Structural Decorations in Viruses

Unraveling Acquired Functional Structures in Icosahedral RNA Virus Capsids

HAN WANG
Abstract


Viruses have a profound impact worldwide, posing challenges to animal welfare, agriculture, human health, and the ecosystem. This thesis examines the realm of non-enveloped icosahedral double-stranded (ds)RNA and single-stranded (ss)RNA viruses through three studies. In Paper I, we employed a reverse genetics approach to generate recombinant dsRNA totivirus-like viruses—which negatively impact fisheries and the economy—unraveling the intricate relationships between viral genes and life cycles. Our reverse genetic method has proven essential for generating infectious totivirus-like virus particles, allowing for a nuanced exploration of viral behaviors. Understanding these behaviors has the potential to help in developing effective virus control approaches. In Paper II, we elucidated the previously unknown capsid structure, uncovering the intriguing acquired features of a dsRNA megabirnavirus—Rosellinia necatrix megabirnavirus 1-W779 (RnMBV1)—through cryogenic electron microscopy single-particle analysis. RnMBV1, a fungal virus, has potential applications in controlling white root rot, a plant disease that causes substantial economic losses. Insights into this viral structural information can enhance our ability to leverage this fungal virus for economic and agricultural benefits. In Paper III, we obtained the capsid atomic models of a Marnaviridae ssRNA virus: Chaetoceros socialis forma radians RNA virus 1. Additionally, we generated a structure-based phylogeny using viral protein structures predicted by AlphaFold2; this was done to enhance our understanding of algal virus-host specificity. As harmful algal blooms (HABs) pose global threats to ecology and the economy, Chaetoceros algae have emerged as a contributing factor. Certain Marnaviridae viruses exhibit specific infection patterns in Chaetoceros, thereby influencing the occurrence and mitigation of HABs. Studies on Marnaviridae viruses collectively provide insights into the interactions between algal viruses and their hosts, paving the way for utilizing marine algal viruses to address HAB-related challenges. Together, our functional and structural analyses will contribute to a broader understanding of both dsRNA and ssRNA viruses, their behaviors, and their potential applications in addressing economic, agricultural, ecological, and healthcare issues.

Keywords: Viruses, RNA viruses, capsid, structure, cryo-EM

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Dedicated to all those who have ever supported me, lifted my spirits, and provided solace throughout this profound journey.

Your steadfast encouragement has been the guiding force that fueled my perseverance. Thank you for being the unwavering pillars of strength and the well-springs of inspiration in my life.
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<th>Description</th>
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<tbody>
<tr>
<td>2D</td>
<td>two-dimensional space</td>
</tr>
<tr>
<td>3D</td>
<td>three-dimensional space</td>
</tr>
<tr>
<td>BAC</td>
<td>bacterial artificial chromosome</td>
</tr>
<tr>
<td>BTV</td>
<td>Bluetongue virus</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>CP</td>
<td>capsid protein</td>
</tr>
<tr>
<td>CPE</td>
<td>cytopathic effect</td>
</tr>
<tr>
<td>CrP</td>
<td>crown protein</td>
</tr>
<tr>
<td>Cryo-EM</td>
<td>cryogenic electron microscopy</td>
</tr>
<tr>
<td>CsfrRNAV</td>
<td>Chaetoceros socialis forma radians RNA virus 1</td>
</tr>
<tr>
<td>CtenRNAVII</td>
<td>Chaetoceros tenuissimus RNA virus type II</td>
</tr>
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<td>DENV</td>
<td>Dengue virus</td>
</tr>
<tr>
<td>DMV</td>
<td>double-membrane vesicles</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dsRNA</td>
<td>double-stranded RNA</td>
</tr>
<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
</tr>
<tr>
<td>GLV</td>
<td>Giardia lamblia virus</td>
</tr>
<tr>
<td>HAB</td>
<td>harmful algal bloom</td>
</tr>
<tr>
<td>HAV</td>
<td>Hepatitis A virus</td>
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<tr>
<td>HBV</td>
<td>Hepatitis B virus</td>
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<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HvV190S</td>
<td>Helminthosporium victoriae virus 190S</td>
</tr>
<tr>
<td>ICTV</td>
<td>International Committee on Taxonomy of Viruses</td>
</tr>
<tr>
<td>IMNV</td>
<td>Infectious myonecrosis virus</td>
</tr>
<tr>
<td>IPNV</td>
<td>Infectious pancreatic necrosis virus</td>
</tr>
<tr>
<td>kbp</td>
<td>kilobase pairs</td>
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<tr>
<td>m7G</td>
<td>7-methylguanosine</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>MV</td>
<td>Measles virus</td>
</tr>
<tr>
<td>NTP</td>
<td>nucleoside triphosphate</td>
</tr>
<tr>
<td>OmRV</td>
<td>Omono River virus</td>
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<tr>
<td>PBV</td>
<td>picobirnavirus</td>
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<tr>
<td>PcV</td>
<td>Penicillium chrysogenum virus</td>
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<td>PCV-1</td>
<td>Pepper cryptic virus 1</td>
</tr>
<tr>
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<td>Description</td>
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<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>PMCV</td>
<td>Piscine myocarditis virus</td>
</tr>
<tr>
<td>PsV-F</td>
<td>Penicillium stoloniferum virus F</td>
</tr>
<tr>
<td>RdDp</td>
<td>RNA-dependent DNA polymerase</td>
</tr>
<tr>
<td>RdRp</td>
<td>RNA-dependent RNA polymerase</td>
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<tr>
<td>RDV</td>
<td>Rice dwarf virus</td>
</tr>
<tr>
<td>RMSD</td>
<td>Root-mean-square deviation</td>
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<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RnMBV1</td>
<td>Rosellinia necatrix megabirnavirus 1-W779</td>
</tr>
<tr>
<td>RnQV1</td>
<td>Rosellinia necatrix quadrivirus1</td>
</tr>
<tr>
<td>RT</td>
<td>reverse transcription</td>
</tr>
<tr>
<td>RT-qPCR</td>
<td>quantitative reverse transcription polymerase chain reaction</td>
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<tr>
<td>SARS-CoV-2</td>
<td>severe acute respiratory syndrome coronavirus 2</td>
</tr>
<tr>
<td>ScV-L-A</td>
<td>Saccharomyces cerevisiae virus L-A</td>
</tr>
<tr>
<td>ScV-L-BC</td>
<td>Saccharomyces cerevisiae virus L-BCLa</td>
</tr>
<tr>
<td>SPA</td>
<td>single-particle analysis</td>
</tr>
<tr>
<td>SsMBV1</td>
<td>Sclerotinia sclerotiorum megabirnavirus 1</td>
</tr>
<tr>
<td>ssRNA</td>
<td>single-stranded RNA</td>
</tr>
<tr>
<td>T</td>
<td>Triangulation number</td>
</tr>
<tr>
<td>TVV</td>
<td>Trichomonas vaginalis virus</td>
</tr>
<tr>
<td>VF</td>
<td>virus factory</td>
</tr>
<tr>
<td>VP</td>
<td>viral protein</td>
</tr>
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<td>ZIKV</td>
<td>Zika virus</td>
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</table>
1. Introduction

Viruses play a significant role in the world, infecting a wide range of organisms and causing notable economic and health challenges. With its widespread distress and substantial economic losses, the recent pandemic underscored the impact of viruses. Some viruses also have a significant impact on animal welfare, agriculture, human health, and the ecosystem. Given these circumstances, it is crucial to carry out virus research to prepare for and prevent potential future pandemics or ecological issues. Identifying the structurally significant features that play a crucial role in controlling viral replication is essential. However, this task is not straightforward, and the adoption of structure-based approaches is necessary.

Viruses have developed functional configurations by adorning prominent structural features on their surfaces. The central aim of our present studies is to identify these acquired structural characteristics by using a structural evolution approach and to comprehend their crucial structure–function relationships. The primary focus of my research is on non-enveloped icosahedral viruses with single-stranded (ss) and double-stranded (ds)RNA. This thesis pursues both molecular and structural biology objectives: 1) to generate an infectious DNA clone of a dsRNA totivirus-like virus to evaluate its acquired functional structures and 2) to determine capsid structures to identify the general and acquired capsid features in ssRNA and dsRNA viruses using cryogenic electron microscopy single-particle analysis (cryo-EM SPA).

The introduction is organized into three sections: Section 1.1, titled “Viruses,” provides essential foundational knowledge on virus classes and life cycles. Section 1.2, “Double-stranded RNA viruses,” gives information about non-enveloped icosahedral dsRNA viruses, including their typical lifestyle, known capsid functions and structures, and potential evolution-driven structural traits relevant to their functions. Section 1.3, “Marine algal single-stranded RNA viruses,” delves into non-enveloped icosahedral ssRNA viruses, specifically picorna-like viruses infecting bloom-forming algae species. This section explores their ecological impact, common transmission mechanisms, and fundamental capsid structure. Both sections 1.2 and 1.3 conclude with an exploration of the current scientifically significant yet unresolved questions that were investigated in this thesis.
1.1 VIRUSES

Viruses are the smallest entities that infect living organisms and have diverse host tropisms. Although some types of viruses infect prokaryotes, this thesis focuses on those viruses that infect eukaryotes. Viruses replicate by using host cellular resources, and their replication can lead to cell death, eventually damaging the organism and causing virus-driven diseases. Typically, a virus is comprised of an exterior protein capsid shell, interior functional proteins, and a nucleic acid genome (DNA or RNA). The viral genome encodes capsid proteins (CPs) and interior functional proteins. Some viruses also have envelopes that originate from the host cellular membrane. Most known viruses range in size from 10 to 300 nm and have varying shapes and morphologies.

