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All-Atom Virus Simulations

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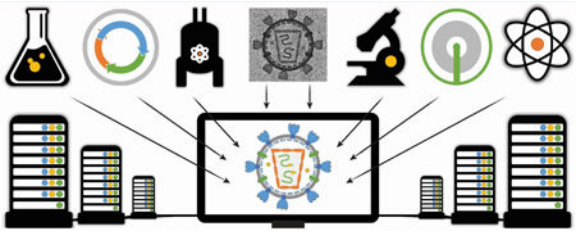
Abstract

The constant threat of viral disease can be combated by the development of novel vaccines and therapeutics designed to disrupt key features of virus structure or infection cycle processes. Such development relies on high-resolution characterization of viruses and their dynamical behaviors, which are often challenging to obtain solely by experiment. In response, all-atom molecular dynamics simulations are widely leveraged to study the structural components of viruses, leading to some of the largest simulation endeavors undertaken to date. The present work reviews exemplary all-atom simulation work on viruses, as well as progress toward simulating entire virions.

Graphical Abstract:



Introduction

Viruses are a constant threat to human health, as well as the health of our domesticated animals and agriculture. Often, viral infections are challenging to treat due to limited therapeutic options. Although vaccines are effective at preventing or reducing the severity of viral disease, protective vaccines against fewer than 20 different viruses are currently available (WHO). Further, the high mutation rates of some viruses necessitate that vaccines be updated annually, or that new antiviral treatments be constantly realized to continue disease management in chronically infected individuals.

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The rational development of novel prophylactic and therapeutic interventions against viral pathogens depends on detailed knowledge of virus structure, infection cycle processes, and the conformational dynamics that link structures to their functional roles in these processes. All-atom molecular dynamics (MD) simulations, often referred to as the “computational microscope,” remain the only method capable of elucidating the dynamical properties of biomolecules within their physiological environments at full chemical resolution [1]. Modern supercomputers and MD simulation codes enable researchers to investigate the nuances of virus structure and dynamics, even for very large components of virus structure [2].

Most all-atom MD simulation studies of viruses have focused on their protein-based components, particularly envelope proteins, viroporins, capsids, and accessory proteins or enzymes. Some work has leveraged MD simulations to construct complete atomistic models of viral proteins or their assemblies by integrating experimental data. Other work has taken advantage of MD simulations to characterize, under native conditions, the chemical-physical properties of such structures through analysis of their dynamics and influence on surrounding solvent. The overarching goals of virus simulation endeavors are to reveal critical aspects of virus structure or mechanistic insights into their function that can be targeted for novel disease treatments. Here, exemplary all-atom simulations of viruses are briefly reviewed, and progress toward simulating entire virions at atomistic detail is discussed.

Envelope Proteins

The first stage of viral infection is cell entry. Often, this process begins with adhesion of the virus to the host cell via receptor binding. In enveloped viruses, receptor binding is mediated by membrane-embedded surface proteins, which typically also represent the major antigens of the virus and may participate in virus-host membrane fusion. Other envelope proteins play a role in viral egress. The motivation to elucidate the molecular determinants of receptor recognition, as well as the antigenic and fusogenic properties of envelope proteins, have rendered them of great interest for study with MD simulations.

A broadly-studied viral envelope protein is the influenza hemagglutinin (HA) trimer (Fig. 1a), which is responsible for cellular adhesion and membrane fusion. Much work on HA has been aimed at understanding its specificity for host cell receptor glycans, which is an important factor in the avian-human transmission barrier [6, 7]. Characterization of receptor binding may support the development of adhesion-inhibiting drugs.

All-atom MD simulations have been applied to numerous crystal complexes of HAs with receptor analogues to characterize binding mode dynamics, as well as to study recognition and specificity based on bound receptor conformations and interaction profiles [8, 9, 10, 11, 12]. Recent modeling and simulation work has also revealed that HA receptor specificity may depend on extended glycan structure and the ability to accommodate bidentate binding to biantennary glycans [13, 14]. MD simulations have been further employed to evaluate the effects of HA mutations that alter receptor binding and specificity [15, 16] and to study HA-antibody recognition and escape [17, 18].

