Controlled Integration of Gold Nanoparticles and Organic Fluorophores Using Synthetically Modified MS2 Viral Capsids

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Supporting Information

General Procedures and Materials:

Unless otherwise noted, all chemicals were purchased from commercial sources and used as received without further purification. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F_{254} plates with visualization by ultraviolet (UV) irradiation at 254 nm and staining with potassium permanganate. Purifications by flash chromatography were performed using EM silica gel 60 (230-400 mesh). Chromatography solvents were used without distillation. All organic solvents were removed under reduced pressure using a rotary evaporator. Water (dd-H_2O) used in all procedures was deionized using a NANOpure^{TM} purification system (Barnstead, USA). All unconjugated gold colloid solutions were purchased from Ted Pella, Inc (Redding, CA). Bis(*p*-sulfonatophenyl)phenylphosphine dehydrate dipotassium salt (BSPP) was purchased from Sigma Aldrich (St. Louis, MO). All oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Coverslips (25 mm, number 1.5 thickness) were purchased from Warner Instruments (Hamden, CT), catalog number: 64-0715 (CS-25R15). Lab-tek chamber slides (8-well, glass) were obtained from Nunc (Rochester, NY). Neutravidin was purchased from Invitrogen (Carlsbad, CA) and stored as 1 mg/mL aliquots. Biotin-PEG-maleimide 5 kDa was purchased from Nanocs (Boston, MA). Poly-L-Lysine (20 kDa) grafted to 2 kDa PEG (PLK-PEG) and Poly-L-lysine (20 kDa) grafted with PEG (2 kDa) and PEG-Biotin (3.4 kDa) was purchased from SuSoS (Dübendorf, Switzerland) and stored at 1 mg/mL in HEPES buffer, pH 5.3.

Instrumentation and Sample Analysis:

NMR. ^{1}H and ^{13}C spectra were measured with a Bruker AVB-400 (400 MHz) spectrometer. ^{1}H NMR chemical shifts are reported in δ in units of parts per million (ppm) relative to $CDCl_3$ (δ 7.26, singlet). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (quintet), or br-s (broad singlet).

Mass Spectrometry. Electrospray Ionization Liquid Chromatography/Mass Spectrometry (LC/MS) analysis was performed using an API 150EX system (Applied Biosystems, USA) equipped with a Turbospray source and an Agilent 1100 series LC pump. Protein chromatography was performed using a Phenomenex Jupiter^{TM} 300 5µ C18 300 Å

reversed-phase column (2.0 mm x 150 mm) with an MeCN: dd-H_2O gradient mobile phase containing 0.1% formic acid (FA). Protein mass reconstruction was performed on the charge ladder with Analyst software (version 1.3.1, Applied Biosystems). Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) was performed on a Voyager-DE^{TM} system (PerSeptive Biosystems, USA). Prior to MALDI-TOF MS analysis, samples were desalted using C18 ZipTip® pipet tips (Millipore, USA). Oligonucleotide samples were co-crystallized using a 3-hydroxypicolinic acid: ammonium citrate solution (45 mg/mL:5 mg/mL in 4.5:5.5 MeCN:dd-H_2O).

Gel Analyses. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a Mini-Protean apparatus from Bio-Rad (Hercules, CA) with 10-20% gradient polyacrylamide gels (BioRad, CA), following the protocol of Laemmli.[1] All samples were mixed with SDS loading buffer in the presence of 1,4-dithiothreitol (DTT) and heated to 95 °C for 10 min to ensure complete denaturation. Gels were run at 120 V for 80 min. Commercially available molecular weight markers (Bio-Rad) were applied to at least one lane of each gel for assignment of molecular masses. For fluorescent protein conjugates, a UV-backlight was used for visualization. Gels were then stained with either Coomassie Brilliant Blue (Bio-Rad) or SYPRO® ruby protein gel stain from Molecular Probes™ (Eugene, OR). Coomassie stained protein gels were imaged on an EpiChem3 Darkroom System (UVP, USA). SYPRO® ruby stained gels were visualized on a Typhoon 9410 variable mode imager (Amersham Biosciences) (exc. 532 nm and em. 610 nm).

Dynamic Light Scattering (DLS). DLS measurements were obtained using a Malvern Instruments Zetasizer Nano ZS. Samples were filtered through a 0.22 μm centrifugal filter unit (Millipore Corporation, Billerica, MA) prior to data collection. Data plots are shown as size distribution by number, which weighs large and small particles equally. Diameters were calculated from an average of three measurements.

Transmission Electron Microscopy (TEM). TEM images were taken at the University of California Berkeley Electron Microscope Laboratory (UCB EML) using a FEI Tecnai 12 transmission electron microscope (TEM) with 120 kV accelerating voltage. Samples were prepared by pipetting 5 μL onto Formvar-coated copper mesh grids (400 mesh, Ted Pella, Redding, CA) for 5 min, followed by rinsing with 8 μL of dd-H_2O. The grids were then exposed to 8 μL of a solution of uranyl acetate (15 mg/mL in dd-H_2O) for 90 s as a negative stain. Excess stain was then removed and the grids were allowed to dry in air.

Total Internal Reflection Fluorescence Microscopy. Single particle fluorescence imaging was performed on a Nikon Ti-E/B (Tokyo, Japan) inverted microscope equipped with a Nikon 100x Apo TIRF 1.49 NA objective lens and an Epi/TIRF illuminator. Static images were recorded with a Hamamatsu (Hamamatsu City, Japan) Orca-R2 interline charge coupled device (CCD) camera. The sample was illuminated with the 488 nm line of a Spectra Physics (Santa Clara, CA) 177g argon-ion laser, which was controlled using an acousto-optic tunable filter from Solamere (Salt Lake City, UT). The excitation light was directed to a Chroma (Bellows Falls, VT) ZT488rdc dichroic mirror, which directed the light to the sample. Emission light was filtered by a Chroma ET500lp long-pass filter and a Chroma ET525/50m band-pass filter. Images were acquired using Micro-Manager microscopy software.[2]

Spinning Disk Confocal Microscopy. A spinning disk confocal head (Yokogawa CSU-X1-M1N-E, Solamere, Salt Lake City, UT) was custom fit to the microscope and camera described in the previous section. A T405/488/568/647 multiline dichroic (Semrock, Rochester, NY) in the spinning disk head was used to direct excitation light to the sample. Emission light was filtered with an ET525/50M (Chroma, Bellows Falls, VT) in a custom-mounted filter wheel (ASI FW-1000, Eugene, OR). Images were captured using a 1024x1024 pixel electron-multiplying CCD camera (Andor iXon3 888, Belfast, Ireland) using MicroManager software, as above.[2]

Fluorescence Lifetime Measurements and Fluorescence Correlation Spectroscopy. Fluorescence lifetime and fluorescence correlation spectroscopy measurements were acquired on a customized Nikon TE2000E inverted fluorescence microscope with a Nikon 100X Apo TIRF NA 1.49 objective lens. The microscope was modified to include an additional dichroic mirror (Chroma Technology Corp., Rockingham, VT) and a Nikon PFS system, which maintains the focal position. Illumination was provided with a 479 nm pulsed diode laser (LDH-P-C-485, PicoQuant, Berlin, Germany) set to a repetition rate of 10 MHz. Fluorescence emission light was collected through the objective and passed through a custom notch filter (Semrock, Rochester, NY) to remove any scattered laser light. The emitted light was then passed through a 50 μm confocal pinhole (Thorlabs, Newton, NJ), a 492 nm long pass filer and a 550 nm short pass filter. The emission light was split by a 50/50 beamsplitter onto two avalanche photodiodes (APDs) (SPCM-AQRH-16, Perkin-Elmer, Canada). This configuration allowed cross-correlation of the signal to remove contribution of the after-pulsing effect of the detectors to the correlation function.

