Published in final edited form as:

ACS Nano. 2018 June 26; 12(6): 5323–5332. doi:10.1021/acsnano.8b00069.

Defects and Chirality in the Nanoparticle-Directed Assembly of Spherocylindrical Shells of Virus Coat Proteins

Cheng Zeng^{†,¶}, Guillermo Rodriguez Lázaro[‡], Irina B. Tsvetkova[†], Michael F. Hagan[‡], and Bogdan G. Dragnea[†]

[†]Department of Chemistry, Indiana University, Bloomington, IN 47405, U.S.

[‡]Department of Physics, Brandeis University, Waltham, MA 02453, U.S.

Abstract

Virus coat proteins of small isometric plant viruses readily assemble into symmetric, icosahedral cages encapsulating non-cognate cargo, provided the cargo meets a minimal set of chemical and physical requirements. While this capability has been intensely explored for certain virus-enabled nanotechnologies, additional applications require lower symmetry than that of an icosahedron. Here we show that the coat proteins of an icosahedral virus can efficiently assemble around metal nanorods into spherocylindrical closed shells with hexagonally close-packed bodies and icosahedral caps. Comparison of chiral angles and packing defects observed by *in situ* atomic force microscopy with those obtained from molecular dynamics models offers insight into the mechanism of growth, and the influence of stresses associated with intrinsic curvature and assembly pathways.

Keywords

virus-like particles; chirality; defects; packing; nanoparticle-directed assembly

The ability to place chemical, photonic, magnetic, or electronic subunits in deterministic fashion and with molecular accuracy is a long-standing goal of nanotechnology, and an enabling factor towards property control in scale-dependent materials. In recent years, a broad selection of viruses has provided excitement and inspiration from this perspective, as nature's multifunctional nanomaterials.^{1–11} Viruses achieve astonishing architectural accuracy *via* rapid, highly efficient self-assembly. Thus, using virus shell templating to construct arrays of coupled elements is appealing, especially when the desired effect is sensitive to the distance between active elements and when the coupling range is limited to $\lesssim 1$ nm to a few tens of nm. For example, researchers have used two different rod-like viruses to explore energy transfer in chromophore arrays organized on virus templates which mimic

hagan@brandeis.edu, dragnea@indiana.edu, Phone: +123 (0)123 4445556., Fax: +123 (0)123 4445557.

[¶]Current address: SEAS, Harvard University

Supporting Information Available

Definition of Gaussian curvature and mean curvature. Histogram of AFM measured ring defect locations relative to the proximal VLP end. The classes of assembly morphologies observed in simulations for assembly around long nanorods and fraction of assembly outcomes as a function of nanorod radius for three different capsomer preferred curvature values. This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

light harvesting complexes.^{12,13} Other examples include dipole-dipole coupling effects in magnetic¹⁴ and biophotonic¹⁵ virus-like particles.

The effect of symmetry on the density of states of collective excitations provides an exciting opportunity for molecular nanomaterials. Symmetry control allows harnessing coherence in collective excitations, and thereby accessing unique and interesting properties.^{16,17} However, while it is known that virus coat proteins are sufficiently flexible to accommodate the different local symmetry environments within a polyhedral shell,^{18,19} it is unclear if the proteins of, say, an icosahedral virus, could form defect-free polyhedral packings of reduced symmetry. Indications that this might be the case come from early experiments with the brome mosaic virus (BMV), a small, single-stranded RNA, icosahedral plant virus, whose coat protein was observed to encapsulate calf-thymus DNA into bacilliform particles.²⁰ More recently, tubular sheets formed from the coat protein of cowpea chlorotic mottle virus (CCMV) have also been observed upon assembly on stiff, double-stranded DNA.²¹ The phase diagram of pure (template-free) CCMV coat protein assemblies was shown to comprise a variety of polymorphs, depending on ionic strength and pH, including tubular structures.²² However, the details of protein packing in these complexes remain unknown. Coarse-grained molecular dynamics simulations have suggested that the coat proteins of icosahedral virus shells could form spherocylindrical particles, albeit under non-optimal conditions of temperature or protein concentration during assembly.²³ Many insightful theoretical and computational studies have modeled assembly on spherical templates,^{24–35} and Monte Carlo simulations of packings of hard, attractive spheres on a prolate spheroid surface were shown to recapitulate a few of the structures found in elongated virus capsids.³⁶ However, experiments have yet to identify conditions in which proteins designed to inhabit a space with Gaussian curvature (see Supporting Information for definition) pack into closed shells of lower symmetry, and locally zero Gaussian curvature.

Here we show that the BMV coat protein can be efficiently directed by metal nanorod templates to assemble into stable spherocylindrical shells. These elongated shells have icosahedral caps and cylindrical sides. The template-directed approach allows for the observation of features of molecular packing by *in situ* atomic force microscopy, including chirality and the presence of defects as a function of aspect ratio. Coarse-grained simulations recapitulate many of the experimental observations and indicate that the interplay between the preferred curvature of the subunit and inhomogeneous curvature of the nanorod template plays a key role in determining the mechanism of growth, the presence of packing defects, and chirality. However, there are differences between experimental results and computational predictions for the relationship between complete shells and nanorod dimensions and chiral angles. Based on these differences, we discuss features of BMV proteins that may play a key role in the assembly process.

Results and discussion

When solution conditions are favorable, virus assembly occurs through spontaneous association of free proteins and nucleic acid, followed by organization into a final proteinaceous shell of precise stoichiometry and symmetry encapsulating the genome.³⁷ In many instances, the process can be recapitulated *in vitro* with non-cognate nucleic acid,²⁰

linear and branched polymers,³⁸ and even droplets³⁹ and solid nanoparticles⁴⁰ as cargo, provided interactions between cargo and shell are modeled after the dominant interactions in wild-type viruses.^{33,41,42}

Since metal nanorods (NRs) are a type of nanoparticle of significant interest for plasmonics, there is a significant body of work concerning their synthesis and synthetic control of length and aspect ratio.⁴³ Nanorod-directed virus-like particle (VLP) assembly has not been explored until now. For this study, we have adopted the NR preparation protocol by Ye *et al.*,⁴⁴ which allows tuning the nanorod length while roughly keeping the diameter of the cylindrical part commensurate with the luminal cavity in native BMV (~8 nm radius for a T=3 shell). We have explored the assembly of BMV coat protein shells around Au nanorods coated with a monolayer of carboxylated triethylene glycol (TEG), which provides the required charge for neutralization and anchoring of the flexible N-termini of the protein subunits onto the template surface.⁴⁵ We have tested two types of nanorods of different sizes: “short” NRs (9.5±0.7 nm wide and 29.6±3.8 nm long), and “long” NRs (14.9±3.5 nm wide and 56.4±5.8 nm long), Fig 1.

We obtain high assembly efficiency for spherocylindrical VLPs, as previously observed for spherical nanoparticle-templated spherical VLPs, albeit at different protein concentrations (see Methods). Inspection of TEM micrographs indicates that the coat proteins form monolayers of the same approximate thickness (~5 nm) as the shell of wtBMV. However, unlike spherical VLPs, spherocylindrical VLPs sometimes exhibit defects in their shells, which in projection TEM micrographs appear as vacancy defects, Figs. 2 (A) and 3.

