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Chapter 4

Archaeal Host Cell Recognition and Viral Binding of HFTV1 to its *Haloferax* Host

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Abstract

Viruses are highly abundant and the main predator of microorganisms. Microorganisms of each domain of life are infected by dedicated viruses. Viruses infecting archaea are genomically and structurally highly diverse. Archaea are undersampled for viruses in comparison with bacteria and eukaryotes. Consequently, the infection mechanisms of archaeal viruses are largely unknown, and most available knowledge stems from viruses infecting a select group of archaea, such as crenarchaea. We employed Haloferax tailed virus 1 (HFTV1) and its host, *Haloferax gibbonsii* LR2-5, to study viral infection in euryarchaea. We found that HFTV1, which has a siphovirus morphology, is virulent, and interestingly, viral particles adsorb to their host several orders of magnitude faster than most studied haloarchaeal viruses. As the binding site for infection, HFTV1 uses the cell wall component surface (S)-layer protein. Electron microscopy of infected cells revealed that viral particles often made direct contact with their heads to the cell surface, whereby the virion tails were perpendicular to the surface. This seemingly unfavorable orientation for genome delivery might represent a first reversible contact between virus and cell and could enhance viral adsorption rates. In a next irreversible step, the virion tail is orientated toward the cell surface for genome delivery. With these findings, we uncover parallels between entry mechanisms of archaeal viruses and those of bacterial jumbo phages and bacterial gene transfer agents.

Importance: Archaeal viruses are the most enigmatic members of the virosphere. These viruses infect ubiquitous archaea and display an unusually high structural and genetic diversity. Unraveling their mechanisms of infection will shed light on the question if entry and egress mechanisms are highly conserved between viruses infecting a single domain of life or if these mechanisms are dependent on the morphology of the virus and the growth conditions of the host. We studied the entry mechanism of the tailed archaeal virus HFTV1. This showed that despite “typical” siphovirus morphology, the infection mechanism is different from standard laboratory models of tailed phages. We observed that particles bound first with their head to the host cell envelope, and, as such, we discovered parallels between archaeal viruses and non-model bacteriophages. This work contributes to a better understanding of entry mechanisms of archaeal viruses and to a more complete view of microbial viruses in general.

Keywords: Haloarchaea, *Caudoviricetes*, archaeal virus, viral adsorption, infection mechanism, *Haloferax*, *Archaea*, viral entry

1 Introduction

Archaeal viruses represent the most unexplored part of the virosphere [1,2]. Archaea are ubiquitous microorganisms that colonize very diverse parts of our planet. They make up a considerable part of the biodiversity in the oceans, play important roles in biochemical cycling, and can live in hot springs or hydrothermal vents with temperatures around the boiling point, and they also grow on the human skin and are found in the human gut [3-5]. Archaea are evolutionarily more closely related to eukaryotes than to bacteria, although they are prokaryotic. They have unique properties, such as the archaeal cell envelope composition, which consists of ether-linked lipids with a glycerol-1-phosphate backbone, in contrast to the ester-based lipids with a glycerol-3-phosphate backbone in bacteria and eukaryotes [6]. Whereas bacteria are usually covered in a peptidoglycan layer of murein, archaea lack murein and are instead nearly always wrapped in a surface (S)-layer consisting of glycosylated protein [7]. As a consequence, archaeal viruses face different challenges to enter a host cell from bacterial or eukaryotic viruses [8]. Archaeal viruses are highly diverse both with regard to their sequences and their structures. Some archaeal viruses have unique shapes, such as that of a bottle, spindle, or spiral, while the others have morphologies that can also be found for viruses of bacteria and/or eukaryotes, such as tailless icosahedral or a head-tail morphology [9]. As archaeal viruses are understudied, it is still an open question if entry and egress mechanisms are conserved within all archaeal viruses or if such mechanisms mimic those of known bacterial viruses. Specifically, the entry mechanism of archaeal viruses is not well understood [8,10,11]. Only a handful of receptors have been identified, and they include the S-layer protein or adhesive pili that are presented at the cell surface [12-14]. The limited available information stems mainly from viruses infecting members of the Crenarchaeota. The entry mechanisms of double-stranded (dsDNA) tailed bacteriophages have been studied in detail, such that for multiple phages the receptors on the host surface have been identified, as well as the virion proteins that are essential for entry [15]. Genome sequences of tailed dsDNA archaeal viruses are quite diverse. As a result, it is difficult to predict archaeal virus gene function based on bacteriophage genomics [16]. Since the cell envelopes of archaea and bacteria also differ significantly, entry mechanisms used by bacteriophages might not function in archaea. Therefore, unraveling of entry mechanisms of archaeal viruses relies on experimental approaches.

We aimed to explore the entry mechanism of archaeal tailed double-stranded DNA viruses, which are the most numerous archaeal virus isolates known today [2,16]. For this, we selected the haloarchaeon *Haloferax gibbonsii* LR2-5 and Haloferax tailed virus 1 (HFTV1, the family *Haloferuviridae*, the order *Kirjokansivirales*, and the class *Caudoviricetes*), which has a siphovirus-like morphotype with a head connected to a long noncontractile tail [16-18]. This is the first and only available virus isolated from a *Haloferax* host and it serves as a model for haloarchaeal virus-host studies. HFTV1 was isolated together with its host from the hypersaline Lake Retba in Senegal [17]. The genome sequence of *H. gibbonsii* LR2-5 is 3.8 MB and revealed that LR2-5 does not contain a CRISPR-Cas antiviral defense mechanism, which might explain why it is one of the few *Haloferax* strains susceptible to viral infection [18,19]. LR2-5 has the typical rod shape of haloarchaea and is motile in early exponential phase, whereas cells round up and lose their motility in the stationary phase [18]. HFTV1 specifically infects *H. gibbonsii* LR2-5, and closely related strains such as *H. gibbonsii* Ma2.38 and *Haloferax volcanii* H26 are not susceptible to HFTV1 [17,18]. In this study, we found that HFTV1 uses the highly abundant S-layer protein as binding site and that it absorbed unusually fast in comparison to other haloarchaeal viruses. Electron microscopy revealed that HFTV1 can bind either with the head or the tail to the cell surface.

2 Results

2.1 Effect of Salinity and High Temperature on HFTV1 Stability

We tested the impact of various NaCl concentrations (0 to 5 M) on the stability of the HFTV1 particle and on the efficiency of infection. HFTV1 particles were stable independent of NaCl concentrations (Figure 1A). After 2 hours, 3×10^{11} plaque-forming units (PFU)/mL were still detectable in 0 M NaCl, which was at the same level as the control in high salt. After 24 hours, infectivity was 2×10^{11} PFU/mL. This shows that HFTV1 is very stable in low to almost saturated salt concentrations (4 to 5 M NaCl, also including 113 mM MgCl_2 , 108 mM MgSO_4 , and 71 mM KCl). Therefore, NaCl concentrations have no effect on infectivity. In general, the viral stock titer (on average 5×10^{11} PFU/mL) remained unchanged over a 6-month period (see Figure S1 in the supplemental material).

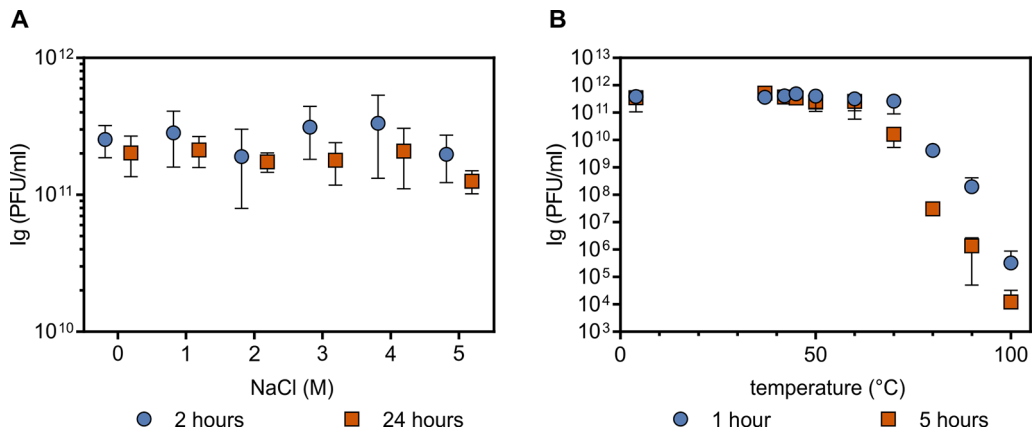
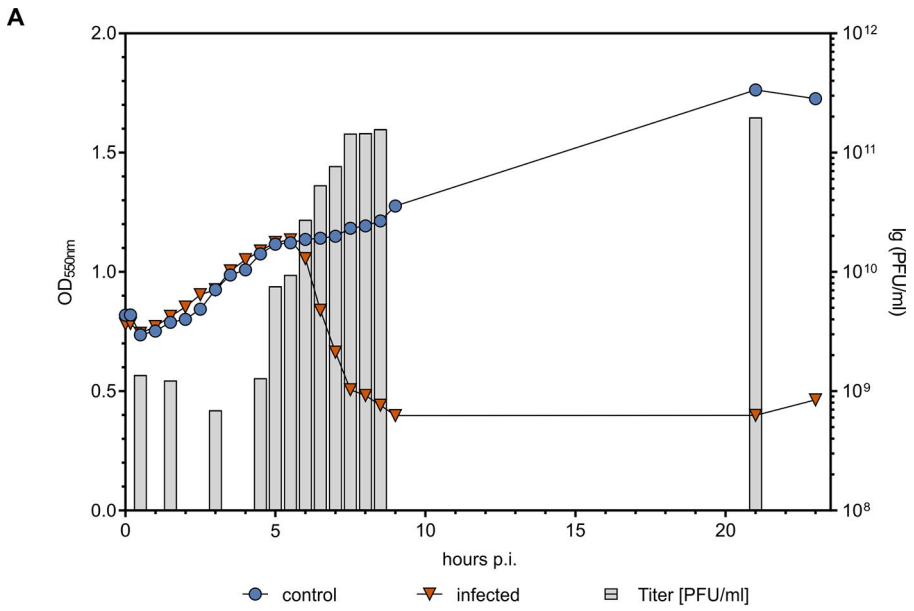


Figure 1. HFTV1 stability. (A) Infectivity at low and high NaCl concentrations. HFTV1 particles were diluted 1000 times in MGM medium with different concentrations of NaCl and incubated for 2 or 24 hours before the infectivity of the sample was determined by plaque assay. Error bars represent the standard deviation from three biological and technical replicates. (B) Temperature stability. HFTV1 virus lysate was exposed to varying temperatures for 1 hour or 5 hours. Subsequently a plaque assay was performed to determine the number of infectious viral particles. Error bars represent the standard deviation from four independent experiments. If the error bars are not visible, the deviation could not be resolved graphically.

