Supporting Information

Scaffold properties are a key determinant of the size and shape of self-assembled virus-derived particles

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Methods

VLPs production. SV40 VP1 VLPs were produced in *Spodoptera frugiperda* (Sf9) insect cell and nuclear extract containing VLPs were harvested as previously described (*1*). VLPs were purified by equilibrium sedimentation in CsCl gradient.

Nucleic acids production. We used the following nucleic acids in the assembly reactions: Supercoiled circular dsDNA were plasmids minicircle (2.4 kbp) and pGL3-control (5.2 kbp) purified from an agarose gel using Qiagen Qiaquick gel extraction kit. Longer RNA substrates were prepared by *in vitro* transcription using Ambion MegaScript T7 and SP6 kits. Linearized pCC1 plasmid was used as a template for 3200 nt CCMV RNA1, pBR322 and pBR322-GFP plasmids for 8000 and 11000 nt RNAs, and Xenopus elongation factor DNA for 1900 nt RNA. 3200 nt ssDNA was prepared from the bacteriophage M13 truncation mutant, DH11SF1SK+ bluescript, with VCSM13 helper phage. F^+ *E. coli* were infected with phage at a moi of 0.001 and incubated for 24 hr. Progeny phage was isolated from media. Bacteria were centrifuged at 5000 g for 10 minutes. Phage was precipitated by addition of NaCl to 0.2 M and PEG8000 to 4% followed by a 15 minute incubation and centrifugation at 6,000 g for 10 minutes. The resulting pellet was brought up in TE buffer and the ssDNA isolated by phenol:chloroform extraction.

Dissociation of VLPs. For assembly experiments, pentamers were obtained by dissociating VLPs by two cycles of dialysis, each 1.5 hours, first against 20 mM Tris-Cl, pH 8.9, 50 mM NaCl, 2 mM DTT, 5 mM EDTA at 4 ºC followed by dialysis against 20 mM Tris-Cl, pH 8.9, 50 mM NaCl, 2 mM DTT, 2 mM EDTA at 4 ºC. The dialysate was centrifuged at 13,000 rpm for 30 minutes at 4 ºC to sediment aggregated protein. Clarified supernatant was quantified by UV

absorbance using an extinction coefficient of e_{280} = 32890 $M^{-1}cm^{-1}$ per VP1 monomer. This extinction coefficient was based on amino acids composition (*2*) .

Assembly reaction. Disassembled VP1 pentamers were mixed with assembly buffer x2 (300 mM NaCl, 100 mM MOPS pH 7.2, nucleic acid at appropriate concentration). Reactions were incubated at room temperature (20 °C) for 15 min.

Sucrose gradients. Samples of assembled SV40 (260 µl) were loaded on a ~13ml 10-60% linear sucrose gradient in assembly buffer; gradients were prepared using a gradient master. Gradients were centrifuged for 2 hours at 40,000 rpm in a Beckman SW40 swinging bucket rotor. Discrete bands of nucleoprotein complex scattered light when illuminated the centrifuge tube was illuminated from the bottom. Bands were extracted by side puncture with a syringe.

Analysis of nucleoprotein complexes. For visualization of samples by transmission electron microscopy (TEM), samples were adsorbed to freshly glow discharged formvar-carbon coated copper grid for 3 minutes and stained with 2% uranyl acetate. Samples were visualized on JEOL JEM-1010 transmission electron microscope equipped with CCD camera.

Size exclusion chromatography was performed using Bio SEC-5 columns (7-ml and 14-ml) with a 500Å pore (Agilent Technologies) connected to a Shimadzu HPLC. Reaction mixtures were injected onto columns pre-equilibrated in assembly buffer (20mM MOPS, pH 7.2 150mM NaCl). Peaks were observed by absorbance using a diode array detector, and integrated using Shimadzu software.

