Review

Structural Virology: The Key Determinants in Development of Antiviral Therapeutics

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Review

Structural Virology: The Key Determinants in Development of Antiviral Therapeutics

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Abstract: Structural virology has emerged as the foundation for the development of effective antiviral therapeutics. It is pivotal in providing crucial insights into the three-dimensional frame of viruses and viral proteins at atomic or near atomic-level resolution. Structure-based assessment of viral components, including capsids, envelope proteins, replication machinery, and host interaction interfaces, are instrumental in unravelling the multiplex mechanisms of viral infection, replication, and pathogenesis. The structural elucidation of viral enzymes, including proteases, polymerases, and integrases, has been essential in combating viruses like HIV, SARS-CoV-2, and influenza. Techniques including X-ray crystallography, Nuclear Magnetic Resonance spectroscopy, Cryo-electron Microscopy, and Cryo-electron Tomography have revolutionized the field of virology and significantly aided in the discovery of antiviral therapeutics. The ubiquity of chronic viral infections, along with the emergence and re-emergence of new viral threats necessitate the development of novel antiviral strategies and agents, while the extensive structural diversity of viruses and their high mutation rates further underscore the critical need for structural analysis of viral proteins to aid antiviral development. This review highlights the significance of structure-based investigations for bridging the gap between structure and function, thus facilitating the development of effective antiviral therapeutics, vaccines, and antibodies for tackling emerging viral threats.

Keywords: Structural virology; antiviral therapeutics; viral proteins; viral replication enzymes; X-ray crystallography; nuclear magnetic resonance (NMR); cryo-EM; emerging and re-emerging viruses; rational drug design; bioinformatics

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1. Introduction

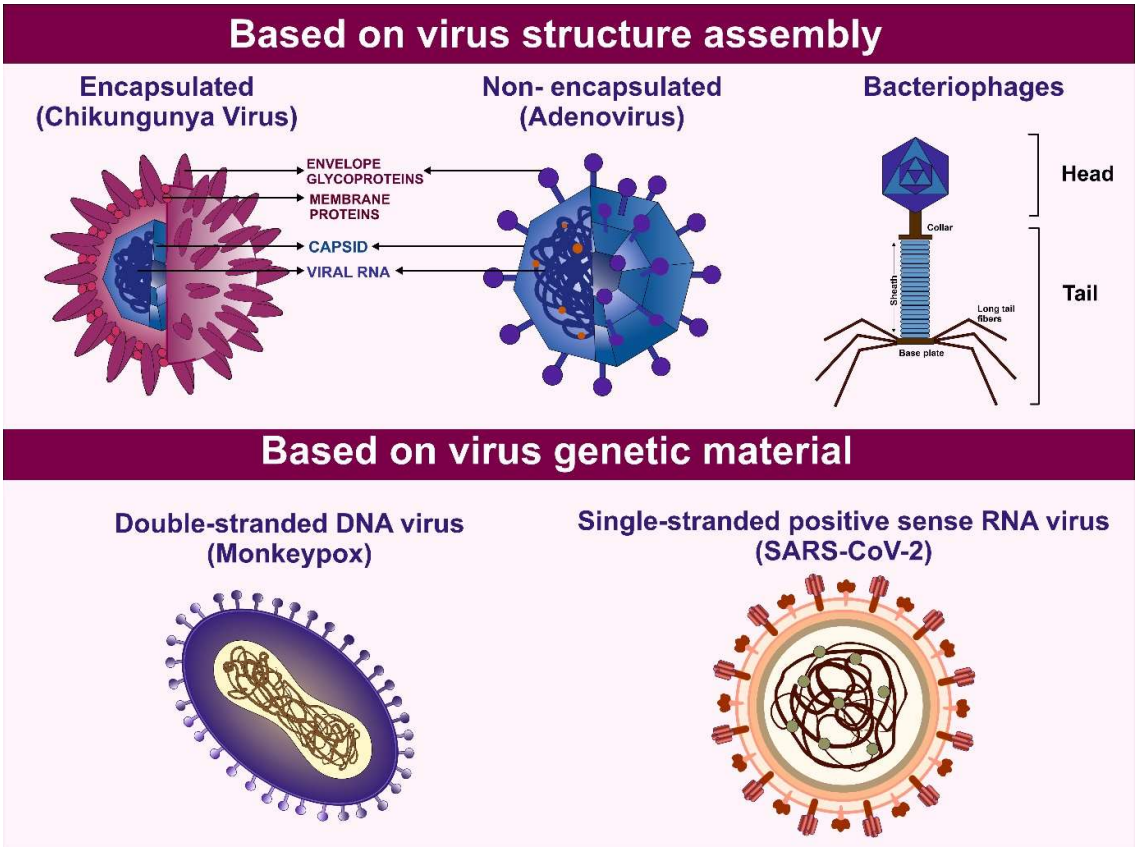

Figure 1. Graphical representation of various viruses exhibiting diverse characteristics based on their structural composition and genetic organization. The figure above highlights examples of various virus types based on their diverse features.

Viruses constitute a diverse group of sub-microscopic infectious agents that are reliant on the host cells' metabolism to replicate. They lack cellular structures and possess a genome composed of deoxyribonucleic Acid (DNA) or ribonucleic acid (RNA), which can be single-stranded or double-stranded. The genome can range from 3000 to over 1,000,000 nucleotides, and virus size can vary from 10 to 1000 nm [1] (Figure 1). According to the International Committee on Taxonomy of Viruses (ICTV), there are 314 families of viruses as of 2024 [2]. Viruses replicate via a series of complex steps, including initial attachment to the host cell, followed by entry, subsequent uncoating, genome replication, protein synthesis, virion assembly, and release of viral particles. Viral infection can thereby hijack the cellular machinery and disrupt cellular metabolism. These perturbations can manifest as a broad range of pathological outcomes for the host organism and even death [1]. Owing to the wide variety of viruses and the diversity of hosts, including bacteria, blue-green algae, fungi, plants, insects, and vertebrates, viruses pose a threat of infection across the three cellular domains of life- Archaea, Bacteria, and Eukarya.

Archaeal viruses can be broadly divided into archaea-specific viruses and cosmopolitan archaeal viruses, classified into 12 and 5 families, respectively. Most known archaeal viruses have been isolated from extreme environments, from hyperthermophiles or hyperhalophiles. These viruses are known to encode anti-clustered regularly interspaced short palindromic repeats (CRISPR) proteins. It is suggested that the viruses play a significant role in ocean biogeochemical cycling [3,4]. Bacteriophages infect bacteria and exhibit ubiquitous distribution in the environment and diverse genomes. They exhibit lytic or lysogenic life cycles and can facilitate horizontal gene transfer, playing

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a crucial role in microbial ecology and evolutionary dynamics. Temperate phages can form a mutually beneficial relationship with their host. Phages have played a significant role in developing several molecular biology techniques, including CRISPR-Cas (CRISPR-associated protein) system for genome editing. They have recently been utilized in phage display technology and as phage therapy to combat antimicrobial resistance [5,6]. Amongst eukaryotes, protists, including amoebae, ciliates, and flagellates, can be infected by protist-infecting viruses. Giant viruses (GVs) such as Mimivirus belonging to phylum *Nucleocytoviricota* have genome and particle sizes comparable to prokaryotes and small eukaryotes [7,8]. Algal viruses, such as chloroviruses and phaeoviruses infecting *Chlorella* and brown algae, respectively, influence host evolution via predator-prey selection and genetic exchange, thereby affecting host fitness and microbial community composition. The infections can lead to aquatic "viral shunt," i.e., alteration of organic matter composition and distribution [9]. Viruses that infect fungi are known as mycoviruses and are classified into 23 families and the genus *Botybirnavirus*. Mycoviruses infecting plant pathogenic fungi are the primary research focus due to their potential to act as biocontrol agents against the host fungi. They are reliant on hyphal anastomosis for intracellular spread and lack an extracellular transmission mechanism, limiting cross-strain spread [10]. Plant viruses pose a significant threat to agriculture and food security and can potentially cause pandemics and epidemics globally [11,12]. They are predominantly RNA viruses transmitted via vectors such as aphids, nematodes, whiteflies, fungi, or mechanical injury. Inside the plant host, they employ plasmodesmata and vasculature to spread internally, exhibiting symptoms such as mosaics, chlorosis, stunting, and wilting. Notable examples include Tobacco mosaic virus (TMV), Potato virus Y (PVY), and Cucumber mosaic virus (CMV) [11,12].

Animal viruses infect an extensive range of hosts, including vertebrates and invertebrates such as insects [1]. They display significant structural and genetic diversity and are categorized into several families. Insect viruses include families such as Baculoviridae and Iridoviridae, which are known to infect lepidopteran larvae and other insects, respectively [13]. These viruses can be vital for pest management and agriculture. Moreover, arthropods serve as vectors for the transmission of arboviruses, such as members of Flaviviridae, Togaviridae, and Nairoviridae**,** to other animals, including humans [14–16]. Infection with animal viruses can manifest into numerous disease pathologies, including localized and systemic infection in wild and domestic animals. Members of Orthomyxoviridae (e.g., influenza viruses) [17,18] and Rhabdoviridae (e.g., rabies virus- RABV) [19] have caused substantial disease burden and mortality. A subset of animal viruses is represented by human viruses, which include notable pathogens, such as members of Coronaviridae (e.g., Severe Acute Respiratory Syndrome Coronavirus 2- SARS-CoV-2) [20,21], Orthomyxoviridae (e.g., influenza viruses) [17,18], Herpesviridae (e.g., herpes simplex virus- HSV) [22–25], Papillomaviridae (e.g., human papillomavirus- HPV) [26–28], Retroviridae (e.g., human immunodeficiency virus- HIV) [29,30], and Picornaviridae (e.g., poliovirus) families [31,32]. Seven identified human oncoviruses, including Epstein-Barr virus (EBV), human T-cell leukemia virus type 1 (HTLV-1), hepatitis B virus (HBV), HPV, hepatitis C virus (HCV), Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8), and Merkel cell polyomavirus (MCV or MCPyV) account for causing an estimated 12-15% of cancers globally [33]. Additionally, the zoonotic spillover from animals to humans or reverse zoonosis from humans to animals, potentially facilitated by wildlife farming and trade, is of great concern [34,35]. Examples of zoonoses include rabies and avian influenza. All known coronaviruses (HCoVs) are believed to have originated in animals, with five of the seven HCoVs originating in bats [36]. Most recently, the Coronavirus disease 2019 (COVID-19) outbreak is believed to have transmitted from bats to humans. Therefore, adopting a One Health approach, considering human, animal and environmental health, for disease prevention and control is imperative.

Throughout history, viral infections have periodically emerged as epidemics and pandemics, resulting in significant loss of life. It has been estimated that there have been at least 14 influenza pandemics since 1500, including the Russian flu (1889-1893), Spanish flu (1918-1920), Asian flu (1957-1959), Hong Kong flu (1968-1970), and the first influenza pandemic of the 21st century, Swine flu (2009-2010) [37,38]. The ongoing HIV/ AIDS pandemic (1981-present) has claimed millions of lives

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[39,40]. Severe acute respiratory syndrome coronavirus (SARS-CoV) [41], Middle East respiratory syndrome coronavirus (MERS-CoV) [42], and SARS-CoV-2 [20,21] are distinct coronaviruses that emerged in 2002, 2012, and 2019, respectively. On 11 March 2020, the World Health Organization (WHO) declared COVID-19, caused by SARS-CoV-2, a pandemic, which raised an alarming situation and caused ~7 million fatalities worldwide [43]. Other significant outbreaks include Smallpox epidemics in the 17th century [44], polio epidemics in the 20th century [45], frequent outbreaks of Ebola [46], Dengue fever [47], yellow fever [48], Zika [49], Measles [50], Chikungunya [51], Japanese encephalitis [52], West Nile fever [53] and rabies [19,54]. As many RNA viruses are emerging and re-emerging viruses, they can evolve and reappear in the future with mutations [55,56]. The frequent viral outbreaks and lack of effective treatment and vaccination strategies underscore the urgent need to identify and develop antiviral therapeutics and advanced drug discovery for preparedness against future viral pandemics.