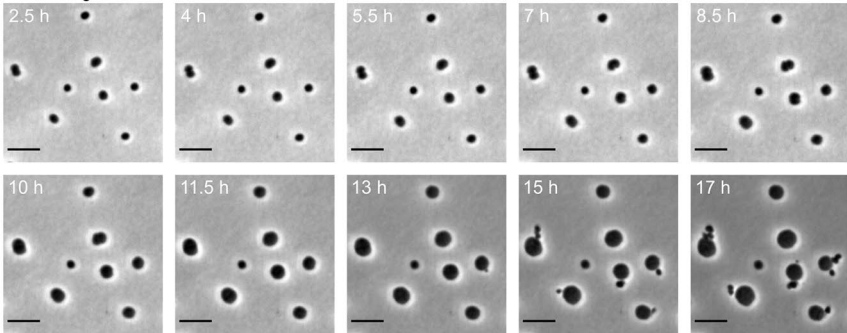
After incubation of HFTV1 at various temperatures ranging from 50°C to 100°C, we observed that the infectivity remained unchanged up to 70°C. The infectivity decreased within 1 to 5 h when the temperature was higher than 80°C (Figure 1B). At this temperature, the infectivity decreased 6 orders of magnitude but was not completely lost even at 100°C.

Thus, HFTV1 tolerates both large and small amounts of NaCl and temperatures up to 70°C, at least for relatively short time periods. These properties render the virus very robust to the changing environmental conditions in its natural habitat. This finding is in line with the previously detected wide global distribution of archaeal tailed viruses in different environments [16,20]. We decided to use a temperature of 37°C in further infection experiments.



B

Haloferax gibbonsii LR2-5 infected with HFTV1



C

Haloferax gibbonsii LR2-5 (uninfected control)

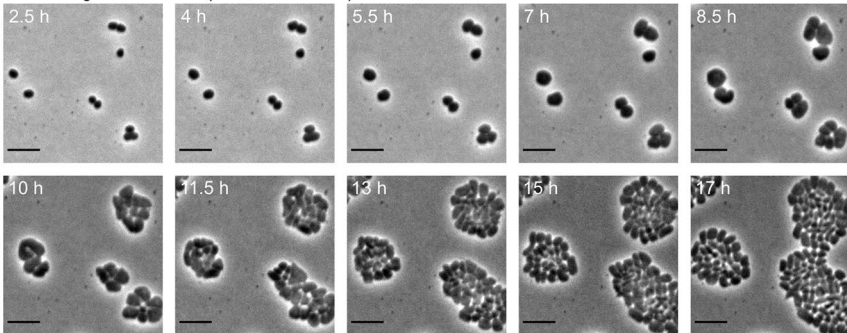


Figure 2. HFTV1 life cycle and time-course of infection in *Haloferax gibbonsii* LR2-5. (A) Single-step growth curve of HFTV1. *H. gibbonsii* cells were grown to logarithmic phase (OD_{550} of 0.8, $\sim 9.0 \times 10^8$ CFU/mL) and infected with an MOI of 10 at 37°C. To remove unadsorbed viral particles, the cells were washed thoroughly 20 min post infection (p.i.) and transferred to fresh medium at 37°C. The number of free viruses was monitored by plaque assay. (B) Time-laps microscopy of *H. gibbonsii* cells during HFTV1 infection. (C) Time-lapse microscopy of uninfected control cells. Cells were grown on an agarose pad supplemented with casamino acids in a thermomicroscope set at 45°C. Selected phase contrast images show cells 2.5–17 h p.i. (1.5 h intervals) after infection with HFTV1 (B) or uninfected control cells (C). HFTV1 infected cells increase strongly in size while they are not observed dividing. First lysis of infected cells was observed between 11–13.5 hours p.i. indicated by white arrows. The uninfected control cells started to divide after 8.5–11 hours incubation. Scale bars 5 μ m.

2.2 Virus Infection Leads to Increase in Host Cell Volume

We observed that HFTV1 forms clear plaques of 2 to 4 mm in diameter on host lawns of *H. gibbonsii* LR2-5, which is indicative of a lytic infection. This is similar as to what is observed for other tailed haloarchaeal viruses releasing their progeny by host cell lysis [21]. We determined the length of the infection cycle by performing a one-step growth experiment using a multiplicity of infection (MOI) of 10 (Figure 2A) (Figure S2). The extracellular virus titer rose after 6 hours post infection (hpi) indicating the length of the latency period. The infection resulted in a large number of progeny, and the average burst size was about 65 viruses per infected cell, resulting in typical titers of 7×10^{10} PFU/mL in the culture lysate. Around 6 hpi, also a drop in the optical density (OD) of the culture was measured. The number of viable cells was reduced over 3 orders of magnitude 21 hpi from 7×10^9 CFU/mL to 2×10^6 CFU/mL. Infected cells were followed by phase-contrast time-lapse microscopy (Movie S1), which revealed that cells first increase considerably in size (Figure S3), before they finally burst at 11.5 to 13 hpi. Snapshots taken at different stages of the infection showed that infected cells (Figure 2B), in contrast to noninfected cells (Figure 2C), were no longer dividing (Movie S2). Their size doubled their volume such that cells immediately prior to virus release had diameters that were ~ 1.5 -fold larger than control cells. Such an increase in cell volume has been observed previously for the crenarchaeal *Sulfolobus tengchongensis* spindle-shaped virus 2 (STSV2). However, in that case, the diameter increased to even more to 20-fold that of control cells [22]. Lysis of the LR2-5 cells in time lapse-microscopy was characterized by sudden leakage of cytoplasm in small burst events (Movie S1).

2.3 HFTV1 is a Fast Adsorbing Virus, and its Receptor is Highly Abundant

We used the so-called “inverted” viral adsorption assay to measure the rate by which particles attach to the cell surface, which relies on the measurement of the decrease of viral particles in the media, after “pulsing” the cells with viruses [23]. The adsorption assay showed that the binding of HFTV1 to host cells is extremely efficient and synchronized. Saturated adsorption occurs within the first 3 minutes after infection, resulting in 90% of the virions being bound to the host cells (Figure 3A). The adsorption rate constant calculated at 10 min postinfection was $1.9 \times 10^{-9} \text{ mL min}^{-1}$.

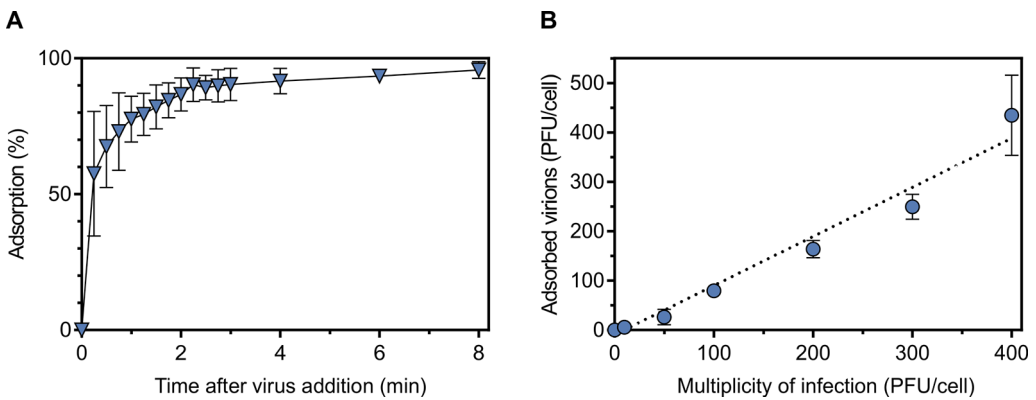


Figure 3. Adsorption efficiency of HFTV1 to *H. gibbonsii* cells. (A) To determine the adsorption rate of HFTV1, *H. gibbonsii* LR2-5 cells were grown to the mid logarithmic phase (OD_{600} 1.0; $\sim 2 \times 10^9$ CFU/mL) and infected with HFTV1 using an MOI of 0.001 at 37°C. The number of unbound virus particles was determined after 0–8 minutes post infection by plaque assay. Error bars represent standard deviation from three experiments. (B) To determine if the receptor can be saturated, a constant number of *H. gibbonsii* LR2-5 cells (OD_{600} 0.65–0.85, $\sim 1 - 6 \times 10^8$ CFU/mL) were infected with HFTV1 using varying MOIs ranging from 0.001 to 400. Number of unadsorbed particles present in the supernatant was determined by plaque assay 30 min post infection and compared to the number of particles in a cell-free control. Error bars represent standard deviation from three independent experiments. If the error bars are not visible, the deviation could not be resolved graphically.

The rate of attachment of HFTV1 particles was not affected by preinfection with HFTV1 (Fig. S7A), indicating that superinfection exclusion of HFTV1 against subsequent infecting HFTV1 particles is not occurring.

To determine whether the receptor of HFTV1 is abundant or rarely exposed on the host cell surface [24], we performed receptor saturation experiments by infecting *H. gibbonsii*

LR2-5 cells with HFTV1 at different MOI values from 0.001 to 400. Subsequently, the number of free particles remaining in the supernatant was determined and compared with the number of virions added to the cell-free control (Figure 3B). Even samples infected with an MOI of 400 showed that about 30 min postinfection, ~400 viruses were bound to the cells, and saturation was observed. This indicates that the receptor mediating the primary interaction between HFTV1 and *H. gibbonsii* is very abundant.

