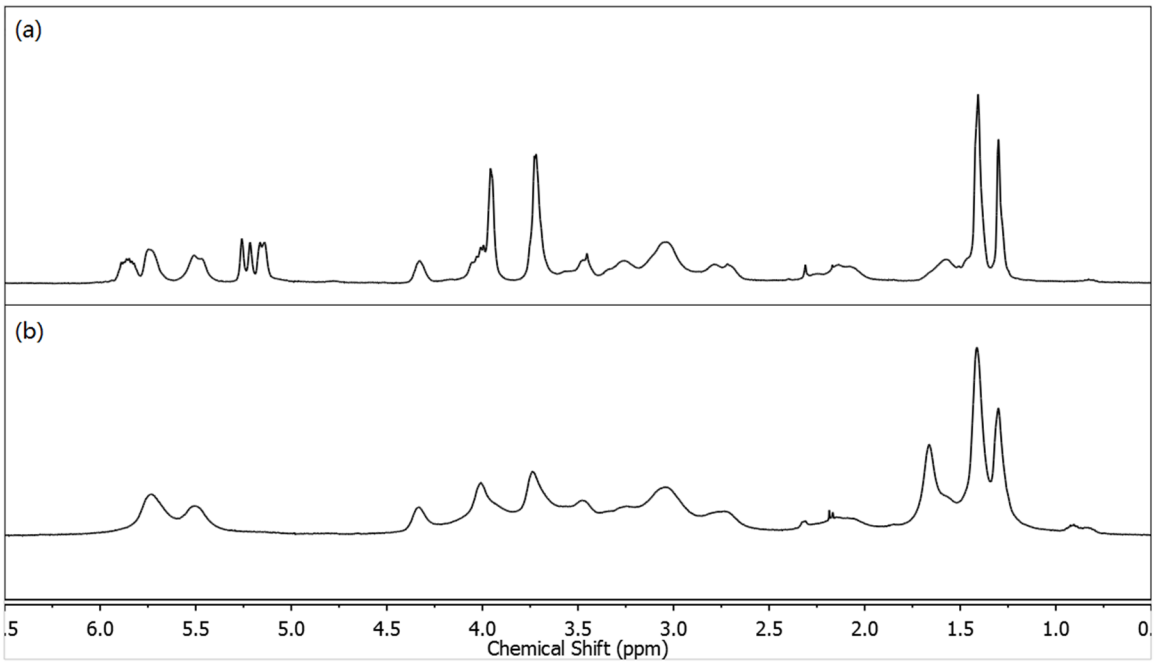

Figure S8. (a) ^1H NMR spectrum of the parent linear polymer (allylated polymer) of multivalent ONP^M-NH_2. (c) ^1H NMR spectrum of the multivalent ONP^M-NH_2 before dihydroxylation.

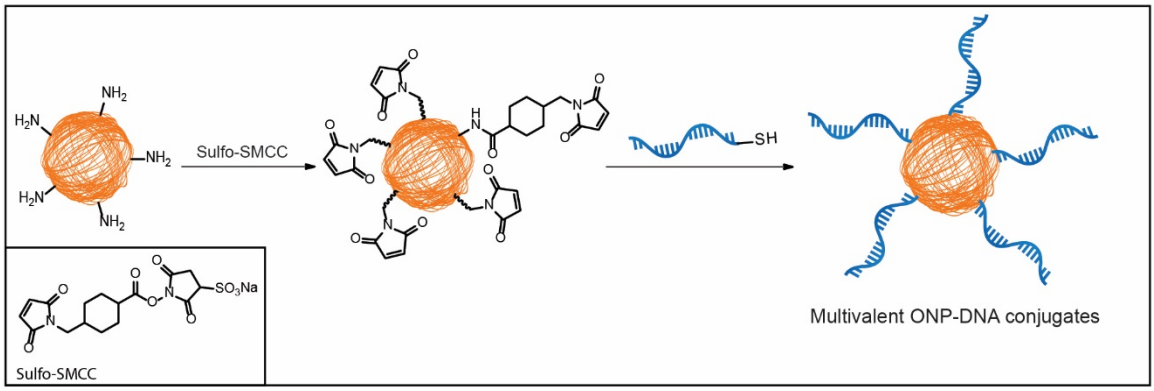

Figure S9. Cartoon scheme showing the SMCC chemistry used for conjugating thiol-DNA onto multivalent ONP-NH_2.

