Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Self-assembled controllable virus-like nanorods as templates for construction of one-dimensional organic-inorganic nanocomposites

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Content

Experimental section

1. Construction of DNA templates with controllable lengths

T7 promoter-based TMV DNA templates were carried out on full-length TMV DNA clones suitable for *in vitro* transcription based on the standard molecular cloning procedure. Based on the position of original assembly site (OAS), TMV constructs for less than 6395 bp were directly amplified from the TMV U1 clone with the polymerase chain reaction methods. For the 400 nm TMV-like assembly construction with more than 6395 bp, a new TMV vector was needed. An exogenous fragment GUS gene was amplified from the original plasmid *pGusi*-AM and double-enzymatically cutted by *Pst I* and *Sal* I (Fig. S1). The GUS gene was then ligated into the *Pst I-Sal* I stie in the modified TMV U1 vector containing six multiple cloning sites (5710-5749 bp). The recombinant vector (T7-TMV-GUS) was used to amplify the TMV templates more than 6395 bp. This new vector resulted in a complete stretch of the viral genome and extended the particle length up to 400 nm. Therefore, all DNA templates at diverse lengths were controllably attained from TMV U1 and T7-TMV-GUS vectors with the method of polymerase chain reaction (PCR). All DNA templates were amplified by suitable primers overlapping a certain length of TMV RNA genome containing OAS sequence.

Four major DNA templates for respective 100 nm, 200 nm, 300 nm and 400 nm were amplified by Transfast^{TM} *pfu* enzyme (TransGen Biotech, 2-4 kb/min) with the T7 promoter-attached PCR primers (**Table S1**) according to the following procedure: 95 ℃ pre-denaturing for 3 min, 30 cycles of 95 ℃ denaturing for 20 s, 56 ℃ annealing for 30 s and 72 ℃ extension for X min (X denoted 1 min, 2 min, 3 min, 4 min for 2130 bp, 4323 bp, 6395 bp, 8040 bp, respectively), 72 ℃ final extension for 5 min. The PCR products were purified by GeneJET PCR Purification kit (ThermoFisher Scientific) and detected in 1% agarose.

Table S1. The primers used in the study for DNA templates at different lengths

Name	Sequences	Amplification length/region
TMV-100F	TAATACGACTCACTATAGGGCAGGAACACAATAGCAATTACAGAT	2130 bp (3405-5534 bp)
TMV-100R	CGAAACTTTGCAAGCCTGATCGACA	
TMV-200F	TAATACGACTCACTATAGGGTAACCACATTCGAACATACCAGGCG	4323 bp (1257-5579 bp)
TMV-200R	CTATTTTTCCCTTTGCGGACATCA	
TMV-300F*	TAATACGACTCACTATAGGGTATTTTTACAACAATTACCAACAA	6395 bp (1-6395 bp)
TMV-300R*	TGGGCCCCTACCGGGGGTAACG	
TMV-400F*	TAATACGACTCACTATAGGGTATTTTTACAACAATTACCAACAA	8040 bp (1-8040 bp)
TMV-400R*	TGGGCCCCTACCGGGGGTAACG	

*TMV-300F/R and TMV-400F/R are the same primer sequence with the unique difference in its amplification region.
The underline denotes the T7 promoter sequence.

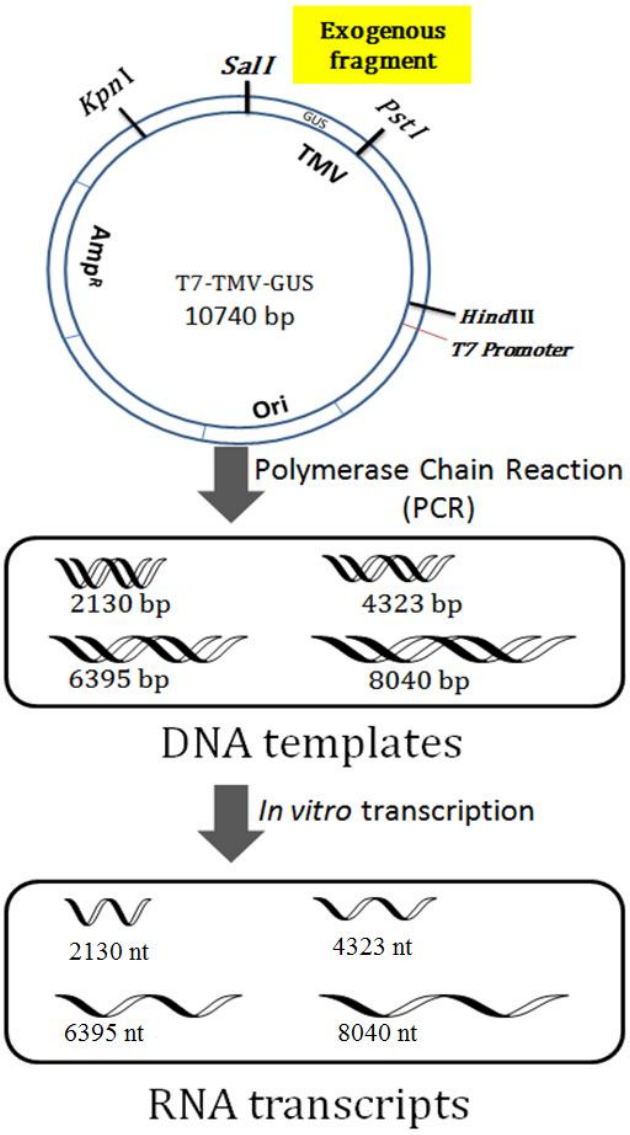


Fig. S1 A strategy for synthesis of size-controllable RNA transcripts at different lengths. Base on the OAS in TMV RNA genome (5443-5518 nt), all the DNA templates including the OAS region at the 3' end were amplified from the native and modified TMV vector (TMV U1 and T7-TMV-GUS) by PCR based on different primer pairs. The final length of PCR fragments was 2130 bp, 4323 bp, 6395 bp and 8040 bp, corresponding to assembled RNA of 2130 nt, 4323 nt, 6395 nt and 8040 nt, respectively, for TMV-like assemblies' length of 100 nm, 200 nm, 300 nm and 400 nm, respectively.