The study of three-dimensional (3D) structures of proteins has been recognized as crucial to expediting drug discovery. It offers insights into the shape of targets, hydrophobic and hydrophilic behaviours of macromolecules, and their interactions with substrates. Structural biology techniques are employed to study the key components of viruses, including structural proteins, replication proteins, and host interaction sites, thereby bridging the gap between viral structure and function, playing a pivotal role in shaping the development of antiviral therapies. The present review provides a comprehensive summary of structure-based investigations in the field of virology that lead to the identification and development of antiviral therapeutics and advanced drug discovery and explores the potential of structural virology in addressing emerging viral threats.

2. Exploring the 3D Protein Landscape: Structural Biology Techniques

Structural biology aims to understand the 3D structure of biological macromolecules, including proteins. These techniques have been employed in the study of viruses for close to a century. It not only furthers our understanding of life's molecular machinery but also enhances our ability to design targeted therapeutic interventions against disease. Researchers can gain valuable insights into biochemical activities and mechanisms by solving protein complex structures, which can be instrumental in drug design, and biotechnology. These methods have helped us visualize the molecular world and expose dynamic and transient stages of proteins [57]. The evolution of structural biology from basic chemical analysis to advanced imaging techniques mirrors scientific inquiry and technology. Chemical degradation and conventional optical microscopy initially provided limited information regarding molecular composition and structure. However, the discovery of X-ray crystallography by the pioneering work of Max von Laue and William Henry Bragg in the early 20th century provided atomic-level resolution and paved the way for structure-guided molecular biology [58,59].

2.1. X-ray Crystallography

X-ray crystallography has been instrumental in antiviral research by enabling the analysis of high-resolution atomic details of crystallized proteins and complexes by interpreting the diffraction patterns. [60–62]. TMV was the first virus to be crystallized by Wendell Stanley in 1935. It was demonstrated that the infectivity of the virus was retained in crystalline form. He was awarded the Nobel Prize in Chemistry in 1946 [63,64]. The virus structure was described in detail for the first time by Bernal and Fankuchen, who examined TMV suspension via X-ray diffraction [65]. The first atomic-resolution structure of a virus was provided in a pioneering study by Harrison et al., revealing an icosahedral arrangement of 180 capsid protein subunits of the Tomato bushy stunt virus (TBSV) at 2.9 Å resolution [66]. Parallelly, Aaron Klug and colleagues determined the structure of the TMV protein disk at a resolution of 2.8 Å [67] and revealed the structure of nucleosome core particle at 7 Å resolution [68]. Aaron Klug was awarded the Nobel Prize in Chemistry in 1982 for his development of crystallographic electron microscopy and his structural elucidation of biologically important nucleic acid-protein complexes [64]. With the development of therapeutics against HIV and HCV, X-

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ray crystallography became pivotal in antiviral and vaccine research in the late 20th century. Protein crystallization faces challenges such as limited solubility, unresolved protein dynamics, and chemical heterogeneity, complicating structural determination and drug discovery. Additionally, X-ray crystallography offers only static snapshots of molecules, potentially missing important dynamic interactions. The limitations of X-ray crystallography, especially its reliance on crystalline crystals, spurred further advancements [69,70]. Despite these limitations, the impact of X-ray crystallography on fields such as biochemistry, pharmacology, and virology has been profound. The primary advantage of the technique over others is its ability to provide highly detailed atomic resolution structures, essential for understanding the precise molecular mechanics of biological processes. It has enabled the detailed mapping of the interaction sites for drug molecules, providing a foundation for rational drug design and a deeper understanding of fundamental biological processes. Overall, X-ray crystallography remains a vital method in structural biology, complemented by newer techniques that provide insights into the structures of non-crystallizable molecules [71].

2.2. Nuclear Magnetic Resonance (NMR)

NMR spectroscopy was developed as a complement to study chemicals, including biomolecules in solution, revealing insights into their conformational flexibility. NMR spectroscopy has evolved from a chemical analysis tool to a fundamental technique in structural biology since the mid-20th century. Advances in technology, higher magnetic field strengths, and computational methods during the 1970s and 1980s allowed NMR to determine the structures of proteins in solution, providing dynamic molecular insights and study of dynamics and interactions within hosts [72]. The technique's ability to reveal protein dynamics and atomic interactions opened new avenues for the study of protein folding, enzyme activity and ligand interaction critical to viral pathogenesis and lifecycle, and eventually identifying antivirals to target the viral antigen proteins. NMR serves as a distinctive investigative tool for obtaining atom-resolved information regarding the structural and dynamic characteristics of highly flexible and disordered proteins, such as intrinsically disordered proteins (IDPs). In contrast to the more compact structures of globular protein domains, IDPs significantly influence NMR observables, necessitating the customization of NMR experiments for their study. In this context, ^{13}C direct detection NMR has emerged as a valuable instrument for the characterization of IDPs/IDRs at an atomic resolution [73]. NMR is, therefore, uniquely suited for examining physiological states and complex biological processes. However, it is limited by its applicability mainly to smaller proteins (up to about 35 kDa), the requirement of large sample amounts, and extensive time [73]. Its spectral complexity demands high expertise for data interpretation, presenting challenges in high-throughput environments. Despite its limitations, NMR has profoundly impacted structural biology. The conjugation of X-ray crystallography and NMR aids in a deeper understanding of the protein structures [70]. NMR spectroscopy aids antiviral drug discovery by identifying ligand-protein interactions, optimizing drug properties, detecting false positives, and supporting multidisciplinary approaches. NMR spectroscopy can help accelerate the design of antiviral drugs. It has been instrumental in studying the HCV non-structural proteins, including protease, helicase, and polymerase, optimizing drug properties, and validating hits from screening and identifying peptidomimetics against HCV non-structural protein (NS3) serine protease [74].

2.3. Transmission electron microscopy (TEM)

Electron microscopy is used to visualize the ultrastructure of specimens using focused electron beams. Helmut Ruska made significant contributions to the field of virology by visualizing viruses in the 1930s. In the next decade, he detailed the sub-microscopic structures of various viruses, including poxviruses, TMV, varicella-zoster virus (VZV), and bacteriophages primarily employing TEM [75]. During the 1940s, TEM was employed for the diagnosis of smallpox and chicken pox [76]. In 1959, a negative staining method for high-resolution electron microscopy of viruses was developed [77]. TEM has since been used to understand viral structures, virus-host interaction studies, vaccine

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development, mutation monitoring, nanomedicine imaging, and diagnostics [78]. The challenges of TEM, such as uneven specimen staining and staining-induced distortions, were overcome when the first successful implementation of cryo-electron microscopy (cryo-EM) was reported.

2.4. Cryo-electron microscopy (cryo-EM)

Cryo-EM methodology acquires images of specimens cooled at cryogenic temperatures, aiding in the visualization of proteins, viruses, and complexes in their native state [79]. Its advent revolutionized the field of structural biology as it allowed the study of large protein complexes and fleeting protein states that are hard to crystallize. The technique gained popularity as it facilitates the visualization of biomolecules in their native, hydrated conformations and can achieve near-atomic resolutions comparable to X-ray crystallography without crystallization. With more sensitive detectors and better image processing tools, cryo-EM has become the standard for structural studies of viruses and components such as viral capsids, membrane proteins, and protein complexes [80]. This has provided invaluable insights into viral assembly, infection mechanisms, and interactions with host cells, directly impacting the development of antiviral drugs and vaccines [81]. One of the key advantages of Cryo-EM over other techniques is its ability to study complex and large biomolecular assemblies at near-atomic resolutions, enabling the study of membrane proteins, large protein complexes, and viruses. Additionally, Cryo-EM can capture snapshots of multiple conformational states of a molecule, providing a dynamic perspective on the functional mechanisms. For instance, the structure of RNA polymerase determined using Cryo-EM has advanced the understanding of the dynamic behaviour of the enzyme [82]. The technique is not without challenges, including the need for expensive, high-maintenance equipment and the requirement for significant computational resources to process large datasets. Moreover, achieving the highest resolutions often necessitates many images and averaging to obtain the 3D structures, which can be time-consuming to collect and analyze. Nevertheless, it enabled unprecedented molecular insights, facilitating structure-guided therapeutic design and driving continual advancements in deciphering intricate biological processes [83].

2.5. Small Angle X-ray Scattering (SAXS)

Building upon the principles of X-ray crystallography, SAXS offers a complementary approach by allowing the study of macromolecules in solution, providing insights into their size, shape, and conformational changes. Unlike X-ray crystallography, which requires the formation of crystals and primarily gives high-resolution static structures, SAXS can analyze samples that are difficult to crystallize and provides low-resolution data on flexible and dynamic assemblies in near-native conditions [84]. SAXS is particularly advantageous for examining large complexes and conducting rapid screenings of samples under various conditions, making it a valuable tool in cases where X-ray crystallography is not feasible. SAXS has been essential in studying IDPs, revealing 3D structures of aggregates and identifying different stages of protein aggregation due to their flexible domains and smaller size [85]. Conversely, for detailed atomic resolution structures necessary for precise molecular interactions, X-ray crystallography remains the superior technique [86].

2.6. Cryo-electron tomography (Cryo-ET)

Leaning on the capabilities of cryo-EM, cryo-ET helps study structural biology further by providing detailed 3D visualizations of cells and viruses in their native environment. While cryo-EM offers revolutionary insights into individual proteins and complexes, cryo-ET extends this by allowing scientists to examine the spatial organization and interactions within entire cells or tissues at near-atomic resolutions. In the early 2000s, cryo-ET provided the first visualization of HIV-1 envelope glycoproteins (Env) on the virion surface, revealing their unique tripod-like structure [87]. Subsequent studies have further elucidated Env's conformational dynamics, aiding in the design of broadly neutralizing antibodies and vaccines [88]. This makes Cryo-ET a superior technique for

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understanding complex viral infection mechanisms and cellular architecture dynamics, as it captures biological processes *in situ* without the need for sample sectioning or markers, providing a more comprehensive and realistic view of molecular biology [89].

2.7. Emerging Techniques

Together, these techniques complement each other, and ongoing and future advances in technologies such as X-ray Free Electron Laser (XFEL) imaging are broadening the horizons of structural biology as these may disclose new biomolecular behaviour, especially in cells and in reaction to inhibitors [90]. Advancements in computational biology have enabled simulations that predict protein folding and dynamics based on known sequences. Techniques such as molecular dynamics (MD) simulations complement experimental data by providing insights into conformational changes over time. AlphaFold 3 is the latest iteration of Google DeepMind's artificial intelligence (AI) tool that offers unparalleled accuracy in predicting 3D protein structures and complex biomolecular assemblies, including protein-nucleic acid and protein-small molecule complexes. In 2024, the Nobel Prize for Chemistry was awarded to David Baker for computational protein design, and to Demis Hassabis and John M. Jumper (Google DeepMind) for protein structure prediction [91].

