VIRUSES' STRUCTURAL CHANGES UPON INFECTION OF A HOST CELL

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Foreword

This Bachelor thesis has been written to fulfill the requirements of the Biology Bachelor degree. Both my Bachelor's project and thesis have been completed in the Electron Microscopy group of the Groningen Biomolecular Sciences and Biotechnology Institute (GGB) of the University of Groningen.

First, I would like to thank prof. dr. E.J. Boekema for allowing me to complete my project and thesis in his research group and for the guidance he has given me. I would also like to thank dr. G.T. Oostergetel for his supervision.

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1 INTRODUCTION

Viruses are biological entities that are considered to exist somewhere in between life-forms and chemicals. Viruses cannot reproduce by themselves, they need to make use of the reproductive machinery of cells. Viruses are cellular parasites that can infect nearly all organisms. As viruses are able to infect such a wide range of organisms, they have developed a wide structural variety.
The structural details of the viruses determine which organisms viruses are able to infect. In order for successful infection to take place, the virus must be able to attach itself to and penetrate a target cell. During this process structural changes on the virus's surface take place.

In this paper I will focus on the structural changes that take place on the viral surface from the moment of attachment of the virus to the host cell until penetration of the host cell. As the structure of viruses is so varied, I have chosen to focus on just three types of viruses: a bacteriophage, a non-enveloped virus and an enveloped virus.

After a short introduction of the discovery and characteristics of viruses, the objective of this paper will be presented. Next, the methods used to study viruses' structures will be covered briefly. In continuation, different methods that viruses use to attach to and enter cells will be described. Then, with the knowledge obtained about the attachment and penetration mechanisms of the selected viruses, I will discuss how the viruses' overall structure is affected during attachment and penetration. Afterwards I will account for the differences in viral attachment and penetration strategies. I will complete this paper with my conclusions.

2 VIRUSES, AN INTRODUCTION

Viruses were first detected around the end of the 19th century, long before it was actually possible to visualize them. In 1883 Adolf Mayer, a German scientist, was working on what today is known as the tobacco mosaic virus when he discovered that the disease caused by the tobacco mosaic virus could be transmitted from plant to plant by rubbing the sap from sick plants to healthy ones. However, he wrongly concluded that the disease was caused by very small bacteria.
Decades later, after conducting further experiments, the Dutch microbiologist Martinus Beijerinck concluded that the disease caused by the tobacco mosaic virus was caused by a much smaller and simpler entity than a bacterium. Because of his discoveries and conclusions, Beijerinck is credited to be the first scientist to raise the concept of a virus. [5]

Another important step in the study of viruses was made by Wendell Stanley, an American scientist who, in 1935, discovered that the tobacco mosaic virus could be crystalized. The discovery that this virus could aggregate into regular crystals, unlike the simplest cells, raised questions about the nature of these entities. [3]

Now that much more is known about viruses, scientists agree that viruses are not living organisms. Instead, they are considered to be a part of an undefined area between life-forms and chemicals. The smallest viruses are in the range of 20nm, whereas the biggest known virus is in the range of several hundred nanometers. They basically consist of genetic material enclosed in a protein coat and sometimes a membranous envelope. The genetic material present in viruses can be either in the form of double stranded DNA, single stranded DNA, double stranded RNA or single stranded RNA. [14] The protein shell in which the viral genetic information is kept in known as a capsid. Most animal viruses have either an icosahedral or a helical capsid. The capsid is in some cases enveloped in a lipid bilayer containing viral spikes proteins. [22]

3 OBJECTIVE

In this study I will focus on virus-host cell interaction during the first stages of viral infection, specifically on the question: what are the structural changes that take place on the viral surface from the moment of attachment of the virus to the host cell until penetration of the host cell? Because of the large variety in viral structures resulting in an extensive range of structural changes during infection of the host cell, I have chosen to study three model viruses.
To make the study structured, I will address the following five sub-questions: What are the basics of viral infection?; What methods can be used to study virus-host cell interactions?; What methods do viruses use to attach to and penetrate host cells?; How do virus-host cell attachments and penetration methods influence the overall structure of the virus?; How can one account for the differences in viral attachment and entry strategies?

4 THE BASICS OF VIRAL INFECTION

Viruses can only reproduce in host cells. They lack both the essential metabolic enzymes and the gear to make proteins, forcing them to rely on the reproductive and protein synthesizing equipment of the host cell. The first step that needs to take place for successful viral infection is the recognition of the target cell and the attachment of the virus to the target cell's surface. Viruses recognize their host cells by a so called 'lock and key' mechanism. The proteins on the surface of the virus must 'fit'

specific receptor molecules on the outside of the host cell. The molecules used by the virus during virus-host cell interaction can be divided into different categories and vary considerably. Some of these molecules are only used to physically attach the virus particles to the cell (these interactions can be relatively non-specific), whereas other molecules can activate endocytic pathways in the host cell enabling the virus to enter the cell and/or transmit the signals necessary for the induction of conformational changes leading to membrane fusion and penetration. [21,22,16]
Once the virus has made its way into the host cell, it sets in motion the manufacturing of many new viral entities. The nature of the virus's genetic information determines in which way the host cell is induced to create new viral particles. The overall picture, however, is quite similar. The virus's genetic information is delivered to either the nucleus or the cytoplasm of the host cell so that viral replication can start. In short, viruses do not only use the host cell for replicating their genetic material, but viruses also use host cells to produce the necessary coating and accessory protein needed to build a new, complete and functional virus. After this entire process the new viruses have to exit the host cell. Depending on the structure of both the virus and the host cell, this can be done by either budding or breakage of the host cell.

5 WHAT METHODS CAN BE USED TO STUDY VIRUS-HOST CELL INTERACTIONS

Virus-host cell interactions are an interesting yet difficult field to study, especially when focusing on the structural changes that take place on the virus's surface during infection. The entry of a virus into the host cell is a frequently visualized phase of the virus's life cycle. [12]

When studying virus host cell interactions it is useful to determine the viruses' structure during various stages. The structure of a virus prior to infection can be determined by x-ray crystallography. By crystalizing the viral particles and exposing them to x-rays, an atomic model of the virus's surface can be created, allowing the identification of both the arrangement and the components of the viral surface. However, this technique does not provide any information about which proteins in the virus's surface interact with the host cell surface. For the identification of these interacting proteins other techniques must be used, these techniques will not be further discussed.