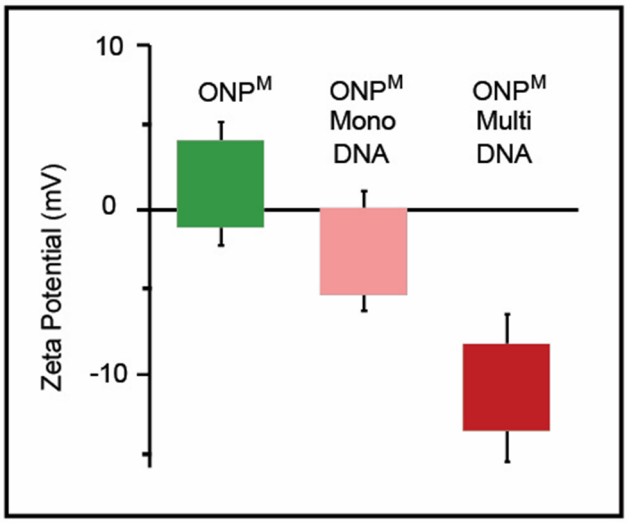

Figure S10. Zeta-potential comparison of ONPs with different numbers of DNA strands attached.

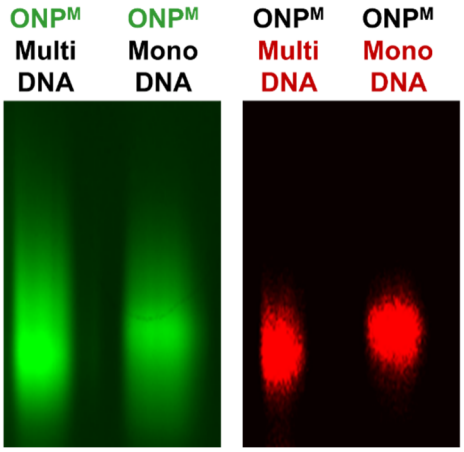

Figure S11. 1% Agarose gel images of mono- and multi-DNA-ONP conjugates.

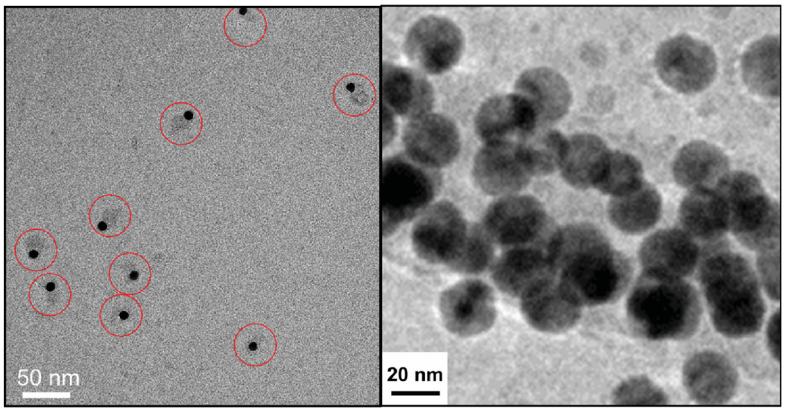

Figure S12. TEM visualization of two assembly modes of ONP-DNA conjugates with AuNPs functionalized with complimentary DNA strands. The image on the left is adapted from our recent report (DOI: 10.1021/jacs.7b00065).