3. Exploring viral structural proteins

Structural proteins are the first to engage with the host receptors during an infection. Structural proteins form the virus's architecture, comprise a protective outer shell for the genetic material (nucleocapsid), a lipid bilayer containing embedded viroporins (membrane proteins) encasing the capsid, and external proteins facilitating interactions between the virus and host cells (envelope proteins). These proteins are essential for the virus as they depend on these to bind to host receptors, take over host cells, and establish their replication machinery [92]. Adherence to host cells is an essential preliminary phase in the infection process for numerous viruses. Proteins facilitating these interactions have been identified as critical therapeutic targets. For example, The H3 protein in Monkeypox virus (MPXV) is crucial for viral adherence through its interaction with cell-surface heparan sulfate (HS) [93]. Likewise, the E2 protein is crucial for attachment in alphaviruses. Hence, it becomes an essential target for virus entry inhibition [94]. Following attachment, viral infiltration via endocytosis is frequently aided by structural proteins. This process is crucial for virus entry and genome release [95]. In alphaviruses, the E1 protein is pivotal for membrane fusion, facilitating virus entry. As the viral infection progresses, the virus hijacks the host system for the assembly and budding of viruses [96]. Structural proteins are also essential for forming new viral particles within infected cells and their subsequent egress (budding) to infect more cells. For instance, the 6K protein in alphaviruses, while its function is not entirely elucidated, is believed to contribute to viral budding and enhance membrane permeability [97]. Additionally, numerous viruses possess an internal nucleocapsid core that encases the viral genome. The capsid protein (Cp) constitutes the primary element of the nucleocapsid in many viruses; it engages with the viral RNA and establishes the core structure [98]. Encapsulating the viral genome within the protective protein shell necessitates precise interactions between structural proteins and the viral genetic material. In Coronaviridae, the replication and transcription of the viral genome are primarily conducted by the replicase; however, other factors, including viral structural proteins and host proteins, have also been implicated. The coronavirus nucleocapsid protein functions as an RNA chaperone, facilitating template switching in the synthesis of sgRNA [99]. Via attachment, these viruses make critical interactions with the host receptors, which eventually hijack the host cells and develop the virus assembly line. Hence, blocking these interactions of viral structural proteins and host receptors is a crucial step for developing antivirals as entry inhibitors [100]. A structure-guided approach for identifying host receptors that interact with viral proteins, as well as identifying critical residues for viral antigen and host receptor interaction, can act as a therapeutic target covering a range of viruses [101]. Many therapeutics in the

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form of antivirals and antibodies have been identified against viral structural proteins based on structure-guided approach and have been in use (Table 1).

Table 1. List of FDA approved therapeutics against viral structural proteins.

Enveloped Glycoprotein						
Protein name	**Virus (family)**	**Name of Therapeutic**	**Type**	**Brand name**	**Experimental Method**	**PDB ID**
N1 neuraminidase	Influenza A Virus (Orthomyxoviridae)	Zanamivir	Antiviral	Relenza	X-ray diffraction	3CKZ,
N8 neuraminidase	Influenza A Virus (Orthomyxoviridae)	Zanamivir	Antiviral	Relenza	X-ray diffraction	2HTQ
N1 Neuraminidase	Influenza A Virus (Orthomyxoviridae)	Oseltamivir	Antiviral	Tamiflu	X-ray diffraction	3CL0, 2HU4
hemagglutinin (HA)	Influenza A Virus (Orthomyxoviridae)	Umifenovir	Antiviral	Arbidol	X-ray diffraction	5T6N, 5T6S
Neuraminidase (NA)	Influenza A Virus (Orthomyxoviridae)	Laninamivir octanoate	Antiviral	Inavira	X-ray diffraction	3TI4
N8 neuraminidase	Influenza A Virus (Orthomyxoviridae)	Peramivir	Antiviral	Rapivab	X-ray diffraction	2HTU
Glycoprotein 120	HIV (Retroviridae)	412d	Antibody (Human)	N/A	X-ray diffraction	2QAD
glycoprotein (GP) trimer	Ebola virus (Filoviridae)	Atoltivimab, maftivimab and odesivimab	Antibody (Human)	Inmazeb (REGN-EB3)	Cryo-EM	7TN9
glycoprotein (GP) trimer	Ebola virus (Filoviridae)	mAb100 and mAb114	Antibody (Human)	Ansuvimab	X-ray diffraction	5FHC
Spike protein (S)	SARS-CoV-2 (Coronaviridae)	LY-CoV555	Antibody (Human)	Bamlanivimab	X-ray diffraction	7KMG
Spike RBD	SARS-CoV-2 (Coronaviridae)	Ly-Cov1404	Antibody (Human)	Bebtelovimab	X-ray diffraction	7MMO
Spike RBD	SARS-CoV-2 (Coronaviridae)	azd8895 and azd1061	Antibody (Human)	Cilgavimab	X-ray diffraction	7L7E
Spike RBD	SARS-CoV-2 (Coronaviridae)	CA1 and CB6	Antibody (Human)	Etesevimab	X-ray diffraction	7C01
Spike RBD	SARS-CoV-2 (Coronaviridae)	REGN10933 and REGN10987	Antibody (Human)	Masavibart	Cryo-EM	6XDG
Spike RBD	SARS-CoV-2 (Coronaviridae)	Ct-P59	Antibody (Human)	Regdanvimab	X-ray diffraction	7CM4
Spike RBD	SARS-CoV-2 (Coronaviridae)	GAR05 and GAR12	Antibody (Human)	Sotrovimab	X-ray diffraction	7T72
Spike RBD	SARS-CoV-2 (Coronaviridae)	DMAbs 2130 and DMAbs 2196	Antibody (Human)	Tixagevimab	Cryo-EM	8D8Q

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Spike protein (S)	SARS-CoV-2 (Coronaviridae)	REGN10933 and REGN10987	Antibody (Human)	REGEN-COV	X-ray diffraction	7M42
Fusion Glycoprotein (F)	respiratory syncytial virus (RSV)	MEDI8897	Antibody (Human)	Palivizumab	X-ray diffraction	5UDC
gp41 subunit (Envelope glycoprotein)	HIV-1	T20	Peptide	Enfuvirtide	X-ray diffraction	5ZCX
Capsid Protein						
Protein name	**Virus (family)**	**Name of Therapeutic**	**Type**	**Brand name**	**Experimental Method**	**PDB ID**
HBV Capsid	hepatitis B virus (HBV) (Picornaviridae)	Lenacapavir (DBT1)	Antiviral	Sunlenca	Cryo-EM	6WFS
HBV capsid assembly	hepatitis B virus (HBV) (Picornaviridae)	AT-130	Antiviral	-	X-ray diffraction	4G93
HBV capsid assembly	hepatitis B virus (HBV) (Picornaviridae)	HAP18	Antiviral	-	X-ray diffraction	5D7Y
Membrane proteins						
Protein name	**Virus (family)**	**Name of Therapeutic**	**Type**	**Brand name**	**Experimental Method**	**PDB ID**
Membrane protein (M2)	Influenza A Virus (Orthomyxoviridae)	Amantadineb	Antiviral	-	X-ray diffraction	3C9J
Membrane protein (M2)	Influenza A Virus (Orthomyxoviridae)	Rimantadine	Antiviral	Flumadine	X-ray diffraction	6BKL, 6US9

a Approved for use in Japan, not FDA approved; b Discontinued as a drug.

3.1. Envelope glycoproteins

Virus envelope glycoproteins are critical structural components that facilitate viral entry into host cells. These proteins play a pivotal role in the infection process by interacting with host cell receptors and enabling viral fusion with the host membrane [102–104]. Envelope glycoproteins can be classified based on their functions into categories such as Spike (S), Envelope (E), or Membrane (M) glycoproteins. S proteins, which protrude from the virus's surface, are particularly important for attachment to host cells. For example, the S protein of coronaviruses like SARS-CoV-2 is essential for receptor binding and membrane fusion. Each glycoprotein variant has specific structural features that determine its unique functions. For instance, S proteins often contain receptor-binding domains that enable them to interact with host cell receptors, while some envelope proteins facilitate membrane fusion [105]. Influenza viruses feature hemagglutinin (HA) and neuraminidase (NA) proteins, which regulate the processes of viral entry and exit from host cells [106]. In contrast, HIV employs the gp160 membrane protein, which splits into gp120, responsible for receptor binding, and gp41, which aids in membrane fusion [107]. Similarly, SARS-CoV-2 uses its spike (S) proteins to attach to the Angiotensin-converting enzyme 2 (ACE2) receptor on human cells, leading to infection [108] Viral fusion peptides are essential for virus to interact and perform endocytosis in host cell [109]. To further blend with the host system, glycosylation helps enveloped viruses evade immune detection by

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masking viral epitopes with host-derived glycans, making it challenging for antibodies to identify and neutralize the virus [110]. In other cases, viral envelope proteins are reported to undergo conformational changes that assist in virulence, as observed in SARS-CoV-2 [111] and DENV [112].

When viral envelope proteins are detected, the immune system initiates a defense response. While envelope proteins may successfully trigger an immune response, this response is sometimes insufficient to neutralize the virus. For instance, vaccine development for HCV has shown limited effectiveness in generating a robust antibody response [113]. Viruses have developed sophisticated mechanisms to evade these defenses. They suppress interferon (IFN) signaling, inhibit the actions of IFN-stimulated gene products, and disrupt the communication between IFNs and other cellular pathways. These strategies allow viruses to avoid immune detection and maintain their infectivity [113]. Interleukin 10 (IL-10) plays an essential role in supporting CVB4 infection by modulating the immune response to favor viral survival. Interestingly, the antiviral agent Umifenovir has been shown to downregulate IL-10 expression, thereby disrupting the virus's ability to exploit this pathway. This interaction underscores the importance of IL-10 in viral pathogenesis and highlights the potential of targeting its regulation as an effective strategy to treat CVB4 infections [114] (Table 1).

To escape antiviral treatments, viruses undergo mutations that allow them to target host receptors while avoiding detection by antivirals [115]. Most of these mutations occur in envelope glycoproteins, e.g., the Omicron variant of concern exhibits 37 mutations in its spike protein, which facilitates entry into host cells. Many of these mutations are in two key domains targeted by neutralizing antibodies: the receptor-binding domain (RBD) and the N-terminal domain (NTD). Despite these changes, some therapeutic antibodies, such as 309 (PDB: 7TLY), retain neutralizing activity against Omicron [116]. The conserved region in antigenic site IV in flank of RBD, is conserved due to which the antibody can retain its neutralizing activity [117]. In other cases, envelope proteins can evade immune defenses by binding to antibodies without being neutralized. This phenomenon is observed in the Dengue virus (DENV), where antibody binding does not neutralize the virus, leading to a condition called antibody-dependent enhancement (ADE) of infection. ADE occurs during secondary infections and exacerbates disease severity [118,119]. Due to structural similarity between the serotypes, the host responds to virus with antibodies generated based on previous infection. These antibodies may not bind to neutralizing sites such as fusion loop in DENV or receptor recognition domains which are conserved across virus serotypes [120]. Display of antigens in a non-infectious manner can help in conferring immunity without compromising the host system with infection. Currently, Dengvaxia is the only vaccine developed against the DENV, and it is effective primarily during secondary infections, as attenuated viruses display the envelope proteins (membrane and envelope glycoproteins) using a different viral vector (yellow fever virus) for the host to generate an immune response but may not lead to infection [121]. Limitations arise as this vaccine cannot be used to immunize prior to primary infection and is only recommended in case of secondary infection [122]. To combat infections with high mutation rates, such as SARS-CoV-2, targeting highly conserved epitope regions within the antigen is essential. This approach minimizes the risk of immune evasion through mutations [123].

