Methods:

Plasmid preparation:

In vitro technology:

Maturation protein and coat protein (CP) dimer were synthesized (IDT) as gene blocks and transformed into pACYC-DUET using Golden Gate standard procedure. To create the variant lacking the maturation protein, one-step polymerase chain reaction (PCR) was conducted (primers were purchased from IDT).

In vivo technology*:*

The plasmid from the *in vitro* system was used for the expression of the MS2 CP and maturase protein. An additional plasmid encoding for the RT and the r_Oligo was also used – see table 1 for sequences. The r_Oligo was engineered either by Gibson standard protocol or by one-step PCR (primers were purchased from IDT).

Purification of MS2 CP dimers using Ni beads columns for *in vitro* assembly and MS2 VLPs for *in vivo* assembly:

Plasmids were transformed into E. coli BL21 (DE3) cells (Novagene) or DH10β (NEB-C3019I) according to the manufacturer's instructions, and the bacterial cells were grown in LB broth Miller (Difco) containing Chloramphenicol (35 µg/mL) (Gold Biotechnology, USA) at 37°C to OD_{600} = 1.7. 100µl of the bacterial culture were diluted into 500 mL TB broth containing Chloramphenicol (35 µg/mL) and cultivated at 37°C to OD_{600} = 0.6-0.8. Protein expression was induced by addition of 2 mM isopropyl-L-thio-D-galactopyranoside (Gold Biotechnology) at 37°C. The culture was cultivated at 37°C for 16 hours. The cell suspension was centrifuged at 4000 × g for 20 minutes at 4 °C and cells were resuspended in 4mL sonication buffer (100mM NaH_2PO_4, 600mM NaCl, pH = 8.0, all from Sigma-Aldrich), complete EDTA-free protease Inhibitor (Roche),200U of DNase I and 200µL of 10mg/mL RNase A. Cells were lysed using ultrasonic disruption for a total of 120 seconds, 20% amplitude (Disintegrator Sonicator W385). To eliminate cell debris, the lysed bacterial suspension was centrifuged at 4000g for 15-30 minutes at room temperature, the supernatant containing His-tagged MS2 CP dimers / His-tagged MS2 VLPs was filtered through a 0.22-µm syringe filter (Merk). Filtered supernatant was mixed in 1:1 ratio with 2×concentrated binding buffer (100mM NaH2PO4, 600mM NaCl, 30mM imidazole, pH=8.0, all Sigma-Aldrich). Subsequently, His-tagged MS2 dimers/ His-tagged MS2 VLPs were purified using Ni beads gravity column loaded with high performance Ni beads (GE healthcare). In brief, the column was pre equilibrated with 50mL of binding buffer (50mM NaH_2PO_4, 300mM NaCl, 15mM imidazole, pH=8.0, all Sigma-Aldrich). The His-tagged MS2 dimers / His-tagged MS2 VLPs bound to the column and were subsequently washed with 200 mL washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, 30 mM imidazole, pH= 8.0, all Sigma-Aldrich). His-tagged MS2 dimers/ His-tagged MS2 VLPs were eluted from the column in a final volume of 10mL with elution buffer (50mM NaH_2PO_4, 300mM NaCl, 200mM imidazole,

pH=8.0, all Sigma-Aldrich). Eluate containing His-tagged MS2 dimers / His-tagged MS2 VLPs was desalted using amicon 10 kDa columns (Merk) and converted to STE buffer (10 mM Tris, 100 NaCl, 1 mM EDTA, pH=7.5, all Sigma-Aldrich). For VLPs encapsulating tweezer, STE buffer did not contain EDTA.

***In vitro* assembly of MS2 VLPs encapsulating Malachite Green aptamer (MGA)**

DNA light-up aptamers generate fluorescence only in the presence of a specific fluorgene and only when the aptamer's secondary structure remains active.38, 39 Upon degradation of the aptamer or a change in its secondary structure, the fluorescent signal dramatically decreases. Malachite Green DNA aptamer (MGA), which specifically binds Malachite Green molecule, was chosen.