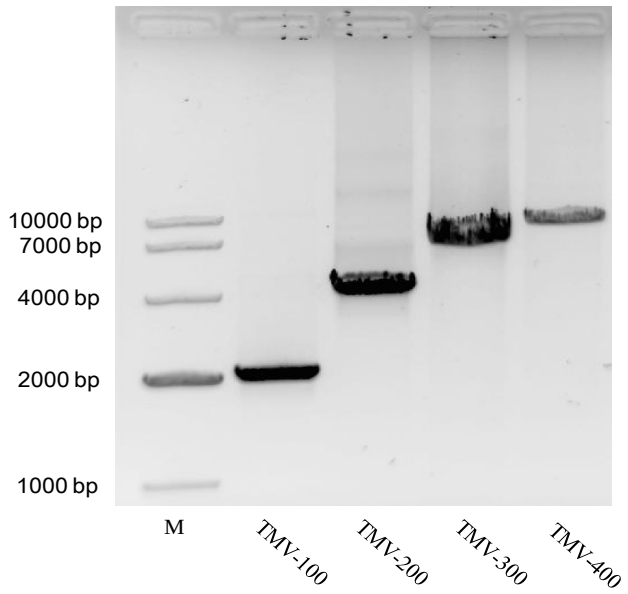


Fig. S2 Four DNA templates for TMV-like nanoparticles at different lengths of 100 nm (TMV-100), 200 nm (TMV-200), 300 nm (TMV-300) and 400 nm (TMV-400). The purified PCR fragments were electrophored in 1% agarose in 0.5×TBE buffer at 100 V for 1 hour and detected by the Gel Doc XRi instructment (Bio-Rad). DNA ladder was 10000 bp, 7000 bp, 4000 bp, 2000 bp, 1000 bp (top to bottom).

2. *In vitro* transcription

All assembled RNAs were *in vitro* transcribed from the purified PCR products with the TranscriptAid T7 High Yield Transcription kit (ThermoFisher Scientific). 1 μg DNA template was transcribed for 2 hours at 37 ℃ in a 20 μL reaction mixture containing 4 μL 5 × TranscriptAid reaction buffer, 8 μL NTPs (10 mM ATP, 10 mM CTP, 10 mM GTP, 10 mM UTP) and 2 μL TranscriptAid enzyme mixture. The transcription products were mixed with 2 μL EDTA (0.5 M, pH 8.0) and then purified by equal volume of phenol/chloroform/isoamylalcohol (25:24:1) for two times, equal volume of chloroform for two times, followed by two volumes of ethonal for RNA precipitation and 50 μL RNase-free water for dissolution. All the transcripts were electrophored in 1% agarose gel in 0.5 × TBE buffer at 100 V for 30 min. As shown in Fig. S3, the RNA bands were visualized and imaged by the Gel Doc XRi instructment (Bio-Rad) and processed with the Quantity One software.

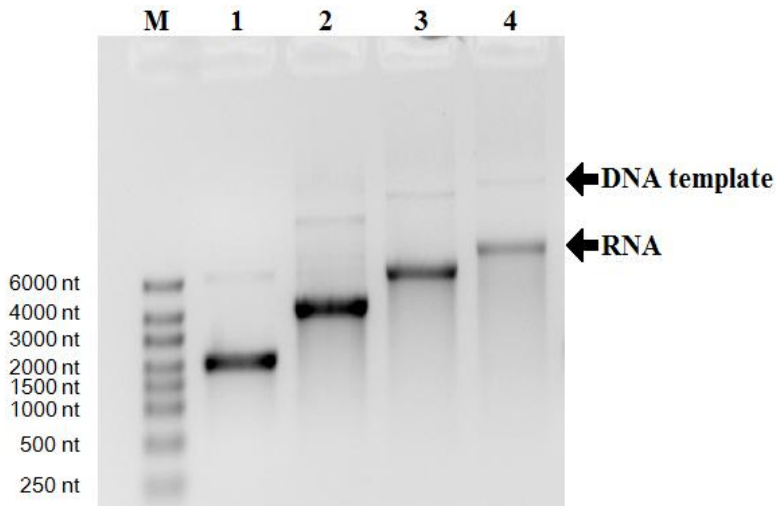


Fig. S3 Agarose gel of *in vitro* transcribed RNA. Four RNA transcripts of 2130 nt (lane 1), 4323 nt (lane 2), 6395 nt (lane 3) and 8040 nt (lane 4) in length were electrophored in 1% agarose in TBE buffer at 100 V for 30 minutes. The DNA templates could be also detected in the agarose gel. The RNA marker (M) was 6000, 4000, 3000, 2000, 1000, 500 and 250 nt, respectively. The length of transcripts of 2130 nt, 4323 nt, 6395 nt and 8040 nt, corresponded to TMV-like assemblies of 100 nm, 200 nm, 300 nm and 400 nm, respectively.