Samples were deposited on Lab-tek chamber slides as droplets. A focal point was chosen at which fluorescence intensity did not change despite significant changes in the focus knobs, indicating that we were observing fluorescence emission from the solution.

The detectors and the pulsed laser were connected to a PicoQuant TimeHarp 200 time-correlated single photon-counting (TCSPC) card, which collects signal from the APDs through a universal router (PicoQuant PRT 400, TTL SPAD router). The power of each laser was measured at 5 μW before entering the optical path of the microscope and was checked periodically. No observable change in excitation power was noted throughout the course of the experiment. The autocorrelation function was calculated using a multiple-tau algorithm.[3]

Experimental Procedures:

I. Preparation of gold nanoparticles (AuNPs) for reassembly:

Phosphination of AuNPs (5 nm and 10 nm diameter). Phosphination of AuNPs (5 nm and 10 nm diameter) was carried out following a previously reported procedure.[4] In a typical reaction, bis(*p*-sulfonatophenyl)phenylphosphine dehydrate dipotassium salt (BSPP, 5 mg) was mixed with AuNPs (10 mL) and vigorously stirred at rt overnight. Solid NaCl was then added to the AuNP solution until the color turned from deep red to purple. The solution was then centrifuged (5000 rpm) for 10 min, yielding a pellet of nanocrystals. The supernatant was carefully removed and the pellet redissolved in an aqueous solution of BSPP (25 mg in 100 mL dd-H_2O). The redissolved nanocrystal solutions were combined and then precipitated through the

addition of methanol, until the color again changed from deep red to purple. The solution was again centrifuged (5000 rpm) for 10 min and the supernatant was removed. The nanocrystal pellet was again dissolved in an aqueous solution of BSPP, and stored at room temperature until use. The AuNP concentration was determined by recording the absorbance at 520 nm, and relating the absorbance values to concentration via Beer's Law ($\epsilon_{520\ nm} = 9.3*10^6\ M^{-1}cm^{-1}$ and $\epsilon_{520\ nm} = 8.1*10^7\ M^{-1}cm^{-1}$ for 5 nm and 10 nm AuNPs, respectively).[5,6]

Phosphination of AuNPs (15 nm, 20 nm and 40 nm diameter). BSPP (5 mg) was added to a solution of AuNPs (10 mL) and stirred vigorously overnight. The AuNP solution was then concentrated through multiple rounds of centrifugal filtration with a 100 kD MWCO centrifugal filter (Millipore), and diluted in an aqueous solution of BSPP (25 mg in 100 mL dd-H_2O). The AuNP concentrations were determined by recording their absorbance at 524 nm (15 nm AuNPs), 525 nm (20 nm AuNPs), and 528 nm (40 nm AuNPs), and relating their absorbance values to concentration via Beer's Law ($\epsilon_{524\ nm} = 2.4*10^8\ M^{-1}cm^{-1}$, $\epsilon_{525\ nm} = 6.4*10^8\ M^{-1}cm^{-1}$, and $\epsilon_{528\ nm} = 7.7*10^9\ M^{-1}cm^{-1}$ for 15 nm, 20 nm, and 40 nm AuNPs, respectively).[5,7,8]

Alkanethiol oligonucleotide modification of BSPP-stabilized AuNPs. Alkanethiol oligonucleotide modification of BSPP-stabilized AuNPs was achieved using a modified version of a previously reported procedure.[4,7]

The oligonucleotide sequence identity was as follows:

R: 5'-/5ThioMC6-D/TTT-TTT-TTA-CAT-GGG-TAA-TCC-TCA-TGT-3'

Lyophilized oligonucleotides containing a 5' thiol modifier [5'-$(CH_2)_6$-S-S-$(CH_2)_6$-phosphodiester bond-oligonucleotide-3'] purchased from IDT technologies (Coralville, IA) were resuspended in dd-H_2O to yield a final concentration of 100 μM. The disulfide functionality was cleaved by incubation with DTT (0.1 M) at rt in 100 mM phosphate buffer pH 8.0 for 1 h. Excess DTT was removed by passing the solution through a commercially available gel filtration column pre-equilibrated with dd-H_2O (NAP 5 column, GE Healthcare). It is important to note that 90% of the recommended volume was applied to the column for elution, to prevent excess DTT from remaining in the oligonucleotide solution. Fractions collected from the gel filtration column that contained oligonucleotides were combined and immediately added to BSPP-stabilized AuNPs (9 nM) to yield a final oligonucleotide concentration of 10 μM. The resulting solution was then incubated at rt for 20 min. The concentration of NaCl was increased by increments of 0.05 M to a final concentration of 0.1 M through successive additions of 2 M NaCl. Following each addition of 2 M NaCl, the alkanethiol oligonucleotide-modified AuNP solution was sonicated for 10 s, followed by agitation at rt for 20 min. After the final concentration of 0.1 M NaCl was reached, the AuNP solution was allowed to incubate at rt overnight. Excess oligonucleotides were removed through multiple rounds of centrifugal filtration with a 100k MWCO centrifugal filter (Millipore), and diluted in 50 mM phosphate buffer, pH 7.0. The final AuNP concentration was approximated by measuring the absorbance at 520 nm, 520 nm, 524 nm, and 528 nm for 5 nm, 10 nm, 15 nm, and 20 nm diameter AuNPs, respectively.

II. Expression and Interior Surface Modification of MS2-T19*p*AF-N87C (prior to reassembly):

Cloning and expression of MS2 mutants. Bacteriophage MS2 T19*p*AF plasmid production and growth has been previously reported.[9,10] The Peter Schultz lab (Scripps Research Institute, La Jolla, CA) provided the tRNA- and tRNA-synthetase-encoding plasmids necessary for *p*-aminophenylalanine (*p*AF) incorporation.[11] A yield of ~10 mg/L was obtained for MS2-*p*AF19 following two purification rounds. For the MS2 sample shown in **Figure 1**, position 87 was mutated into a cysteine using the following forward and reverse primers to allow for the incorporation of a maleimide dye through a cysteine alkylation reaction.

Forward: 5' – AGCCGCATGGCGTTCGTACTTATGTATGGAACTAACCATTC – 3'
Reverse: 5' – GAATGGTTAGTTCCATACATAAGTACGAACGCCATGCGGCT – 3'

Growth and purification of T19*p*AF N87C MS2 was identical to that of T19*p*AF MS2.[9,10]

Interior Surface Modification of T19*p*AF N87C MS2. For the MS2 samples shown in **Figure 1** and **Supporting Information Figure S3**, the general procedure for the cysteine alkylation reaction of T19*p*AF N87C MS2 with an Alexa Fluor 594 c5-maleimide (Invitrogen, Carlsbad, CA) was performed as reported previously.[9]

III. Disassembly and reassembly of T19*p*AF MS2 around AuNPs:

Disassembly and reassembly of T19*p*AF MS2 around AuNPs (10 nm diameter). Bacteriophage MS2 T19*p*AF was first subjected to disassembly conditions, using a method previously described.[12,13] In a typical disassembly reaction, 150 μM T19*p*AF MS2 in 50 mM phosphate buffer, pH 7.2, containing 100 mM NaCl (200 μL) was combined with two volumes of cold glacial acetic acid (400 μL) and kept on ice for 30 min. The mixture was then centrifuged for 20 min (13,000 rpm at 4 ˚C). Following centrifugation, the supernatant was removed and applied to a gel filtration column (NAP-5, GE Healthcare) pre-equilibrated with 1 mM acetic acid. Fractions were collected from the gel filtration column and their absorbance values at 280 nm were recorded. The fractions containing MS2 were combined on ice and used immediately.

