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**Principles of Virus Structure**

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**Glossary**

- **Ångstrom** A unit of length equal to one hundred-millionth of a centimeter to measure wavelengths and distances (1 Å = 0.1 nm).
- **Capsid** The protein shell of a virus particle.
- **Capsomere** The basic structural unit of a capsid which can self-assemble to form the complete capsid.
- **Envelope** A host derived lipid membrane separating the genome from the viral capsid.
- **MCP** Major capsid protein.
- **Nucleocapsid** The protein capsid and the genome.
- **Spike** Glycoprotein surface projections of varying length at regular intervals on a viral surface.
- **Structural proteins** Proteins present in the virus particle.
- **Virion** A virus particle capable of infecting a host cell.
- **VLP** Virus like particle (VLP) consisting of the outer capsid without the genome.

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**Introduction**

Viruses are nanomolecular assemblies tailored to parasitize a wide variety of hosts ranging from prokaryotes to eukaryotes. A virus particle consists of a genome, which can comprise either single- or double-stranded RNA or DNA molecule(s) protected by a protein or a proteolipid outer capsid. Viruses insert their genome into a host organism where they can replicate and assemble. They therefore have a strong influence on protein adaptation and are key drivers of molecular evolution (Enard et al., 2016). Viruses occur in distinct shapes, sizes and symmetries designed for effective transmission of their genetic material. They are classified based on the type and organization of the encapsulated genome and the presence of a lipid envelope derived from the host cell surrounding the viral nucleocapsid.

Viruses occur in three predominant shapes: filaments, spheres and pleomorphic (Fig. 1). The spheres are 20-sided regular icosahedra. The size of icosahedral viruses varies from a diameter of 170 Å (porcine circovirus) to 5000 Å (mimivirus). However, pithovirus and pandoravirus, the largest known viruses, are oval and measure about 10,000 Å and 15,000 Å in size, respectively. Mimivirus, a double-stranded DNA (dsDNA) virus with a genome size of 1.2 megabase pairs has an icosahedral shape with a diameter of 5000 Å and is associated with 1200 Å-long fibers (Klose et al., 2010; Raoult et al., 2004; Xiao et al., 2005, 2009). Most plant viruses are small and are either filamentous or polygonal, as are also many bacterial viruses (Johnson and Speir, 1997; Rossmann, 2013; Solovyev and Makarov, 2016). The larger and more complex dsDNA containing bacteriophages, which infect E. coli, combine both filamentous and polygonal shapes (Ackermann, 1999). For example, the T4 bacteriophage is composed of an icosahedral head (1150 Å long, 210 Å in diameter) (Fokine et al., 2004) with an associated 1000 Å rod-shaped long tail (Kostyuchenko et al., 2005), a 460 Å-diameter baseplate and six 1450 Å-long tail fibers (Kostyuchenko et al., 2003). Structures such as these are unique to bacteriophages. Animal viruses, however exhibit extreme variations in size and shape. For example, paroviruses are icosahedrons and measure about 180–250 Å in diameter. In contrast, members of Podoviridae and Togaviridae are about 250–750 Å in diameter and are either polygons, filamentous or pleomorphic.

The viral genome encodes structural proteins, which constitute the virus particle and non-structural proteins essential for the replication of the virus inside the host cell. These proteins aid in several stages of a typical virus life cycle that includes host cell recognition, replication, assembly, maturation and the release of progeny virus particles. The capsid protects the viral genome against harsh conditions like variation in pH, temperature and degradation by enzymes during infection. The capsid also plays a role in host cell recognition and entry. Although the number and nature of the capsid building blocks vary among viruses, there are certain common principles of capsid organization that apply to most viruses.

Several excellent reviews on the principles of virus structure organization have been published over the years (Caspar and Klug, 1962; Harrison, 2007; Johnson and Speir, 1997; Klug and Caspar, 1960; Lee and Johnson, 2003; Prasad and Schmid, 2012; Rossmann, 2013; Rossmann and Johnson, 1989). Here, we give a short history of structural virology, a discussion of virus symmetry and the methods used to study virus structures followed by the current understanding of the principles of virus architecture. We will be discussing a series of example virus structures to show: (1) how virus capsid subunits are assembled, (2) what triggers their disassembly to release their genome, (3) why certain protein folds occur frequently in virus capsids, (4) how viruses recognize and enter host cells, and (5) how structures aid in the development of vaccines and anti-viral therapeutics.

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**General Principles of Capsid Architecture**

The first significant development in structural virology was in 1935, when Stanley crystallized the infectious particles of tobacco mosaic virus (TMV) (Stanley, 1935) and subsequently Bawden and Pirie crystallized tomato bushy stunt virus (TBSV)
In 1941 Bernal and Fankuchen used X-ray diffraction to establish the size and shape of TMV and TBSV particles and presented the first detailed description of any virus structure (Bernal and Fankuchen, 1941a,b). They established that the TMV virus particles consisted of a protein capsid that protected the viral genome. This work was then extended to near-atomic resolution by Franklin (Franklin, Holmes, 1958; Franklin et al., 1957), Holmes (Holmes et al., 1975), Stubbs (Stubbs et al., 1977), and Bloomer (Bloomer et al., 1978; Champness et al., 1976). The description of the assembly of TMV capsid subunits around the RNA genome was among the foremost studies of virus capsid self-assembly (Butler and Klug, 1972).

In 1953, James Watson and Francis Crick reached their ground-breaking conclusion, using Franklin’s X-ray diffraction images, that DNA exists in the form of a three-dimensional (3D) double helix with the two strands connected by hydrogen bonds between the nucleotides (Watson and Crick, 1953b). They also postulated the ‘Central Dogma’, which implied that the information flow in biological systems is from DNA to RNA to protein (Watson and Crick, 1953a). They observed that a much larger genome than observed in all known viruses would be required to code for a protein of sufficient size to completely cover the virus genome. Thus, the capsid must be constructed of multiple copies of the same protein. As each of these subunits have the same sequence and therefore the same structure, the environment of each subunit must be the same. Hence, they suggested that the capsid structure of viruses have to be regular polyhedrons. Of the five possible polyhedra, the icosahedron would have the greatest number of subunits and would, therefore, represent the most conservative way of using the least amount of genome to code for the largest possible virus (Crick and Watson, 1956; Crick and Watson, 1957). This was verified by Caspar (Caspar, 1956), who observed in an X-ray crystallographic study of TBSV that there appeared to be rotation axes at angles to each other consistent with icosahedral symmetry. The icosahedral symmetry in TBSV was also confirmed using electron microscopy (Horne et al., 1959; Williams and Smith, 1958).

