



Ice nucleation by viruses and their potential for cloud glaciation

Michael P. Adams^{1, #}, Nina S. Atanasova^{2, 3, #}, Svetlana Sofieva^{2, 3}, Janne Ravantti³, Aino Heikkinen^{2 +}, Zoé Brasseur⁴, Jonathan Duplissy^{4, 5}, Dennis H. Bamford^{3, *} & Benjamin J. Murray^{1 *}

¹ - Institute for Climate and Atmospheric Science, School of Earth and Environment, University of Leeds, Leeds, UK

5 ² - Finnish Meteorological Institute, Helsinki, Finland

³ - Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Finland

⁴ - Institute for Atmospheric and Earth System Research / Physics, Faculty of Science, University of Helsinki, Finland

⁵ - Helsinki Institute of Physics, University of Helsinki, Helsinki, Finland

10 # Authors contributed equally to the work

+ Now at the Institute for Molecular Medicine Finland, HiLIFE, University of Helsinki, Helsinki, Finland

Correspondence to: Dennis.Bamford@helsinki.fi and B.J.Murray@leeds.ac.uk

Abstract. In order to effectively predict the formation of ice in clouds we need to know which subsets of aerosol particles are effective at nucleating ice, how they are distributed and where they are from. A large proportion of ice-nucleating particles (INPs) in many locations are likely of biological origin, and some INPs are extremely small being just tens of nanometers in size. The identity and sources of such INPs are not well characterized. Here, we show that several different types of virus particles can nucleate ice, with up to about one in twenty million virus particles able to nucleate ice at -20°C. In terms of the impact on cloud glaciation, the ice-nucleating ability (the fraction which are ice nucleation active as a function of temperature) taken together with typical virus particle concentrations in the atmosphere lead to the conclusion that virus particles make a minor contribution to the atmospheric ice-nucleating particle population in the terrestrial influenced atmosphere. However, they cannot be ruled out as being important in the remote marine atmosphere. It is striking that virus particles have an ice-nucleating activity and further work should be done to explore other types of viruses for both their ice-nucleating potential and to understand the mechanism by which viruses nucleate ice.

15
20
25



1 Introduction

The formation of ice in clouds is critically important for the planet's radiative balance and our prediction of future changes in climate with increased greenhouse gas concentrations (Vergara-Temprado et al. 2018; Tan, Storelvmo, and Zelinka 2016). Ice-nucleating particles (INPs) have the potential to cause supercooled liquid cloud droplets, present in mixed-phase clouds, to freeze at temperatures greater than homogenous freezing, which can drastically alter cloud properties such as albedo, composition and lifetime (Murray et al. 2012; Hoose and Möhler 2012; Kanji et al. 2017). Despite the potential importance of INPs, there is still a lack of knowledge regarding their characteristics, sources, and ultimately their temporal and spatial distribution around the globe.

Our current knowledge of atmospheric INPs (under mixed-phase cloud conditions) suggests a number of potentially important aerosol types, including mineral dust, marine organics and terrestrial bioaerosols (DeMott et al., 2010; Kanji et al., 2017). The characteristics and source regions for mineral dust are relatively better understood than other potentially important INPs, and mineral dust from both high (Sanchez-Marroquin et al. 2020; Tobo et al. 2019) and low latitude sources is thought to be the dominant INP around much of the globe at temperatures < -20 °C. Marine organics and terrestrial bioaerosols have both been demonstrated to play a major role in the global INP burden, but the nature of these INPs are less well understood than that of mineral dust. Marine organics are of particular importance in remote marine regions where there is little mineral dust (T. W. Wilson et al. 2015; Burrows et al. 2013). Terrestrial bioaerosols are thought to outcompete mineral dust in the terrestrial mid-latitudes at temperatures > -20 °C, however their source(s) and nature are at present poorly understood (Conen et al., 2016; McCluskey et al., 2018; O'Sullivan et al., 2018; Vergara-Temprado et al., 2017).