We have investigated the frequency of vacancy occurrence as a function of NR dimensions. Fig. 2 (B) shows a map of occurrence of apparent defect-free and vacancy-carrying particles generated from width vs length bivariate histograms. The map shows qualitatively different behavior for short and long nanorods. For short nanorods we observe separation of defect-free from vacancy-carrying particles based on nanorod length, while long nanorods exhibit overlapping distributions of defect-free and vacancy-carrying VLPs. Interestingly, for long nanorods vacancies are most frequently located at a distance of ~30 nm from one end of the VLP, Fig. 2 (C). The spatial distribution of defects for short nanorods was not possible to ascertain, since intact VLPs are the dominant species for short rods.

To examine the nature of defects, we have imaged single VLPs by intermittent contact atomic force microscopy (AFM) in liquid, Fig. 3. Solution conditions were the same throughout this work. Geometric parameters (NR aspect ratio, and length) were varied either, in ensemble average form, by changing synthesis conditions, or by natural variation due to the stochastic fluctuations in nucleation/growth. Depending on probe sharpness, sufficient spatial resolution can be attained on individual VLPs to distinguish single capsomers in a lattice. This allowed us to determine that the cylindrical portions of the VLPs are formed of a lattice of close-packed hexameric capsomers, while the spherical caps have structures suggestive of a quasi-equivalent icosahedral arrangement.^{18,19} In Fig. 3 B and C, the capsomer arrangements on the spherical caps are consistent with a T=3 structure, while the size and the capsomeric arrangement of the VLP in Fig. 3 D is consistent with a T=3 structure on the bottom cap (height: ~27 nm) and a T=4 structure on the upper cap (height:

~ 32nm). The diameter (measured as height difference) of the particle in Fig. 3 E is consistent with a T=4 cap structure. This is the most frequent outcome for long NR VLPs.

Most types of packing defects are ring-like scars and gaps oriented perpendicular to the long axis. Fig. 3 F–H shows examples of particles with one or two such ring defects. Consistent with the TEM observation in Fig. 2 C, ring defects are most frequently observed 25 – 30 nm from an end (Fig. SI-1). Moreover, in many particles with a ring defect, the lattice orientation differs from one side of the defect to the other. As discussed later, these observations suggest that assembly may start on end-caps rather than on the cylindrical portion of the NR.

We observe multiple orientations of the hexameric lattice relative to the long axis of the NR template, which we quantify by determining θ as the smallest angle between any of the lattice vectors and the long axis (Fig. 4 D). For $0^\circ < \theta < 30^\circ$, the hexagonal array is chiral, while it is achiral for $\theta = 0^\circ$ or $\theta = 30^\circ$. Following Luque *et al.*, who theoretically studied the optimal structures of elongated capsids, we distinguish between two achiral geometries, “zigzag” ($\theta = 0^\circ$), “ring” ($\theta = 30^\circ$), and chiral or “skew” geometries ($0^\circ < \theta < 30^\circ$).^{46,47}

Fig. 4 presents histograms of chiral angles from particles separated in two categories: 1) complete; i.e., without an observable vacancy defect, and 2) incomplete, which exhibit one or more defects. Note that the latter category includes particles with cylindrical sheets of different chiral angles, separated by an extended, ring defect. Due to the small total number of particles that could be measured it is difficult to discriminate finer features in the histograms, but most defect-free particles have the ring geometry ($\theta = 30^\circ$), whereas the chiral angle is more broadly distributed among particles with defects. Thus, there is evidence of a correlation between chiral lattices and generation of defects during assembly.

We note that the symmetry of the cap relative to the NR axis determines the chiral angle in a way that is potentially important for the shell stability. When the 5-fold cap axis is collinear with the cylinder axis, cap pentamers are located farther away from the area of zero Gauss curvature, and presumably less strained, than when a 3-fold cap symmetry axis is collinear with the cylinder axis, Fig. 4. Moreover, the dihedral angle between two adjacent capsomers depends on the lattice orientation relative to the NR axis. The simulations described next suggest that this feature can reduce the effects of a mismatch in the mean curvature of the NR and virus subunit.

To understand the interplay between template geometry and capsomer assembly, we developed a coarse-grained computational model for assembly on a spherocylindrical template. We initially modeled capsomers as spherical particles with short-range attractions between pairs of capsomers and between capsomers and the nanorod surface, thus neglecting the preferred curvature of capsomer assemblies. Simulations of this model with long nanorods exhibited nucleation and growth of low-defect hexameric arrays on the cylindrical portion of the nanorod, with accumulation of defects at nanorod ends. These results are inconsistent with the experimental observations described above, which show that defects primarily occur away from nanorod ends. Since the end-caps and cylindrical portion of the sphero-cylinder have different curvatures (both mean and Gaussian), this discrepancy

suggests that the preferred curvature of capsomer assemblies plays an important role in the experimental outcomes.

We therefore modified the model to include a preferred radius of curvature, R_{capsomer} , for capsomer assembly. We represent capsomers by conical subunits formed from stacked pseudoatoms (Fig. 5), with spherically symmetric interaction potentials between pseudoatoms. Attractive interactions (represented by a Morse potential) between pseudoatoms drive lateral association of capsomers, while excluded volume interactions (represented by a repulsive Lennard-Jones potential) favor assembly of curved shells. The six pseudoatoms within each cone are placed in a straight line at uniform separation 0.8 nm, so that the cone height (the distance between the centers of the first and last bead) is $h = 4$ nm. The bead radii are given by $r_n^{\text{eq}} = R_{\text{capsomer}}(1 + nh/5) \sin \alpha/2$ with $n = 0 \dots 5$ (see Methods for further details). The preferred curvature radius of these shells, R_{capsomer} , is tuned by the cone angle of the model capsomers (Fig. 5). Adsorption of capsomers onto the nanoparticle surface is driven by a short-range attraction (represented by a Morse potential) between the bottom of each cone and the nanorod surface. To match the experiments, we consider interaction parameters that lead to a nanoparticle-directed assembly regime, with strong adsorption onto the nanoparticle and weak capsomer-capsomer interactions that preclude assembly in the bulk. In particular, we set the well-depth of the capsomer-nanorod interaction to $\epsilon_{\text{nc}} = 0.3 k_B T$ (which results in a average binding energy of $6.65 k_B T$ /capsomer since each capsomer interacts with many nanoparticle pseudoatoms), and the well-depth for each of the attractive capsomer-capsomer interactions to $\epsilon_{\text{cc}} = 2.0 k_B T$, with $k_B T$ the thermal energy.

To compare computational and experimental results, we first focus on assembly around short nanorods. We simulated assembly around NRs with the range of experimental lengths, $L_{\text{nr}} \in [20, 30]$ nm. Since the experiments considered a narrow range of NR radii, $R_{\text{nr}} \in [4.5, 6]$ nm, we fixed the simulation NR radius at $R_{\text{nr}} = 5$ nm, for which the experiments had the most length variation. The preferred curvature radius of individual BMV capsomers, R_{capsomer} is not known, since it could differ from the size of wild-type capsids if the assembly process generates strain. Therefore, we considered a range of preferred curvature radii $R_{\text{capsomer}} \in [5, 10]$ nm. Note that the results are expected to depend on both preferred curvature and bending modulus. The latter can be derived from interaction parameters as described in ref.⁴⁸ Its value was close to $16 k_B T$, throughout this work.