Notably, HA is glycosylated, with *N*-glycans contributing around 20% of its molecular weight [6]. All-atom simulations of glycosylated HAs have been used to probe the influence of glycosylation on receptor binding [19], to provide insight into accessibility of glycan-remodeling enzymes [20], and to characterize interactions between HA and lung surfactant protein D, an innate immune lectin that neutralizes influenza A via attachment to HA glycans [21].

HA initiates fusion of the viral envelope and host membranes following cell adhesion and endocytosis, when endosomal acidification triggers it to undergo a conformational change. The mechanism of HA-mediated membrane fusion has been extensively studied with simulation approaches, including characterization of fusion peptide dynamics, interactions with the host membrane, and structural effects of reduced pH [22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32].

The secondary surface antigen of the influenza A virus is the neuraminidase (NA) tetramer (Fig. 1a), which cleaves host cell glycans to allow escape of progeny virions. Several compounds that mimic the native substrate of NA and bind to inhibit its action have been licensed for use as antiviral drugs for the treatment of influenza A (i.e., tamivir and zanamivir) [33]. The majority of simulation work on NA has focused on exploiting it as a drug target. In some cases, all-atom simulations have been employed to characterize the conformational dynamics of NA from the perspective of designing novel drug compounds or elucidating the determinants of ligand recognition and inhibition [34, 35, 36, 37]. In other cases, simulations of NA have been used as part of computational screening protocols to discover new druggable hot spots or inhibitor leads [38, 39, 40]. Additional studies have provided insights into the mechanisms of drug resistance [41, 42, 43], the role of calcium in NA stability and drug binding [44], and the structural basis for increased virulence of NA stalk-deletion mutants [45].

Another well-studied envelope protein is the human immunodeficiency virus type 1 (HIV-1) envelope (Env) trimer (Fig. 1b and 2d), which mediates cellular adhesion and membrane fusion. All-atom MD simulations have been employed to characterize the conformational properties of Env, particularly its V3 variable domain, a critical determinant of CD4 receptor binding [46, 47]. Other simulation work on Env has focused on identifying correlated motions that may underlie allosteric communication networks within the protein [48, 47, 49, 50]. The transmembrane domain and fusion peptide of Env have also been extensively investigated with MD simulations to explore conformational variability and membrane interactions, providing insights into the mechanism of membrane fusion [51, 52, 53, 54, 55, 56].

Importantly, Env is heavily glycosylated, with *N*-glycans accounting for roughly 50% of its molecular weight [57]. These glycans form a shield around Env, cloaking it from the host immune system, and may also influence the binding affinity of CD4. The glycan shield is of great interest as a target for broadly-neutralizing antibody and vaccine design [57], and can only be accurately described with simulations that encompass chemical detail. Several all-atom MD studies have investigated the effects of glycosylation on the conformation and dynamics of Env variable domains V1, V2, and V3, as well as implications for receptor and

antibody binding [58, 59, 60]. More recent work has used computational modeling and simulation to produce fully glycosylated Env trimers, with the goals of characterizing the dynamical behavior of the glycan shield and determining its impact on antibody elicitation, recognition, and binding [50, 61, 62, 63].

Beyond influenza A and HIV-1, all-atom MD simulations have also been applied to study the envelope proteins of several other viruses. Such work has included investigations of the effects of varying pH on the dengue virus [64, 65, 66, 67, 68, 69] and vesicular stomatitis virus (VSV) [70] envelope proteins, structure and host membrane interactions for the Ebola virus [71, 72, 73] and herpes simplex virus (HSV) [74] envelope protein fusion peptides, solution dynamics of human parainfluenza virus type 3 (HPIV-3) hemagglutinin-neuraminidase [75], and conformational flexibility in the hepatitis C virus (HCV) E2 envelope protein [76, 77].

Viroporins

Enveloped viruses can encode another class of membrane-embedded proteins, called viroporins, that play diverse roles in their respective viral infection cycles. Viroporins are small, hydrophobic proteins that oligomerize within the envelope to form hydrophilic channels, capable of transporting ionic species and small molecules [78]. The essential activities of these channels, their druggability, and potential as models to understand transport mechanisms within the human cell, have rendered them of long-standing interest for study with MD simulations.