3. *In vitro* self-assembly of TMV-like nanoparticles

The TMV-like nanoassemblies were reconstituted by using the capsid protein of the TMV U1 strain from the systematic tobacco plants (Fig. S4). The original TMV suspension (10 mg/ml) was then diluted to 2 mg/ml for capsid protein purification, followed by repeated dialysis and centrifugation. The final capsid protein concentration was 20 mg/ml in 0.1 M phosphate buffer (pH 7.0). Prior to the particle reconstitution, the capsid protein at 20 mg/ml was incubated to form 20S discs at 20 ℃ in 0.1 M phosphate buffer (pH 7.0) for up to 12 hours, and the 20S discs were diluted to 1 mg/ml for the subsequent reconstitution. The 10 μL reconstitution mixture contained 8 μL capsid protein (1 mg/mL) and 2 μL RNA (0.2 mg/ml). The assembly reaction was incubated at 20 ℃ overnight. For disaggregate protein-alone assemblies, the assembly solution was mixed with four-fold Tris buffer (10 mM, pH 8.0) and incubated at 4 ℃ for 1 hour.

A drop of virion-assemble solution was placed onto a carbon-coated copper grid, incubated for 3 minutes and removed with the filter paper. Afterwards, samples were negatively stained with a 2% uranyl acetate solution by floating the grid on a drop of stain and removing after 1 min. The particles were observed and imaged in a JEM-2100F

transmission electron microscope operated at 200 kV and equipped with an Oxford EDX analyzer. The images were processed with the Image J software. The sample of 400 nm TMV-like assemblies was diluted to 0.1 mg/ml in a solution of 0.1 M phosphate buffer (pH 7.0). The sample was deposited over the mica plate to incubate for 30 min. Prior to imaging, the mica plate was gently rinsed with pure water (Millipore) and later vacuum-dried. AFM imaging was performed in Nanoscope IV (Veeco) controller. The gained images were processed and analyzed with the Nanoscope5.31r1 software.

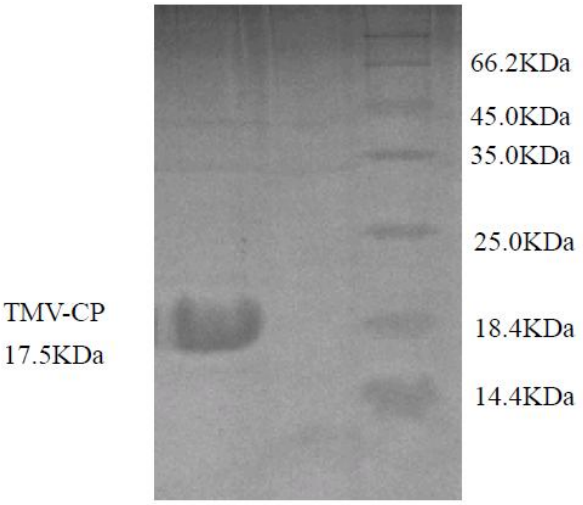


Fig. S4 The purity of capsid protein (CP) of the TMV U1 strain extracted from the systematic tobacco plants. The TMV CP purification procedure was performed according to Fraenkel-Conrat's glacial acetic acid methods. The final CP protein was detected in 10% SDS-PAGE. The protein marker was 66.2, 45.0, 35.0, 25.0, 18.4, 14.4 KDa (top to bottom).

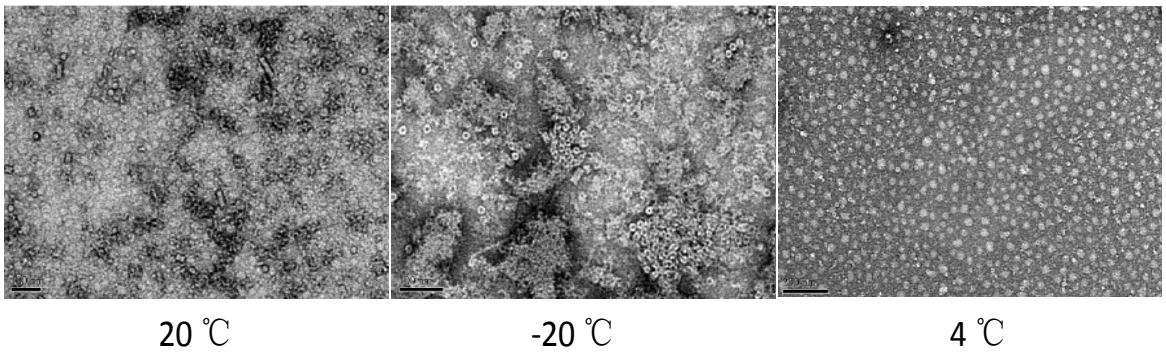


Fig. S5 TEM characterization of disassembly of TMV CP under different temperatures. The TMV CP was firstly incubated to form 20S discs overnight and then diluted with 10 mM Tris-HCL (pH 8.0). The dilution aliquot was placed at 20 ℃, -20℃ and 4℃ to disaggregate for 1 hour. The treated samples were imaged by TEM.

