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Design of *in vitro* Symmetric Complexes and Analysis by Hybrid Methods Reveal Mechanisms of HIV Capsid Assembly

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Abstract

Unlike the capsids of icosahedral viruses, retroviral capsids are pleomorphic, with variably curved, closed fullerene shells composed of ~250 hexamers and exactly 12 pentamers of the viral CA protein. Structures of CA oligomers have been difficult to obtain because the subunit-subunit interactions are inherently weak, and CA tends to spontaneously assemble into capsid-like particles. Guided by a cryoEM-based model of the hexagonal lattice of HIV-1 CA, we used a two-step biochemical strategy to obtain soluble CA hexamers and pentamers for crystallization. First, each oligomer was stabilized by engineering disulfide cross-links between the N-terminal domains of adjacent subunits. Second, the cross-linked oligomers were prevented from polymerizing into hyperstable, capsid-like structures by mutations that weakened the dimeric association between the C-terminal domains that link adjacent oligomers. The X-ray structures revealed that the oligomers are comprised of a fairly rigid, central symmetric ring of N-terminal domains encircled by mobile C-terminal domains. Assembly of the quasi-equivalent oligomers requires remarkably subtle rearrangements in inter-subunit quaternary bonding interactions, and appears to be controlled by an electrostatic switch that favors hexamers over pentamers. An atomic model of the complete HIV-1 capsid was then built using the fullerene cone as a template. Rigid-body rotations around two assembly interfaces are sufficient to generate the full range of continuously varying lattice curvature in the fullerene cone. The steps in determining this HIV-1 capsid atomic model exemplify how structural biology can be leveraged by the use of hybrid methods, a powerful approach for exploring the structure of pleomorphic macromolecular complexes.

Keywords

HIV-1 capsid; hybrid methods; engineered disulfide bonds; electron microscopy; X-ray crystallography

Introduction^{1; 2; 3}

During the late stages of their replication cycle, retroviruses assemble as “immature” virions composed of 2000–4000 copies of the virally encoded Gag polyprotein, which are radially

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arranged, adherent to the inner leaflet of the viral membrane and closely associated as a hexagonal, paracrystalline lattice with a unit cell spacing of ~80 Å (Figure 1(b, d) ^{4; 5; 6; 7; 8}. The HIV-1 Gag protein is composed of four independently folded domains that are demarcated by flexible regions (Figure 1(a)). The N-terminal domain of Gag corresponds to the mature MA protein ^{9; 10}, which targets Gag to assembly sites at the plasma membrane and facilitates incorporation of the envelope glycoproteins into the assembling virion. MA is myristoylated and has a basic patch of amino acids that mediate attachment to the inner leaflet of the viral envelope ^{11; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22; 23}. The mature CA protein is formed by independently folded N- terminal and C-terminal domains, designated as CA^{NTD} and CA^{CTD}, connected by a flexible linker ^{24; 25; 26; 27}. These domains mediate the Gag-Gag contacts in the immature shell and mature capsid. In addition, HIV-1 Gag, in particular, contains two spacer peptides, called SP1 and SP2, which respectively demarcate the CA/NC and NC/p6 domains. Residues at the CA/SP1 boundary have been predicted to adopt an α -helical secondary structure that participate in forming Gag-Gag contacts in the immature shell ^{28; 29; 30; 31; 32; 33}. Analogous regions have been identified in biochemical and structural analyses of other orthoretroviruses ^{34; 35; 36}, suggesting that structural roles of the CA/SP1 boundary residues are conserved. The NC protein contains two zinc fingers that bind the RNA genome ^{37; 38; 39; 40}. High-affinity NC-RNA interactions enable specific recognition and packaging of two copies of the genome, whereas low-affinity interactions promote Gag assembly ^{41; 42; 43}. HIV-1 Gag contains an additional region at its C-terminus, called p6, which is specific to lentiviruses. The p6 sequence lacks a defined tertiary structure, but contains two peptide motifs (called “late domains”) that function as docking sites for the cellular ESCRT machinery ⁴⁴, which facilitates the release of newly assembled virions from the cell surface ^{45; 46; 47; 48; 49}. (Other orthoretroviral Gag proteins harbor late domains with equivalent functions, but at different positions in the Gag primary sequence.)

Cleavage of the Gag polyprotein in “immature” retroviruses results in dramatic morphological changes that result in “mature” infectious particles

Concomitant with budding and release from an infected cell, virions undergo a process called “maturation,” during which the viral protease cleaves the Gag polyprotein into its component domains ⁵⁰, resulting in dramatic morphological rearrangements ^{5; 6; 51; 52; 53; 54} (compare Figure 1(b) and (d) with (c) and (e)). Cleavage of Gag and assembly of the mature capsid are essential for infectivity. Consequently, understanding the mechanisms that guide maturation and assembly of the mature capsid not only provide insight into the design principles of retroviral capsids but may also provide clues for novel therapeutic strategies.

After Gag cleavage, MA remains associated with the viral membrane ^{5; 6}. Gag cleavage disassembles the immature lattice and liberates the subunits of the mature capsid (the CA protein) from the central region of the Gag precursor. CA is the last mature protein to be released as a result of sequential processing of Gag’s proteolytic sites. The flexible linker between CA^{NTD} and CA^{CTD} is not cleaved by the viral protease, and ~1500 copies of CA oligomerize to form a closed “mature” capsid that surrounds NC/RNA complex.

Although a precise understanding of the molecular transformations that occur during maturation must await a high-resolution structure of the immature Gag lattice, deuterium exchange and mutagenesis studies indicate that the two CA domains (CA^{NTD} and CA^{CTD}) utilize the same or highly overlapping interfaces to mediate interactions in both the immature and mature lattices ^{55; 56; 57; 58}. It is likely then that the tertiary structures of these two domains are largely preserved in the Gag polyprotein and mature CA, except for residues in the immediate vicinity of the proteolytic sites ^{30; 32; 59; 60}.

Proteolytic cleavage at the N-terminal end of CA releases the protein from the upstream MA domain and results in refolding of the first 13 residues of HIV-1 CA from an extended conformation into a β -hairpin (Figure 2(c)). The β -hairpin is stabilized by a salt bridge between the new Pro1 imino group and a conserved acidic residue (Asp51 in HIV-1) ^{26; 61}. Formation of the β -hairpin probably destabilizes the immature CA^{NTD} hexamer and facilitates proper juxtaposition of the adjacent domains in the mature hexamer conformation ^{60; 61; 62; 63}. Interestingly, it appears that folding of the β -hairpin does not proceed immediately after cleavage of the MA/CA junction, but after the final proteolytic processing step at the CA/SP1 junction ⁵⁸. Thus, cleavage at the CA C-terminus appears to be the dominant maturation switch. Indeed, mutagenesis of this region is particularly detrimental to capsid assembly and virus infectivity ^{28; 64; 65; 66}. Proteolysis of the CA/SP1 junction is expected to unravel the putative helical bundle in this region that helps stabilize the immature lattice, and indeed, the last 11 residues of CA appear to be disordered in the mature capsid ^{67; 68}.

Studies of other orthoretroviruses have revealed comparable structural switches at the CA termini. For example, folding of the β -hairpin during maturation appears to be a general switch, because N-terminal extensions shift the *in vitro* assembly phenotypes of HIV-1, RSV and MPMV CA proteins from mature-like particles to immature-like particles ^{61; 63; 69; 70}. Interestingly, spacer peptides upstream of the CA domains of RSV and MPMV appear to make important protein-protein interactions in the immature lattice that are not present in HIV-1 ^{71; 72; 73}. The best-characterized example is the “p10” region immediately upstream of RSV CA, which folds into a helix and packs against the globular CA^{NTD} domain ⁷⁴. This interaction is important for RSV Gag hexamerization and lattice assembly ^{72; 73}. Assembly regions downstream of CA, which are analogous to the SP1 spacer in HIV-1, have also been identified in MLV, RSV and MPMV Gag, even though these proteins lack a bona fide spacer between their CA and NC domains ^{34; 35; 36}. Indeed, pillar-like densities below the CA^{CTD} hexamer layer are also visible in cryotomographic reconstructions of immature MPMV and RSV particles, although the MPMV pillar appears much smaller than in either HIV-1 or RSV ³⁵. These examples highlight both the degree of conservation in the assembly principles of orthoretroviruses and the structural/mechanistic variations that different viruses use to reach the same endpoint.