TR DNA MGA and TR-DNA oligonucleotides were purchased from IDT. Stock DNA solutions were prepared by dissolving lyophilized DNA in ultra-pure water and stored at -20°C until further use. Following purification, CP dimer concentration was measured using Bradford assay (Biorad) according to the manual instructions. 45µL of 2mg/mL CP dimers were taken and Trimethylamine N-oxide (TMAO) (Sigma-Aldrich) was added to a final concentration of 0.5M and the appropriate oligonucleotide was added to a final concentration of 5µM. The assembly reaction was carried in 4°C for 36-48 hours.

***In vitro* assembly of MS2 VLPs encapsulating tweezer**

The DNA nanotweezer contain cytosine-rich regions that act as proton sensors. Under acidic conditions, the cytosine-rich oligonucleotides associate to form i-tetraplex, also known as an i-motif. The i-motif comprises two parallel-stranded paired duplexes in an antiparallel orientation and is intercalated.[43] The i-switch tweezers comprise three oligonucleotides: O1, O2, and O3 (For complete sequences see sTable 1).[42] O1 and O2 are hybridized onto sites adjacent to O3, leaving only one-base gap which serves as a hinge. In addition, O1 and O2 contain cytosine-rich overhangs, each forming one-half of an i-motif. Under acidic conditions, these overhangs are protonated and a full intramolecular i-motif is formed, resulting in the closing of the DNA nanotweezer.

Tweezer assembly: stock DNA solutions (IDT) were prepared by dissolving lyophilized DNA in ultra-pure water and stored at -20°C until further use. 5µM of O1, O2 and O3 variants were mixed in equimolar ratios in STE buffer. The resultant solution was heated to 90°C for 5 minutes and cooled to room temperature over an hour. Tweezers assemblies were added to CP dimers at a final concentration of 1µM and VLPs assembly was performed as mentioned above.

Fluorescence measurements and DnaseI treatment

All fluorescence measurements were conducted using Tekan SPARK plate reader.

VLPs encapsulating MGA: Following *in vitro* assembly or VLPs purification, Malachite Green (Sigma-Aldrich) was added to the samples at a final concentration of 5µM, followed by an incubation at 37°C in 250 RPM for 15 minutes. 20µL of each sample were transferred to 384 black plate (Greiner) and fluorescence was measured at 618nm

excitation and 650nm emission. Then, 2µL of DnasI buffer and 1µL of DnaseI were added to each sample and incubated at 37°C for one-hour. Following incubation, the samples were again measured using plate reader as mentioned above.

VLPs encapsulating Tweezer: During the assembly process, the nanotweezer are added to the MS2 CP dimers in excess, to ensure the removal of the excess DNA nanotweezer following VLP assembly, 5µL of DnaseI buffer and 2µL of DnaseI were added to all samples and incubated at 37°C for one hour. Following incubation, to ensure the removal of excess nucleotides, fluorophores, and quenchers, VLPs were filtered using amicon 10 kDa columns (Merk) and the buffer was converted to 20 mM potassium phosphate buffer of desired pH containing 100 mM KCl. Samples were then transferred to 384 wells black plate (Greiner) and fluorescence was measured at 630nm excitation and 655nm emission using plate reader. Either 1% HCl or 1M NaOH were added until the measured pH reached 5.3 or 7.3, respectively. The samples were then measured using plate reader as mentioned above.

