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Multiscale modelling and simulation of viruses

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In recent years, advances in structural biology, integrative modelling, and simulation approaches have allowed us to gain unprecedented insights into viral structure and dynamics. In this article we survey recent studies utilizing this wealth of structural information to build computational models of partial or complete viruses and to elucidate mechanisms of viral function. Additionally, the close interplay of viral pathogens with host factors — such as cellular and intracellular membranes, receptors, antibodies, and other host proteins — makes accurate models of viral interactions and dynamics essential. As viruses continue to pose severe challenges in prevention and treatment, enhancing our mechanistic understanding of viral infection is vital to enable the development of novel therapeutic strategies.

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Introduction

Viral diseases continue to impose a severe burden on human health and agricultural production [1], and pose special challenges for the development of effective therapies [2]. Viral pathogens occur in a variety of complexity and size. Simple virions typically contain a genome that is only a few kilobases in length enclosed in a homopolymeric protein capsid with dimensions of a few tens of nanometers. Complex, enveloped viruses can contain a variety of viral proteins, lipid bilayers, internal capsids, potentially segmented, single-stranded or double-stranded RNA and DNA genomes of over a megabase in length, and may reach hundreds of nanometers in diameter. Gaining an understanding of the molecular mechanisms underpinning viral infections provides us with crucial information to advance our therapeutic toolkit against this important class of pathogens. Advances in cryo-electron microscopy (cryoEM) have enabled us to observe viral structures in unprecedented detail [3], and in combination with X-ray crystallography of individual viral components, allow for (near)-atomic resolution reconstruction. These structures often now also serve as the starting point for computational modelling of viral dynamics. The most widely used technique to this end is molecular dynamics (MD) simulation, based on classical, non-polarizable force fields. As of today, describing full viruses (and their respective complexes with therapeutics, antibodies, or host cell components) in atomistic detail is computationally challenging but possible, thanks in particular to the development of specialized hardware such as GPUs and custom-built chips [4–6], in combination with improved MD parallelization algorithms [7]. Moreover, simplified coarse-grained (CG) models are now routinely used to reduce the number of interactions that need to be calculated while retaining essential structural and dynamic characteristics [8,9]. Thus, simulations have been used to elucidate a number of viral phenomena including the assembly of capsids, the permeability of viral shells, or the interaction of viral proteins with lipid bilayer envelopes and endocytosis. In this article we aim to highlight recent advances in computational structural biology of viral systems and summarize their key findings with regard to both virus function and antiviral therapy.