As infection starts, the virus's surface changes. As these interactions often involve non-symmetrical components and the partial attachment of the virus to the host cell, x-ray crystallography cannot be used anymore. For the visualization of the virus as it interacts with the host cell, electron cryo-tomography (cryo-ET) is a good option. For example, by preparing a sample and fixating it in the middle of the infection process, one can obtain a snapshot of the virus as it attaches itself to the host cell. [10]

In electron tomography 3D information of organelles and macromolecules that are not easily crystalized is collected on the level of 2 to 5nm resolution. [1] It is the highest resolution 3D imaging technique available at the moment [19]. In electron tomography a series of two-dimensional (2D) images of the same specimen is recorded. Each one of these images is recorded from a different angle, providing a series of pictures in each of which a different view of the specimen is visible. This is done by recording images at constant intervals while tilting the sample over a range of up to 70°. By combining these images a 3D reconstruction of the specimen can be constructed. [10]

Out of the many embedding techniques available, a very appropriate one for studying viruses-host cell interactions is cryo. In cryo-ET cells are rapidly frozen: they are embedded in an amorphous ice layer. The advantage of doing this is that it is likely to be a reliable representation of the living state of the sample. It does, however, as all techniques also come with drawbacks, such as low contrast [19]. Several studies about the structure of viruses before, during and after infection have been conducted with the help of cryo-ET, helping to provide a more detailed model of what happens during the initial steps of viral infection.

6 WHAT METHODS DO VIRUSES USE TO ATTACH TO AND PENETRATE HOST CELLS

Structure-wise viruses are very varied, so I will focus on three viruses, each of which represent a category of viruses: Epsilon15 (bacteriophage), reoviruses (non-enveloped virus) and HIV-1 (enveloped virus). Bacteriophages specialize in infecting bacteria. Non-enveloped (lacking an envelope) and enveloped viruses (having an envelope, consisting of a lipid bilayer with its corresponding proteins) affect eukaryotes.

6.1 Bacteriophages (Epsilon15)

Bacteriophages, the most abundant biological entities known on Earth, are viruses that specialize in infecting bacteria. [11] Bacteriophages have adapted their machinery to be able to penetrate the outer layers of bacterial cells. The structure of bacteriophages differs from other viruses as they are composed of a head and a tail. The tail is used to recognize, attach and penetrate a host cell. This machinery is so efficient that in many cases one single bacteriophage is enough to infect one bacterial cell. [17,2]

Studies conducted by W. Jiangin et al. 2006 and J. T. Chang et al. 2010 on the bacteriophage epsilon 15 (ε15), a bacteriophage that infects *Salmonella*, has provided visualization of the steps that take place upon infection. This was done by using single particle electron cryo-microscopy (cryo-EM), electron cryo-tomography (cryo-ET) and 3D subvolume averaging. ε15's head has an icosahedral shape and one of the vertices is occupied by six tailspikes, which are connected to a central tail hub. The tailspikes are made out of gp20 and consist of two domains separated by a bend. The tail hub is located at the center of the tailspikes and has a six-fold symmetry. It does not only anchor the tailspikes, but it also serves as a lid for the portal opening. The portal is located just below the tail hub (Figure 2).
The structures that were visualized provide insight into the different stages of infection. Infection starts with the attachment of ε15 onto the surface of *Salmonella*. Then a tunnel is formed through which the genetic information of the virus can enter the host cell (Figure 1). When looking at the cryo-ET pictures, a few structures can be recognized, namely the virus's capsid, its DNA, the core, the portal, the tailspike and the hub (Figure 2). Of these structures, mainly the portal, tailspikes and the hub play a role when it comes to the initial virus-host cell interaction. The infection process starts with the random collision of the viral particle with the future host cell, thus the tailspikes have a chance to recognize the bacterial lipopolysaccharides (LPS). The tailspikes are also responsible for cleaving the O-side chain of LPS, aiding the digestion of this layer and thus allowing the particle to move closer to the bacterial membrane. As the outer membrane is reached another cell receptor is activated, resulting in the conformational change of the viruses' tail vertex. Then the portal and tail

hub form a channel that eventually manages to pass the peptidoglycan layer and the inner membrane of the bacteria, allowing the entry of the capsids content into the bacteria. Once the bacteriophage's content has made its way into the bacteria, the channel closes so that the content of the cytoplasm does not start leaking and the empty capsid is left attached to the bacterial surface. [9,13]

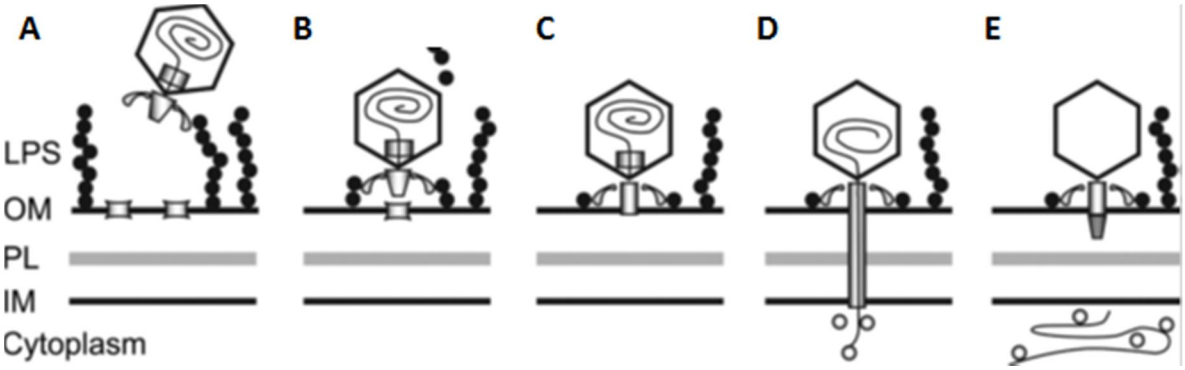

Figure 1: Schematic model of bacteriophage infection. (A) Tailspikes of the bacteriophage come into contact with bacterial lipopolysaccharides (LPS). (B) LPS is digested allowing the virus to reach the outer cell membrane (OM). (C) Tail hub opens. (D) A tube is formed across the periplasmic space, allowing the bacteriophage to inject its contents into the bacteria. (E) After emptying its contents, the tube is sealed, staying behind empty on the cell surface. PL stands for peptidoglycan layer and IM for inner membrane. [9]