Mature retroviral capsids are pleomorphic and adopt multiple shapes

The mature capsids of orthoretroviruses adopt different preferred shapes, and are primarily cones in HIV-1 and lentiviruses, cylinders in betaretroviruses (e.g., MPMV), and polyhedral or “spherical” in others (e.g., RSV, MLV) ⁷⁵ (Figure 2a). The various shapes are a consequence of the distribution of the pentamers within a curved hexagonal lattice: “spherical” capsids have the 12 pentamers distributed randomly, cylinders have 6 pentamers at each end of a tube, and cones have 5 pentamers at the narrow end and 7 at the wide end (Figure 2(b)) ^{53; 54; 76; 77}.

The architecture of the mature capsid of HIV-1 can be described by the geometric principles of fullerene shells ^{78; 79; 80; 81}. The body of the capsid is a cone-shaped two-dimensional lattice of CA hexamers. By analogy to quasi-equivalent icosahedral viruses, the hexagonal capsid lattice incorporates 12 pentamers to form a closed shell. The pentamers are also composed of CA, and are distributed asymmetrically across the capsid shell. Thus, the capsid itself is globally asymmetric even though it is composed of locally symmetric building blocks.

The CA N-terminal domain (CA^{NTD}) and C-terminal domain (CA^{CTD}) of various retroviruses have a conserved tertiary structure in spite of minimal sequence homology

The CA^{NTD} is an arrowhead shaped domain composed of seven α -helices (numbered 1–7)^{26; 59; 60} (Figure 2(c)), and the CA^{CTD} is a globular domain composed of a short 3_{10} helix, a highly conserved sheet-turn-helix element termed the major homology region (MHR), and four α -helices (numbered 8–11)^{25; 30} (Figure 2(d)). The isolated CA^{CTD} is a dimer in solution^{25; 82; 83}, and high-resolution X-ray and NMR structures of the isolated CA^{CTD} dimer have identified at least two dimerization modes (side-by-side and domain-swapped, with at least 3 possible side-by-side configurations)^{25; 30; 82; 83; 84}.

Hybrid methods: visualizing the HIV capsid from all angles

As a result of the pleomorphic morphology and lack of icosahedral symmetry of retroviral capsids^{5; 6}, it is not possible to crystallize intact capsids and determine their high-resolution structures by X-ray crystallography, as is the case for viruses that manifest strict icosahedral symmetry. Although full-length CA can be expressed and purified in milligram quantities, the flexible linker between CA^{NTD} and CA^{CTD} and the inherently weak interactions within CA hexamers have also thwarted 3D crystallization. In addition, expressed CA tends to spontaneously assemble into capsid-like particles, further confounding crystallization. A “divide and conquer” hybrid approach has therefore been used to gain insight into mechanisms of retroviral capsid assembly (Figure 3). For example, the individual CA^{NTD} and CA^{CTD} domains have been separately expressed and solved by X-ray crystallography and NMR spectroscopy (Figure 2(c) and (d)). Extensive mutagenesis, proteolysis and mass spectrometry studies have implicated specific residues that mediate assembly, which can be mapped onto the high-resolution structures of the CA^{NTD} and CA^{CTD} domains.

Electron cryomicroscopy and cryotomography of mature virions and capsids of HIV-1^{53; 54; 85} and RSV⁷⁷ have revealed the overall dimensions and capsid morphologies and shown that the hexamer-to-hexamer spacing in the mature lattice is 90–100 Å⁸⁵. The mature CA hexamer is distinct from the immature Gag hexamer, and therefore the inter-subunit interactions mediated by CA^{NTD} and CA^{CTD} must rearrange during maturation.

To a large extent, our understanding of the quaternary interactions in the mature capsid is derived from analyses of *in vitro* model systems that are assembled from purified recombinant CA proteins^{68; 86; 87; 88; 89; 90}. These assemblies recapitulate the local symmetry of the building blocks but are also globally symmetric, and are thus more amenable to biochemical and structural analyses (Figure 4). For example, helical tubes composed of CA hexamers have provided low-resolution views of the hexagonal lattice^{80; 83}, intermediate-resolution two-dimensional crystals of the hexagonal CA lattice have revealed elements of secondary structure⁶⁸, and icosahedral assemblies of RSV CA allowed the first visualization of the CA pentamer^{91; 92}. Biochemical and mutagenesis experiments have established that these *in vitro* assemblies faithfully recapitulate the lattice interactions of CA subunits in mature capsids^{55; 56; 61; 76; 88; 90}. Docking of high-resolution structures of the CA^{NTD} and CA^{CTD} domains into the cryoEM maps has provided working atomic models that have enabled further mutagenesis to stabilize CA oligomers.

Disulfide cross-linking of CA guided by a cryoEM-based atomic model

Wild-type full-length HIV-1 CA assembles *in vitro* into cylinders with helical symmetry^{80; 86}. In contrast, a single point mutation, R18A, shifted the assembly phenotype into spheres, cones, and cylinders – the three mature capsid shapes observed in

retrovirions⁷⁶. Replacement of R18 with a large hydrophobic residue (i.e., Val, Ile, Leu, Phe) generated CA proteins that assembled predominantly as spheres^{67; 68}. By the use of sparse matrix crystallization conditions, a buffer was identified in which CA assembled as large spheres up to ~2.5 μm in diameter. When applied to a continuous carbon EM grid, these large spheres collapsed and flattened, and behaved as well-ordered 2D crystals, exhibiting diffraction beyond 10 $\text{\AA}$ resolution. Electron cryocrystallography yielded a 3D map of full-length HIV-1 CA with an in-plane resolution of 9.0 $\text{\AA}$. Rods of density could be readily identified as α -helices and served as fiducials for docking high-resolution structures of the CA^{NTD} and CA^{CTD}. This cryoEM based model served as the basis for designing disulfide crosslinks to stabilize the CA hexamer.

Strategy for screening disulfide crosslinks using an *in vitro* assembly assay

A critical aspect of disulfide crosslinking of oligomeric complexes is the ability to detect crosslinks that interfere with formation of the native complex or the generation of spurious crosslinks resulting from disulfide exchange. As noted, CA assembles as long tubes in 1 M NaCl and near neutral pH that faithfully mimic the hexagonal portion of the HIV-1 capsid lattice^{76; 80; 85; 86; 87; 88} (Figures 4 and 5). Within the assembled cylinders, one or more of the engineered cysteine pairs would be positioned optimally to form intermolecular disulfide bonds and, upon oxidation, create a covalently linked hexamer. Our expectation was that subsequent incubation at low ionic strength would release individual disulfide-bonded hexamers (Figure 5).

A two-step biochemical strategy for generating soluble CA hexamers and pentamers for X-ray crystallography

Guided by the cryoEM-based atomic model⁶⁸, a panel of cysteine mutants was selected using a distance filter of ≤ 6 $\text{\AA}$ between C β -C β atoms⁶² (Figure 6(a)). Within the assembled tubes, if the cysteine pairs were at the appropriate distance and geometry, then they should spontaneously oxidize and form disulfide bonds. The assembly reactions were performed in a highly reducing assembly environment that discouraged formation of spuriously cross-linked species and promoted disulfide bonding only if the local geometry was favorable. Nonreducing SDS-PAGE gels demonstrated that several mutants migrated as ladders of cross-linked n-mers, with n=1–6, with minimal formation of higher molecular weight species (Figure 6(b)). This indicates that disulfide bond formation was being driven by the non-covalent intersubunit interactions within the assembled particles and not by random, nonspecific association of soluble protein, which would lead to higher molecular weight aggregates. As a negative control, the cysteine pair Q13C/E45C had an expected separation of ~10 $\text{\AA}$, and indeed, this mutant migrated exclusively as monomers.

A construct with cysteine substitutions for A14 and E45 displayed almost complete formation of 6-mers. In fact, this mutant assembled into cylinders that were hyperstable and remained intact even at low ionic strength, which would normally disintegrate tubes formed by wild-type CA. Consequently, a second biochemical step was introduced to weaken the extended lattice while leaving the hexamer-stabilizing interfaces unchanged. This was accomplished by introducing two mutations (W184A and M185A) within helix 9, which forms the CA^{CTD} dimer interface^{25; 55} (Figure 7(b)). Since the CA^{CTD} dimer interface does not directly stabilize the hexamer, the W184A and M185A mutations were not expected to affect formation of the cross-linked A14C/E45C hexamers. CA containing the mutations A14C/E45C/M184A/M185A could be isolated as a homogenous population of crosslinked

hexamers, and two different crystal forms yielded high-resolution X-ray structures that faithfully matched the cryoEM density map (Figure 8(g)).