Known INPs of biological origin include bacteria, fungi, pollen and marine organics amongst others (Kanji et al. 2017). Bacteria and fungi exhibit ice nucleation due to the presence of ice-nucleating proteins (Green and Warren 1985; Pouleur et al. 1992; Lindow, Army, and Upper 1982), whilst the ice-nucleating ability of pollen has been linked to polysaccharides (Pummer et al. 2012; Dreischmeier et al. 2017). Marine organic INPs, associated with sea spray, are thought to be biogenic and are often smaller than 0.22 μm , but it is currently not clear exactly what these ice-nucleating particles are and there may be multiple marine INP types (Creamean et al., 2019; DeMott et al., 2016; Irish et al., 2017, 2019; Schnell et al., 1975; Wang et al., 2015; Wilson et al., 2015). Compared to non-biological INPs, some microorganisms such as specific bacteria or fungi nucleate ice at relatively high temperatures; for example, the best-studied ice nucleating bacterium, *Pseudomonas syringae*, can nucleate ice at temperatures up to -2 °C (Morris et al., 2004; Morris et al., 2013). Despite the ice nucleation potential of primary biological aerosol particles, recognized since 1970s (Schnell et al. 1976), the global distribution and sources of biological INPs remain poorly understood (Murray et al. 2012; Kanji et al. 2017). Hence characterizing the ice nucleating ability of the various categories of biological aerosol particles is important.

In bacteria, membrane proteins are thought to interact with water and impose order in supercooled water in such a way as to promote nucleation of ice. Pandey et al. (2016) demonstrated that in the case of *P. syringae* patterned hydrophilic-hydrophobic



regions due to the interactions of amino acids belonging to the membrane protein led to the increased ordering of water molecules coupled with efficient removal of thermal energy from the surrounding water molecules into the bacterial cell. This mechanism could potentially protect microorganisms at sub-zero temperatures and preserve their viability and infectivity in the atmosphere (Wilson, Grogan, and Walker 2012; Morris, Monteil, and Berge 2013). Whether or not a bacterium has the potential to produce ice-nucleating proteins is dependent on the presence of an ice nucleation gene. At present, eight ice-nucleating proteins are known and reviewed in the protein database UniProt, each with an associated gene (protein IDs: O33479, P06620, Q47879, P16239, O30611, P09815, P20469, P18127). It is thought that a single functional ice nucleation protein gene in bacteria is both necessary and sufficient for ice nucleation activity. The INA of a bacterium that has a gene for the ice-nucleating protein in its genome depends on the expression of the gene (i.e., if the protein coded by the gene is actually produced by the bacterium), the integration of the protein into the outer membrane of the bacterial cell and stabilization of the protein complex by the surrounding membrane constituents.

Viruses are a presently under-studied source with respect to their potential as atmospheric biological INPs. Very little is known about viruses in the atmosphere in general, and even less about their potential to influence cloud properties through cloud glaciation. The only studies we are aware of in which the ice-nucleating ability of a virus was examined was that of Junge & Swanson (2008) (who studied the polar Colwellia phage virus) and Cascajo-Castresana et al. (2020) who studied a series of common proteins and a single virus. The former found that these virus particles did not nucleate ice in their experimental system. The latter observed ice nucleation activity in the Tobacco mosaic virus (TMV), a plant virus that infects the family of Solanaceae such as tobacco, tomato, or pepper. TMV was shown to be above the baseline of the buffer solution it was suspended in, and it was noted in the study that whilst TMV had a lower onset freezing temperature of other samples in the study (a range of proteins), when normalized to cumulative active site density it was more active.

Compared to bacteria and other micron-sized, single-celled microorganisms, viruses are considerably smaller (from ~25 nm in diameter; except for the nucleocytoplasmic large DNA viruses that are cellular size). The small size of virus particles means that their atmospheric lifetime has the potential to be on the order of many days to weeks in the atmosphere, although this will depend on the size of the particles that they are internally mixed with. This is considerably longer than the lifetime of larger biological particles, especially those larger than ~10 μm , which have lifetimes of only hours (Grythe et al. 2014; Reche et al. 2018) and therefore have atmospheric abundances which decrease rapidly during transport (Hoose, Kristjánsson, and Burrows 2010).