To combat viral infections, entry inhibitors play a critical role by targeting viral envelope proteins and preventing infection [124]. These inhibitors have shown significant efficacy against influenza, one of the most studied viruses in this context. Several potent, Food and Drug Administration (FDA)-approved inhibitors, such as Zanamivir [125], Oseltamivir [126], and Laninamivir octanoate [127], effectively target envelope glycoproteins of influenza virus and block its entry into the host [128] (Table 1). Glycoproteins also have significant potential in vaccine development. When applied appropriately, they can act as antigens and stimulate immune responses, as seen in respiratory syncytial virus (RSV) subunit vaccines for pregnant women and the elderly [129]. Virus like particles (VLPs) encoding envelope glycoproteins have been effective for multiple purposes, including as diagnostic tools and vaccines [130,131]. With emerging applications, it has been possible to identify antivirals based on entry sites and inhibit virus entry into the host.

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3.2. Viroporins

Viroporins are small, hydrophobic proteins encoded by viruses. They oligomerize within host cell membranes, forming hydrophilic pores that disrupt cellular processes [132]. Many viruses incorporate membrane proteins into their lipid bilayer envelopes, which are essential for viral entry, assembly, release, and structural integrity. These proteins act as scaffolds, supporting other viral proteins and encapsulating viral DNA [133]. Viroporins facilitate viral budding by interacting with viral proteins and host cell membranes. They also influence membrane fluidity and fusion processes critical for viral entry, although they do not directly bind to receptors like spike or envelope proteins [134].

Viroporins were first identified in 1992 with the discovery of the ion channel activity of the M2 protein of the influenza A virus [135]. Since then, numerous viroporins with diverse structural and functional characteristics have been discovered in various viruses. These proteins are classified into two main groups, class I and class II, based on the number of transmembrane domains they possess. Subclasses are further defined by their position within the membrane [136]. Despite their importance, the study of viroporins as potential targets for antiviral drugs remains challenging. This is due to the lack of reliable 3D structures, difficulties in functional characterization, and the absence of direct, verifiable binding between inhibitors and viroporins [137]. Nevertheless, while the presence of viroporins may not always be critical for viral survival, their absence significantly weakens the virus [138].

Viroporins perform diverse functions across different virus families, reflecting adaptations to specific hosts and biological environments. Membrane proteins contribute to viral envelope stabilization and may also participate in cell signaling and immune evasion. Currently, Amantadine [139], and Rimantadine [140] are the only FDA-approved antivirals targeting the influenza M2 protein. Amantadine binds with the N-terminal of M2 channel protein for inhibition [141]. It is also reported to have antiviral efficacy against chikungunya virus ion channel [142], showcasing potential as broad spectrum viroporin inhibitor. Although, Rimantadine has shown to have antiviral activity against Influenza A, it is reported to show little activity against influenza B virus and drug resistant variants arise within few days after dosage [143]. These drugs elicit antiviral responses, although many other viroporin-targeting therapies remain in pre-clinical and clinical development [144] (Table 1). Advances in cryo-EM have enabled high-resolution imaging of viroporins in their native, membrane-bound conformations. Structural studies reveal the dynamic properties of viroporins, including structural changes during viral entry and membrane fusion [145].

3.3. Capsid

Capsids are protective structures in viruses that shield viral genomic material until it enters a host cell [146]. Their primary function is to protect the viral genome from enzymatic degradation by host enzymes and enable its transfer into host cells [147]. Upon entry, the capsid may either disassemble to release the genome for replication or remain intact, allowing transcription within the capsid, depending on the architecture with different virus types [148]. Capsid proteins vary in shape among virus families, containing both major and minor structural proteins. These geometries, studied through models such as Caspar-Klug nomenclature [149] and Alpha shape theory [150], explain assembly patterns in large molecular systems. Computational methods play a vital role in analyzing capsid dynamics, assembly, and interactions with lipid membranes [151]. Capsids are promising antiviral targets due to their essential role in viral infectivity. Disrupting capsid formation or stability can hinder replication [152–156]. Virus-specific non-structural proteins involved in capsid assembly provide opportunities for selective antiviral therapies [157,158]. The nucleocapsid (N) protein of SARS-CoV-2 constitutes a pivotal structural component of the virion, facilitating the encapsulation of the viral RNA into a ribonucleoprotein (RNP) complex and mediating key processes in viral replication and propagation. The C-terminal domain of the N protein (N-CTD) is indispensable for genome packaging, serving a critical role in the stabilization of the RNP assembly, whereas the RNA-binding site is located in the N-terminal domain (NTD) [159,160]. The first capsid

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structure identified was that of the TMV [161]. In the 1950s, Rosalind Franklin visualized TMV's rod-shaped capsid using X-ray crystallography, revealing its protein and RNA organization [162]. Early structural studies employed techniques such as electron microscopy and X-ray crystallography [163,164]. Modern advances, including cryo-EM, have enabled high-resolution imaging of capsids in near-native states, providing insights into structural changes during viral life cycles. Capsid inhibitors are designed to disrupt capsid assembly or disassembly, blocking viral replication. For example, Lenacapavir (marketed as Sunlenca) targets HIV capsids, interfering with multiple replication stages [165]. Immunotherapy strategies also target capsid proteins to elicit immune responses that neutralize viruses. Vaccines often incorporate capsid proteins to stimulate protective immunity [166]. Antivirals based on a structural approach, such as pleconaril, inhibit picornavirus (enteroviruses and rhinoviruses) even with differences in the amino acid sequence of the capsid proteins [167] (Table 1).

4. Exploring viral non-structural proteins

Non-structural proteins are encoded by viral genomes but are not part of the structural components of the virus. These proteins may function to facilitate viral replication or partake in the regulation of replication and assembly. Viral replication enzymes are a subset of viral non-structural proteins encoded by the virus to facilitate the replication and transcription of the viral genome within host cells. Viral replication enzymes, including proteases, polymerases, integrases, and helicases, play crucial roles in synthesizing viral RNA or DNA, enabling genome amplification and the production of new viral progeny. Antiviral drug discovery and repurposing majorly focus on these enzymes (Table 2).

Table 2. List of FDA approved drugs targeting viral replication enzymes.

Viral Replication enzymes						
Target protein		**Virus (Family)**	**Drug**	**Brand name**	**Experimental Method**	**PDB ID**
Protease	**HIV protease**	HIV (Retroviridae)	Saquinavir (SQV)	Invirase[a]	X-ray diffraction	1HXB
			Ritonavir (RTV)	Norvir	X-ray diffraction	1HXW
			Indinavir (IDV)	Crixivan[a]	X-ray diffraction	1HSG
			Nelfinavir (NFV)	Viracept	X-ray diffraction	1OHR
			Lopinavir (LPV)	Kaletra, (combination with ritonavir)	X-ray diffraction	1MUI
			Atazanavir (ATV)	Reyataz	X-ray diffraction	2AQU
			Darunavir (DRV)	Prezista	X-ray diffraction	1T3R
		HIV-1 (Retroviridae)	Amprenavir (APV)	Agenerase[a]	X-ray diffraction	1HPV
					Neutron diffraction	4JEC

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			Tipranavir (TPV)	Aptivus	X-ray diffraction	1D4S
	HCV NS3/4A protease	HCV genotype 1 (Flaviviridae)	Telaprevir (TVR)	Incivek[a]	X-ray diffraction	3SV6
			Boceprevir (BOC)	Victrelis[a]	X-ray diffraction	3LOX
			Simeprevir (SMV)	Olysio[a]	X-ray diffraction	3KEE
			Vaniprevir (VPV)	Vanihep, in combination with ribavirin + PegIFNα-2b	X-ray diffraction	3SU3
		HCV genotype 1 and 4 (Flaviviridae)	Asunaprevir (ASV)	Sunvepra[b]	X-ray diffraction	4WF8
			Grazoprevir (GZR)	Zepatier	X-ray diffraction	3SUD
		HCV genotype 1 to 4 (Flaviviridae)	Voxilaprevir (VOX)	Vosevi	X-ray diffraction	6NZT
			Glecaprevir (GLE)	Mavyret	X-ray diffraction	6P6L
	Main proteases (M pro or 3-chymotrypsin-like proteases (3CL pro)	SARS-CoV-2 (Coronaviridae)	Nirmatrelvir (NMV)	Paxlovid (combination with ritonavir)	X-ray diffraction	7SI9
			Ensitrelvir (ENS)	Xocova[b]	X-ray diffraction	7VU6
Polymerase	**DNA-dependent DNA polymerase (DdDp)**	HSV (Herpesviridae)	Foscarnet (PFA)	Foscavir	Cryo-EM	8EXX
			Acyclovir (ACV)	Zovirax	Cryo-EM	8V1T
	Reverse Transcriptase (PT)	HIV (Retroviridae)	Stavudine (d4T)	Zerit[a]	X-ray diffraction	6AMO
			Lamivudine (3TC)	Epivir, Combivir (combination with Zidovudine), Trizivir (combination with	X-ray diffraction	6KDJ

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				Zidovudine and abacavir)		
			Tenofovir disoproxil fumarate (TDF)	Viread	X-ray diffraction	1T05
			Doravirine (DOR)	Pifeltro	X-ray diffraction	4NCG
					Cryo-EM	7Z2G
		HIV-1 (Retroviridae)	Nevirapine (NVP)	Nevirapine (generic)	X-ray diffraction	1FKP
					Cryo-EM	7KJX
			Delavirdine (DLV)	Rescriptor[a]	X-ray diffraction	1KLM
			Efavirenz (EFV)	Efavirenz (generic)	X-ray diffraction	1FK9
					Cryo-EM	7KJW
			Etravirine (ETR)	Intelence	X-ray diffraction	1SV5
			Rilpivirine (RPV)	Edurant	X-ray diffraction	2ZD1
					Cryo-EM	7Z2D
			Dapivirine (DPV)	DapiRing[b]	X-ray diffraction	1S6Q
	RNA-dependent RNA polymerase (RdRp)	Influenza A and B viruses (Orthomyxoviridae)	Baloxavir (BXA)	Xofluza	X-ray diffraction	6FS6
		SARS-CoV-2 (Coronaviridae)	Remdesivir (RDV)	Veklury	Cryo-EM	7BV2
			Molnupiravir (MOV)	Lagevrio[b, c]	Cryo-EM	7OZU
			Favipiravir (FVP)	Avigan[b]	Cryo-EM	7CTT
Integrase (IN)	**Retroviral IN**	HIV (Retroviridae)	Dolutegravir (DTG)	Tivicay, Triumeq (combination), Dutrebis (combination)	X-ray diffraction	3S3M
					Cryo-EM	8FN7
			Bictegravir (BIC)	Biktarvy (combination)	Cryo-EM	6PUW
	HSV-1 TK	HSV-1 (Herpesviridae)	Idoxuridine (IDU)	Dendrid	X-ray diffraction	1KI7

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Thymidine kinase (TK)			-5-substituted 2′-deoxyuridine analogue, substrate of TK			
			Brivudine (BVDU) 5-substituted 2′-deoxyuridine analogue, substrate of TK	Zostex[b]	X-ray diffraction	1KI8
		HSV (Herpesviridae)	Penciclovir (PCV)	Denavir	X-ray diffraction	1KI3
			Acyclovir (ACV)	Zovirax	X-ray diffraction	2KI5
	VZV TK	VZV (Herpesviridae)	Brivudine (BVDU) 5-substituted 2′-deoxyuridine analogue, substrate of TK	Zostex[b]	X-ray diffraction	1OSN

[a] Discontinued; [b] Approved in some countries, not FDA approved; [c] Emergency use authorization (EUA) by FDA.