Due to the possibility of perturbation of the CA structure by introduction of the disulfide, we determined the X-ray structure of a second disulfide pair. Even though this mutant (A42C/T54C) showed incomplete formation of 6mers (Figures 6(b) and 8(d)), the structure could be refined to 1.9 Å resolution. Furthermore, the structures of the (A14C/E45C) and (A42C/T45C) mutants superimposed with a root-mean-square deviation of only 0.4 Å on equivalent backbone atoms. Given this near identity of the independent structures, the non-mutated portions were combined to generate an atomic resolution model for the native hexamer (Figure 8(h) and (j)).

Note that the N21C/A22C mutant displayed a strong pentamer band (Figures 6(b) and 9(b)), and by the same strategy as described above, the X-ray structure of the CA pentamer was solved at 2.5 Å resolution (Figure 9(c)–(f)).

Helix-capping hydrogen bonds function as molecular pivots between the CA^{NTD} and CA^{CTD} domains and enable rigid-body rotations

In the assembled CA lattice, each CA^{CTD} domain packs against the CA^{NTD} domain from the adjacent subunit, via an intermolecular set of interactions called the NTD-CTD interface^{56; 68; 93} (Figure 10). A critical feature of the NTD-CTD interface is that it contains a set of direct protein-protein hydrogen bonds, each of which is a helix capping interaction, and connects a flexible polar side chain in one domain with a helix terminus in the other domain^{67; 94}. These helix-capping hydrogen bonds act as molecular pivots for limited rigid-body rotations of CA^{CTD} relative to CA^{NTD} (indicated by red double-headed arrow in Figure 10(b)). In this manner, the NTD-CTD interface restricts the movement of the CA^{CTD} dimers relative to the CA^{NTD} rings. Each dimer can only rotate about a fixed axis that is approximately parallel to the plane of the ring, and this flexion allows each CA^{NTD} ring to adopt slightly different bite angles relative to its neighbors. The NTD-CTD interface therefore has the essential function of channeling the native flexibility of the CA protein into a mechanism for generating variable lattice curvature. This feature is common to the hexamer and pentamer, since an identical set of interactions is found in both capsomers^{67; 94}.

The CA^{NTD} hexamer and pentamer are stabilized by 18 and 15 helix bundles, respectively

The CA^{NTD} hexamer is a 6-fold symmetric ring that is organized around an 18-helix barrel composed of the first three helices of each subunit^{67; 68; 95} (Figure 8(g) and (i)). The quaternary organization of the CA^{NTD} pentamer and hexamer are quite similar, and the pentamer subunits form a 5-fold symmetric ring organized around a 15-helix barrel^{91; 92; 94} (Figures 9(c) and (e)).

Even though the packing angles between adjacent subunits are different in the hexamer and pentamer, the subunits in each oligomer display a similar 3-helix repeat unit – stabilized by essentially identical hydrophobic interactions⁹⁴ (Figure 11(c)). The repeating interaction is a 3-helix bundle formed by length-wise packing of helix 2 from one subunit against helices 1 and 3 of the adjacent subunit (Figure 11(a) and (b)). In HIV-1, the center of the 3-helix bundle contains a small hydrophobic core composed of aliphatic sidechains (Figure 11(c)), whereas interactions at the periphery are mediated by polar residues. Direct electrostatic protein-protein contacts are conspicuously absent, and essentially all the inter-subunit hydrogen bonds are bridged by water molecules⁶⁷.

The CA hexamer and pentamer are quasiequivalent

Switching between the CA^{NTD} hexamer and pentamer follows the general model for quasi-equivalence as originally proposed by Caspar and Klug⁹⁶. Alternative packing of subunits in the two oligomers occurs by a simple rotation of adjacent subunits, about an axis that appears to coincide with the precise center of the 3-helix repeat unit⁹⁴ (Figure 11). Residues at the center of the 3-helix repeat unit can therefore maintain essentially the same hydrophobic packing interactions in both oligomers, and only subtle rearrangements in the hydrogen bonding interactions at the inner and outer rims of the CA^{NTD} rings are required.

ARG18 is functions as an electrostatic switch that disfavors pentamer formation

The energetic landscape of hexamer vs. pentamer assembly appears to be controlled by an electrostatic switch^{91; 94}. In the case of HIV-1 CA, an almost universally conserved arginine residue (Arg18) occupies an annulus at the centers of both the hexamer and pentamer (Figure 12(c) and (d)). Juxtaposition of like charges creates electrostatic repulsion, which is greater for the pentamer because the arginine residues are closer to each other in the 5-fold ring⁹⁴. Indeed, substitution of Arg18 with uncharged residues promotes pentamer formation *in vitro*^{68; 76}. Electrostatic destabilization of the pentamer is most probably counterbalanced by local assembly rules and cooperativity, such that a pentamer is integrated into the assembling capsid lattice only at a position where incorporation of a hexamer is incompatible with the local lattice curvature.

CA^{CTD} domains form dimeric linkers between hexamers in the lattice

The CA^{NTD} hexamers and pentamers are linked into a continuous lattice by symmetric CA^{CTD} dimers (Figure 7), with side-by-side interacting subunits (i.e., not domain-swapped). The affinity of the mature HIV-1 CA^{CTD} dimer is measurable in solution, with a dissociation constant (K_d) of $\sim 10^{-5}$ M^{25; 83}. Characterized CA proteins of other orthoretroviruses remain monomeric in solution^{97; 98; 99}, but also form analogous dimers under conditions that promote capsid assembly^{100; 101}. The CA^{CTD} dimer interface is mediated by symmetric packing of helix 9 across the dyad, and hydrophobic interactions between the 3₁₀ helix of one subunit and helix 9 of the other^{25; 30; 83}. The lattice-stabilizing dimer interface is the same as the solution dimer interface^{68; 83}, implying that the basic assembly unit of the mature capsid is composed of two CA subunits linked by CA^{CTD}.

Rigid-body rotation at the CA^{CTD} dimer interface

Biochemical and structural analyses also indicate that the CA^{CTD} domain is conformationally flexible^{68; 82; 84; 102; 103; 104}. For example, comparison of X-ray^{25; 30} and NMR⁸³ structures of full-affinity CA^{CTD} dimers reveal slight variations in the structure of the dimerization helix, which occur at both the tertiary level (in terms of helix 9 packing against other helices in the same domain) and quaternary level (in terms of the helix 9 crossing angle across the dimer dyad) (Figure 12(b)). Flexibility of the CA^{CTD} dimer might have functional implications, since twisting at the dimer interface may also dictate lattice curvature (indicated by red double-headed arrows in Figure 10(b))^{68; 103}. Indeed, computational modeling of the HIV-1 capsid suggests that proper formation of the pentameric declinations requires a flexible dimer interface⁹⁴.

A cryoEM reconstruction of helical tubes composed of HIV-1 CA hexamers indicates that adjacent CA^{CTD} domains surrounding the 3-fold axes of the hexagonal lattice may also interact via extended polar residues⁸³. This interaction is not observed in structures of

flattened lattices of CA⁶⁸, and therefore appears to specifically stabilize subunit packing in a curved lattice. In the assembled capsid, it is likely that interactions across the 3-fold interface vary in response to different degrees of rotation at the NTD-CTD interface^{67; 83; 91}.

Rigid-body rotations around two assembly interfaces are sufficient to generate the full range of continuously varying lattice curvature in the fullerene cone

Having on hand a complete gallery of high-resolution structures of the building blocks allowed us to build an atomic model of the HIV-1 capsid using the fullerene cone as a template (Figure 13). A key characteristic of the HIV-1 fullerene cone capsid is that the curvature of the hexagonal CA lattice changes continuously^{78; 80} (Figure 13(b)). This is most easily appreciated by considering the bite angle between adjacent hexamers. The bite angles change continuously, and range from around 135° between two hexamers connected to the same pentamer at the ends of the cone to around 180° in more flat regions at the body of the cone (Figure 13(c)).

In our modeling, the NTD hexamers, NTD pentamers and CTD dimers were treated as rigid bodies. Within the body of the cone, the CA subunits were arranged on a hexagonal lattice with a unit cell spacing of ~93 Å. The full range of variable lattice curvature in this region could be modeled by introducing small rigid-body rotations across the NTD-CTD interfaces, as observed in the independent views of the CA hexamers observed in different crystal forms (Figure 9(b)), while keeping the NTD-NTD hexamerization and CTD-CTD dimerization interfaces constant (Figure 12(b) and (e)). The CA protein has the requisite flexibility for creating such a variably curved lattice, because the CA^{NTD} and CA^{CTD} domains are linked by a flexible stretch of 4 amino acid residues. Indeed, NMR analyses of full-length CA proteins in solution demonstrate that the two domains can rotate almost independently of each other^{97; 98}. In the assembled lattice, the relative orientations of the CA^{NTD} rings and CA^{CTD} dimers can also vary^{67; 83; 91}.

