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Mechanisms of allostery at the viral surface through the eyes of molecular simulation

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Abstract

The outermost surface layer of any virus is formed by either a capsid shell or envelope. Such layers have traditionally been thought of as immovable structures, but it is becoming apparent that they cannot be viewed exclusively as static architectures protecting the viral genome. A limited number of proteins on the virion surface must perform a multitude of functions in order to orchestrate the viral life cycle, and allostery can regulate their structures at multiple levels of organization, spanning individual molecules, protomers, large oligomeric assemblies, or entire viral surfaces. Here, we review recent contributions from the molecular simulation field to viral surface allostery, with a particular focus on the trimeric spike glycoprotein emerging from the coronavirus surface, and the icosahedral flaviviral envelope complex. As emerging viral pathogens continue to pose a global threat, an improved understanding of viral dynamics and allosteric regulation will prove crucial in developing novel therapeutic strategies.

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Introduction

Viruses are infectious particles on the very border of the definition of life. They generally consist of genetic material and a protective proteinaceous capsid. Some also have an additional external envelope layer composed of a membrane with embedded glycoproteins. Despite this ostensibly simple organisational plan, structural proteins that form the outermost viral surface layers carry the burdens of host recognition, attachment, entry, reassembly, maturation, exit, and immune evasion. While traditionally thought of as static, immovable structures, it is now apparent that viral surface layers are in fact dynamic, with functional regulation often linked to substantial conformational changes. The delicate balance between regulation — linked to environmental factors such as pH, binding of interaction partners, or proteolysis events — and function-performing conformational changes hinges upon rigorous structural cross-talk across viral surface proteins and their assemblies. In this context, allostery is a universal property of proteins to sense environmental perturbations and induce a shift in the energy landscape, resulting in a redistribution of possible conformational states [1]. Allosteric signalling relies on communication networks between regulatory sites and response sites, even if the two are a long distance apart. This multi-step control system ensures precision in regulation, and viral surface layers adeptly use such allosteric regulatory mechanisms to guarantee the correct sequence of functional conformational changes is carried out in the appropriate compartments at the right moments [2].

Molecular dynamics (MD) simulations represent a powerful tool to address the dynamics of allosteric signalling in proteins. Simulating viral surface layers is challenging given their size and often requires access to extremely powerful high-performance computers [3—5]. Such issues may be bypassed by considering a smaller fragment of the viral surface [6,7], utilising advanced and/or AI-driven sampling methods [8,9], or by representing the surface layer using coarse-grained (CG) modelling [10—12]. The latter may be achieved via numerous strategies, such as by representing the solvent implicitly or partially [13], by grouping atoms into lower-resolution beads [14,15], or by a combination of both [16]. The particle-based CG approach can be pushed to even lower resolutions [17], in which case the fitting parameters for bead interactions can be informed by atomistic simulations [18] or experiments [12,19,20]. In such cases, viral capsids, together with some envelopes, are built from a few types of interlaced protein

units arranged in a low-level symmetry, which makes them convenient to model using shape-based CG approaches [21,22]. However, because of their inherent lack of detail, CG methods may be inadequate in describing subtle allosteric communication networks and their signalling effects, especially if those occur within a single protein subunit. Simulation studies might therefore opt for multiple levels of system description. With such a multiscale strategy, the systems can then be described at various levels of magnification and resolution, where low-resolution simulations of viral particles are supplemented with higher-resolution simulations of the smaller segments [9,23,24].