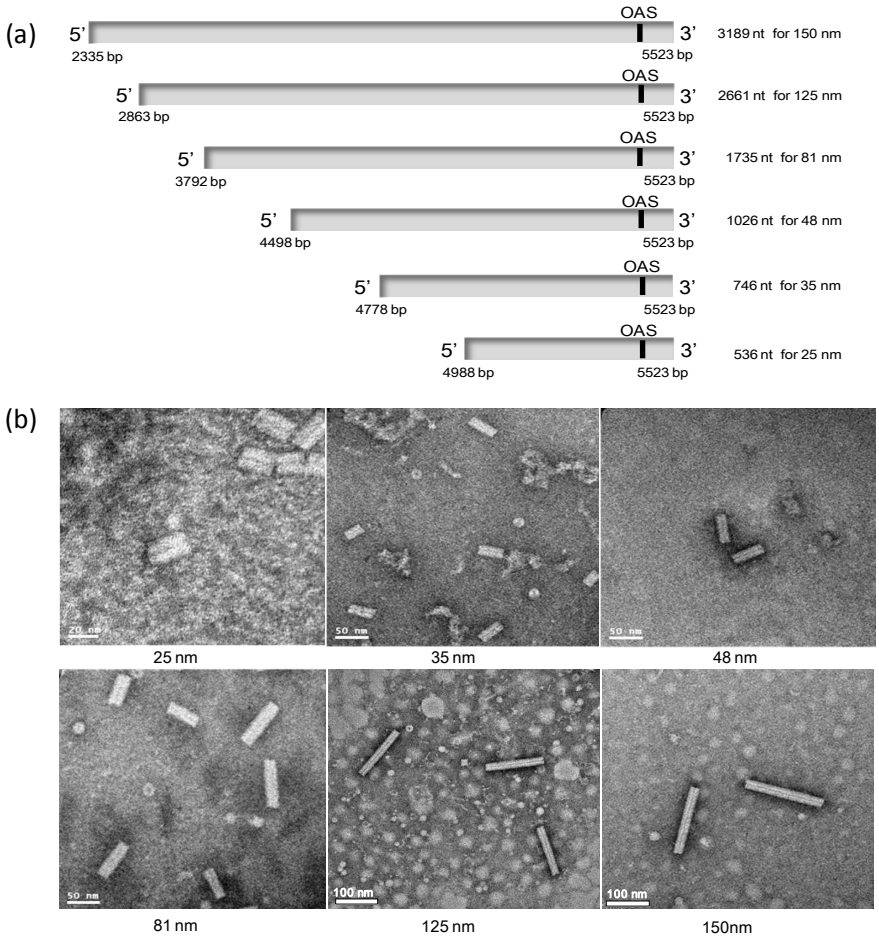


Fig. S6 (a) A schematic description of TMV-like assembly at diverse lengths with the OAS region at the 3' terminus. The length of DNA templates could be determined by the fixed reverse primer (including OAS region) at the 3' terminus and different forward primers at the 5' terminus. The ideal TMV-like rod length could be calculated from the TMV DNA sequence length. (b) TEM characterization of TMV assembly at diverse lengths.

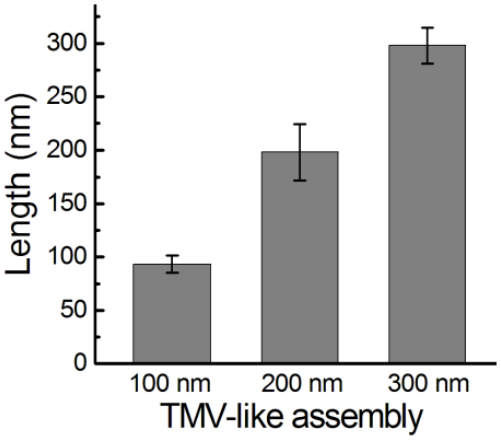


Fig. S7 The statistical graph of different TMV-like assemblies. The total sampling number was 386, 112, 382 for each dimension of 100 nm, 200 nm, 300 nm, respectively.

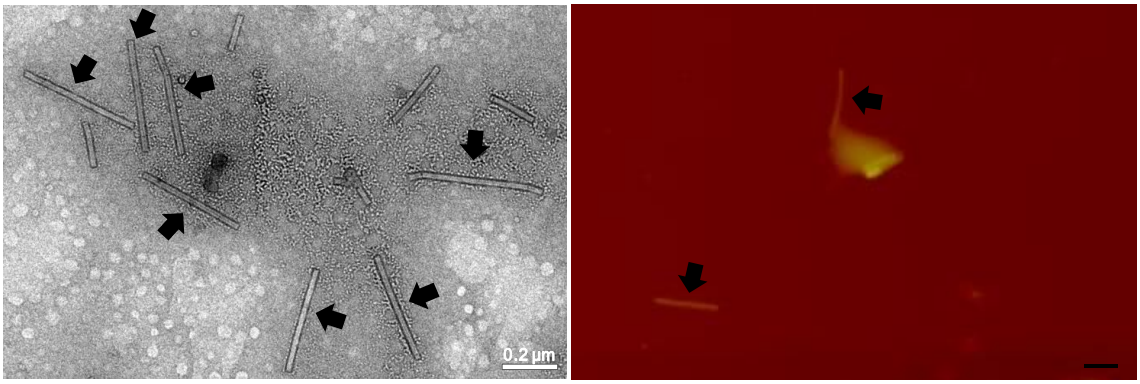


Fig. S8 The 400 nm virus-like assembly was characterized by TEM (a) and AFM (b). The bar length was 0.2 μm. The black arrow indicated the 400 nm virus-like assembly.

4. Nuclease resistance experiments

After the TMV CP and RNA reconstitution, the nuclease resistance test was performed to detect the reconstitution efficiency. The reaction mixture component was listed as follows: 2 μL 10 × micrococcal nuclease buffer, 10 μL recontituion mixture, 7 μL RNase-free water and 1 μL micrococcal nuclease (20 U/μL). The reaction mixture was incubated at 25 ℃ for 30 min, followed by the reaction termination with 1 μL EDTA (0.5 M, pH 8.0). The nuclease-treated nanopaticles were then mixed with 1% SDS and equal volume of phenol/chloroform/isoamylalcohol (25:24:1) for two times, equal volume of chloroform for two times, followed by two volumes of ethanol for RNA precipitation and 10 μL RNase-free water for dissolution. The protected RNA by capsid protein could be detected in 1% agarose gel to demonstrate whether the TMV RNA was packaged by capsid proteins.