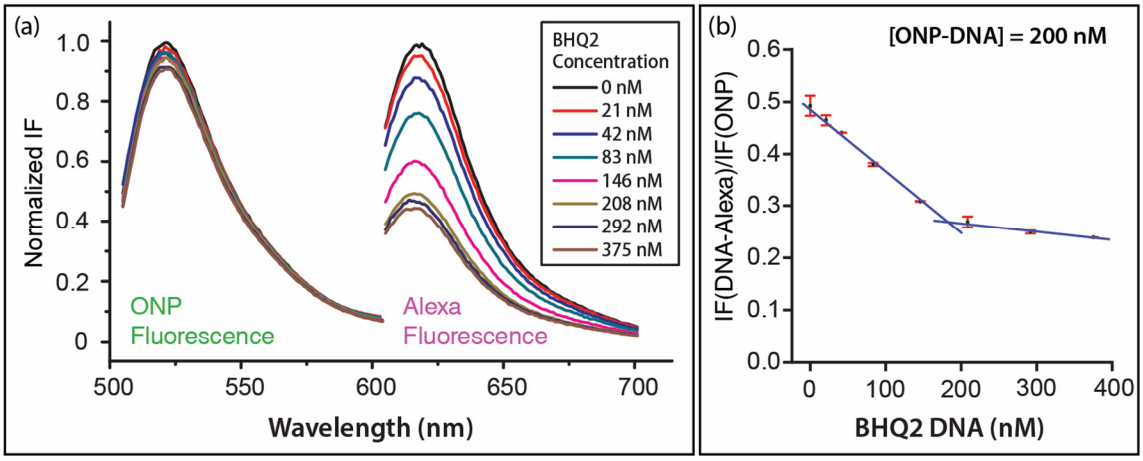

Figure S13. Fluorescence quenching of the ONP^{M}-DNA adenosine probe. (a) Combined ONP^{M} and DNA fluorescence emission spectra of the ONP^{M}-DNA adenosine probe in response to adenosine molecules of different concentrations. With the increase of the adenosine concentration, the DNA fluorescence intensity increases while the ONP^{M} fluorescence intensity almost remains constant, indicating the ratiometric property of the probe. (b) Plot of fluorescence ratio of ONP^{M}-DNA adenosine probe against complimentary BHQ2-DNA concentration. The fluorescence ratio of DNA and ONP decreased with the increase of BHQ2-DNA concentration and reached plateau at [BHQ2-DNA] = ca. 200 nM, which was consistent with the concentration of ONP^{M}-DNA (200 nM) in the system. This confirmed the formation of adenosine ratiometric sensor with a 1:1 NP/reporter ratio.