In addition to supermicron entities such as bacteria, submicron sized biological particles have also been shown to be effective ice nucleating particles (O'Sullivan et al. 2015). For example, it has been shown that there are biological INPs belonging to fungal and pollen samples at sizes below 200 nm (Pummer et al. 2012; Fröhlich-Nowoisky et al. 2015). Fertile soil samples when dispersed in water and filtered, have also been shown to have a significant number of ice-nucleating particles below 200 nm (O'Sullivan et al. 2015; Hill et al. 2016). O'Sullivan et al. (2015) showed that some ice nucleation persisted in fertile soil



90 samples filtered to 1000 kDa, however ice nucleation above $-10\text{ }^{\circ}\text{C}$ was removed by these filtrations. Decayed plant litter was shown to have comparable INP concentrations before and after filtration through 200 nm filter pores, and retained a fraction of these INPs when further filtered through 20 nm filter pores (Vali et al. 1976). Ice-nucleating particles below 200 nm were measured in North American Arctic snow samples and in precipitation from North China temperate grassland (Du et al. 2017; Rangel-Alvarado, Nazarenko, and Ariya 2015). The snow samples were shown to be of biological origin and subsequently
95 tested for virus-like structures, of which none was observed. Despite this, the authors stated they could not preclude viruses as a potential explanation for the observed ice-nucleating activity, based on the size of the INPs and their likely origin. Measurements of INPs in the Arctic sea surface microlayer showed that most of the observed ice nucleation (in the immersion mode) was caused by particles between 0.02 and 0.2 μm in size and were heat labile; viruses were suggested as a potential explanation (Irish et al. 2017; T. W. Wilson et al. 2015). Atmospheric measurements made in the Arctic showed the presence
100 of atmospheric INPs in the size range 150 - 340 nm (Creamean et al., 2019; Creamean et al., 2018). Size resolved measurements made in a boreal forest in Hyttiälä, Finland showed an instance in which INPs in the size range 250 - 500 nm dominated the atmospheric INP burden at temperatures $> -22\text{ }^{\circ}\text{C}$, whilst measurements made at near surface level locations in the U.K showed INPs present at sizes below 250 nm (Porter et al. 2020). There is a growing body of evidence that suggests there is a reservoir of currently unidentified biological particles in the fine mode ($<250\text{ nm}$) present in soil/plant life, the oceans and the
105 atmosphere. In this study, we test the hypothesis that viruses are a potential candidate for the source of these fine mode INPs.

It has been estimated that there are $\sim 10^{31}$ virus particles in the biosphere (Whitman, Coleman, and Wiebe 1998), with approximately 10^7 virus particles per ml of seawater, 10^8 - 10^9 per ml in marine surface sediments (Suttle 2007; 2005) and 10^8 - 10^9 per gram of soil in different types of terrestrial environments (Srinivasiah et al. 2008). Numerous studies indicate that there are approximately 10-100 times more viruses compared to their host cells in any given environment (Srinivasiah et al. 2008;
110 Cai et al. 2019). With respect to viral abundance in the atmosphere, there is at present a dearth of knowledge. Rastelli et al. (2017) measured the viral abundance in both the seawater microlayer and the aerosol phase directly above using a bubble generator system designed to mimic wave breaking in open seawater. Virus concentrations for seawater and sampled air were 5×10^{11} virus particles m^{-3} and 0.3 - 3.5×10^5 virus particles m^{-3} , respectively. Virus particles were measured from outdoor air samples using a filter based technique taken at a University campus, with the atmospheric virus particle concentration being
115 measured as $1.2 \pm 0.7 \times 10^6$ virus particles m^{-3} (Prussin, Garcia, and Marr 2015). The spatial and temporal variability of airborne viruses were investigated in a series of different locations (residential district, forest and an industrial complex), with concentrations of 1.7×10^6 to 4.0×10^7 virus particles m^{-3} being measured (Whon et al. 2012). Overall, the range of outdoor virus concentrations recorded in the literature range between 0.3×10^5 to 4.0×10^7 virus particles m^{-3} . It is likely that these numbers do not represent the full variability of virus particle concentration due to the scarcity of measurements.