Simulations can elevate our understanding of the allosteric mechanisms in viruses, with even highly simplified "toy models" [25] instrumental in substantiating the idea that controlled assembly of icosahedral surfaces must proceed through allosteric conformational switching [26]. An understanding of such allostery is a prerequisite for rational antiviral therapeutics design, as amply demonstrated by efforts to target the homodimeric core proteins (Cp) of hepatitis B virus (HBV), for which multiple classes of Cp allosteric modulators (CpAMs) have been developed to disrupt capsid assembly. Simulations of HBV assembly intermediates emphasized the importance of allosteric networks [27,28] and rationalized alternative mechanisms of CpAMs in inducing distinct Cp conformational states [29,30], while CpAM-induced correlated motions that may communicate with and thereby disrupt sites crucial for genome packaging were evident in simulations of the entire HBV capsid [31]. Similarly, large-scale simulations of entire HIV-1 capsids revealed spatially correlated patterns of strain across the lattice induced by binding of native cofactors [32], something which could conceivably be modulated for antiviral targeting. In the search for novel druggable sites, so-called "flooding" simulations in the presence of small organic probes such as benzene [33,34] have emerged as a useful tool for uncovering cryptic pockets in viral surface proteins with potential for allosteric modulation [35].

In the following sections, we discuss the concept of viral surface layers as allosterically regulated machines with prospects for therapeutic intervention, and the role that molecular modelling and simulations spanning multiple scales have recently played in advancing the field. We focus in particular on two classes of enveloped viruses with a significant global disease burden, namely coronaviruses and flaviviruses.

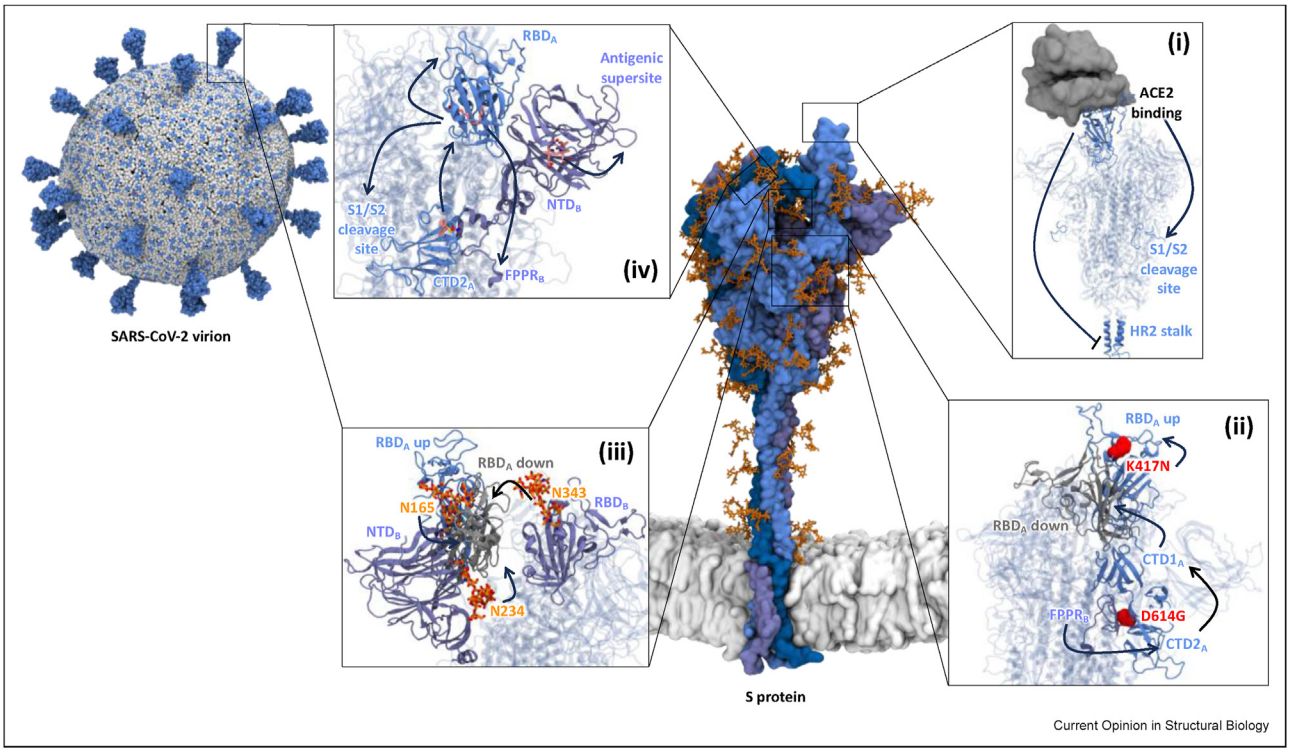
Allosteric communication in the SARS-CoV-2 spike glycoprotein