4.1. Protease

Virus-encoded proteases catalyse the cleavage of specific peptide bonds in viral polyprotein precursors or in cellular proteins, allowing them to function. Over the years, preclinical investigations have focused on these proteases due to their essential role in virus replication. The determination of 3D crystal structures of retroviral proteases began in 1989 with the elucidation of the RSV protease structure, followed by the HIV-1 protease structure [168]. Additionally, the structure of HIV-1 protease was modeled using known eukaryotic aspartic protease structures as templates. The breakthrough in structure-based drug design for viral proteases was enabled when the enzyme and substrate binding sites of HIV-1 protease (aspartyl protease) were analyzed via X-ray crystallography [169–171]. A number of peptide inhibitors (PIs) were designed based on the transition state mimetic concept [172]. The Hoffmann–La Roche drug Saquinavir (Ro 31-8959, Invirase) was the first FDA-approved drug (1995) of HIV-1 protease. The interaction of the drug with the protease was studied by X-ray crystallography [173]. This was followed by Ritonavir (Norvir) 's approval. X-ray structures guided the development, and computational design was incorporated to augment binding and pharmacokinetics [174,175]. Indinavir (Crixivan) was the next HIV protease drug to be approved by the FDA in 1996. Its development was driven by molecular modeling and X-ray crystallographic studies [176–178]. Nelfinavir (Viracept) design employed iterative co-crystallographic analyses of

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protease-bound peptidic inhibitors followed by substitution of parts of the inhibitors with non-peptidic functional groups. It was the first protease inhibitor authorized for pediatric use (1997) [178–180]. The other approved HIV antivirals, discovered predominantly employing XRD, include Atazanavir (Reyataz), Darunavir (Prezista), Fosamprenavir (Lexiva), Lopinavir-Ritonavir (Kaletra) and Tipranavir (Aptivus) [61]. In 2013, a joint X-ray/neutron structure HIV-1 protease in complex with now discontinued drug, Amprenavir was determined. The structural data collected by neutron diffraction revealed the precise localization of hydrogen atoms within the active site, which disclosed that some hydrogen bonds may be weaker than previously inferred from non-hydrogen interatomic distances. This insight could prove useful for the development of enhanced protease inhibitors [181] (Table 2). NMR has significantly contributed to the development and optimization of many HIV protease inhibitors. Of these, it one notable drug is Ritonavir. Two years post-market launch, certain batches failed to meet dissolution standards. Investigations revealed the presence of an additional crystal Form II other than Form I. Solid-state characterization techniques, NMR and IR confirmed the existence of two distinct crystalline forms (polymorphs) of ritonavir. However, solution-state analysis demonstrated that both forms dissolve to yield identical molecular structures. Therefore, either form could be employed for manufacture provided complete dissolution [182].

The NS3 protein of HCV comprises two distinct functional domains: an N-terminal serine protease domain and a C-terminal RNA helicase domain. NS4a peptide binds to NS3 and serves as co-factor for polyprotein maturation. The NS3/4a complex imparts proteolysis of the HCV polyprotein. HCV PIs are designed to inhibit this proteolytic activity [183]. The crystal structure of NS3 was published in 1996, revealing a trypsin-like fold and a structural zinc binding site [184], and the crystral structure of NS3/4a complex revealed NS4A peptide intercalates within a β sheet of the enzyme core [185]. The solution NMR structure of N-terminal protease of NS3 published in 1998 revealed insights into its activation and catalytic mechanism [186]. Initial investigations into the suppression of NS3/4A protease concentrated on the identification and optimization of peptide-based inhibitors [183,187]. Boceprevir and Telaprevir (Table 2) were discovered via structure-based drug design. both of which belong to the ketoamide class of molecules. They feature a ketoamide group that forms a reversible covalent bond with the enzyme's catalytic serine residue, thereby inhibiting the enzymatic activity [183]. This was followed by the discovery of several inhibitors via structure-guided drug design. High-resolution crystallographic data served as the basis for discovering small molecule inhibitors (Table 2). In 2012, a highly conserved new allosteric pocket at the HCV protease and helicase domain interface was identified via crystallographic fragment-based screening and proposed as a drug target [188].

The main proteases (M pro), also termed 3-chymotrypsin-like proteases (3CL pro) and Papain-like protease (PLpro), are cysteine proteases encoded by SARS-CoV-2. Mpro (nsP5) and PLpro (nsP3) co-translationally process pp1a and pp1ab polyproteins into mature non-structural proteins (nsPs), making them attractive drug targets [189,190]. Each protomer of Mpro, a homodimer, consists of three domains (I, II, and III). The substrate-binding pocket is located in the interdomain cleft between domains I and II, where a non-canonical catalytic dyad, Cys145-His41, mediates proteolytic cleavage at 11 distinct sites on pp1a and pp1ab. The substrate-binding sites of SARS-CoV-1 and SARS-CoV-2 Mpro exhibit 100% sequence homology; inhibitors of the former were also screened for the latter [191]. X-ray structures of the unliganded SARS-CoV-2 Mpro and its complex with an α-ketoamide inhibitor were reported, which provided the basis for development of improved inhibitors [192]. These two studies resulted in the development of the inhibitor Nirmatrelvir (PF-07321332) [191]. The FDA approved Paxlovid (a dual-therapy of Nirmatrelvir and Ritonavir) on May 25, 2023 as an oral antiviral pill [193,194] (Table 2). Ensitrelvir (S-217622), the first oral noncovalent and nonpeptidic SARS-CoV-2 Mpro PI clinical candidate, was discovered via virtual screening and optimization of the hit compound using a structure-based drug design strategy (SBDD). X-ray constructure of the enzyme and PI provided insights on binding and interaction [195] (Table 2). In another study, Cryo-EM structure of polyprotein bound and apo form of Mpro highlighted the flexible nature of the active site [196].

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4.2. Polymerase

Virus encoded polymerases are enzymes the catalyse the synthesis of nucleic acid, either DNA or RNA for replication of viruses, making them key target for antiviral research [197–199]. Different types of polymerases include RNA-Dependent RNA Polymerase (RdRp), RNA-Dependent DNA Polymerase or Reverse Transcriptase (RT), DNA-Dependent DNA Polymerase (DdDp), and DNA-Dependent RNA Polymerase (DdRp) [200].

HSV DNA polymerase (UL30) possesses polymerase activity, intrinsic 3′-5′ exonuclease activity, and ribonuclease (RNase) H activity. The first structure of HSV DNA polymerase was published in 2006. It has five conserved structural domains including an NH2-terminal, 3′-5′ exonuclease, thumb, fingers and palm domains and an additional pre-NH2-terminal domain at the N-terminal end [201,202]. Cryo-EM structures published in 2024 revealed the dynamics of the UL30 during DNA synthesis and proof-reading [203]. Viral DNA polymerase cryo-EM structures elucidated how Pol and UL42 bind DNA for processive synthesis, with Pol adopting multiple closed state conformations in the absence of nucleotides. Structures were not elucidated at the time of approval, but recently drug-bound (Foscarnet and Acyclovir) and drug-resistant mutant analyses indicated that resistance mutations alter conformational dynamics rather than drug binding, clarifying selectivity mechanisms [204].

The mature HIV-1 RT is a heterodimer comprising two subunits: the larger p66 subunit (560 amino acids) and the smaller p51 subunit, derived from the first 440 residues of p66. While the p66 subunit harbors the active polymerase and RNase H domains, the p51 subunit is believed to adopt a structural role with analogous subdomains (fingers, palm, thumb, and connection) differing in relative arrangement to support the heterodimer's functional integrity. Stavudine, Lamivudine and Tenofovir disoproxil fumarate are nucleoside reverse transcriptase inhibitors (NRTIs) that bind to the active site of polymerase on activation. These drugs were developed based on mechanism of action; however, the crystal and cryo-EM structures were elucidated later [205,206] (Table 2). Discovered in the late 1980s, non-nucleoside reverse transcriptase inhibitors (NNRTIs) include five approved anti-HIV drugs: nevirapine, delavirdine, efavirenz, etravirine, and rilpivirine. Dapivirine is approved in several countries. They function as noncompetitive inhibitors by engaging the allosteric site of HIV-1 reverse transcriptase (RT), approximately 15 Å from its catalytic domain. This interaction induces conformational alterations that disrupt the enzyme's catalytic function, thereby inhibiting viral replication. The hydrophobic binding site of HIV-1 RT and NNRTIs were identified through compound library screening and structural biology analysis. NNRTIs target HIV-1, while HIV-2's structural features confer innate resistance [205–208] (Table 2).

The influenza virus RdRp is a heterotrimeric enzyme composed of PA, PB1, and PB2 subunits. Transcription involves a "cap-snatching" process, wherein nascent capped host RNA transcripts are bound by the PB2 subunit, cleaved by the cap-dependent endonuclease (CEN) of the PA subunit, and utilized as primers by the PB1 subunit for viral mRNA synthesis [209,210]. Reported cryo-EM structure provide a basis for understanding the enzyme’s activity [211]. FDA approved baloxavir acid and its prodrug Baloxavir Marboxil, which target the conserved active site of the PA proteins in influenza A and B viruses, were developed by rational drug design leveraging the two-metal pharmacophore framework initially established for Dolutegravir. Recognizing that both HIV integrase and CEN utilize divalent metal ions as cofactors for endonuclease activity, DTG's metal-chelating scaffold was adapted for CEN inhibition. Crystal structures have been elucidated to study the interaction [209,210] (Table 1).

SARS-CoV-2’s multi-subunit RdRp complex is composed of a catalytic subunit nsP12 and the accessory subunits, nsP7 and nsP8 [212]. The nsP12 subunit is composed of a nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain, an interface domain, and a catalytic domain at the C-terminus. These domains collectively mediate key functions, including the catalysis of phosphodiester bond formation between nucleoside triphosphates (NTPs), as well as template binding, entry, primer-template release, and polymerization. The drug remdesivir was approved by FDA in 2020 [213]. While docking and molecular dynamics simulations revealed the dynamic

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interactions and binding pockets, the cryo-EM structures of SARS-CoV-2 RdRP complex with Remdesivir revealed its incorporation into the nascent RNA strand, stalling elongation and provided a rational template for drug design [214–216]. In 2021, FDA issued an emergency use authorization (EUA) for Molnupiravir, a broad-spectrum ribonucleoside analog, originally developed to treat influenza, and discovered via screening a library of compounds [217,218]. Favipiravir, another inhibitor of influenza RdRP [216] was approved for SARS-CoV-2 treatment in several countries [219]. Cryo-EM structures of the three inhibitors bound to RdRp provide crucial data like mechanism of enzyme catalysis and base-pairing pattern with inhibitors (Table 2). These drugs are designed to mimic natural nucleotides and incorporate themselves into the growing viral DNA or RNA chain, thereby terminating viral replication.