SEC-MALLS experiments were conducted using Bio SEC-5 column in line with a Wyatt DAWN HELEOS II light scattering detector and a Wyatt Optilab Rex refractive index detector. The system was calibrated using 65 kDa Bovine Serum Albumin (Sigma). Capsid samples of 25-50 μL, at 1 μg μL^{-1} protein concentration, were resolved by SEC at a flow rate of 0.3 mL min^{-1}. The SEC-MALLS system was pre-equilibrated in 150 mM NaCl, 50 mM MOPS pH 7.2. Weight-averaged molecular mass and radius determinations were calculated using ASTRA software (Wyatt).

Cryo-electron microscopy. The specimens for cryo-EM were frozen in vitrified ice and imaged by the well-established procedures. Very briefly, a 3.5 μl drop of reassembled SV40 VP1 on 1900 nt RNA was applied on a glow-discharged Quantifoil® holey-carbon grid (R2/2), blotted with filter paper for 4 s, and plunged into a cryo-container containing liquid ethane cooled by liquid nitrogen. The process described above was performed by FEI Vitrobot™. The frozen specimen was then transferred to a Gatan 626DH cryo-holder and kept at a low temperature environment (≤ -176 ºC) for subsequent processing. Low-dose images (≤ 20 e^{-} $Å^{-2}$) were collected on a Gatan UltraScan™ 4000 4k x 4k CCD camera at a nominal magnification of x60,000 (equal to a pixel size of 0.18 nm) by a JEOL-3200FS EM operated at 300 kV with an in-column energy filter slit adjusted to 20-eV. Images were taken at multiple defocuses to compensate for the effect from the contrast transfer function of the EM.

Image processing. Images were visually inspected and selected based on the criteria of suitable particle concentration, optimal ice thickness, and minimal specimen drift. Only those images that fulfilled these standards were analyzed. A total of 4386 particles were semi-

automatically boxed from 254 images using e2boxer.py.(*3*) An *ab initio* random model method was used to generate an initial model using 150 particles. The initial model clearly showed a *T*=1 surface lattice comprised of 12 pentamers.(*4*) The origins and orientations searches were carried out iteratively using AUTO3DEM (v4.02) until a stable 3-D reconstruction had been achieved.(*5*) Only the phase information of the contrast transfer function was corrected. A final 3-D reconstruction was obtained from 3511 particles at the resolution of 7.2 Å using a Fourier shell correlation cutoff of 0.5 as the criteria. The model was visualized and analyzed in Chimera.(*6*)

Modeling atomic coordinates. The initial structural modeling used the crystal structure from *T*=7 SV40 particle (PDB entry 1SVA). The atomic coordinates contain 6 VP1 structures: 5 VP1 from hexavalent pentamer and 1 VP1 from pentavalent pentamer. The VP1 from pentavalent pentamer was symmetrized to generate the atomic model of pentavalent pentamer, which was subsequently used to fit into the cryo-EM density map of *T*=1 SV40. Initially, the pentamer was rigidly and manually fit into the related position using cryo-EM density as guidance, and was further refined based on the cross correlation between the atomic model and the cryo-EM density map. Once the optimized result was achieved, icosahedral symmetry was imposed on 1 VP1 subunit to generate the pseudo-atomic model for *T*=1 particle. To analyze the pentamer-pentamer interaction, we broke the atomic model between residues Tyr^{299} and Pro^{300}, treated the C-terminal arm (residues Pro^{300}-Gln^{355}) as one rigid body, and used the short helix (Ile^{301}-Arg^{312}) as a register to model the structure.