Capsid stability and permeability

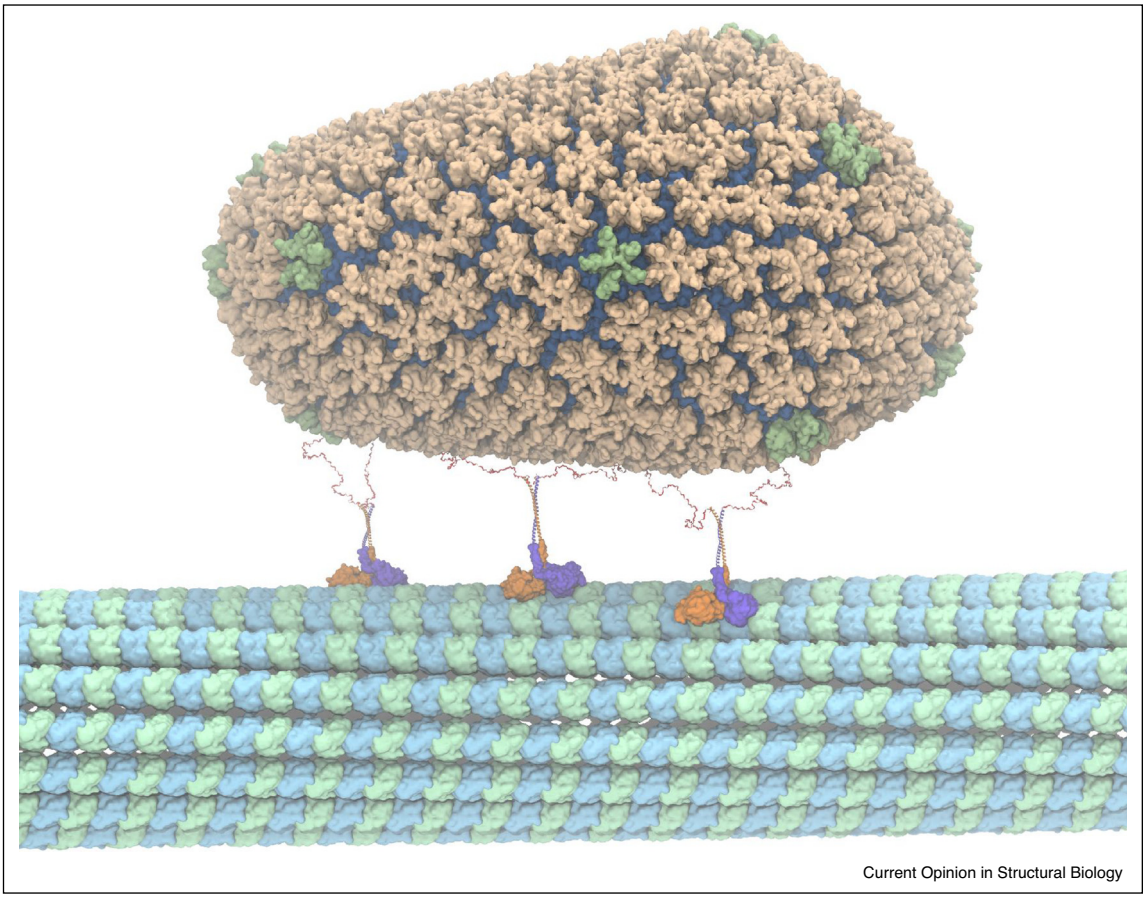
The first atomic-resolution simulation of a complete virus was of the ~17 nm diameter satellite tobacco mosaic virus (STMV) [10]. Over 10 ns, the icosahedral capsid was stable only in the presence of an artificially modelled RNA molecule. This contrasted with other capsids such as that of poliovirus [11], indicating that the dependence of capsid nucleation upon genomic material may vary from virus to virus. A dense internal chloride layer was reported for simulations of the ~20 nm diameter porcine circovirus type 2 (PCV2) [12] and ~27 nm diameter MS2 bacteriophage [13], which may mimic the genome [14]. In fact, the functional role of capsid ion distributions and permeability has been a primary focus of numerous studies. Ions may be 'sensed' by viral capsids to transition along the life cycle to, for example, trigger genome release. 1 μs atomistic simulations of the ~17 nm diameter satellite tobacco necrosis virus (STNV) revealed capsid opening upon the loss of structural calcium ions, representative of the plant cytoplasmic environment [14], while a multiscale study of the ~30 nm diameter Triatoma Virus (TrV) indicated that the opening of uni-directional proton pores within the capsid occur in the presence of high chloride concentrations,

concomitant with alkalinization inside insect cells [15]. More generally, it has been noted that viral capsids can act as semipermeable layers with distinct selectivity. A 1.2 μs simulation of an HIV-1 lattice system comprising ~64 million atoms revealed a permeation rate of chloride ions twice that of sodium, which was hypothesized to be important for nucleotide translocation [16•]. Conversely, in a 1.1 μs simulation of the ~36 nm diameter hepatitis B virus (HBV) sodium permeated around five times faster than chloride via highly acidic triangular pores, leading to the concentration of sodium ions on the interior surface, speculated to be important in extrusion of the basic C-terminal domain capsid protein tails for cell signaling [17•]. Meanwhile, in simulations of poliovirus [18] or PCV2 [19] no significant ion exchange was observed. Bidirectional translocation of water molecules was typically observed in all of these simulations, but as pointed out by Hadden *et al.* [17•], the measured exchange rates span several orders of magnitude that do not correlate with capsid morphology, highlighting the mechanistic diversity of viral capsids.