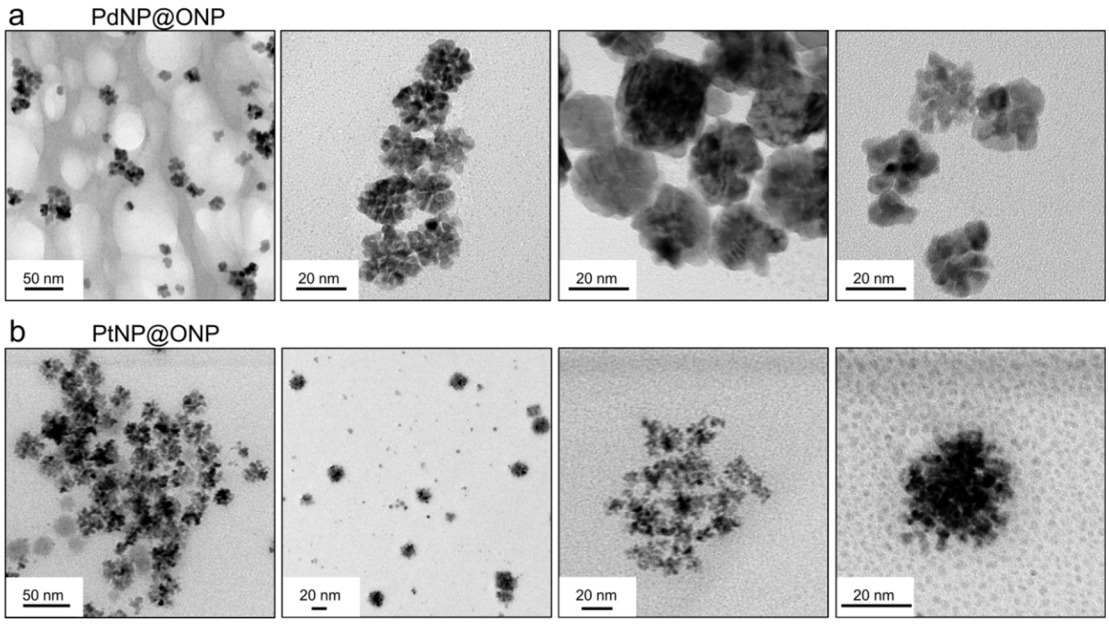

Figure S14. TEM images of PdNP (a) and PtNP (b) prepared on the template of ONP^{M}.

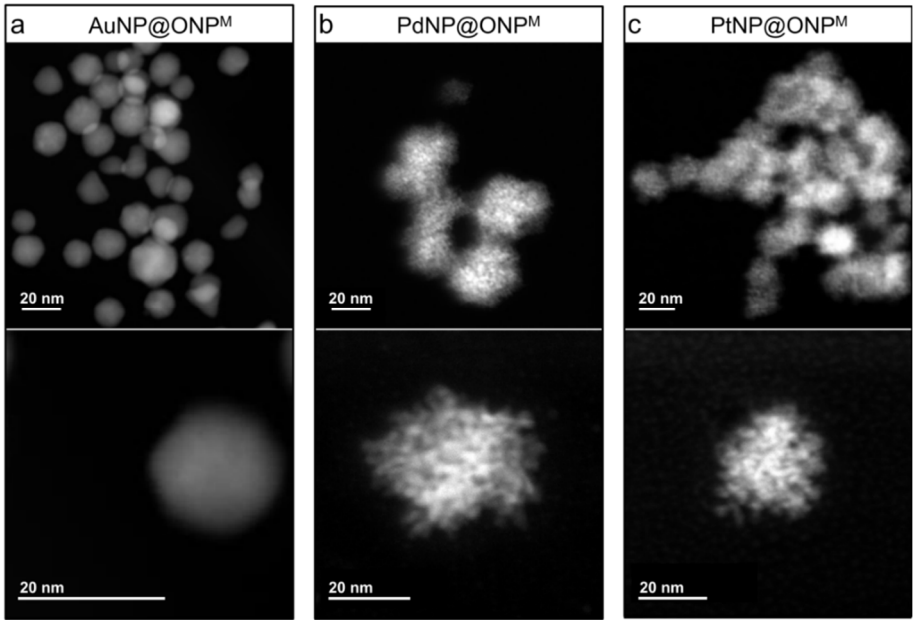

Figure S15. High-angle annular dark-field scanning TEM (HAADF-STEM) images of (a) AuNP, (b) PdNP, and (c) PtNP prepared on the template of ONP^{M}.