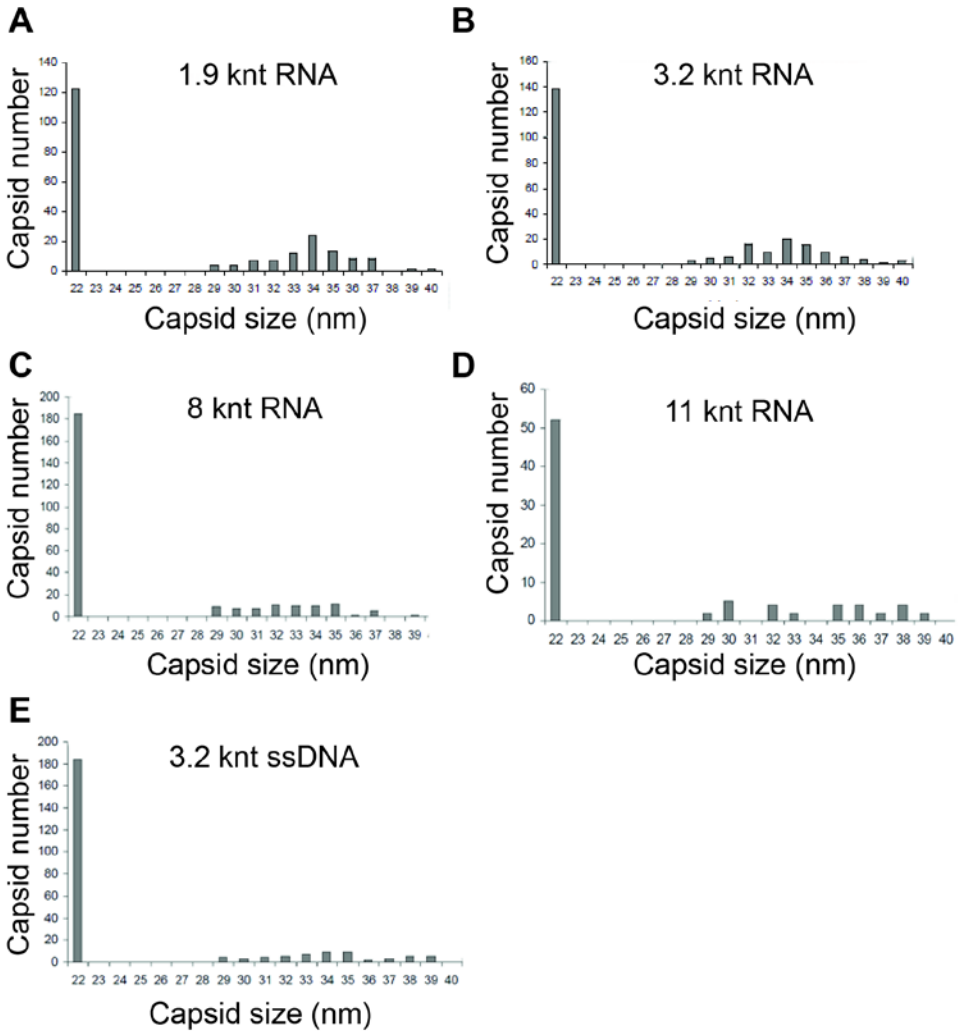

Figure S1. Sizes distributions for capsids assembled on ssRNA from 1.9 to 11 knt. Assembly products were visualized under TEM, the capsids were counted, and their sizes were measured. Assembly on 1.9 and 3.2 knt RNA resulted in ~58% of uniform 22 nm capsids and 42% of heterogeneous particles, mostly 32-37 nm (A and B) with a peak of particle number at 34 nm. Assembly on longer RNA, 8 and 11 knt, also resulted in the mixture of heterogeneous capsids, with similar distribution of large heterogeneous particles (C and D), however the proportion of the uniform 22 nm particles was higher, closer to 70%. Remarkably, assembly on 3.2 knt ssDNA had sizes distribution similar to long RNA, with ~70% of 22 nm particles.