1.1.1 Classifications

Viruses are classified into seven groups (Baltimore classification, Figure 1.1.1), based on their genome types, strandedness, sense, and manner of messenger RNA (mRNA) synthesis[^1].

**DNA viruses**

DNA viruses have a genome composed of DNA, that is replicated by DNA polymerase. They are classified into groups I, II, and VII (Figure 1.1.1). The genome can be either dsDNA or ssDNA. The dsDNA viruses use either the host cellular or their own acquired DNA polymerase for replication[^2][^3]. Some dsDNA viruses such as the hepatitis B virus (HBV) use its specialized RNA-dependent DNA polymerase (RdDp, also called reverse transcriptase) for replication[^4] (Figure 1.1.1). Except for those viruses in the Anelloviridae family that are negative-strand (-) ssDNA viruses, most ssDNA viruses have positive-strand (+) genomes and share a replication mechanism similar to that of dsDNA viruses[^5].

**RNA viruses**

As their genetic material, RNA viruses carry RNA inside the capsid shell. They are classified into groups III, IV, V, and VI (Figure 1.1.1). The genome is usually single-stranded but can also be double-stranded. The dsRNA viruses encode RNA-dependent RNA polymerase (RdRp) as part of their replication machinery[^6]. The ssRNA viruses can be further classified into +ssRNA and -ssRNA. In a +ssRNA virus, the genome serves as an mRNA and first encodes RdRp for following protein expression; while for a -ssRNA virus, a +ssRNA has first to be synthesized from the -ssRNA genome by its intrinsic RdRp[^2]. Retroviruses are an exception because they have RdDp that transcribes their
RNA genome into DNA, giving them a replication mechanism similar to that of DNA viruses\(^7\) (Figure 1.1.1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Example</th>
<th>Genetic Material Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>dsDNA</td>
<td>dsDNA \rightarrow mRNA</td>
</tr>
<tr>
<td>Group II</td>
<td>+ssDNA</td>
<td>+ssDNA \rightarrow dsDNA \rightarrow mRNA</td>
</tr>
<tr>
<td>Group III</td>
<td>dsRNA</td>
<td>dsRNA \rightarrow mRNA</td>
</tr>
<tr>
<td>Group IV</td>
<td>+ssRNA</td>
<td>+ssRNA \rightarrow mRNA</td>
</tr>
<tr>
<td>Group V</td>
<td>-ssRNA</td>
<td>-ssRNA \rightarrow +ssRNA \rightarrow mRNA \rightarrow mRNA</td>
</tr>
<tr>
<td>Group VI</td>
<td>+ssRNA-RT</td>
<td>+ssRNA \rightarrow dsRNA \rightarrow mRNA \rightarrow mRNA</td>
</tr>
<tr>
<td>Group VII</td>
<td>dsDNA-RT</td>
<td>dsDNA-RT \rightarrow +ssRNA \rightarrow dsRNA \rightarrow dsDNA \rightarrow mRNA</td>
</tr>
</tbody>
</table>

*Figure 1.1.1. Baltimore classification of viruses. Viruses are classified into seven groups, and -ssDNA viruses are not included here. Each group includes an exemplar virus. The smallpox virus belongs to the Poxviridae family. Parvovirus belongs to the Parvoviridae family. HBV belongs to the Hepadnaviridae family. Rotavirus belongs to the Sedoreoviridae family. Coronavirus is from the Coronaviridae family. MV, the measles virus, is from the family Paramyxoviridae. HIV, the human immunodeficiency virus, belongs to the family Retroviridae. RT: with reverse transcriptase or RdDp.*
1.1.2 Life Cycle
Viruses can have different life cycles. Some viruses that infect unicellular eukaryotes, such as protozoan or yeast viruses, transmit to other cells during host cell division and mating. These viruses only have an intracellular phase. On the other hand, viruses that can infect multicellular eukaryotes often have both intracellular and extracellular phases; they use different strategies for infection, genome replication, and capsid assembly.

1.1.2.1 Viruses in Unicellular Hosts
Certain viruses, such as the yeast Saccharomyces cerevisiae virus L-A (ScV-L-A) and the Trichomonas vaginalis viruses (TVVs), which exist within unicellular hosts, tend to stay inside the host cell and are spread to new cells through cytoplasmic exchange, sporogenesis, or hyphal anastomosis\(^8\)\(^–\)\(^10\). However, some protozoan viruses have also developed the ability to transmit extracellularly. For instance, Giardia lamblia virus (GLV), which belongs to the Totiviridae family, can infect G. lamblia extracellularly\(^1\)\(^1\). Therefore, these viruses may resemble those viruses in multicellular hosts and have multiple stages in their life cycles as described below.

1.1.2.2 Viruses in Multicellular Hosts
Viruses that infect multicellular eukaryotes usually undergo multiple stages in their life cycles. They replicate and assemble during the intracellular phase. In this section, I introduce the main representative viral life-cycle stages, along with some examples.

**Entry**
Viruses can be transmitted through airborne droplets or particles, body fluids, and blood\(^1\)\(^2\). After host cell attachment, they enter the cells through various mechanisms, with differences between enveloped and non-enveloped viruses. In both cases, the goal is to deliver the viral genetic material into the host cell, where replication and the production of new viral particles can occur\(^1\)\(^3\).

Enveloped viruses typically enter host cells via direct membrane fusion or endocytosis. Inside an endosome, the virus may fuse with the endosomal membrane to release its genetic material into the cytoplasm. Most non-enveloped viruses are also internalized into host cells through endocytosis. Once inside an endosome, viruses can escape to the cytoplasm using various strategies\(^1\)\(^3\). Some non-enveloped viruses, particularly those with rigid capsids, use direct penetration for entry (e.g., picornaviruses); they create channels in the host cell membrane or directly inject their genetic material into the cell\(^1\)\(^4\). The specific mechanisms and strategies employed vary among viruses, hence emphasizing the adaptability and diversity of viral entry mechanisms.
Genome replication
After infecting a host, viruses begin to replicate using the host’s replication and translation machinery. DNA viruses require access to the host’s DNA polymerase to replicate their genomes, which they can achieve by invading the nucleus. However, some viruses, such as poxviruses, are unique in that they have their own DNA polymerase, enabling them to replicate directly in the cytoplasm. Host ribosomes are then recruited to translate viral transcripts into proteins, which is followed by the assembly of the viral capsid and packaging of the genome. For RNA viruses, most replication occurs in the host cytoplasm, where the genome functions as mRNA or as a template for mRNA synthesis. However, some ssRNA viruses, such as orthomyxoviruses (e.g., influenza viruses), require replication in the host’s nucleus.

Host’s innate immune response and dsRNA
Most living creatures have an innate immune response that can be triggered by dsRNA or dsRNA intermediates, which help reduce the risk of infection. However, viruses have developed different strategies to avoid this response. One of these is to create virus factories (VFs) that are responsible for replication and morphogenesis, which can improve the efficiency of viral replication and assembly and protect the viruses from the host’s immune response. In addition, dsRNA viruses replicate by “intraparticle genome synthesis”; that is, dsRNA viruses can replicate within the capsid.

Virus factories
A VF is a platform for viruses to carry out replication and morphogenesis intracellularly. These structures—often several microns in diameter—comprise remodeled cell membranes and contain functional compartments that are responsible for virus replication, assembly, and egress. DNA viruses rely on the host cell nucleus for replication, while most RNA viruses replicate in the cytoplasm, making the structure of their VFs better understood.

VFs have different morphologies (e.g., viroplasm, spherules, vesicles, and tubes) and compositions. Viroplasms are electronic dense cytoplasmic inclusions where the replication and assembly of some large DNA viruses (Poxviridae), dsRNA viruses (Reoviridae), and -ssRNA viruses (Filoviridae) occur. Many viruses create spherules as VFs for their replications. Spherules are single-membrane invaginations in organelles (Figure 1.1.2.2), ranging in size from 50 to 400 nm. The endoplasmic reticulum (ER) is the major and most common eukaryotic cell organelle for viruses to construct VFs. For example, flaviviruses such as hepatitis C virus (HCV), dengue virus (DENV) or Zika virus (ZIKV), coronaviruses, and picornaviruses, which are +ssRNA viruses, have VFs built with single ER-derived spherules or double-membrane vesicles (DMV).
Golgi complex and mitochondria also contribute to VF modeling and virus morphogenesis in these viruses (Figure 1.1.2.2). The invaginated spherules and DMVs can hide the viral dsRNA or dsRNA intermediate, thus helping the viruses evade their hosts’ immune responses\(^{22,30}\).