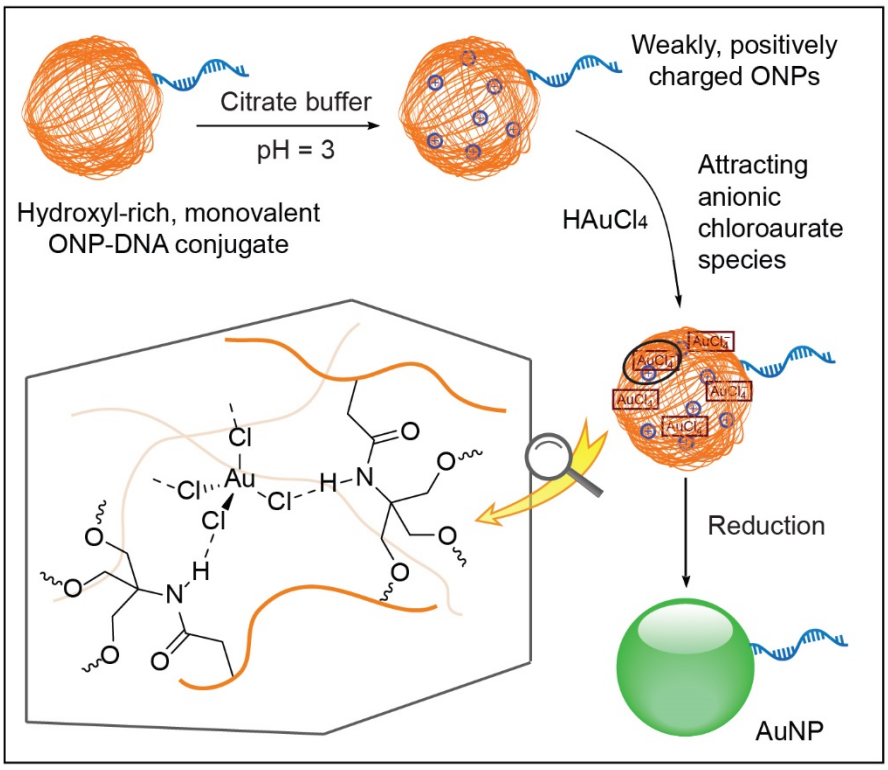

Figure S16. A proposed mechanism of templated AuNP formation on organic nanoparticles, based on the report of Beer et al. PdNPs and PtNPs likely formed in a similar way.