VLPs encapsulating MGA expressed in *E.coli:* VLPs expression was induced as described above. 1mL of the bacterial culture was taken and Malachite Green was added to a final concentration of 5µM to each sample. Then, the bacterial culture was incubated for 30min at 37°C, 250RPM. Following incubation, the bacterial culture was centrifuged at high speed for 1min, supernatant was discarded and 1mL of Phosphate buffered saline (PBS) was added for washing. The washing step was repeated for 3 times. Following the washing steps, the bacterial culture was diluted to OD600~0.2. Then, 200µL of each sample was transferred to 96-wells µclear plate (Greiner). OD600 and fluorescence was measured 618nm excitation and 650nm emission using plate reader. The fluorescence results were normalized by dividing the fluorescence signal in the OD signal. Furthermore, DH10β cells culture was used as a blank and the signal from this control sample was reduced from all the other samples.

Transmission electron microscopy (TEM)

TEM imaging was performed by applying 10µL samples onto 400-mesh copper grids covered by a carbon-stabilized Formvar film (SPI, West Chester, PA, USA). The samples were allowed to adsorb for 2 min before excess fluid was blotted off. Negative staining was then achieved by depositing 10µL of 2% uranyl acetate on the grid for 2 min before blotting off excess fluid. Micrographs were recorded using a Tecnai 12 electron microscope (FEI, Tokyo, Japan) operating at 120 kV.

Cryogenic temperature transmission electron microscopy (cryo-TEM)

MS2 VLPs were purified from DH10β cells as described above and kept at 4°C for 2 weeks – 6 weeks. Cryogenic transmission electron microscopy (cryo-TEM) imaging was performed on a Thermo-Fisher Talos F200C, FEG-equipped high resolution-TEM, operated at 200kV. Specimens were transferred into a Gatan 626.6 cryo-holder and equilibrated below -170 °C. Micrographs were recorded by a Thermo-Fisher Falcon III direct detector camera, at a 4k x 4k resolution. Specimens were examined either at TEM-

bright field mode using and objective aperture and defocusing, or at TEM nanoprobe mode using volta phase plates for contrast enhancement. Imaging was performed at a low dose mode of work to minimize the exposure of the imaged area to electrons. Images were acquired using the Tem Imaging and Acquisition (TIA) software.

Cryo-TEM specimens were prepared in a controlled environment vitrification system (CEVS) (Bellare et al., 1988). Since the system under study is aqueous, preparation was done in the temperature-controlled chamber with humidity at saturation, to prevent evaporation of volatiles (Talmon, 2015). Temperature was kept constant at 25 °C. A drop of the solution was placed on a carbon-coated perforated polymer film, supported on a 200 mesh TEM grid, mounted on a tweezers. The drop was turned into a thin film (preferably less than 300 nm) by blotting away excess solution with a metal strip covered with a filter paper. The grid was then plunged quickly into liquid ethane at its freezing point (-183 °C). Prior to specimen preparation, grids were plasma etched in a PELCO EasiGlow glow-discharger (Ted Pella Inc., Redding, CA) to increase their hydrophilicity.

ssDNA purification, polymerase chain reaction and gel electrophoresis

ssDNA purification from supernatant: following expression of VLPs in DH10β cells, cells were sonicated and centrifuged (as described above). 200µL were taken from the supernatant and ssDNA was purified from the supernatant using the DNA Clean & Concentrator-5 (Uncapped) kit (Zymo). The ssDNA was purified according to the manufacture instructions with the exceptional of the first step; following the addition of the DNA binding buffer the samples were incubated at 95°C for 20min. Then, the concentration of the ssDNA was measured using nanodrop.

ssDNA purification from VLPs: following expression and purification of MS2 VLPs (as described above),100µL of VLPs were taken and ssDNA was purified from the supernatant using the DNA Clean & Concentrator-5 (Uncapped) kit (Zymo). The ssDNA was purified according to the manufacture instructions with the exceptional of the first step; following the addition of the DNA binding buffer the samples were incubated at 95°C for 20min. Then, the concentration of the ssDNA was measured using nanodrop.

ssDNA calculation for 1L of bacteria: following the determination of the ssDNA concentration, the total amount of the ssDNA was calculated by multiplying the amount of DNA in the total starting volume of either supernatant or VLPs. The total amount was than divided to the total volume of bacteria which was used in the assay.