For reassembly, the final MS2 coat protein dimer concentration was approximated by recording the absorbance at 280 nm and assuming eighty percent reassembly efficiency. The AuNP concentration was approximated by measuring the maximum absorbance and relating that value to the concentration via Beer's law. In a typical reassembly reaction, AuNPs (final ratio of 1:1 AuNPs: MS2 capsids) were combined with two thirds volume of 100 mM phosphate buffer, pH 7.2 containing 100 mM NaCl. The final one third volume comprised MS2 coat protein dimer solution. The solution was then kept at 4 ˚C for 40 h to allow for reassembly. Following reassembly, a saturating amount of NaCl was added directly to the solution. The reassembly reaction was then applied to a gel filtration column (NAP-5, GE Healthcare), pre-equilibrated with 10 mM phosphate buffer, pH 7.2. Samples were then concentrated through multiple rounds of centrifugal filtration with a 100k MWCO centrifugal filter (Millipore), and diluted in 10 mM phosphate buffer, pH 7.2. The reassembly was confirmed through dynamic light scattering, transmission electron microscopy, and native agarose gel electrophoresis (2.5% agarose, 50% glycerol loading buffer, 0.5x TBE running buffer, pH 8.0, 2 h at 40 V, on ice). A portion of the

sample was then run on an SDS-PAGE gel with standard concentrations of MS2. The concentration of the reassembled sample was then determined by optical densitometry using ImageJ software.

If desired, the MS2 samples containing 10 nm AuNPs were also able to be purified via PEG precipitation (0.5 M NaCl, 10 w/v% PEG-6k) for 1 h at 4 °C. MS2 samples were isolated through centrifugation at 5000 rpm for 20 min. The supernatant was carefully removed and the precipitate was resuspended in 50 mM phosphate buffer, pH 7.0. The resuspended sample was then passed through a gel filtration column pre-equilibrated with 50 mM phosphate buffer, pH 7.0 (NAP-5, GE Healthcare).

IV. Preparation of MS2-DNA constructs shown in main text:

Alkylation of thiol oligonucleotides. Oligonucleotides were purchased with thiol groups installed at either the 5' or 3' end as designated below. The oligonucleotide sequence identities were as follows:

3 bp hairpin: 5'-/5AmMC6/-TTT-ATG-GCA-CGC-CCT-TTT-TTC-CGC-GTG-CCA-T-/Thio-C3-SS/-3'

12 bp hairpin: 5'-/5AmMC6/-TTT-TTT-TTT-TTT-CGG-CAC-GCC-CTT-TTT-TCC-GCG-TGC-CG-/Thio-C3-SS/-3'

24 bp hairpin: 5'-/AmMC6/-TTT-TTT-TTT-TTT-CCC-TTT-CCC-TTT-GTC-GGC-ACG-CCC-TTT-TTT-CCG-CGT-GCC-GAC-/Thio-C3-SS/-3'

complementary hairpin: 5'-/5ThioMC6-D/-GGC-ACG-CGG-AAA-AAA-GGG-CGT-GCC-3'

Alkylation of thiol oligonucleotides was performed as previously described.[14] Alexa Fluor 488 c5 maleimide (Invitrogen) was stored as a 20 mM stock in DMSO at -20 ˚C until use. The following sequences were modified with Alexa Fluor 488 c5 maleimide: 3 bp hairpin, 12 bp hairpin, and 24 bp hairpin. Aliquots of 10 mM biotin maleimide 5k PEG in DMSO were stored at -20 ˚C until use. The following sequences were modified with biotin maleimide 5k PEG: complementary hairpin. Commercially available gel filtration columns (NAP 5, GE healthcare) were used to separate the small molecule or PEG from DNA (3 bp hairpin, 12 bp hairpin, 24 bp hairpin, and complementary hairpin). The DNA samples were then lyophilized and re-suspended in 50 mM phosphate buffer, pH 8.0. All reactions were monitored by MALDI-TOF-MS.

General procedure for the addition of *o*-nitrophenol to oligonucleotides. DNA (3 bp hairpin, 12 bp hairpin, and 24 bp hairpin) was labeled with *o*-nitrophenol following labeling of the 3' thiol with AF 488 maleimide dyes. A typical reaction was as follows: DNA at a concentration of 300 µM was reacted with *o*-aminophenol succinimidyl ester (60-120 eq.) in 1:1 solution of DMF and 50 mM phosphate buffer, pH 8.0. The reaction mixture was briefly vortexed and allowed to react at rt for 1.5 h. DNA samples were then filtered through commercially available 0.22 µm centrifugal filter units (Millipore Corporation, Billerica, MA). Commercially available gel filtration columns (NAP 5, GE healthcare) were then used to separate the small molecule from DNA. A 100 mM stock of $Na_2S_2O_4$ in 200 mM phosphate

buffer, pH 6.5 was added to the purified DNA mixture at a final concentration of 10 mM and allowed to react for 10 min. This yielded the *o*-aminophenol labeled oligonucleotides which were further purified through multiple rounds of gel filtration columns, lyophilized, and resuspended in 50 mM phosphate buffer, pH 7.0 to a final concentration of ~1 mM. DNA hairpin formation was then promoted by heating the DNA samples to 95 °C followed by cooling to 4°C at 1°C/5 min. DNA samples were held at 4 °C prior to attachment to MS2.

General procedure for *o*-aminophenol-DNA conjugation to MS2. The general procedure for the conjugation of DNA to MS2 was followed as reported previously.[9] Reassembled T19*p*AF MS2 (20 μM) and *o*-aminophenol-containing oligonucleotide (200 μM) were combined in 50 mM phosphate buffer, pH 7.0, containing 150 mM NaCl. The solution was then combined with $NaIO_4$ (1 mM), briefly vortexed and allowed to react at rt for 5 min. For a 50 μL reaction, the reaction was then quenched by the addition of *tris*(2-carboxyethylphosphinehydrochloride (TCEP, 10 μL of 330 mM). The pH of the TCEP solution was adjusted to 7.0 prior to addition. The resulting sample was then purified using a gel filtration column (NAP-5, GE healthcare). The eluent from the column was then concentrated through successive centrifugal filtrations using 100k molecular weight cutoff filters (Millipore) to remove excess DNA. It is critical for all TIRF, confocal, and lifetime measurements that excess fluorescently-labeled DNA is removed; this can either be achieved through successive centrifugal filtrations or purification on a non-denaturing agarose gel. A portion of the sample was then run on an SDS-PAGE gel. Optical densitometry was then used to determine the percent DNA modification.

Incubation of MS2 and MS2 (10 nm AuNPs) for 12 bp hairpin and 24 bp hairpin sequences with stabilizing strands. Stabilizing oligonucleotide sequences were dissolved in 50 mM phosphate buffer pH 7.0 to a concentration of 300 μM.

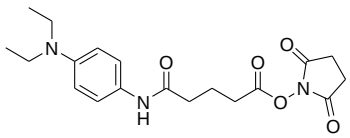
The sequence identities of the stabilizing sequences were as follows:

12-s hairpin: 5'-AAA-AAA-AAA-AAA-3'

24-s hairpin: 5'-AAA-GGG-AAA-GGG-AAA-AAA-AAA-AAA-3'

In a typical incubation, MS2-DNA (~10 μM monomer), NaCl (300 μM), and stabilizing DNA (~50 μM) were combined to a final volume of 30 μL in 50 mM phosphate buffer, pH 8.0. This solution was cycled from 37 °C to 4 °C at 0.5 °C/min, and then held at 4 °C for 2-10 h.