**Exit**

Once progeny viruses are assembled, they exit host cells in two ways: through cytolytic mechanisms or nonlytic pathways\(^{13,31}\). Nonlytic pathways include exocytosis (virus budding) and cell-to-cell transport\(^{31–33}\). Virus budding can take place in organelles and cell plasma membranes\(^{13,34,35}\). Although most non-enveloped viruses are believed to escape from cells through cytolytic egress\(^{13}\), such as apoptosis or necrosis, some exceptions exist. Some non-enveloped dsRNA viruses exit cells using extracellular vesicles, such as exosomes in rotaviruses\(^{36}\) and certain picornaviruses\(^{37}\) and secretory autophagosomes in polioviruses\(^{38,39}\).

*Figure 1.1.2.2. Overview of virus factories in eukaryotic cells. Except for some viruses like *Baculoviridae* and *Polyomaviridae* viruses that build their VFs inside the nucleus (nuclear VFs), other viruses mainly have their VFs in various forms in the cytoplasm (cytoplasmic VFs) with the recruitment of different organelles. Viruses from the *Poxviridae*, *Reoviridae* or *Filoviridae* form viroplasm in cytoplasm, with the recruitment of mitochondria. Some viruses like nodavirus, *Togaviridae* viruses, tombusvirus, tymovirus, and *Flaviviridae* viruses form single-membrane spherules originated from different organelles. Viruses like *Coronaviridae*, *Flaviviridae* and *Picornaviridae* viruses form DMVs in either endoplasmic reticulum or Golgi. *Bunyaviridae* viruses have tube-like VFs that are in the Golgi apparatus.*
1.2 DOUBLE-STRANDED RNA VIRUSES

The dsRNA viruses have a genome consisting of dsRNA (Group III in the Baltimore classification, Figure 1.1.1). These viruses have both non-segmented and segmented genomes, with the number of segments ranging from 1 to 12. There are two clades of dsRNA viruses: the Duplornaviricota and Pisuviricota phyla. There are currently 12 approved families and one genus—Birnaviridae, Chrysoviridae, Megabirnaviridae, Quadriviridae, Totiviridae, Spinareoviridae, Sedoreoviridae, Partitiviridae, Curvulaviridae, Amalgaviridae, Picobirnaviridae, and Cystoviridae—and the Botybirnavirus genus—that fall under the dsRNA virus classification. All dsRNA viruses were assigned to these two phyla, excluding Birnaviridae viruses, which were not assigned to any order or phylum. The Chryso-, Megabirn-, Quadri-, and Totiviridae viruses are “toti-like” dsRNA viruses belonging to the order Ghabrivirales. The Spinareo- and Sedoreoviridae viruses are “reo” dsRNA viruses from the Reovirales order. The Partiti-, Curvula-, Amalga-, and Picobirnaviridae viruses are “durna” dsRNA viruses from the order Durnavirales. Cystoviridae viruses are “cysto” dsRNA viruses and are part of the Mindivirales order. The Botybirnavirus has not yet been assigned to any family.

1.2.1 Host Spectra and Transmission

The life cycles of dsRNA viruses are diverse and depend on their hosts. This section describes their host spectra, social impacts, and transmission capabilities.

Host spectra and social impacts

The dsRNA viruses infect a variety of hosts and, thus, have enormous effects on fisheries, agriculture, animal welfare, food manufacturing, and human health\(^{40-43}\). They can infect both prokaryotes and eukaryotes, yet most infect eukaryotes. Cystoviruses, like Pseudomonas virus phi6, are the only dsRNA virus genus that infect bacteria\(^{44}\). ScV-L-A, a representative member of the Totiviridae family, infects unicellular yeast\(^{45}\). On the one hand, some dsRNA viruses can be problematic. For instance, the infectious bursal disease virus is a well-characterized Birnaviridae virus known to infect young chickens and turkeys\(^{46,47}\). Bluetongue virus (BTV) and rotaviruses belong to the Sedoreoviridae family and infect livestock and humans, respectively\(^{48,49}\). BTV causes an acute disease called bluetongue, which is highly fatal in ruminants and leads to economic loss\(^{48}\). Rotaviruses cause gastroenteritis among infants and young children worldwide\(^{49}\). Infectious myonecrosis virus (IMNV) causes 70% mortality in shrimp\(^{40}\), and Piscine myocarditis virus (PMCV) causes cardiomyopathy syndrome in Atlantic salmon\(^{50}\). On the other hand, some dsRNA viruses can be utilized as viro-control or virotherapy tools, such as Rosellinia necatrix megabirnavirus 1-W779 (RnMBV1) from the Megabirnaviridae.
family$^{41}$. RnMBV1 can confer hypovirulence to its host fungi, which helps control the plant disease called white root rot$^{51}$. In addition, the GLV that infects the parasite *G. lamblia* can potentially be applied to control giardiasis$^{52}$.

**Host transmission ability**

Members of the Birna-, Spinareo-, Sedoreo-, and Picobirnaviridae families infect multicellular eukaryotes like humans or animals and have gained extra-cellular transmission ability$^{49,53–57}$. Members of the Partiti-, Chryso-, Quadri-, Toti-, and Megabirnaviridae families, which infect unicellular and simple eukaryotes, such as fungi and protozoa$^{41,45}$, as well as some plants$^{58}$, have various strategies for transmission. For example, totivirus ScV-L-A is transmitted to new cells only intracellularly, thus lacking a host-entry strategy$^{59}$. Unlike ScV-L-A, which remains intracellular, some totivirus-like dsRNA viruses resemble toiviruses but infect multicellular hosts or gain cell-to-cell or extra-cellular transmission ability. These toivirus-like dsRNA viruses are unsigned and infect a broad range of eukaryotic hosts, including arthropods such as shrimps$^{40}$, insects (e.g., mosquitoes, flies, ants, mosses)$^{60–62}$, vertebrates such as fish$^{50,63}$, and plants$^{58}$.

### 1.2.2 Capsid Functions

Viral capsids play a pivotal role in their life cycles. The primary function of a viral capsid is to enclose and protect the viral genetic material, ensuring its safe delivery to host cells, aiding in viral replication, and facilitating the

![Figure 1.2.2](image1.png)

*Figure 1.2.2.* Intraparticle genome synthesis mechanism in a non-enveloped icosahedral dsRNA virus. The process has been well documented in *Sedoreoviridae* viruses like rotavirus. However, the mechanism in other icosahedral dsRNA viruses remains unclear. The replication complex, consisting of at least one RdRp, is embedded within the capsid of the dsRNA virus. Pores are commonly found at each 5-fold axis (5f) of the icosahedral vertex. The RdRps inside the capsid may synthesize nascent +ssRNAs by utilizing incorporated NTPs through these pores, leading to their subsequent release.
infection process\textsuperscript{64,65}. Specifically, viral capsids play a role in interactions with host cell receptors, the viral mRNA capping process, and immune evasion\textsuperscript{66,67}. In this section, three putative capsid functions are described. First, many dsRNA viruses exhibit pores on their capsids, and the pores are involved in intraparticle genome synthesis. Second, capping strategies are employed for viral mRNA stabilization. Third, dsRNA viruses use decoys to evade the immune system.

**Pore and intraparticle genome synthesis**

In section 1.1.2.2, it is highlighted that dsRNA viruses utilize intraparticle genome synthesis as a strategy to evade the host’s immune system. To enable this process, their capsid shells feature pores that serve a crucial role\textsuperscript{24}. These pores facilitate the influx of nucleoside triphosphates (NTPs) and the egress of newly transcribed +ssRNA\textsuperscript{67,68} (Figure 1.2.2).

**Capping strategy and host mRNA degradation**

Many dsRNA viruses utilize mRNA capping strategies with their own or cellular capping machinery or by stealing the cap structures from cellular mRNAs, enabling them to obtain a 5’ cap structure\textsuperscript{45,69}. For example, 	extit{Sedoreoviridae} dsRNA viruses employ a single, multifunctional CP for capping. Specifically, λ2 in mammalian orthoreovirus\textsuperscript{70}, viral protein (VP)4 in the case of BTV\textsuperscript{71}, and VP3 from rotavirus\textsuperscript{72}, function as a capping protein. This arrangement ensures the efficient transfer of the RNA substrate from one domain to the next within the protein.

This acquisition is vital for initiating the transcription and translation of viral genes within host cells, safeguarding mRNA from cellular exonucleases\textsuperscript{73}. In contrast, uncapped mRNA molecules undergo fast degradation within host cells and might be recognized as foreign elements, leading to the activation of innate antiviral immune responses by the host cell\textsuperscript{73}. These strategies collectively aim to ensure the efficient translation of viral proteins and promote the viral replication process.

**Viral mRNA cap-snatching**

Eukaryotic mRNA molecules typically have a 5’ cap structure comprising a 7-methylguanosine (m7G) linked to the first nucleotide of the mRNA via a 5’-5’ triphosphate bridge. This cap structure is critical for the efficient translation, stabilization, and transport of mRNAs in eukaryotic host cells\textsuperscript{74}.