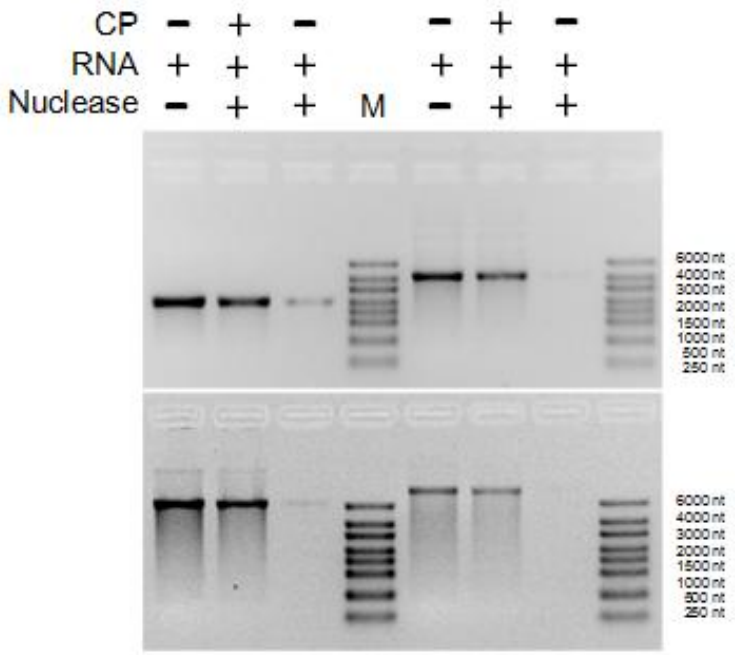
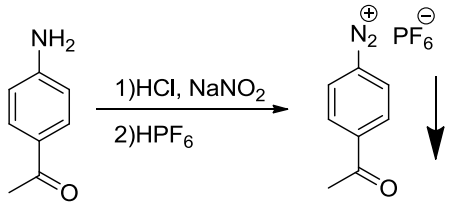


Fig. S9 Nuclease resistance assay of different nanoparticle reconstitution. The length of four transcripts for 100 nm, 200 nm, 300 nm and 400 nm was 2130 nt (left top), 4323 nt(right top), 6395 nt (left bottom) and 8040 nt (right bottom), respectively. The RNA was electrophored in 1% agarose in 0.5×TBE buffer at 100 V for 30 min.

5. Synthesis of the labelling molecule



1

4-Aminoacetophenone (862 mg, 6.4 mmol) was dissolved in 45 mL 12 N HCl at 0 ℃. Then $NaNO_2$ (1.31 g, 19.2 mmol) solution was added at 0-5 ℃. After 2 h reaction, 60% HPF_6 in water (1.2 mL, 8.2 mmol) was added at 0 ℃ and stirred for 2 h. Then the mixture was further stirred at room temperature for 30 min. The product was collected by filtration and washed with ice-cold water, yielding a light grey solid **1** (408.6 mg, 22%). ^{1}H NMR (600 MHz, ^{d6}DMSO), δ 8.80 (d, J = 8.7 Hz, 2H), 8.40 (d, J = 8.7 Hz, 2H), 2.70 (s, 3H). ^{13}C NMR (151 MHz, ^{d6}DMSO), δ 196.3, 144.6, 133.2, 130.5, 130.0, 119.4, 115.0, 27.2. ^{19}F NMR (376 MHz, ^{d6}DMSO) δ -70.29 (d, $J_{P\text{-}F}$ = 711.2 Hz). ^{31}P NMR (243 MHz,

^{d6}DMSO), δ -144.23 (h, $J_{P\text{-}F}$= 711.2 Hz). HRMS (ESI): m/z $[M]^+$ calcd. for $C_8H_7N_2O$: 147.0553; found: 147.0557.

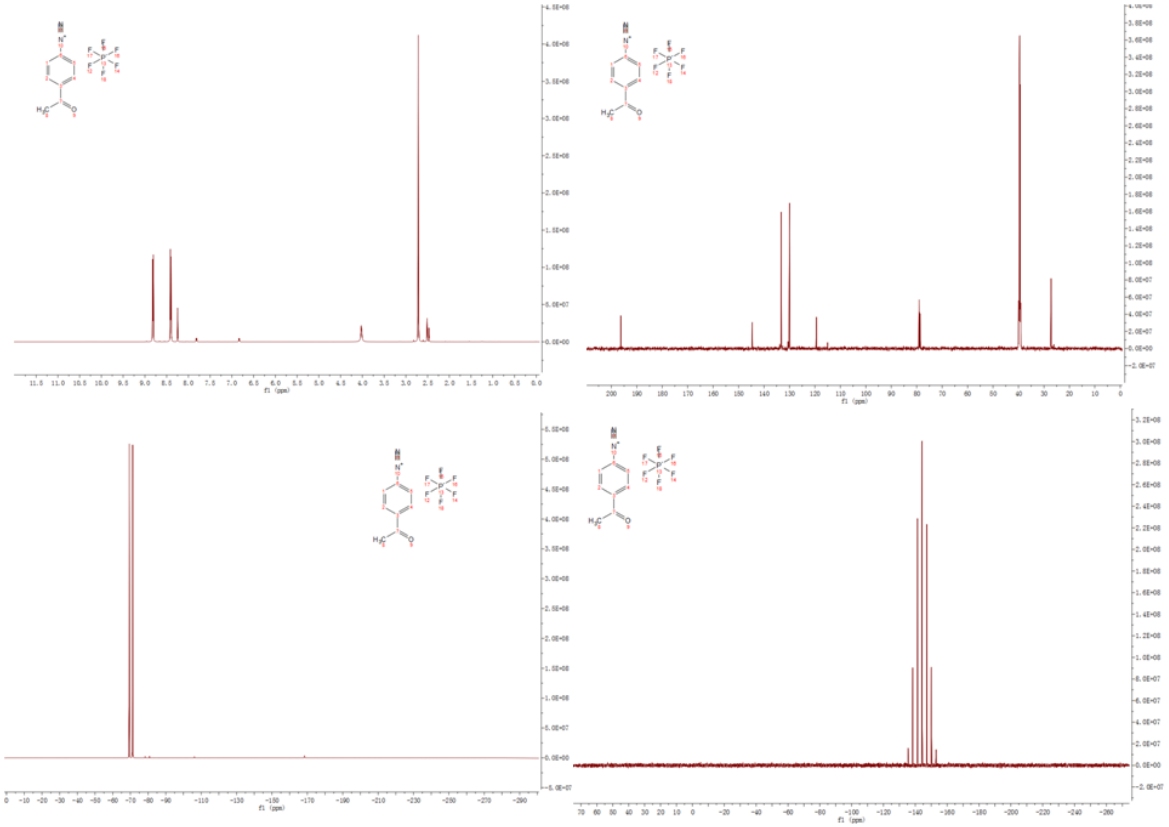


Fig. S10 ^{1}H-, ^{13}C-, ^{19}F-, and ^{31}P-NMR spectra of **1**.