Icosahedral Symmetry

Caspar and Klug provided the first evidence for the presence of icosahedral symmetry by analyzing the X-ray diffraction patterns of TBSV and turnip yellow mosaic virus (TYMV). An icosahedron has 12 vertices with 5-fold rotational symmetry, 20 triangular faces...
with 3-fold rotational symmetry and 30 edges with 2-fold rotational symmetry (Fig. 2). This allows the placement of 60 identical subunits decorated with capsid proteins with equivalent contacts between them. However, there are many spherical viruses where the number of capsid subunits far exceed the 60 subunits required by icosahedral symmetry. To address this question, Caspar and Klug proposed an extension to Watson and Crick’s strict icosahedral symmetry concept in viruses (Caspar and Klug, 1962). They suggested that capsid subunits in an icosahedron might adapt to slightly different inter-subunit interfaces and introduced the principle of quasi-equivalence for larger virus structures with more than 60 capsid subunits. They predicted a virus assembly process in which the subunits assembled into hexagonal arrays. This would give rise to a scheme where an icosahedral virus can be constructed from pentamers of triangular faces (pentons) forming the 12 vertices and hexamers of triangular faces (hexons) covering the 20 icosahedral faces. The assumption that the monomers that make a hexamer would have quasi-equivalent environments increased the number of subunits in an icosahedron from 60 to 60 T, with T representing the triangulation number.

The triangulation number (T) is the number of quasi-similar subunits per icosahedral asymmetric unit with possible values of T, because of geometric restrictions, dictated by the equation $T = h^2 + hk + k^2$. Here, h and k are the number of hexons between pentons along the h and k axes of a hexagonal array, where h and k are either zero or positive integers. To further elaborate, the formation of an icosahedron can be visualized using a uniform hexagonal lattice, where each hexagon can be decomposed into six equilateral triangles. The hexagonal lattice is constructed with h and k axes crossing at 60° angles. A pentagon can be constructed in 3D by removing one triangle from the hexagon (Figs. 2 and 3). By arbitrarily choosing one of the 5-fold vertices as the origin (h, k = 0, 0),
the positions h and k of the neighboring 5-fold vertex closest to the origin gives the T-number of that icosahedron. For example, T = 1 represents an icosahedron with the closest 5-fold vertex positioned at either h = 1 and k = 0 or at h = 0 and k = 1. T = 3, however, represents an icosahedron with the closest 5-fold vertex positioned at h = 1 and k = 1 (Fig. 3). Thus, a simple icosahedron with 60 identical subunits would have T = 1 symmetry. The structures of tobacco bushy stunt virus (TBSV) (Harrison et al., 1978) and southern bean mosaic virus (SBMV) (Abad-Zapatero et al., 1980), the first near-atomic resolution structures of icosahedral viruses, had T = 3 quasi-symmetries consistent with the predictions of Caspar and Klug (subsection “Non-enveloped Icosahedral RNA Viruses”). Although the amino acid sequence of the capsid proteins was rather different, both structures were very much alike with three identical protein subunits constituting the icosahedral asymmetric unit. The first animal virus structure, that of human common cold virus strain 14 (HRV14) (Erickson et al., 1983; Rossmann et al., 1985), mimicked the structures of TBSV and SBMV plant viruses. However, in HRV14, the three independent subunits in the icosahedral asymmetric unit were different proteins, referred to as viral proteins VP1, VP2, and VP3 (subsection “Non-enveloped Icosahedral RNA Viruses”).

Based on the equation T = h² + hk + k², only certain T-numbers are allowed. Therefore, the icosahedra can be further divided into three classes (Caspar and Klug, 1962; Prasad and Schmid, 2012). These classes are calculated using the formula T = P², where P is the largest common divisor between h and k. The first class, P = 1 (T = 1, 4, 9, 16, 25, 36 etc.), where h is greater than or equal to 1 and k = 0, the lattice lines run parallel to the edges of the triangular icosahedral facet. In the second class, P = 3 (T = 3, 12, 27, 48, 75, 108 etc.), the icosahedra have T numbers along the line that bisects the h and k axes with h = k. The third class, called the skew class, includes icosahedra with other allowed T numbers (7, 13, 19, 21, 28, 31 etc.) with the possibility of having enantiomorphic configurations. In T = 7 icosaheda, the asymmetric unit is made of 7 proteins giving a total of 420 capsid proteins per virus capsid. However, depending on the values of h and k, the capsid can either have a configuration of dextro (d) for h = 1, k = 2 or laevo (l) for h = 2, k = 1. For example, polyomavirus has a non-enveloped capsid with T = 7d pseudo-symmetry because it is only composed of pentamers and lacks the expected hexamers in its capsid whereas Rotavirus, a non-enveloped, icosahedral virion with a double capsid structure has a T = 13l symmetry for the outer capsid and T = 1 for the inner capsid. Another form of icosahedron, called the prolate icosahedron where the icosahedron is stretched along one axis, is found in viruses like T-even bacteriophages (Fokine et al., 2004; Rao and Black, 2010). Here, the icosahedron has a central cylindrical body made of 10 triangular faces with two caps at the bottom and the top. The prolate icosahedra are characterized by the combination of a T number and an elongation number called Q according to the formula n = 30(T + Q), where T is the triangulation number of the central body, Q is any positive integer and n refers to the number of subunits. The caps follow the triangulation T, whereas the main cylindrical body follows the Q number. When T = Q, the icosahedra becomes isometric, when Q > T, the icosahedron is referred to as prolate and when Q < T, the icosahedra is referred to as oblate.

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**Fig. 3** The construction of an icosahedron from arrays of hexagons. T = 3 structure of turnip yellow mosaic virus, where (A) shows the icosahedral capsid assembly in TYMV (PDB ID: 2FZ2) and (B) the planar sheet of twenty equivalent triangles superposed on the hexagonal lattice. The T = 3 arrangement with h = 1 and k = 1 is indicated by a yellow arrow. (C) Schematic showing a planar sheet of 20 triangles which can then be folded up to form a closed icosahedron. Figures (A) and (B) were prepared using the Viper data base.
Helical Symmetry

Helical symmetry is another prevalent symmetry occurring in the capsids of numerous plant viruses, tails of bacteriophages, and flexible capsids of negative-stranded RNA genome containing viruses. Helical organization of capsids in plant viruses has the advantage that it imposes no limitations on the size of the packed RNA genome compared to icosahedral viruses which are limited by their internal volume. Helical symmetry combines rotation and translation, giving rise to a screw axis where the pitch of the helix (P) is defined as the axis rise. A helical virus structure is described by the location of subunits with respect to the helix axis, the number of subunits per helix turn (n) and the axial rise per subunit (h).