In some dsRNA viruses, RdRp lacks the capping ability. To overcome this limitation, these viruses recruit CPs and employ a cap-snatching mechanism during transcription. The specific mechanisms of cap-snatching can vary among different dsRNA viruses. For example, yeast ScV-L-A acquires a 5’ cap structure for its mRNA transcript through cap-snatching\textsuperscript{75}. This process involves transferring the m7G cap structure from the host mRNA to the 5’-end of the viral transcript. This adaptation allows these viruses to achieve
efficient transcription and translation within host cells, underscoring their intricate strategies for exploiting host cell machinery to their advantage. In addition, the decapped host mRNAs generated from the cap-snatching mechanism are likely to divert the host mRNA’s degradative machinery.

Decoy and empty particles
Many dsRNA viruses produce noninfectious empty particles without enclosing any genome inside. They have structures or components that mimic infectious virus particles. These particles may act as “decoys” and serve various purposes, such as diverting the host’s immune responses or interfering with the host’s antiviral defenses. However, they can also be dead-end products or direct precursors of mature particles.

1.2.3 Capsid Structure
The dsRNA viruses have a general rule for capsid shell structures and assembly, but some also own extra decorated structures that support their life cycles.

General capsid structures
Except for cystoviruses, which are enveloped, most dsRNA viruses are non-enveloped but have one or more layers of capsid shells. They share commonalities in overall capsid structures. Except for viruses belonging to the Birnaviridae family, all classified dsRNA viruses encompass an icosahedral capsid single shell with a triangulation number \( (T) = 1 \) symmetry. The capsid is essentially built with 60 asymmetric homodimers or heterodimers (e.g., Quadriviridae) of CPs comprising \( \alpha \)-helix-rich \( \alpha + \beta \)-fold structures. ScV-

![Figure 1.2.3.1. Totivirus ScV-L-A capsid and Gag CP model. A) Full capsid model. One Gag dimer (subunits A and B) is circled. Numbers indicate icosahedral symmetry axes. B) Atomic model of a Gag dimer (PDB ID: 1m1c). The pentagon, triangle and ellipse indicate the 5-, 3- or 2-fold axis, respectively.](image-url)
L-A is a typical $T=1$ virus; it infects the unicellular yeast *Saccharomyces cerevisiae*, belongs to the family *Totiviridae*, and has a non-enveloped, icosahedral virion. It has a capsid shell comprising 60 asymmetric homodimers of Gag CP, which conforms to the general structural features of dsRNA viruses (*Figure 1.2.3.1*)\(^{45}\). *Birnaviridae* viruses have an icosahedral capsid shell structure with $T=13$ symmetry, consisting of 60 asymmetric units comprising 13 CPs with a jelly-roll fold\(^{46}\). In addition, the *Cysto-*, *Spinareo-*, and *Sedoreoviridae* viruses possess double-layered capsid shells, including a totivirus-like inner $T=1$ shell and birnavirus-like outer $T=13$ shell\(^{82,83}\). Capsid shells pack their dsRNA genome together with RdRps inside as replication complexes\(^{84}\).

**Acquired capsid structures in evolution**

Some dsRNA viruses that infect multicellular hosts, such as totivirus-like viruses, exhibit multiple structural features that have evolved to suit their extracellular life cycles\(^{67}\). Apart from CPs, these viruses often have additional proteins on their capsid surfaces in the form of spikes, turrets, or protrusions. Some CPs form intermolecular interactions with adjacent proteins through terminal extensions. Capsid pores and channels that function in intraparticle genome synthesis have commonly been observed, while in some icosahedral dsRNA viruses from multicellular hosts, the pores are obstructed. These features, including capsid surface characteristics, extended termini, and different pore conformations, may all play crucial roles in the viral life cycle related to viral infections.

**Extra features on the capsid surface**

Many dsRNA viruses might have acquired surface features for various reasons. Unlike totivirus ScV-L-A, which has no apparent surface structural feature\(^{45}\) (*Figure 1.2.3.1*), totivirus-like viruses, such as Omono River virus (OmRV) (*Figure 1.2.3.2*) and IMNV, feature previously unrecognized proteins over the 5-fold axes of the capsid surface\(^{62,85}\). OmRV infects mosquitoes and has protrusion proteins atop the 5-fold axes, and it appears that the protrusion proteins are likely implicated in viral infection\(^{62}\). OmRV also has protruded surface loops compared with phylogenetically relevant *Totiviridae* viruses, such as ScV-L-A (*Figure 1.2.3.1*)\(^{67}\). IMNV, the pathogen of penaeid shrimp, also exhibits fiber-like protrusion at each 5-fold axis of the capsid surface that is likely involved in cell entry\(^{85}\). In addition, some picobirnaviruses (PBV) have protruded surface arches/loops (*Figure 1.2.3.2*). These surface arches/loops can likely provide insertion sites, which function in cell entry or particle stabilizations\(^{54,86}\). *Reovirales* viruses utilize their multilayers of capsids to strengthen their infection capabilities\(^{87}\). The surface spikes, fibers, or turrets in some mammalian and avian reoviruses from the genus *Orthoreoviruses* under the *Spinareoviridae* family are likely connected with receptor binding\(^{88}\). In the family *Partitiviridae*, Penicillium stoloniferum virus F (PsV-F) has a surface arch functioning that enhances capsid stability and particle assembly,
and Pepper cryptic virus 1 (PCV-1) possesses some disordered protrusion apexes (Figure 1.2.3.2) that enable symbiotic relationships between viruses and hosts.89,90

Terminal extensions

Intermolecular interactions, such as terminal arm extensions, can increase the contact surface and are therefore a stabilizing strategy for viral particles. ScV-L-A and TVV2 from the Totiviridae family have no terminal extension, while Saccharomyces cerevisiae virus L-BCLa (ScV-L-BC) has a C-terminal domain swap between the A subunits69,76,77. OmRV has a C-terminal arm in each B subunit that interlocks with two adjacent A subunits, fastening the decamer62,67. Rice dwarf virus (RDV), from the Sedoreoviridae family, has its subunit B N-terminus inserted into the adjacent A subunit91. Chrysoviridae Penicillium chrysogenum virus (PcV) has an arm at the N-terminus that contributes to intersubunit interactions78. Partitiviridae PsV-F has N- and C-terminal extensions that mediate intermolecular interactions within the capsid89. Picorbirnaviridae PBV has an extended N-terminus that swaps between subunits and helps stabilize capsid assembly86. Quadriviridae Rosellinia necatrix quadrivirus1 (RnQV1) has a hook-like C-terminus in the P2 subunit that extends further to the adjacent P4 subunit and therefore stabilizes pentamers79 (Figure 1.2.3.3). Robust capsid-stabilizing strategies like terminal extensions seem to be unnecessary for protozoan/yeast viruses such as ScV-L-A and TVV2. Less robust interactions are sufficient for these viruses to conceal the genome inside the capsid from the host’s immune defense76. Even though terminal extension is one of the main strategies for capsid stabilization, there are other approaches. For example, TVV2 has a thicker capsid than that of reovirus and “thumb” protrusions that help increase its capsid lateral contact area76.

Pore conformation and obstruction

Pores can vary in size, location, structure, and state. Typically, pores are found at each 5-fold vertex, although some viruses have them at each 3-fold axis77. Amino acid residues surrounding the pores are often positively charged, such as arginine or lysine, which can attract NTPs and help coordinate the negatively charged +ssRNA, which is made up of a phosphate backbone. The RdRp can attach itself near the pore inside the capsid, while +ssRNA is synthesized close to the pore to minimize potential secondary structure formation76,84. When infecting unicellular hosts, dsRNA viruses may have open pores that are large enough to allow NTPs to enter and for nascent transcribed +ssRNA to exit45,77,92. For example, PcV particles have pores that are approximately 11-Å in diameter and 26-Å long, allowing for the release of synthesized +ssRNA transcripts78. In some cases, viral capsids have pores, but they may not be large enough. However, the capsid’s structural components are flexible, so conformational changes may occur to provide enough pore size to allow gene release79,89,93. However, in some dsRNA viruses that infect
multicellular eukaryotes, pores are closed or obstructed\textsuperscript{67,86}. The 10-Å diameter 5-fold pores in OmRV particles are typically blocked by surface protrusion proteins that sit on top of the pores\textsuperscript{62}. However, the function of the pore obstruction has not yet been clarified.

Figure 1.2.3.2. Protrusion or proteins on viral capsid surfaces in non-enveloped icosahedral dsRNA viruses. All maps were generated from atomic models by Chimera 1.16, using the \textit{molmap} command with a 3.5 Å resolution cut-off, and are radially color coded (Å). PDB IDs: OmRV, 7cz6 (protrusion protein), 7d0k (CPs); PBV, 2vf1; PCV-1, 7ncr; PsV-F, 3es5.
1.2.4 Remaining Questions in the Structural Acquisition of Icosahedral dsRNA Viruses

As outlined in section 1.2.3, the acquired structural features have been identified in some dsRNA viruses, while functional studies including molecular or cellular approaches, still need to be completed. It is crucial to establish a tool or platform, such as one employing reverse genetics, that can produce recombinant wild-type and mutated viruses. This step is essential in expanding and validating the existing structural observations and hypotheses. Section 2.1.1 gives a brief overview of the reverse genetics approach and describes existing examples. Section 2.2.1 (Paper I) provides a comprehensive summary and description of my endeavors in developing reverse genetics, focusing on a non-enveloped icosahedral dsRNA totivirus-like virus, OmRV.