The lattice curvature is most pronounced at the insertion sites of the pentameric declinations (Figures 12(a) and 13(a)). In modeling the pentameric declinations, we found that the CTD dimers must span different distances when connecting hexamers to hexamers and hexamers to pentamers (Figure 12(a)). Surprisingly, these distances were in close correspondence to the dimensions of two independently determined X-ray and NMR CTD dimer structures (2KOD and 1A43) (Figure 12(b)). Although the structures were solved from slightly different protein constructs, both retained the dimerization affinity of full-length CA. However, each exhibited a distinct subunit packing geometry across the dimer dyad (Figure 12(b)). Therefore the 2KOD dimer was used to connect hexamers to hexamers, and the 1A43 dimer was used to connect hexamers to pentamers. Models wherein the NTD rings were connected by either dimer alone displayed significant backbone clashes (2KOD) or relatively large separations (1A43) between subunits surrounding the declinations (not shown). This suggests that rotation or slippage at the CTD-CTD interface may be a mechanistic element of capsid assembly.

It is remarkable that our simple modeling approach, which allowed just two types of rigid-body rotation between the building blocks, produced a fullerene model wherein essentially all the subunits displayed reasonable packing geometries. On the basis of this analysis, we concluded that CA assembly entails flexibility at both the NTD-CTD interface and the dimer interface to generate the constantly varying lattice curvature in the HIV-1 capsid⁹⁴.

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Abbreviations

βME	β-mercaptoethanol
2D	two-dimensional
3D	three-dimensional
CA^{CTD}	C-terminal domain of CA
CA^{NTD}	N-terminal domain of CA
cryoEM	electron cryomicroscopy
HIV-1	human immunodeficiency virus type 1
HTLV	human T-cell leukemia virus
MLV	murine leukemia virus
RSV	Rous sarcoma virus

References

1. Ganser-Pornillos BK, Yeager M, Sundquist WI. The structural biology of HIV assembly. *Curr Opin Struct Biol.* 2008; 18:203–17. [PubMed: 18406133]
2. Ganser-Pornillos, BK.; Yeager, M.; Pornillos, O.; Rossmann, MGaR; VB, editors. *Viral Molecular Machines*. Springer; 2011. *Assembly and Architecture of HIV*. in press
3. Goff, S.; Knipe, David; Peter Howley, AEDG.; Lamb, Robert; Martin, Malcolm; Roizman, Bernard; Straus, Stephen. *Retroviridae: The Retroviruses and Their Replication*. Lippincott Williams & Wilkins; Philadelphia, PA: 2007.
4. Carlson LA, Briggs JA, Glass B, Riches JD, Simon MN, Johnson MC, Muller B, Grunewald K, Kräusslich HG. Three-dimensional analysis of budding sites and released virus suggests a revised model for HIV-1 morphogenesis. *Cell Host Microbe.* 2008; 4:592–9. [PubMed: 19064259]
5. Fuller SD, Wilk T, Gowen BE, Kräusslich HG, Vogt VM. Cryo-electron microscopy reveals ordered domains in the immature HIV-1 particle. *Curr Biol.* 1997; 7:729–38. [PubMed: 9368755]
6. Yeager M, Wilson-Kubalek EM, Weiner SG, Brown PO, Rein A. Supramolecular organization of immature and mature murine leukemia virus revealed by electron cryo-microscopy: implications for retroviral assembly mechanisms. *Proc Natl Acad Sci U S A.* 1998; 95:7299–304. [PubMed: 9636143]
7. Briggs JA, Riches JD, Glass B, Bartonova V, Zanetti G, Kräusslich HG. Structure and assembly of immature HIV. *Proc Natl Acad Sci U S A.* 2009; 106:11090–5. [PubMed: 19549863]
8. Wright ER, Schooler JB, Ding HJ, Kieffer C, Fillmore C, Sundquist WI, Jensen GJ. Electron cryotomography of immature HIV-1 virions reveals the structure of the CA and SP1 Gag shells. *EMBO J.* 2007; 26:2218–26. [PubMed: 17396149]
9. Massiah MA, Starich MR, Paschall C, Summers MF, Christensen AM, Sundquist WI. Three-dimensional structure of the human immunodeficiency virus type 1 matrix protein. *J Mol Biol.* 1994; 244:198–223. [PubMed: 7966331]
10. Hill CP, Worthylake D, Bancroft DP, Christensen AM, Sundquist WI. Crystal structures of the trimeric human immunodeficiency virus type 1 matrix protein: implications for membrane association and assembly. *Proc Natl Acad Sci U S A.* 1996; 93:3099–104. [PubMed: 8610175]

11. Göttinger HG, Sodroski JG, Haseltine WA. Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1. *Proc Natl Acad Sci U S A*. 1989; 86:5781–5. [PubMed: 2788277]
12. Spearman P, Wang JJ, Vander Heyden N, Ratner L. Identification of human immunodeficiency virus type 1 Gag protein domains essential to membrane binding and particle assembly. *J Virol*. 1994; 68:3232–42. [PubMed: 8151785]
13. Zhou W, Parent LJ, Wills JW, Resh MD. Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 Gag protein which interacts with acidic phospholipids. *J Virol*. 1994; 68:2556–69. [PubMed: 8139035]
14. Spearman P, Horton R, Ratner L, Kuli-Zade I. Membrane binding of human immunodeficiency virus type 1 matrix protein in vivo supports a conformational myristyl switch mechanism. *J Virol*. 1997; 71:6582–92. [PubMed: 9261380]
15. Paillart JC, Göttinger HG. Opposing effects of human immunodeficiency virus type 1 matrix mutations support a myristyl switch model of Gag membrane targeting. *J Virol*. 1999; 73:2604–12. [PubMed: 10074105]
16. Ono A, Demirov D, Freed EO. Relationship between human immunodeficiency virus type 1 Gag multimerization and membrane binding. *J Virol*. 2000; 74:5142–50. [PubMed: 10799589]
17. Tang C, Loeliger E, Luncsford P, Kinde I, Beckett D, Summers MF. Entropic switch regulates myristate exposure in the HIV-1 matrix protein. *Proc Natl Acad Sci U S A*. 2004; 101:517–22. [PubMed: 14699046]
18. Ono A, Ablan SD, Lockett SJ, Nagashima K, Freed EO. Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane. *Proc Natl Acad Sci U S A*. 2004; 101:14889–94. [PubMed: 15465916]
19. Saad JS, Miller J, Tai J, Kim A, Ghanam RH, Summers MF. Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly. *Proc Natl Acad Sci U S A*. 2006; 103:11364–9. [PubMed: 16840558]
20. Li H, Dou J, Ding L, Spearman P. Myristoylation is required for human immunodeficiency virus type 1 Gag-Gag multimerization in mammalian cells. *J Virol*. 2007; 81:12899–910. [PubMed: 17881447]
21. Datta SAK, Zhao Z, Clark PK, Tarasov S, Alexandratos JN, Campbell SJ, Kvaratskhelia M, Lebowitz J, Rein A. Interactions between HIV-1 Gag molecules in solution: an inositol phosphate-mediated switch. *J Mol Biol*. 2007; 365:799–811. [PubMed: 17098251]
22. Dalton AK, Ako-Adjei D, Murray PS, Murray D, Vogt VM. Electrostatic interactions drive membrane association of the human immunodeficiency virus type 1 Gag MA domain. *J Virol*. 2007; 81:6434–45. [PubMed: 17392361]
23. Dalton AK, Murray PS, Murray D, Vogt VM. Biochemical characterization of rous sarcoma virus MA protein interaction with membranes. *J Virol*. 2005; 79:6227–38. [PubMed: 15858007]
24. Gamble TR, Vajdos FF, Yoo S, Worthylake DK, Houseweart M, Sundquist WI, Hill CP. Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell*. 1996; 87:1285–94. [PubMed: 8980234]
25. Gamble TR, Yoo S, Vajdos FF, von Schwedler UK, Worthylake DK, Wang H, McCutcheon JP, Sundquist WI, Hill CP. Structure of the carboxyl-terminal dimerization domain of the HIV-1 capsid protein. *Science*. 1997; 278:849–53. [PubMed: 9346481]
26. Gitti RK, Lee BM, Walker J, Summers MF, Yoo S, Sundquist WI. Structure of the amino-terminal core domain of the HIV-1 capsid protein. *Science*. 1996; 273:231–5. [PubMed: 8662505]
27. Momany C, Kovari LC, Prongay AJ, Keller W, Gitti RK, Lee BM, Gorbalenya AE, Tong L, McClure J, Ehrlich LS, Summers MF, Carter C, Rossmann MG. Crystal structure of dimeric HIV-1 capsid protein. *Nat Struct Biol*. 1996; 3:763–70. [PubMed: 8784350]
28. Kräusslich HG, Fäcke M, Heuser AM, Konvalinka J, Zentgraf H. The spacer peptide between human immunodeficiency virus capsid and nucleocapsid proteins is essential for ordered assembly and viral infectivity. *J Virol*. 1995; 69:3407–19. [PubMed: 7745687]
29. Accola MA, Hoglund S, Göttinger HG. A putative α -helical structure which overlaps the capsid-p2 boundary in the human immunodeficiency virus type 1 Gag precursor is crucial for viral particle assembly. *J Virol*. 1998; 72:2072–8. [PubMed: 9499062]