In TMV, one of the earliest and best characterized helical virus structures, the genomic RNA of 6400 bases is encapsidated by 2140 identical viral capsid proteins giving rise to a rod-like virion, 3000 Å long and 180 Å in diameter with a central hole of 20 Å in radius (Fig. 4). After the preliminary structural studies of TMV using X-ray fiber diffraction to 2.9 Å (Namba et al., 1989a), a more recent cryoEM structure has been determined to a resolution of 3.3 Å (Ge and Zhou, 2011). In TMV the capsid protein subunits form a right-handed helix with a pitch of 23 Å and 16 and 1/3rd subunits in each turn. Each capsid subunit interacts with three nucleotides. The structure of the TMV genome follows the symmetry of the capsid providing details on capsid-RNA interactions and the assembly of the virus.

Methods for Studying Virus Structures

The principal techniques used to study intact virus structures are X-ray crystallography, X-ray fiber diffraction, cryo-electron microscopy (cryoEM) and cryo-electron tomography (cryo-ET). To date, according to the statistics on the VIPERdb database (Carrillo-Tripp et al., 2009) about 404 virus structures have been determined using X-ray crystallography and 345 structures have been determined using cryoEM. Major accomplishments in understanding the structure of viruses were closely associated with methodological, technological and computational advances in X-ray crystallography and cryoEM and developments in molecular virology techniques.

X-ray crystallography has been the most successful technique for determining the structure of individual icosahedral viruses since the 1970s. The first of the structures determined using crystallography were of icosahedral plant viruses (Abad-Zapatero et al., 1980;
high resolution models of the virus capsid were derived by exploiting cryoEM and X-ray crystallography-based hybrid techniques. This required common to obtain cryoEM electron potential maps of virus samples to a resolution better than 3 Å. Recent development of direct electron detectors as well as other technological advances in cryoEM has made it easier and more.

Champness (1974; Champness et al., 1984) followed by more complex animal and human viruses. X-ray fiber diffraction was used to study viruses with helical symmetry. A key issue in X-ray crystallography is the solution of the phase problem, which in the case of viruses is helped enormously by the presence of non-crystallographic symmetry (NCS). Several crystallographic milestones that have also assisted in solving many significant virus structures have been (1) the use of high intensity synchrotron radiation for automated collection of diffraction images (Rossmann, 1979, 1984, 1999; Rossmann et al., 1979); (2) determining the orientation of virions in the unit cell (Rossmann and Blow, 1962; Tong and Rossmann, 1990); (3) using non-crystallographic symmetry based methods to solve the phase problem (Akerwall et al., 1972; Rossmann and Blow, 1963) and the use of NCS averaging for improving the phases (Buehner et al., 1974; Champness et al., 1976). If, for instance, isomorphous replacement had been used to obtain a 3.5 Å structure, then averaging that structure will increase the quality of the phases of the current map which can then be used to extend the resolution of the map (usually about one reciprocal lattice point in all directions). The slightly improved map can be used again for averaging and so forth.

The above real space considerations can also be expressed in reciprocal space and by doing so give insight into why phasing can be very successful by gradual resolution increase at each stage by less than one reciprocal lattice point given knowledge of the NCS. This had been under considerable dispute until the structure of HRV14 in 1985 (Arnold and Rossmann, 1986; Rossmann et al., 1985) when the initial 5 Å resolution isomorphous replacement phases were extended to a beautiful 3 Å resolution map using NCS (Rossmann et al., 1992a,b).

Electron microscopy of negatively stained virus samples was among the first techniques used to recognize virus shapes and sizes (Brenner and Horne, 1959; Horne et al., 1959). Subsequent development of computational techniques to reconstruct the 3D structure of viruses from 2D projection images (Crowther et al., 1970) and sample freezing techniques (Adrian et al., 1984; Dubochet et al., 1988) to preserve the native structure, revolutionized intact virus structure determination using cryoEM (Haas and Rossmann, 1970). For the next three decades, many virus structures were determined to a resolution of 25 Å. However, the more recent development of direct electron detectors as well as other technological advances in cryoEM has made it easier and more common to obtain cryoEM electron potential maps of virus samples to a resolution better than 3 Å. Prior to this, pseudo-atomic models of the virus capsid were derived by exploiting cryoEM and X-ray crystallography-based hybrid techniques. This required high resolution structures of viral components, obtained by using X-ray crystallography to be fitted into the low resolution cryoEM capsid map of the whole virus (Kuhn et al., 2002; Zhang et al., 2002). CryoEM has the advantage that it can be used where crystal growth is limiting especially with heterogeneous samples of enveloped viruses and viruses in complex with antibodies, receptors or anti-viral compounds. For example, crystals of West Nile virus diffracted to about 16 Å resolution (Kaufmann et al., 2010), whereas cryoEM gave a map to a resolution of 10 Å (Zhang et al., 2013). In the case of pleomorphic viruses such as Rubella (Battisti et al., 2012; Mangala Prasad et al., 2013; 2017a), cryo-electron tomography (cryo-ET) has been the method of choice to study the 3D structure. Tomography uses a series of collected images, where the microscope stage has been tilted to give a series of 2D projections that can be combined to form a 3D image (Frank, 1992; Lucic et al., 2005; Subramaniam et al., 2007). However, the resolution limitation for this technique (in the absence of averaging) has been about 20 Å because of the need to reduce the dose of each image to be able to collect a 3D data set before the sample has been destroyed by repeated exposure to electrons.

Structural Folds of Capsid Proteins

One of the characteristic features of viral capsid proteins lies in the folded topology of the capsid monomers, allowing them to assemble into large, symmetrical and geometrically sophisticated architectures (Chapman and Liljas, 2003; Cheng and Brooks III, 2013). The canonical jelly-roll β-barrel, as a core structural motif, is the most common capsid fold occurring in virus shells of varying sizes (Abad-Zapatero et al., 1980; Chelvanayagam et al., 1992; Harrison et al., 1978; Johnson and Chiu, 2000). Other common protein folds occurring in viruses are the Greek key β-barrel with six strands (Choi et al., 1991a; Tong et al., 1992,1993), the helix bundle (Bloomer et al., 1978; Conway et al., 1997; Gamble et al., 1997), the immunoglobulin-like fold (Rey et al., 1995) and the HK97 fold (Suhansky and Teschke, 2015). The structurally unique capsid proteins are also functionally unique in terms of their folding topology because of the evolutionary constraints imposed by protein-protein interactions and are most likely a consequence of geometric requirements of the building blocks to form cage-like assemblies. Here, we describe the commonly occurring virus capsid folds.