2.4 HFTV1 Binds to Cells with an Unusual Orientation with a Tail Pointing Outward from the Cells

In order to observe the binding process of HFTV1 by electron microscopy, highly pure and infectious viral particles were produced. Purification of HFTV1 virions was optimized by changing the sucrose gradient from 5 to 20% (wt/vol) [17] to 10 to 40% (wt/vol) and using optimized time to separate viruses by rate-zonal centrifugation. The purification of polyethylene glycol-NaCl-precipitated viruses in a linear 10 to 40% (wt/vol) sucrose gradient resulted in two blue and one grey light-scattering band (Figure S4A) that were separated from most of other sample components absorbing at 280 nm (Figure S4B). Most of the infectivity was found in the lower blue band (a total of 80% of infectivity in three peak fractions [Figure S4C]) resulting in high specific infectivity of $\sim 1 \times 10^{13}$ PFU/mg protein (Figure S4D). Purifying the viruses further by equilibrium centrifugation in CsCl resulted in a single sharp light-scattering band and occasionally also a minor upper band with low infectivity. Recovery of the total amount of infectious viruses was >10% compared to that of the lysate, and the yield of the CsCl-purified viruses was 1 to 2 mg per L of lysate (n=3). The specific infectivity of CsCl-purified virus was $\sim 1\text{--}3 \times 10^{13}$ PFU/mg protein (n = 3). The transmission electron microscopy (TEM) and SDS-PAGE analysis of the protein profiles of the purified viruses confirmed the purity of the sample (Figure 4A to C). TEM analysis showed that the purified HFTV1 sample was very homogeneous and devoid of impurities such as archaeella or cell debris (Figure 4A and B). In addition, the majority of the particles showed DNA-filled heads (Figure 4A), in contrast to the majority of DNA-devoid particles that were obtained with the original purification method [17]. It is noteworthy that this method separates the infectious viruses efficiently and produces significantly better-quality material with specific infectivity that was 4 orders of magnitude higher than previously reported [17], allowing the particles to be used in infection experiments.

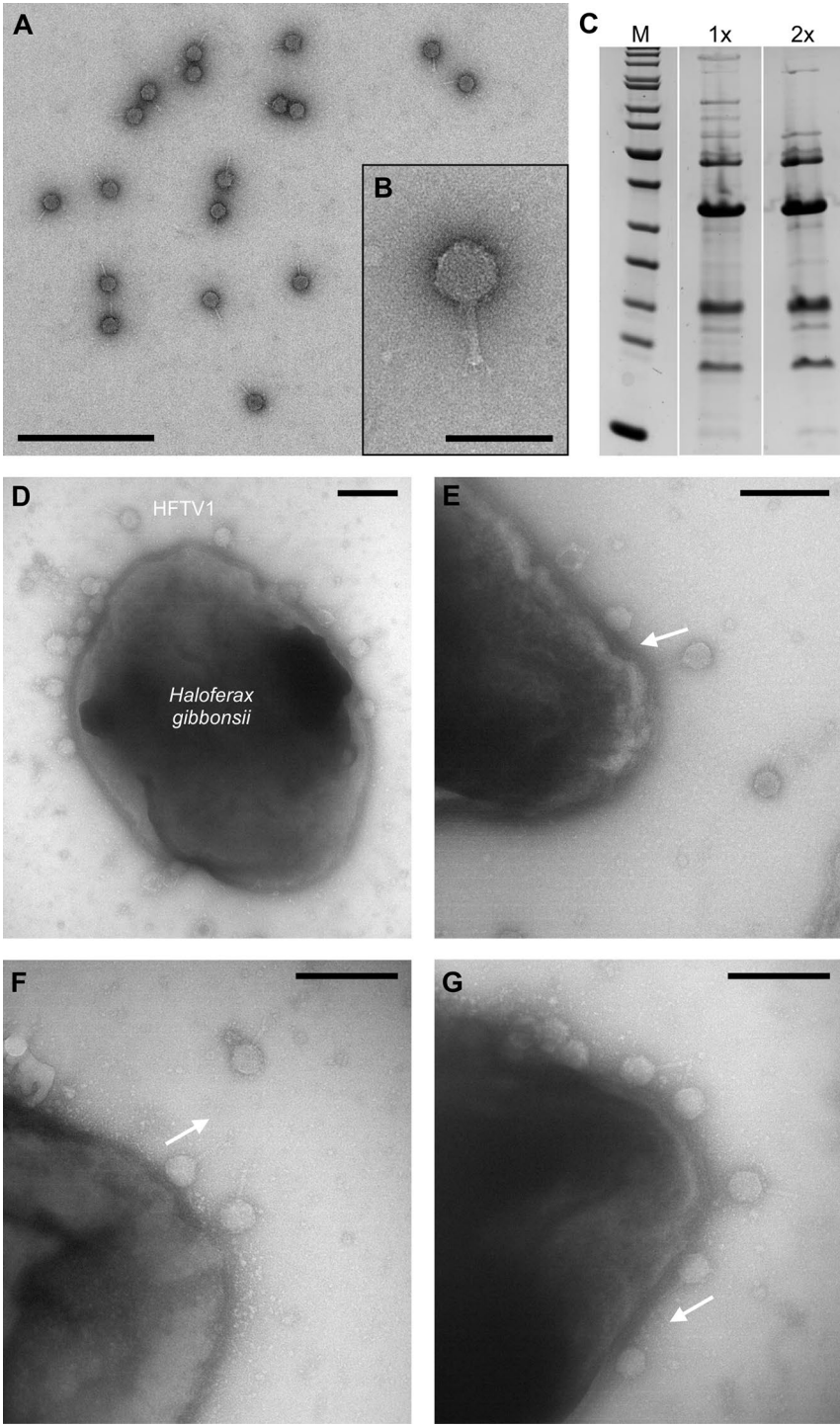


Figure 4. TEM of HFTV1 purification and particle binding to *Haloferax gibbonsii*. (A) Transmission electron microscopy of negatively stained 2 x purified HFTV1. Scale bar = 500 nm. (B) Scale bar = 100 nm. (C) Major protein bands of 1 x (purified by precipitation and rate zonal ultracentrifugation in sucrose) and 2 x (purified by precipitation, rate zonal ultracentrifugation in sucrose, and equilibrium ultracentrifugation in CsCl) purified HFTV1 particles. Molecular mass marker (M) ranges from 200 kDa to 10 kDa (Thermo Scientific™ #26614). (D-G) Adsorption of HFTV1 to *Haloferax gibbonsii* LR2-5 cells. Scale bars = 200 nm. (D) TEM observation showed particles attach with random orientations. Particles were observed binding (E) with their tails towards the cell surface, (G) with their heads, or (F) in parallel to the surface. Orientation of tails of HFTV1 are indicated with white arrows pointing towards the tail tip. The majority (50–70%) of HFTV1 particles were examined attaching to the cell surface head side.

To visualize the binding of HFTV1 to the host cell surface, we used TEM to observe negatively stained *H. gibbonsii* LR2-5 cells mixed with HFTV1 in different MOIs. Directly after adding the virus to the host cell, viral binding was observed. Numerous particles with full heads were visible at the cell surface of *H. gibbonsii* LR2-5 (Figure 4D). HFTV1 bound to the cell all over the surface and did not accumulate at specific sites (Figure 4D). Curiously, the largest fraction (50 to 70%) of the viral particles was found bound directly with the DNA-filled head to the cell surface. Their tails were parallel or even perpendicular to the host cell surface (Figure 4E to G). This observation is in contrast to the visualized binding events of most tailed phages, which are typically orientated with the tail and tail fibers toward the cell surface.

2.5 Escape Mutants Implicate one of Two S-layer Proteins as the Primary Receptor

To identify the HFTV1 receptor, we isolated “escape-mutants” of the HFTV1 host strain, which are cells that survived a viral infection and were no longer susceptible to renewed HFTV1 infection. *H. gibbonsii* LR2-5 cells were challenged with HFTV1 in liquid culture, and the resulting lysate was plated to isolate resistant cells. Single colonies were colony purified, regrown, and rechallenged with HFTV1 by spot assay (Figure S6). Three LR2-5 strains did not support HFTV1 plaque formation and were sent for whole-genome sequencing. Analysis of the sequences of three escape-mutants: Ω 48, Δ 16K, and Ω 15, revealed clear differences from the sequence of the wild-type HFTV1 -susceptible strain (Figure 5). All changes were found in the region around the gene *HfgLR_11210*, which encodes one of the two S-layer proteins of *H. gibbonsii* LR2-5. S-layer proteins are the

major cell wall components of haloarchaea, and several haloarchaea, such as *H. gibbonsii* LR2-5 encode two different S-layer proteins [18]. In the escape mutants $\Omega 15$ and $\Omega 48$, small 15-bp and 48-bp in-frame tandem duplications, respectively, had occurred in the gene *HfgLR_11210*. This region encodes a threonine-rich motif of the S-layer protein (237 to 246 amino acids). Mutant $\Delta 16K$ has a large 16-kb, affecting more than 90% of the *HfgLR_11210* gene and also several other adjacently located genes (Figure 5). These include *pilA3* and *pilA4*, as well as several currently nonannotated genes, followed by *dppF3* and *dppA3* encoding parts of an ABC transporter system, *rnhA1*, which encodes a This mode RNase, and *maeB2* (putative malate dehydrogenase). Analysis of the susceptibility of the three mutants to HFTV1 by spot assays showed that strains were able to grow in liquid medium (Figure S5) and on plates in a layer of soft agar. Cells in a soft layer showed no lysis after infection with HFVTV1 (Figure S6). There were reads mapping to the HFTV1 sequence in $\Omega 15$ and $\Omega 48$ sequences, while these were completely absent in $\Delta 16K$ (Table S1). *HfgLR_11210* encodes an S-layer glycopeptide, which is part of the cell wall of LR2-5 [18]. We conclude that this S-layer glycopeptide is likely the receptor for HFTV1 and that the binding between HFTV1 and S-layer glycopeptide probably can be hampered by the small insertions around positions 237 to 246. We hypothesize that a very inefficient adsorption may still take place. These few adsorption events will still result in viral replication and are thus responsible for the detected HFTV1 reads in these mutants. In case of $\Delta 16K$, where almost the complete S-layer glycopeptide gene is deleted, presumably, no adsorption can take place, and thus, no HFTV1 reads are detected. This is also consistent with our observation that the binding to $\Delta 16K$ was reduced to 50 to 60%, showing a significant effect of the S-layer glycoprotein gene deletion on adsorption of HFTV1 (Figure S7B). Due to the low sequence similarity of *HfgLR_11210* to the S-layer of *H. volcanii*, of which the structure has been solved [25], it was not possible to predict if the small amino acid insertions were in a particular domain of the S-layer protein structure. The deletion of one of the S-layer proteins is not lethal for *H. gibbonsii* LR2-5, and the $\Delta 16K$ escape-mutants grow at the same rate as the original strain. We cannot exclude that under variable environmental conditions (i.e., low salinity, low nutrients, biofilm formation), there might be a fitness burden for this escape mutant with only one S-layer protein.