There is still room to expand our knowledge of dsRNA virus structures. So far, only one megabirnavirus, RnMBV1, has been classified, but its capsid
structure remains unknown. RnMBV1 infects multicellular fungi and has gained a horizontal cell-to-cell transmission ability. It is interesting to investigate whether this virus also acquires structural features that are mentioned in section 1.2.3. Section 2.1.2 provides a brief overview of the development and benefits of cryo-EM SPA, focusing on new virus structure determination. Subsequently, section 2.2.2 (Paper II) succinctly presents our structural analyses of the RnMBV1 capsid and its recognized features.
1.3 MARINE ALGAL SINGLE-STRANDED RNA VIRUSES

To date, ssRNA viruses (Groups IV, V, and VI in the Baltimore classification, Figure 1.1.1) have been categorized into various families based on their genetic materials, replication mechanisms, and host preferences. Notable ssRNA virus family examples include *Coronaviridae* (e.g., SARS-CoV-2), *Flaviviridae* (e.g., DENV and ZIKV), *Filoviridae* (e.g., Ebola virus) and *Picornaviridae* (e.g., poliovirus). These ssRNA viruses constitute a diverse and significant group of pathogens with profound implications for human and animal health, agriculture, and ecosystems. The ssRNA viruses have high mutation rates and genetic variability, which often result in the emergence of new strains and variants. These can pose challenges and influence the efficacy of current vaccines and antiviral treatments.

Many human ssRNA pathogens come from within the *Picornaviridae* family and are responsible for diseases such as the common cold (rhinoviruses), poliomyelitis (poliovirus), and hepatitis A (hepatitis A virus, HAV). Understanding the intricate biology of these viruses and developing effective control strategies are crucial for managing these diseases. However, one question arises: How did this diverse group of pathogenic ssRNA viruses originate? It is possible that some of them evolved from ancient groups of algal picorna-like ssRNA viruses that continue to thrive in oceans. Hence, studying marine algal picorna-like viruses is crucial for understanding the capsid functions observed in *Picornaviridae* viruses affecting humans and animals. Additionally, these viruses play a significant role in shaping the dynamics of algae species, thereby exerting a substantial impact on the marine ecosystem. This section explicitly focuses on marine algal ssRNA picorna-like viruses, which share phylogenetic similarities with *Picornaviridae* viruses and can significantly impact ecosystems and economies.

1.3.1 Harmful Algal Blooms

Algae are diverse and include various types such as diatoms (e.g., *Chaetoceros*), dinoflagellates, and others. These are the foundational building blocks of various aquatic food chains and food webs. However, algae populations may undergo unchecked growth and form harmful algal blooms (HABs) under favorable conditions. Some algae produce toxins and other certain nontoxic algae can deplete water oxygen, causing fish die-offs and posing risks to animals and even humans. Therefore, it is a persistent request to deal with HABs.

**Causes and current precautions for HABs**

Although numerous factors contribute to the occurrence of HABs, the precise factors that trigger them remain inadequately studied. HABs have a natural
origin, yet human activities that disrupt ecosystems may contribute to their heightened frequency and intensity. Factors such as heightened nutrient inputs and pollution, changes in food web dynamics, modifications to water flow patterns, and the influence of climate change all contribute to this phenomenon. Addressing HABs involves a multifaceted approach, such as the reduction of nutrient pollution, early monitoring and detection, water quality improvement, regulatory measures, and so on. These strategies collectively aim to prevent, mitigate, and manage the detrimental effects of HABs on aquatic ecosystems, human health, and the environment but they remain challenging.

**Algae Chaetoceros**

*Chaetoceros* is a genus of diatoms and is mainly accommodated in marine waters worldwide. *Chaetoceros* species exist worldwide and are the most abundant species of marine planktonic diatoms. *Chaetoceros* diatoms have over 200 classified species and can cause algal blooms. For instance, *Chaetoceros tenuissimus* and *Chaetoceros socialis f. radians* are two species in this genus and both can cause HABs.

1.3.2 Marine Algal ssRNA Viruses

Marine algal ssRNA viruses are a group of viruses that specifically infect marine algae; these viruses can regulate the algal populations and nutrient cycling, and thus, they are a crucial component of marine ecosystems. Algal ssRNA viruses are highly diverse and are classified into various families, such as *Marnaviridae*; each family may infect specific algal hosts. The presence of these viruses may help control algal populations, preventing the overgrowth of certain algal species that can lead to HABs with negative ecological and economic consequences (*Figure 1.3.2.1*).

**Marnaviridae viruses**

*Marnaviridae* viruses are a group of +ssRNA algal viruses that can regulate the dynamics of HABs; they belong to the order of *Picornavirales*, thus resembling viruses in this order. Indeed, large-scale genomic studies have indicated that a great number of uncharacterized picorna-like viruses exist in the ocean. These viruses exhibit a limited host range, and their infection has a significant impact on their specific hosts. Accumulated evidence suggests that the strain-specific lysis caused by *Marnaviridae* viruses infecting eukaryotic phytoplankton has a transformative effect on phytoplankton communities, including algal blooms. Hence, there is a keen interest in identifying structural characteristics within these picorna-like *Marnaviridae* viruses that influence their transmission. The aim is to devise strategies to leverage these features for the viro-control of HABs.
The following section describes the structural findings of a picorna-like **Marchnnaviridae** Chaetoceros tenuissimus RNA virus type II (CtenRNAVII) compared with those of well-studied **Picornaviridae** viruses in the order **Picornavirales**.

![Figure 1.3.2.1. Regulation of HABs by marine algal viruses within one year. The prevalence of a specific group of algal viruses (X or Y) leads to a reduction in the corresponding host algae population (X or Y).](image)

**Common structural features of Picornaviridae viruses**

The order **Picornavirales** encompasses viruses infecting vertebrates (**Picornaviridae**), invertebrates (**Dicistroviridae**, **Polycipiviridae**, and **Iflaviridae**), protists (**Marnaviridae**), and plants (**Secoviridae**). It includes many notable viruses that are highly related to human health, such as poliovirus, rhinovirus, and HAV.

The virions of **Picornaviridae** viruses share commonalities. They are non-enveloped, icosahedral, and approximately 30 nm in diameter. The capsids have pseudo-$T=3$ symmetry and encase their +ssRNA genome. The capsid is built with 60 copies of each of four structural proteins, that is, VP1, VP2, VP3, and VP4; VP1 has a receptor-binding site and a notable surface feature called the “canyon” ([Figure 1.3.2.2](image))118, which is an inaccessible depression around the 5-fold axis and is thought to help the viruses escape from the binding of host antibodies119.
Unique structural features of a Marnaviridae virus

CtenRNAVII was first isolated from the Hiroshima Bay in Japan. The virion exhibits pseudo-$T=3$ icosahedral symmetry. The capsid comprises VP1, VP2, and VP3 as the outer surface and a short peptide VP4 inside the capsid. It is the first structure in the Marnaviridae family providing important structural information about this group of viruses. As mentioned above, Picornaviridae viruses have a canyon structure surrounding each 5-fold axis. However, the VP1 of CtenRNAVII has a CD-loop that blocks the supposed canyon, resulting in no canyon structure (Figure 1.3.2.2). In addition, VP1 possesses an EF-loop whose function remains unclear. It was previously thought that VP1 plays a crucial role in viral infection and host specificity. Therefore, these distinctive structural characteristics are likely vital for the primordial infection.

![CtenRNAVII and Polioviruses Diagram](image-url)
mechanisms of algal Marnaviridae viruses. The CtenRNAVII and primordial groups of human Picornaviridae viruses, such as HAV, exhibit a domain swap in the VP2 N-terminus. This swap may represent an ancestral feature that is conserved among Marnaviridae viruses99.

1.3.3 Remaining Questions in the Structural Functions of Marnaviridae Viral Capsids

Unlike Picornaviridae viruses, picorna-like Marnaviridae viruses do not have a canyon structure, implying that they possess a different infection mechanism. Nonetheless, it is still unclear how each Marnaviridae virus infects its alga host in a host-specific manner and which surface structures are implicated. The host specificity of the Marnaviridae virus is crucial for precise viro-control, hence enabling the targeted regulation of a specific algae species that is responsible for HABs. Section 2.2.3 (Paper III) presents our latest structural findings of a Marnaviridae virus that were found using cryo-EM SPA method: Chaetoceros socialis forma radians RNA virus 1 (CsfrRNAV).
2. Present Investigations

2.1 KEY METHODS

In my three papers, I primarily utilized two techniques: reverse genetics system, which involves generating infectious clones of a dsRNA virus, and cryo-EM SPA, which is used for viral structure reconstruction. This section provides an overview of these two techniques’ contemporary applications.