Jelly-Roll β-Barrel

Both TBSV and SBMV, the first determined icosahedral virus structures, were similar with respect to their capsid structures and organization. It was then obvious that the two virus capsid structures with no apparent sequence similarity must have diverged from a common ancestral fold, later termed as a ‘jelly-roll’ fold by Jane S. Richardson in 1981 (Richardson, 1981), reflecting its resemblance to a Swiss roll cake. The jelly-roll fold is also referred to as a wedge shaped fold, an RNA virus capsid β-barrel or a β-sandwich (Cheng and Brooks, 2013).

A jelly-roll fold consists of eight antiparallel β-strands that are named from B to I along the polypeptide sequence (Fig. 5). The two four-stranded sheets that form the opposite sides of a β-barrel consist of strands BIDG and CHEF, folded such that strand B packs opposite strand C, I opposite H, etc. The end strands almost form a closed barrel structure, however there is no hydrogen-bonding between the end strands of facing sheets. A single tight turn between strands E and F leads to the pairing of strands D and G, C and H, and B and I, which then roll up, Swiss cake style. Therefore, the BI and DG pairs combine to form the BIDG sheet, and their NCS (Rossmann et al., 1984).
Fig. 5  Virus capsid structure folds. (A) and (B) show the jelly-roll β-barrel fold in satellite tobacco mosaic virus (STMV) (PDB ID: 4OQ9). The β-sheets BIDG are colored green and the β-sheets CHEF are colored golden yellow. (B) The T = 1 icosahedral assembly of the subunits to form the STMV virus capsid. The icosahedral asymmetric unit is drawn as a black triangle while the 2-, 3- and 5-fold symmetry axes are drawn in red. (C) Immunoglobulin fold in the domain-III (blue) of ZIKV envelope protein E (PDB ID: 6C08). Domain I, domain II and the membrane associated helices are colored red, yellow, and gray, respectively. (D) The serine protease-like fold in the capsid of Sindbis virus (PDB ID: 1SVP) and (E) the four-helix bundle in TMV (PDB ID: 1VTM) are shown in rainbow colors (blue to red) from N- to C-terminus. (F) The HK97 coat protein in the immature state (PDB ID: 3E8K). The common secondary structural elements observed in HK97 fold are labeled.
the CH and EF pairs combine to form the CHEF sheet. However, variations to the common core might occur in different viruses. There may be one or two additional strands like the β-strand A in TBSV outside the capsid’s common fold. The length of the strands might also differ depending on triangulation number and capsid architecture. The largest variations occur in the size of the loops connecting the β-strands. In some viruses, these external loops come together to form antigenic sites (Rossmann et al., 1985) and in others the loops account for the increase in capsid size (McKenna et al., 1992a,b). Most of the jelly-roll folds are about 180 amino acids and can be as big as 580 amino acids in parvoviruses (Tsao et al., 1991).

A large number of viruses build their capsids using either single or double jelly-roll fold capsid subunits. Double jelly-roll proteins have presumably evolved from single jelly-roll proteins by gene duplication. Single jelly-roll capsid folds are found primarily in many icosahedral RNA and DNA viruses. However, the majority are positive-sense single-stranded RNA viruses and the only dsDNA virus with single jelly-roll folds are the members of Papillomaviridae and Polyomaviridae. The double jelly-roll capsid proteins are found exclusively in dsDNA viruses spanning a large capsid size range. Most members of the double jelly-roll fold group are icosahedral, such as Poxviridae and Ascoviridae have oval or brick-shaped mature virions.

The Immunoglobulin-like Fold

The immunoglobulin fold is found in a vast variety of proteins of different functions such as antibodies, cellular adhesion molecules, the receptor tyrosine kinase and many other molecules. The canonical immunoglobulin fold consists of a 7-stranded antiparallel β-sheet sandwich within which there is extensive hydrogen bonding. One example in a virus is one of the domains of a flavivirus envelope glycoprotein (E) (Rey et al., 1995). The flavivirus protein E consists of three domains: The N-terminal domain-I, which is an antiparallel β-barrel quite different from the jelly-roll fold, a β-sheet rich domain-II and the C-terminal domain-III with an immunoglobulin fold. In flavivirus E protein domain-III, the three- (β-sheet CFG) and four- (β-sheet ABED) member sheets face each other and are commonly linked by a disulfide bond. The domain topology resembles a Greek key (Fig. 5).

The Serine Protease Fold

The serine protease fold is observed in chymotrypsin, many other proteases and viral proteins. It is again an anti-parallel Greek key β-barrel with six-strands. Examples of viruses with this domain as core protein can be found in the capsid proteins of the members of Togaviridae such as Chikungunya virus (CHIKV) (Sun et al., 2013b), Sindbis virus (Choi et al., 1991a), Ross River virus, Rubella (Mangala Prasad et al., 2013) and Eastern equine encephalitis virus (Hasan et al., 2018b) (Fig. 5). In Sindbis virus, the capsid polypeptide is folded into two similar ‘Greek key’ β-barrel domains with the substrate site situated between the domains. The capsid structure of Sindbis virus identified Ser215, His141 and Asp163 as the essential catalytic triad as in other serine proteases (Choi et al., 1991a).

The Four-helix Bundle

The four-helix bundle is a common motif in which antiparallel α-helices are packed side by side with a close packed hydrophobic core (Fig. 5). It is also found in a wide range of proteins with diverse functions like ferritin, various cytochromes, cytokines, growth hormones etc. Viral examples include the coat protein of TMV, capsid of hepatitis B and the C-terminal domain of the HIV-1 capsid (Bloomer et al., 1978; Conway et al., 1997; Gamble et al., 1997). Each capsid subunit of TMV consists of 158 amino acids assembled into a four-helix bundle, which are joined by a loop proximal to the virion axis. Each coat protein interacts with 3 RNA nucleotides (Namba et al., 1989b).

The HK97 Fold

HK97 capsid fold is termed after the first capsid protein structure solved by X-ray crystallography of bacteriophage HK97 (Helgstrand et al., 2003; Suhannovsky and Teschke, 2015; Wikoff et al., 2000). Most of the viral capsids using the HK97-fold are found in bacteriophages. Additionally, this fold is also found in the capsids of Herpes Simplex virus type 1, murine cytomegalo-virus, pseudorabies virus and the rhesus monkey rhadinovirus. Viruses using the HK97-fold share unexpected similarities beyond the fold of their coat proteins including the architecture of their virions, an internal scaffolding protein-mediated assembly, active dsDNA packaging, and capsid maturation events (Suhannovsky and Teschke, 2015).