V. Preparation of MS2-DNA constructs shown in Supporting Information Figures S9-S13:



***4-(*4-diethylamino-phenylcarbamoyl)-butyric acid succinimidyl ester.** The synthesis of this compound was performed as reported previously.[10] To an oven-dried round bottom flask equipped with a Teflon stir bar was added 4-(*N*,*N*-diethylamino)aniline hydrochloride (500 mg, 2.49 mmol) and dichloromethane (30 mL) followed by triethylamine (0.348 mL, 2.49 mmol). Glutaric anhydride (284 mg, 2.49 mmol) was then added and the resulting solution was stirred at rt for 30 min. *N,N'*-dicyclohexylcarbodiimide (479 mg, 2.49 mmol) and *N*-hydroxysuccinimide (287 mg, 2.49 mmol) were then added, and the stirring was continued for 1 h at rt. The reaction was then gravity filtered to remove precipitate and concentrated under reduced pressure. The resulting black oil was dissolved in dichloromethane, gravity filtered, and applied directly to a silica gel column (20% - 100% EtOAc: Hexanes). Fractions were collected and concentrated yielding a light green oil, 217 mg (23 %) that was dissolved in 2.3 mL of dimethylsulfoxide and frozen for storage until use. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), δ 7.18 (d, 2H, *J* = 9 Hz), δ 6.43 (d, 2H, *J* = 9 Hz), δ 3.15 (q, 4H, *J* = 7 Hz), δ 2.63 (br-s, 4H), δ 2.52 (t, 2H, *J* = 7 Hz), δ 2.23 (t, 2H, *J* = 7 Hz), δ 1.97 (p, 2H, *J* = 7 Hz), δ 1.07 (t, 6 H, *J* = 7 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.6, 169.4, 168.4, 144.8, 127.1, 121.9, 112.2, 44.4, 34.9, 30.6, 25.5, 20.8, 12.3.

General procedure for the addition of phenylene diamine to oligonucleotides. The general procedure for the addition of phenylene diamine to oligonucleotides was reported previously.[9] The oligonucleotide sequence identity was as follows:

A: 5'-/5AmMC6-D/TTT-TTT-TTT-TTT-TAT-GTT-AGC-GTA-ATG-TGA-ATT-TGT-GTG-3'

A typical reaction was as follows: DNA at a concentration 300 μM was reacted with 4-(4-diethylaminophenylcarbamoyl) butyric acid succinimidyl ester (60-120 eq.) in 1:1 solution of DMF and 50 mM pH 8.0 phosphate buffer. The reaction mixture was briefly vortexed and allowed to react at rt for 1.5 h. Commercially available gel filtration columns (NAP 5, GE healthcare) were then used to separate the small molecule from the DNA. The DNA sample was then lyophilized overnight and re-suspended in 10 mM phosphate buffer, pH 7.2.

General procedure for phenylene diamine-DNA conjugation to MS2. The general procedure for the conjugation of DNA to MS2 was followed as reported previously.[9] Reassembled T19*p*AF MS2 (20 μM) and phenylene diamine-containing oligonucleotide (200 μM)were combined in 50 mM phosphate buffer, pH 7.0, containing 150 mM NaCl. The solution was then combined with $NaIO_4$ (5 mM), briefly vortexed and allowed to react at rt for 1 h. For a 100 μL reaction, the reaction was then quenched by the addition of *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP, 10 μL of 500 mM). The pH of the TCEP solution was adjusted to 7.0 prior to addition. The resulting sample was then purified using a gel filtration column (NAP-5, GE healthcare). The eluent from the column was then concentrated through successive centrifugal filtrations using 100k molecular weight cutoff filters (Millipore) to

remove excess DNA. A portion of the sample was then run on an SDS-PAGE gel. Optical densitometry was then used to determine the percent DNA modification.

Alkylation of thiol oligonucleotides. Oligonucleotides were purchased with thiol groups installed at either the 5' or 3' end as designated below.

The oligonucleotide sequence identities were as follows:

3 bp A': 5'-CAC-ACA-AAT-TCA-CAT-TAC-GCT-AAC-ATA-AAA-AAA-AAA'-/Thio-C3-SS/-3'

12 bp A': 5'-CAC-ACA-AAT-TCA-CAT-TAC-GCT-AAC-ATA-/Thio-C3-SS/-3'

24 bp A': 5'-CAC-ACA-AAT-TCA-CAT-/Thio-C3-SS/-3'

surface 24 bp A': 5'-/5ThioMC6-D/-CAC-ACA-AAT-TCA-CAT-3'

Alkylation of thiol oligonucleotides was performed as previously described.[14] Alexa Fluor 488 c5 maleimide (Invitrogen) was stored as a 20 mM stock in DMSO at -20 ˚C until use. The following sequences were modified with Alexa Fluor 488 c5 maleimide: 3 bp A', 12 bp A', and 24 bp A'. Aliquots of 10 mM biotin maleimide 5k PEG in DMSO were stored at -20 ˚C until use. The following sequences were modified with biotin maleimide 5k PEG: surface 24 bp A'. DNA (3 bp A' and 12 bp A' sequences) was isolated using the ethanol precipitation protocol of Shah, et al.[15] Commercially available gel filtration columns (NAP 5, GE healthcare) were used to separate the small molecule or PEG from DNA (24 bp A' and surface 24 bp A' sequences). The DNA samples were then lyophilized overnight and re-suspended in 50 mM phosphate buffer, pH 8.0. All reactions were monitored by MALDI-TOF-MS.

Incubation of MS2 (10 nm AuNPs)-A, MS2-A with (3 bp A' + AF 488, 12 bp A' + AF 488, 24 bp A' + AF 488). Stabilizing oligonucleotide sequences were dissolved in 50 mM phosphate buffer pH 7.0 to a concentration of 100 μM.

The sequence identities of the stabilizing sequences were as follows:

12-s: 5'-AAA-AAA-AAA-AAA-3'

24-s: 5'-TAC-GCT-AAC-ATA-AAA-AAA-AAA-AAA-3'

In a typical incubation, MS2-DNA (~10 μM monomer), NaCl (300 μM), DNA-fluorophore (~30 μM), and stabilizing DNA (~20 μM) were combined to a final volume of 30 μL in 50 mM phosphate buffer pH 8.0. This solution was cycled from 37 ˚C to 4 ˚C at 0.5 ˚C/min 5 times, and then held 4 ˚C for 2-10 h. Following incubation, the solution was concentrated into 0.5x TBE buffer, pH 8.0, in 100k MWCO spin filters (Millipore) at 4 ˚C to remove some excess DNA-fluorophore and buffer exchange for agarose gel electrophoresis. These samples were then run on an agarose gel (1% agarose, 50% glycerol loading buffer, 0.5x TBE running buffer pH 8.0, 80 V, 1 h, on ice). The gel was then visualized under ultraviolet

light, and bands containing MS2 were extracted, cut into small pieces and placed into a gel extraction spin column (Freeze 'N Squeeze, Biorad, Hercules, CA). Following the addition of 200 mM phosphate buffer pH 7.0 (200 µL), the extraction spin columns were kept at 4 ˚C for 1 h. The spin columns were then centrifuged for 10 min (5000 rpm). The flow through was then applied to 100k MWCO spin columns and spun for 10 min (5000 rpm). This process was repeated three additional times; the combined samples were then stored at 4 ˚C and imaged within 12 h.