30. Worthylake DK, Wang H, Yoo S, Sundquist WI, Hill CP. Structures of the HIV-1 capsid protein dimerization domain at 2.6 Å resolution. *Acta Crystallogr D Biol Crystallogr*. 1999; 55:85–92. [PubMed: 10089398]
31. Liang C, Hu J, Russell RS, Roldan A, Kleiman L, Wainberg MA. Characterization of a putative α -helix across the capsid-SP1 boundary that is critical for the multimerization of human immunodeficiency virus type 1 Gag. *J Virol*. 2002; 76:11729–37. [PubMed: 12388733]
32. Newman JL, Butcher EW, Patel DT, Mikhaylenko Y, Summers MF. Flexibility in the P2 domain of the HIV-1 Gag polyprotein. *Protein Sci*. 2004; 13:2101–7. [PubMed: 15238640]
33. Morellet N, Druillennec S, Lenoir C, Bouaziz S, Roques BP. Helical structure determined by NMR of the HIV-1 (345–392)Gag sequence, surrounding p2: implications for particle assembly and RNA packaging. *Protein Sci*. 2005; 14:375–86. [PubMed: 15659370]
34. Cheslock SR, Poon DTK, Fu W, Rhodes TD, Henderson LE, Nagashima K, McGrath CF, Hu WS. Charged assembly helix motif in murine leukemia virus capsid: an important region for virus assembly and particle size determination. *J Virol*. 2003; 77:7058–66. [PubMed: 12768025]
35. de Marco A, Davey NE, Ulbrich P, Phillips JM, Lux V, Riches JD, Fuzik T, Ruml T, Kräusslich HG, Vogt VM, Briggs JA. Conserved and variable features of Gag structure and arrangement in immature retrovirus particles. *J Virol*. 2010; 84:11729–36. [PubMed: 20810738]
36. Keller PW, Johnson MC, Vogt VM. Mutations in the spacer peptide and adjoining sequences in Rous sarcoma virus Gag lead to tubular budding. *J Virol*. 2008; 82:6788–97. [PubMed: 18448521]
37. Morellet N, Jullian N, De Rocquigny H, Maigret B, Darlix JL, Roques BP. Determination of the structure of the nucleocapsid protein NCp7 from the human immunodeficiency virus type 1 by 1H NMR. *EMBO J*. 1992; 11:3059–65. [PubMed: 1639074]
38. Morellet N, de Rocquigny H, Mely Y, Jullian N, Demene H, Ottmann M, Gerard D, Darlix JL, Fournie-Zaluski MC, Roques BP. Conformational behaviour of the active and inactive forms of the nucleocapsid NCp7 of HIV-1 studied by 1H NMR. *J Mol Biol*. 1994; 235:287–301. [PubMed: 8289249]
39. South TL, Blake PR, Sowder RC 3rd, Arthur LO, Henderson LE, Summers MF. The nucleocapsid protein isolated from HIV-1 particles binds zinc and forms retroviral-type zinc fingers. *Biochemistry*. 1990; 29:7786–9. [PubMed: 2261434]
40. Summers MF, Henderson LE, Chance MR, Bess JW Jr, South TL, Blake PR, Sagi I, Perez-Alvarado G, Sowder RC 3rd, Hare DR, et al. Nucleocapsid zinc fingers detected in retroviruses: EXAFS studies of intact viruses and the solution-state structure of the nucleocapsid protein from HIV-1. *Protein Sci*. 1992; 1:563–74. [PubMed: 1304355]
41. Jouvenet N, Simon SM, Bieniasz PD. Imaging the interaction of HIV-1 genomes and Gag during assembly of individual viral particles. *Proc Natl Acad Sci U S A*. 2009; 106:19114–9. [PubMed: 19861549]
42. Miyazaki Y, Garcia EL, King SR, Iyalla K, Loeliger K, Starck P, Syed S, Telesnitsky A, Summers MF. An RNA structural switch regulates diploid genome packaging by Moloney murine leukemia virus. *J Mol Biol*. 2010; 396:141–52. [PubMed: 19931283]
43. D'Souza V, Summers MF. Structural basis for packaging the dimeric genome of Moloney murine leukaemia virus. *Nature*. 2004; 431:586–90. [PubMed: 15457265]
44. Hurley JH, Emr SD. The ESCRT complexes: structure and mechanism of a membrane-trafficking network. *Annu Rev Biophys Biomol Struct*. 2006; 35:277–98. [PubMed: 16689637]
45. VerPlank L, Bouamr F, LaGrassa TJ, Agresta B, Kikonyogo A, Leis J, Carter CA. Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). *Proc Natl Acad Sci U S A*. 2001; 98:7724–9. [PubMed: 11427703]
46. Garrus JE, von Schwedler UK, Pornillos O, Morham SG, Zavitz KH, Wang HE, Wettstein DA, Stray KM, Cote M, Rich RL, Myszkowski DG, Sundquist WI. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell*. 2001; 107:55–65. [PubMed: 11595185]
47. Martin-Serrano J, Zang T, Bieniasz PD. HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nat Med*. 2001; 7:1313–9. [PubMed: 11726971]