The HK-97 fold is characterized by having an N-arm (sometimes z-helical rich), an E-loop with variable lengths that is a two-stranded anti-parallel β-sheet (β2 and β3), a peripheral P-domain with a long helix called the “spine helix” and an unusually long β-sheet; and an A-domain (axial domain) with a central β-sheet referred to as the β-hinge surrounded by short helices and loops (Fig. 5).
Capsid Assembly

Non-Enveloped Icosahedral RNA Viruses

The first icosahedral RNA plant virus structures, TBSV (Harrison et al., 1978; Winkler et al., 1977) and SBMV (Abad-Zapatero et al., 1981), had similar capsid folds (jelly-roll fold) and organization with three independent subunits (A, B, and C) in the icosahedral asymmetric unit arranged around a quasi-3-fold axis (as predicted by Caspar and Klug). These structures clearly established that virus capsids with more than 60 subunits were organized based on a triangulated icosahedral lattice as described by Caspar and Klug (Caspar and Klug, 1962). The capsid is composed of 180 capsid subunits and exhibited a \( T = 3 \) capsid organization with rings of pentons and hexons. The assembly of these viruses occurred via AB and CC dimers. However, the AB dimer had a disordered amino-terminal \( \beta \)-strand compared to the one of the CC dimer, which was ordered, giving the two types of dimers a different curvature on the virus surface (Fig. 6).

The first animal virus structure was that of HRV14 (Arnold et al., 1987; Erickson et al., 1983), which mimicked the structures of TBSV and SBMV plant viruses. HRV14 belongs to the family of picornaviruses, which are small icosahedral animal viruses of about 300 Å diameter with a positive-sense eight thousand base RNA genome. However, while in the plant viruses the three protein
subunits in the icosahedral asymmetric unit were identical, the icosahedral asymmetric unit of HRV14 was composed of three independent subunits of the viral proteins: VP1, VP2, and VP3 (and VP4 which is internal in the mature virus) (Fig. 6). The capsid is composed of 60 copies of VP1, VP2, and VP3 (and internal VP4) arranged with pseudo $T = 3$ icosahedral symmetry. Nevertheless, each subunit had the same fold referred to as the "jelly-roll" fold. Shortly after the determination of the HRV14 structure and its extraordinary evolutionary implication, Hogle was able to determine the structure of poliovirus serotype 1, using the same phase extension technique as had been pioneered for HRV14 (Hogle et al., 1985). This further confirmed the remarkable similarity of simple plant and animal RNA viruses.

The structure determination of HRV14 was not only a significant advancement in the method used for the analysis of X-ray diffraction data but was also informative of the functions required by the virus for infecting a cell and for replication. Viral escape mutations for a series of neutralizing monoclonal antibodies were all found to be on the virus surface and thus were likely the site of antibody binding (Sherry et al., 1986; Sherry and Rueckert, 1985). The surface of HRV14 had a deep depression ("canyon") around each pentameric vertex (Rossmann et al., 1985). None of the MAb binding sites were in the canyon. This, therefore, suggested that the canyon might be the site where cellular receptor molecules could bind without experiencing any inhibiting viral mutations (Rossmann et al., 1985; Rossmann and Palmenberg, 1988). Thus, by hiding the receptor binding site in the canyon (which would require a long thin receptor molecule), the virus could evade the host’s immune system while still being able to bind to the same receptor molecule (Rossmann, 1989). Subsequently it was established that most HRVs use intercellular adhesion molecule 1 (ICAM1) as receptor (Greve et al., 1989; Staunton et al., 1989). In one of the earlier applications of cryoEM, Rossmann and colleagues verified that indeed ICAM1 bound into the canyon as predicted (Olson et al., 1993).

Another significant discovery related to the structure of HRV14 was that a series of anti-rhino-viral compounds bound into a hydrophobic pocket within VP1 (Smith et al., 1986). This pocket was below and close to the canyon. It was shown that binding one of the antiviral compounds being developed by the Sterling Winthrop Company caused an enlargement of the pocket and a change of structure in the canyon, explaining why the binding of these compounds inhibited cellular attachment. Furthermore, it was found that this pocket was occupied by a small stabilizing lipid in HRVs and poliovirus. Thus, binding of the infectious virus to ICAM1 would displace the stabilizing "pocket factor" and prepare the virus for the release of its genome into the cytoplasm of the cell containing the ICAM1 (Filman et al., 1989; Oliveira et al., 1993; Rossmann, 1994; Smyth et al., 1995). These results stimulated extensive work by the ViroPharma Company to create an inhibitor that was effective over a wide range of HRV serotypes, resulting in the development of "Pleconaril" (Hayden et al., 2003; Xiao et al., 2011). Although this compound was successful in clinical trials it put women on contraceptive hormones at risk of conception. Thus, the drug never reached the pharmaceutical market.

### Enveloped Icosahedral RNA Viruses

Many major viral pathogens such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), influenza viruses, herpes simplex virus (HSV), coronaviruses as well as various poxviruses, alphaviruses and flaviviruses contain host-derived lipid membranes studded with glycoproteins. Among these, members of Herpesviridae (ex: HBV) and Herpesviridae have an internal icosahedral capsid shell surrounded by an amorphous tegument and a lipid envelope embedded with glycoproteins. Flaviviruses and alphaviruses, however, have an icosahedral outer shell and an internal lipid membrane separating the outer glycoprotein shell from the genome (Mukhopadhyay et al., 2005).

Alphaviruses have a positive-sense ssRNA genome that is about 11 kb in length and codes for 9 proteins (Kuhn, 2007). The 3’ end of the genome is transcribed for subsequent translation into a polyprotein precursor containing the three structural proteins PE2 (the precursor of E3 and E2), E1, and the capsid protein. The structures of a number of alphaviruses have been determined using cryoEM to resolutions better than 10 Å, including the structure of Chikungunya virus-like particles to 5.3 Å resolution (Sun et al., 2013b), Venezuelan equine encephalitis virus to 4.6 Å resolution (Zhang et al., 2011a), Eastern equine encephalitis virus to 4.4 Å resolution (Hasan et al., 2018b) and Sindbis virus to 3.5 Å resolution (Chen et al., 2018). Alphaviruses have an external diameter of about 700 Å and are icosahedral with quasi-$T = 4$ symmetry (Fuller, 1987; von Bonsdorff and Harrison, 1975; von Bonsdorff and Harrison, 1978). They have a nucleocapsid core that is completely surrounded by a lipid envelope, derived from a host membrane, into which is embedded an icosahedral array of glycoproteins. A single virus particle contains 240 copies each of the E1 and E2 glycoproteins in the mature virus (in addition 240 copies of E3 in the immature virus) that form 20 “i3” spikes situated on the icosahedral 3-fold axes and 60 quasi-3-fold “q3” spikes at general positions (Cheng et al., 1995; Zhang et al., 2002). There are 240 copies of the capsid protein on the internal cytoplasmic side of the viral lipid membrane, arranged as 12 pentamers about the 5-fold vertices and 30 hexamers about the icosahedral 2-fold vertices, consistent with the $T = 4$ symmetry of the glycoprotein on the external side of the membrane. The capsid protein of alphaviruses is similar to trypsin-like proteases and participates in the assembly process (Choi et al., 1991b) (Fig. 7).