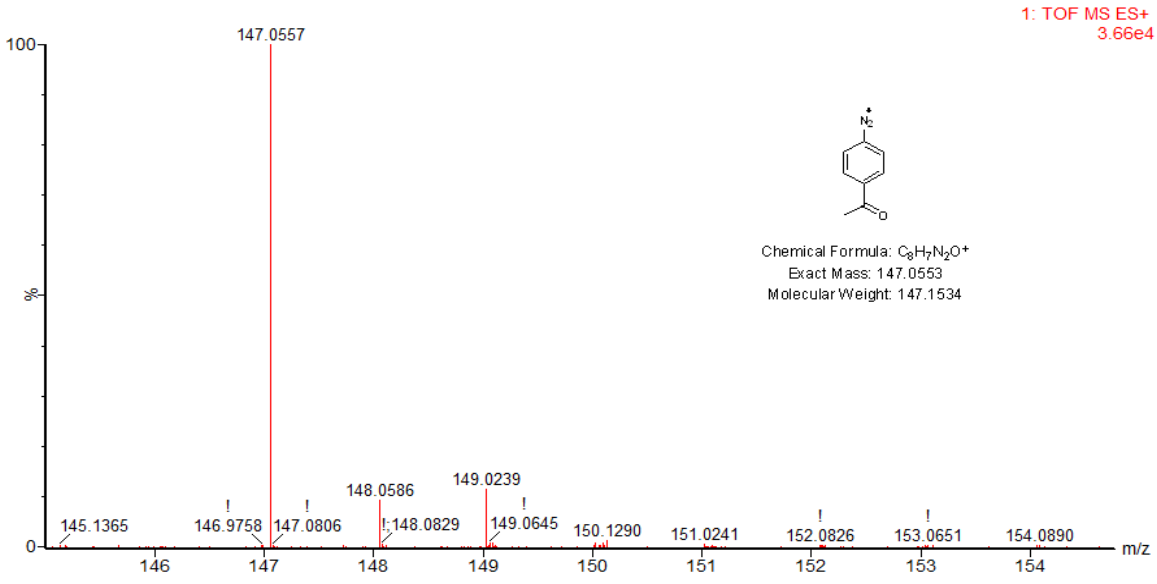


Fig. S11 HRMS spectra of **1**.

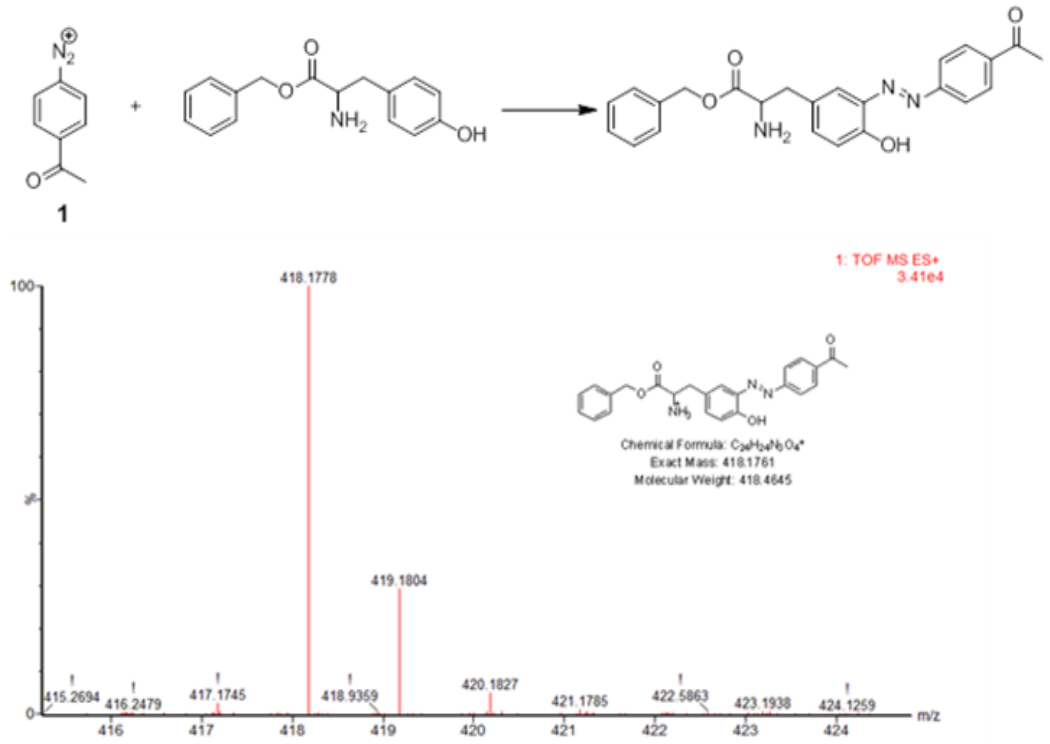


Fig. S12 The reaction of **1** with Tyr-containing small molecule and the HRMS spectra of the reaction product. 10 mM **1** and 10 mM Tyr-containing small molecule were incubated in 50 mM PBS (pH 8.0, containing 30% CH_3CN) for 1 h at room temperature. Then the reaction mixture was submitted into ESI-MS without purification.