120 Despite the large number of virus particles measured in various environments there are a relatively small number of different particle structures a virion (an infective virus particle) can have. This is due to physical constraints of protein fold space that make up the virus particle architecture (Abrescia et al. 2012). Structurally similar viruses can have different host organisms



and different geographical source locations (Bamford 2003; Saren et al. 2005; Atanasova et al. 2012) . There are several observations of virus isolates with high genome identity originating from spatially distant environments (Atanasova et al. 2015; Pietila et al. 2012; Saren et al. 2005; Tschitschko et al. 2015). We have chosen virus particles for this study that represent several different symmetric or asymmetric virus architecture types, icosahedral, icosahedral with internal lipid membrane, icosahedral enveloped and lemon-shaped. As it would be beyond the realms of feasibility to test even a minute fraction of the 1031 different viruses in the biosphere, we took the approach that we believe allows us to investigate the maximum parameter space and test the hypothesis that virus architecture/structure controls ice-nucleating ability. In this study we present the ice-nucleating ability of viruses with these different architecture types, demonstrate the potential of different structural components in viruses to nucleate ice, and attempt to estimate the potential of viruses as a class of atmospheric ice-nucleating particles.

2 Methods

2.1 Virus growth, purification and production of Phi6 subviral particles

Virus particle suspensions were produced under carefully controlled conditions which resulted in suspensions of high purity; the details of this preparation are given here. Bacterial and archaeal strains and viruses used in this study are listed in Table S1. Bacterial host strains were aerobically grown in Luria-Berthani broth at 28 °C for *Pseudomonas syringae* pathovar *phaseolicola* HB10Y, and *P. syringae* LM2489, and at 37 °C for *Escherichia coli* HMS174 and *E. coli* C122 strains. Archaeal host strains were aerobically grown in 23 % Modified Growth Medium (MGM) at 37 °C (Nuttall and Smith 1993).

Here, 1× signifies once purified particles and 2× twice purified, concentrated virus sample. Bacteriophages PRD1 and Phi6 were 1× purified as described in Bamford et al. (1995). The 2× purification of PRD1 was performed (Bamford et al. 1995; Lampi et al. 2018). The PRD1 particles devoid of DNA (procapsids) were collected after 1× purification, during which the DNA containing particles sediment further along the sucrose gradient compared to the empty procapsids. The 1× purified Phi6 was further purified to 2× by density gradient ultracentrifugation in 20-70 % sucrose in 20 mM K-phosphate buffer pH 7.2 with 1 mM MgCl₂ (designated here as K-phosphate buffer) followed by concentration as described in Bamford et al. (1995). Viruses Phi8, Phi12, Phi13 and, Phi2954 were produced and precipitated according to Qiao et al. (2010), and the 1× purification was performed by rate-zonal ultracentrifugation in 5-20 % sucrose gradients in K-phosphate buffer, Sorvall AH629 rotor, 24 000 rpm, 50 min, 15 °C, followed by concentration using differential ultracentrifugation, Sorvall T865 rotor, 34 000 rpm, 3 h, 10 °C. All other viruses were purified to 1× preparations according to protocols described in Eskelin et al. (2019) (for PhiX174), Pietilä et al. (2009) (for HRPV-1), Pietila et al. (2012) (for HRPV-6), Demina et al. (2016) (for HCIV-1) and Bath et al. (2006) (for His1).

Phi6 subviral particles were prepared according to Bamford et al. (1995), modified by Eskelin & Poranen (2018) (for butylated hydroxytoluene treated particles). Phi6 NC were prepared by adding 1 % final concentration of Triton X100 to 1× purified Phi6 particles in K-phosphate buffer and incubating 30 min at 22 °C. The treated particles were collected by ultracentrifuge,



155 Ti1270 rotor, 30 000 rpm, 4 h, 15 °C. Particles were flushed three times with and resuspended in 0.5 ml K-phosphate buffer overnight at 5 °C.

The protein concentration of viral and subviral particles was measured by the Bradford assay using bovine serum albumin as a standard (Bradford 1976). Virus samples were analyzed by sodium dodecyl sulfate- 16% polyacrylamide gel electrophoresis (SDS-PAGE) (Olkkonen and Bamford 1989) to visualize viral protein profiles.