The E1 glycoprotein of CHIKV forms three β-barrel domains (I, II, and III). The CHIKV E2 glycoprotein is arranged into three immunoglobulin-like domains A, B and C (Gilbert et al., 2002; Li et al., 2010; Voss et al., 2010). Domain A contains the receptor binding site, domain B is at the distal end of each spike protecting the fusion loop on DII of E1 and domain C is situated closest to the viral membrane. The E1 glycoprotein contains a hydrophobic fusion loop that is responsible for membrane fusion with an endosomal membrane when initiating infection. The E2 protein can bind to cellular receptors and protects the E1 fusion loop at neutral pH. During the initial stages of infection, the host cellular receptor is recognized by the surface glycoprotein E2.
Once the virus has been internalized, the low pH of the endosome causes the virion to undergo an irreversible conformational change resulting in the disassociation of E2 from E1 and the formation of E1 trimers. Upon E2 disassociation, the fusion loop then binds to and fuses with the endosomal membrane. Finally, the viral genome is released into the host cytoplasm, where replication of new viral particles can begin (Kielian and Rey, 2006; Lescar et al., 2001; Lu et al., 1999; Marsh and Helenius, 1989).

The presence of the lipid causes some heterogeneity, making it often difficult to crystallize these viruses (Harrison et al., 1992). Instead the structure of many of these larger, lipid containing viruses have been determined by using cryoEM to provide a lower resolution structure of the virus and higher resolution X-ray crystal structures of the components. The latter could then be fitted into the EM structure of the whole virus. A particularly good early example was the determination of the mature dengue virus structure (Kuhn et al., 2002). However, more recently it has been possible to determine the structure of lipid containing viruses to about 3 Å resolution with the development of direct electron counting detectors for electron microscopes and general

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**Fig. 7**  $T = 4$ icosahedral capsid architecture in immature Chikungunya virus (CHIKV).  
(A) Electron potential map of immature CHIKV viewed down an icosahedral 2-fold axis. The icosahedral asymmetric unit is drawn as a black triangle and 2-, 3- and 5-fold symmetry elements are represented as oval, triangle and pentagon, respectively. The four subunits of icosahedral asymmetric units are labeled from 1 to 4 in white text.  
(B) Internal capsid shell of immature CHIKV.  
(C) Central cross-section of (A) with the represented components colored as labeled.  
(D) The fitting of E1-E2-E3 and C coordinates into the region outlined in (C).  
Figure adapted from Yap, M.L., Klose, T., Urakami, A., et al., 2017. Structural studies of Chikungunya virus maturation. Proceedings of the National Academy of Sciences of the United States of America 114, 13703–13707.
improvements to microscopes and techniques. For instance, the structure of mature Zika virus (ZIKV) was determined to about 3 Å resolution using cryoEM (Sevvana et al., 2018), thus also confirming the earlier hybrid techniques.

Similar to other flaviviruses, ZIKV is an enveloped, single-stranded, positive-sense RNA virus (Hasan et al., 2018a; Sevvana et al., 2018; Sirohi et al., 2016). The 11 kb RNA genome is translated into a long polyprotein. It is post-translationally cleaved by host and viral proteases into three structural proteins: pre-membrane (prM), envelope (E), and the capsid (C) as well as seven nonstructural proteins (Sirohi and Kuhn, 2017). The E and prM proteins form a protective capsid around the genome. The E-protein mediates the assembly of virus, virus entry and fusion with host membrane and contains putative receptor binding sites. It forms a complex with prM in the endoplasmic reticulum shortly after its synthesis. The E-prM complex is arranged as 60 trimeric spikes ($T = 3$) on the surface of the immature virus (Mangala Prasad et al., 2017b). The pr domain of prM prevents premature fusion to membranes during virus production. Immature virions undergo pH-induced conformational changes to form the mature virion. Mature virus particles are formed after the pr domain is cleaved from the prM protein by furin in the trans-Golgi network. The icosahedral mature ZIKV consists of 180 copies of E and M proteins arranged in 60 asymmetric units (quasi-$T = 3$). Each icosahedral asymmetric unit consists of three E-M oligomers. Three parallel dimers from adjacent asymmetric units form a “raft”. Therefore, there are 30 rafts arranged in a herringbone pattern on the surface of the mature virus (Fig. 8). Flavivirus membrane fusion is initiated by E-protein interaction with a potential receptor molecule and the endocytosis of the virus. This is followed by low pH-induced conformational changes in the endosome, exposure of the fusion loop and its interaction with the endosomal membrane. The E-proteins then rearrange into trimeric structures (fusogenic trimer) leading to the fusion of viral and endosomal membrane and subsequent release of viral genome into the host cytosol.

Though, most of the flavivirus structures have icosahedral symmetry, a recent study has shown that immature Kunjin and Zika viruses are not accurately icosahedral (Therkelsen et al., 2019). Their nucleocapsid core approaches close to one side of the external glycoprotein shell, indicating the presence of a special, unique region that may have been created while interacting with glycoproteins to promote budding from the endoplasmic reticulum (ER) in infected cells and may be required for genome packaging in flaviviruses. Furthermore, on the opposite face on the exterior glycoprotein surface, there is a departure from exact icosahedral symmetry that may reflect the final step in budding. Similar asymmetric properties may have been overlooked in the usual averaging procedures assumed in the study of most essentially icosahedral viruses.