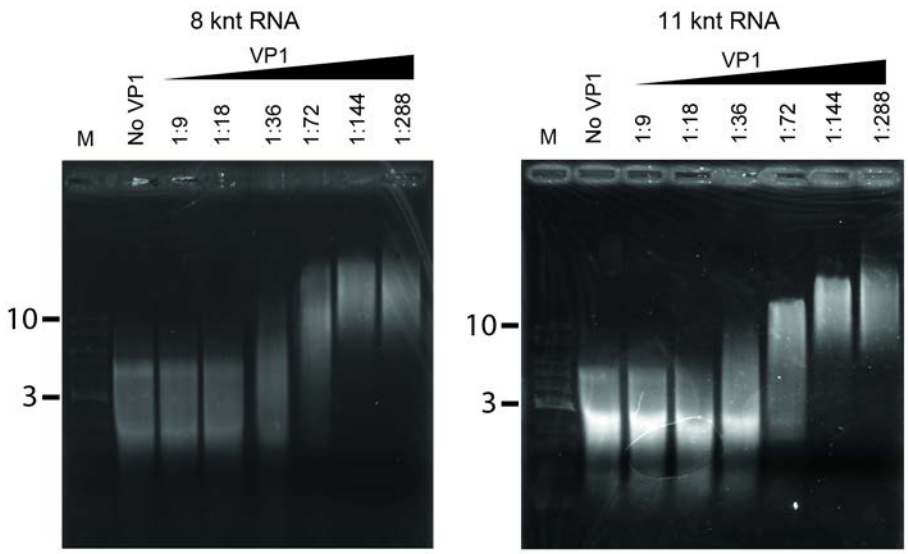

Figure S2. EMSA results of 8 and 11 knt RNA. EMSA analysis showing titration of 8 and 11 knt RNA substrates with increasing molar ratios of VP1. The reaction products were analyzed on 0.6% agarose gels and stained with EtdBr.

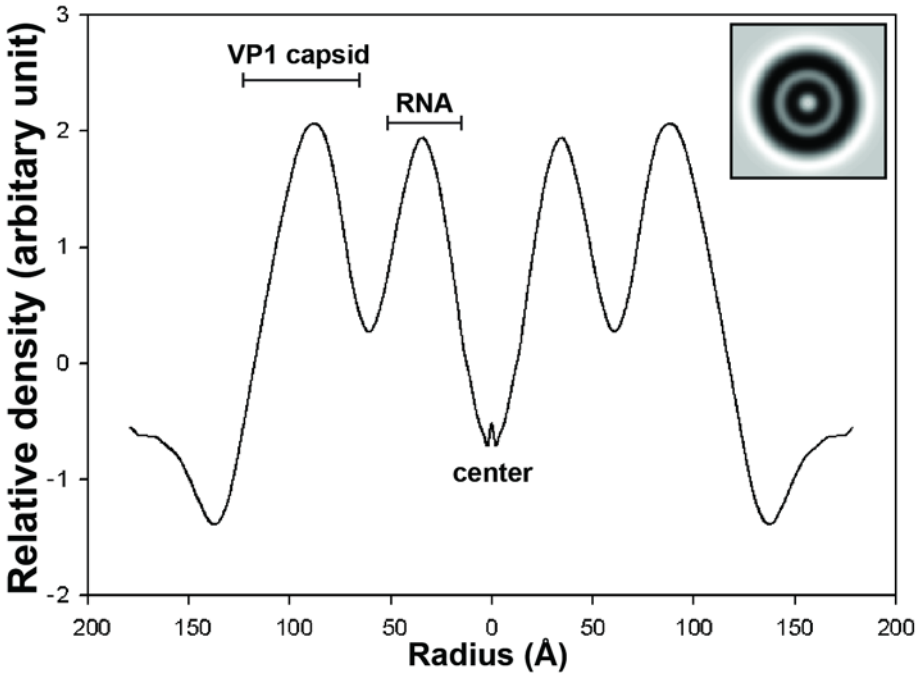

Figure S3. One-dimensional radial density profile of a rotationally averaged particle calculated from the translationally aligned images. Inset shows the rotationally, translationally averaged image. Note that the density between capsid and RNA shells is higher than the center.