4.3. Integrase

HIV integrase (IN) mediates the insertion of viral DNA into host chromosomal DNA, employing two consecutive reactions named 3′-processing (3-P) and strand transfer (ST). The cleavage of Pol polyprotein by HIV protease leads to the generation of IN, a 32-kDa enzyme composed of three domains: the amino-terminal (NTD), catalytic core (CCD), and carboxy-terminal (CTD). The atomic structures of these domains were resolved using X-ray crystallography or solution NMR spectroscopy [220–222]. The IN active site at the CCD domain contains the highly conserved DDE motif, responsible for coordinating two Mg (II) ions. The substantial spatial distance between the enzyme active site in the CCD domain and both the NTD and CTD regions suggests a high degree of multimeric organization within the IN enzyme. Within infected cells, integrase (IN) is predominantly localized in the cytoplasm as a component of the viral pre-integration complex (PIC). The minimal functional subunit of the PIC, the Intasome (INT), catalyzes the integration, a process specific to retroviruses [223]. Combination antiretroviral therapy (cART) comprises four pharmacological classes: (i) NRTIs, (ii) NNRTIs, (iii) PIs, and (iv) integrase strand transfer inhibitors (INSTIs) [224,225]. Since approval, INSTIs have assumed a pivotal role in HIV antiretroviral therapy (ART) due to favourable clinical attributes like high antiviral potency with expedited reductions in HIV RNA levels, absence of significant drug-drug interactions and cross-resistance to other drugs [226].

All INSTIs share structural similarity to experimental compound 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)propenone (5-CITEP) characterized by the pivotal diketoacid (DKA) moiety. It represents the first integrase inhibitor successfully co-crystallized with the CCD, with electron density confirming interactions with residues D64, D116, and E152, as well as additional contacts involved in host DNA docking [227–229]. Further studies revealed that PICs are predominantly assembled via coordination with two Mg(II) ions during viral DNA integration, one of these metal ions is coordinated by residues D64 and D116, whereas the other is coordinated by residues D116 and E152. DKA-containing compounds maintain antiviral activity provided their divalent metal ion chelation capacity is retained. Raltegravir and Elvitegravir are first generation INSTIs. The widespread use of Raltegravir since its approval in 2007 resulted in the rapid emergence of viral variants harboring mutations within the IN-CCD domain [228]. Second generation INSTIs include Dolutegravir [230], Bictegravir, and Cabotegravir. Elucidation of crystal structure revealed enhanced structural flexibility of Dolutegravir improved the drug's integration into the enzyme active site, increasing efficacy against mutations triggered by first-generation INSTIs [229,231] (Table 2). Since 2018, WHO has recommended use of Dolutegravir as the preferred first- and second-line HIV treatment [232]. Bictegravir and Cabotegravir exhibited superior genetic barrier to resistance [228]. Cryo-EM structures of second generation INSTIs bound to INTs and drug-resistant INTs, further revealed mechanism of inhibition and resistance and may aid in development of better drugs [233,234].

X-ray and cryo-EM structures demonstrated that allosteric IN inhibitors (ALLINIs) disrupt catalytic activity of the enzyme by binding to allosteric sites [235,236]. Lens Epithelium-Derived Growth Factor/p75 (LEDGF/p75) is a cellular factor that enhances host DNA interaction with functionally active PICs by binding to the integrase binding domain (IBD) at the C-terminus IN, via

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coordination with residues D366, V408, I365, and F406. LEDGINs target this interaction and affects IN multimerization and catalytic activity. These can be developed via rational drug design and show no cross-resistance with INSTIs [235,237,238]. Multimerization-selective integrase inhibitors (MINIs) act by promoting aberrant IN multimerization. IN-RT RNase H inhibitors and INI-LEDGF/p75-IN interaction disruptors are examples of dual-acting inhibitors that simultaneously act on different and/or multiple targets in an additive or synergistic manner to confer antiviral effect [235].

4.4. Thymidine kinase (TK)

Thymidine kinase (TK) genes of alpha-herpesviruses are virulence-related genes encoding key kinases in the nucleoside salvage pathway. The HSV-1 TK enzyme phosphorylates four nucleosides and various nucleoside analogues. Hence, this enzyme is a pivotal target in antiviral therapeutic strategies [239]. The crystallographic structures of HSV-1 TK bound to its endogenous substrate deoxythymidine (dT) and the guanosine analogue Ganciclovir were elucidated and published in 1995 [240,241]. Three 5-substituted 2′-deoxyuridine analogues (Idoxuridine, Trifluridine, and Brivudine [BVDU]) have been approved as antiviral drugs. These analogues mimic natural substrate, facilitating phosphorylation. These phosphorylated antiviral agents interfere with viral DNA replication (Table 2). Acyclic guanosine analogues like Acyclovir and Penciclovir are phosphorylated by TK to monophosphate form. Cellular enzymes convert the compound to its active triphosphate form, which is then incorporated into viral DNA, halting replication [241] (Table 2). Mutations in TK gene can result in resistance to these inhibitors [242].

4.5. Methyltransferase (MTase)

In viral systems, MTases facilitate the synthesis of the 5′ cap-0 structure, thereby enhancing the virus's ability to evade the host's innate immune defenses [243]. nsP1 of alphaviruses exhibits S-adenosyl-l-methionine (SAM)-dependent methyltransferase (MTase) and m7GTP transferase (GTase) activities that are membrane-binding dependent and are essential for viral capping [244]. Solution NMR and cryo-EM structures provide insights into the membrane binding and enzyme capping mechanism [245,246]. Berbamine hydrochloride (BH), ABT199/venetoclax (ABT), ponatinib (PT), and selective estrogen receptor modulators (SERMs) are reported to inhibit nsP1 of CHIKV and SINV [247][248][249].

The plus-strand RNA genome of flaviviruses is capped with a 5′ terminal Cap 1 structure (m7GpppAmG). The flaviviruses encode one methyltransferase, located at the N-terminal portion of the NS5 protein, responsible for catalyzing both guanine N-7 and ribose 2′-OH methylations [250]. X-ray structures aided in elucidation of the catalytic site and drug-binding pockets [250–254]. Ribavirin, aurintricarboxylic acid, sinefungin, S-adenosyl homocysteine (SAH), Compound 10, herbacetin (HC) and caffeic acid phenethyl ester (CAPE) are reported as DENV MTase inhibitors [255–258]

SARS-CoV-2 MTases, nsp10/nsp16 and nsp14, facilitate viral RNA capping. Nsp14 catalyzes the 7-methylation of the 5′-cap guanosine, while nsp16 with nsp10, mediates the 2′-O-methylation of the ribose, thereby finalizing the cap structure. X-ray and cryo-EM structures were solved for both enzymes [259]. Several adenosine-like inhibitors and non-nucleoside inhibitors have been reported against nsp14 and nsp16 [260].

4.6. Helicases

Virus-encoded helicases are exploited by certain viruses to unwind DNA or RNA duplexes, a process driven by hydrolysis of ATP [261]. Prominent examples include a domain of HCV-NS3 [262], vaccinia nucleoside triphosphate phosphohydrolase-II (NPH-II) [263], SARS-CoV-2 Nsp13 helicase [264], Simian virus 40 (SV40) TAg protein [265], HPV E1 protein [266], DENV NS3 helicase [267], MPXV E5 helicase [268], and N-terminal helicase of CHIKV nsP2 [269]. Inhibitors are reported to target the helicase-mediated ATP hydrolysis and nucleic acid binding [270]. X-ray [271–277], cryo EM [268,278], NMR [279], and structure prediction [280] data have been reported for several viruses.

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Helicase enzymes are essential during multiple stages of genome replication, rendering them compelling antiviral targets, particularly given the extensive structural data available. Despite significant efforts to develop helicase-targeting antivirals, clinical advancement has been hindered by challenges related to cytotoxicity, bioavailability, and pharmacokinetics [270,281]. As of now, no virus-encoded helicase inhibitors have received FDA approval.

5. Host-targeted antivirals:

Protein-protein interactions (PPIs) are fundamental to any virus infection. Detailed understanding of protein interactions is essential for understanding viral pathogenesis. Many host proteins act as receptors for viruses, mediating attachment and entry (Figure 2). C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) act as co-receptors for the entry of HIV-1. Antagonists of these co-receptors are being utilized as antiviral strategies. Drug candidates like Aplaviroc, Cenicriviroc and Vicriviroc have shown efficacy in inhibiting HIV-1 replication. Available structural data for CCR5 and CXCR4 can prove to be valuable for improving inhibitor design and therapeutic potential [282–284]. Structure of the CCR5 chemokine receptor with FDA approved inhibitor, Maraviroc has been reported [285]. Ibalizumab, a CD4-directed post-attachment inhibitor was the first monoclonal antibody to be approved for the treatment of HIV-1 infection [286,287].

Additionally, glycosylation, autophagy, actin polymerization, fatty acid biosynthesis, programmed ribosomal frameshifting (PRF) and proteolytic cleavage are some of the host-mediated metabolic processes that can be targeted for antiviral development. Key pathways that can be targeted include host lipid pathway, host glycolytic pathways, host ubiquitination pathways, polyamine metabolic pathway, host nucleoside synthesis pathway, cytokine signalling and inflammatory pathways, and stress granule (SGs) machinery (Figure 3). [288,289]. SGs are assemblies of stalled mRNA and proteins that form in response to cellular stress, such as viral infection. Viral proteins like N-protein of SARS-CoV-2 and nsP3 of CHIKV in virus-infected cells recruit SG proteins G3BP1 (Ras GTPase-activating protein SH3-domain-binding protein 1) and G3BP2. This interaction suppresses SG formation, enhancing virus replication and assembly of new virions. Therefore, antivirals targeting the host protein G3BP can aid in SG-mediated antiviral response [290,291]. Viruses establish virus–host protein interactions to exploit cellular machinery essential for critical stages such as entry, genome replication, translation, assembly and release [292–294]. Increased resistance to antiviral agents and the need for broad-spectrum antiviral therapeutics have steered efforts to targeting proviral host factors and cellular mechanisms [288,295–298].

Viral infection can lead to the activation of various innate immune responses, with interferons (IFNs) playing a central role. IFNs, particularly IFN-α, have been utilized in antiviral therapies for infections like hepatitis B and C, with ongoing efforts to enhance therapeutic efficacy. Pegylated interferon afla 2b (PegIFNα-2b), interferon alfacon 1 (CIFN), pegylated interferon alfa 2b + ribavirin (PegIFNα-2b+RBV), pegylated interferon afla 2a (PegIFN-α2a) are some of the interferon therapeutics that are approved against HCV infections. Podofilox (PDX) is an antimitotic drug that interrupts cell division, approved for HPV-related diseases. Imiquimod (IQM) stimulates cytokines and sinecatechins (SINE) is an immunomodulatory drug approved against HPV-related diseases [61].

Cyclophilins (Cyps), key cellular factors playing role in transcription regulation, immune response, protein secretion, and mitochondrial function. Cyclophilin A (CypA), a mediator of cyclosporin A's (CsA) immunosuppressive effects, also supports the replication of multiple viruses, including HIV-1, HCV, and influenza. Interaction of CypA with viral proteins, facilitates virus replication, as seen with HIV-1, HCV, influenza virus, HCV, VSV, vaccinia virus, SARS-CoV, rotavirus (RV), and HPV. Cyclophilin inhibitors, such as alisporivir and NIM811, are reported to display potent antiviral activity against HIV and HCV [270].