Small Icosahedral ssDNA Viruses

Two examples of small icosahedral ssDNA viruses are φX174 (McKenna et al., 1992b), which belongs to the Microviridae family, with a single-stranded circular DNA and paroviruses with a linear genome. Paroviruses have a 5000 nucleotide ssDNA genome, and typically have a $T = 1$ icosahedral capsid that is about 260 Å in diameter (Fig. 9). Most mammalian paroviruses have 60 subunits of VP1, VP2, and VP3 in the viral capsid. Many parovirus structures such as human B19 virus-like particles, canine parovirus, feline parovirus, porcine VLP’s and murine VLP’s were determined to near atomic resolution using X-ray crystallography (Agbandje et al., 1993; Kaufmann et al., 2005; 2008; 2004; Simpson et al., 2000; Simpson et al., 2002; Tsao et al., 1991;
Adeno-associated parvoviruses (AAV-2) are of particular interest because of their potential use in gene therapies (DiMattia et al., 2012; Hueffer et al., 2003b; Lerch et al., 2012; Padron et al., 2005; Walters et al., 2004; Xie et al., 2002). The capsid proteins of parvoviruses have large insertions on their jelly-roll structure. These account for most of the interactions between neighboring subunits as well as receptor interactions (Chapman and Rossmann, 1996; Tsao et al., 1992). For example, canine and murine parvoviruses can use transferrin (TfR) receptor for binding and infection (Hueffer et al., 2003a). However, the TfR molecule only binds to a few of the 60 available binding sites on the virus thereby inducing asymmetry in the icosahedral virus (Hafenstein et al., 2007).

Bacteriophage FX174 is a tailless phage that contains a single-stranded, closed and circular DNA with about 5000 nucleotides (Dokland et al., 1999; 1998; McKenna et al., 1992b). The T = 1 mature icosahedral virus consists of four structural proteins, J, F, G, and H. The capsid of 260 Å diameter is formed by 60 F subunits. The 12 pentameric spikes formed by 60 copies of the G protein rise about 30 Å above the capsid surface and both F and G subunits have jelly-roll folds. There are 12 copies of the H protein, which form a tail tube similar to the tail tube of the common tailed dsDNA phage during infection for the insertion of DNA into the host cell (Sun et al., 2013a). Structural studies shed light on the assembly of FX174, which proceeds via virally encoded scaffolding proteins (B and D) both of which are discarded when assembly is complete (Fig. 10).

**Large Icosahedral dsDNA Viruses**

Nucleocytoplasmic large dsDNA viruses (NCDLVs) infecting a wide variety of organisms’ span members of the Asfaviridae, Ascoviridae, Marseilleviridae, Mimiviridae, Pandoraviridae, Phycodnaviridae, Iridoviridae, and Poxviridae. Most NCDLVs are roughly icosahedral in shape. However, members of Poxviruses, Ascoviruses, and Pandoraviruses are pleomorphic. CryoEM reconstructions of several icosahedral NCDLVs infer similar capsid organization with large T numbers (Andreani et al., 2017; Cherrier et al., 2009; Klose et al., 2016; Okamoto et al., 2018; Reteno et al., 2015; Xiao et al., 2017; Yan et al., 2005; 2006; 2009; Zhang, et al., 2011b). An inner membrane that separates the genome from the external capsid is observed in most NCDLVs except Faustoviruses, which have an internal capsid.

The capsomers in icosahedral NCDLVs are arranged in trisymmetrons (20 triangular arrays) and pentasymmetrons (12 pentagonal arrays) (Fig. 11) (Klose and Rossmann, 2014). The capsid is assembled from pseudo-hexagonal, closely arranged trimeric capsomers where each subunit is formed by a double jelly-roll fold protein. Each capsomere has a diameter of about 75–85 Å and a height of 75 Å. Because of their size range, the capsomeres can have two orientations related by a six-fold rotation. First, the capsomeres within each trisymmetron can have the same orientation or, second, they can be rotated by 60° relative to neighboring capsomeres which creates cleavage planes between neighboring trisymmetrons (Wrigley, 1969). The five-fold vertices are formed by a pentameric single jelly-roll fold protein. The pentasymmetrons have a constant size and consist of 30 trimeric double jelly-roll capsomeres and one pentameric single jelly-roll protein. It was postulated that this constant size is ideal to relieve the strain induced around the five-fold axis for icosahedral NCDLVs with large diameters.

Another characteristic feature of NCDLVs is the presence of minor capsid proteins found underneath the major capsid proteins towards the inside of the virus capsid as can be seen in high resolution structures of faustovirus (Klose et al., 2016), Chilo iridescent virus (CIV) and Paramecium bursaria chlorella virus 1 (PBCV-1) (Zhang et al., 2011b). The minor capsid proteins...
support the outer capsid shell, connect the neighboring trisymmetrons and/or connect the outer capsid to the inner membrane. Some NCLDVs like mimivirus (Xiao et al., 2005,2009; Klose et al., 2010) and PBCV-1 also have a unique vertex where the membrane becomes separated from the capsid. Many NCDLVs have decoration proteins and surface fibers attached to the viral capsid. Mimivirus is covered by a dense layer of surface fibers except near the unique vertex called the stargate, which is tightly associated with the adjacent capsomers.

One of the most studied NCDLVs is PBCV-1, where a recent near-atomic resolution structure identified several of the minor capsid proteins and cast light on the capsid assembly pathway (Fang et al., 2019). PBCV-1 has a 330 kbp genome that encodes 416 proteins, of which 149 different proteins were identified in the mature virion. The nucleocapsid is surrounded by a membrane, which in turn is surrounded by a roughly icosahedral capsid shell giving it an overall size of 190 nm. It also has a unique vertex with a spike structure required for host entry. The outer shell consists of the MCP (Vp54) and the penton protein P1. Each tri- and pentasymmetron contains 66 (MCP) and 31 (30 MCP + 1 P1) capsid proteins respectively, arranged in an icosahedral lattice with a triangulation number of $T = 169d$. The high resolution cryoEM structure led to the identification of the penton protein, P1, and 13 minor capsid proteins (named P2 through P14). The minor capsid proteins form a hexagonal network below the outer capsid shell. Most of the minor capsid proteins are primarily located at the interface between neighboring capsomeres. The size of the viral capsid is determined by a tape-measure protein, P2, and the zip protein P11 which plays a crucial role in cementing neighboring symmetrons (Fig. 11).

Faustovirus, on the other hand uses two concentric capsid shells to protect its genome (Klose et al., 2016). Consequently, two distinct types of particles were observed in cryoEM images of purified virus. The larger icosahedral particle constituting the outer and inner capsids measured about 2600 Å in diameter, whereas the smaller particles with only an inner capsid measured between 1600–1900 Å. The capsomere on the outer capsid is arranged with a triangulation number of $T = 277$ ($h = 7$ and $k = 12$).
icosahedral asymmetric unit is composed of one penton protein and 276 MCPs. Each tri- and pentasymmetron of the outer capsid shell contains 360 \((\text{MCP})\) and 31 \((30 \text{MCP} + 1 \text{P1})\) proteins, respectively \((\text{Fig. 11})\).