Fig. 6 shows snapshots of typical assembly configurations, and a phase diagram for the most frequent outcomes in terms of defect presence as a function of nanorod length and R_{capsomer} . The shapes of the packing defects and the phase diagram qualitatively recapitulate the observations by TEM and the results of Fig. 2B. In particular, we observe a transition from defect-free assemblies to defective assemblies with vacancies at a threshold nanorod length. The capsomers are able to form complete ellipsoidal capsids around the shortest nanorods, but are incompatible with the elongated ellipsoid required to encapsulate longer nanorods. Consequently, the capsomers typically assemble into two partial capsids (Fig. 6B).

The threshold nanorod length at which defective capsids appear increases with R_{capsomer} . The distribution of assembly outcomes is most consistent with experimental results for the

preferred curvature corresponding to the wild-type BMV capsid, $R_{\text{capsomer}}=8.0$ nm. However, simulations on longer nanorods suggest that the relationship between capsomer and NR curvatures is more complex.

We simulated the assembly around long nanorods for nanoparticle dimensions similar to those used in experiments, $R_{\text{nr}} = 8 - 12$ nm and $L_{\text{nr}} = 61 - 69$ nm. Interestingly, for capsomer radii below $R_{\text{capsomer}} = 9.5$ nm, most simulations resulted in incomplete VLPs. To observe complete VLPs the radius of the cone had to be increased to $R_{\text{capsomer}} \gtrsim 13$ nm, on a NR template with $R_{\text{nr}} = 8$ nm, Fig. 7. However, in this range of capsomer radii the simulations reproduce all of the outcomes observed in experiments (Figs. 3 and 7). The distributions of different simulation outcomes are shown for three capsomer radii in Supplemental Information (Fig. SI-2).

From simulations over a range of $R_{\text{capsomer}} \in [5, 20]$ nm and $R_{\text{nr}} \in [6, 16]$ nm, we find that assembly morphology primarily depends on the ratio of the preferred subunit curvature radius to the NR radius $\gamma_{\text{nr}} \equiv R_{\text{capsomer}}/R_{\text{nr}}$. In Ref.⁴⁸ we derive a continuum expression for the bending energy of a triangular lattice with spontaneous curvature on an arbitrary surface, which shows that assembly depends on two forms of curvature mismatch. The bending energy is (per unit surface area of the template)

$$U_{\text{bend}} = \kappa \left[\frac{1}{2} (2H - c_0)^2 + (H^2 - K) \right], \quad (1)$$

with κ the bend modulus, $H = (c_1 + c_2)/2$ the mean curvature of the template with c_1 and c_2 its two principal curvatures, $K = c_1 c_2$ the template Gaussian curvature, and $c_0 = 2/R_{\text{capsomer}}$ the spontaneous curvature of the capsomers. The first term on the right hand side of Eq. (1) is the standard Helfrich Hamiltonian⁴⁹ for deviations between the preferred mean curvatures of the template and subunit, while the second term $(H^2 - K) = (c_1 - c_2)^2/4$ accounts for a mismatch between the curvature anisotropy of the template and capsomers. Setting the mean curvatures of the spherical and cylindrical portions of the NR as $H_{\text{sphere}} = 1/R_{\text{nr}}$ and $H_{\text{cyl}} = 1/2R_{\text{nr}}$, noting that the Gaussian curvature is $K = 1/R_{\text{nr}}^2$ on the spherical portion but $K = 0$ on the cylinder, we find that there is a threshold curvature ratio $\gamma_{\text{nr}}^* = 8.5$ above which assembly is favorable on the cylinder and below which assembly is favorable on the sphere.

This prediction roughly matches the simulation morphologies that we observe over the range of $R_{\text{capsomer}} \in [5, 20]$ nm and $R_{\text{nr}} \in [6, 16]$ nm. For capsomers with curvatures for which assembly is sufficiently favorable on the cylindrical portion of the template ($\gamma_{\text{nr}} \gtrsim 8.5$, Fig. 7A) particles are complete, while capsomers which favor higher curvature ($\gamma_{\text{nr}} < 8.5$, Figs. 7 B,C) tend to assemble well on the end caps but exhibit incomplete domains and vacancies on the cylinder. Comparison of this result with our experiments on NRs and the curvature radius of a wild type BMV capsid (≈ 8 nm) suggests that BMV capsomers have greater flexibility in the geometry of their interactions than accounted for in our model. In particular, the model considers only a single subunit preferred curvature radius, while the BMV hexamers may have a larger preferred radius than BMV pentamers. Also due to having only a single subunit radius, the model does not capture the tendency of VLPs to exhibit T=4 structures on

the end caps. Despite this limitation of the model, we find it can qualitatively explain additional features of the experimental observations.

We next analyzed the results of the computational model to investigate the relationship between curvature mismatch and lattice orientation. We focused on a nanorod dimensions $R_{nr} = 8$ nm, $L_{nr} = 61$ nm and varied the capsomer preferred curvature over the range $\gamma_{nr} \in [1.2, 2]$. We find that there is a strong correlation between curvature ratios and the chiral angle θ . For capsomers whose curvature matches the spherical cap better than the cylinder ($\gamma_{nr} < 8/5$, Fig. 8B) most particles have chiral angles close to the ring configuration ($\theta \approx 30^\circ$). In contrast, below the threshold curvature mismatch ($\gamma_{nr} > 8/5$, Fig. 8A) the hexagonal lattice favors alignment with the cylinder axis (close to the zigzag configuration, $\theta = 0^\circ$).

There are similarities and differences between these computational results and the experiments. The simulations predict a preferred angle of $\approx 30^\circ$, as observed in experiments, for a curvature ratio $\gamma_{nr} < 8/5$ when the capsomer curvature matches the spherical cap better than the cylinder. We show next that the simulation kinetics also more closely match the experiments in this regime. The key difference is, as discussed above, that the simulations do not reproduce the experimental observation of complete shells for this degree of curvature mismatch. In addition, the computational VLPs do not favor the T=4 structure on the end caps that many experimental VLPs appear to exhibit. We discuss possible implications of these differences between simulation and experiment below.

The very sharp distribution of defect location along the NR length is an intriguing feature of our experiments, Fig. 2C. Analysis of dynamical assembly pathways in simulations suggests that two factors contribute to this outcome, one kinetic and the other thermodynamic. For all simulation parameters, the initial adsorption of capsomers is rapid and homogeneous on the NR, Fig. 9A. However, the location at which adsorbed subunits subsequently assemble depends sensitively on the ratio of the preferred mean curvature of the subunits to the template curvature. When the subunit mean curvature $H_{capsomer}$ is similar to the curvature of the end-cap ($R_{capsomer} = R_{nr}$) and thus larger than the curvature of the cylindrical surface, nucleation preferentially occurs on end-caps. Growth proceeds rapidly until the endcap is covered, but slows on the cylindrical region because strain begins to build. For sufficient curvature mismatch, assembly eventually stops. Typically, an independent nucleation event occurs at the other end-cap, resulting in a ring-shaped grain boundary where the two growth fronts meet, distant from the ends.

The location of preferred nucleation can be tuned by varying the ratio of curvatures, Fig. 9. For subunit curvatures with nearly equal mismatch to the cylinder or sphere ($\gamma_{nr} \approx 8/5$, Fig. 9B), nucleation occurs uniformly on the NR, whereas a closer match to the cylinder results and preferential nucleation on the NR shaft ($\gamma_{nr} \approx 8/5$, Fig. 9B).