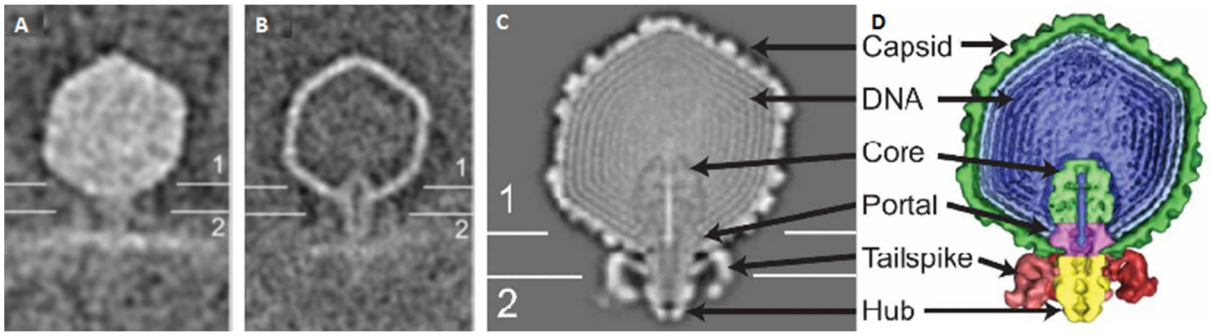

Figure 2: Electron cryo-tomography slice of epsilon 15. (A) Full virus particle attached to a host cell, showing structural details. (B) Empty virus particle attached to host cell. (C) Slice through the center of the particle showing the different parts. (D) Same as C but showing the particles in color [9]

6.2 Non-enveloped viruses (Reoviruses)

Non-enveloped viruses lack a lipid bilayer. Their outer layer is a protein capsid. Non-enveloped

viruses infect eukaryotes. One of the interesting characteristics some of these viruses, such as reoviruses, have is that they keep their viral membrane "penetrating protein" in a dormant, yet stable state. It is in a dormant state because it is bound to a so called "protection protein". This is done to protect the "penetrating proteins" from harsh environments. An essential step during viral infection is the transition of these "penetrating proteins" from the dormant to the active state. If this transition is successful it results in the so called 'infectious subvirion particle' (ISVP). [8]

An interesting group of viruses to study are reoviruses, a group of small animal and plant RNA viruses, whose RNA is located in a two concentric icosahedral capsid. Of these viruses' ISVP state, a structure with an estimated resolution of 3.3Å has been determined by electron cryo-microscopy (Figure 3). [25] The transition to the ISVP conformation is triggered by the exposure of the viral particle to proteases; this can for example take place in the intestinal lumen or in endocytic vacuoles. Once in the active state, the "penetrating proteins" enable the virus to enter the cell by either forming a small pore or by locally disturbing the lipid bilayer. [8] The capsid is mainly made out of six proteins: λ1, λ2, σ2, μ1, σ3 and σ1. Three of these proteins contribute greatly to the structure of the capsid: λ1, λ2 and σ2. Out of these λ1 is the major core protein, forming the icosahedral shell. σ2 helps stabilizing λ1. λ2 forms tower-like structures through which the virus's genetic material can exit. The other three proteins (μ1, σ3 and σ1) are exposed and contribute to the entry of the virus. μ1 is the actual viral penetrating agent. σ3 serves as a protector protein for μ1 (they form $\mu1_3$- $\sigma3_3$ complexes) and σ1 mediates the attachment of the virus onto cellular receptors. The first step in the reovirus entry procedure is the attachment of σ1 onto the cell surface receptors. This step can be performed in both the dormant and the ISVP state. [25,18] It has been observed that when reoviruses are in the ISVP state they can increase membrane permeability. Having seen this only when the virus is in the ISVP state suggests that one of the core proteins that is lost or modified during the transition from the dormant state to the ISVP state (μ1 or σ3) is responsible for the increase in membrane permeability. Further assays show that μ1 is indeed the main reovirus's penetrating protein. [7] On the ISVP surface there are about 600 copies of μ1. As IVPS particles are highly hydrophilic they have the tendency to remain dispersed when in solution. Because of this and because of the finding that when ISVP is incubated in physiological temperatures it can undergo structural transformations, K. Chandran et al. (2002) suggested that ISVP particles can be present in two conformations: ISVP and ISVP* (Figure 4 and Figure 5). In this model ISVP does not mediate membrane penetration, ISVP* does. In the ISVP* state μ1 is present in a hydrophobic membrane seeking conformation. [6]