The most widely-studied viroporin, from a computational perspective, is the influenza A matrix protein 2 (M2) ion channel (Fig. 1a), which transports protons across the viral envelope following endosomal acidification to prepare the particle for host membrane fusion and subsequent genome release [79]. All-atom MD simulations have been broadly employed to study M2 channel activation, gating, and proton permeation [80, 81, 82, 83, 84, 85, 86], as well as investigate the relationship between pH and channel conductance [87, 88] and internal water structure [89]. Adamantane drug compounds (i.e., amantadine and rimantidine) are known to inhibit M2 channel activity [79]. Additional all-atom MD simulation work on M2 has been aimed at characterizing drug binding and channel inhibition [84, 85, 90], probing the mechanisms of drug resistance [91, 92], and designing new inhibitors against drug-resistant mutants [93, 94].

Other viroporins that have been examined using all-atom MD simulations include the viral protein U (Vpu) of HIV-1 (Fig. 1b and 2a). Various early modeling and simulation studies of Vpu [95] led to evaluation of its preferred oligomeric state and all-atom investigation of its dynamics and channel activity [96]. All-atom simulations were also applied to develop a model for the Paramecium bursaria chlorella virus type 1 (PBCV-1) Kcv potassium channel to probe structure-function relationships [97, 98] and elucidate its mechanism of ion transport [99]. More recently, all-atom simulations were employed to determine the structure of the hepatitis C virus (HCV) p7 viroporin monomer [100] and to model various channel oligomer states, enabling characterization of their dynamics and conductance properties [101, 102, 103, 104, 105].

Capsids

Many viruses package their genome within a remarkable protein shell called a capsid. Often, capsids play key functional roles in delivering the viral genome to the host cell nucleus, rendering them of great interest as drug targets. Capsids generally represent the largest protein components of virus structures, and simulations of intact capsids have accounted for the most substantial all-atom virus simulations yet published [106, 2]. Work by Perilla et al. has clearly demonstrated the need for chemical detail to facilitate accurate simulation studies of virus capsids as drug targets [107].

The first all-atom simulation of a complete virus capsid was based on satellite tobacco mosaic virus (STMV, Fig. 1c and 2g) and encompassed a landmark particle count of 1 million atoms with solvent [108]. Both an empty and genome -containing capsid were investigated over 10–13 ns. With packaged RNA, the simulated capsid maintained structural integrity and exhibited only minor deviations from icosahedral symmetry. Without RNA, the simulated capsid rapidly broke symmetry and began to implode. The results of the work strongly suggest that RNA is responsible for nucleating assembly of STMV virions.

Prior to work on STMV, simulations of complete capsids were approximated using rotational symmetry boundary conditions, which exploit the icosahedral symmetry of capsid structures to model, at least in effect, a complete capsid using only the capsid asymmetric unit [106]. As time has progressed, all-atom simulations of intact capsids have become increasingly accessible. Notably, such work has included characterization of the stress-response of the southern bean mosaic virus (SBMV) capsid [109], validation of multi-scale modeling approaches based on the Sesbania mosaic virus (SeMV) capsid [110], derivation of a complete atomic model for the rabbit hemorrhagic disease virus (RHDV) capsid [111], and examination of the interplay between solvent and ions and the porcine circovirus type 2 (PCV2) capsid [112, 113].

The first all-atom simulation of a virus capsid to explore the microsecond timescale was based on an empty model of satellite tobacco necrosis virus (STNV), which encompassed 1.2 million atoms with solvent [114]. Like many non-enveloped plant viruses, STNV exhibits specific binding sites for divalent ions, likely calcium, and has been shown experimentally to undergo structural expansion unless these ions are bound. Simulations with and without bound calcium confirmed that the former maintain a smaller diameter and the latter swell. The results of the work produced an atomistic model for the swollen STNV capsid, which was not previously available, as well as an atomistic description of the dynamics underlying the swelling process.