We have thus seen that the geometric constraints imposed by the NR template may lead to architectures that are very different from the minimum free energy structures in absence of constraints. There are other interesting examples, of natural viruses, in which such geometric constraints are believed to lead to low-symmetry, nonequilibrium, packings, for instance, the elongated (conical, cylindrical, and asymmetric) capsids of the human immunodeficiency

virus (HIV).⁵⁰ In this regard, Zandi and co-workers have considered using a mean-field theory approach the important role played by RNA-capsid protein interaction in the selection and stability of different possible capsid shapes.⁵¹ Nguyen *et al.* have used continuum elastic theory to show that the conical shape is the most stable shape only in conditions of fixed volume and/or fixed spanning length, as those imposed by the spherical membrane of the immature HIV particle.⁵² We note that, although some of the VLPs we have studied have an asymmetric structure with a T=3 cap at one end and a T=4 cap at the other, there are some important characteristics that differentiate them from HIV conical capsids. In our asymmetric spherocylindrical structures with a T=4 at one end and a T=3 at the other, the shell is formed of two icosahedral-capped cylindrical tubes of different radii, separated by a ring defect. In HIV, there is a smooth change of curvature corresponding to a conical shape as one moves from the narrow end towards the broad end. Moreover, an extended defect in HIV is often observed which has the form of a seam or a flap parallel to the long axis of the particle. In our case, when a defect is observed, it is better described as a ring perpendicular to the long axis. As a possible explanation for these differences we will note that unlike BMV, which readily forms empty capsids of indistinguishable structure from those of wild type particles and thus has a well-defined preferred curvature, it is unclear what the preferred radius of curvature of HIV Gag or CA is.⁵³ Assembly results in HIV are very polydisperse, and wild-type Gag does not assemble into empty particles in absence of nucleic acid.⁵⁴

Conclusion

In conclusion, this work demonstrates the symmetric assembly of icosahedral virus coat proteins in elongated polyhedral structures, following a metal nanorod template approach. We show that the spherocylindrical packings are chiral, that chirality is controlled by the relative magnitudes of subunit intrinsic curvature, and the template mean curvature and curvature anisotropy, and that the probability of packing defects correlates with the chiral angle. The preferred curvature of the protein subunits plays an important role in the formation of grain boundary defects; with guidance from numerical simulations, we propose an explanation for this behavior based on curvature-frustrated growth.

Materials and methods

Gold nanorod template preparation

Gold nanorods were synthesized following well described method of seed-mediated growth in the presence of hexadecyltrimethylammonium bromide(CTAB) with 5-bromosalicylic acid as aromatic additive for size control.⁴⁴ After the synthesis, gold NRs were purified from CTAB excess and, in order to provide a negative surface charge, functionalized by ligand exchange of CTAB with carboxylate-terminated thiolalkylated tetraethyleneglycol (TEG) ligand.⁴⁵ NR particles were purified from TEG ligand excess by ultra-centrifugation and subsequent wash with water. After purification, NR samples were imaged by TEM and sized. Size distributions were obtained from at least 300 particles from a few areas of the TEM grid.

Preparation of virus-like particles

BMV proteins were prepared according previously described protocols⁴⁵ and stored in TKM buffer (1 M KCl, 0.005 MgCl₂ and 0.01 M Tris-HCl at pH 7.4) at 4 °C. Nanoparticle encapsulating VLPs were assembled according a two step protocol with adjustment of protein/NR core ratio according to surface area of NR to be covered with capsid. Briefly, NRs were mixed with the capsid protein in TKM buffer while the total protein concentration in the mixture was kept constant at 0.5 mg/ml. The mixture was transferred to a dialysis unit and dialyzed against TNKM buffer (0.05 M Tris-HCl, 0.05 M NaCl, 0.01 M KCl, 0.005 M MgCl₂, pH 7.4) overnight and then against SAMA buffer overnight (50 mM NaOAc, 8 mM Mg(OAc)₂, pH 4.6).

Virus-like particle characterization by transmission electron microscopy

VLPs were examined by negative stain TEM. Specimens were prepared by placing 10 μ L drop of a 10 $\times$ diluted stock solution onto a carbon-coated copper grid. After 10 min, the excess solution on the grid was removed with filter paper. A 10 μ L portion of 2% uranyl acetate was used to stain for 10 min. Excess solution was removed by blotting with filter paper. Images were acquired at an accelerating voltage of 80 kV on a JEOL JEM1010 transmission electron microscope equipped with a Gatan UltraScan 4000 CCD camera and analyzed with the ImageJ Processing Toolkit to estimate VLP diameters. For size and morphology characterization, we measured 150–300 VLPs per batch.

Atomic force microscopy

AFM images were acquired with a Cypher AFM (Asylum Research, Santa Barbara, U.S.A.) in AC mode. Both newly-cleaved HOPG and mica substrates were used in this study. High imaging resolution was achieved utilizing BioLever Mini cantilevers (Olympus, Tokyo, Japan) with a nominal tip radius of curvature of 9 nm. A 30 min UV treatment was applied to the tip before experiment. To start imaging, a droplet of 50 μ L of 10 $\times$ diluted from the initial VLP solution at 0.5 mg/ml was first deposited onto the newly-cleaved substrate surface. After 10 min incubation, the droplet was washed 3 times with 25 μ L SAMA buffer with a pipette, to remove unadsorbed particles. Another 50 μ L of filtered SAMA buffer was used to pre-wet the UV-treated tip mounted in the droplet cantilever holder. AFM images were acquired with a mean imaging force of $\sim$ 100 pN. Images were typically processed with a masked first-order flattening to correct uneven substrate effects, and then rendered in 3D for better display.

Computational

Model overview—To understand the interplay between template geometry and capsomer assembly, we developed a coarse-grained computational model, adapted from Chen *et al.*⁵⁵ and Lazaro *et al.*⁴⁸ Each capsomer is represented by a conical subunits formed pseudoatoms (beads) with spherically symmetric interactions. For simplicity, both hexamer and pentamer capsomers are represented by the same particle; the capsomer coordination numbers emerge organically from the assembly dynamics. Each model capsomer consists of an array of six equally spaced beads of increasing radius, characterized by the cone angle α (Fig. 5a). In contrast to Chen *et al.*,⁵⁵ the cones are truncated, so that the attractions drive assembly into

hollow shells resembling viral capsids, with a preferred curvature determined by the cone angle α . By performing bulk simulations (with no nanorod), we found that for $\alpha = 33.1^\circ$ subunits assemble into monodisperse shells containing 32 subunits arranged with $T=3$ icosahedral symmetry, with inner radius $R_{\text{capsomer}} \approx 9.5\text{nm}$. These structures thus match the size and organization of the native BMV capsid⁵⁶ (Fig. 5c). To allow for the possibility that the intrinsic preferred curvature of individual BMV proteins differs from the curvature of the $T=3$ capsid (i.e., if capsomers are under elastic stress in the capsid), we considered a broad range of cone angles, leading to a range of preferred curvature radii $R_{\text{capsomer}} \in [4, 21]\text{nm}$.