The spike (S) glycoprotein (Figure 1) which protrudes from the SARS-CoV-2 envelope surface is vital to the spread of COVID-19, and is a major target for prophylaxis and therapeutics development. The S protein is a large homotrimer comprised of two subunits: S1, which is involved in binding to the human angiotensin converting enzyme 2 (ACE2) receptor, and S2, which is crucial for membrane fusion. The receptor binding domain (RBD) is the first point of contact between the S protein and the host cell. The RBD alternates between the "up" conformation, which exposes its receptor binding motif (RBM) for binding to ACE2, and the "down" conformation, whereby the RBM is tucked away. Cleavage at the S1/S2 and S2' sites triggers a large conformational change that reveals a fusogenic sequence driving entry into the host cell. A wealth of structural data have spurred numerous computational studies, including simulations of full-length S protein in its native viral membrane [6,36–39] and millisecond-timescale sampling of S protein opening [40].

At the CG resolution, models of the whole virion were developed incorporating parameters derived from all-atom simulations of individual viral proteins [12] and mesoscale simulations to provide initial guesses of protein organisation [23]. AI-guided workflows driving whole virus simulations in atomic detail used knowledge obtained from smaller-scale simulations of viral components to gain insights into RBD opening, glycan shielding, and ACE2 interactions [9,41]. Tan et al. presented a comprehensive analysis at the single-residue level suggesting that S protein opening is governed by an intricate network of allostery [42]. Correspondingly, numerous simulation studies have shown that the conformational transition of RBD is tightly controlled via complex allosteric networks spanning the S protein (Figure 1). Steered MD simulations of down-to-up RBD opening revealed that multiple residues in the S1 C-terminal domains (CTD1 and CTD2) linking the RBD and the S2 subunit show long-distance coupled motions [43]. These include residue D614, whose mutation to glycine is associated with enhanced infectivity [44]. Mapping the free energy landscape of RBD opening demonstrated that mutations around the hinge between the RBD and CTD1 facilitate RBD opening, suggesting this region could allosterically modulate the conformational transition [45]. Congruently, analyses of the S-ACE2 complex based on an elastic network model converged towards an allosteric modulation region around CTD1 [46]. CG modelling of the S protein identified the CTD1 domain as an allosteric bridge between the RBD and the fusion peptide proximal region (FPPR) [47]. Crucially, cryo-electron microscopy (cryo-EM) structures of the prevalent D614G S protein variant showed that FPPR packs directly against a loop in the neighbouring CTD2 domain, whose order-disorder dynamics correlate with RBD opening [44].

There appear to be allosteric networks within the RBD that optimize the conformation of RBM for ACE2 binding (Figure 1), as indicated by microsecond timescale atomic-resolution simulations of RBD-ACE2

Figure 1



Allostery in SARS-CoV-2 S protein. (Top left) SARS-CoV-2 envelope rendered from a CG model developed by Yu et al. [12]. **(Centre)** Spike model embedded within viral membrane developed by Zuzic et al. [6]. Four types of allosteric communication are illustrated: **(i)** ACE2 binding affects the dynamics of the cleavage site and stalk region; **(ii)** the down-to-up RBD transition is allosterically modulated by distal domains and mutations; **(iii)** glycans facilitate RBD opening; and **(iv)** ligand-bound cryptic pockets.