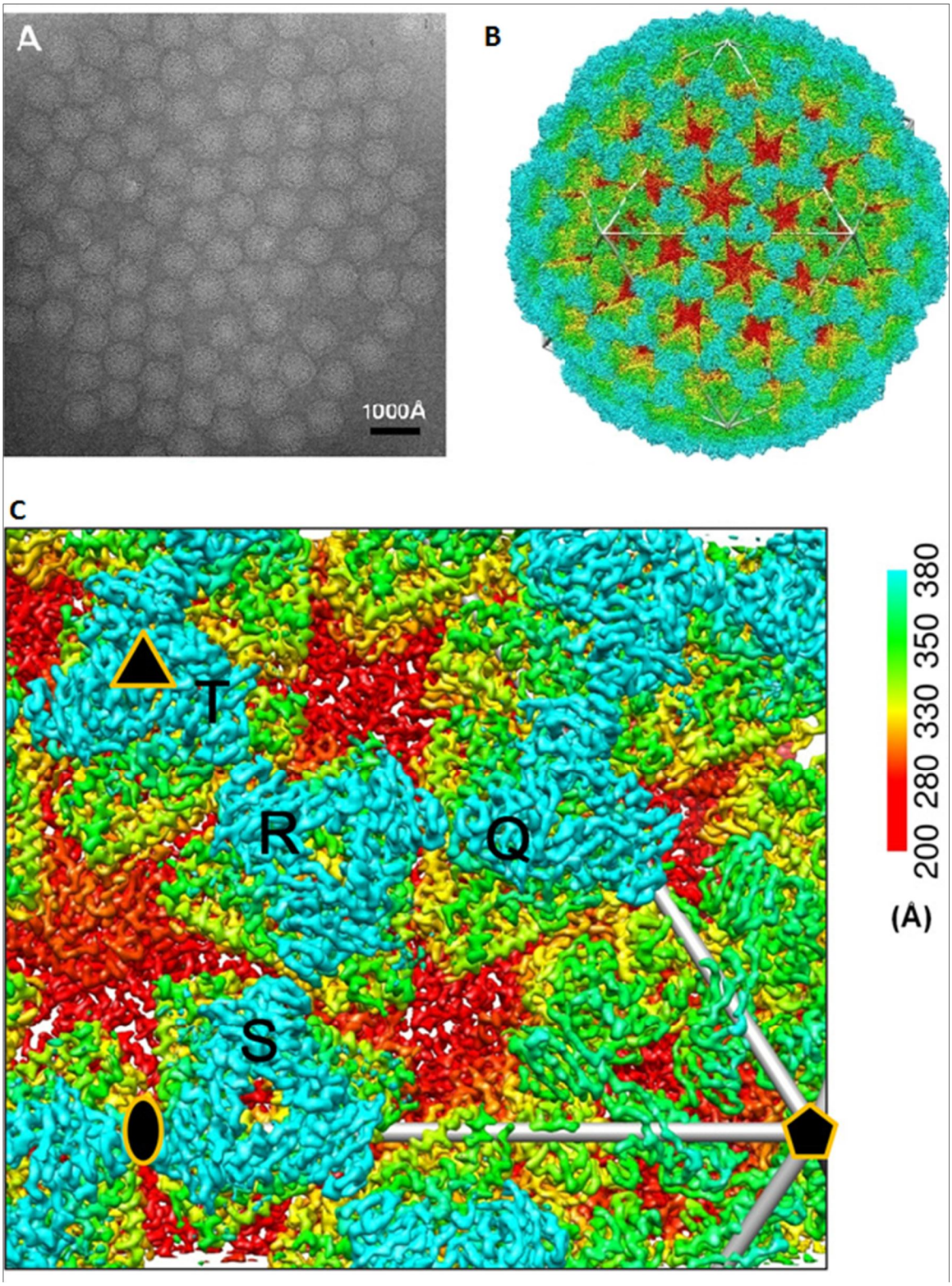

Figure 3: Cryo-electron microscope projection of an reovirus Infectiuos Subvirons particle (Aquareovirus). (A) Particles suspended in vitrous ice at a 1.2µm underfocus (no carbon support film). (B) Density map. Colors were chosen according to radius. (C) Enlarged view of B. T, R, S and Q show the trimers. The elipse, triangle and pentagon show the two-, three- and five- fold axis. [25]

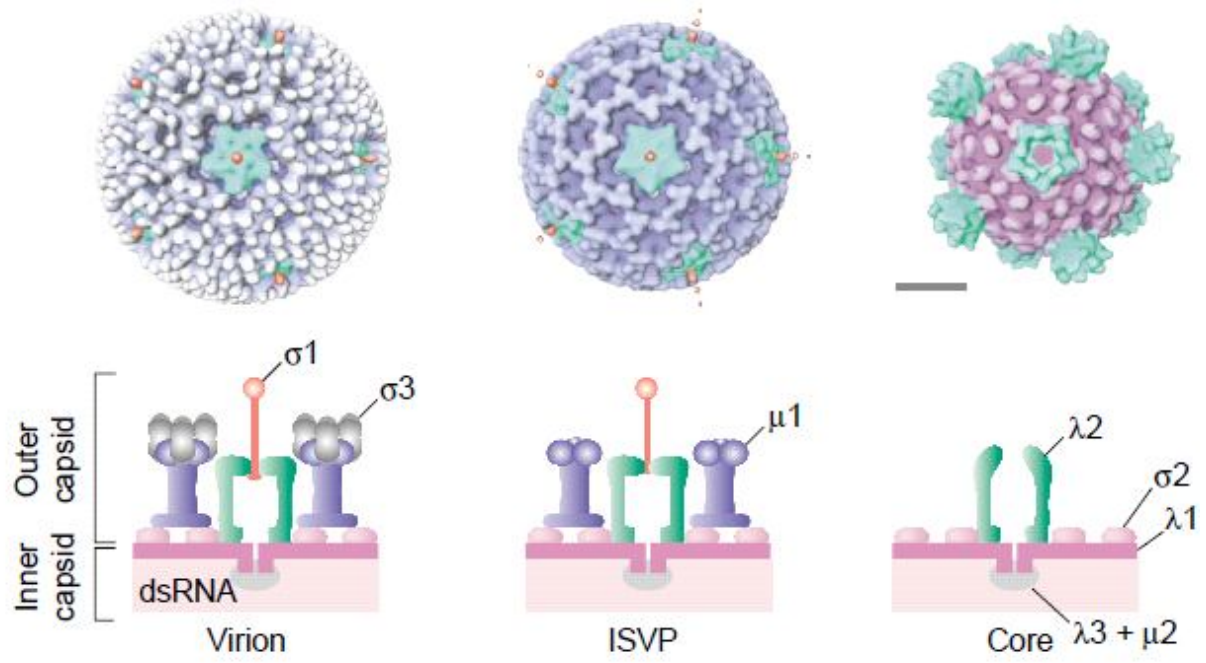

Figure 4: Non-enveloped virus capsid organization. (scale bar is 20nm). [7]