Simulations meet experiment in defining novel viral architectures and complexes

With ongoing advances in structural biology, myriad data are frequently obtained for different conformational states or assemblies, and at varying resolutions. In such cases, MD can help integrate them into unified models or ensembles, as in, for example, prediction of the structure of cylindrical Ebola virus lattice filaments [20] or assessment of capsid mechanostability [21,22] MD flexible fitting (MDFF) was developed as a means to dynamically fit atomic structures into experimental density maps, enabling elucidation of several virus capsid structures [23] including mature HIV-1 tubular lattice assemblies [24]. Subsequently, biochemical and biophysical studies were combined with spontaneous binding simulations spanning tens of microseconds for different higher-order HIV-1 lattice assemblies, indicating how restriction factor MxB [25] recognizes the capsid at regions where three capsid hexamers meet to inhibit HIV infection [26], while a 1.25 μs simulation of a pentamer-of-hexamers bound to the antiviral PF-3450074 [27] revealed that the drug may modulate functional allosteric pathways within the capsid

Figure 1



The HIV-1 capsid recruits kinesin for trafficking through the cytoplasm towards the cellular nucleus. The molecular details of the interactions among the capsid, kinesin and tubulin have been established using a combination of atomistic simulations and experimental methods [29•]. Figure courtesy of Juan R Perilla.

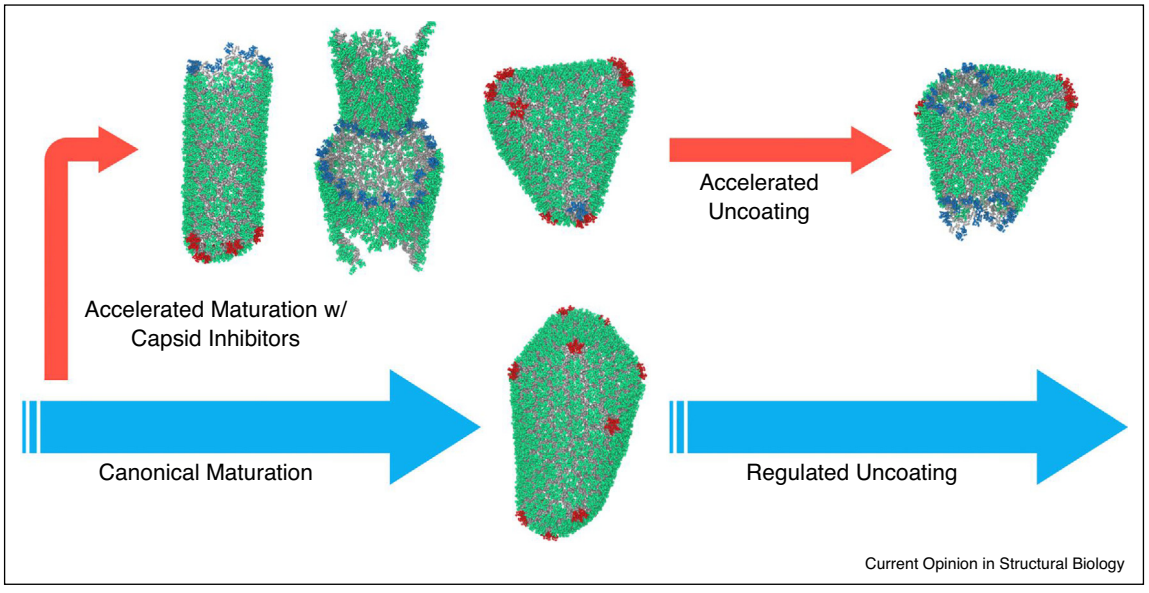
assemblies [28]. A recent joint biochemical and theoretical effort also showed how kinesin-1 adaptor protein FEZ1 binds to the positively charged central pore of HIV-1 capsid hexamers, with spontaneous translocation of FEZ1 poly-glutamate stretches into the pore observed during 2 μs atomistic simulations, thereby explaining how the virus is targeted for trafficking to the nucleus via the microtubule network [29•] (Figure 1).