complexes [48]. Sequence analysis and network mapping of compensatory mutations in S protein variants also suggested that K417N acts as a positive allosteric modulator for ACE2 binding [49]. It is noteworthy that some of the many N-glycans decorating the S protein [50–52] may allosterically modulate RBD opening. Simulations uncovered the essential role of N343 glycans in initiating opening of the S protein [8,53], with N165 and N234 glycans inserting between the RBD and neighbouring N-terminal domain (NTD) to stabilise the up conformation [13,37,54]. Finally, the receptor binding event itself appears to send allosteric signals to prime the S protein for the next steps of infection, by increasing the dynamics of the S1/S2 cleavage site while simultaneously reducing the dynamics of the stalk region to stabilise the S-ACE2 complex, as revealed by MD simulations and hydrogen-deuterium exchange mass spectrometry [55]. Congruently, mutations in progressively emerging variants appear to stabilise the S protein stalk and alter the dynamics of the S1 domain to enhance receptor recognition or viral entry [56].

Uncovering spike's repertoire of allosteric pockets

Collectively, the S protein is a hub for multiple allosteric communications, some of which present opportunities for development of therapeutics against COVID-19 (Figure 1). Cryo-EM structures of the S protein identified cryptic pockets on the RBD and NTD bound to hydrophobic molecules such as linoleic acid [57], polysorbate detergent [58], haem [59], and metabolites [60]. A multidisciplinary approach incorporating simulations and free-energy calculations revealed these pockets also bind to Gram-negative bacterial lipopolysaccharide to boost host inflammatory reactions [61,62]. Dynamical non-equilibrium MD simulations showed that removal of lipid from the RBD pocket results in a variant-dependent allosteric response on the RBM and antigenic supersite of the neighbouring NTD [63], suggesting a role in virulence [64]. A metainterference cryo-EM approach identified a cryptic pocket in an intermediate state of the RBD transition [54]. Benzene flooding simulations of S protein uncovered a novel cryptic pocket underneath a loop at residues 617–628 in

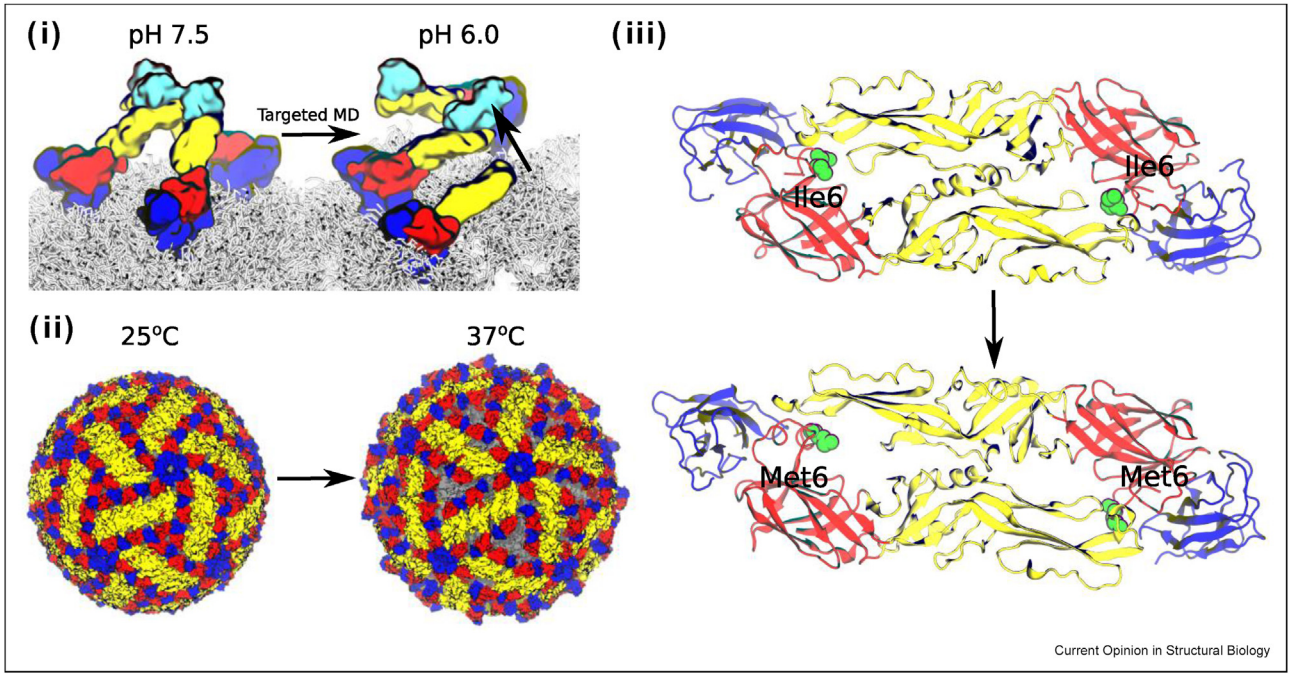
CTD2 [6]. The RBD conformational transition is allosterically correlated to the order-disorder conformational dynamics of this CTD2 loop, which is linked to promotion of premature S1 shedding, making it an attractive target for drug design [44]. Cryo-EM and simulations of common cold human coronavirus HKU1 spike revealed that the binding of a sialoglycan to a cryptic pocket on its NTD-equivalent domain allosterically triggers opening of its RBD-equivalent domain [65], suggesting that allosteric pocket modulations are a universal feature of (beta)coronaviruses.