Tailed Bacteriophages

The structure of dsDNA bacteriophages is unique in that most of them have tails attached to a special pentameric vertex of their capsids \((\text{Fokine and Rossmann, 2014})\). The unique vertex in most bacteriophages is occupied by a dodecameric portal protein, which forms a channel for genome packaging during virion assembly and subsequent exit during infection. Tailed bacteriophages belong to the order \text{Caudovirales}, which are further divided into three families: \text{Myoviridae} \((\text{i.e., T4 and } \phi92 \text{ have long, straight contractile tails})\), \text{Siphoviridae} \((\text{i.e., lambda and HK97} \) have long flexible non-contractile tails, and \text{Podoviridae} \((\text{i.e., T7} \) have short non-contractile tails).

Capsid heads of tailed bacteriophages come in a large variety of sizes and range in diameter from about 400–1700 Å with either icosahedral or prolate heads. The capsid contains linear double-stranded DNA packaged to a high density of about 500 g/l and exerts an internal pressure of tens of atmospheres on the capsid walls. The capsid precursor, called the procapsid or prohead, is assembled at the portal vertex by copolymerization of the MCP and scaffolding proteins. The scaffolding protein regulates the correct geometry of the capsid. The prohead consists of the portal protein, the outer MCP, the internal scaffolding protein and in some cases other minor capsid proteins. The scaffolding protein and the internal core are degraded in the procapsids of many phages by head maturation proteases, therefore making space for DNA packaging. The genome is packaged by a DNA translocation motor via the portal vertex and is ATP-dependent. DNA packaging is followed by large structural rearrangements, an increase in internal volume and attachment of other proteins (for example decoration proteins) to form the mature capsid. After DNA packaging, the DNA translocation motor is removed and the capsid assembly is completed by sealing the portal gate, the

\text{Fig. 11}  \text{Capsid organization in NCLDV}s. (A) Topology plot and (B) cartoon representation of the double jelly-roll fold of the MCP in faustovirus. The secondary structure elements are labeled. (C) \(T = 277\) and \(T = 64\) capsid organizations of faustovirus external and internal capsid shells respectively. (D) CryoEM map of the overall structure of the PBCV-1 capsid. The boundaries of neighboring trisymmetrons and pentasymmetrons are outlined in black. (E) The cryoEM density of the minor capsid proteins and the penton proteins after subtracting the density for MCP. Each protein is depicted in a different color as indicated to the right of the image. The \(h\) and \(k\) indices are labeled on the icosahedron in panels \(C\) and \(D\). The radial color gradient scale bar is in Å. Figures adapted from: (A), (B), and (C) Klose, T., Reteno, D.G., Benamar, S., et al. 2016. Structure of faustovirus, a large dsDNA virus. Proceedings of the National Academy of Sciences of the United States of America 113, 6206–6211. (D) and (E) Fang, Q.L., Zhu, D.J., Agarkova, I., et al. 2019. Near-atomic structure of a giant virus. Nature Communications 10, 388.
attachment of head completion proteins and the assembly of the tail. Bacteriophage tails are designed to recognize host cells, penetrate the cell envelope and deliver the genome. Their length varies from $B_100 \text{ Å}$ in *Podoviridae* to $B_8000 \text{ Å}$ in some *Siphoviridae*. Most tails of the members of *Myoviridae* and *Siphoviridae* consist of a tail tip complex for host cell recognition, a tail tube, which makes a conduit for genomic DNA with a contractile sheath around it in some phages and the terminator proteins near the tail-head interface (Fig. 12).

One of the most studied bacteriophage structures is of T4 bacteriophage, which infects *E. coli*. The dsDNA genome encodes about 40 structural proteins. The mature virus consists of an 1150 Å-long and 850 Å-wide prolate head encapsidating the genomic DNA. The head is attached to a 1000 Å-long and 210 Å-diameter cylindrical contractile tail and is terminated with a 460 Å-diameter baseplate (Fokine *et al.*, 2004; Kostyuchenko *et al.*, 2003) (Fig. 12). The baseplate is associated with six 1450 Å-long fibers (Aksyuk *et al.*, 2009; Kostyuchenko *et al.*, 2005). The distal ends of the long tail fibers (made of gp37) recognize the host cell (Bartual *et al.*, 2010) and then transfer the signal to the baseplate to unfold the short tail fibers (gp12) (Thomassen *et al.*, 2003; van Raaij *et al.*, 2001). The short tail fibers subsequently bind to the lipopolysaccharides on the surface of the *E. coli* host. The unfolding of the short tail fibers results in large conformational changes of the dome-shaped baseplate to a star-shape as the tail sheath (gp18) starts to contract (Aksyuk *et al.*, 2009; Aksyuk and Rossmann, 2011; Crowther *et al.*, 1977; Kostyuchenko *et al.*, 2003; Leiman *et al.*, 2004). The tip of the tail tube (gp5) punctures the cell, enters the periplasm and digests the cell wall (Brownie *et al.*, 2012; Kanamaru *et al.*, 2002). The genome is then ejected into the host’s cytoplasm by the tail tube. In phage T4 the pentameric protein forming 11 of the 12 vertices (gp24) has an HK97 fold, which has a high sequence similarity to T4 MCP (gp23) (Jiang *et al.*, 2003; Morais *et al.*, 2005).

**Conclusion**

Viruses are complex organisms that build extraordinary structures out of a limited number of building blocks. It is remarkable that the principles established by Watson and Crick (Watson and Crick, 1953b) and expanded by Caspar and Klug (Caspar and Klug,
1962) in the 1960s have been validated and still hold true for most virus structures discovered to date. Furthermore, it is worth noting that the large variety of virus structures that have been described in the scientific literature so far utilize a fairly small number of protein folds, most of which have been covered in this review. Improvements in sequencing technologies and less stringent isolation techniques have led to an immense increase in the number of viruses that have been described in the literature and it will be interesting to see if this increase eventually correlates with the discovery of new folds or new assemblies. It is already becoming clear that the future of structural virology will be dominated by cryoEM. Most of the recently determined virus structures were solved using this technique and one would expect this number to continue increasing. X-Ray crystallography will still play an important role, but will likely be confined to solving the structures of smaller components of virions or assemblies. In addition, cryoEM and cryoET will allow us to look into deviations of the strict symmetry requirements imposed onto most viral structures to date and help us to learn more about assembly pathways and unique structural components that might otherwise be overlooked. This might show us that viruses are not that perfect after all and may lead to important biological discoveries and potentially new therapeutic approaches.

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