2.2 Search for ice nucleation motifs

160 Currently, there are eight referenced ice nucleation proteins identified from bacterial cells according to the public protein database (UniProt, <https://www.uniprot.org/>). The ice nucleation motifs (INMs) predicted based on these genes are short protein sequences conserved in this protein family. They are abundant for the ice nucleation proteins (IN proteins), but scarce in the rest of the bacterial genomes. The group of motifs specific for a protein family can serve as a functional fingerprint indicating similarities in structure and function. It was previously determined that INM3 corresponds to the clathrate structure
165 part of the protein responsible for ice nucleation activity in bacterial IN proteins (Gurian-Sherman and Lindow 1993; Kajava and Lindow 1993).

The INMs were acquired from SPRINT, an interface for PRINTS data bank of protein family fingerprints. SPRINT is a public domain database currently maintained at the University of Manchester (<http://130.88.97.239/dbbrowser/sprint/>). The INMs can be found in SPRINT by identifier ICENUCLEATN. All known ice nucleation motifs in IUPAC (International Union
170 of Pure and Applied Chemistry) nomenclature are listed in Table S2. Since some of the putative viral proteins are not fully characterized, we used protein INMs from SPRINT to build generalized nucleotide motifs. The annotated viral genomes were acquired from the National Centre for Biotechnology Information (NCBI) genome database (Table S3).

Ice nucleation motifs were searched for in the viral genomes using MEME (Multiple Em for Motif Elicitation) Suite 5.1.0. (Bailey et al. 2009). The search was performed using MCAST (Motif Cluster Alignment Search Tool) and FIMO (Find
175 Individual Motif Occurrences)-tools (Grant et al., 2011; Bailey & Noble, 2003). MCAST searches for input motifs in the query sequence for statistically significant clusters of non-overlapping occurrences. FIMO, in turn, searches for individual motif occurrences in the sequences, each motif independently. Each found occurrence was scored with p-value. The p-score thresholds for significant findings were set to 0.0001.

Putative IN-proteins were determined in the viral genomes based on the repetitiveness of IN motif occurrences in the
180 sequences, as well as the total INM coverage. The INM coverage is calculated from the total length of the protein sequence matching the INM sequences compared to the total length of the protein. The INMs were annotated to the sequences using Artemis 17.0.1 and the protein alignments were performed using Muscle 3.8.425 and visualized using Geneious Prime 2020.1.1. All the potential IN proteins are listed in Table 1.



2.3 Ice nucleation experiments

185 Samples for analysis of the ice nucleating activity of virus particles were prepared by diluting 1× or 2× purified virus particles to specific buffer solutions (Table S1) so that the final concentration of pfu/ml was 10^{10} - 10^{12} . Subviral particles were used without dilution. Virus host strains were collected by centrifugation (Eppendorf, 13 000 rpm, 5 min, 22 °C), diluted into the same buffer as the virus (Table S1), centrifuged (Eppendorf, 13 000 rpm, 5 min, 22 °C) and resuspended into buffer according to Table S1.

190 Ice nucleation experiments were carried out using the μ L-NIPI (nucleation by immersed particles instrument) (Whale et al. 2015). In brief, the μ L-NIPI analysis involved pipetting 1 μ L droplets (in which sample material was suspended) onto a hydrophobic-coated glass cover slip atop an aluminium cold stage, and cooled. Viral samples were vortexed for 30 seconds prior to being pipetted to ensure the particles were evenly distributed through the suspension. Droplet freezing was recorded using a camera, with the freezing temperature of each droplet recorded.

195 The cumulative fraction of droplets frozen on cooling to a temperature, $f_{ice}(T)$ is defined by:

$$f_{ice}(T) = \frac{n_{ice}(T)}{N_{tot}} \quad (1)$$

Where $n_{ice}(T)$ is the cumulative number of droplets frozen on cooling to T and N_{tot} is the total number of droplets. The cumulative number of active sites per particle, $n_n(T)$, was calculated according to equation 2:

$$n_n(T) = \frac{-\ln(1-f_{ice}(T))}{n_v} \quad (2)$$

200 Where n_v is the number of virus particles per 1 μ L droplet. The cumulative active sites per unit mass of material, $n_m(T)$ is defined by:

$$n_m(T) = \frac{-\ln(1-f_{ice}(T))}{m_v} \quad (3)$$

Where m_v is the mass of virus particles per droplet.