We represent nanorods by rigid bodies consisting of thousands of partially overlapping beads. Each nanorod has a cylindrical body with L_{nr} and radius R_{nr} (Fig. 5b) and two hemispherical caps with radius R_{nr} , so the total spherocylinder length is given by $L_{\text{nr}} = L_{\text{cyl}} + 2R_{\text{nr}}$.

We randomly distribute beads on the spherocylindrical surface with a high and roughly homogeneous density $\rho=13$ beads/ nm^2 , so that the cones interact with a homogeneous surface. The bottom bead of each subunit is attracted to every nanorod surface bead through a Morse potential. Interaction potentials

In all simulations we fix the height of the cone and the diameter of the outermost bead to correspond to the dimensions of BMV hexamers. We set the unit of length by setting the cone height (the distance between the centers of the first and last bead) to 4nm. The bead sizes set the cone angle α as follows. We consider a spherical shell of capsomers, with the innermost bead of each capsomer located at a radial distance from the shell center R_{capsomer} . The outer cone bead is located at a distance $R_{\text{out}} = R_{\text{capsomer}} + h$, and we set $h = 4\text{nm}$, so that the edge-to-edge height is approximately 5 nm, the height of BMV capsid proteins. Four more beads are uniformly distributed between the inner and outer beads, and the bead radius is given by $r_n^{\text{eq}} = R_{\text{capsomer}}(1+n/5) \sin \alpha/2$ with $n = 0 \dots 5$. Each of the four interior beads ($n = 1 \dots 4$) in a capsomer interacts with its counterpart (bead with the same index) in a nearby capsomer through a Morse potential (Eq. (2)). In addition, all pairs of beads that do not interact through a Morse potential and are not on the same capsomer experience excluded volume interactions (Eq. (3)).

The potential between pairs of capsomers, U_{cc} , consists of two contributions. The attractive interaction between pairs of interior beads ($n = 1 \dots 4$) with the same index is modeled by a Morse potential, with the equilibrium distance of the potential depending on the bead radius, r_n^{eq} defined above:

$$U_{\text{cc}}^{\text{att}} = \sum_{i,j} \sum_{n=1}^4 \epsilon_{\text{cc}} \left(e^{-2\alpha_n(r_{ij,n} - 2r_n^{\text{eq}})} - 2e^{-\alpha_n(r_{ij,n} - 2r_n^{\text{eq}})} \right) \quad (2)$$

with i and j indexing capsomers, n indexing beads within a capsomer, $r_{ij,n}$ the distance between the pair of beads with index n in capsomers i and j , $\epsilon_{\text{cc}} = 0.3k_B T$ and $\alpha_n = (12.0/r_n^{\text{eq}})$, and cutoff radius $r_n^{\text{cut}} = 2r_n^{\text{eq}} + 1.5$

All pairs of cone beads not subject to the Morse potential and not on the same subunit experience excluded volume interactions represented by a repulsive WCA potential⁵⁷

$$U_{cc}^{ex} = \sum_{i,j < i} \sum_{n \neq m} 4\epsilon_{ex} \left[\left(\frac{\sigma_{nm}}{r_{ij,nm}} \right)^{12} - \left(\frac{\sigma_{nm}}{r_{ij,nm}} \right)^6 \right] \quad (3)$$

with $r_{ij,nm}$ the distance between bead n in capsomer i and bead m in capsomer j , $\epsilon_{ex} = 1.0$, and the cutoff distances $r_{nm}^{cut} = \sigma_{nm} = r_n^{eq} + r_m^{eq}$.

Likewise, the interaction potential between the nanorod and capsomers, U_{nc} , can be separated into attractive and repulsive terms. The bottom beads of the capsomers experience attractive interactions with nanoparticle beads, described by a Morse potential:

$$U_{cc}^{att}(r) = \sum_{i=1}^{N_{nr}} \sum_{j=1}^{N_{cap}} \epsilon_{nc} \left(e^{-2\alpha_{nc}(r_{ij,0} - 2r_{nc}^{eq})} - 2e^{-\alpha_{nc}(r_{ij,0} - 2r_{nc}^{eq})} \right) \quad (4)$$

with the sum running over all N_{nr} nanorod beads and the bottom beads of all N_{cap} capsomers, $r_{ij,0}$ the distance between nanorod pseudoatom i and the bottom bead of capsomer j , and $r_{nc}^{eq} = 0.5$. The well-depth parameters are set to $\epsilon_{nc} = 2.0k_B T$ for beads on the cylindrical portion of the NR, and $\epsilon_{nc} = 1.96k_B T$ for beads on the endcap to ensure a uniform driving force for subunit adsorption everywhere (see **simulations** below), and the interaction width parameter is set to $\alpha_{nc} = 3.0$.

The 3 outermost beads of each capsomer, at positions $\{\mathbf{r}_n\}$ with $n = 3 \dots 5$, also also experience excluded volume interactions with all nanorod pseudoatoms:

$$U_{nc}^{ex} = \sum_{i=1}^{N_{nr}} \sum_{j=1}^{N_{cap}} \sum_{n=3}^5 4\epsilon_{ex} \left[\left(\frac{\sigma_n^{nr}}{r_{ij,n}} \right)^{12} - \left(\frac{\sigma_n^{nr}}{r_{ij,n}} \right)^6 \right] \quad (5)$$

with $r_{ij,n}$ the distance between nanorod pseudoatom i and bead n of capsomer j , $\epsilon_{ex} = 1.0$ and cutoff radius $r_n^{cut} = \sigma_n^{nr} = r_{nc}^{eq} + r_n^{eq}$.

Simulations

We performed simulations in HOOMD,⁵⁸ taking advantage of its highly efficient GPU acceleration.⁵⁹ All physical units are expressed relative to the basic units of energy $k_B T$, mass m , and length σ . To compare to experimental results, we set the length scale $\sigma = 1\text{ nm}$ so that the height of model capsomers roughly corresponds to the height of BMV proteins. The diameter of the outermost bead $d = 7.7\sigma$ is then approximately equal to the BMV capsomer diameter ($\approx 8\text{ nm}$).

To match the experiments, we considered interaction parameters ($\epsilon_{nc, cyl} = 2.0k_B T$, $\epsilon_{cc} = 0.3k_B T$) that lead to a nanoparticle-directed assembly regime, with strong adsorption onto the nanoparticle and assembly of capsomers in bulk rare due to the weak capsomer-capsomer interactions. Each nanorod assembly simulation contained one nanorod and 300 capsomers in a cubic box with side length 130nm. The ratio of free and adsorbed capsomers always remained above 5:1, so that the kinetics were not significantly influenced by depletion of free capsomers.

To ensure that the nanoparticle-capsomer interaction was homogeneous over the nanoparticle surface, we performed a set of exploratory simulations in which we compared the density of adsorbed capsomers on the cylindrical and spherical surfaces. For these simulations, we turned off all capsomer-capsomer interactions to avoid the effects of preferred capsomer curvature. We found that adsorption on the spherical surface was slightly higher, most likely due to the finite-size of nanoparticle surface beads. In particular, while the nanoparticle surface bead density is set constant at the radius corresponding to the surface bead centers, the capsomer bottom beads experience a slightly different interaction geometry due to the different curvatures of the cylindrical and spherical surfaces. We counteracted this effect by slightly weakening the interaction between capsomers and nanoparticle surface beads on the spherical endcaps, setting $\epsilon_{nc, sph} = 0.98\epsilon_{nc, cyl}$. Empirically we found that this led to approximately equal adsorption densities on the cylindrical and spherical portions of the nanoparticle.