The empty poliovirus capsid was studied over a timescale of 200 ns, based on a system of 6 million atoms with solvent [115]. Simulation results revealed that the capsid functions as a semipermeable membrane structure, translocating water molecules, but not ions, in equilibrium across its surface. Further, the solution pressure on the interior of the capsid was found to be negative, owing to electrostatic interaction of solution electrolytes with the charged capsid surface. The results of the work demonstrate that the capsid plays an

essential role in maintaining an environment conducive to the stable accommodation of its RNA genome and associated counterions.

The empty hepatitis B virus (HBV) capsid was simulated for 1.1 μs based on a model of the complete assembly domain, derived from crystallography and molecular modeling [116]. The simulation encompassed 6 million atoms with solvent and revealed that the capsid undergoes notable asymmetric distortion at equilibrium. Further, the capsid exhibited a fivefold preference to translocate sodium over chloride through its triangular pores, and induced the localization of sodium along its interior surface. The results of the work implicate the triangular pores as the extrusion site of the capsid's carboxy-terminal domain tails, which contain cellular signals, and suggest a mechanism by which the capsid could modulate signal exposure based on tail phosphorylation. Simulations have also been used to investigate the effects of small-molecule drugs on HBV capsid morphology [107] and to determine the structural basis for enhanced assembly and drug resistance in HBV capsid mutants [117].

An all-atom structure of the human immunodeficiency virus type 1 (HIV-1) capsid (Fig. 1b and 2e) was derived by combining results from cryo-EM and NMR experiments with computational modeling and data-guided MD simulations [118]. This landmark simulation endeavor, encompassing 64 million atoms with solvent, overcame the challenges of HIV-1 capsid size and asymmetric architecture to generate the first chemically complete model. The results of the study revealed key structural elements defining pentamer-hexamer and hexamer-hexamer interfaces within the capsid and produced a much-needed platform to enable investigation of capsid function and targeted drug development. Notably, the all-atom model has been used to study capsid interactions with the host cell factors cyclophilin A [119, 120] and MxB [121], which regulate and restrict HIV-1 viral infectivity, respectively.

In a subsequent study, the empty HIV-1 capsid model was simulated for 1.2 μs, setting the current published record for the most substantial all-atom simulation ever performed [122]. The simulation revealed that the capsid translocates chloride across its surface at twice the rate of sodium through ion-specific channels, which may relate to the translocation of nucleotides during reverse transcription. Further, the capsid exhibited complex dynamics, including collective motions that divide the structure into two hemispheres and suggest a mechanism for capsid uncoating, as well as oscillatory surface waves at four different frequencies that may underlie emergent properties of the capsid.

Immature Particle Lattices

Retroviruses such as HIV-1, human T-cell leukemia virus type 1 (HTLV-1), and Rous sarcoma virus (RSV) initially assemble as immature particles from Group-specific antigen (Gag) polyproteins. During maturation, proteolytic processing of Gag yields cleavage products that reassemble to produce mature virions [123]. All-atom simulations proved invaluable in determining the structures of the immature HTLV-1 [124] and RSV [125] Gag lattices, as well as the HIV-1 capsid protein-SP1 peptide maturation intermediate (CA-SP1) [126]. Notably, simulations revealed that the CA-SP1 six-helix bundle exists in a dynamic helix-coil equilibrium, and that maturation-inhibiting drugs and mutations act by stabilizing

a helical form of the bundle [126]. MD studies of CA-SP1 bound to inositol phosphates showed that these small molecules facilitate the formation of the six-helix bundle and, thus, promote assembly of the Gag lattice [127]. Simulations of the RSV lattice model were used to determine the effects of charge distribution and mutations on Gag assembly [128].