VI. Imaging procedures:

Preparation of glass coverslips for TIRF and confocal microscopy. Coverslips were cleaned by sonication in a 50:50 (v/v) mixture of isopropanol and water. They were then dried and further cleaned by plasma treatment for 5 min in a Harrick Plasma PDC-32G plasma cleaner. They were then assembled with an Attofluor cell chamber (Invitrogen, Carlsbad, CA) and 0.25 mL poly-L-lysine PEG with PLK-PEG-biotin (500:3 ratio of the two solutions by volume) was added. After 30 min, the samples were rinsed five times with 5 mL of 1xTAE-Mg^{2+} (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate, pH 8.0) buffer each time. Neutravidin was added to a final concentration of 0.1 mg/mL and incubated for 10 min. Excess neutravidin was rinsed with ten 5 mL rinses with 1xTAE-Mg^{2+} buffer. The sample was incubated for an additional 30 min and then rinsed with five 5 mL portions of 1xTAE-Mg^{2+} buffer. PEG-biotin labeled DNA was added to approximately 10 nM final concentration and incubated for 10 min. Again, excess DNA was rinsed away with five 5 mL portions of 1xTAE-Mg^{2+} buffer.[16] Capsid solutions were added until an appropriate density was achieved.

TIRF image collection. Samples were checked for fluorescence contamination by finding focus using reflection interference contrast microscopy and then examining the sample with the same imaging conditions as described below. In general, the level of observable particles before adding our capsid sample was very low: most fields of view contained at most 1-2 particles.

TIRF angle was empirically optimized for the first sample we examined on a given day, and kept constant between samples. The angle was adjusted until few diffusing species could be seen during stream acquisition. Since coverslips may differ in thickness (although the observations did not indicate that re-optimization was necessary), images were sometimes collected from the same sample at de-optimized TIRF angles and little difference was observed in molecular brightness. The stage was then moved to a new area and a series of images was collected with an exposure time of 240 ms. The camera was set to bin pixels on chip 2 by 2, providing a pixel size of 0.1284 µm.

These settings provided sufficiently high signal to noise ratio to resolve individual capsids, as described in the image analysis section. Regions of interest were chosen without prior inspection to avoid photobleaching before data collection.

TIRF image analysis. Each image set was analyzed separately using ImageJ software. Images were loaded into the program as a stack, background subtracted, and the threshold range was determined (image\adjust\threshold\default). The threshold range for each stack was set as twenty five percent of the smallest maximum through the largest maximum. Images that contained few or no particles (less than 10) were removed from each stack. The particles were

then analyzed by selecting the size as greater than or equal to 3 square pixels and the circularity was selected to be 0.90 – 1.00 (analyze\analyze particles). The area, mean, and integrated intensity were recorded for each particle spot in the stack of images. Mean intensity histograms shown in Figure 4, Supporting Information Figures S5, and Supporting Information Figure S11 were fit to a Gaussian curve, as shown as a black curve in the plots using OriginPro 8 software.

Preparation of glass plates for lifetime measurements. Glass 8-well plates were incubated with 0.5 M NaOH for 1 h at rt. The NaOH solution was then removed, and a solution of BSA in phosphate buffered saline was added to the wells. Following overnight incubation at 4 °C, the BSA solution was removed, the plates were rinsed once with 1x TAE-Mg^{2+} buffer, and dried prior to sample addition. All prepared glass plates were used within 3 h of preparation.

Analysis of fluorescence lifetime data. The instrument response function was measured at approximately one nanosecond, while the measured decay curves have lifetimes on the order of a few nanoseconds. Consequently, a simple semi-log plot with linear regression was not sufficient to extract lifetime information. Thus, the decay curves were fit via an iterative non-linear least squares method that takes into account the instrument response function as measured from a colloidal sample. The samples containing AuNPs scattered light strongly, and the signal due to scattering was incorporated into the fitting to correctly account for the signal shape. Measurement error was calculated as the standard deviation between three successive acquisitions. The error of the fitting procedure was also evaluated and included in the stated errors by calculating the standard deviation of the fitted time constants derived from randomized starting parameters. The following is the fitting function used.

$$f(t) = g(t) * [A \cdot H(t - t_0) \cdot e^{\frac{-(t-t_0)}{\tau}} + B \cdot \delta(t - t_0)]$$

Where g(t) = the measured instrument response function, H(t) = the Heaviside step function that defines t_0 for the fluorescence decay, δ(t) = the Dirac delta function that accounts for the scattered light, A = amplitude of the fluorescence component, t_0 = time zero for the fluorescence decay, τ = decay time for the fluorescence component, B = amplitude of the scattered light, and the * denotes a numerical convolution.

Effect of TIRF microscopy versus confocal microscopy on brightness measurements. The effect of small differences in the distance of capsids from the surface as a result of gold functionalization was considered in regards to the effect of these differences on measured fluorescence intensity, since the intensity of excitation light decays exponentially as a function of distance from the surface. The following equation relates intensity of the excitation light to the distance from the surface:[17]

$$I(z) = I(0)e^{-z/d}$$

I(z) is the intensity at a given distance from the surface. *I(0)* refers to the intensity at the surface. The distance from the surface is represented as *z*, and the penetration depth is represented by *d*. The penetration depth is given by:[17,18]

$$d = \frac{\lambda}{4\pi}\sqrt{\frac{1}{n_1^2 sin^2(\alpha) - n_2^2}}$$

The critical angle for the glass/water (n_1 = 1.52 n_2 = 1.33) interface is approximately 61°. This gives a penetration depth by the above calculation of 216 nm and would result in a difference of $e^{-1/216}$ or 0.5% in excitation light intensity for a difference in distance of 1 nm. Of course, it has been suggested that the depth of the evanescent field is not the only contribution to the z-selectivity of TIRF microscopy.[17,19] In addition, the collection light efficiency of fluorophores nearer the surface is enhanced if a very high numerical aperture objective (NA > 1.4) is used. This behavior can be approximated using a single exponential with lower penetration depth. In a system similar to ours, but using an objective with a NA of 1.45 (instead of 1.49 in our study), the penetration depth was found to be 125 nm. The resulting difference of intensities between fluorophores separated by 1 nm would be $e^{-1/125}$ or 0.8%. Assuming an even smaller penetration depth of 60 nm, the difference in intensities between the aforementioned fluorophores would be expected to be approximately 1.6%. These differences cannot explain the differences in fluorescence intensity reported in the manuscript.