Capsomers were initialized at random positions and orientations, except that there were no overlaps between pairs of capsomer-capsomer or capsomer-nanorod pseudoatoms. Capsomer positions and orientations were evolved using the HOOMD Brownian dynamics algorithm for rigid bodies, while the nanorod was fixed. We used a timestep $t = 0.005$, and we performed $\sim 1.5 \times 10^7$ timesteps in each simulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

The authors thank the U.S Army Research Office for support under award # W911NF-17-1-0329 (to BD) and the National Institutes of Health, Award Number R01GM108021 from the National Institute of General Medical Sciences (GRL and MFH), the Brandeis Center for Bioinspired Soft Materials, an NSF MRSEC, DMR-1420382 (GRL). Computational resources were provided by the NSF through XSEDE computing resources (XStream, Maverick, Bridges, Comet) through award number MCB090163, and the Brandeis HPCC which is partially supported by the Brandeis MRSEC.

References

1. Young M; Debbie W; Uchida M; Douglas T Plant Viruses as Biotemplates for Materials and Their Use in Nanotechnology. *Annu. Rev. Phytopathol* 2008, 46, 361–384. [PubMed: 18473700] –
2. Steinmetz NF; Cho C-F; Ablack A; Lewis JD; Manchester M Cowpea Mosaic Virus Nanoparticles Target Surface Vimentin on Cancer Cells. *Nanomedicine (London,U. K.)* 2011, 6, 351–364.–
3. Minten IJ; Claessen VI; Blank K; Rowan AE; Nolte RJM; Cornelissen JJLM Catalytic Capsids: the Art of Confinement. *Chem. Sci* 2011, 2, 358–362.–

4. Dang X; Yi H; Ham M-H; Qi J; Yun DS; Ladewski R; Strano MS; Hammond PT; Belcher AM Virus-Templated Self-Assembled Single-Walled Carbon Nanotubes for Highly Efficient Electron Collection in Photovoltaic Devices. *Nat. Nanotechnol* 2011, 6, 377–384. [PubMed: 21516089] –
5. Judd J; Ho ML; Tiwari A; Gomez EJ; Dempsey C; Van Vliet K; Igoshin OA; Silberg JJ; Agbandje-McKenna M; Suh J Tunable Protease-Activatable Virus Nanonodes. *ACS Nano* 2014, 8, 4740–4746. [PubMed: 24796495] –
6. Park H; Heldman N; Rebentrost P; Abbondanza L; Iagatti A; Alessi A; Patrizi B; Salvalaggio M; Bussotti L; Mohseni M; Caruso F; Johnsen HC; Fusco R; Foggi P; Scudo PF; Lloyd S; Belcher AM Enhanced Energy Transport in Genetically Engineered Excitonic Networks. *Nat. Mater.* 2015, 15, 211–216. [PubMed: 26461447] –
7. Malyutin AG; Easterday R; Lozovyy Y; Spilotros A; Cheng H; Sanchez-Felix OR; Stein BD; Morgan DG; Svergun DI; Dragnea B; Bronstein LM Viruslike Nanoparticles with Maghemite Cores Allow for Enhanced MRI Contrast Agents. *Chem. Mater* 2015, 27, 327–335.–
8. Delalande L; Tsvetkova IB; Zeng C; Bond K; Jarrold MF; Dragnea B Catching a Virus in a Molecular Net. *Nanoscale* 2016, 6, 205–207.–
9. Rother M; Nussbaumer MG; Renggli K; Bruns N Protein Cages and Synthetic Polymers: A Fruitful Symbiosis for Drug Delivery Applications, Bionanotechnology and Materials science. *Chem. Soc. Rev* 2016, 45, 6213–6249. [PubMed: 27426103] –
10. Chen Z; Li N; Li S; Dharmawardana M; Schlimme A; Gassensmith JJ Viral Chemistry: The Chemical Functionalization of Viral Architectures to Create New Technology. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol* 2016, 8, 512–534. [PubMed: 26663821] –
11. Brillault L; Jutras PV; Dashti N; Thuenemann EC; Morgan G; Lomonossoff GP; Landsberg MJ; Sainsbury F Engineering Recombinant Virus-like Nanoparticles from Plants for Cellular Delivery. *ACS Nano* 2017, 11, 3476–3484. [PubMed: 28198180] –
12. Miller RA; Presley AD; Francis MB Self-Assembling Light-Harvesting Systems from Synthetically Modified Tobacco Mosaic Virus Coat Proteins. *J. Am. Chem. Soc* 2007, 129, 3104–3109. [PubMed: 17319656] –
13. Nam YS; Shin T; Park H; Magyar AP; Choi K; Fantner G; Nelson KA; Belcher AM Virus-Templated Assembly of Porphyrins into Light-Harvesting Nanoantennae. *J. Am. Chem. Soc* 2010, 132, 1462–1463. [PubMed: 20078048] –
14. Usselman RJ; Walter ED; Willits D; Douglas T; Young M; Singel DJ Monitoring Structural Transitions in Icosahedral Virus Protein Cages by Site-Directed Spin Labeling. *J. Am. Chem. Soc* 2011, 133, 4156–4159. [PubMed: 21388197] –
15. Wen AM; Infusino M; De Luca A; Kernan DL; Czapar AE; Strangi G; Steinmetz NF Interface of Physics and Biology: Engineering Virus-Based Nanoparticles for Biophotonics. *Bioconjugate Chem* 2015, 26, 51–62.–
16. Scholes GD; Fleming GR; Chen LX; Aspuru-Guzik A; Buchleitner A; Coker DF; Engel GS; van Grondelle R; Ishizaki A; Jonas DM; Lundeen JS; McCusker JK; Mukamel S; Ogilvie JP; Olaya-Castro A; Ratner MA; Spano FC; Whaley KB; Zhu X Using Coherence to Enhance Function in Chemical and Biophysical Systems. *Nature* 2017, 543, 647–656. [PubMed: 28358065] –
17. Dragnea B Virus-Based Devices: Prospects for Allopoiesis. *ACS Nano* 2017, 11, 3433– 3437. [PubMed: 28441712]
18. Caspar DLD; Klug A Physical Principles in Construction of Regular Viruses. *Cold Spring Harbor Symp. Quant. Biol* 1962, 27, 1–24. [PubMed: 14019094] –
19. Johnson JE; Speir JA Quasi-Equivalent Viruses: A Paradigm for Protein Assemblies. *J. Mol. Biol* 1997, 269, 665–675. [PubMed: 9223631] –
20. Bancroft J The Self-Assembly of Spherical Plant Viruses. *Adv. Virus Res.* 1970, 16, 99–134. [PubMed: 4924992]
21. Mukherjee S; Pfeifer CM; Johnson JM; Liu J; Zlotnick A Redirecting the Coat Protein of a Spherical Virus to Assemble into Tubular Nanostructures. *J. Am. Chem. Soc* 2006, 128, 2538–2539. [PubMed: 16492029] –
22. Lavelle L; Gingery M; Phillips M; Gelbart WM; Knobler CM; Cadena-Nava RD; Vega-Acosta JR; Pinedo-Torres LA; Ruiz-Garcia J J. *Phys. Chem. B*