Simulating viral plasticity and assembly

In recent years, MD simulations have proven useful in elucidating conformational rearrangements in viral systems, ranging from subtle motions associated with infection and drug resistance, to capsid assembly, maturation, and endocytosis [30]. Structural fluctuations including 'surface waves' and long-range collective dynamics were observed in simulations of the complete HIV-1 capsid, and proposed to play allosteric functions in viral infection [16•]. Both local motions and global asymmetric distortions were noted for the HBV capsid, which may be important during maturation or viral transport through nuclear pores [17•]. These simulations were also mined to explain the mechanistic basis for resistance to small-molecule antivirals in naturally occurring HBV mutants [31] while follow-up simulations of the HBV shell indicated how the small-molecule drug HAP1 may misdirect assembly by modifying its global quaternary morphology [27].

Immature retrovirus lattice structures, whose assembly are essential during the early stages of the replication cycle, have also been the focus of numerous computational studies. In the first simulation of an immature HIV-1 capsid, a multiscale approach was taken to study the virion envelope and Gag polypeptide, which contains subdomains corresponding to the matrix (MA), capsid (CA), and nucleocapsid (NC) proteins, as well as other smaller fragments including spacer (SP) peptides, that are subsequently cleaved during maturation. The highly conserved CA monomer structure is composed of an N-terminal domain (NTD) and C-terminal domain (CTD), both of which are predominantly α-helical, separated by a flexible linker. The simulations revealed key interactions responsible for maintaining hexagonal symmetry, and suggested how Gag mutants distort the CA domain bundle structure and hence lattice assembly [9]. Extensive atomistic simulations of HIV-1 CTD-SP1 hexamers indicated that the bundle is stabilized by the binding of inositol phosphates at its hexameric center [32], and that it exists in a dynamic helix-coil equilibrium that may be shifted towards the helical state by certain drugs or mutations to inhibit maturation [33]. Simulations based on an integrative model of the immature Rous sarcoma virus (RSV) lattice further revealed that the Gag lattice requires components upstream and downstream of the NTD and CTD CA layers for stability [34] including a poorly conserved flexible loop (FL) in the CA NTD [35].