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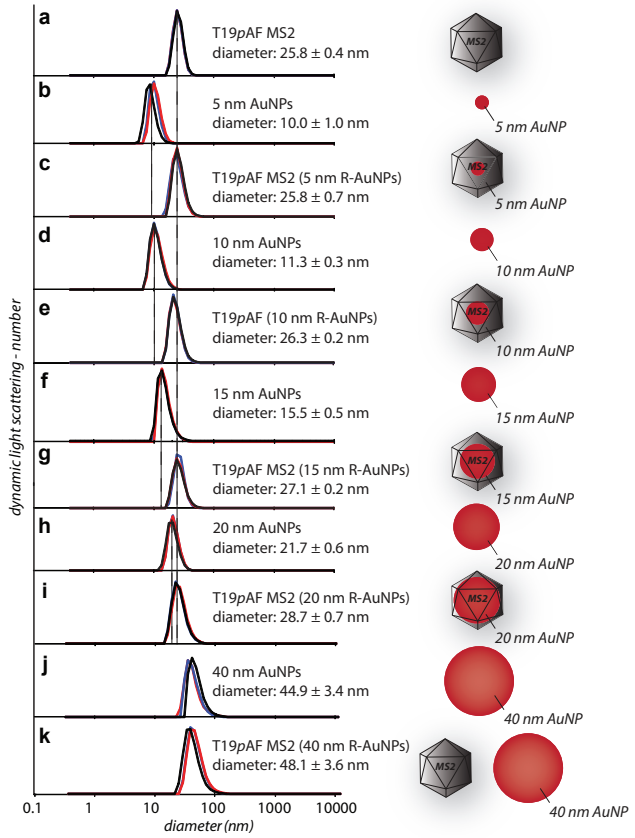
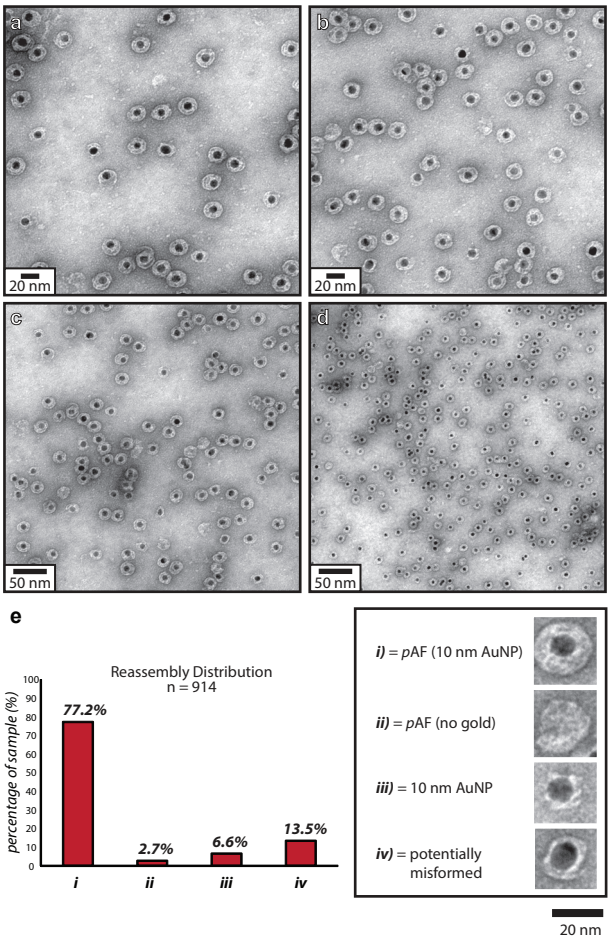
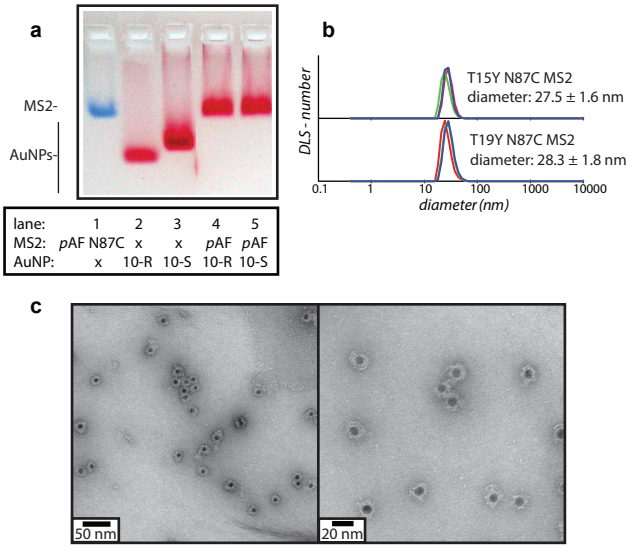
Supporting Information Figures



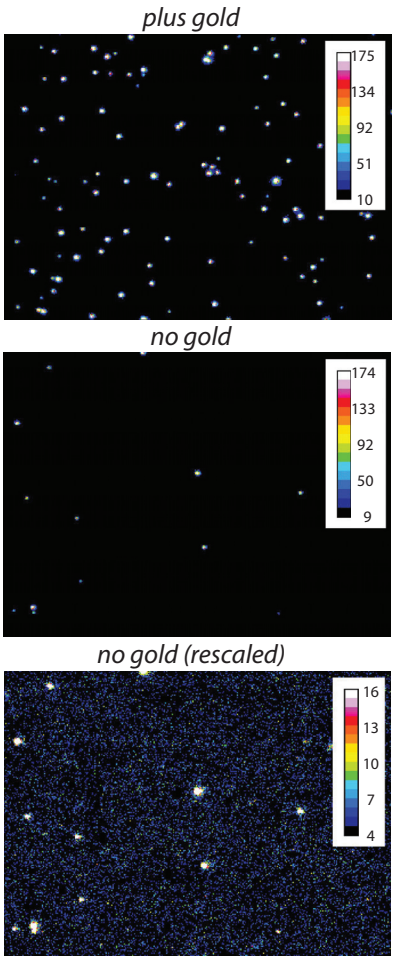
Supporting Information Figure S1. Dynamic light scattering by number data for (a) T19*p*AF MS2, (b) 5 nm, (d) 10 nm, (f) 15 nm, (h) 20 nm, and (j) 40 nm AuNPs, as well as T19*p*AF MS2 subjected to reassembly conditions in the presence of **R**-DNA modified (c) 5 nm, (e) 10 nm, (g) 15 nm, (i) 20 nm, and (k) 40 nm AuNPs. T19*p*AF MS2 reassembled in the presence of 5 nm, 10 nm, 15 nm, and 20 nm AuNPs resulted in average diameters similar to T19*p*AF MS2 not subjcted to reassembly conditions. These values were different from their respective AuNPs. As a control, T19*p*AF MS2 was subjected to reassembly conditions in the presence of 40 nm AuNPs. This sample (k) had a similar diameter to the 40 nm AuNPs (j). TEM analysis of this sample revealed no protein shell surrounding the metal particles.



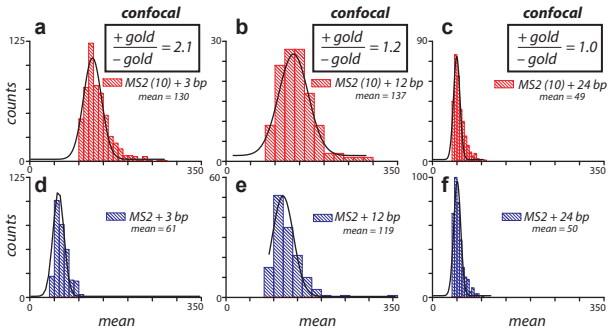
Supporting Information Figure S2. (a) - (d) Additional transmission electron micrograph images of T19*p*AF MS2 reassembled around 10 nm AuNPs. (e) The distribution of reassembled species for T19*p*AF MS2 subjected to oxidative coupling with DNA. Representative images are also shown for *(i)* T19*p*AF MS2 reassembled around R-DNA modified 10 nm AuNPs (77.2%), *(ii)* T19*p*AF MS2 reassembled without 10 nm AuNPs (2.7%), *(iii)* free 10 nm AuNPs, and *(iv)* 10 nm AuNPs with a potentially misformed protein shell (n = 914).



Supporting Information Figure S3. T19*p*AF MS2 reassembled around 10 nm AuNPs modified with alternative oligonucleotide sequences. (a) A native agarose gel of MS2 (lane 1), 10 nm **R**-DNA AuNPs (lane 2), 10 nm **S**-DNA AuNPs (lane 3), MS2 reassembled around 10 nm **R**-DNA AuNPs, and 10 nm **S**-DNA AuNPs (lanes 4 and 5, respectively). The electrophoretic mobility of MS2 reassembled around **R**-DNA and **S**-DNA 10 nm AuNPs is the same as MS2, which indicated that the AuNPs were encapsulated by MS2. (b) Alternative MS2 mutants reassembled around **R** DNA-modified 10 nm AuNPs. Dynamic Light Scattering (DLS) of T15Y N87C MS2 and T19Y N87C MS2 reassembled with **R**-DNA 10 nm AuNPs revealed a diameter similar to that of wild-type genome-free MS2. (c) Transmission Electron Micrograph images of T15Y N87C MS2 (left) and T19Y N87C MS2 (right) confirmed the DLS results and indicated the reassembly of MS2 around 10 nm AuNPs.