48. Pornillos O, Alam SL, Davis DR, Sundquist WI. Structure of the Tsg101 UEV domain in complex with the PTAP motif of the HIV-1 p6 protein. *Nat Struct Biol.* 2002; 9:812–7. [PubMed: 12379843]
49. Wollert T, Wunder C, Lippincott-Schwartz J, Hurley JH. Membrane scission by the ESCRT-III complex. *Nature.* 2009; 458:172–7. [PubMed: 19234443]
50. Pettit SC, Sheng N, Tritch R, Erickson-Viitanen S, Swanstrom R. The regulation of sequential processing of HIV-1 Gag by the viral protease. *Adv Exp Med Biol.* 1998; 436:15–25. [PubMed: 9561194]
51. Nermut MV, Frank H, Schafer W. Properties of mouse leukemia viruses. 3. Electron microscopic appearance as revealed after conventional preparation techniques as well as freeze-drying and freeze-etching. *Virology.* 1972; 49:345–58. [PubMed: 4115401]
52. Yoshinaka Y, Luftig RB. Murine leukemia virus morphogenesis: cleavage of P70 in vitro can be accompanied by a shift from a concentrically coiled internal strand (“immature”) to a collapsed (“mature”) form of the virus core. *Proc Natl Acad Sci U S A.* 1977; 74:3446–50. [PubMed: 410020]
53. Briggs JA, Grünewald K, Glass B, Forster F, Kräusslich HG, Fuller SD. The mechanism of HIV-1 core assembly: insights from three-dimensional reconstructions of authentic virions. *Structure.* 2006; 14:15–20. [PubMed: 16407061]
54. 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; 346:577–88. [PubMed: 15670606]
55. von Schwedler UK, Stray KM, Garrus JE, Sundquist WI. Functional surfaces of the human immunodeficiency virus type 1 capsid protein. *J Virol.* 2003; 77:5439–50. [PubMed: 12692245]
56. Lanman J, Lam TT, Barnes S, Sakalian M, Emmett MR, Marshall AG, Prevelige PE Jr. Identification of novel interactions in HIV-1 capsid protein assembly by high-resolution mass spectrometry. *J Mol Biol.* 2003; 325:759–72. [PubMed: 12507478]
57. Lanman J, Lam TT, Emmett MR, Marshall AG, Sakalian M, Prevelige PE Jr. Key interactions in HIV-1 maturation identified by hydrogen-deuterium exchange. *Nat Struct Mol Biol.* 2004; 11:676–7. [PubMed: 15208693]
58. Monroe EB, Kang S, Kyere SK, Li R, Prevelige PE Jr. Hydrogen/deuterium exchange analysis of HIV-1 capsid assembly and maturation. *Structure.* 2010; 18:1483–91. [PubMed: 21070947]
59. Tang C, Ndassa Y, Summers MF. Structure of the N-terminal 283-residue fragment of the immature HIV-1 Gag polyprotein. *Nat Struct Biol.* 2002; 9:537–43. [PubMed: 12032547]
60. Kelly BN, Howard BR, Wang H, Robinson H, Sundquist WI, Hill CP. Implications for viral capsid assembly from crystal structures of HIV-1 Gag(1–278) and CA(N)(133–278). *Biochemistry.* 2006; 45:11257–66. [PubMed: 16981686]
61. von Schwedler UK, Stemmler TL, Klishko VY, Li S, Albertine KH, Davis DR, Sundquist WI. Proteolytic refolding of the HIV-1 capsid protein amino-terminus facilitates viral core assembly. *EMBO J.* 1998; 17:1555–68. [PubMed: 9501077]
62. Pornillos O, Ganser-Pornillos BK, Banumathi S, Hua Y, Yeager M. Disulfide bond stabilization of the hexameric capsomer of human immunodeficiency virus. *J Mol Biol.* 2010; 401:985–95. [PubMed: 20600115]
63. Gross I, Hohenberg H, Huckhagel C, Kräusslich HG. N-terminal extension of human immunodeficiency virus capsid protein converts the in vitro assembly phenotype from tubular to spherical particles. *J Virol.* 1998; 72:4798–810. [PubMed: 9573245]
64. Gross I, Hohenberg H, Wilk T, Wiegers K, Grattinger M, Muller B, Fuller S, Kräusslich HG. A conformational switch controlling HIV-1 morphogenesis. *EMBO J.* 2000; 19:103–13. [PubMed: 10619849]
65. Li F, Goila-Gaur R, Salzwedel K, Kilgore NR, Reddick M, Matallana C, Castillo A, Zoumplis D, Martin DE, Orenstein JM, Allaway GP, Freed EO, Wild CT. PA-457: a potent HIV inhibitor that disrupts core condensation by targeting a late step in Gag processing. *Proc Natl Acad Sci U S A.* 2003; 100:13555–60. [PubMed: 14573704]

66. Müller B, Anders M, Akiyama H, Welsch S, Glass B, Nikovics K, Clavel F, Tervo HM, Keppler OT, Kräusslich HG. HIV-1 Gag processing intermediates trans-dominantly interfere with HIV-1 infectivity. *J Biol Chem.* 2009; 284:29692–703. [PubMed: 19666477]
67. Pornillos O, Ganser-Pornillos BK, Kelly BN, Hua Y, Whitby FG, Stout CD, Sundquist WI, Hill CP, Yeager M. X-ray structures of the hexameric building block of the HIV capsid. *Cell.* 2009; 137:1282–92. [PubMed: 19523676]
68. Ganser-Pornillos BK, Cheng A, Yeager M. Structure of full-length HIV-1 CA: a model for the mature capsid lattice. *Cell.* 2007; 131:70–9. [PubMed: 17923088]
69. Rumlova-Klikova M, Hunter E, Nermut MV, Pichova I, Ruml T. Analysis of Mason-Pfizer monkey virus Gag domains required for capsid assembly in bacteria: role of the N-terminal proline residue of CA in directing particle shape. *J Virol.* 2000; 74:8452–9. [PubMed: 10954545]
70. Campbell S, Vogt VM. In vitro assembly of virus-like particles with Rous sarcoma virus Gag deletion mutants: identification of the p10 domain as a morphological determinant in the formation of spherical particles. *J Virol.* 1997; 71:4425–35. [PubMed: 9151833]
71. Knejzlík Z, Smékalová Z, Ruml T, Sakalian M. Multimerization of the p12 domain is necessary for Mason-Pfizer monkey virus Gag assembly in vitro. *Virology.* 2007; 365:260–70. [PubMed: 17490704]
72. Joshi SM, Vogt VM. Role of the Rous sarcoma virus p10 domain in shape determination of gag virus-like particles assembled in vitro and within *Escherichia coli*. *J Virol.* 2000; 74:10260–8. [PubMed: 11024160]
73. Phillips JM, Murray PS, Murray D, Vogt VM. A molecular switch required for retrovirus assembly participates in the hexagonal immature lattice. *EMBO J.* 2008; 27:1411–20. [PubMed: 18401344]
74. Nandhagopal N, Simpson AA, Johnson MC, Francisco AB, Schatz GW, Rossmann MG, Vogt VM. Dimeric rous sarcoma virus capsid protein structure relevant to immature Gag assembly. *J Mol Biol.* 2004; 335:275–82. [PubMed: 14659756]
75. Vogt, VM. Retroviral virions and genomes. In: Coffin, JM.; Hughes, SH.; Varmus, HE., editors. *Retroviruses*. Cold Spring Harbor Laboratory Press; New York: 1997. p. 27-70.
76. Ganser-Pornillos BK, von Schwedler UK, Stray KM, Aiken C, Sundquist WI. Assembly properties of the human immunodeficiency virus type 1 CA protein. *J Virol.* 2004; 78:2545–52. [PubMed: 14963157]
77. Butan C, Winkler DC, Heymann JB, Craven RC, Steven AC. RSV capsid polymorphism correlates with polymerization efficiency and envelope glycoprotein content: implications that nucleation controls morphogenesis. *J Mol Biol.* 2008; 376:1168–81. [PubMed: 18206161]
78. Ganser BK, Li S, Klishko VY, Finch JT, Sundquist WI. Assembly and analysis of conical models for the HIV-1 core. *Science.* 1999; 283:80–3. [PubMed: 9872746]
79. Jin Z, Jin L, Peterson DL, Lawson CL. Model for lentivirus capsid core assembly based on crystal dimers of EIAV p26. *J Mol Biol.* 1999; 286:83–93. [PubMed: 9931251]
80. Li S, Hill CP, Sundquist WI, Finch JT. Image reconstructions of helical assemblies of the HIV-1 CA protein. *Nature.* 2000; 407:409–13. [PubMed: 11014200]
81. Heymann JB, Butan C, Winkler DC, Craven RC, Steven AC. Irregular and semi-regular polyhedral models for Rous sarcoma virus cores. *Comput Math Methods Med.* 2008; 9:197–210. [PubMed: 19122884]
82. Ivanov D, Tsodikov OV, Kasanov J, Ellenberger T, Wagner G, Collins T. Domain-swapped dimerization of the HIV-1 capsid C-terminal domain. *Proc Natl Acad Sci U S A.* 2007; 104:4353–8. [PubMed: 17360528]
83. Byeon IJ, Meng X, Jung J, Zhao G, Yang R, Ahn J, Shi J, Concel J, Aiken C, Zhang P, Gronenborn AM. Structural convergence between cryo-EM and NMR reveals intersubunit interactions critical for HIV-1 capsid function. *Cell.* 2009; 139:780–90. [PubMed: 19914170]
84. Bartonova V, Igonet S, Sticht J, Glass B, Habermann A, Vaney MC, Sehr P, Lewis J, Rey FA, Kräusslich HG. Residues in the HIV-1 capsid assembly inhibitor binding site are essential for maintaining the assembly-competent quaternary structure of the capsid protein. *J Biol Chem.* 2008; 283:32024–33. [PubMed: 18772135]
85. Briggs JA, Wilk T, Welker R, Kräusslich HG, Fuller SD. Structural organization of authentic, mature HIV-1 virions and cores. *EMBO J.* 2003; 22:1707–15. [PubMed: 12660176]