2.1.1 Reverse Genetics of Viruses

Section 1.2.3 emphasizes that the capsids in totivirus-like viruses have evolved unique and functionally significant structural attributes as part of their strategies for survival, encompassing transmission and replication. To investigate how viral genes are related to these capsid features and their functional changes, it is essential to employ robust reverse genetic tools to generate infectious clones that enable genome-wide manipulation. There are two commonly employed reverse genetics systems. The first involves synthesizing full-length genomic RNA transcripts from a linear DNA template in vitro, which is followed by introducing these synthesized RNA transcripts into host cells using lipofection or electroporation (the RNA transcript-based method). The second involves transfecting plasmid constructs or bacterial artificial chromosomes (BAC) that encode a full-length cDNA, including the RdRp gene in the promoter/terminator region. The RNA transcripts are then generated from the plasmids/BAC using host RNA polymerases (plasmid/BAC-based method).

Reverse genetics systems have already been effectively applied to create infectious clones for various viruses. For instance, the RNA transcript-based method has been applied to generate infectious clones of a broad range of viruses, including the HAV, coronaviruses, and flaviviruses\textsuperscript{121–125}. The different infectious clones of coronaviruses have also been created via the plasmid/BAC-based approach\textsuperscript{126–128}. As for dsRNA viruses, infectious clones for multi-segmented \textit{Reovirales} viruses have been produced using both the RNA transcript-based and plasmid-based techniques, for studying the protein functions and pathogenesis of some genera such as orthoreoviruses\textsuperscript{129–131}, orbiviruses\textsuperscript{132–136}, and rotaviruses\textsuperscript{137–141}. Furthermore, the infectious particles for a segmented dsRNA virus in the \textit{Birnaviridae} family were also successfully generated using the RNA transcript-based method\textsuperscript{142}. However, in the case of
non-segmented dsRNA viruses, such as totivirus-like viruses, few cases were reported. Therefore, the development of infectious clones for non-segmented totivirus-like viruses is technically and biologically imperative in the field of virology.

2.1.2 Cryogenic Electron Microscopy Single-Particle Analysis

In recent years, cryo-EM techniques have undergone rapid developments and are now firmly established as essential tools for structural biologists. These advances encompass several technical breakthroughs, such as the integration of direct electron detectors, which are complemented by algorithms designed to correct beam-induced movement and specimen drift. Direct electron detectors combine the benefits of conventional charge-coupled device cameras and photographic films, allowing for a fast readout. Additionally, automated data collection strategies and the introduction of image analysis software have also significantly contributed to the widespread adoption of cryo-EM for various applications in the field of structural biology. Cryo-EM has become the primary method of structural analysis in many biological sample studies.

![Workflow of cryo-EM SPA for a non-enveloped icosahedral virus sample.](image)

Cryo-EM SPA becomes a powerful technique for visualizing and analyzing the three-dimensional (3D) structure of viruses at a resolution of 2–4 Å. This method allows us to study the architecture and organization of viral particles without crystallization. It can capture the structure of viruses in their near-native and hydrated state, allowing for the study of conformational changes and dynamic processes. It also enables in situ structural studies in viruses, such as examining the structure of the CP-RdRp complex at a 5-fold vertex to explore the virus’s replication process within the capsid. The general procedures for cryo-EM SPA include sample preparation, data collection, particle picking, 2D classification, 3D reconstruction, modeling, and refinement (Figure 2.1.2). Samples are usually applied on a holey grid and are flash-frozen using liquid ethane, which helps place particles in a thin film of vitreous ice. This process protects samples from radiation.
damage and dehydration, which is beneficial for subsequent data processing\textsuperscript{154}. Numerous 2D electron micrographs, each displaying diverse particle projections, are captured from individual sample grids and are subjected to micrograph preprocessing. Particles of interest are selected through manual or automated methods within the software. The chosen particles are subsequently extracted from each micrograph and categorized according to various orientations and conformations. Enhanced signals result from averaging multiple individual particles. Particles within high-quality 2D classes are then chosen and utilized for the reconstruction of a 3D map, which is followed by subsequent refinement (\textit{Figure 2.1.2})\textsuperscript{154,155}. 
2.2 KEY RESULTS
This section comprises three subsections, each of which is dedicated to providing background information and the major research findings for my three papers.

2.2.1 First Infectious cDNA Clone Generation of a dsRNA Totivirus-Like Virus (Paper I)
We successfully created the initial infectious cDNA clone for OmRV, a dsRNA totivirus-like virus. In addition, we successfully recovered a transmission-impaired OmRV T365A mutant. These achievements open possibilities for applying this methodology to uncover the functions of essential components, ranging from proteins to specific amino acid residues, in dsRNA totivirus-like viruses and Totiviridae viruses.

Totiviridae viruses
There are five approved genera within the family Totiviridae: Giardiavirus, Leishmaniavirus, Totivirus, Trichomonasvirus, and Victorivirus. These viruses, such as GLVs, Leishmania RNA virus 1, TVVs, and Helminthosporium victoriae virus 190S (HvV190S), mainly infect fungi and parasitic protozoa. Many members, including ScV-L-A, ScV-L-BC, and TVVs of the Totiviridae family, remain intracellular and transmit to other host cells during cell division. However, GLVs have acquired an extracellular transmission ability similar to totivirus-like viruses. Their virion is mostly 40 nm in diameter, and the capsid shell encloses a single 4.6–7.0 kbp dsRNA genome, which typically encodes a CP and a CP-RdRp complex via a -1 ribosomal frameshift.

Totivirus-like viruses
OmRV is a representative totivirus-like virus. It has a 40 nm virion encompassing a non-segmented 7.6 kbp dsRNA genome that resembles other members in the Totiviridae family. OmRV is insect-borne and is genetically related to another arthropod-borne virus: IMNV. To date, two strains—OmRV-AK4 and -LZ—have been isolated and characterized. Both empty and full viral particles are observed within these strains. OmRV demonstrates the ability to infect multicellular hosts and possesses an extracellular phase in its lifecycle. OmRV-LZ has its capsid surface adorned with a protrusion protein, which is thought to play a role in the infection process. However, this protrusion protein is absent in the capsids of other related Totiviridae viruses. Additional structural features, including terminal extensions and obstructed capsid pores, have been observed, even though their functions remain unverified. Therefore, a molecular approach such as reverse genetics is a valuable tool for further investigation.
Recombinant OmRV/IC-wt particles resemble the native OmRV strain
The first isolated strain, OmRV-AK4, served as a gene sequence template. The plasmid containing the full-length infectious cDNA clone was designed and generated (OmRV-full-length/pACYC177) (Figure 2.2.2-A). OmRV wild-type infectious clone particles (OmRV/IC-wt) were generated from C6/36 Aedes mosquito cells using an RNA transcript-based method. A severe cytopathic effect (CPE) induced by OmRV/IC-wt infection was observed, which was similar to that of the native OmRV-AK4 strain (Figure 2.2.2-B). Subsequently, OmRV/IC-wt particles were purified through sucrose gradient ultracentrifugation and subjected to examination via cryo-EM. Like OmRV native particles, the recombinant OmRV/IC-wt particles have a similar morphology (Figure 2.2.2-C).

Figure 2.2.2. Illustration summary of Paper I. A) Full-length OmRV/IC-wt plasmid constructed in low-copy-number pACYC177 vector with SP6 as an in-vitro transcription promoter and two OmRV-AK4 gene fragments. B) CPE of C6/36 cells after OmRV/IC-wt infection. C) Cryo-EM raw images of purified OmRV/IC-wt and native OmRV-AK4 particles. D) Propagation curves of OmRV/IC-wt and -T365A mutant after the transfections of their +ssRNAs in C6/36 cells. ns: No significant difference. *Statistically significant difference, P<0.05.
Mutation T365A affects OmRV propagation

In addition to OmRV/IC-wt, a mutant OmRV/IC-T365A, was engineered using the same approach, here incorporating mutagenesis. The significance of T365 as a crucial amino acid residue bridging a hydrogen bond between OmRV capsid and protrusion proteins suggests that its mutation has the potential to hinder the interactions between these proteins. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed to monitor viral particle propagation. The results obtained through RT-qPCR revealed a disparity in the propagation rates between OmRV/IC-wt and OmRV/IC-T365A, as depicted in Figure 2.2.2-D. The mutation partially diminished the propagation capability of OmRV particles in mosquito cells.
2.2.2 Capsid Structure of a Megabirnavirus RnMBV1 (Paper II)

We performed cryo-EM SPA and obtained the first atomic models of dsRNA RnMBV1 particles at a resolution of 3.2 Å. Based on the structures, three main features were identified: 1) CP protrusion domain and extra surface crown proteins (CrPs); these may be required for viral transmissions; 2) extra-long extensions on the C-terminus of CPs that possibly stabilize viral particle assembly; and 3) 5-fold obstructed pores that are required for NTP intake and synthesized genome release. These structural features might play essential roles in RnMBV1’s viral life cycles. Understanding these roles will enable us to apply this virus as a fungi-induced white root rot disease control.