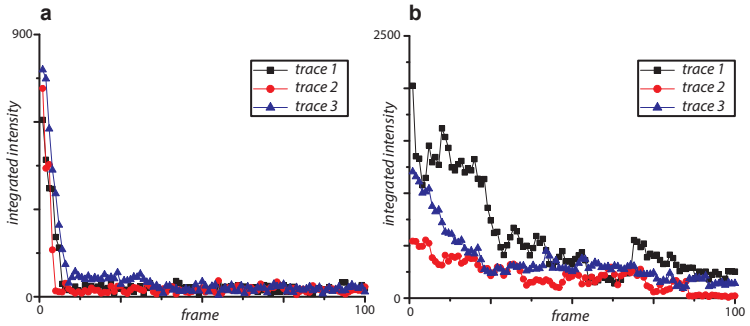
Supporting Information Figure S4. Larger version of the images shown in Figure 3(b).



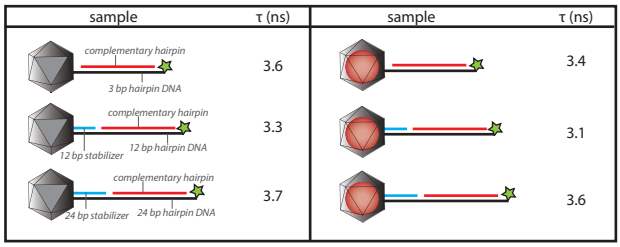
Supporting Information Figure S5. Mean intensity histograms determined from confocal microscopy images for (a) 3 bp hairpin, (b) 12 bp hairpin, and (c) 24 bp hairpin MS2 capsids with gold as well as (d) 3 bp hairpin, (e) 12 bp hairpin, and (f) 24 bp hairpin MS2 capsids without gold samples. The black curves are gaussian fits to the histogram data. These results are similar to those obtained with TIRF microscopy.

Supporting Information Table S1. TIRF microscopy results of (+gold/–gold) ratios for multiple capsid sample preparations. Samples 1, 2, and 3 shown in the table are different preparations of the hairpin DNA MS2 construct described within the main text. The percent MS2 modifications for sample 1 are ~60 strands per capsid (33%), ~45 strands per capsid (25%), and ~34 strands per capsid (19%), for 3, 12, and 24 bp separation, respectively. The percent MS2 modifications for sample 2 are ~34 strands per capsid (19%), ~9 strands per capsid (5%), and ~ 9 strands per capsid (5%), for 3, 12, and 24 bp separation, respectively. The percent MS2 modifications for sample 3 are ~25 strands per capsid (14%), ~16 strands per capsid (9%), and ~11 strands per capsid (6%) for 3, 12, and 24 bp separation, respectively. The * denotes that the fluorophore DNA hairpin strand was not pre-formed prior to attachment to the capsid. In all cases, fluorescence enhancement is observed. However, these data point to critical parameters in this construct to obtain reproducible intensity enhancements (percent MS2 modification and preformation of the hairpin DNA prior to attachment).

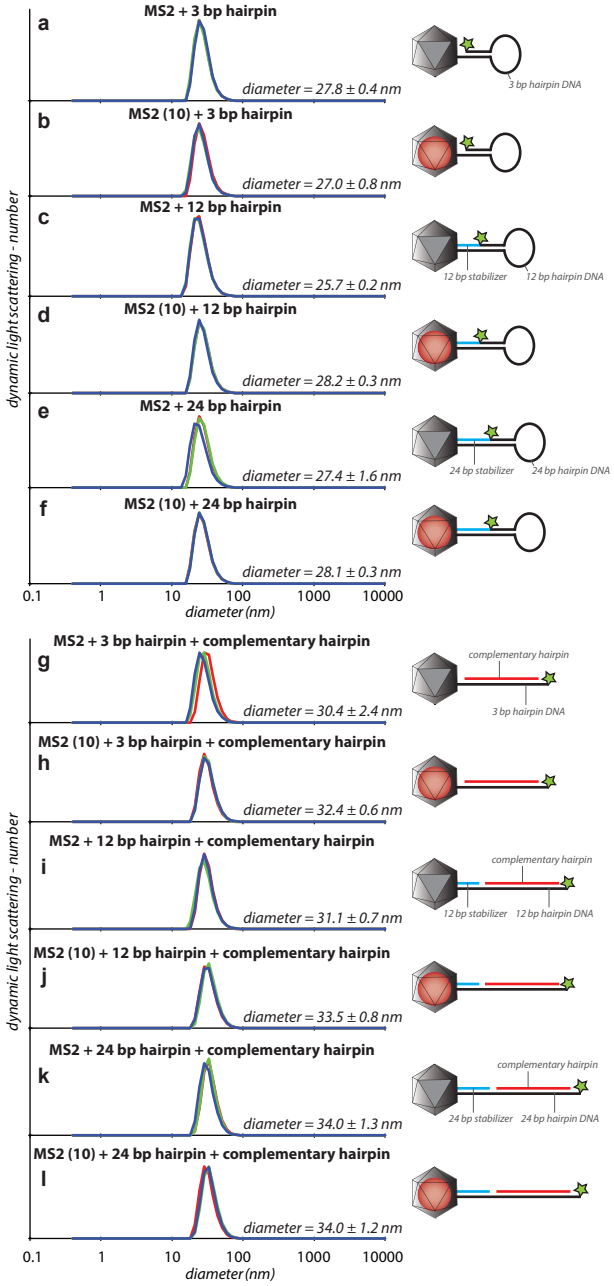
	3 bp distance	***12 bp distance***	***24 bp distance***
sample 1 *(shown in text)*	2.2	1.2	1.0
sample 2	3.9	3.4	2.0
sample 3*	5.4*	1.8*	3.1*



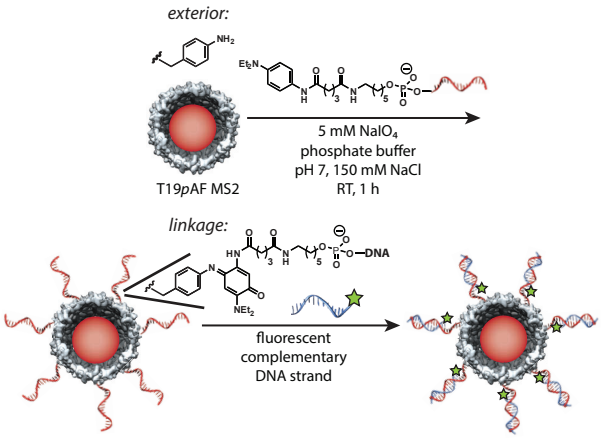
Supporting Information Figure S6. TIRF microscopy movie traces of integrated intensity versus time for a) representative traces of single fluorophores, versus b) representative traces of multi-fluorophore containing capsids. The single dye traces show the fluorescence bleaches to background within a few frames whereas the multiple fluorophore containing capsids bleach slowly over 100 frames. We have observed that if excess fluorescently-labeled DNA remain in solution upon TIRF or confocal microscopy imaging, little to no effect will be observed when comparing samples with and without gold for all distances.