Viral Enzymes

Some viruses encode and package enzymes necessary to carry out the processes that drive their infection cycles. All-atom MD simulations of the HIV-1 protease (Fig. 1b and 2b), which is responsible for proteolytic cleavage of Gag prior to maturation, revealed the motion of the enzyme's active-site flaps, whose opening and closing are considered essential to function [129, 130, 131, 132, 133, 134]. Further, studies of the West Nile virus (WNV) NS3 protease identified a conformational selection mechanism used to identify its substrate [135]. Additionally, all-atom simulations were instrumental in elucidating the mechanism by which non-nucleoside reverse transcription inhibitors (NNRTIs), an important class of therapeutics against HIV-1, interfere with the virus' reverse transcripase [136] (Fig. 1b and 2c), which synthesizes DNA from the RNA template.

Toward All-Atom Simulations of Complete Virions

As experimental methods, particularly cryo-EM and cryo-ET, continue to provide greater detail on virus composition and architecture, and as supercomputers and simulation codes continue to offer greater performance for the study of large biological systems, the computational community becomes poised to study the dynamics of complete virions within physiological environments. Nonetheless, the paucity of accurate, full-length, and fully atomistic models for some major components of virus structure presents a significant challenge to the derivation and investigation of all-atom virions.

Importantly, the lack of accurate all-atom models for encapsidated genome structures is the foremost limiting factor for the construction of complete virions. While the work of Freddolino et al. on genome-containing STMV (Fig. 1c) represents the only all-atom simulation of a complete virion performed to date [108], the genome structure employed was not based on the actual STMV RNA sequence. Similarly, a complete pariacoto virus (PaV) virion derived using molecular modeling included an RNA model based on an artificial RNA sequence [137]. For both of these virions, limitations in determining genome organization from crystal structures, which typically rely on icosahedral averaging, led to inaccuracies in the resulting RNA models [138, 139]. Recently, Zeng et al. used computational methods to generate the first all-atom model for the complete structure of any virus using the natural genome sequence, that of STMV containing realistic RNA [5].

Moving forward, modeling and simulation will continue to play an essential role in the determination of all-atom structures for genome-containing capsids, particularly through approaches that integrate experimental data from techniques such as NMR and cryo-EM [140, 141]. Although simulation studies of the intact HBV capsid have shown that the inherent flexibility of some virus structures may limit the ability to obtain true atomic (1–2 $^{\circ}$A) resolution with cryo-EM [116], advances in image processing are enabling high-

resolution asymmetric reconstructions capable of producing m o r e authentic descriptions of encapsidated genome, such as those obtained for the immature HBV particle and bacteriophage MS2 [142, 143]. The availability of more complete structural information, along with improved understanding of genome organization within capsids [144] and integrative modeling approaches [1] that employ increasingly accurate protein and nucleic acid force fields [145, 146], will support new simulation studies of all-atom virions in the near future, particularly for non-enveloped viruses.

All-atom simulations of enveloped viruses will face the additional challenge of treating the envelope membrane lipid bilayer. Due to size, complex lipid compositions, and the inclusion of membrane-embedded surface proteins, viral envelopes require careful consideration to model and equilibrate [147]. Further, enclosing a virus capsid in a fully atomistic envelope will significantly increase system size and simulation expense, particularly given the inclusion of solvent. Figure 2 emphasizes the dramatic size disparity between several protein-based components of HIV-1 that have been previously investigated with all-atom simulations and a model of the intact envelope membrane (Fig. 2f). The envelope necessarily dwarfs the HIV-1 capsid, the simulations of which currently represent the most far-reaching accomplishment of computational virology reported for structures of atomic detail [118, 122]. Following construction of a complete HIV-1 virion, it is clear that substantial supercomputing power will be required to simulate such a colossal biological assembly at chemical resolution, especially over timescales sufficient to allow meaningful study.

In the meantime, three viral envelopes have been investigated using coarse-grained simulation methods, including that of an immature HIV-1 particle [148], influenza A virus [149], and dengue virus [150] While the immature HIV-1 particle included its viral matrix component, the influenza A virion did not explicitly include matrix protein 1 (M1, Fig. 1a). The general lack of high-resolution structures for viral matrix assemblies represents another limiting factor in the construction of realistic all-atom virions.