Allosteric control of the icosahedral flaviviral shell

Flaviviruses encompass numerous human pathogens such as dengue, yellow fever, and Zika viruses. The mature flavivirus outer shell is made up of envelope (E) and membrane (M) proteins, arranged with icosahedral symmetry and embedded within a lipid bilayer. The E protein is divided into three domains (DI, DII, and DIII) with DII contributing to virus-mediated membrane fusion inside endosomes. The flaviviral life cycle is tightly regulated by host environmental factors, coordinated via multiple E/M protein conformational transitions propagated over long distances across the entire viral envelope to induce alternative particle morphologies. Multiscale simulations in combination with cryo-EM and other integrative biophysical approaches have provided substantial insights into these dynamic processes [66].

Immature flavivirus particles consist of trimeric spikes of E and precursor M (prM) proteins. These undergo large structural rearrangements into E protein dimers resulting in "smooth" viral particles when localized inside the low-pH environment of the trans-Golgi network, exposing furin protease sites on prM [67]. The cleaved pr molecule remains attached to the E protein in the low-pH environment, capping the fusion loop and inhibiting fusogenicity until the release of the virus from the cell. Interestingly, exposure to anti-prM antibodies accelerates maturation, which can enhance infection and worsen patient outcomes. Multiscale MD simulations were used to rationalize this phenomenon, by driving the entire viral envelope along the pathway of maturation between conformational intermediates resolved by cryo-EM [68] (Figure 2). Thus, pr molecules on E/pr complexes were shown to unbind from a specific "regulator" E protein across all asymmetric units on the viral envelope early on during maturation progression. Furthermore, superposed pr-bound antibodies further contributed to this E/pr disassembly, and were predicted to help in dislodging pr molecules to expose the E protein fusion loop during global