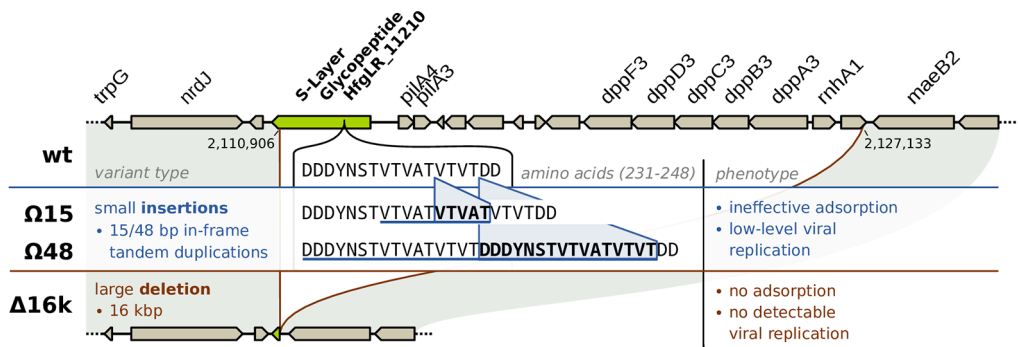


Figure 5. Sequence changes in *H. gibbonsii* LR2-5 mutants causing resistance to HFTV1 infection. Shown is a 25 kilobase region on the *H. gibbonsii* LR2-5 main chromosome for the wildtype (wt, top) and escape mutants Ω15, Ω48 and Δ16k. Mutants Ω15 and Ω48 each carry a small insertion, 15 and 48 bases, respectively in *hfgLR_11210* which is one of two S-layer glycoproteins of *H. gibbonsii* LR2-5 (indicated in green). The changes at the protein level (amino acids in bold) caused by these tandem duplications are highlighted by blue triangles. Mutant Δ16k contains a 16.2 kilobase deletion affecting more than 90% of the coding region of *hfgLR_11210*.

3 Discussion

Haloferuviridae is a small and diverse archaeal viral family belonging to the order *Caudoviricetes* [16]. Haloferuvirus HFTV1 is the only virus isolate infecting a *Haloferax* host [17,19] making it an interesting model to study the entry and infection mechanism of an archaeal virus in high detail.

HFTV1 particles can cope with temperatures between 4°C to 70°C, which is a much wider temperature distribution than the temperature from the isolation site in hypersaline Lake Retba, Senegal, where the average temperature is 25°C [26], but within the range of other viruses of haloarchaea [27,28]. HFTV1 was tolerant to a broad NaCl concentration surviving also at “zero salt”. It was reported before that tailed haloarchaeal viruses (non-lipid containing) are usually more resistant to changes in ionic strength, whereas membrane-containing viruses are more sensitive to changing NaCl concentrations (with enveloped viruses being the most sensitive) [29]. Generally, haloarchaeal viruses can withstand a wider range of NaCl concentrations than their hosts [28,29].

HFTV1 particles adsorb to the host cell within minutes. This makes HFTV1 several orders of magnitude faster than most haloarchaeal viruses studied (Table 1). So far, only

one haloarchaeal virus, VOLN27B, is known to adsorb to its host *Haloarubrum* sp. strain LN27 in less than a minute [27]. The known adsorption rate constants of haloarchaeal viruses range from $\sim 10^{-10}$ to 10^{-13} mL min⁻¹, in contrast to viruses of crenarchaea or bacteria that have adsorption rate constants of $\sim 10^{-9}$ to 10^{-10} mL min⁻¹ (Table 1). The current hypothesis is that the high ionic strength under which haloarchaeal viruses infect might result in a natural slower adsorption than viruses that infect in nonsaline environments [30]. Alternatively, it was also discussed whether the low adsorption rates of haloarchaeal viruses are due to differences in the surface structures of bacteria and archaea [29]. However, the fast adsorption of HFTV1 now shows that slow binding of most haloarchaeal viruses cannot solely be attributed to the high salinity conditions, and other factors might play a role.

Table 1. Adsorption rate of different archaeal viruses and bacteriophages

	Virus	Host	Morphology	Adsorption rate [mL/min]	Reference
Euryarchaeal viruses	HFTV1	<i>Haloferax gibbonsii</i> LR2-5	Icosahedral non-contractile tail	1.8×10^{-9}	This study
	HHTV-1	<i>Haloarcula hispanica</i>	Icosahedral non-contractile tail	2.9×10^{-13}	[29]
	His1	<i>Haloarcula hispanica</i>	Lemon-shaped	1.9×10^{-12}	[51]
	His2	<i>Haloarcula hispanica</i>	Pleomorphic	5×10^{-12}	[52]
	HRPV9	<i>Haloarubrum</i> sp. SS7-4	Pleomorphic	8.5×10^{-11}	[53]
	HHIV-2	<i>Haloarcula hispanica</i>	Icosahedral with internal membrane	3.7×10^{-12}	[23]
	HCIV-1	<i>Haloarcula californiae</i>	Icosahedral with internal membrane	5.7×10^{-11}	[28]
Crenarchaeal viruses	STIV1	<i>Sulfolobus solfataricus</i>	Icosahedral with membrane	2×10^{-9}	[54]
	SIRV2	<i>Sulfolobus islandicus</i>	Rod-shaped	2×10^{-8}	[14]
	SMV1	<i>Sulfolobus islandicus</i>	Spindle-Shaped	7×10^{-9}	[55]
	SSV9	<i>Sulfolobus islandicus</i>	Spindle-shaped	8.4×10^{-11}	[56]

	Virus	Host	Morphology	Adsorption rate [mL/min]	Reference
Bacteriophages	T1	<i>Escherichia coli</i>	Icosahedral non-contractile tail	3×10^{-9}	[57]
	T2	<i>Escherichia coli</i>	Icosahedral non-contractile tail	2.1×10^{-9}	[58]
	φ6	<i>Pseudomonas phaseolicola</i>	Enveloped	3×10^{-10}	[59]
	PM2	<i>Pseudoalteromonas espejiana</i>	Icosahedral with internal membrane	2×10^{-10}	[60]
	SCTP-1	<i>Salicola sp.</i> PV3	Icosahedral non-contractile tail	3.4×10^{-10}	[29]

We show that HFTV1 likely uses an S-layer glycoprotein as receptor. The composition of the archaeal cell envelope varies considerably between archaeal species. The sole structural cell wall components of haloarchaea and many crenarchaea are S-layer proteins. In the case of the well-studied crenarchaeon *Sulfolobus acidocaldarius*, the S-layer consists of two structurally distinct proteins, SlaA and SlaB, which together form an ordered 2-dimensional paracrystalline sheet around the cell [31]. The membrane-proximal face of the S-layer consists of tripod-like SlaB trimers. The SlaB trimers support the outer canopy of the S-layer, which is formed by an array of tightly interwoven boomerang-shaped SlaA dimers [31]. Deletion of genes encoding SlaB leads to mutant cells with partial S-layers consisting solely of SlaA. It was shown that SlaB-depleted strains, were less susceptible for *Sulfolobus* Spindle-shaped Virus (SSV) infection, suggesting that SlaB is the receptor for this virus [32]. In contrast, when SlaA is deleted, the S-layer does not assemble anymore, and cells are deformed. Thus, SlaA and SlaB, both have different functions in the *Sulfolobus* S-layer.

The S-layer of *H. volcanii* consists of a hexagonal array of tightly interacting S-layer proteins that contain immunoglobulin-like domains [25]. There is only one S-layer protein encoded, and it is lipid anchored in the cell membrane, in contrast to the S-layer protein of *Sulfolobus*, which is anchored by a transmembrane domain [33,34]. Interestingly, some haloarchaea, such as *H. gibbonsii*, encode two or more different S-layer proteins (In LR2-5, HfgLR_11210, and HfgLR_04635). These proteins in *H. gibbonsii* LR2-5 have a similar amino acid sequence, and likely do not have such structurally dissimilar functions as the crenarchaeal SlaA and SlaB. It is not known if both LR2-5 genes are redundant,

as their high sequence similarity might suggest. In addition, it is unclear if individual cells express only one of the genes or if S-layers in these organisms consist of a mixture of both proteins. At least for *H. gibbonsii* LR2-5, we have previously determined by mass-spectrometry that both proteins are expressed [18]. However, as we have used cell pellets for this analysis, we cannot detect S-layer differences at the single-cell level, and thus, there might be individual cells that only express one S-layer protein.

Deletion of *hfgLR_11210* results in viable *H. gibbonsii* LR2-5 cells, suggesting that a bona fide or partial S-layer is still being formed by the HfgLR_04635 protein, which can (partially) replace the function of HfgLR_11210. Deletion of *hfgLR_11210* results in the strain becoming resistant to HFTV1 infection, which shows that the S-layer protein (HfgLR_11210) encoded by *H. gibbonsii* LR2-5 is an essential receptor for HFTV1. As only *hfgLR_11210* is essential for HFTV1 infection, downregulation or deletion of this gene might even be a strategy of the host to escape viral infection. The multiple insertions that we observed in the gene *hfgLR_11210* could also indicate a mutational hot spot around the position encoding amino acids 237 to 246 in HfgLR_11210. Analysis of metagenome sequences of the original environment might give insight into this hypothesis.

Little is known about the receptors used by other haloviruses. The only other receptor of a haloarchaeal virus that has been identified previously is that of Halorubrum pleomorphic virus 6 (HRPV-6). This virus, like HFTV1, uses the S-layer of its host, *Halorubrum* sp. SS7-4, as receptor. Binding of HRPV-6 to the S-layer leads to activation of the viral fusion protein and results in virus-cell membrane fusion and genome delivery [12]. Since HFTV1 is a tailed virus without a lipid envelope, viral fusion with the cell membrane is not the likely mechanism of entry for this virus.