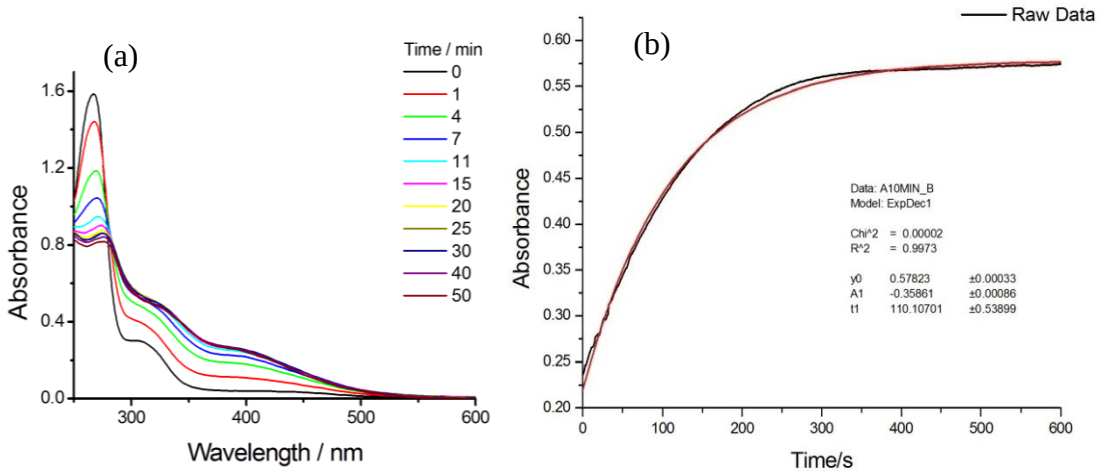


Fig. S13 (a) Time-dependent UV-Vis absorbance spectra of **1** (100 μM) upon treatment with Tyr-containing small molecule (100 μM) in 50 mM PBS at pH 7.0, containing 30% CH_3CN) at room temperature. The different reaction time is indicated inset. (b) The absorbance at 400 nm versus reaction time of **1** (100 μM) upon treatment with Tyr-containing small molecule (1 mM) in PBS (50 mM, pH 7.0) at room temperature. The red line represents the best fitting with single-exponential function.

6. One-pot surface thiolylation of TMV

To achieve the thiolylation on the surface of TMV, a tyrosine-specific linker **1** (1 mM) was introduced onto the TMV-like particle (5 mg/mL) at moderate condition (pH 7.0), with ketone group left on the other end. The ketone group could react with the amino group of cysteamine (1 mM) specifically to form C=N bonds, which were *in situ* reduced by $NaBH_3CN$. The cysteamine provided the free thiol group for the subsequent Michael addition-based fluorescent labelling or thiol-based mineralization.

Before the post-labelling of TMV-SH, the suspension was placed in a centrifugal filtration device (3K, Millipore) and eluted by 0.1 M phosphate buffer (pH 7.0) to remove excess interfering molecules. This procedure did not damage the TMV-like rod structure. The thiol-labelled dye CPM (1 mM) was incubated with the TMV-SH suspension at 37 ℃ for 30 min. After removing small molecules, the resulted solution was electrophored in 1% agarose gel in TBE buffer at 100 V for 2 hours. The agarose gel was firstly imaged under the UV light to detect whether the TMV-like particle was labelled. After the fluorescence imaging, the agarose gel was stained by Coomassie brilliant blue for 1 hour and destained by the glacial acetic acid/alcohol/water mixture (1:1:8). The TMV-like nanoparticle bands were visualized and imaged by the Gel Doc XRi instructment (Bio-Rad) and processed with the Quantity One software.

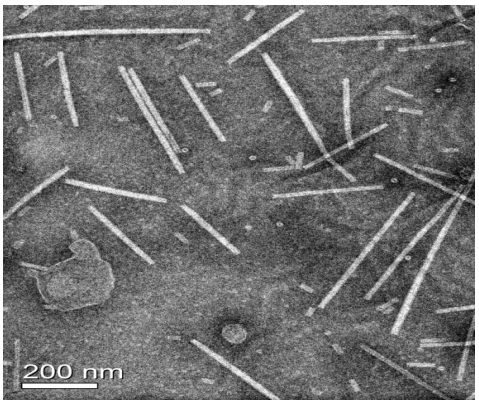


Fig. S14 TEM image of the thiol-labelled TMV (TMV-SH). TMV-SH was ultrafiltrated at 13,000 rpm to remove excess small molecules for subsequent post-labelling process. The ultrafiltrated TMV-SH rods were imaged by TEM for rod completeness analysis.

7. Surface coating of virus-like assemblies

A suspension of TMV-like assemblies was thiolylated according to the methods as described above. The resulted nanoparticles provided 1D template for the subsequent post-modification on the surface for Au and CdS mineralization. 1 mg/mL TMV-like rods was incubated in a MES bufffer (0.1 M, pH 7.0) with 10 mM $CdCl_2$ for 6 hours, followed by mounting on the parafilm-covered glass plate in a sealed container. H_2S was controllably produced to slowly diffuse into each droplet on the parafilm for 1 hour until the light yellow color appeared. The droplet was transferred onto the copper grids for enough binding. For thiol-labelled TMV-like rods, 1 mg/mL suspension was incubated in a buffer with 10 mM $CdCl_2$ for 30 min, followed by H_2S treatment as described above. To achieve the gold plating on the TMV surface, 1 mM chloroauric acid ($HAuCl_4$) was incubated with 1 mg/mL thiol-labelled suspension for 30 min. The coated nanorods were used directly for TEM images without negatively staining.

