

DNA-templated CMV Viral Coat Protein Assemble Into Nanotubes

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Preparation of 500bp double-stranded DNA:

The 500 bp double-stranded DNA was amplified from the template of pBR322 (Takara Biotechnology, Dalian) plasmid by Polymerase Chain Reaction with the forward primer: 5'-CCC TTA TGT TAC GTC CTG-3' and the reverse primer: 5'-TGG TGT AGA GCA TTA CGC-3'. The amplification was carried out using the following thermal cycling profile: 94°C for 3 min, followed by 30 cycles of amplification (94°C for 30 s, 55°C for 30 s, 72°C for 30 s). Finally, the reaction was maintained at 72°C for 2 min. The PCR products were purified using DNA fragment purification kit. The pBR plasmid was bought from Takara Biotechnology Co. Ltd. (Dalian, China); the primers were synthesized by Saibaisheng Biocompany (Beijing, China); the DNA purification kit was purchased from Tiangen Biocompany (Beijing, China).

Fluorescent microscopy image of FAM-labeled DNA directed CP assembly

FAM (6-carboxyfluorescein) labeled DNA sequence: 5'
FAM-GGGTTGCCCCGGTCCTTTTTTAAACCCCCCCCCAAGGAAGGGAAAAA
AAA-3' was annealed with its complementary strand and applied into the normal
assembly process (500 bp DNA as starting material, as described in manuscript). As
the assembled structure is longer than micrometer, its morphology could be observed
on Fluorescent Microscopy (Leica DMI 6000). As shown in Figure. S1, similar
images has been observed with bright field mode and fluorescent mode (excited at
494 nm and detected at 518 nm). This result demonstrated that the assembly of CP is
based on the DNA templates.

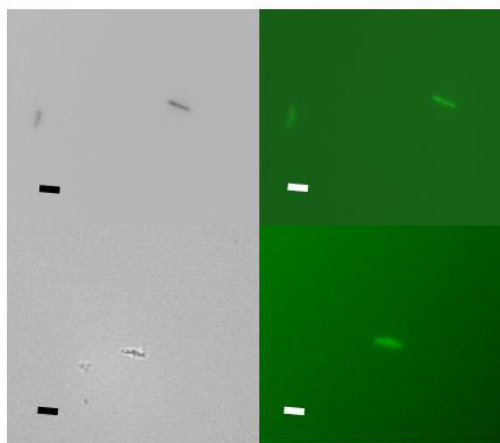


Figure S1. Represented optical (left) and the corresponding fluorescence (right) images of CP assembly templated by FAM-labeled DNA. The scale bars are 2 μ m.

CMV CPs observed under Transmission Electron Microscopy (TEM):

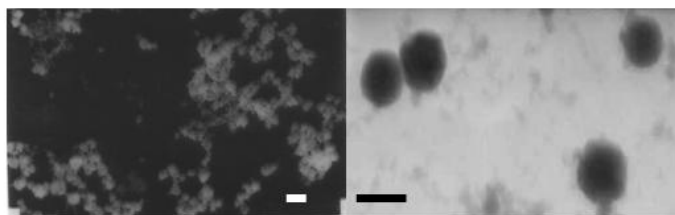


Figure S2. TEM images of pure CMV CPs. The scale bar is 20 nm (left) and 50 nm (right).

The CMV CPs was applied to the assembly process without DNA (20 mM Tris-HCl, 150 mM NaCl, pH 7.5). The mixture was then applied onto copper grids coated with amorphous carbon film and then negatively stained with 2% uranyl acetate. As shown in Figure S2, there is no tubular or fibril structures observed. Without the DNA template, the CPs can self-assemble into virus-shaped nanoparticles.

TEM images of the nanotubes formed at a DNA/CP ratio of 1:1

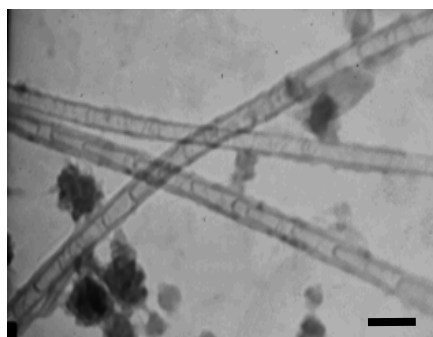


Figure S3. TEM images of nanotubes formed by DNA/CP=1:1. Scale bar is 50 nm. Conditions are same as Figure 2 but with the ratio of one DNA base pair to one protein. As shown in Figure S3, there are assembled nanotubes, the small particles accompanied could be the virus-shaped nanoparticles self-assembled by superfluous CPs.

CMV CP expression and purification protocol:

Buffers used are as follows:

solution buffer (500 ml): 50 mM TrisHCl (3.94 g) pH 8.0, 25% Sucrose (125 g), 1 mM NaEDTA (1ml stock solution [0.5 M]), 0.1% NaAzide (1.5 ml stock solution [30%]), 10 mM DTT (770 mg);

lysis buffer (500 ml): 50 mM TrisHCl (3.94 g) pH 8.0, 1% Triton X-100 (5 ml), 0.1% Na deoxycholate (5 ml stock solution [10%]), 100 mM NaCl (2.93 g), 0.1% NaAzide (1.5 ml stock solution [30%]), 10 mM DTT (770 mg);

washing buffer: 50 mM Tris HCl (3.94 g) pH 8.0 with 0.5% Triton X-100 (2.5 ml), 100 mM NaCl (2.93 g), 1 mM NaEDTA (1 ml stock solution [0.5 M]), 0.1% NaAzide (1.5 ml stock solution [30%]), 1 mM DTT (77 mg).