As the S-layer is essential for adsorption, we assume that binding of the S-layer might be the first step in genome delivery. Analysis with electron microscopy showed that HFTV1 particles can bind to the surface both with their heads and with their tails. It is possible that the S-layer is the only receptor with which both parts of the virion interact or that head and tail interact with two distinct receptors. Adsorption where virions are orientated with their heads toward the cell envelope and their tails perpendicular to it seems unfavorable for genome delivery, which we assume would occur through the tail tube, as is the case for other dsDNA tailed viruses infecting bacteria. Indeed, most tailed dsDNA viruses bind with their tail tubes to the cell surface prior to genome delivery. However, some cases of virion head binding have also been observed, specifically in

non-model viruses. For example, jumbo phages PTm1 and PTm5 infecting the bacterium *Tenacibaculum maritimum* have lytic life cycles with a latent period of 90 min [35]. Viral particles have flexible fiber-like head appendages of 50 to 100 nm long. TEM observations on phage adsorption to the bacterial cell surface showed particles that seem to adsorb head fiber first on the cell during short incubation times (15 or 25 min), and the usual tail-first adsorption was observed later [35].

Such time-dependent reorientation of the particle is also observed for gene transfer agents (GTAs). These are bacteriophage-like genetic exchange elements that resemble small DNA bacteriophages, and which transfer random pieces of the producer's cell's genome to recipient cells [36]. Small phage-like particle RcGTA produced by *Rhodobacter capsulatus* looks like a tailed bacteriophage and the capsid head is decorated by triangular spikes that are needed for binding to the capsule, which is a polysaccharide layer at the outside of the bacterial cell envelope. RcGTA particles attach to cells in random orientations. In the model described for RcGTA-mediated DNA delivery, RcGTA particles attach to the cell surface by the head spikes and the particle reorients by the binding of tail fibers to outer membrane receptors [37,38]. Next, the particle attaches to the membrane by putative receptor-binding domains of the baseplate, which is followed by penetration of the outer membrane [37,38].

We hypothesize that HFTV1 might also reorient the particle during initial step of the entry in a time-dependent manner. We assume that the capsid head might contain specific structures, such as spikes or turrets, which have been observed in the heads of several bacteriophages [39-43] and have already been described for an haloarchaeal tailed HSTV-1 podovirus (the family *Shortaselviridae*) [44]. Indeed, specific turrets at the HFTV1 head might be visible (Figure 4B). These structures might undergo specific and reversible interactions with the cell surface of *H. gibbonsii* LR2-5. A likely target might be the S-layer protein encoded by *HfgLR_11210*, but it might be equally likely that there is an interaction with glycans that also make part of the cell envelope. Next, the particle reorients with help of the short tail fibers, which eventually leads to a specific and irreversible interaction of the base plate of HFTV1 with the S-layer protein. This event is followed by genome delivery, which we assume is by ejection of the viral dsDNA genome via the tail tube (Figure 6).

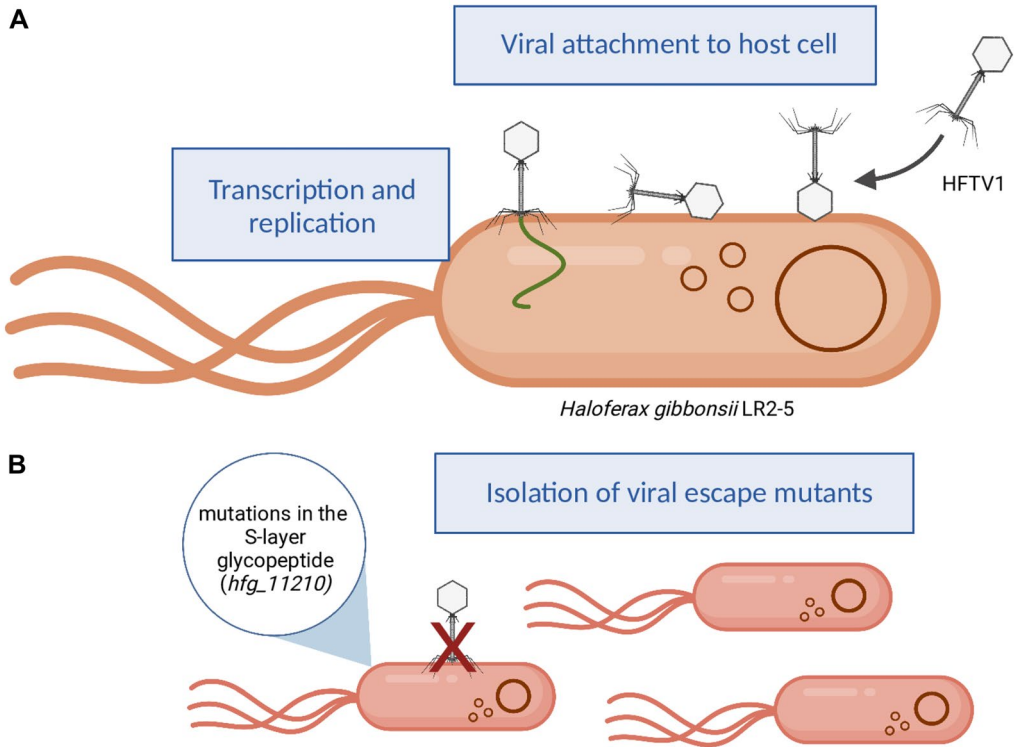


Figure 6. Model of HFTV1 binding to *Haloferax gibbonsii* LR2-5 cells. (A) HFTV1 adsorbs to the host cell within 3 min of infection. Viral particles attach to the host cell surface in random orientations (right side of the cell). For example, HFTV1 heads bind the cell and the HFTV1 tails are perpendicular or parallel to the surface. Next, particles reorientate and attach to the S-layer by their tail (left). This is followed by viral genome (green) ejection and intracellular transcription and translation. Brown circles indicate the circular host genome and host megaplasmids. (B) Alterations in the S-layer glycopeptide Hfg_11210 of *H. gibbonsii* LR2-5 lead to escape mutants that are no longer susceptible to HFTV1. Figure created with BioRender.com.

This mode of binding to the host cell surface might increase the chances of successful genome delivery, as the orientation with which the viral particle first gets in contact with the cell surface is irrelevant for attachment to the cell. We assume that the head-first binding of HFTV1 might be responsible for the unusually fast adsorption rates measured for this virus in comparison with other haloarchaeal viruses. The actual genome delivery is not directly coupled with the adsorption, and it could still be possible that fast irreversible head-first binding is followed by a longer period of tail-first binding before

genome delivery takes place. Future studies that include high-resolution microscopy techniques will be necessary to test this model.

In conclusion, although the overall morphology of HFTV1 is similar to other members of the class *Caudoviricetes*, its mechanism of adsorption to the cell is rather unusual and likely involves interaction with two distinct parts of the virion, both the head and tail. Interestingly, this mode of adsorption and host recognition is not unique to archaeal viruses and has also been observed for some bacteriophages and GTAs. This demonstrates that this mechanism might be conserved among diverse mobile genetic elements like viruses and GTAs of bacteria and archaea and more common than previously thought. This also highlights the importance of studying new model viruses in order to uncover the full diversity of microbial viral infection strategies.

4 Material & Methods

4.1 Virus and Archaeal Strain and their Growth Conditions

Haloferax gibbonsii LR2-5 and HFTV1 [17] were cultured as described previously [45-47]. For details, see Text S1 in the supplemental material.

4.2 Plaque Assay and Preparation of Virus Stock

For preparation of virus stock, semiconfluent plates were produced using the double-layer method [17,18]. Plaque and spot assays were performed as described previously [18]. For details, see Text S1.

4.3 HFTV1 Stability

The temperature stability of HFTV-1 was examined by incubating small aliquots (500 μ L) of HFTV1 stocks at different temperatures ranging from 4°C to 100°C. After 1 h or 5 h of treatment in a thermoshaker, the infectivity was determined by plaque assay.

To test the effects of NaCl concentration on HFTV1 infectivity, virus stock was diluted 1:1,000 in 23% MGM medium that contained variable concentrations of NaCl (0 to 5 M) and a constant concentration of 113 mM MgCl₂, 108 mM MgSO₄, and 71 mM KCl, (61 mM Tris-HCl, pH 7.5). After 2 h and 24 h of incubation at room temperature, the number of infective particles was determined with plaque and spot assays.

4.4 Adsorption Assay and Constant Calculation

Haloferax gibbonsii LR2-5 cells from the mid-logarithmic growth phase were infected at a multiplicity of infection (MOI) of 10^{-3} and viral adsorption was monitored by plaque assay as described previously [23]. For details, see Text S1.

4.5 Receptor Saturation Assay

H. gibbonsii LR2-5 was grown to an OD at 600 nm (OD_{600}) of 0.65 to 0.85 (1×10^8 to 6×10^8 CFU/mL) and infected using MOIs from 0.001 to 400 at 37°C. At 30 min postinfection, cells were removed by centrifugation ($15,000 \times g$, 2 min, 4°C), and the number of nonadsorbed particles in the supernatant was determined by plaque assay and compared to the amount of virus present in a cell-free control (MGM medium).

4.6 Superinfection Assay

H. gibbonsii LR2-5 cells (OD_{600} of 0.6) were infected with HFTV1 using an MOI of 10 and incubated at 37°C (with shaking, 140 rpm) for 1.5 h. Cells were washed twice with 37°C warm 23% (wt/vol) MGM medium in two rounds of centrifugation ($4,000 \times g$, 20 min, 30°C). Subsequently, infected cells were subject to a second round of infection using an MOI of 0.1. The number of unadsorbed viral particles in the supernatant was determined by plaque assay and compared to a cell-free control and cells that underwent only one round of infection at MOI of 0.1.

4.7 Infection Assay and Virus Life Cycle

The life cycle of HFTV1 was investigated by infecting *H. gibbonsii* LR2-5 culture with a cell density of OD_{550} of 0.8 and $\sim 8.5 \times 10^8$ CFU/mL at mid-exponential growth phase using an MOI of 10. The turbidity of infected and uninfected culture was monitored at OD_{550} . Samples were collected at several time points postinfection. Cells and cellular debris were removed by centrifugation ($4,000 \times g$, 20 min, 4°C) and supernatants were analyzed by plaque assay.