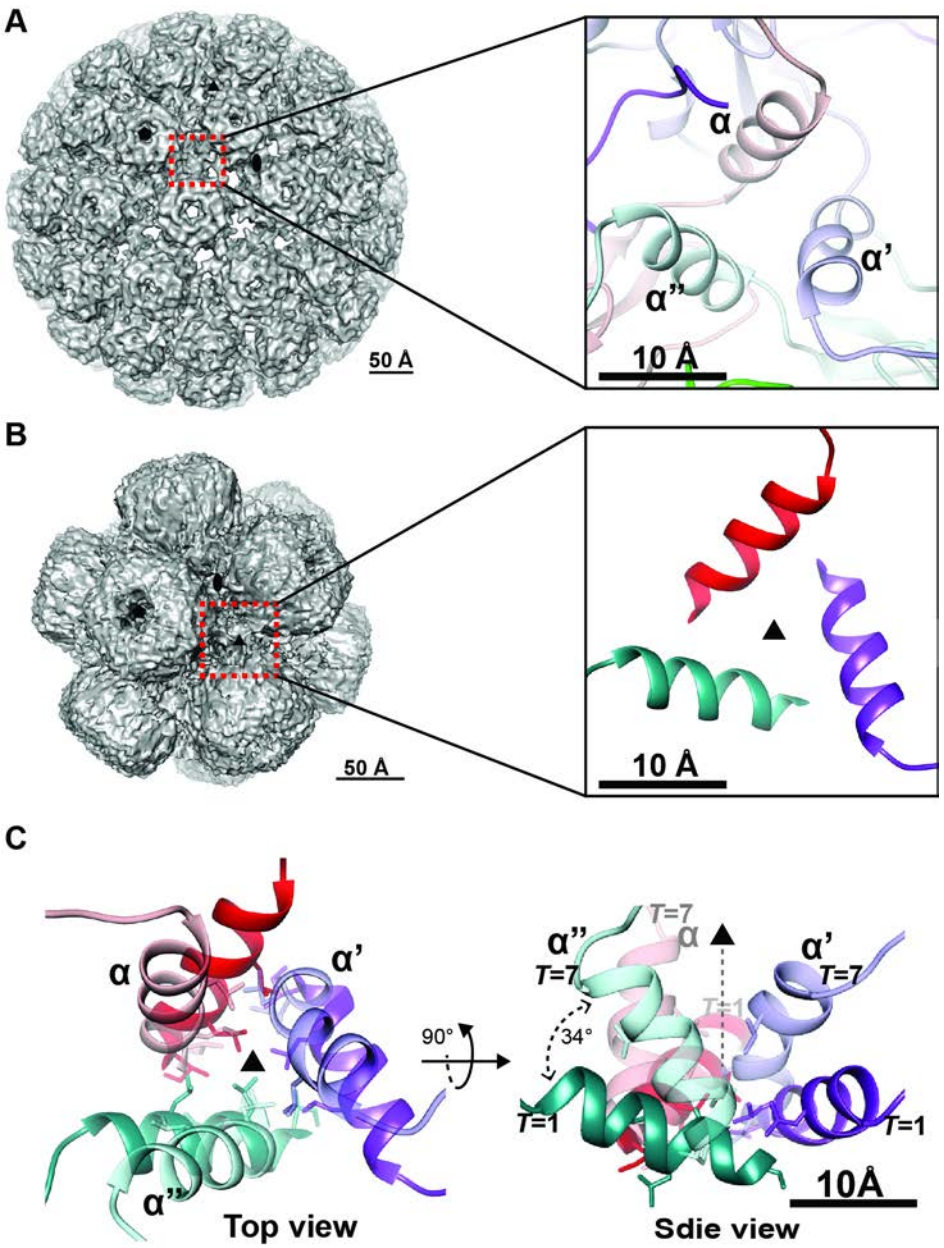

Figure S4. Comparison of inter-pentameric interactions between *T*=1 to *T*=7 SV40 particles. (A) Isosuface rendering of *T*=7 SV-40 particle generated from low-pass filtered the atomic structure (PDB 1SVA) to 10Å resolution. (B) Cryo-EM 3-D reconstruction of *T*=1 SV-40 particle. Areas used for the close-up views are marked by the rectangle. Oval, triangle, and pentagon indicate locations of twofold, threefold and fivefold axes, respectively. (C) Superimposition of α- α'-α" interaction from *T*=7 particle on the inter-pentameric interaction at

threefold location of the *T*=1 particle. Light color denotes C-arms from *T*=7 particle while dark color denotes *T*=1 particle. Note that in both cases the center maintains the hydrophobic interaction from the peripheral C-arms.

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