**Rosellinia necatrix and white root rot**

White root rot is a destructive fungal disease that affects a variety of woody and herbaceous plants including fruit trees (e.g., apple and pear), woody ornamentals, vines, and various crops like avocado, coffee, and citrus\(^1\). It is caused by several species of the multicellular fungus *Rosellinia*, with *Rosellinia necatrix* (*R. necatrix*) being the most notorious pathogen\(^1\). Above-ground symptoms may include the yellowing or browning of leaves, dieback of branches, and poor growth. Below the ground, the roots become discolored and decayed, turning white, which gives the disease its name\(^1\). White root rot is favored by cool and humid conditions, with the disease becoming more severe in areas with high humidity and frequent rainfall. White root rot can have a significant economic impact, particularly in the agricultural and horticultural industries, because it can lead to the loss of valuable crops and trees\(^1\). Addressing white root rot involves enhancing soil drainage, refraining from planting vulnerable hosts in areas where the disease is prevalent, and using fungicides\(^1\). Despite these efforts, managing the disease poses significant challenges\(^4\).

**Megabirnaviridae viruses and RnMBV1**

Viruses in the family *Megabirnaviridae* have recently been characterized and recognized as dsRNA fungal viruses. RnMBV1, which is named from an *R. necatrix* W779 field strain, is the exemplar strain within the *Megabirnaviridae* family\(^1\). There are two more unassigned members: Sclerotinia sclerotiorum megabirnavirus 1 (SsMBV1) and Rosellinia necatrix megabirnavirus 2 (RnMBV2)\(^1\). RnMBV1 is a potential candidate for controlling white root rot disease because it can confer hypovirulence to *R. necatrix*\(^4\); however, more molecular and structural investigations are needed.

RnMBV1 has an approximately 52 nm icosahedral virion that encloses two genome segments: dsRNA-1 (8.9 kbp in length) and -2 (7.2 kbp in length). The dsRNA-1 encodes CPs and RdRps, while dsRNA-2 encodes proteins whose functions are unknown. The CP-RdRp fusion product can be expressed through -1 ribosomal shifting\(^4\). Previously, a low-resolution (15.7 Å) cryo-
EM model was reported, with many structural details remaining unclear. Therefore, an atomic model at a higher resolution can enable an in-depth study of this virus.

Capsid composition and surface features

The capsid of RnMBV1 virions comprises 60 chemically identical but asymmetric CP subunits (CP_A and CP_B) and surface CrPs. The CPs are assembled in a $T=1$ icosahedral symmetry. Each CP_A proximally surrounds while CP_B distally surrounds each 5-fold axis. Three CP_Bs build a trimer. Five CP_A and B dimers build a 5-fold complex (Figure 2.2.3.1-A).

Protrusion domains were found in each CP on the capsid surface of RnMBV1 (Figure 2.2.3.1-B). This protrusion feature also exists in multicellular Quadriviridae RnQV1 and totivirus-like OmRV but is absent in some other structural-related yeast or protozoan viruses like Totiviridae ScV-L-A and Chrysoviridae PcV. It is elusive but of interest to investigate the roles that the protrusion domains play in the viral life cycle.

CrPs sit over the CP_B trimers surrounding the 3-fold axes (Figure 2.2.3.1-A). Although the CrPs in RnMBV1 have no sequentially related proteins, the feature of extra capsid surface proteins is present in several dsRNA viruses.

Figure 2.2.3.1. Capsid composition and protrusion domain. A) Full capsid model and structure of CPs (light yellow or purple) and CrP (yellow, orange, or red) of RnMBV1. B) Surface protrusion domains that are highlighted in red. Numbers indicate icosahedral symmetry axes. The pentagon, triangle and ellipse indicate the 5-, 3- or 2-fold axis, respectively.
infecting multicellular hosts, including OmRV\textsuperscript{62}. With the loss of CrPs, the viruses are viable but fail to confer hypovirulence to their hosts\textsuperscript{51}.

**Terminal extensions**

As discussed in section 1.2.3, many dsRNA viruses have acquired terminal extensions as a viral particle stabilization strategy. In RnMBV1, both the C-termini of CP\_A and \_B are extended and interact with up to three adjacent subunits (Figure 2.2.3.2-A). RnMBV1 is larger than the most known $T=1$ icosahedral dsRNA viruses; therefore, the C-terminal arms significantly contribute to the contact area between subunits and help stabilize the viral capsid.

**Pore conformations**

As mentioned in section 1.2.3, the life cycles of dsRNA viruses necessitate the presence of pores. In the case of RnMBV1, an obstructed 5-fold pore was noted (Figure 2.2.3.2-B). This pore is constructed from 10 Arg residues and a segment of the C-terminal arm within CP\_B. Even though Arg residues partially block the pore, a 16-Å small room remains, as depicted in Figure 2.2.3.2-B. This space and the positively charged Arg residues enable the intake of NTPs and the release of +ssRNA transcripts.

*Figure 2.2.3.2. C-terminal arm interlockings between subunits and obstructed pore conformation. A) C-terminals arm interlocking system within a 5-fold complex (left) and close-up view within four adjacent subunits (right). B) Different pore representations: electrostatic potential (left), structural organization of 10 Arg residues surrounding a 5-fold axis (middle), and cross section of the pore (right). CP\_As and CP\_Bs are colored light yellow and purple, respectively.*
2.2.3 Capsid Structure of an Algal ssRNA CsfrRNAV (Paper III)

We employed cryo-EM SPA to reconstruct 3D atomic models of both full (3.0 Å) and empty (3.1 Å) CsfrRNAV particles. By conducting structural comparisons, we identified shared and distinctive features among various Marnaviridae viruses. This analysis offers valuable insights into the transmission mechanisms common to Marnaviridae viruses as well as those specific to each. Additionally, we utilized AlphaFold2 to predict the structures of other Marnaviridae VP1 proteins, hence showing the potential application of AlphaFold2 in generating a structure-based phylogeny. The generated structure-based phylogeny can be utilized for a comprehensive classification of the Marnaviridae virus, based on their host specificity.

Overall capsid structure

The capsid shell exhibits pseudo-T=3 icosahedral symmetry and consists of three VPs: VP1, VP2, and VP3 (Figure 2.2.4.1-A). VP1s assemble around the 5-fold axis to create pentamers, while VP2s form dimers, and each trio of

![Overall capsid and VP1 structure. A) Presentation of the complete CsfrRNAV full capsid atomic model (left) and the inside view of a 5-fold complex (right). The 5-, 3-, and 2-fold axes are denoted by a pentagon, triangle, and ellipse, respectively. VP1, VP2, and VP3 are colored pink, blue, and light green, respectively. B) Display of VP1 distinctive loops (CD-loop in blue, E_E2-loop in red, and EF-loop in green) in three orientations.](image-url)
VP2-VP3 complexes surrounds a 3-fold axis (Figure 2.2.4.1-A). All VPs show jelly-roll folds (Figure 2.2.4.2-A). The capsid surface features three distinct VP1 loops: CD-loop, E₁E₂-loop, and EF-loop (Figure 2.2.4.1-B).

**Empty capsid structure**
The overall diameter of empty CsfrRNAV particles is approximately 318 Å, which is slightly larger than that of full particles, which measure around 311 Å in diameter. The structures of VP1, VP2, and VP3 are largely conserved between full and empty capsids. The primary difference lies in the absence of the VP2 N-terminal domain swap in the empty capsid (Figure 2.2.4.2-A and -B). These findings suggest a less tightly assembled structure in the empty capsid, and the role of the VP2 N-terminal domain may be associated with genome packaging. A 3D variability analysis of empty particles also reveals

*Figure 2.2.4.2. Structural comparison between full and empty capsids. A) Superimpositions of VP1, VP2, and VP3 between full and empty capsids. B) Overview of full and empty particles and the N-terminal swap (red) difference shown in VP2 complexes. VPs in full or empty particles are colored pink or blue, respectively.*
diverse mobile patterns, including rocking, tilting, and expansion, particularly in the 5-fold complexes.

**Commonalities and differences between CsfrRNAV and CtenRNAVII**

The structural configurations of the VPs are largely conserved and similar between CsfrRNAV and CtenRNAVII, with the most notable variations occurring in VP1s (*Figure 2.2.4.3*). In the full CsfrRNAV capsid, the VP2 N-terminal domain swap, VP1 CD-loop, and EF-loop exhibit consistency. Regarding VP1 structures, CsfrRNAV displays a distinct orientation of the E₁E₂-loop compared with that of CtenRNAVII (*Figure 2.2.4.3*). This suggests a potential correlation between the E₁E₂-loop conformational changes and the host specificity of these algal viruses. Additionally, CsfrRNAV lacks VP4, indicating that VP4 might not be indispensable for the life cycle of all *Marnaviridae* viruses.

*Figure 2.2.4.3.* Structural differences in VP1 between *Marnaviridae* viruses. Left: Superimposed structures of VP1s (CsfrRNAV in pink, CtenRNAVII in gray). Right: Root-mean-square deviation (RMSD) per residue map for CsfrRNAV VP1 to that of CtenRNAVII. The CD- and E₁E₂-loops are circled, emphasizing regions with the high variation or local RMSD values. Local RMSD is indicated from blue to red, representing low to high values (Å).