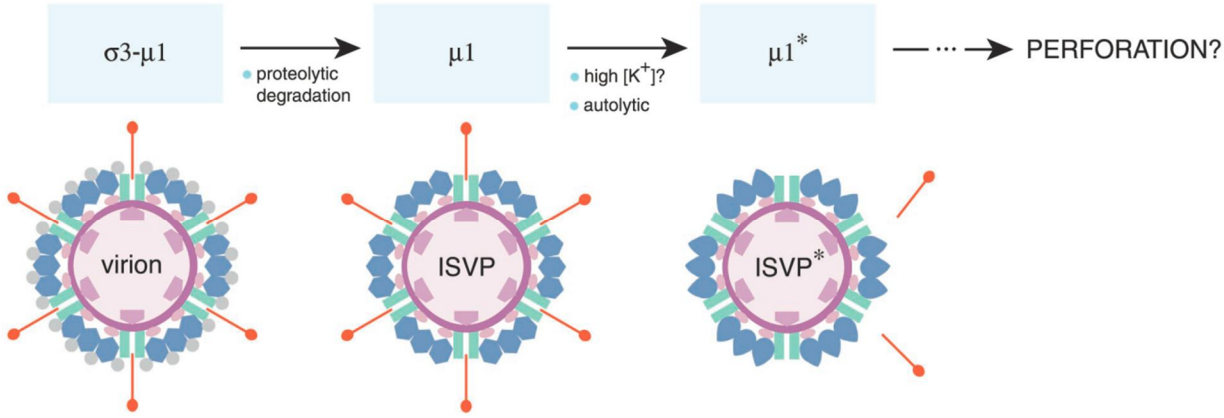

Figure 5: Schematic drawing of the transformation from viron to ISVP to ISVP*. [6]

6.3 Enveloped viruses (HIV-1)

The outer layer of enveloped viruses consists of a lipid bilayer, this enables the viruses to enter host cells by means of membrane fusion. Membrane fusion can take place under two different conditions: at neutral pH at the surface of the cell or at low pH in an endosomal compartment. This process is aided by 'viral fusion proteins'. These fusion proteins fall into three different structural categories.

Although these proteins vary in their mode of activation, they are thought to undergo comparable conformational changes as fusion proceeds. Fusion proteins are located on the surface of the viral particle and are present in an active state, meaning that they are always ready for interacting with host cells. In order for fusion to take place, the protein must be triggered by environmental factors. Some of these triggers can be low pH, receptor binding and a combination of both low pH and receptor binding.

After triggering, proteins on the virus's surface take on a pre-hairpin intermediate conformation, which is able to insert itself into the target membrane. So far this membrane inserted intermediate has only been observed as a homotrimer. The following steps are thought to include the involvement

of several other prehairpins, resulting in the formation of a stable and compact 'rod-like trimer-of-hairpins' (Figure 6, Figure 8). This last conformation is thought to be very stable, thus making it able to overcome the huge energy barrier required to merge the membranes. For all the three classes of hairpins the final conformation is made out of a trimer. In short, during these steps the bilayers are brought closer and closer to each other, resulting in hemifusion. Hemifusion is the step during which small parts of the outer layer of the bilayers are fused together, while the inner layers are still intact. After hemifusion a small opening is formed. This small opening becomes larger, forming a pore that allows the entry of the virus's genetic material into the host cell (Figure 6). [21,22,16] So far electron cryo-microscopy has been used to visualize this process in HIV-1 (Figure 7). However, the pre-hairpin intermediate has not been visualized yet.[23]

In HIV-1, a well-studied example of an enveloped virus, viral entry is mediated in great lengths by two glycoproteins: gp41 and gp120 (Figure 8 A-B). Gp41 and gp120 are present on the surface of HIV-1 as a 'mushroom shaped' trimer of heterodimers, forming a spike (Figure 8 C-D). This trimeric structure is anchored to the membrane by gp41. The number of gp120/gp41 trimers varies from viral particle to viral particle, ranging from around 10 to 100 trimers per virion. Viral entry is initiated by the binding of gp120 with CD4, a protein on the target cell's surface. Gp120 has two domains, the "inner domain" and the "outer domain", (Figure 8 A). The "outer domain" is responsible for the interactions with CD4.These high affinity interactions enable the virus to attach itself to the cell surface and trigger structural changes that eventually lead to the insertion of the ectodomain of gp41 into the target cell's membrane, making gp41 the so called fusion peptide. Gp41 has three domains: the ectodomain, which is able to insert itself into the membrane due to its hydrophobic amino acid terminal, the transmembrane domain and the cytoplasmic domain. [20, 24, 4, 15]
In short, after the triggering of the gp120-gp41 complex, conformational changes take place that enable the insertion of gp41 into the membrane leading to membrane fusion and entrance of HIV-1 into the cell (Figure 9 and Figure 10).

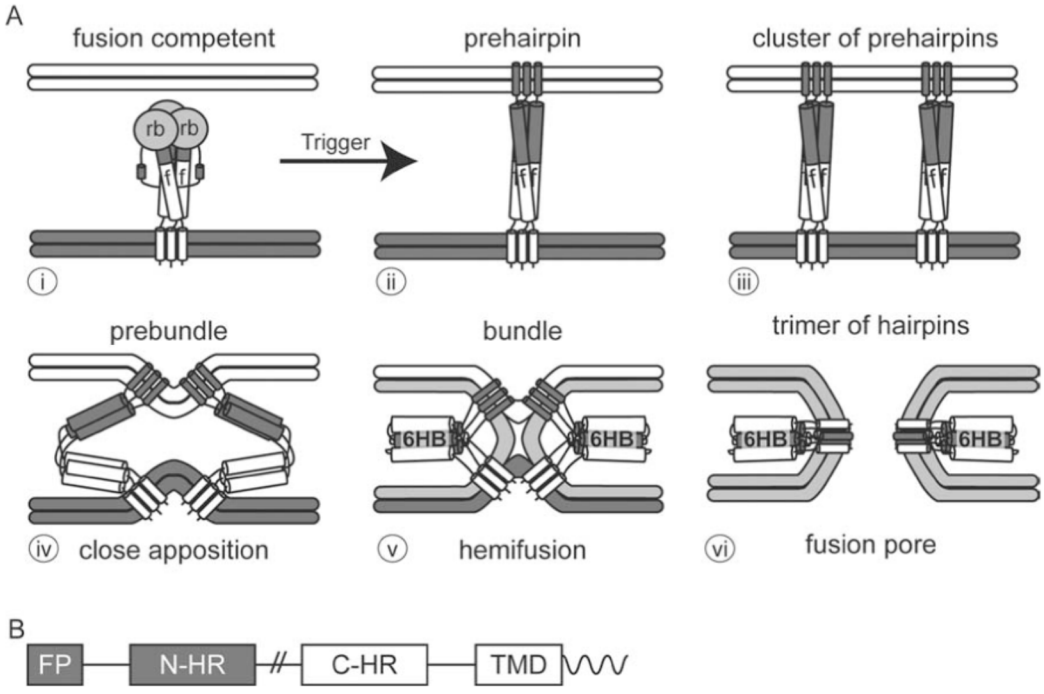

Figure 6: The common trimer-of-hairpins pathway of type I membrane fusion. [21]