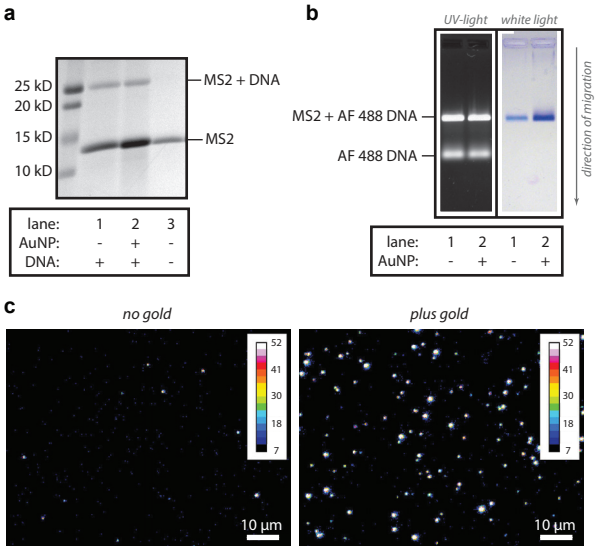
Supporting Information Figure S7. Fluorescence lifetime analysis of MS2-DNA conjugates. Fluorescence lifetime data are tabulated for three MS2 distances with and without gold incubated with a DNA sequence complementary to the hairpin loop. Presumably, the complementary hairpin moves the fluorophore away from the capsid, reducing the effect of the capsid and the interior AuNP on the fluorescence lifetime for all distances. Error on lifetime measurements is approximately 0.1 ns.



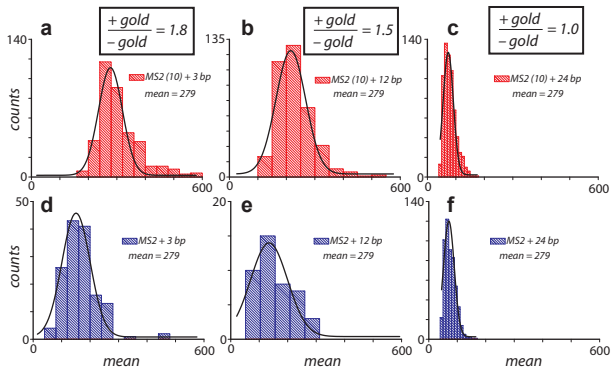
Supporting Information Figure S8. Dynamic light scattering by number data for (a) 3 bp hairpin MS2, (b) 3 bp hairpin MS2 plus gold, (c) 12 bp hairpin MS2, (d) 12 bp hairpin MS2 plus gold, (e) 24 bp hairpin MS2, and (f) 24 bp hairpin MS2 plus gold. Dynamic light scattering by number for MS2 samples incubated with a complementary hairpin sequence for (g) 3 bp hairpin MS2, (h) 3 bp hairpin MS2 plus gold, (i) 12 bp hairpin MS2, (j) 12 bp hairpin MS2 plus gold, (k) 24 bp hairpin MS2, and (l) 24 bp hairpin MS2 plus gold.



Supporting Information Figure S9. Overall synthetic strategy for constructing fluorophore-AuNP conjugates for an alternative construct to the one described within the main text. For exterior surface modification, phenylene diamine-containing DNA can be attached to exterior anilines on T19*p*AF MS2 using a $NaIO_4$-mediated oxidative coupling reaction. The exterior DNA can then be incubated with a complementary fluorescent DNA strand to yield the desired conjugate. Details of the preparation can be found in the Supporting Information text.



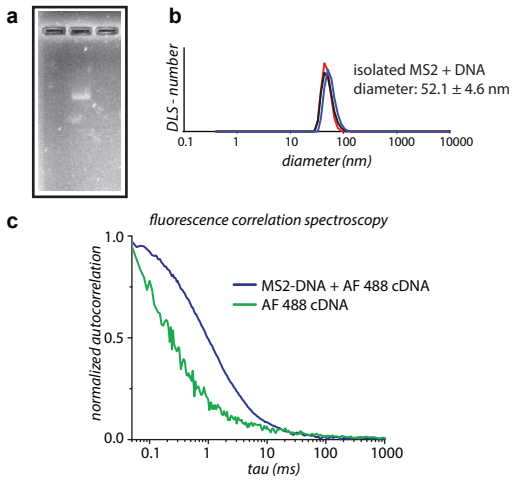
Supporting Information Figure S10. Gel and representative TIRF images for the alternative construct described in the Supporting Information text. (a) MS2-DNA conjugates were analyzed by SDS-PAGE followed by Coomaissie staining. Samples in lanes 1 and 2 were reacted with **A** DNA, and display a gel shift corresponding to the MS2-DNA conjugate. Lane 1 contains MS2 without gold and Lane 2 contains MS2 with gold. Lane 3 was not reacted with **A** DNA. The DNA modification was determined to be approximately 15% by optical densitometry for both the plus gold and minus gold case. (b) MS2-DNA conjugates incubated with complementary fluorescent DNA and a stabilizing strand were purified by native agarose gel electrophoresis. The gel was then visualized under UV-light (left), which revealed the MS2-fluorescent DNA conjugate as well as the free fluorescent DNA. The gel was also Coomaissie stained to confirm the presence of MS2 in both the minus gold and plus gold case. The MS2-containing bands were extracted and these samples were visualized in (c) by TIRF microscopy for the minus gold (left) and plus gold (right) case. The preparation of the samples and glass slides for TIRF microscopy is described in Supporting Information text.



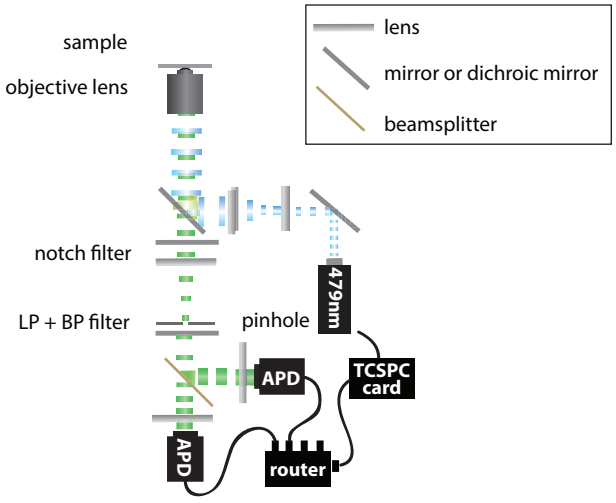
Supporting Information Figure S11. Mean intensity histograms determined from TIRF microscopy images for the alternative construct outlined in Supporting Information Figure S9. Shown are (a) 3 bp, (b) 12 bp, and (c) 24 bp MS2 capsids with gold as well as (d) 3 bp, (e) 12 bp, and (f) 24 bp MS2 capsids without gold samples. The black curves are gaussian fits to the histogram data.

control	τ (ns)	sample	τ (ns)	sample	τ (ns)
	4.1		3.2		2.9
	4.2		3.7		3.0
	4.2		4.0		3.3

Supporting Information Figure S12. Fluorescence lifetime data for the alternative construct outlined in Supporting Information Figure S9 and the Supporting Information text. Lifetimes are tabulated for three control samples, as well as the three distances with and without gold. Error on lifetime measurements is approximately 0.1 ns.



Supporting Information Figure S13. Analysis of MS2-DNA samples extracted from a native agarose gel for the alternative construct described in the Supporting Information text. (a) MS2-DNA plus AF 488 cDNA samples extracted from a native agarose gel were resubjected to an additional native gel. The gel indicated that the capsids were assembled and that there were few excess AF 488 cDNA oligos in solution. (b) Dynamic light scattering analysis of MS2 samples extracted from native agarose gels indicated an average diameter of 52.1 ± 4.6 nm (expected = 52.7 nm, assuming 0.33 nm per Franklin-Watson-Crick base pair). (c) Fluorescence correlation spectroscopy (FCS) autocorrelation function of MS2-DNA plus AF 488 cDNA as well as AF 488 cDNA. The FCS data suggested that the capsids were assembled and that few unhybridized AF 488 DNA strands remained in solution.



Supporting Information Figure S14. Fluorescence correlation spectroscopy (FCS) and fluorescence lifetime setup. A description of the setup can be found in the Supporting Information text.