Expression:

Full-length CP gene of 218 amino acid residues (GenBank ID AB008777) of CMV was PCR-amplified by the forward primer: 5'-AACCATGGACAAATCTGAATCAACC-3' and the reverse primer: 5'-TTGGATCCTCAAACCTGGGAGCACCCCAGACGTGGG-3'. The PCR products were gel-purified, restricted enzyme digested with NcoI and BamHI, and ligated with NcoI and BamHI restricted pET11d (Novagen, USA). *E. coli* BL21 (DE3) Rosseta (Novagen) was used as a host strain for the production of recombinant protein. Cultures were grown at 37°C by inoculating a 5 ml overnight culture into 2 L LB medium containing ampicillin (10 mg/L). Incubation was continued until the culture reached an OD600 of between 0.4 and 0.6, at which point expression was induced by

the addition of 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG). After 4 h incubation, cells were harvested by centrifugation at 4000g for 10 min. The cells pellets were then stored at -20°C .

Purification of inclusion body:

All purification steps were performed on ice or at 4°C . The pellets (crude inclusion body) were re-suspended in solution buffer and then homogenized by sonication, followed by lysis buffer. The protein products were washed by centrifugation for 3 times by washing buffer, each for 20 min at 11,000g.

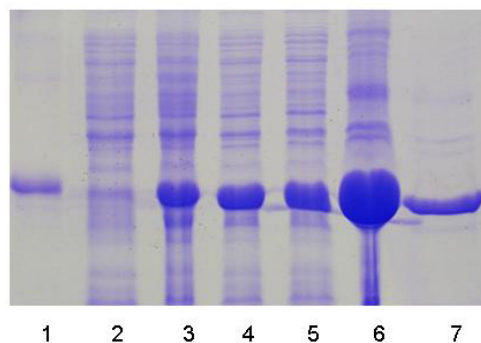


Figure S4. Recombinant CMV CP induced-expression and inclusion body purification in *E. coli*. Lane 1: 29Kda pre-stained protein marker; lane 2: uninduced and lane 3: IPTG induced total bacterial proteins; lane 4: crude inclusion protein; lane 5 and 6 are the inclusion body protein after washing; lane 7: the final washed inclusion body.

Protein refolding process:

Buffers used are as follows:

refolding buffer (250 ml): 100 mM Tris-HCl (3.925 g) pH 8.0, 400 mM L-arginine-HCl (21 g), 2 mM NaEDTA (1 ml stock solution [0.5 M]), 0.5 mM oxid. glutathione (0.077 g), 5 mM red glutathione (0.385 g);

guanidine solution (100 ml): 3M guanidine-HCl (28.7 g); [SIGMA, G-4630] pH 4.2, 10 mM sodium acetate (0.082 g) keep at room temperature, 10 mM NaEDTA (2 ml stock: 0.5 M);

Protease inhibitors (stock solutions): PMSF, 100 mM (174.2 mg in 10 ml 2-propanol, stored at RT); [SIGMA P-7626], Pepstatin, 2 mg/ml (5 mg in 2.5 ml DMSO, aliquots stored at -20°C); [SIGMA P-4265]; Leupeptin, 2 mg/ml (5 mg in 2.5 ml deionized H₂O, aliquots stored at - 20°C);

Exchange buffer (500ml): 20 mM Tris HCl (1.57 g) pH 8.0, 50 mM NaCl (1.46 g).

Procedure:

With the above mentioned process, a quantity of CP inclusion bodies was achieved. In an effort to obtain soluble and re-natured proteins, the CP inclusion bodies were solved in 2 M urea solution and then guanidine solution was added into. 2 ml 100 mM PMSF, 100 µl 2 mg/ml pepstatin and 100 µl 2 mg/ml leupeptin were mixed with 200 ml refolding buffer. The protein solution was injected into above prepared refolding buffer which was circumgyrating with high speed. The refolding process was then carried out for 8h at 4°C with lower circumgyrating speed. To achieve higher concentrations, the refolded protein solution was ultra-filtered using Ultrafree-CL

(10,000 MW) filters (Millipore). Washing with 10 times diluted exchanger buffer, and finally solved in the exchange buffer to a final concentration at 50 $\mu\text{g}/\text{ml}$.

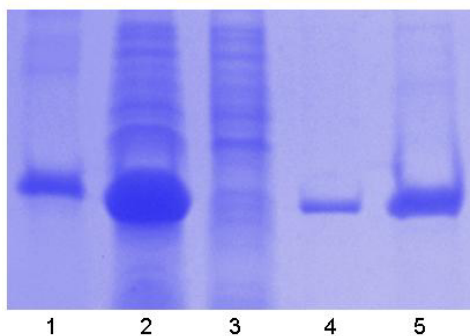


Figure S5. Re-natured CMV CP Lane 1: 29Kda pre-stained protein marker; lane 2: induced CP; lane 3: un-induced CP; lane 4: re-natured CP; lane 5: higher concentration CP in exchanger buffer (final assembly buffer)