23. Nguyen HD; Reddy VS; Brooks CL III; Brooks CL Invariant Polymorphism in Virus Capsid Assembly. *J. Am. Chem. Soc* 2009, 131, 2606–2614. [PubMed: 19199626] –
24. Fantoni R; Salari JW; Klumperman B Structure of Colloidosomes with Tunable Particle Density: Simulation versus Experiment. *Phys. Rev. E* 2012, 85, 061404.
25. Luque A; Reguera D; Morozov A; Rudnick J; Bruinsma R Physics of Shell Assembly: Line Tension, Hole Implosion, and Closure Catastrophe. *J. Chem. Phys* 2012, 136, 184507. [PubMed: 22583300]
26. Zandi R; Reguera D; Bruinsma RF; Gelbart WM; Rudnick J Origin of Icosahedral Symmetry in Viruses. *Proc. Natl. Acad. Sci. U. S. A* 2004, 101, 15556–15560. [PubMed: 15486087] –
27. Paquay S; Kusumaatmaja H; Wales DJ; Zandi R; van der Schoot P Energetically Favoured Defects in Dense Packings of Particles on Spherical Surfaces. *Soft Matter* 2016, 12, 5708–5717. [PubMed: 27263532] –
28. Hagan MF Controlling Viral Capsid Assembly with Templating. *Phys. Rev. E* 2008, 77, 051904.
29. Elrad OM; Hagan MF Mechanisms of Size Control and Polymorphism in Viral Capsid Assembly. *Nano Lett* 2008, 8, 3850–3857. [PubMed: 18950240] –
30. Fejer SN; Chakrabarti D; Wales DJ Emergent Complexity from Simple Anisotropic Building Blocks: Shells, Tubes, and Spirals. *ACS Nano* 2010, 4, 219–228. [PubMed: 20055436] –
31. Williamson AJ; Wilber AW; Doye JPK; Louis AA Templated Self-Assembly of Patchy Particles. *Soft Matter* 2011, 7, 3423–3431.–
32. Hagan MF A Theory for Viral Capsid Assembly around Electrostatic Cores. *J. Chem. Phys* 2009, 130, 114902. [PubMed: 19317561]
33. Kusters R; Lin H-K; Zandi R; Tsvetkova I; Dragnea B; van der Schoot P Role of Charge Regulation and Size Polydispersity in Nanoparticle Encapsulation by Viral Coat Proteins. *J. Phys. Chem. B* 2015, 119, 1869–80. [PubMed: 25562399] –
34. Lin H-K; van der Schoot P; Zandi R Impact of Charge Variation on the Encapsulation of Nanoparticles by Virus Coat Proteins. *Phys. Biol* 2012, 9, 066004. [PubMed: 23114290]
35. Yu N; Ghosh A; Hagan MF Faceted Particles Formed by the Frustrated Packing of Anisotropic Colloids on Curved Surfaces. *Soft Matter* 2016, 12, 8990. [PubMed: 27748486]
36. Chen T; Glotzer SC Simulation Studies of a Phenomenological Model for Elongated Virus Capsid Formation. *Phys. Rev. E* 2007, 75, 51504.
37. Casjens S Virus structure and assembly; Jones & Bartlett Pub, 1985.
38. Hu Y; Zandi R; Anavitarte A; Knobler CM; Gelbart WM Packaging of a Polymer by a Viral Capsid: the Interplay between Polymer Length and Capsid Size. *Biophys. J* 2008, 94, 1428–36. [PubMed: 17981893] –
39. Chang CB; Knobler CM; Gelbart WM; Mason TG Curvature Dependence of Viral Protein Structures on Encapsidated Nanoemulsion Droplets. *ACS Nano* 2008, 2, 281–6. [PubMed: 19206628] –
40. Aniagyei SE; DuFort C; Kao CC; Dragnea B Self-Assembly Approaches to Nanomaterial Encapsulation in Viral Protein Cages. *J. Mater. Chem* 2008, 18, 3763– 3774. [PubMed: 19809586] –
41. Kivenson A; Hagan MF Mechanisms of Capsid Assembly around a Polymer. *Biophys. J* 2010, 99, 619–628. [PubMed: 20643082] –
42. Lin H-K; van der Schoot P; Zandi R Impact of Charge Variation on the Encapsulation of Nanoparticles by Virus Coat Proteins. *Phys. Biol.* 2012, 9, 66004.
43. Schmucker AL; Harris N; Banholzer MJ; Blaber MG; Osberg KD; Schatz GC; Mirkin CA Correlating Nanorod Structure with Experimentally Measured and Theoretically Predicted Surface Plasmon Resonance. *ACS Nano* 2010, 4, 5453–5463. [PubMed: 20738131] –
44. Ye X; Jin L; Caglayan H; Chen J; Xing G; Zheng C; Doan-Nguyen V; Kang Y; Engheta N; Kagan CR; Murray CB Improved Size-Tunable Synthesis of Monodisperse Gold Nanorods through the Use of Aromatic Additives. *ACS Nano* 2012, 6, 2804– 2817. [PubMed: 22376005] –
45. Tsvetkova IB; Dragnea B **In** Methods Mol. Biol; Orner B, Ed.; 2015; Chapter 1.**In**
46. Luque A; Reguera D The Structure of Elongated Viral Capsids. *Biophys. J* 2010, 98, 2993–3003. [PubMed: 20550912] –

47. Luque A; Zandi R; Reguera D Optimal Architectures of Elongated Viruses. *Proc. Natl. Acad. Sci. U. S. A* 2010, 107, 5323–5328. [PubMed: 20212146] –
48. Lázaro GR; Mukhopadhyay S; Hagan MF Why Enveloped Viruses Need Cores-The Contribution of a Nucleocapsid Core to Viral Budding. *Biophys. J* 2018, 114, 619–630. [PubMed: 29414708] –
49. Helfrich W Elastic properties of lipid bilayers: theory and possible experiments. *Z. Naturforsch., C: J. Biosci* 1973, 28, 693–703.
50. Benjamin J; Ganser-Pornillos BK; Tivol WF; Sundquist WI; Jensen GJ Three-Dimensional Structure of HIV-1 Virus-Like Particles by Electron Cryotomography. *J. Mol. Biol* 2005, 346, 577–588. [PubMed: 15670606] –
51. Erdemci-Tandogan G; Wagner J; van der Schoot P; Zandi R Role of Genome in the Formation of Conical Retroviral Shells. *J. Phys. Chem. B* 2016, 120, 6298–6305. [PubMed: 27128962] –
52. Nguyen TT; Bruinsma RF; Gelbart WM Continuum Theory of Retroviral Capsids. *Phys. Rev. Lett* 2006, 96, 078102. [PubMed: 16606144]
53. Saxena P; He L; Malyutin A; Datta SAK; Rein A; Bond KM; Jarrold MF; Spilotros A; Svergun D; Douglas T; Dragnea B Virus Matryoshka: A Bacteriophage Particle-Guided Molecular Assembly Approach to a Monodisperse Model of the Immature Human Immunodeficiency Virus. *Small* 2016, 12, 5862–5872. [PubMed: 27634413] –
54. Crist RM; Datta SAK; Stephen AG; Soheilian F; Mirro J; Fisher RJ; Nagashima K; Rein A Assembly Properties of Human Immunodeficiency Virus Type 1 Gag-Leucine Zipper Chimeras: Implications for Retrovirus Assembly. *J. Virol* 2009, 83, 2216–2225. [PubMed: 19073719] –
55. Chen T; Zhang Z; Glotzer SC A Precise Packing Sequence for Self-Assembled Convex Structures. *Proc. Natl. Acad. Sci. U. S. A* 2007, 104, 717–722. [PubMed: 17215354] –
56. Wang Z; Hryc CF; Bammes B; Afonine PV; Jakana J; Chen D-H; Liu X; Baker ML; Kao C; Ludtke SJ; Schmid MF; Adams PD; Chiu W An Atomic Model of Brome Mosaic Virus Using Direct Electron Detection and Real-Space Optimization. *Nat. Commun.* 2014, 5, 4808. [PubMed: 25185801]
57. Weeks JD; Chandler D; Andersen HC Role of Repulsive Forces in Determining Equilibrium Structure of Simple Liquids. *J. Chem. Phys* 1971, 54, 5237–5247.–
58. Anderson JA; Lorenz CD; Travesset A General Purpose Molecular Dynamics Simulations Fully Implemented on Graphics Processing Units. *J. Comput. Phys* 2008, 227, 5342–5359.–
59. Nguyen TD; Phillips CL; Anderson JA; Glotzer SC Rigid Body Constraints Realized in Massively-Parallel Molecular Dynamics on Graphics Processing Units. *Comput. Phys. Commun* 2011, 182, 2307–2313.–