4.8 Production and Purification of HFTV1 Particles

To purify HFTV1, *H. gibbonsii* LR2-5 was grown to mid-exponential phase to an OD_{550} of 1.2 and then infected at an MOI of 10. HFTV1 particles were produced in liquid culture by infecting logarithmically growing LR2-5 cells at OD_{550} of 1.2 (1.9×10^9 CFU/mL) using an MOI of 10. After the lysis, the cells were removed by centrifugation ($10,800 \times g$,

30 min, 5°C). Alternatively, the viruses were purified from a virus stock. Viruses were precipitated with two-step polyethylene glycol (PEG)-NaCl precipitation as described previously [17]. For details, see Text S1.

4.9 Time-laps Microscopy

Haloferax gibbonsii LR2-5 cells were grown in Casamino Acids (CA) medium containing 18% salt water (SW). Sample preparation and light microscopy was carried out in a similar fashion as described in [48]. Cells were imaged on an agarose pad with nutrients at $\times 100$ magnification using an Axio Observer.Z1 (Zeiss) inverted microscope equipped with a heated (45°C) XL-5 2000 incubator running ZEN software. Cells were recorded with 10- to 15-min time-lapse movies. Microscopy images were processed to analyze cell shapes using Fiji and the MicrobeJ plugin [49,50]. For details, see Text S1.

4.10 Transmission Electron Microscopy

Samples (5 μ L) of $2\times$ purified HFTV1 or *H. gibbonsii* LR2-5 cells from early exponential phase ($OD_{600} \sim 0.1$) infected with purified HFTV1 (MOI of 150) were adsorbed onto Formvar carbon-coated copper grids for 1 min and stained with 2% (wt/vol) uranyl acetate for 20 s. Imaging was performed with a Hitachi 7800 TEM (120 kV), equipped with a LaB6 filament and coupled to an Emsis Xarosa complementary metal oxide semiconductor (CMOS) camera (Emsis GmbH, Muenster, Germany).

4.11 Isolation and Sequencing of HFTV1-resistant Mutants of *H. gibbonsii* LR2-5

Resistant mutants were selected from *H. gibbonsii* LR2-5 liquid cultures after infection with HFTV1. Cells from mid-log phase (OD_{600} of 0.65) were infected at an MOI of 10, and cultures were incubated at 37°C for 24 h. The lysate was plated in different dilutions on 20% MGM plates and incubated at 37°C for 7 days to isolated individual resistant mutants. Single colonies were purified by streaking on a new plate three times. Resistance to HFTV1 was confirmed by spot-on-lawn assays as described previously [18].

4.12 Annotation of Genomic Variants in Escape Mutants

DNA was extracted as described previously [18] and subjected to Illumina sequencing. Reads were mapped to the *H. gibbonsii* LR2-5 genome, and single and small nucleotide variants were identified. For details, see Text S1.

Data Availability: The three Illumina sequencing libraries supporting the findings of this study are openly available from the European Nucleotide Archive (study, accession no. PRJEB53889; samples, accession nos. ERS12284977, ERS12284978, and ERS12284979).

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Supplemental Material

Supplemental Text

Virus and Archaeal Strain and their Growth Conditions

Haloferax gibbonsii LR2-5 and HFTV1 [1] were aerobically grown at 37°C in modified growth medium (MGM) [2] containing artificial salt water (SW) [2,3]. A 30% (v/v) stock of SW was produced and diluted to make the working media. Broth, solid agar, and soft-agar media contained 23%, 20%, and 18% (w/v) SW, yeast extract (0.1% w/v Oxoid) and peptone (0.5% w/v Oxoid), respectively. Agar (Bacto-Agar) was added to prepare solid (14 g/liter) or top-layer (4 g/Liter) media. *Hfx. gibbonsii* cells used for electron microscopy experiments were grown in selective CA (casamino acids) medium [4] prepared with 18% (w/v) SW buffered with 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (pH 7.0) and supplemented with casamino acids (Difco) to a final concentration of 0.5% (w/v).

Plaque Assay and Preparation of Virus Stock

An appropriate dilution series of HFTV1 stock was prepared using test tubes containing 5 mL of MGM broth. The virus was spread with its host cells using the double layer method. Dense host culture (300 μ L) was mixed with 100 μ L of appropriate virus dilution and 3 mL of melted soft agar and spread over a plate. Control plates with top agar and host cells, without the virus were included. The experiment was performed by using parallel plates. Plates were incubated for 2–3 days at 37°C and the number of infectious viruses i.e. the titer (PFU/mL) was determined.

For spot assay (titration), a mixture of host culture and melted soft agar was pour on a plate and the lawn was solidified and dried at room temperature for approximately 30 minutes. Virus dilutions (10 μ L) were pipetted on the solid lawn. A control spot of medium only was included on each plate. The virus drops were dried for 30 minutes at room temperature prior to incubation for 4 days at 37°C.

For virus stock preparation, semi-confluent plates were produced by plaque assay. The top-layer agar was collected and 2 mL MGM medium was added per a collected plate. The lysate was incubated with aeration for 1.5 h at 37°C. HFTV1 particles were harvested by centrifugation (4,000 \times g, 20 min, 4°C) and stored until use at 4°C.

Time-laps Microscopy

For imaging, cultures were diluted to an OD₆₀₀ of 0.1 and 5 µl cell suspension was spotted on an agarose pad containing nutrients (0.3% [w/v] agar, 18% SW supplemented with casamino acids). After the sample was dried, the pad was flipped up-side down into a Delta T Dish (Bioptechs Inc.) and the dish was closed with a lid to avoid evaporation. The cells were observed at 100x magnification in the Phase contrast mode (PH3) using an Axio Observer.Z1 inverted microscope (Zeiss). The imaging procedure was carried out at 45°C over-night for 16.5 h with image acquisition every 10 or 15 min.

The FIJI/ImageJ plugin MicrobeJ was used to calculate the surface area of the cells from phase-contrast images taken at different time points after infection. MicrobeJ automatically detects and evaluates the particles in the image. The Plugin allows constraining the attributes in the user interface in order to exclude certain particles (dividing or lysed cells) or include missed particles upon reanalysis. Particles with an area outside 0.3 µm² in the field were rejected. With this procedure, the cell area of HFTV1 infected *Hfx. gibbonsii* LR2-5 cells was determined at the indicated times and shown in a boxplot diagram in Figure S3.

Adsorption Assay

Haloferax gibbonsii LR2-5 cells (optical density at 600 nm [OD₆₀₀] = 1.0; 2 x 10⁹ CFU/ml) were infected at a multiplicity of infection (MOI) of 10⁻³ and incubated aerobically at 37°C. HFTV1 infection was monitored in 50 mL reaction vessels. Samples were taken at 0.25, 1, and 2 min intervals and adsorption was stopped by diluting the samples 1:100 in ice-cold broth. The cells were removed by centrifugation, and the reduction in the number of PFUs in the supernatant was determined by plaque assay using *Hfx. gibbonsii* LR2-5 as a host. The adsorption rate constant was calculated as described previously using the formula, $k = 2.3/Bt \times \log_{10}(P_0/P)$ [5] where B represents the concentration of host cells, P₀ represents the concentration of free viruses at time point zero, and P represents the concentration of free viruses at the end of the experiment after a period of time t.

Purification of HFTV1 Particles

First, 4% (w/v) PEG 6000 (no NaCl added due to the high salinity of the medium) was dissolved by magnetic stirring for 1 h at 4°C. After centrifugation (10,800 × g, 40 min, 5°C), more PEG was added to the supernatant to obtain a final concentration of 11% (w/v). After dissolution of PEG and centrifugation (as above), the virus precipitate was dissolved in 18% SW buffer (around 50 times concentrated compared to the original growth volume). The aggregates were removed by centrifugation (6,300 × g, 10 min, 5°C). The viruses were purified by rate zonal centrifugation in 10–40% (w/v) sucrose gradient in Sorvall TH641 rotor (18% SW buffer, 210,000 × g, 1.5 h, 15°C). After centrifugation, the gradient fractions (12 1-mL fractions) were analysed for their infectivity, absorbance (A_{280}), and purity by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). For more pure particles, rate zonal centrifugation was followed by equilibrium (CsCl $r=1.45$ g/mL in 18% SW) and differential centrifugation as described previously [1] (114,000 × g, 2 h, 5°C). Viruses were resuspended in 18% SW buffer.

Protein concentrations were determined by Bradford assay [6] using bovine serum albumin as a control. Specific infectivity (pfu/ mg of protein) was calculated as the ratio of titer to concentration. Proteins were analysed by tricine-SDS-PAGE [7] using 14% and 4% (w/v) acrylamide in separation and stacking gels, respectively.

Annotation of Genomic Variants in Escape Mutants

To obtain genomic DNA of escape mutants, cells of *Hfx. gibbonsii* LR2-5 were grown aerobically at 37°C with shaking (120 rpm) to an OD_{600} of 1.2 in MGM were collected by centrifugation (30 min at 5,000 g, room temperature) and processed for DNA extraction by Eurofins NGS Lab Constance (Constance). Cells were resuspended in ST buffer (1 M NaCl, 20 mM Tris-HCl, pH 7.5). Lysis solution (100 mM EDTA pH 8.0, 0.2% SDS) was then added to the cells and 1 mL ethanol was layered on top of the aqueous solution to form two phases. DNA was spooled onto a capillary at the interface. The DNA was washed in ethanol and resuspended in 500 μ L TE buffer (10 mM Tris-HCl pH 8.0, 10 mM EDTA). DNA was precipitated by adding 50 μ L 3 M sodium acetate (pH 5.2) and 400 μ L isopropanol and collected by centrifugation (16,000 g, 5 min). The pellet was washed with 70% ethanol and dried and the DNA was resuspended in 100 μ L TE buffer. The sample was mixed with RNase A (1 μ L of 30 mg/mL in 50% glycerol, Sigma-Aldrich Cat. R 4642: 45°C for ≥ 1 hour). The DNA was left at 4°C overnight for resuspension. Three

2x150 bp genomic libraries were prepared at Eurofins and sequenced on a NovaSeq 6000 platform.