Figure 2

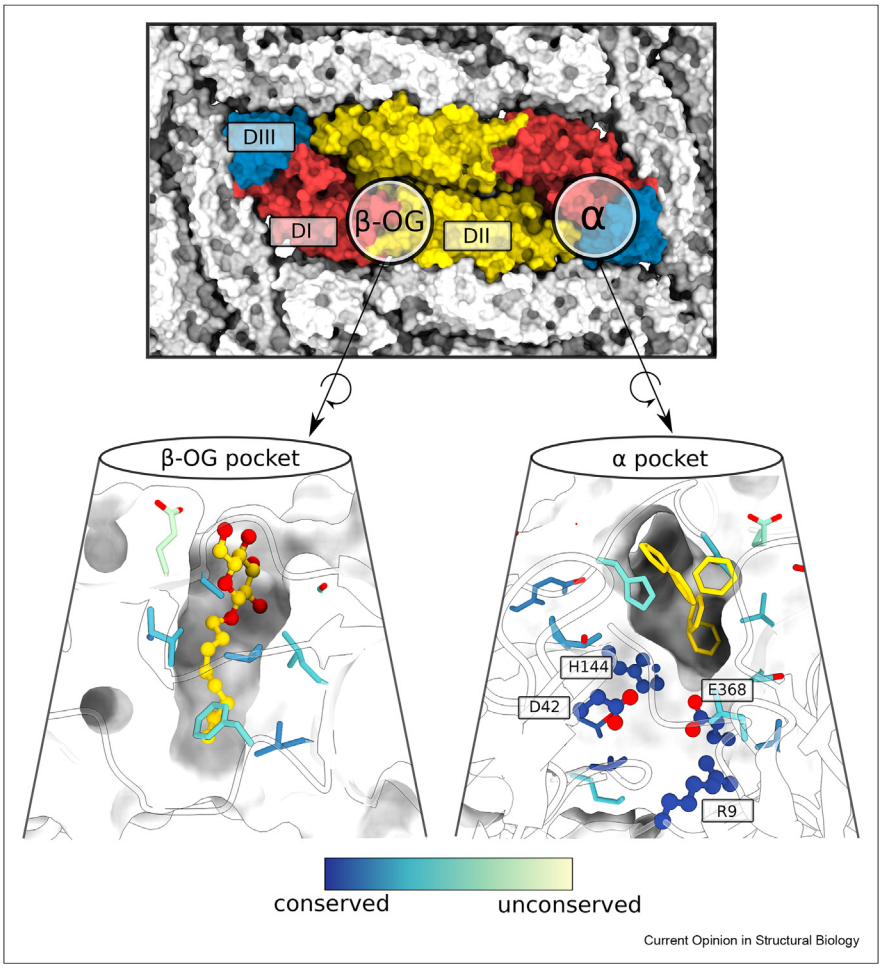


Maturation and flaviviral envelope "breathing" regulated by allosteric mechanisms. (i) Antibody-assisted maturation of icosahedral envelope via loss of pr molecules (cyan) from E proteins (DI in red, DII in yellow, DIII in blue) on the viral lipid bilayer (grey) explored by targeted MD simulations. **(ii)** Temperature-induced "breathing" of mature viral particle. **(iii)** Ile6Met mutation (green) destabilizes E protein dimer interface, allosterically triggering interdomain motions that induce bumpy virus morphology.

protein reorganization of the viral envelope into its mature morphology. Dengue virus-like particles lacking genomic material may also undergo a similar maturation process and hence hold promise as vaccines [69].

Mature dengue virus particles exhibit structural alterations even outside the cell, switching from smooth to "bumpy" morphologies when exposed to mammalian body temperature [70]. Different clinical isolates from patients exhibit variations in the temperature at which this "breathing" phenomenon is observed, corresponding to either normal body temperature (37 °C) or high fever (40 °C) [71]; this may result from selective pressures associated with thermostability and infectivity, but also immunological exposure given different virion morphologies have different antigenic properties. Atomic-resolution simulations were used to study E proteins from different isolates, revealing how single amino acid substitutions induce subtle structural effects — such as a switch in a loop conformation (Figure 2) — leading to inter-domain motions that disrupt symmetry-related icosahedral contacts in the smooth virus particle. This would result in the emergence of bumpy morphologies at a lower temperature threshold [71]. Interestingly, virion breathing was shown to be reversible in the presence of physiological concentrations of divalent cations, with a multiscale simulation strategy demonstrating that Mg^{2+} or Ca^{2+} can break inter-subunit salt bridges at the icosahedral 5-fold vertices to destabilize the bumpy state [72]. The ability to adopt different shapes at varying temperatures adds complexity to the development of therapeutic antibodies and vaccines, though a follow-up study demonstrated that with sufficiently high affinity, antibodies can bind even partially hidden epitopes to neutralize strains regardless of their morphology [73].