The freezing point depression of pure water due to NaCl (i.e. in the buffer solutions) was calculated using:

205
$$\Delta T_F = K_F \cdot b \cdot i \quad (4)$$

where ΔT_F is the freezing depression, K_F is the cryoscopic constant (1.853 K kg mol⁻¹ for water), b molality and i is the Van't Hoff factor (2 for NaCl). Hence, we report the degree of supercooling relative to the melting point of the aqueous saline solution. The correction was typically about 1°C for most virus suspensions (it was around 3°C for an Archea virus which required a very high salt concentration).



210 3 Results

3.1 Ice nucleating ability of virus particles

We studied virus ice nucleation from a virus structural perspective using the nucleation by immersed particle instrument (μ -NIPI) technique (Whale et al. 2015). We examined the ice nucleation activity (INA) of 11 viruses with different particle architectures, in an effort to probe the hypothesis that virus architecture/structure influences the ice-nucleating ability of virus particles. (Figure 1). These viruses included five enveloped Cystoviruses of *P. syringae* hosts with particle diameters of ~85 nm (Phi8, 6, 12, 12 and 2954; Figure 1A), two icosahedral viruses with an internal lipid membrane and particle diameters of ~70 nm (Figure 1B, PRD1 and HCIV-1), one of the icosahedral viruses without the DNA (Figure 1B, PRD1 no DNA), one 30 nm icosahedral virus without lipids (Figure 1B, PhiX174), two enveloped pleomorphic viruses with particle diameter of ~50 nm (Figure 1C), and one lemon-shaped virus (Figure 1D). Phi6-like viruses are commonly used as models for viruses that cause respiratory illnesses like SARS-CoV-2, the causative agent of COVID-19, due to structure similarity. Of the eleven viruses tested, nine showed an INA distinct from the INA of the buffer solution they were suspended in (Figure 1E and S2).

Phi12, an enveloped virus infecting *Pseudomonas syringae*, was found to be the most ice nucleation active virus in our study (in terms of the number of INPs per virus particle, n_n). Phi12 was observed to trigger freezing from -15°C to -21°C , with n_n values between 5×10^{-10} to 5×10^{-8} particles $^{-1}$. The other structurally similar cystoviruses of *P. syringae* were all ice nucleation active, although less so compared to Phi12 (Figure 1E). At this point we address the question of if the host bacterial cells might introduce ice nucleating entities which might be confused with the virus particles. In particular, several ice nucleation active *P. syringae* strains have been described in previous studies (de Araujo et al. 2019). However, none of the *P. syringae* host strains of the viruses used in this study exhibited an INA distinguishable from that of the buffer solution (Table S1, Figure S1a). Also, none of the strains contain a functional *ina*-gene, only partial pseudogenes. This indicates that the INA observed here is solely due to the virus particles. In addition (and more importantly), the virus samples used in the μ -NIPI test were purified according to established virus purification protocols (See Methods) and the purity was verified on sodium dodecyl sulfate-polyacrylamid gel electrophoresis (SDS-PAGE) (Figure S2). Furthermore, a well-established model virus, Phi6, was purified twice and the results of both purifications were examined on the μ -NIPI assay strongly implying that the virus particles are responsible for the observed INA (Figure S3) rather than contamination from the process or host bacteria.

The source of the INA was further studied using two of the best-characterized model viruses, Phi6 of *P. syringae*, and PRD1 of *E. coli*. Regarding Phi6, we used biochemical dissociation to disassemble the virus particles into sub-viral particles (Figure 2). First, the virus spike proteins were removed using butylated hydroxy toluene (BHT), with the resulting particle referred to as Phi6 BHT and the separate spike proteins referred to as Phi6 P3. Secondly, the lipid envelope and the associated proteins were removed using the anionic detergent Triton X100, exposing the nucleocapsid (NC) structure of Phi6 virion (Figure 2A). Each of the sub-viral particles was shown to have an INA distinguishable from the K-phosphate buffer (Figure 2B). Each of the sub-viral components, along with Phi6, was normalized to the mass of particles per volume of sample (n_m). When