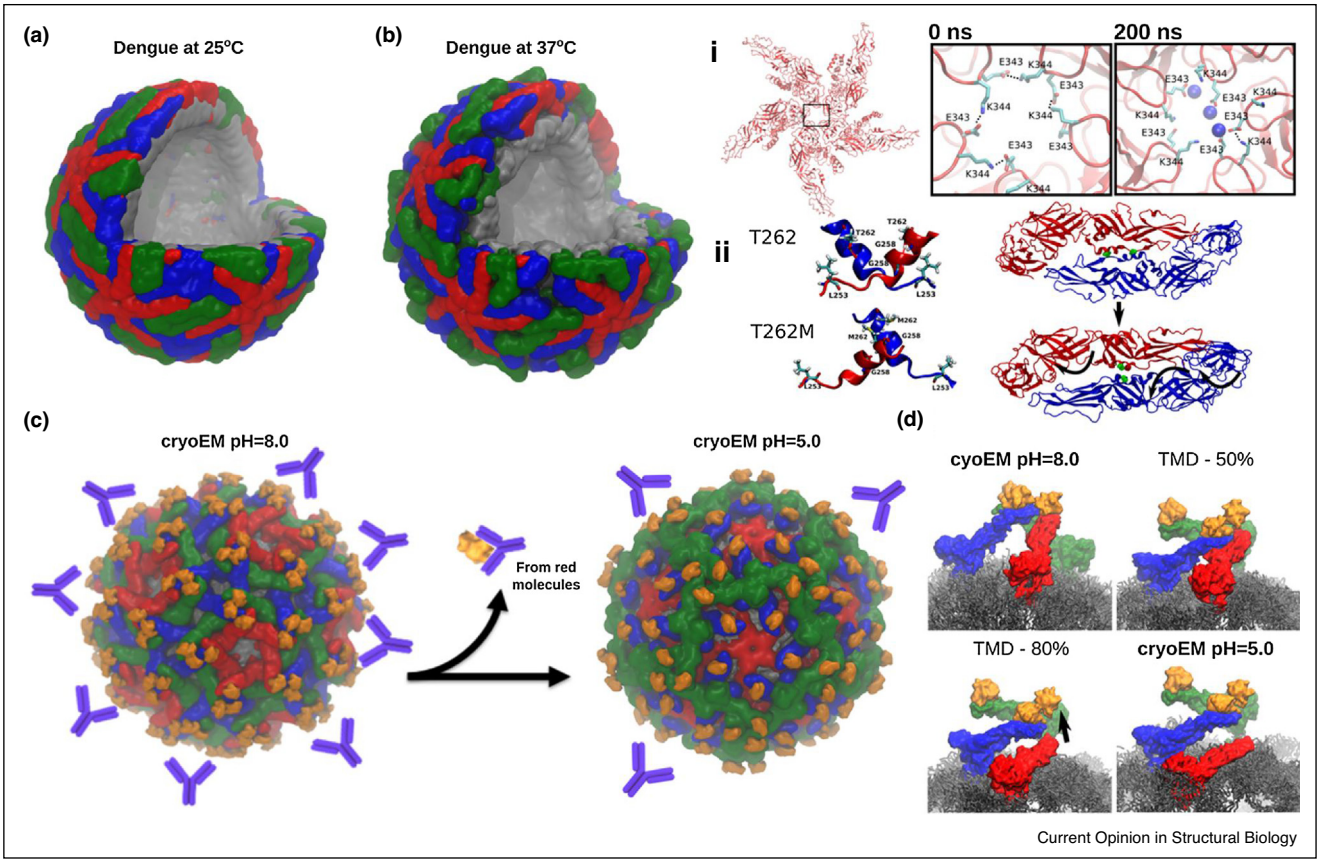
Figure 2



CIs of HIV-1 reduce infectivity by affecting pathways during both assembly in viral maturation and uncoating. An 'ultra-CG' model was developed to study HIV-1 capsid assembly, based on a Cα-resolution representation of CA dimers restrained strategically by elastic networks, with native protein/protein interfaces identified from experimental structures favored by additional attractive interactions [42]. This led to the identification of metastable trimer-of-dimer (TOD) oligomers as key nucleating structures, for which an auxiliary elastic network was implemented to study the effect of CIs which bind to and stabilize such oligomers [43•]. The resultant CA assemblies observed during simulations included hexamers (green), pentamers (red), and the growing edge of the capsid (blue) [43•]. Figure courtesy of Prof. Gregory A. Voth.

Simulating the spontaneous assembly of complete viral shells represents a major ongoing challenge [36]. Highly simplified models have continued to improve, such that many can broadly reproduce assembly/maturation intermediates and final capsid structures, for example, in which constituent proteins [37] or capsomeres [38] are represented by single particles, or by sets of soft spheres fused together into rigid structures [39] as well as simplified representations of host [40] or viral membranes [41]. Of particular note is work from the Voth group in which structurally detailed but 'ultra-CG' models have been used to study the assembly of conical HIV-1 shells. Models for CA dimers were developed, consisting of a Cα-resolution representation restrained strategically by elastic networks, with native inter-CA lattice contacts promoted by additional attractive interactions [42]. Simulations were used to study assembly and uncoating under various conditions, incorporating the effects of CA concentration, molecular crowding, and conformational switching. A reversible, multi-step process of lattice assembly was reported, in which metastable trimer-of-dimers (TODs) were identified as key structures nucleating mature lattice growth [42]. The model was later extended to study the mechanisms of capsid inhibitor (CI) drugs; since crystallographic evidence suggests that some CIs preferentially target an inter-CA pocket to stabilize oligomers, the effect of CI binding was incorporated by introducing a fixed population of TODs maintained via an auxiliary elastic network [43•]. Simulations revealed that CIs can accelerate hierarchical CA self-assembly by increasing the number of accessible, anisotropic assembly pathways, promoting pentameric defects and formation of non-canonical, pleomorphic capsids which may not be able to enclose the viral genome. The CI-bound lattice was also found to be inherently less stable, which may lead to inappropriate disassembly and hence reduce infectivity (Figure 2).