86. Ehrlich LS, Agresta BE, Carter CA. Assembly of recombinant human immunodeficiency virus type 1 capsid protein in vitro. *J Virol.* 1992; 66:4874–83. [PubMed: 1629958]
87. Campbell S, Vogt VM. Self-assembly in vitro of purified CA-NC proteins from Rous sarcoma virus and human immunodeficiency virus type 1. *J Virol.* 1995; 69:6487–97. [PubMed: 7666550]
88. Gross I, Hohenberg H, Kräusslich HG. In vitro assembly properties of purified bacterially expressed capsid proteins of human immunodeficiency virus. *Eur J Biochem.* 1997; 249:592–600. [PubMed: 9370371]
89. Kingston RL, Fitzon-Ostendorp T, Eisenmesser EZ, Schatz GW, Vogt VM, Post CB, Rossmann MG. Structure and self-association of the Rous sarcoma virus capsid protein. *Structure.* 2000; 8:617–28. [PubMed: 10873863]
90. Purdy JG, Flanagan JM, Ropson IJ, Rennoll-Bankert KE, Craven RC. Critical role of conserved hydrophobic residues within the major homology region in mature retroviral capsid assembly. *J Virol.* 2008; 82:5951–61. [PubMed: 18400856]
91. Cardone G, Purdy JG, Cheng N, Craven RC, Steven AC. Visualization of a missing link in retrovirus capsid assembly. *Nature.* 2009; 457:694–8. [PubMed: 19194444]
92. Hyun JK, Radjainia M, Kingston RL, Mitra AK. Proton-driven assembly of the Rous Sarcoma virus capsid protein results in the formation of icosahedral particles. *J Biol Chem.* 2010; 285:15056–64. [PubMed: 20228062]
93. Bowzard JB, Wills JW, Craven RC. Second-site suppressors of Rous sarcoma virus Ca mutations: evidence for interdomain interactions. *J Virol.* 2001; 75:6850–6. [PubMed: 11435564]
94. Pornillos O, Ganser-Pornillos BK, Yeager M. Atomic-level modelling of the HIV capsid. *Nature.* 2011; 469:424–7. [PubMed: 21248851]
95. Mortuza GB, Haire LF, Stevens A, Smerdon SJ, Stoye JP, Taylor IA. High-resolution structure of a retroviral capsid hexameric amino-terminal domain. *Nature.* 2004; 431:481–5. [PubMed: 15386017]
96. Caspar DL, Klug A. Physical principles in the construction of regular viruses. *Cold Spring Harb Symp Quant Biol.* 1962; 27:1–24. [PubMed: 14019094]
97. Khorasanizadeh S, Campos-Olivas R, Summers MF. Solution structure of the capsid protein from the human T-cell leukemia virus type-I. *J Mol Biol.* 1999; 291:491–505. [PubMed: 10438634]
98. Campos-Olivas R, Newman JL, Summers MF. Solution structure and dynamics of the Rous sarcoma virus capsid protein and comparison with capsid proteins of other retroviruses. *J Mol Biol.* 2000; 296:633–49. [PubMed: 10669613]
99. Mortuza GB, Goldstone DC, Pashley C, Haire LF, Palmarini M, Taylor WR, Stoye JP, Taylor IA. Structure of the capsid amino-terminal domain from the betaretrovirus, Jaagsiekte sheep retrovirus. *J Mol Biol.* 2009; 386:1179–92. [PubMed: 19007792]
100. Bailey GD, Hyun JK, Mitra AK, Kingston RL. Proton-linked dimerization of a retroviral capsid protein initiates capsid assembly. *Structure.* 2009; 17:737–48. [PubMed: 19446529]
101. Purdy JG, Flanagan JM, Ropson IJ, Craven RC. Retroviral capsid assembly: a role for the CA dimer in initiation. *J Mol Biol.* 2009; 389:438–51. [PubMed: 19361521]
102. Alcaraz LA, del Álamo M, Barrera FN, Mateu MG, Neira JL. Flexibility in HIV-1 assembly subunits: solution structure of the monomeric C-terminal domain of the capsid protein. *Biophys J.* 2007; 93:1264–76. [PubMed: 17526561]
103. Ternois F, Sticht J, Duquerroy S, Kräusslich HG, Rey FA. The HIV-1 capsid protein C-terminal domain in complex with a virus assembly inhibitor. *Nat Struct Mol Biol.* 2005; 12:678–82. [PubMed: 16041386]
104. Wong HC, Shin R, Krishna NR. Solution structure of a double mutant of the carboxy-terminal dimerization domain of the HIV-1 capsid protein. *Biochemistry.* 2008; 47:2289–97. [PubMed: 18220423]

Figure 1. Organization of immature and mature HIV-1 virions. (a) Schematic tertiary structural model of full-length HIV-1 Gag. Individual domains are in different colors and are labeled on the left. Schematic models of the (b) immature and (c) mature virions. Images of (d) immature and (e) mature virions preserved in vitreous ice. Modified and reprinted from ¹ with permission from Elsevier.

Figure 2.

(a) Morphologies of representative mature capsids of different orthoretroviruses. Images and fullerene models of Moloney murine leukemia virus (Mo-MLV, a gammaretrovirus), Mason-Pfizer monkey virus (MPMV, a betaretrovirus), and HIV-1 (a lentivirus) are shown.¹ In all cases, the capsids are organized as fullerene structures (b) that incorporate 12 pentamers (red) to close the curved hexagonal lattices. The CA N-terminal domain (CA^{NTD}) (c) and C-terminal domain (CA^{CTD}) (d) of various retroviruses have a conserved tertiary structure in spite of minimal sequence homology. Secondary structural elements are displayed in different colors. The NTD has an arrowhead shape, whereas the CTD is more globular.

Figure 3.

Illustration of the “divide and conquer” hybrid approach to structure determination of irregularly-shaped supramolecular complexes, such as the mature HIV-1 capsid. A working atomic model (center) is a synthesis of information derived from a variety of biochemical, biophysical and computational methods. For pleomorphic HIV-1 capsids, the methods (moving clockwise) include biochemical results based on mutagenesis, proteolysis, mass spectrometry, etc., low-resolution (20–50 Å) maps derived by electron cryotomography of authentic virions, (c) intermediate resolution (9–20 Å) cryoEM density maps of *in vitro* assemblies with helical, icosahedral and 2D crystalline symmetry, atomic resolution (1.5–3.5 Å) NMR and X-ray structures of building blocks, and models based on geometric principles and computational analysis.

Figure 4.

Gallery of *in vitro* symmetric assemblies for analysis of pleomorphic retroviral capsids: images of helical tubes, two-dimensional hexagonal crystals, and icosahedral particles. 3D density maps derived by image reconstruction of HIV-1 CA helical tubes⁸⁰, 2D crystals⁶⁸ and RSV icosahedral particles⁹¹. Note that the RSV “spheres” have icosahedral symmetry, and fall into two classes: T=1, composed of 12 CA pentamers, and T=3, composed of 20 hexamers and 12 pentamers⁹¹. Only the T=1 reconstruction is shown.

Figure 5.

Schematic representation of the disulfide crosslinking strategy to obtain soluble HIV-1 CA hexamers for 3D crystallization. The CA^{NTD} and CA^{CTD} are represented by the orange ovals and blue circles, respectively. Tubes assemble at high ionic strength, and disulfide crosslinks between adjacent CA^{NTD} domains (green lines) form under oxidizing conditions. Tubes assembled from wild type CA tubes disassemble at low ionic strength, but the crosslinked tubes were hyperstable. Therefore, two additional, destabilizing mutations were introduced at the CA^{CTD} dimer interface (red X's), so that the crosslinked hexamers could be generated by incubating the tubes at low ionic strength. Note that the approach has an embedded functional check, because hexamer assembly proceeds through a well-validated assembly assay.

Figure 6.

Design of disulfide-stabilized HIV-1 CA hexamers guided by a cryoEM based atomic model. (a) Top view ribbon representation of the 18-helix barrel comprising the CA^{NTD}-CA^{NTD} hexamerization interactions, as seen in the cryoEM model (3dik)^{67; 68}. Each subunit is colored differently. The locations of residues mutated to cysteines are indicated by spheres and are labeled within a single interface (between the yellow and blue subunits). Residue pairs are connected by black lines (broken lines for the Q13/E45 negative control). The A14/E45 and A42/T54 residue pairs are colored red and blue, respectively. (b) Non-reducing SDS-PAGE profiles of assembly reactions of HIV-1 CA double-cysteine mutants. Lanes 1–9 are wild-type HIV-1 CA, Q13C/E45C, A14C/E45C, P17C/R18C, P17C/T19C, P17C/A22C, N21C/A22C, P38C/N57C, and A42C/T54C. Molecular mass markers are labeled on the left, and the positions of cross-linked n-mers are indicated on the right.

Figure 7.

Top view of one sheet of the hexagonal lattice of 3D CA crystals, which recapitulates the hexameric lattice of authentic capsids at its planar limit ⁶⁷. The CA^{NTD} domains are colored orange, and the CA^{CTD} domains are blue. This view emphasizes that CA^{CTD} dimers link adjacent hexamers. (b) Two views of the CA^{CTD} dimers, indicating the positions of W184 and M185 in H9 that were mutated to weaken the dimer interactions in generating soluble hexamers.