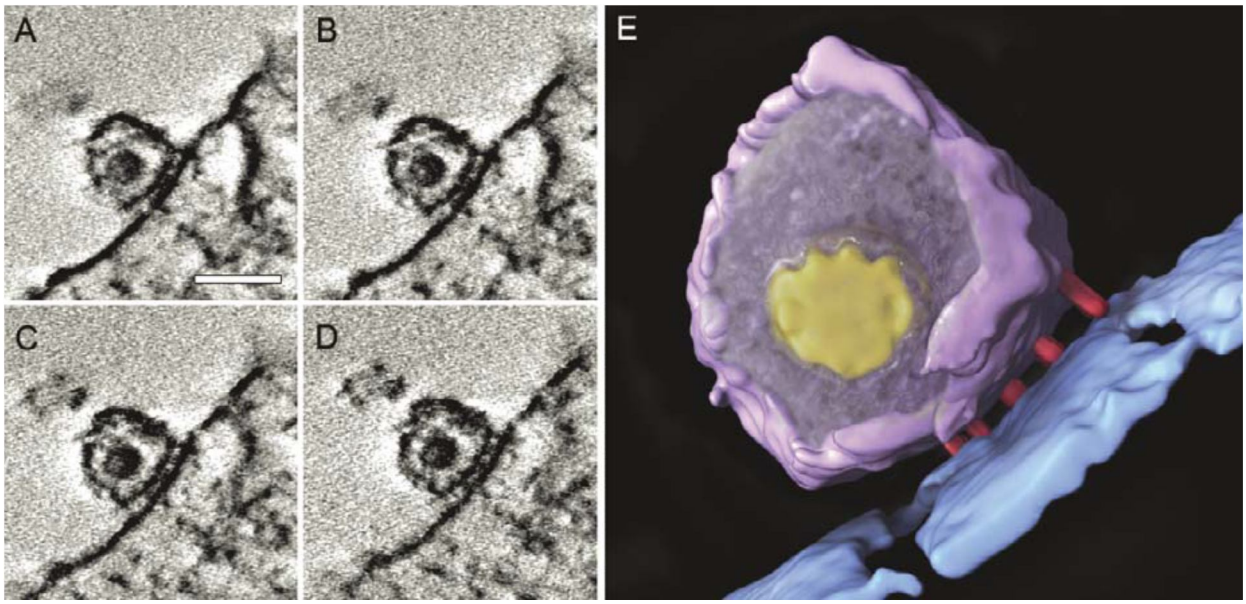

Figure 7: (A-D) tomogram of contact between HIV-1 and T cells, each representing different depths (scale bar is 100 nm). (E) 3D representation of the contact represented in a, b, c and d. [20]