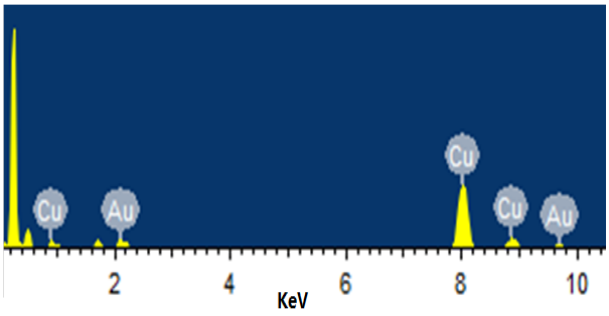


Fig. S15 The energy spectrum of 300 nm Au-coated particles.

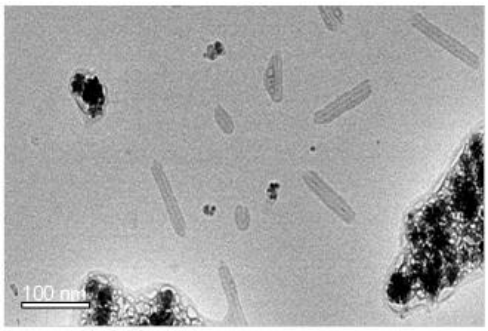


Fig. S16 TEM image of 100 nm CdS-coating TMV-like assembly. The TMV-like assembly without thiol-labelling was firstly incubated with $CdCl_2$ (10 mM) for 6 hours, followed by slow H_2S diffusion to coating CdS precipitate on the surface. The precipitate was transferred into the copper grid for TEM analysis.

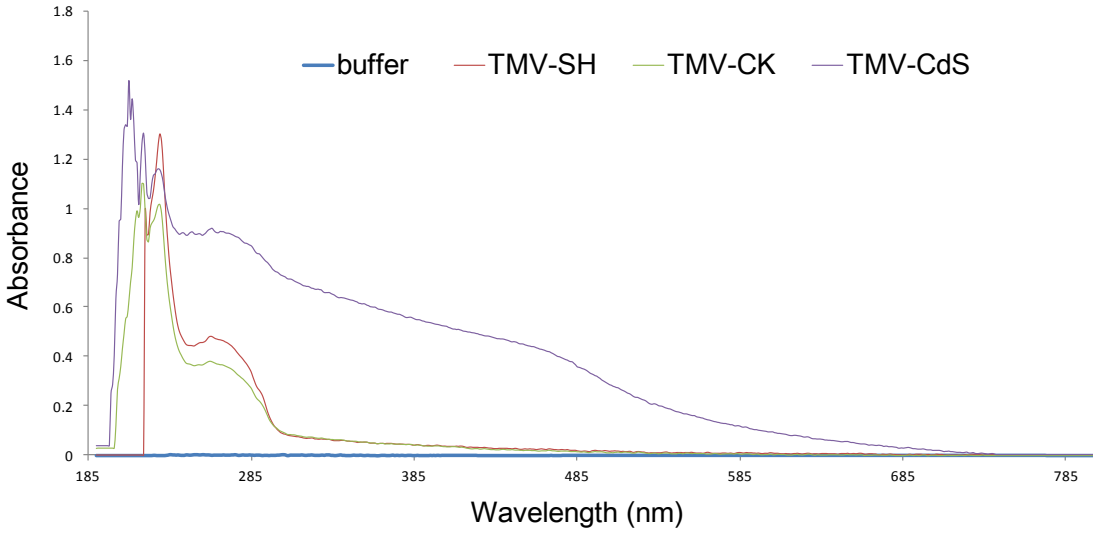


Fig. S17 UV-Vis spectra of 300 nm CdS-coated nanorods. The 300 nm TMV nanorods with thiol-labelling (TMV-SH, 5 mg/ml) was firstly incubated with $CdCl_2$ (10 mM) for 1 hour, followed by slow H_2S diffusion to form CdS-coated TMV nanorods. The control included buffer, intact TMV (TMV-CK) and TMV-SH.

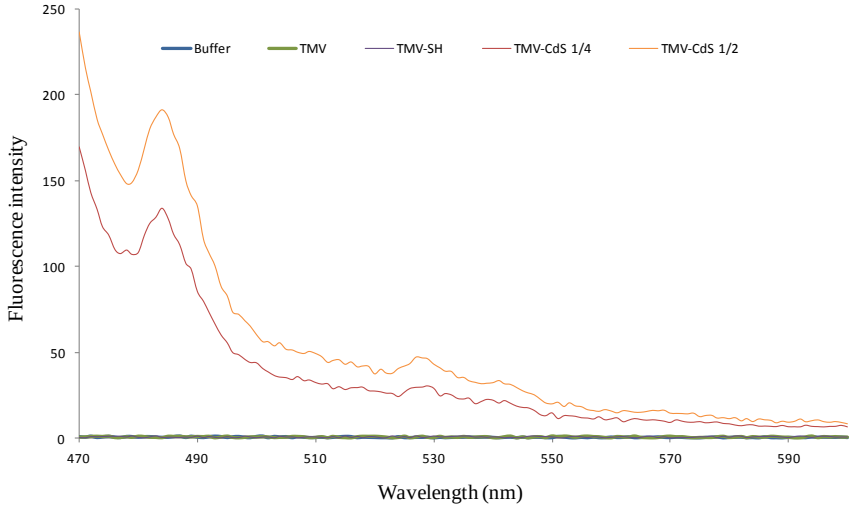


Fig. S18 Fluorescence spectra of 300 nm CdS-coated TMV nanorods under 460 nm excitation. The concentration of nanorods suspension was 10 mg/ml (TMV-CdS 1/2) and 5 mg/ml (TMV-CdS 1/4). Buffer, TMV and TMV-SH were set as the negative control.