**Structural phylogeny using AlphaFold2**

We generated a structural phylogeny based on our experimental data and the other 16 predicted *Marnaviridae* VP1 structures. The result provides insights into the use of AlphaFold2 in host-specificity predictions in the future. Based on all the VP1 models, the E₁E₂-loop exhibits the highest diversity and, thus, is implicated with its host-specific binding mechanism. It is interesting to perform structural predictions by AlphaFold2, which is followed by analysis to reclassify viruses based on their structural features, not just based on their genome-based phylogenetic analysis.
3. Conclusion and Future Perspectives

The three papers in this thesis emphasize the potential impact of integrating structural analyses through cryo-EM with molecular experiments, such as reverse genetics, to explore virus-to-host interactions and viral life cycles. Paper I introduces the potential application of the reverse genetics approach to unveil the functions of crucial components, from proteins to amino acid residues, in non-enveloped icosahedral dsRNA totivirus-like viruses and Totiviridae viruses. Molecular experiments will complement our structural findings, enabling the validation of our structure-based hypotheses. Leveraging reverse genetics, we have generated mutants to elucidate the functions of amino acid residues, particularly those possibly involved in viral intraparticle genome synthesis—an essential aspect for many non-enveloped icosahedral dsRNA viruses. Ongoing molecular and cellular experiments on these mutants will further enhance our understanding of the roles played by amino acid residues in icosahedral dsRNA viruses, providing fundamental insights for future studies in this field.

Paper II presents crucial structural features essential to the life cycle of a fungal icosahedral dsRNA megabirnavirus, RnMBV1, hence offering potential avenues for utilizing this virus to control fungi-induced white root rot disease. Furthermore, a deeper comprehension of icosahedral dsRNA virus capsid structures consistently maintained across different species will strengthen the foundation for future virus studies. Concurrently, an ongoing structural study on the icosahedral dsRNA virus GLV, which is associated with the protozoan pathogen *G. lamblia*, is underway. Unraveling its structural features will provide insights into disease prevention, diagnosis, and treatment related to *G. lamblia*. Together with other ongoing structural studies on pathogenic icosahedral dsRNA viruses, including PMCV, IPNV, and IMNV, we aim to offer valuable biological information for virus applications in viro-control, virotherapy, and vaccine development targeting pathogenic viruses.

Paper III provides insights into viral transmission mechanisms among Marnaviridae viruses, demonstrating the capability of AlphaFold2 to generate a structure-based phylogeny. This approach will be advantageous for addressing HABs by using algal viruses based on their host specificity. In the future, we plan to analyze more algal Marnaviridae viruses using bioinformatic and structural biology approaches to specifically treat HABs through viro-control.
This approach will mitigate both the negative ecological and economic impacts of HABs in the ocean.
Viruses, existing everywhere, play a crucial role globally by infecting a diverse range of organisms, leading to significant economic, ecological, and health challenges. Being the smallest infectious agents, viruses replicate by exploiting their hosts. Viruses display diverse shapes and sizes. Typically, a virus has an outer protein shell, which we call it capsid. The heart of a virus’s life cycle lies in its capsid—a protective cover that houses the genome, the genetic material of the virus. This shell ensures the virus’s safety, facilitates its replication, and aids in its infection.

To understand the correlation between viral genes and the viral life cycles, we utilized reverse genetics, a method enabling the creation of synthetic viruses. Additionally, to visualize viruses, we employed cryogenic electron microscopy (cryo-EM) to capture detailed structures of viral capsid shells.

**What is a dsRNA virus?**

Double-stranded ribonucleic acid (dsRNA) viruses are a group of viruses characterized by a genome comprising two RNA strands. These viruses exert a broad range of impacts due to their diverse life cycles and the ability to infect various hosts, impacting fisheries, agriculture, animal welfare, food production, and human health.

Most dsRNA viruses possess one or more layers of capsids and share many commonalities in their overall capsid structures. The typical architecture of capsids in dsRNA viruses follows a sophisticated design: a single layer constructed from 120 identical protein subunits in an icosahedral (20-sided) arrangement. However, for some dsRNA viruses infecting complex organisms with multiple cells, the capsid may exhibit greater complexity. Throughout evolution, these viruses have acquired intriguing features such as additional proteins or entry-like pores that actively participate in their replication.

**Customization of a dsRNA virus**

We successfully generated the first artificial Omono River virus (OmRV), which is a dsRNA virus. This virus infects mosquitoes, and it has a more complex capsid shell than those of related viruses. Additionally, we successfully created a genetically altered OmRV, intentionally influencing its replication. These achievements will allow us to unravel essential details about the inner relations of these viruses and their genes.
White root rot, fungus, and fungal virus

White root rot, which is a notorious fungal disease impacting various plants, including fruit trees, is primarily caused by the fungus *Rosellinia*, specifically *R. necatrix*. There is hope on the horizon with *Rosellinia necatrix* megabirnavirus 1-W779 (RnMBV1), a dsRNA virus that infects *R. necatrix* and can control white root rot. However, unlocking its full potential requires more information, which can be provided through biological studies.

We got the first structure of the RnMBV1 capsid shell at a good resolution using cryo-EM. RnMBV1 exhibits a complex capsid shell with many interesting features acquired through evolution, likely playing a crucial role in its lifecycle. Understanding these could pave the way for utilizing this virus as a promising control measure against white root rot disease.

Algal bloom, algae, and algal virus

Harmful algal blooms (HABs) emerge when algal populations undergo uncontrolled growth in favorable conditions, posing threats such as fish die-offs and risks to animals, humans, and economic losses. Thus, addressing HABs is an urgent priority.

Algae, representing various types such as diatoms, serve as the foundation of diverse aquatic food chains and webs. *Chaetoceros* species, the most abundant marine planktonic diatoms globally, can cause HABs. Some *Marnaviridae* viruses infect these *Chaetoceros* diatoms and can influence HAB dynamics. These viruses share similarities with picornaviruses, such as the Hepatitis A virus. To harness these algal viruses, a deeper understanding of them is important.

Recently, the capsid structure of a *Marnaviridae* virus—Chaetoceros tenuissimus RNA virus type II (CtenRNAVII)—has provided crucial structural information, highlighting both its similarities and differences from picornaviruses. We have contributed to this knowledge by obtaining capsid structures of another *Marnaviridae* virus—Chaetoceros socialis forma radians RNA virus 1 (CsfrRNAV)—using cryo-EM, thereby revealing the shared and unique features among *Marnaviridae* viruses. Our findings could pave the way for utilizing these algal viruses to control HABs in the future.
5. Sammanfattning på Svenska


För att förstå sambandet mellan virala gener och virusets livscykel använde vi så kallad omvänd genetik, en metod som möjliggör skapandet av syntetiska virus. Dessutom använde vi kryogen elektronmikroskopi (kryo-EM) för att visualisera virus och erhålla detaljerade strukturer på deras kapsid.

Vad är ett dsRNA-virus?
Dubbelsträngat RNA (dsRNA) virus är en grupp virus kännetecknade av ett genom bestående av två RNA-strängar. Dessa virus har vittskild påverkan inom många nischer på grund av sina varierande livscykler och förmågan att infektera olika värdar. De påverkar fiske, jordbruk, djurskydd, livsmedelsproduktion och mänsklig hälsa.


Anpassning av ett dsRNA-virus
Vi har framgångsrikt skapat det första syntetiska Omono River-viruset (OmRV), som är ett dsRNA-virus. Detta virus infekterar myggor och har ett mer komplext kapsidhölje än besläktade virus. Dessutom har vi lyckats introducera genetiska förändringar i detta syntetiska OmRV, med avsikt att påverka dess replikationsförmåga. Dessa framsteg kommer kunna leda oss vidare till nya insikter kring egenskaperna hos viruset och dess gener.
Rotröta, svamp och svampvirus


Vi har för första gången lyckats strukturbestämma kapsiden från RnMBV1 med mycket god upplösning genom att använda metoden kryo-EM. RnMBV1 har ett komplext kapselhölje med flera intressanta evolutionärt förvärvade detaljer som verkar spela avgörande roll i dess livscykel. Den förståelsen kan öppna vägen till att använda detta virus som en kontrollåtgärd mot rotröta.

Algblomning, alger och algvirus

Skadliga algblomningar uppstår när algpopulationer genomgår okontrollerad tillväxt under gynnsamma förhållanden och utgör hot såsom risker för djur, människor, fiskdöd och ekonomiska förluster. Att kunna begränsa algblomningar är därför ett akut problem och av högsta prioritet.


Den nyligt lösta strukturen av kapsiden hos *Marnaviridae*-viruset *Chaetoceros tenuissimus* RNA-virus typ II (CtenRNAVII) gav avgörande strukturell information och belyst både dess likheter och skillnader jämfört med picornavirus. Nu har vi ytterligare kunnat bidra till denna kunskap genom att erhålla kapsidstrukturen av ett annat *Marnaviridae*-virus—*Chaetoceros socialis* forma radians RNA-virus 1 (CsfrRNAV)—med hjälp av kryo-EM, vilket avslöjade gemensamma men även flertalet unika drag hos dessa virus. Våra fynd kan öppna vägen för att använda dessa algvirus för att kontrollera algblomningar i framtiden.
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References


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