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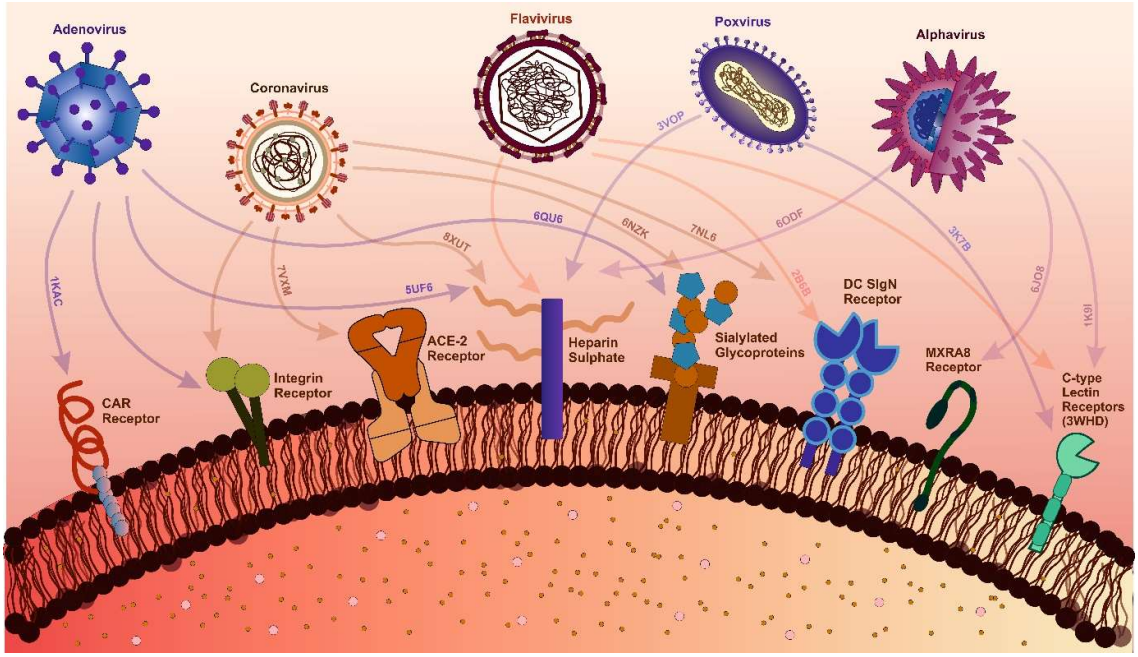

Figure 2. Interactions Between Viral Families and Host Receptors with Structural Insights. The figure highlights key interactions between viral families and host receptors critical for viral attachment and entry. For adenoviruses, the Coxsackievirus and Adenovirus Receptor (CAR) (PDB: 1KAC), heparan sulfate (PDB: 5UF6), and sialylated glycoproteins (PDB: 6QU6) are key receptors. Coronavirus interactions include ACE-2 (PDB: 7VXM), integrins, heparan sulfate (PDB: 8XUT), sialic acid (PDB: 6NK), and the DC-SIGN receptor (PDB: 7NL6) [299,300]. Alphaviruses engage with heparan sulfate (PDB: 6ODF), C-type lectin receptors (PDB: 1K9I), and MXRA8 (PDB: 6JO8) [301]. Similarly, flaviviruses interact with heparan sulfate, DC-SIGN (PDB: 2B6B), and C-type lectin receptors [302,303]. Poxviruses target heparan sulfate (PDB: 3VOP) and C-type lectin receptors (PDB: 3K7B)[304]. PDB identifiers highlight viral protein-host receptor complexes, offering molecular-level insights into receptor diversity and viral entry mechanisms, supported by recent structural and experimental studies.

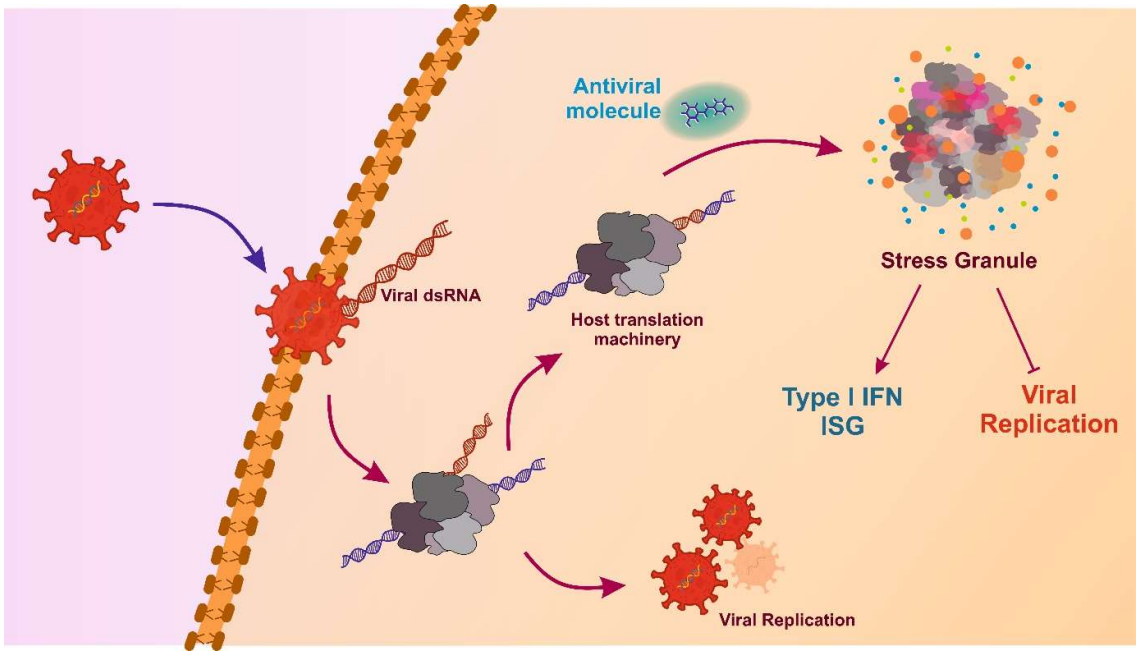

Figure 3. Stress granule formation in response to viral infection. Viruses hijack host cell replication machinery, but antiviral molecules counteract this by promoting stress granule formation, inducing activation of Interferon-

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Stimulated Genes (ISGs), and producing Type I Interferons, directly inhibiting viral replication, thereby creating an antiviral environment that restricts viral survival and propagation.

6. Rational Drug Design

Viral outbreaks, such as COVID-19 and Monkeypox, highlight the challenges posed by viral mutations that enhance immune escape and virulence, potentially leading to pandemics. Predicting the next mutation remains difficult [305]. Therefore, global preparedness is essential to counter future pandemics [306]. The pursuit of advanced, structure-guided treatments through AI-driven technologies is crucial to address viral mutations that could reduce therapeutic efficacy [307]. For instance, after the COVID-19 outbreak, it became evident that a strategic pipeline is required to identify antigenic sites, design non-cytotoxic ligands, and enable mass production and distribution of drugs [308]. Similarly, the Monkeypox outbreak reinforced the need to identify viral targets and develop specific drugs against them [309].

Visualization forms the foundation of rational drug design, enabling researchers to analyze viral structures [310]. This structural understanding facilitates precise drug design based on viral protein architecture, as demonstrated during the COVID-19 pandemic [311]. Unlike traditional methods, which relied on screening drugs for antiviral activity without prior molecular insight, rational drug design employs high-resolution structural techniques—X-ray crystallography, NMR spectroscopy, and cryo-EM—to study viral proteins and processes essential for replication [312]. The process begins by identifying a target viral protein critical to replication or host entry. Structural analyses then reveal active sites and binding pockets where small molecules can inhibit protein function [313]. Using this data, scientists design molecules tailored to fit these pockets, thereby blocking protein activity. This targeted strategy improves efficacy and minimizes side effects by avoiding interactions with non-target proteins.

Rational drug design combines structural and biochemical insights to create highly specific therapies, transforming antiviral treatment approaches. For example, the small molecule Nirmatrelvir targets SARS-CoV-2's main protease, showing high antiviral efficacy [314]. Similarly, analogues like spirolactam, derived from zamnair, improve antiviral potency, while peptides such as VIR250 selectively inhibit the papain-like protease of SARS-CoV-2 [315]. Nanoparticles, like the ICO-RBD nanovaccine, mimic virus-like structures to boost immunogenicity [316]. Future developments in rational drug design promise faster and more precise solutions for emerging pathogens, leveraging computational tools and structural biology advancements.

Rational drug design has transformed structural biology by providing a direct pathway from understanding a virus's atomic structure to developing effective antiviral therapies [317]. This approach has led to the creation of antiviral drugs for diseases such as HIV/AIDS and influenza, where structural studies of viral enzymes and surface proteins play a key role in designing inhibitors. Additionally, this method is vital for quickly developing treatments against emerging viral threats, as demonstrated by the rapid creation of SARS-CoV-2 spike protein inhibitors during the COVID-19 pandemic. AI has further accelerated this process by reducing screening times for antivirals, improving predictive models for binding affinity, and enabling data mapping to track trends in viral mutations and forecast future changes [318,319]. AI technologies have enhanced drug discovery by optimizing, generating, and identifying molecules with drug-like properties [320]. The need for such advancements in rational drug design is clear, as they support the rapid development of therapeutics to combat future pandemics. Ultimately, rational drug design not only deepens our understanding of drug mechanisms but also advances public health by enabling the swift creation of targeted treatments for both existing and emerging viral diseases [321].

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6.1. De novo designing - a targeted approach with improved features

De novo protein design has emerged as a promising strategy to create molecules from scratch, contributing significantly to combating viral infections [322]. Computational pipelines can be developed and deployed for the streamlined development of antibody-based therapeutic interventions against emerging pathogens [323]. Applications include an *in silico* affinity maturation pipeline developed and employed to successfully bioengineer nanobodies with enhanced affinity [324]. With the integration of generative AI, it is now possible to engineer proteins targeting specific protein structures. This advancement also enables personalized therapeutic research tailored to individual patients [325,326]. Large language models, such as PALM-H3, further enhance these capabilities by generating antibodies using pre-trained models, allowing for the de novo synthesis of antibodies [327]. Beyond drug design, vaccines targeting viral proteins can also be developed to present surface glycoproteins and elicit immunization responses. Modelling tools such as SabPred assist further in modelling antibodies and in silico validation of structures [328]. For instance, HIV vaccines displaying envelope glycoproteins have demonstrated strong neutralizing titers, emphasizing the effectiveness of nanoparticle-based strategies [329]. Moreover, generative AI can be utilized to produce antibodies specific to target antigens, enhancing therapeutic applications [330].

Breakthroughs such as AlphaFold have revolutionized protein modeling, enabling the accurate prediction of protein structures [331]. These tools facilitate the identification of protein functions and their interactions with other molecules [332]. Further advancements, like RFdiffusion, simplify the design of protein binders that target specific antigen sites, eliciting neutralizing responses against viruses [333]. Various approaches taken for designing these antivirals are mentioned in Table 3. Rational approach has not only been evident in designing antivirals but antibody and vaccines designing too.

Table 3. List of therapeutics and the rational approaches used in their designing.