Figure 8.

Assembly and analysis of two disulfide-bonded mutants of CA that form soluble hexamers⁶²: A14C/E45C/W184A/M185A (a, c, e) and A42C/T54C/W184A/M185A (b, d, f). (a, b) Negatively-stained EM images of tubes assembled by dialyzing 30 mg/mL protein in 50 mM Tris, pH 8, 1MNaCl, and 2mM β ME. The scale bar represents 500 nm. (c, d) Non-reducing SDS-PAGE profile of cross-linked soluble hexamers of the two constructs. Molecular mass markers are labeled on the left, and the expected positions of cross-linked n-mers are indicated on the right. (e, f) Conformations of the engineered disulfide bonds. (e) Ball-and-stick representation of the A14C/E45C/W184A/M185A mutant, with the rest of the structure in ribbons. Green mesh shows omit mFo-DFc densities for the sulfur atoms contoured at 3σ . The omit map was obtained by setting the sulfur occupancies to zero, introducing random shifts to the remaining atoms (mean displacement = 0.5 Å), and refining the resulting model with simulated annealing. Note that the Cys14 and Cys45 sulfur density peaks have equal magnitude and are 100% oxidized. (f) Disulfide-bonded conformation of Cys42 and Cys54 in the A42C/T54C/W184A/M185A mutant. Note that the sulfur density for Cys54 is clear, whereas Cys42 is weaker and more diffuse. (g, h, i, j) Structure of the disulfide-stabilized HIV-1 CA hexamer. Top view (g) and side view (h) of the hexamer, with the CA^{NTD} colored in orange and the CA^{CTD} in blue. Helices are represented as ribbons. In (g), note the close correspondence between the X-ray structure and the cryoEM density map derived from analysis of 2D hexagonal CA crystals (gray-scale).⁶⁸ Top view (i)

and side view (j) of the hexamer, with each subunit in a different color. The red and blue spheres indicate the locations of the stabilizing Cys14/Cys45 and Cys42/Cys54 disulfides, respectively.

Figure 9. Assembly and analysis of a disulfide-bonded mutant of CA that forms soluble pentamers: P17C/T19C/W184A/M185A⁹⁴. (a) Negative-stain EM revealed spherical particles indicative of insertion of pentamers. (b) Non-reducing SDS-PAGE profile of cross-linked soluble pentamers. Molecular mass markers are labeled on the left, and the expected positions of cross-linked n-mers are indicated on the right. (c, d, e, f) Structure of the disulfide-stabilized HIV-1 CA pentamer. Top view (c) and side view (d) of the pentamer, with the CA^{NTD} colored in orange and the CA^{CTD} in blue. Helices are represented as ribbons. Top view (e) and side view (f) of the pentamer, with each subunit in a different color. The yellow spheres in ((c) indicate the locations of the stabilizing Cys21/Cys22 disulfide.

Figure 10.

Atomic details of the hexamerization interface⁶⁷. (a) Polar and water-mediated contacts. Selected side chains are shown explicitly and labeled. Green mesh shows unbiased $F_O - F_C$ density contoured at $+3\sigma$. These were modeled as water molecules (magenta spheres) in the structure derived from hexagonal crystals. Putative hydrogen bonds are represented by yellow lines. Note that the region around the salt bridge between P1 and D51 (red asterisk), which forms only upon maturation of CA, is particularly water rich. These two residues coordinate water-mediated hydrogen bonds with H12, T48, and Q50. We speculate that the missing E45 side chain (mutated to cysteine in this construct) would participate in this network. (b), (c) and (d) display 13 crystallographically independent high-resolution structures. (b) Helix-capping hydrogen bonds at the CA^{NTD}-CA^{CTD} interface. Relevant side chains are shown explicitly and labeled. Hydrogen bonds are shown as yellow lines. The most critical of these caps appears to be R173 (to helix 3), since it is located in the middle of the hexamerization interface, and is conserved in 1668/1670 sequences in the Los Alamos HIV database, with the remaining two conservatively substituted with lysine. An intermolecular C cap for helix 7 in the blue protomer is not shown. The double-headed red arrow indicates the location of the CA^{CTD} dimer interface. The helix-capping residues shown in (b) act as pivot points for lever-like motions of the CA^{CTD}. Helix-capping hydrogen bonds at energetically favorable distances are maintained, while producing substantial linear displacements at distal regions of the domain. Hydrogen bonds are shown as yellow lines. Capping residues are shown in stick representation, as are side chains for Y145 and R162, which form a pi-cation stack that may be energetically significant (black asterisk). (c) and (d) display top and side views, respectively, of the CA hexamer.

Figure 11.

Quasi-equivalence in the pentameric and hexameric CA^{NTD} rings⁹⁴. Top views of the pentameric (a) and hexameric (b) CA^{NTD} rings, with each subunit in a different color. Subunits in the pentamer and hexamer are shown in darker and lighter shades, respectively. The angles subtended by adjacent domains are shown explicitly for the blue and orange subunits. One of the repeating three-helix units is outlined in black. (c) Close-up view of the pentameric and hexameric repeat units, superimposed on helices 1 and 3 of the blue subunit. The aliphatic residues that form a small hydrophobic core are shown explicitly and labeled. (d) The 'rotation' between adjacent subunits, in going from the hexamer to the pentamer. The approximate position of the rotation axis is indicated by the red dot. Note that this axis is parallel to neither the pentameric nor hexameric symmetry axes.

Figure 12.

Modeling of the pentameric declination (a, b) and ARG18 as an electrostatic switch that disfavors pentamer formation (c, d) ⁹⁴. (a) The CA^{CTD} dimer mediates hexamer-hexamer and pentamer-hexamer interactions and spans two distinct inter-ring distances in a declination. (a) Geometric model of a declination (light blue), with a docked CA^{NTD} pentamer (yellow) and two CA^{CTD} hexamers (orange). The distances that must be bridged by the CA^{CTD} dimer connectors are shown by the black double-headed arrows. (b) Comparison of the X-ray (1A43, blue) and NMR (2KOD, magenta) structures of the isolated, full-affinity CA^{CTD} dimer. Note that the structural variations occur at both the tertiary and quaternary levels, indicating that the CA^{CTD} dimer has a flexible architecture. The structures were superimposed on one subunit (left), to illustrate the differences in dimer dimensions and packing geometry. The black double-headed arrows indicate the center-to-center distances between the helix 8 pairs. The dyads are indicated by filled circles. (c) Arg18 (blue spheres) resides at the centers of the hexamer and pentamer is proposed to function as an electrostatic switch that governs the distribution of hexamers and pentamers. Note the closer proximity of Cα-Cα distances across opposite Arg18 residues in the pentamer (10.9 Å) versus the hexamer 16.5 Å). For comparison, a fully extended arginine sidechain is ~6.5 Å long (mtt180 rotamer). Densities for the Arg18 sidechains are not well defined for the pentamer. In the hexamer structures, the Arg18 sidechains also had poor density in 3H4E, but were defined in 3H47. It is likely that the guanidinium sidechains move to relieve electrostatic repulsion. However, the Arg18 residues in the pentamer should experience greater electrostatic repulsion and have less freedom of movement (due to steric constraints), compared to the hexamer.

Figure 13.

Assembly of an atomic model of the HIV-1 capsid relies on rigid body rotations around the CA^{NTD}-CA^{CTD} and CA^{CTD}-CA^{CTD} assembly interfaces⁹⁴. (a) The fullerene cone⁷⁸ is composed of ~250 hexamers (gray) and exactly 12 pentamers (red), which introduce sharp declinations to close the HIV-1 capsid. (b) A line of hexamers (red) connecting two declinations emphasizes the continuously varying curvature in the hexagonal lattice. (c) Graph of dihedral angles between hexamer planes as a function of cone dimension, as measured along the line of hexamers in (b). (d) A backbone-only model composed of 1,056 CA subunits. The hexamers, pentamers and dimers are colored orange, yellow and blue, respectively. As depicted in (b), the capsid displays a variably curved surface. In the body of the cone, curvature changes continually, which was modeled by subunit flexion at the CA^{NTD}-CA^{CTD} interface (Figure 10(b-d)). Pentamers (yellow) alter the trajectory of the hexagonal lattice and introduce regions of sharp curvature (that is, declinations). Our modeling suggests that formation of the declinations entails a flexible CA^{CTD} dimer. (e) Edge view of the capsid showing a line of connected rings with the CA^{NTD} hexamers colored orange, CA^{NTD} pentamers colored yellow and the CA^{CTD} dimers colored blue.