Figure 3



Cryptic pockets involved in allosteric modulation of flaviviruses. Two allosteric pockets (α and β-OG) on E protein dimer (red, yellow, blue). Zoomed views show both pockets in surface representation, bound ligand(s) in yellow, and surrounding residues in colours corresponding to their conservation score (key, inset). The bottom of the α pocket contains a conserved cluster of ionisable residues (ball and stick representation), labelled for dengue virus serotype 2 sequence.

Allosteric inhibition of the flaviviral envelope

Exposure of mature virions to low pH following endocytosis triggers extensive conformational rearrangements of E protein dimers into trimeric spikes that catalyse fusion, allowing genomic delivery into the cytosol. Targeting small molecules to allosteric sites on the E protein represents a promising antiviral approach focussing on the early stages of the viral life cycle [74]. Early crystallographic studies of dengue virus revealed a cryptic pocket located at the DI-DII interface which opens upon binding of an *n*-octyl-β-D-glucoside (β-OG) detergent molecule (Figure 3) [75]. Extensive efforts have gone into developing viral inhibitors targeting this pocket [76,77], but so far without clinical success [7].

Atomic-resolution simulations of flaviviral rafts — composed of three E protein dimers embedded within a viral membrane model — paired with a benzene flooding approach uncovered a new cryptic site at the DI-DIII interface termed the α pocket [7]. In addition to being highly conserved and exhibiting comparable dynamics across strains, the α pocket contains an interconnected network of ionisable residues (Figure 3). Constant-pH simulations revealed that a gradual drop in pH results in accumulation of positive charge, destabilising the ionisable cluster and leading to separation of DIII from DI, presumed to be the first step towards formation of the fusogenic spike. The highly conserved and functionally relevant nature of the α pocket thus makes it a promising site for targeting of allosteric pan-flaviviral drugs.

Conclusions and outlook

It is tantalising to imagine possible routes of inhibiting viral life cycles using small molecule or antibody-based therapeutics by targeting allosteric and/or cryptic sites on viral surface proteins, either by suppressing the allosteric signal or by causing the signal to misfire. Accurate targeting of such processes hinges upon our understanding of the allosteric mechanisms and their role, increasingly achievable through multiscale simulations of viral surface layers. The integration of multiple structural and biophysical data allows us to build realistic models of viruses [9,12,23,41]. Computational resources have also stretched far beyond what was previously available as exemplified by the advent of exascale computing via the Folding@home network, applied to generate 0.1 s of simulations of the SARS-CoV-2 proteome [40]. Furthermore, advances in AI and machine learning are helping to integrate complex data and accelerate conformational sampling, as demonstrated by simulations of the entire SARS-CoV-2 envelope [9]. Importantly, the increasing trend towards open source data, as shown by various publicly available resources during the COVID-19 pandemic, is helping to accelerate research worldwide. Simulation-aided development of antivirals that bind to allosteric pockets has enormous potential, especially when they are cryptic in nature, as such pockets are under less evolutionary pressure to undergo immune evasion or resistance via mutation. Our understanding of allosteric modulation of viral proteins should therefore be actively leveraged for future development of therapeutics against emerging pathogens.

CRediT author statement

Firdaus Samsudin: Writing — original draft, Writing — review & editing, Funding acquisition. **Lorena Zuzic**: Writing — original draft, Writing — review & editing. **Jan K. Marzinek**: Writing — original draft, Writing — review & editing, Funding acquisition. **Peter J. Bond**: Writing — review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

No data was used for the research described in the article.

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