normalized in this manner, each component spanned approximately the same range in n_m space, $10^2 - 10^4$ (mg^{-1}) across a range of temperatures. The freezing spectrum of each component was similar, with the Phi6 BHT sub-viral components having slightly warmer freezing temperatures than Phi6 at equivalent n_m values, whereas spike proteins (P3 in Figure 2A) had a slightly lower freezing temperature than Phi6. NC was found to be the most IN active sub-viral particle of Phi6 (Figure 2C), freezing approximately 4 °C warmer across the measured n_m range when compared to Phi6. INA is in part related to size (Pummer et al. 2015), hence since the spike proteins are only ~20 nm in diameter, whereas the BHT and NC particles are close to 80 nm. Hence, the difference in activity may be related to size. It is not clear how virus particles behave in the atmosphere, but several environmental stressors can disrupt virus particles exposing their internal parts. The other Phi6 sub-viral particles were also IN active (Figure 2) indicating, that the virus has broad IN potential, either being active as a whole or in a disrupted form. PRD1 was measured for its INA both with and without DNA. The n_n values for PRD1 with and without DNA are shown in Figure 3, and are similar to one another. This result suggests that the presence of PRD1's DNA is not related to the INA of the particles.

To further our understanding of the influence of virus structure on IN activity, we tested six other viruses, four archaeal and two bacterial. Of these six viruses, two archaeal viruses (HRPV1 and HRPV6) were enveloped like Phi6, but lack particle symmetry and an NC structure (Figure 1C). HRPV1 (Figure S1b) was not distinguishable from the Saline buffer (Table S1) it was suspended in, whilst HRPV6 (Figure S1b) was distinguishable from the buffer, but was not distinguishable from its host, and as such are shown as limiting values (Figure S5). Viruses with icosahedral symmetry that contain an internal lipid membrane (PRD1 and HCIV-1, Figure 1B) were also tested to further probe the dependency of viral INA on structure. PRD1, a well-known model virus (Bamford et al. 1995) was shown to be INA with a signal distinguishable from both the K-phosphate buffer and its host (Figure S6), and n_n values comparable to that of the majority of the *P. syringae* viruses (excluding Phi12) (Figure 1E). HCIV-1 did not have an INA distinguishable from the Saline buffer it was suspended in and is thus shown as a limiting value (Figure 1B). Another icosahedral virus, PhiX174, this time without a lipid membrane (Figure 1B) was tested and had n_n values similar to that of PRD1 and the majority of the *P. syringae* viruses. We further studied the INA dependency on virus architecture by studying an asymmetrical lemon-shaped archaeal virus, His1 (Bath et al. 2006). Interestingly, the virus had a higher INA than all the tested viruses, except for Phi12 (Figure 1D-E), indicating that structurally different viruses, symmetric or asymmetric, can be IN active. His1 was shown to be distinguishable from the saline buffer solution (Figure S1b) and its host (Figure S7).

3.2 Genetic analysis of ice active virus particles

The genomes of the 11 viruses included in this study were explored by bioinformatic analysis to further examine the source of IN activity. The ice nucleation activity observed in bacteria is due to protein structures which mimic ice crystal clathrate structure on the cell surface thus facilitating ice crystal formation around the cell (Kajava and Lindow 1993). In viruses, the source of INA might also be manifested due to proteinaceous origin. The possibility of the capsid or membrane proteins in



275 virus particles possessing similar structure and function as explanation for their ice nucleation capacity was explored in this study. This hypothesis was approached using ice nucleation motifs, specific and conserved short sequences in IN proteins, to search for the proteins potentially capable of nucleating ice.

280 Viral proteins with significant INM coverage and presence of INM3 in their sequence were predicted in eight of the viruses (Table 1). According to the results, only Phi13 and PhiX174 did not have potential IN proteins, with His1 having coverage below 15% and so is not shown in the table. Other viruses contained at least one potential protein with INM coverage of 15-50% and obligatory INM3 presence. However, the INA of Phi13 and PhiX174 is similar to the majority of the tested viruses such as Phi6. Similarly, HRPV1 and HCIV1 contain potential IN proteins, but these viruses had the weakest INA of the tested virus particles. Therefore, the presence of INMs in the sequence does not correlate to the capacity to nucleate ice.