Figure 3



Molecular simulations of the flavivirus life cycle. Multiscale models of mature DENV envelope are shown for **(a)** compact and **(b)** 'bumpy' conformations, the latter having been shown to be dependent upon (i) divalent cations and (ii) point mutations. The CG simulations of the whole envelope were generated based on the MARTINI forcefield [8], extended from previous models developed for the smooth virion particle [45]. The all-atom models of the E protein pentamers were back-mapped from the CG simulations of the expanded envelope [53], while *in silico* mutations were introduced into atomic models of the E protein dimers to reveal how amino acid substitutions modulate the temperature-dependency of viral 'breathing' [55•]. **(c)** CryoEM data provided snapshots associated with pr-antibody-dependent maturation, and **(d)** targeted MD simulations based on CG resolution models of the viral envelope revealed the pathways by which dissociation of pr-antibody complexes may be coupled to maturation in ADE [56•].

Simulating viral envelopes

Efforts have also been made to simulate explicit lipid bilayers representing the envelopes of certain viruses [44]. The flavivirus envelope has been of particular interest from a multiscale simulation perspective [45–47]. Numerous joint experimental/computational studies have focused on the processes of membrane fusion [48–50] and remodelling [51] in flaviviruses and related viruses, and on strategies to block these processes [52]. CG simulations of the entire dengue (DENV) particle enabled refinement of the envelope structure composed of 180 envelope (E) and membrane (M) proteins arranged with icosahedral symmetry against cryoEM maps [45]. This was used as a platform to study different aspects of the viral life cycle. For example, the flavivirus envelope has been shown to 'breathe', switching from compact to 'bumpy' conformations in response to an increase in temperature, facilitating infectivity (Figure 3a-b). This is partially reversible only in the presence of divalent cations, and a combination of experiments and multiscale simulations explored the molecular basis for this [53]. Targeted CG simulations of the entire envelope were used to trigger the transition (Figure 3b). Subsequent 'back-mapping' of bumpy virion structures to atomic resolution enabled detailed simulations of E protein pentamers, revealing that divalent cations can 'soak' the pore at the fivefold axes to break inter-chain salt bridges and thereby destabilize the expanded structure (Figure 3bi). The pentameric site has also been targeted by nanoparticles covered with ligands mimicking heparan sulfate proteoglycans [54]. Atomistic simulations further revealed how single amino acid substitutions associated with different viral strains can alter interactions at the E protein dimer interface (Figure 3bii) to modulate the temperature threshold for DENV 'breathing' [55•]. Finally, CG simulations in combination with biophysical experiments have been applied to understand the large-scale conformational changes associated with DENV maturation, and its dependence upon host antibody interactions. Targeted MD simulations of the entire immature DENV envelope were used to explore the transition between intermediates identified by cryoEM (Figure 3d), revealing how antibodies targeting the pr fragment of the precursor membrane (prM) protein may dislodge them to expose a key fusogenic region of the E protein [56•]. This provides a molecular rationale of the phenomenon of antibody dependent enhancement (ADE), which can lead to the most severe cases of dengue pathogenesis.

Conclusions and outlook

Continued success in elucidating previously unknown structures of viruses, or known structures of viruses at different stages of their life cycle, in combination with simulations describing the transition between states and interaction with host components, is expected to fill in many of the current blank spots in our understanding of viral infections. It remains especially challenging to model the structures of viral nucleic acids [10,11,57] as they are rarely organized in clear geometric patterns amenable to cryoEM or crystallographic structure determination. Computational models can make a unique contribution to the understanding of nucleic acid centered processes such as capsid assembly [58–60], packaging [61–64], budding [65], and uncoating [66]. Alternative approaches are emerging with the advent of structure probing by next generation sequencing methods such as SHAPE-MaP [67] or SPLASH [68], which provide information on local genome structure and genome-genome interactions respectively [69•,70,71]. An enhanced comprehension of the precise factors necessary for viral infection and proliferation will allow us to more effectively design therapies against this important and challenging class of pathogens. Moreover, a mechanistic understanding of viruses may assist in the rapid design of more effective vaccines and may prove crucial in combating the threat of global pandemic outbreaks.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Jan K Marzinek: Writing - original draft. **Roland G Huber:** Writing - original draft. **Peter J Bond:** Writing - original draft, Writing - review & editing, Conceptualization, Funding acquisition.

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