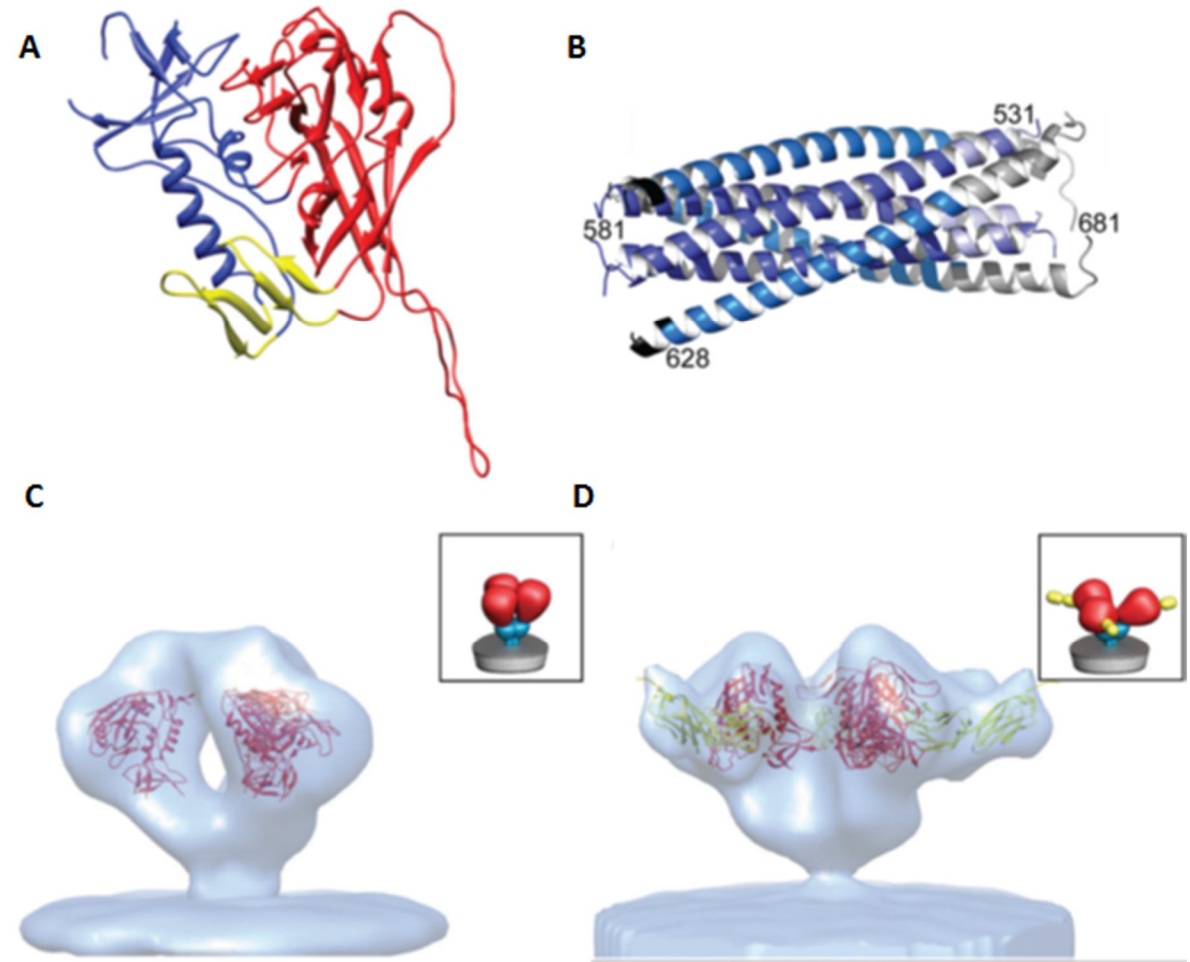

Figure 8: (A) Gp120 as determined by x-ray crystallography. In blue is the inner domain, in red the outer domain and in yellow the bridging domain. (B) Gp41 ectodomain in 6HB conformation as determined by x-ray crystallography. (C-D) Cryo-electron tomography projection of the HIV-1 gp120-gp41 trimer in the closed and open state. Gp120 is shown in red and Gp41 in cyan. (D) Is the open CD4 triggered conformation. CD4 is showed in yellow.[24]

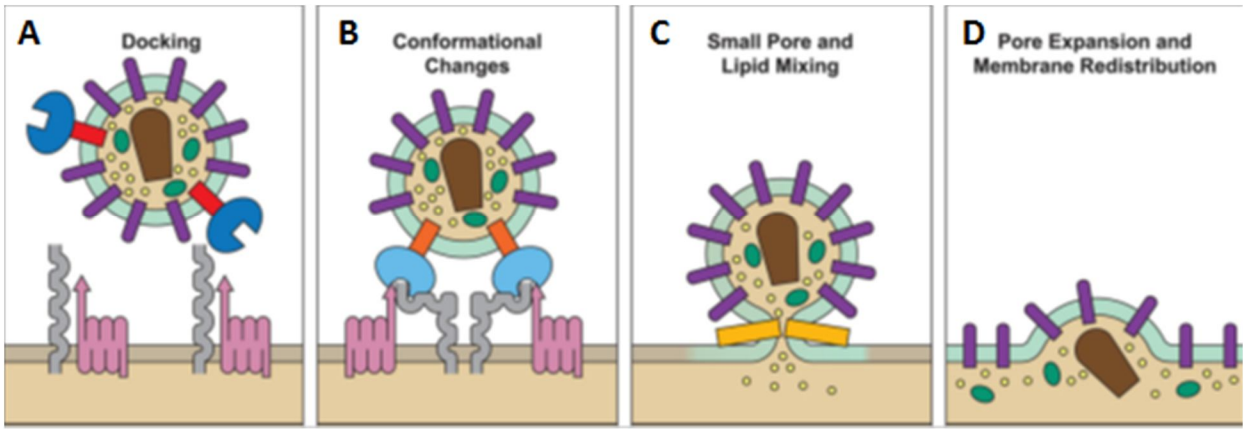

Figure 9: HIV fusion process. Gp120 is dark blue, gp41 is red, accessory proteins (HLA-DR) are in purple, CD4 is grey and CR is pink. The attachment of the virus via gp120-CD4 interactions induces both conformational changes and aggregations of proteins involved in the attachment. This leads to membrane fusion and the entrance of HIV-1 into its host cell. [24]