Name of Therapeutic	Target Virus (family)	Rational designing approach and result	Type	PDB	Ref.
Nirmatrelvir	SARS-CoV-2	A protease inhibitor for SARS-CoV-2 with reported protein-ligand complex, demonstrating its binding to the viral main protease (Mpro), which is crucial for viral replication.	Ligand	7TLL	[334]
Spirolactam	Influenza A	Carbocyclic analogue of zanamivir in which the hydrophilic glycerol side chain is replaced by the hydrophobic 3-pentyloxy group of oseltamivir. This hybrid inhibitor showed excellent inhibitory properties in the neuraminidase inhibition assay	Ligand	4MJV	[335]
VIR 250 and VIR 251	SARS-CoV-2	Designed and biochemically characterized potent inhibitors (VIR250 and VIR251) harboring high selectivity for SARS-CoV-2 PLpro and the related SARSCoV-1 PLpro versus other proteases.	Peptide	6WUU, 6WX4	[336]
Adintrevimab	SARS-CoV-2	The crystallisable fragment (Fc) region of adintrevimab undergoes affinity maturation, leading to two amino acid modifications (S52A and W100B) that enhance the half-life while preserving the normal effector functions of IgG1.	Antibody	7U2E	[337]
RBD-I53-50 nanoparticles	SARS-CoV-2	The nanoparticle vaccines present 60 SARS-CoV-2 spike receptor-binding domains (RBDs) were arranged in a	Nanoparticle Vaccine	-	[338]

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		highly immunogenic configuration, yielding neutralising antibody titers tenfold greater than those elicited by the prefusion-stabilized spike, even when administered at a fivefold lower dosage.			
ICO-RBD nanovaccine	SARS-CoV-2	The immunogenicity of nanovaccines aimed at viral components, including the SARS-CoV-2 receptor binding domain (RBD), is improved by multivalent antigen presentation on nanoparticles. Using icosahedral DNA origami (ICO) as a display particle, we achieve virus-like morphology and diameter for RBD nanovaccines.	Nanoparticle Vaccine	-	[339]
GPC-I53-50NP	Lassa virus (LASV)	The study employs two-component protein nanoparticles to stabilize the Lassa virus glycoprotein complex (GPC) in its trimeric form. These nanoparticles elicited robust antibody responses in rabbits and provided protection to guinea pigs against lethal LASV challenge experiments.	Nanoparticle Vaccine	7SGE	[340]
D25	Respiratory syncytial virus (RSV)	To elicit robust and specific neutralising antibodies (nAbs), immunogens were designed with a focus on the epitopes derived from the prefusion structure of the respiratory syncytial virus (RSV) fusion protein.	Antibody	-	[341]
DS-Cav1-I53-50	Respiratory syncytial virus (RSV)	The approach involves presenting a prefusion-stabilized variant of the F glycoprotein trimer (DS-Cav1) in a repetitive array on the nanoparticle exterior. This two-component nanoparticle scaffold enables the production of highly ordered, monodisperse immunogens that display DS-Cav1 at controllable density.	Nanoparticle Vaccine	-	[342]
Fab 14N4	Respiratory syncytial virus (RSV)	Palivizumab is a humanized monoclonal antibody derived from a murine mAb, designed to target antigenic site II of the RSV fusion (F) protein, a critical target in vaccine development.	Antibody	5J3D	[343]
Casirivimab and Imdevimab	SARS-CoV-2	This cocktail, targeting the SARS-CoV-2 spike protein's receptor-binding domain, received Emergency Use Authorization (EUA) from the FDA in November 2020 for COVID-19 treatment, effectively preventing viral mutation escape.	Antibody	6XDG	[344]
Bamlanivimab and Etesevimab	SARS-CoV-2	A combination targeting the SARS-CoV-2 spike protein was granted EUA in February 2021. These rapidly developed monoclonal antibodies have proven effective in reducing viral load and improving patient outcomes. The Fc region was modified to enhance stability.	Antibody	7KMG, 7C01	[345]
Tixagevimab and Cilgavimab	SARS-CoV-2	This combination, targeting SARS-CoV-2, received EUA for COVID-19 prevention and treatment and includes engineered Fc domains to reduce adverse effects.	Antibody	8D8Q, 7L7E	[346]

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Sotrovimab	SARS-CoV-2	B-cells from a SARS-CoV-infected individual, with an Fc domain modification to extend half-life, received FDA EUA in 2021 for treating mild to moderate COVID-19.	Antibody	-	[347]
Spike protein Epitope-scaffolds[a]	SARS-CoV-2	This study introduces a novel vaccine design strategy targeting conserved regions of the SARS-CoV-2 spike protein, bypassing the mutagenic receptor-binding domain. Using epitope grafting, stable immunogens mimicking these regions' surface features were engineered. Immunogenicity assessments in murine models showed promising results, and the designed epitope scaffolds demonstrated potential diagnostic utility.	Nanoparticle Vaccine	-	[348]
VHH60	SARS-CoV-2	Derived from the FDA-approved nanobody caplacizumab, this neutralizing nanobody binds the receptor-binding domain of the SARS-CoV-2 spike protein with high affinity (2.56 nM). It has shown significant efficacy in inhibiting virus infection in vitro and in vivo, making it a strong candidate for further clinical investigation against COVID-19.	Nanobody	-	[349]

Not clinically approved.

7. Identifying the Threat of Future – a structural approach:

Viruses pose a significant global health threat due to their potential to cause epidemics and pandemics. The WHO R&D Blueprint for Epidemics prioritizes identifying high-risk viruses for early detection, targeted research, efficient resource allocation, and global collaboration (WHO Pathogen Prioritization Report, 2024) [350] (Table 4). “Pathogen X”, an unknown future threat, underscores the need for preparedness. Identifying new pathogens involves robust surveillance, genomic sequencing, and epidemiological studies. Targeting Pathogen X requires focusing on pathogen families, studying prototype pathogens, fostering international collaboration, and investing in R&D [351]. Prioritizing high-risk viruses and preparing for Pathogen X enhances global response capabilities, ultimately saving lives and protecting public health (WHO Pathogen Prioritization Report, 2024) [350]. Having the knowledge of the threat, knowing their structures and with rational approach to designing therapeutics, there is hope to fight the upcoming pandemics with advanced knowledge of structural virology.

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Table 4. Virus families and pathogens identified as high-priority Public Health Emergencies of International Concern (PHEICs) for 2024. The corresponding PDB IDs of structural and non-structural proteins for these "high-risk" viruses are provided, highlighting potential antiviral therapeutic targets. (Source: WHO Pathogen Prioritization Report, 2024).

Family	Priority Pathogens	Virus	Viral protein	PDB ID
Arenaviridae	*Mammarenavirus lassaense*	Lassa virus	Glycoprotein Complex (GPC)	8TYE
			Nucleoprotein	3MWP
			matrix protein Z	5I72
			spike complex	7PVD
			L protein	7OJJ
	Mammarenavirus juninense	Junin virus	GP1 glycoprotein	5NUZ
			Nucleoprotein	4K7E
			L protein	7EJU
			Z protein	7EJU
	Mammarenavirus lujoense	Lujo Virus	spike complex	8P4T
			GP1 domain	6GH8
	Mamastrovirus virginiaense	Astrovirus	capsid spike	7RK2
Coronaviridae	Subgenus Merbecovirus	MERS	Spike Protein	8SAK
			Fusion core	4MOD
			Papain like Potease	4P16
			3CL Protease	4WMD
			nsP1	8T4S
			nsp5 protease	4YLU
			nsP10	5YN5
			nsP13	5WWP
			nsP16	5YN5
			Nucleocapsid N terminal Domain (NTD)	4UD1
			Nucleocapsid C-terminal Dmain	7DYD
	Subgenus Sarbecovirus	SARS-CoV-2	Spike Protein	6M0J
			Envelope Protein	8U1T
			Membrane protein	8CTK
			Nucleocapsid N terminal Domain (NTD)	7ACT
			Nucleocapsid C-terminal Dmain	7O05
			nsP1	7K3N
			nsP2	7msx
			nsP3 (papain like Protease)	6W9C

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			nsP5 (Main protease)	6y2e
			nsP7	7JLT
			nsP8	7JLT
			nsP9	6wxd
			nsP10	5YN5
			nsP12	6NUR
			nsp13	6ZSL
			nsp14	5C8S
			nsp15	6VWW
			nsp16	5YN5
Flaviviridae	*Orthoflavivirus zikaense*	Zika Virus	Envelope Protein	5JHM,
			Membrane Protein	7KCR
			NS5 Methyltransferase	5GP1
	Orthoflavivirus denguei	Dengue Virus	Envelope Protein	1K4R, 7BUD
			Membrane Protein	7BUD
			NS3 protein	5YVW
			NS2B/NS3 Protease	2FOM
			NS5	8T12
	Orthoflavivirus encephalitidis	Japanese encephalitis virus	Envelope Protein	3P54
			Capsid	5OW2
			Membrane Protein	5WSN
			Nsp1 CTD	5O36
			NS5	4K6M
	Orthohantavirus sinnombreense	Sin Nombre virus	Envelope Protein (Gc)	7FGF
			Envelope Protein (Gn)	8AHN
			Nucleocapsid	2IC9
Orthomyxoviridae	*Alphainfluenzavirus Influenzae H1*	Influenza A Virus	Hemagglutinin H1	6CHX
			Hemagglutinin H5	5Z88
			Neuraminidase	4HZY
			RNA Polymerase	6QPG
Paramyxoviridae	*Henipavirus nipahense*	Nipah Virus	Fusion Core	1WP7
			Glycoprotein	2VSM
			Matrix Protein	7SKT
			Nucleoprotein	4CO6
			Phosphoprotein	7PNO
			Polymerase	9IR3
Poxviridae	*Orthopoxvirus variola*	Variola virus	Phosphoprotein	6EB9
			L1 protein	1YPY
			Topoisomerase	3IGC

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	Orthopoxvirus vaccinia	Vaccinia Virus	DNA-dependent RNA polymerase complex	6RFL
			virulence factor F1L	5AJJ
	Orthopoxvirus monkeypox	Monkeypox Virus	H3 envelope protein	5EJ0
			methyltransferase VP39	8B07
Togaviridae	*Alphavirus chikungunya*	Chikungunya virus	E1 & E2 Envelope Glycoproteins	2XFB
			Capsid Protein	5H23
			nsP2 Protease	4ZTB
			nsP3	4TU0
			nsP4	7VB4
	Alphavirus venezuelan	Venezuelan Equine Encephalitis virus (VEEV)	Envelope Glycoprotein	7SFV
			nsP2 Protease	5EZQ

8. Conclusion and future direction:

Structural virology has played an instrumental role in shaping our understanding of viral mechanisms and enabling the development of targeted therapeutics. Key techniques like X-ray crystallography, cryo-EM, and NMR spectroscopy have yielded high-resolution images of viral structures, revealing key molecular targets on enzymes, receptors, and structural proteins. With the ever-increasing data and research on virus-encoded proteins, structural biology has proven to be an indispensable tool for rational design and optimization of antiviral drugs. Additionally, computational methods have become integral to the early stages of drug discovery and development. The review delves into existing and new technological advancements to further the depth of structural understanding of viral proteins, interactions, and the strategies for antiviral therapeutic development.

Although notable strides have been made in the field, the structural data of many of the key proteins are yet to be elucidated. Therefore, it is imperative to develop strategies for obtaining structural data and establishing a link between structure and function. Covid-19 demonstrated the significance of pandemic preparedness to the world. Based on historical precedents of pandemics and epidemics that have plagued the world, debilitated global healthcare systems, and caused substantial mortality and lasting health impact. Covid-19 is unlikely to be the final pandemic. Therefore, continuous efforts from the scientific community to develop antiviral therapeutics against emerging pathogens and pathogens with pandemic potential is the need of the hour. Further investigations are crucial to identify druggable host factors for the development of broad-spectrum therapeutics.

Due to rapid rate of mutation, many viruses are emerging and reemerging pathogens. Mutation prediction, leveraging sequence and structural data along with surveillance data, are now critical in forecasting evolution, aiding to guide the design of adaptive therapeutic strategies that can keep up with the rapid rate of viral evolution. As witnessed during the SARS-CoV-2 pandemic, available and ongoing research to elucidate the protein structures expedited vaccine development, whereas the rapid identification of variants underscored the importance of anticipating viral evolution in real time. In the future, real-time structural surveillance, coupled with AI-powered tools, will be pivotal in swiftly identifying mutations and new pathogens. It will be essential to assess the impact of viral mutations on transmissibility and immune escape.

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