Figure 1:
Transmission electron microscopy (TEM) of Au nanorod and spherocylindrical VLPs. A. “Long” NR sample and associated VLPs. B. “Short” NR sample and associated VLPs.

Figure 2:

A. TEM micrographs of spherocylindrical VLPs encapsulating long (left) and short (right) NR particles. Long NR VLPs are more prone to defects, which are observable as gaps on the cylindrical side. B. Incidence of VLPs showing vacancy defects and defect-free VLPs for short rods (red = defect-free, short NR; green = with vacancies, short NR; orange = defect-free, long NR; cyan = with vacancies, long NR). C. Histogram of distance of the apparent ring defect from the closest NR end (for long NRs).

Figure 3:

Top: AFM images of single spherocylindrical VLPs in liquid provide sufficient spatial resolution to examine capsomer formation and packing. Cylindrical sides are characterized by hexagonal close packing with occasional vacancy defects. Spherical caps contain pentamers. A. Spherical VLP (28 nm diameter) formed around a 12 nm nanoparticle core is included for reference. B. A short NR VLP. C. NR VLP with chiral oligomeric arrangement. D. A VLP with two different widths separated by a defect. This situation may correspond to a T=3 cap at one end and a T=4, at the other. E. VLP of diameter consistent with T=4 caps. Bottom: Images of typical experimental outcomes and simulation outcomes with morphologies corresponding to those seen in experiments: F. Complete particle; G. Particle with one ring defect; H. Particle with two ring defects. Bead colors map the number of neighbors.

Figure 4:

A. Histograms of chiral angle characterizing cylindrical sides for: particles with no observable vacancies (red) and particles with vacancies (blue). B. Two T=3 end cap structures corresponding to 5-fold and 3-fold icosahedron symmetry axes collinear with the cylinder axis. A “zig-zag” lateral structure corresponds to $\theta = 0^\circ$, 5-fold center axis, and the $\theta = 30^\circ$, 3-fold center axis, corresponds to the “ring” lateral structure. C. Similar models for the T=4 end cap structure. In contrast with T=3, in this case: $\theta = 30^\circ$ “ring” has a 5-fold on center axis; $\theta = 0^\circ$, “zig-zag”, has a 3-fold. D. Representation of the side hexagonal lattice, lattice vectors, and chiral angle. Dotted line: orientation of the NR long axis.

Figure 5:

A. Schematic of model capsomers, which are conical particles comprised of stacked spheres. Interparticle attractions are represented by Morse potentials between the four interior spheres, while excluded volume interactions are represented by a repulsive Lennard-Jones potentials. B. The nanorod template is modeled as a spherocylinder. The model includes attractive interactions between the subunit cone tip and the template surface which lead to adsorption. C. In the absence of the nanorod template, isometric shells with icosahedral symmetry assemble spontaneously due to subunit-subunit interactions. Bead colors map the number of neighbors.

Figure 6:

Representative snapshots of defect-free shells (A) and of shells with vacancies (B), assembled around short NRs. Colors indicate the number of nearest neighbors. Parameters are: NR radius $R_{\text{nr}} = 5$ nm, capsomer preferred curvature radius $R_{\text{capsomer}} = 8$ nm, and NR length $L_{\text{nr}} = 22.5$ nm in A, and $L_{\text{nr}} = 22.5$ nm in B. C. For comparison, TEM image examples from our experiments, with bold arrows pointing the presence of defect. D. Phase diagram showing the most frequent outcome (defect-free or with vacancies) of short nanorods as a function of the capsomer radius and NR length.

Figure 7:

Long NR simulations. A. A sufficient match between the preferred curvature of the capsomer and the curvature of the cylindrical template $\gamma_{nr} \gtrsim 8/5$ results in complete particles, such as this example for $R_{\text{capsomer}} = 14$ nm and $R_{\text{nr}} = 9$ nm. B,C. A mismatch between the preferred curvature of the capsomer and the curvature of the cylindrical template $\gamma_{nr} < 8/5$ leads to incomplete particles with ring defects, such as these examples for $R_{\text{capsomer}} = 11$ nm and $R_{\text{nr}} = 9$ nm (B) and $R_{\text{capsomer}} = 8$ nm and $R_{\text{nr}} = 9$ (C).

Figure 8:

Histogram of the chiral angles for particles assembled above and below the threshold curvature mismatch with the cylindrical portion of the nanorod, with $R_{nr} = 8.0$ nm, $L_{nr} = 61.0$ nm: (A) $\gamma_{nr} = 1.63$ and (B) $\gamma_{nr} = 1.50$, based on 75 independent simulations for each parameter set. A. (i) Representative snapshot of a defect-free particle assembled at conditions favoring a ring structure. (ii) Simulation snapshot showing a particle with vacancies. (iii) A typical particle with vacancies obtained in our experiments showing a ring orientation of the hexagonal lattice ($\theta \approx 30^\circ$). B. (i) Representative snapshot of a defect-free particle with a roughly aligned lattice ($\theta = 2.9^\circ$). (ii) Snapshot showing a defect-free particle with $\theta = 10.9^\circ$. (iii) A typical defect-free particle from our experiments showing a roughly aligned orientation of the hexagonal lattice ($\theta \approx 5^\circ$).

Figure 9: Density of adsorbed capsomers as a function of time, for the spherical (blue) and cylindrical (red) regions, for different capsomer curvatures: A. $R_{\text{capsomer}} = 9.5\text{nm}$, B. $R_{\text{capsomer}} = 14.0\text{nm}$, C. $R_{\text{capsomer}} = 21.5\text{nm}$. Each curve represents the average over ten independent simulations. The time at which nucleation occurs is indicated with a dashed line. Spherocylinder dimensions are: $R_{nr} = 9.5\text{nm}$ and $L_{nr} = 40.0\text{nm}$. The lower panels show representative snapshots of an early stage of assembly for the three cases.