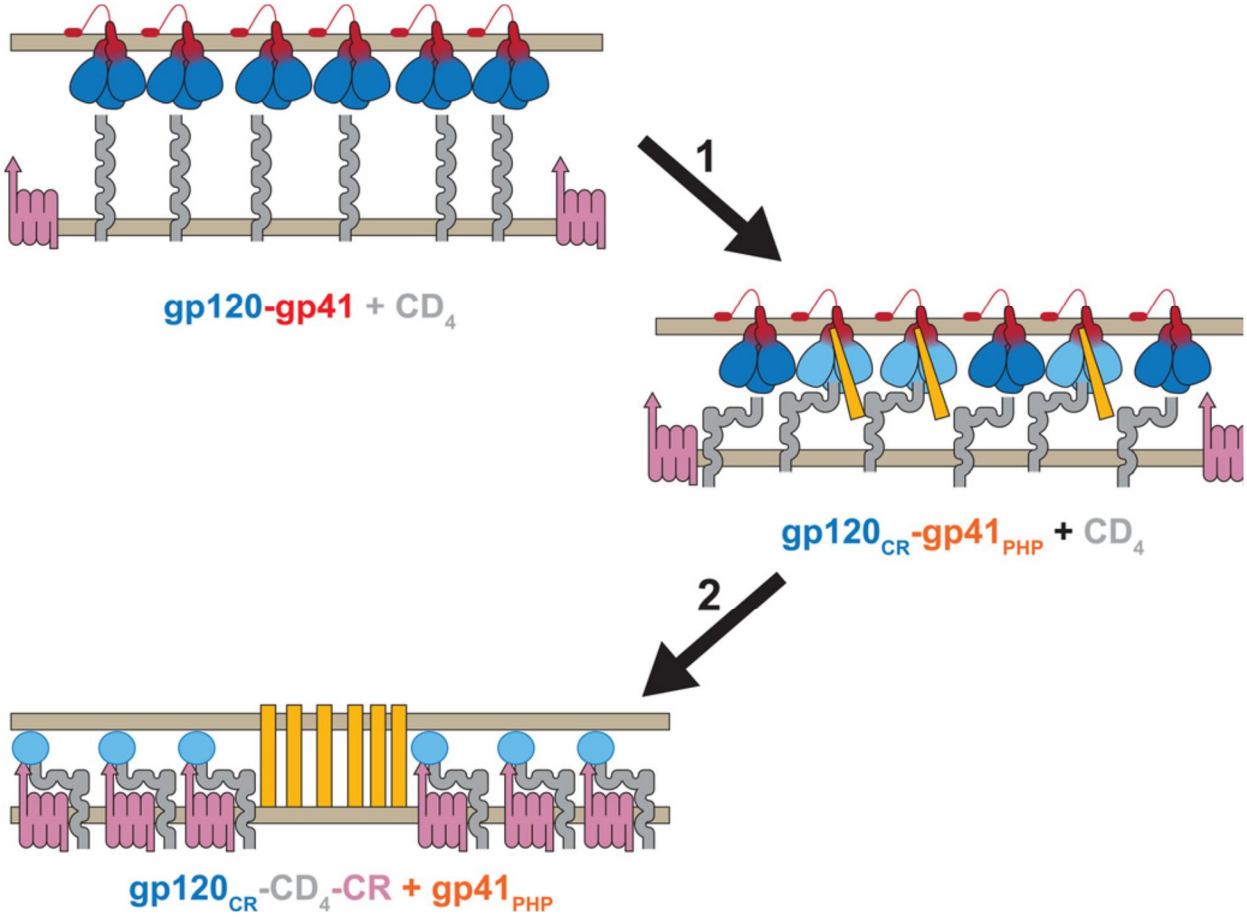

Figure 10: Structural changes of HIV-1 during membrane fusion. In blue is gp120, in red is gp41, in grey CD4 and in pink CR. The first step shows the binding of gp120 to CD4, which induces conformational changes that lead to the formation of the gp41 pre-hairpin intermediate (in yellow). As soon as CR starts, interaction with the complex gp120 disassociates. This is followed by the insertion of gp41 into the target membrane [24].

7 HOW DO VIRUS-HOST CELL ATTACHMENTS AND PENETRATION METHODS INFLUENCE THE OVERALL STRUCTURE OF THE VIRUS

Now that the specific interactions of three structurally different viruses have been discussed, I will proceed to discuss the effects of these interactions on the overall structure of the viral particle's surface.

In the case of the bacteriophage epsilon15, the viral particle is left behind attached to the outside of the bacterium as an empty shell. The head of the virus, where its genetic material is kept, is not engaged in the active penetration. While the head does not undergo any changes, the tail does. Unlike other bacteriophages ε15 does not have a contractile tail, meaning that big structural changes do not take place. However, the tail does undergo small structural changes. In short, in epsilon15 the structural changes that take place on the viral surface during attachment and penetration of the host cell are not significant. Only the tail undergoes structural changes.

In the case of reoviruses, the capsid has a very symmetrical structure and its components form a repeating pattern. As the virus's capsid comes into contact with proteases, the "protection proteins" (σ3) are removed, giving rise to a new pattern throughout the entire surface of the viral particle, and if the model proposed by K. Chandran et al. (2002) is correct, the particle undergoes a second round of overall structural changes, where μ1 and λ2 undergo conformational changes and σ1 is released. σ2 and λ1, which form the main capsid frame, stay in a similar arrangement throughout both rounds of structural changes. In short, the reoviruses' surfaces undergo two rounds of structural changes, one can occur before or after attachment to the host cell and the second round takes place after attachment enabling the virus to enter the host cell.

As enveloped viruses enter their target cells by means of fusion, the viruses go from the original viral state to a non-existing shape, as they melt into the target cells. However, before fusion takes place, the 'fusion proteins' rearrange themselves around the fusion area, changing the surface structure of the virus shortly before the virus fuses with the target cell. In short, HIV-1's surface undergoes small structural changes before merging completely into the host cell membrane and thus becoming non-existing.

8 HOW CAN ONE ACCOUNT FOR THE DIFFERENCES IN VIRAL ATTACHMENT AND ENTRY STRATEGIES

Why bacteriophages possess a different strategy to invade their victims compared to non-enveloped and enveloped viruses is understandable. Bacteria have a very different outer layer than for example mammalian cells. Bacteriophages need to be able to pass through a very tough combination of layers. The bacteriophage's injection mechanism forces its way through the bacterial layers one layer at the time, moving closer and closer to the bacterial cytoplasm.

On the other hand, non-enveloped and enveloped viruses both have the same target cells, but have developed two very different mechanisms to enter their host cells. In other words, they have developed two different strategies to solve the same problem.

Non-enveloped viruses, due to their method of shielding their “penetrating proteins”, have become very good at surviving harsh conditions. In the case of reoviruses, the presence of the bound “protecting protein” (σ3) helps to preserve the infecting capacity of the virus over a range of temperatures [7]. The presence and structure of the reoviruses’ “penetrating proteins” determine how viral attachment and penetration take place.

Enveloped viruses can, due to their lipid bilayer, fuse with their target cells. For the fusion of membranes special proteins are necessary to overcome the big energy barrier that presents the fusion of membranes. These special proteins are present in the lipid bilayers of enveloped viruses. Because of the presence of both the lipid bilayer and the special proteins, enveloped viruses undergo this method of attachment and penetration of host cells.

9 CONCLUSION

Viruses were first identified as viruses in the 20th century, and could only be visualized after the invention of the electron microscope. For more in-depth research, such as virus-host cell interactions, electron cryo-tomography is a very adequate tool to make snapshots of the process of viral attachment to and viral penetration of the host cell.

In this paper I focused on the structural changes that take place on the viral surface during viral attachment to and viral penetration of the target cell. Because of the vast range of viral surface structures I only discussed the structural changes that take place on the viral surface of three different types of viruses: bacteriophages (Epsilon15), non-enveloped viruses (reoviruses) and enveloped viruses (HIV-1). The differences in structure of the viruses lead to different strategies to attach to and penetrate the target cell's membrane.
Bacteriophages have a tail which allows them to pierce through the outer layers of bacteria. The tail is the only part of Epsilon 15 that undergoes structural changes. Non-enveloped viruses' exteriors are composed of a protein capsid. This protein capsid undergoes two rounds of structural changes before penetrating the target cell as a whole. Enveloped viruses enter their host cell by fusing both their membranes together. After small-scale structural changes the virus melts into the target cell.

Differences in the structural changes that take place on the viral surface of Epsilon15, reoviruses and HIV-1 (representing bacteriophages, non-enveloped viruses and enveloped viruses) from the moment of attachment of the virus to the host cell to penetration of the host cell are due to the differences in the viral surface structure of these three viruses, leading to different target cell entry methods. These differences in target cell entry methods lead to the differences in the structural changes that take place on the viral surface of Epsilon15, reoviruses and HIV-1.

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