In addition to suitable numbers and distributions of envelope proteins and viroporins embedded in the viral surface, truly complete and realistic virion models will also incorporate appropriate glycosylation on proteins and glycolipid species, as well as stoichiometric numbers of viral enzymes and accessory proteins encoded and packaged by the respective virus. The availability of in situ structures for intact virions, such as those recently obtained for HIV-1 using cryo-ET [151, 152], will dramatically support future all-atom modeling and simulation efforts. Additionally, experimental characterization of viral envelope lipidomes and improved software and force fields for building and simulating large membrane assemblies [147] are essential advancements that will soon enable all-atom simulations of complete enveloped virions. Undoubtedly, all-atom virions will be among the first large-scale biological systems to be investigated on upcoming exascale supercomputing platforms.

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Highlights:

- Viral protein simulations are among the largest all-atom simulations ever published.
- All-atom simulations reveal the dynamics of viral proteins at chemical resolution.
- All-atom detail is required for accurate simulations, particularly with drugs.
- Data-guided simulations help determine viral structures inaccessible to experiment.
- In situ structures and integrative modeling will enable all-atom simulations of virions.

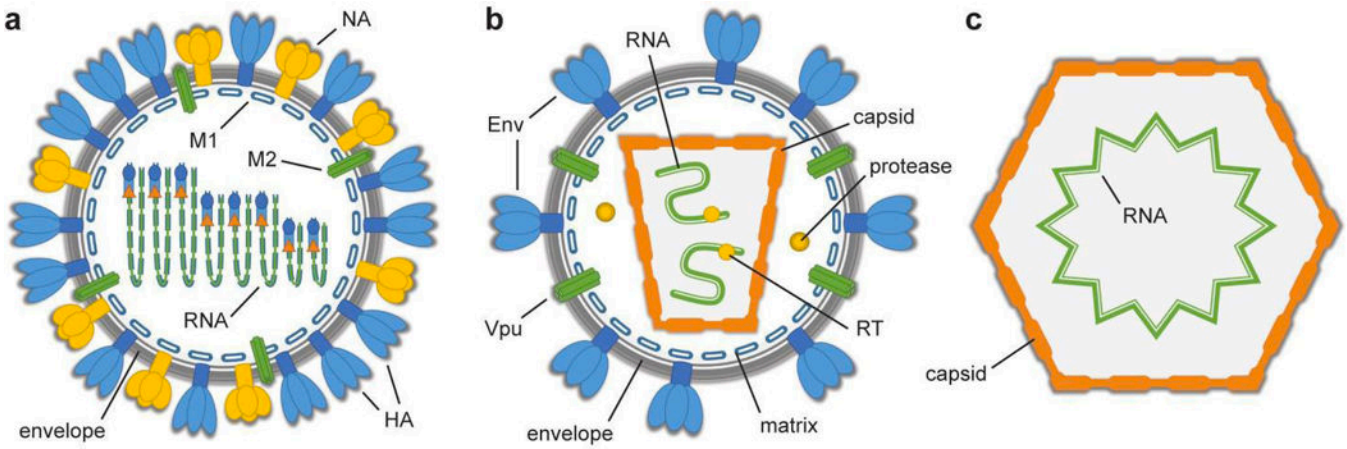

Figure 1:

Schematic illustrations of viruses whose protein components have been heavily studied using all-atom MD simulations. *a.* Influenza A virus virion, average diameter of 120 nm [3]. Hemagglutinin (HA), neuraminidase (NA), and the matrix protein 2 viroporin (M2) are embedded within the viral envelope, which surrounds the matrix protein 1 (M1) assembly and encloses the RNA. *b.* Mature HIV-1 virion, average diameter of 145 nm [4]. The envelope protein (Env) and viroporin (Vpu) are embedded within the viral envelope, which surrounds the matrix protein assembly and encloses the RNA-containing capsid. The virus also carries copies of a protease and reverse transcriptase (RT). *c.* STMV virion, diameter of 17 nm [5]. STMV is a non-enveloped virus, composed only of the RNA-containing capsid.