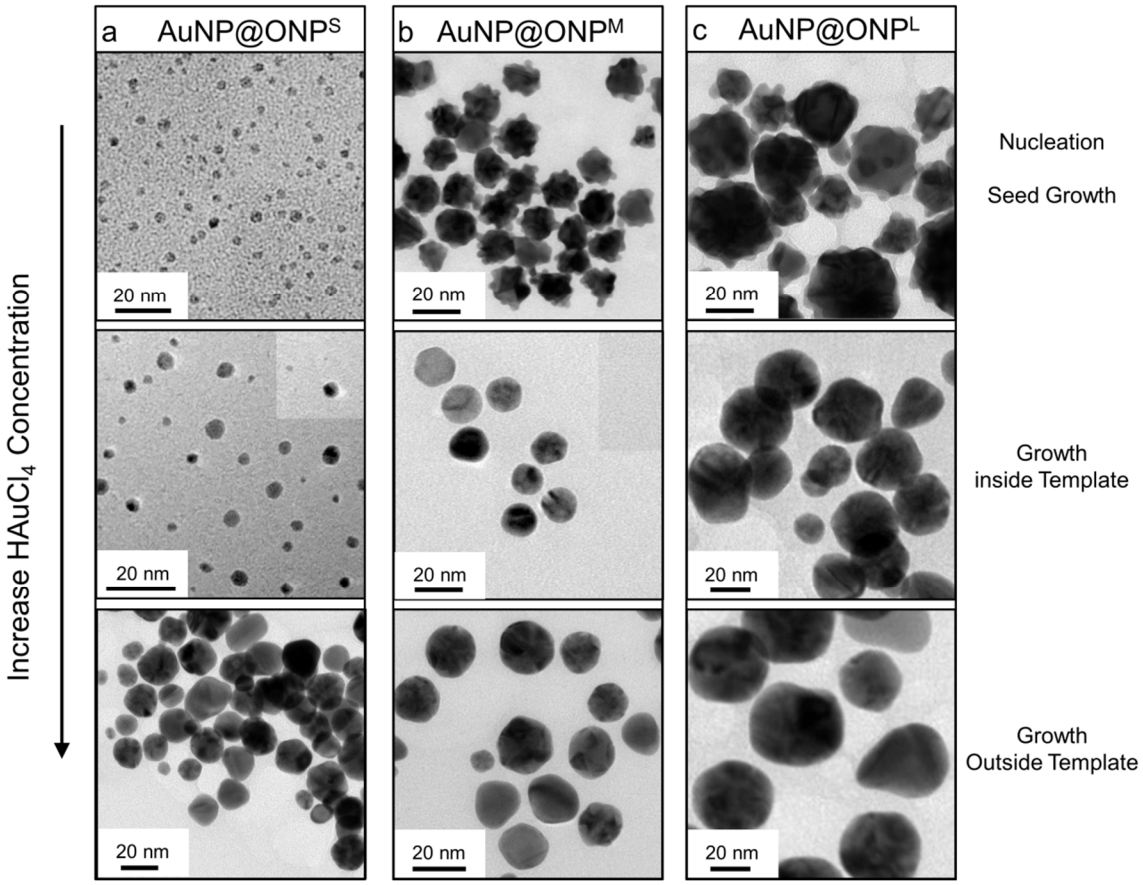

Figure S17. Preliminary mechanistic study of ONP-templated metallization. TEM images of AuNPs synthesized with the combinations of ONP template of different sizes and gold source of different concentrations. A stepwise growth of AuNP on ONP template was observed with a possible mechanism underlying the metallization process proposed as follows: 1. the metallization of ONP template starts with the gold ion nucleation and seed growth. The negatively-charged chloroaurate ions can localize onto positively-charged ONP scaffold where the hydroxyl and amide N-H moieties serve as potential nucleation and seed growth sites, resulting in the formation of AuNP with rough surface and sparsely-packed inner structure. 2. After nucleation and seeding process, Au(0) atoms tend to be deposited onto existing Au nucleation sites because of the low surface energy, filling the gap between neighboring gold sites and forming densely-packed AuNP with round surface. 3. After forming densely-packed AuNP with a similar size to the ONP template, further reduced Au(0) atoms will be deposited onto the surface of AuNP, resulting in the further growth and increased size of the AuNP.

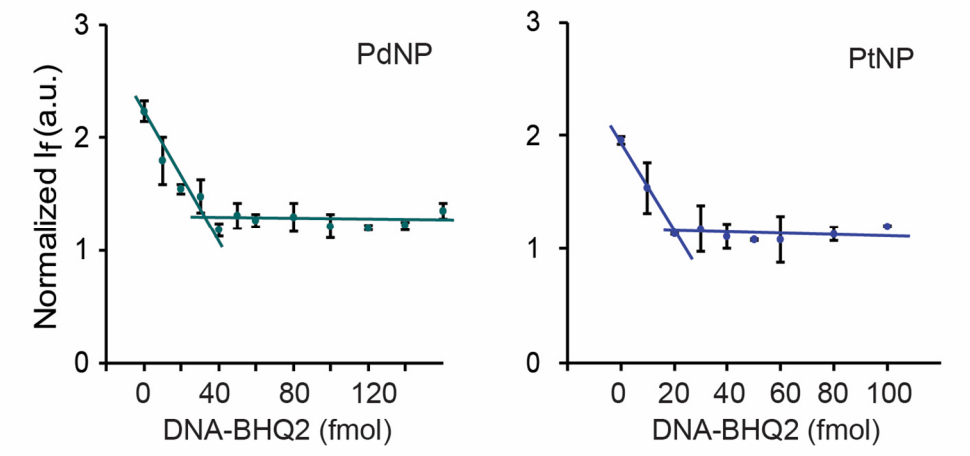

Figure S18. Titration plot of monovalent PdNP@ONP^{M}-DNA and PtNP@ONP^{M}-DNA using BHQ2-DNA.

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