To assess if the resistant clones are escape mutants, we compared sequencing data from the clones to the *Hfx. gibbonsii* reference. We mapped the Illumina reads with minimap2 [8] in short-read mode onto the reference genome for *Hfx. gibbonsii* strain LR2-5 and HFTV1 (NCBI accessions: CP06320 [5-8] and MG550112). Single and small nucleotide variants were called with freebayes ('-p 1 -0 -F 0.2') [9]. After filtering out low quality and low frequency loci, no significant variants were detected. The large deletion in mutant $\Delta 16k$ was identified from a complete lack in coverage in the sequencing depth profile and the small insertions in $\Omega 15$ and $\Omega 48$ (not detected by freebayes due to size) were identified visually in IGV [10] at the same locus as the deletion in $\Delta 16k$. We confirmed the insertions by local reassembly with SPAdes [11] and gene-reannotation with prodigal. Changes on the protein-level in $\Omega 15$ and $\Omega 48$ were analyzed based on multiple sequence alignments generated with MAFFT [12] and visualized with AliView [13]. The residual presence of viral DNA in the samples was analyzed by comparing the coverages across host sequences and the viral genome.

Supplemental References

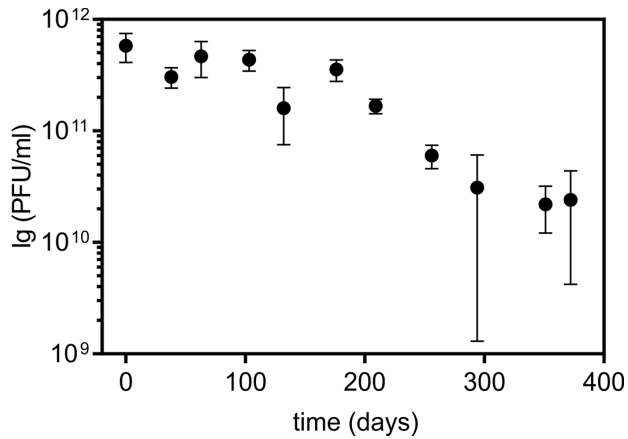
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Supplemental Tables

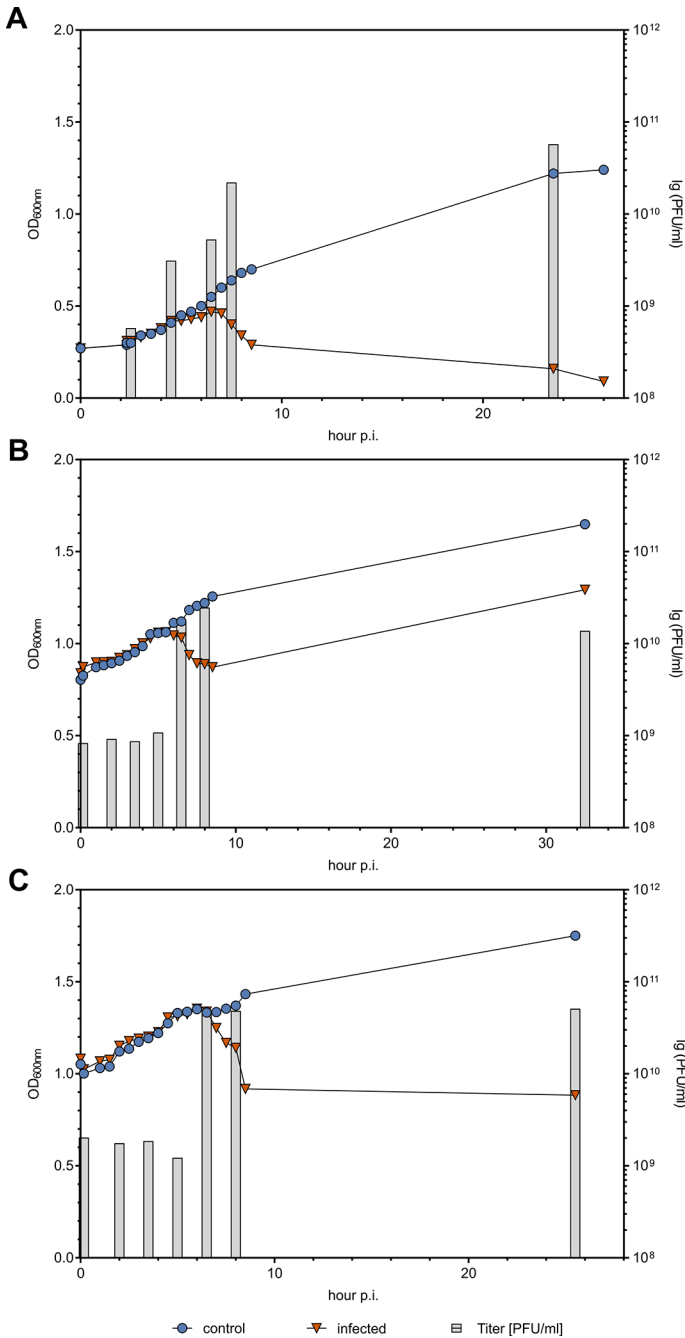
Table S1. Sequence read counts from Illumina Sequencing of *Haloferax gibbonsii* LR2-5 escape mutants

	Sequence	Sequence length	Mean coverage	Median coverage
Ω48	CP063205.1	2999641	216.43	217
	CP063206.1	608598	207.2	207
	CP063207.1	322970	205.88	206
	CP063208.1	65035	303.81	303
	MG550112.1	38059	7944.29	7948
Δ16k	CP063205.1	2999641	372.95	375
	CP063206.1	608598	356.58	358
	CP063207.1	322970	318.6	321
	CP063208.1	65035	634.89	635
	MG550112.1	38059	1.54	1
Ω15	CP063205.1	2999641	440.16	445
	CP063206.1	608598	448.64	452
	CP063207.1	322970	397.4	401
	CP063208.1	65035	671.6	673
	MG550112.1	38059	7895.13	7918

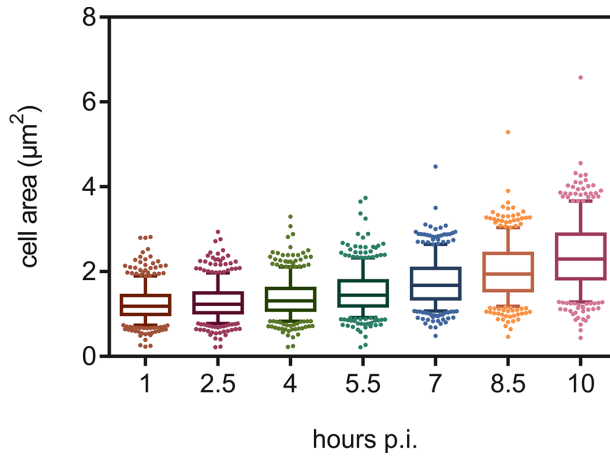
Supplemental Figures



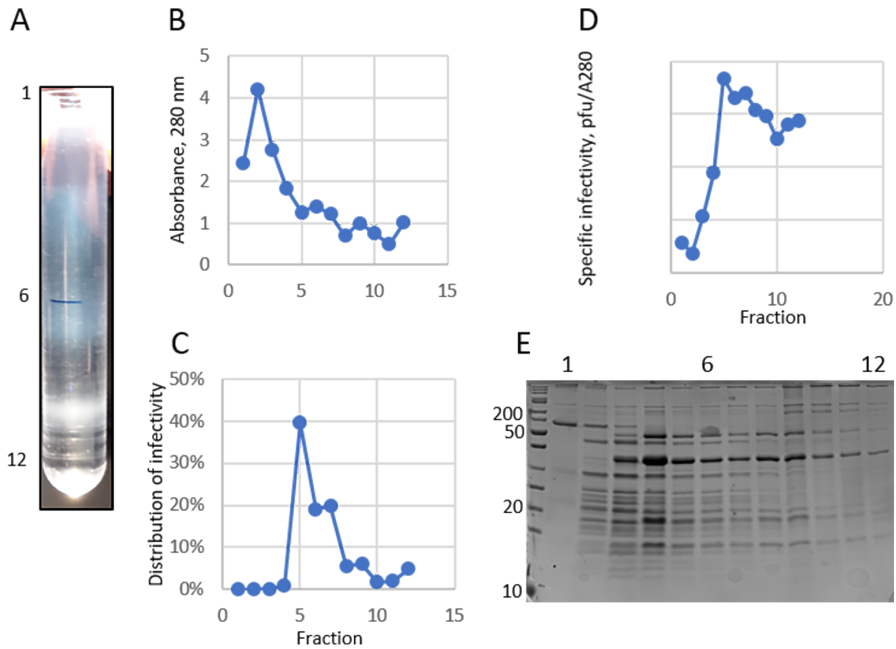
Supplementary Figure S1. HFTV1 is stable for at least 6 months at 4°C. The infectivity of HFTV1 agar stock was monitored over a 12-month period. The stock was stored at 4°C and the titer (PFU/mL) was determined by plaque assay. Scale bars represent two technical replicates.


Supplementary Figure S2.
Single-step growth curves of HFTV1. *H. gibbonsii*

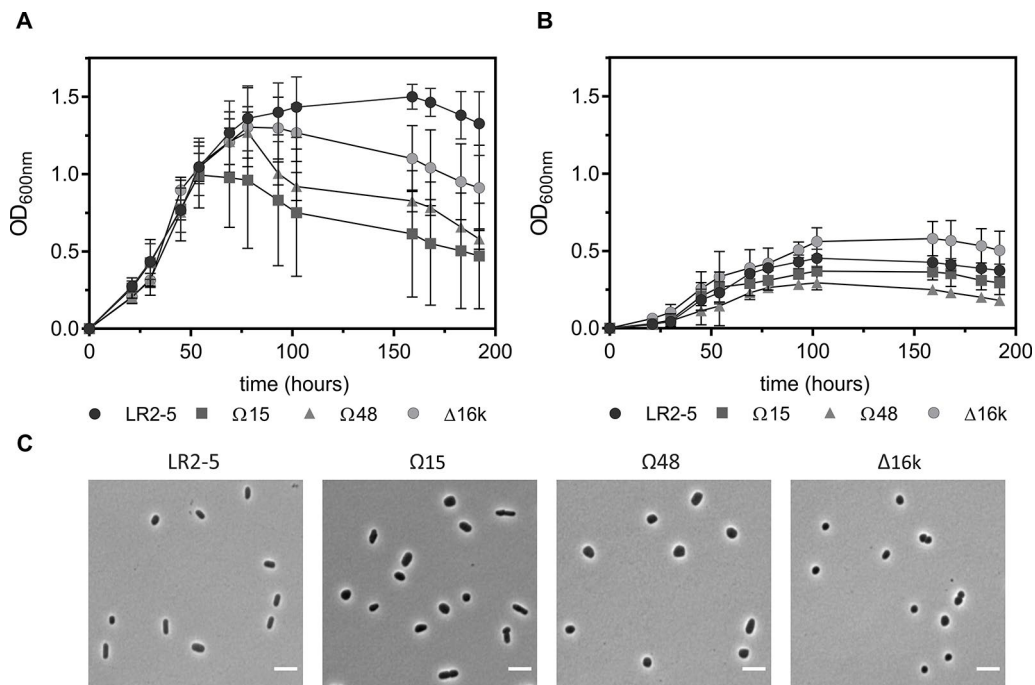
LR2-5 cells were grown to logarithmic phase and infected with an MOI of 10 at 37°C. (A) Cultures infected at OD_{600} of 0.3 ($\sim 1.0 \times 10^7$ CFU/mL), (B) OD_{600} of 0.8 ($\sim 2.0 \times 10^9$ CFU/mL), and (C) OD_{600} of ($\sim 4.0 \times 10^9$ CFU/mL). To remove unadsorbed viral particles the cells were washed thoroughly 20 min post infection (p.i.) and transferred to fresh medium at 37°C. The growth curves of infected (triangles) and uninfected (circles) cultures and the number of free viruses (PFU/mL) are shown.