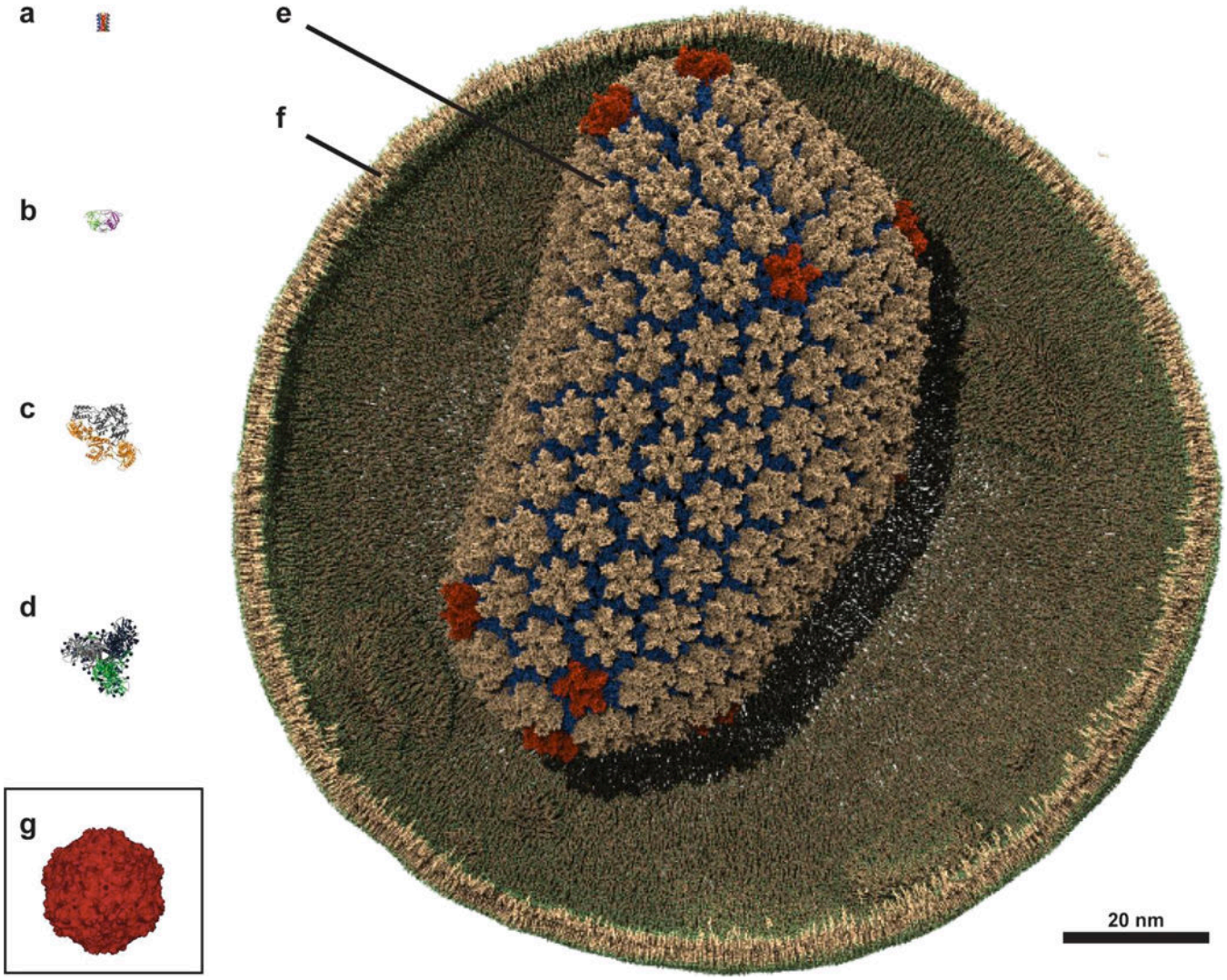

Figure 2:

Graphical size comparison of viral protein components. *a*. HIV-1 viroporin (Vpu), transmembrane domain. *b*. HIV-1 protease. *c*. HIV-1 reverse transcriptase (RT). *d*. HIV-1 envelope protein (Env) ectodomain. *e*. HIV-1 capsid. *g*. HIV-1 envelope membrane. *g*. STMV capsid.