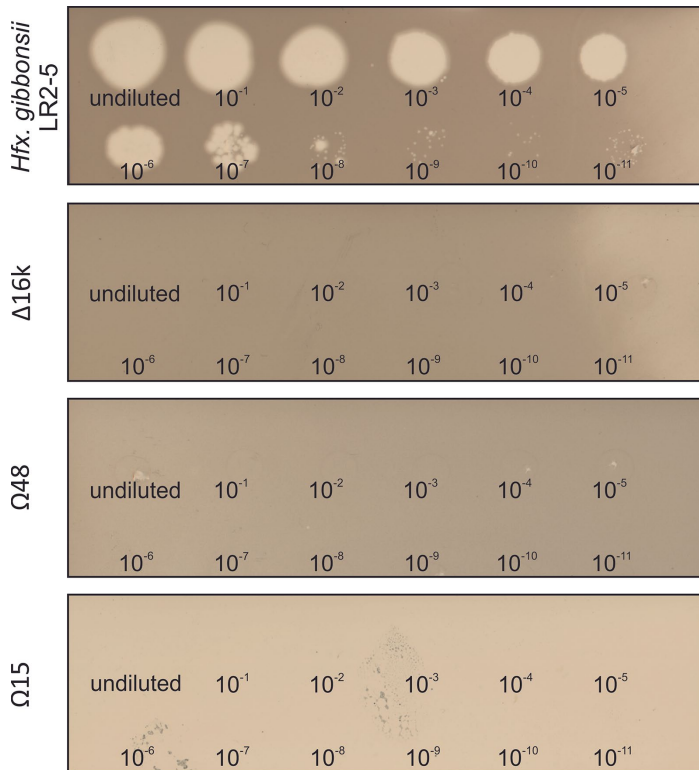
Supplementary Figure S3. Cell size increase during time-course of infection. Box plots showing cell area distributions of *H. gibbonsii* LR2-5 cells infected with HFTV1. Boxes represent values from >600 cells analyzed at 1 to 10 h p.i. (1.5 h intervals). After 10 hpi cell area increased ~1.5 fold. A line within the box marks the median. The upper boundary represents the 75th percentile. Lower whisker represents minimum values and the upper whisker represents maximum values.



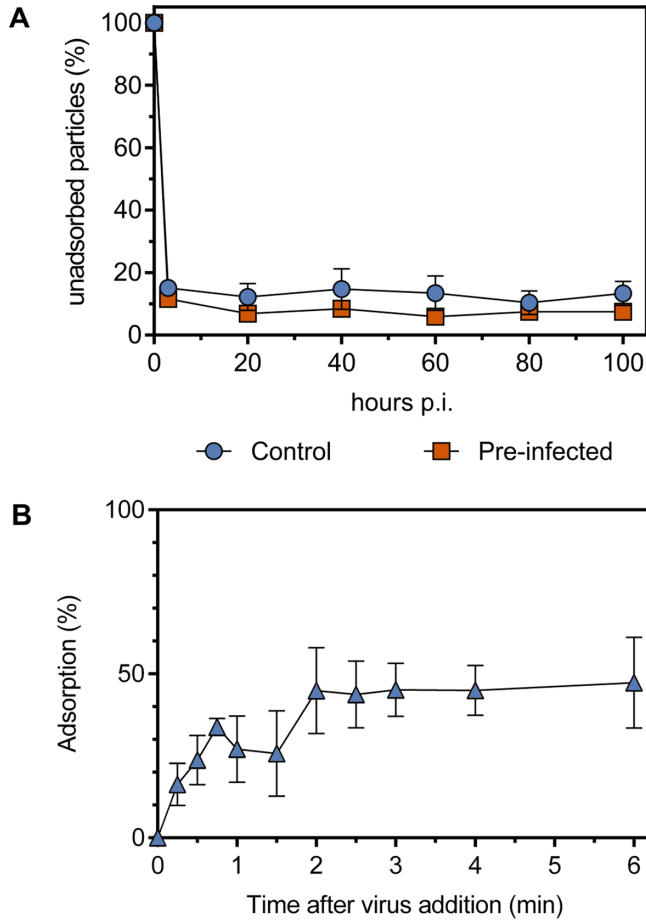
Supplementary Figure S4. Improved purification of HFTV1 particles by rate zonal centrifugation in sucrose. (A) Polyethylene glycol-NaCl precipitated HFTV1 particles were separated in a linear 10-40% (wt/vol) sucrose gradient (18% SW buffer; 210 000 \times g, 1.5 h, 15°C; Sorvall TH641 rotor) and fractionated into 12 fractions (numbering shown on left). Fractions were analysed by their (B) absorbance at 280 nm, (C) infectivity (total PFUs), (D) specific infectivity (PFU/A₂₈₀), and (E) protein content by Coomassie blue stained SDS-PAGE gel. Fraction numbers are shown on top. Mass marker (kDa) is on left.



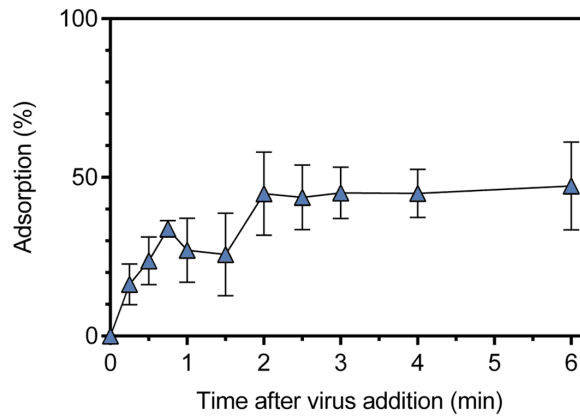
Supplementary Figure S5. Growth of *H. gibbonsii* LR2-5 and its escape mutants in liquid culture. (A) Typical Growth curve of *H. gibbonsii* LR2-5, Ω15, Ω48, and Δ16k in MGM medium with 18% SW. The average optical density was calculated from three independent technical replicates. Error bars represent the standard deviation. (B) Typical Growth curve of *H. gibbonsii* LR2-5, Ω15, Ω48, and Δ16k in CA medium with 18% SW. (C) Phase contrast light microscopy images show the cell shapes of *H. gibbonsii* LR2-5 (OD₆₀₀ of 0.45) and its escape mutants Ω15 (OD₆₀₀ of 0.44), Ω48 (OD₆₀₀ of 0.26), and Δ16k (OD₆₀₀ of 0.31) at mid-exponential growth phases.



Supplementary Figure S6. Susceptibility of *Haloferax gibbonsii* LR2-5 and escape mutants to HFTV1. Spot-on-lawn assay conducted with lawns of *H. gibbonsii* LR2-5, Δ16K, Ω48, and Ω15. Different dilutions of HFTV1 lysate (undiluted 3×10^{11} PFU/mL) were spotted on the respective host lawns and incubated for 4 days. Clearing of the cellular lawn appeared only on *H. gibbonsii* LR2-5 lawns, whereas no indication of cell lysis was observed when HFTV1 lysate was spotted on the host lawns of the escape mutants Δ16K, Ω48, and Ω15.



Supplementary Figure S7. Superinfection exclusion assay. *H. gibbonsii* LR2-5 cells were infected with an MOI of 10 and incubated for 1.5 hours at 37°C. Unbound particles were removed by washing and the cells were subjected to a second round of infection with HFTV1 at an MOI of 0.1. Blue circles show the adsorption kinetics of particles to uninfected control-cells. Orange squares represent the adsorption to cells pre-infected with HFTV1. Error bars represent the standard deviation of three independent experiments. If the error bars are not visible, the deviation could not be resolved graphically.



Supplementary Figure S8. Adsorption efficiency of HFTV1 to the $\Delta 16k$ escape mutant of *H. gibbonsii* LR2-5. (A) The adsorption efficiency of HFTV1 to the $\Delta 16k$ escape mutant of LR2-5 was determined using cells at the mid logarithmic growth phase (OD_{600} of 1.0, $\sim 2 \times 10^9$ CFU/mL) which were infected with HFTV1 using an MOI of 0.001 at 37°C. The number of unbound virus particles was determined at 0 to 6 minutes post infection by plaque assay. Error bars represent standard deviation from three experiments.

Supplemental Movies

Supplementary Movie S1. Phase contrast time-laps imaging of *Haloferax gibbonsii* LR2-5 infected with HFTV1. Cells from early log growth phase (OD_{600} of 0.2) were infected with HFTV1 at an MOI of 10. Cells were grown on an agarose nutrition pad in a thermomicroscope at 45°C and imaged every 10 minutes. Time post infection is indicated in hours.

Supplementary Movie S2. Phase contrast time-laps imaging of non-infected *Haloferax gibbonsii* LR2-5 cells. Cells were grown on an agarose nutrition pad in a thermomicroscope at 45°C and imaged every 15 minutes. Time post infection is indicated in hours.