4 Implications for the atmospheric ice nucleating particle population

285 In order to estimate the INP concentrations associated with virus particles in the atmosphere we have combined the n_n values shown in Fig. 1E and the upper limit of the concentration of viruses in the atmosphere from literature data (taken as 4×10^7 particles m^{-3} ; see discussion in the introduction). It is important to note that we based these virus INP concentrations on the INA of the specific samples which we studied and it may be possible that other virus particles have greater INA. However, since there are a limited number of virus architectures and we test a range of these architectures, we tentatively suggest that that we capture the typical range of INA of virus particles. Also shown on Figure 4 are envelopes showing the range of data from field campaigns in terrestrial (orange) and remote marine/polar environments (green) (see Table S4 for a list of representative measurements included in these envelopes).

295 As discussed in the introduction, in terrestrial environments, mineral dust is thought to be a very important INP type, with marine organics playing a secondary role (Vergara-Temprado 2017). In addition, there is evidence that biological INP play an important role in the terrestrial mid-latitudes (O'Sullivan et al. 2018; Hill et al. 2016; Šantl-Temkiv et al. 2019; Conen, Stopelli, and Zimmermann 2016; Pratt et al. 2009). Figure 4 shows that across the entire temperature spectra relevant for mixed-phase clouds, the concentration of virus INPs are lower than the lowest typical INP concentrations in terrestrial influenced areas. The closest the terrestrial envelope and the virus data points come to overlapping is between -18 °C to -22 °C, at which temperatures the difference in INP concentration is approximately one order of magnitude, i.e. at most they might contribute about 10% of the INP population at around -20 °C. Furthermore, one might expect that in environments where there is a strong source of virus particles, there is also a strong source of other biological materials, hence the influence of virus INP may be overestimated in our simple analysis. Overall, these results suggest that virus INPs generally play a minor role in regions influenced by terrestrial INPs.

300 Remote marine locations are less influenced by active terrestrial sources and thus the INP populations there are different from those of the terrestrial atmosphere (Creamean et al., 2019; DeMott et al., 2016; McCluskey et al., 2018a; McCluskey et al.,



305 2018b). Marine organics and sea spray aerosol have been shown to be INP sources of first order importance in such environments (DeMott et al., 2016; Vergara-Temprado et al., 2017; Wilson et al., 2015). There have been field measurements made in remote marine environments, which have reported remarkably low INP concentrations. McCluskey et al. (2018b) measured INP concentrations in a pristine marine environment at the Mace Head research station in 2015, with INP concentrations as low as 10^{-3} L^{-1} at -20°C . In a separate field campaign, measurements were made in the Southern Ocean,
310 INP concentrations range between 3.8×10^{-4} to 4.6×10^{-3} at -20°C (McCluskey et al., 2018a). Figure 4 shows overlap between the virus INP data points and the marine envelope in the temperature range -15°C to -27°C , with the most active of the virus INPs being approximately $15\times$ higher than the lower limit of the marine envelope at -20°C . Whilst this by no means proves that virus INPs are important in remote marine environment, it indicates they may contribute to the atmospheric INP burden in such regions. However, the lowest INP concentrations in the remote marine environment are most likely associated with
315 periods when the aerosol concentrations were lowest, as a result of the combined effect of precipitation scavenging and weak sources. Under these conditions, virus particles would also presumably be depleted.

5 Summary and conclusions

In this study we show a range of viruses can nucleate ice heterogeneously when immersed in supercooled solution droplets. A selection of virus types with diverse architectures are shown to have ice-nucleating abilities spanning three orders of magnitude
320 at -20°C , when normalized to particle number. We probed the virus ice-nucleating ability dependence on virus particle structure/architecture, showing that for our selected viruses there was not a dependency on virus architecture. Bioinformatic analysis shows that our current knowledge of ice nucleation due to *ina* genes/proteins exhibited by bacterial ice nucleators is likely insufficient to understand why viruses nucleate ice, which can be due to e.g. the overall arrangement of structural proteins making up the virus particles.

325 Our results are based on a small subsample of virus types but include several of the most prominent viral architectures. Nine out of 11 tested viruses were ice nucleation active indicating that several structurally different viruses can have IN potential. In addition, it has been shown previously that a tobacco mosaic virus can also nucleate ice (Cascajo-Castresana et al. 2020). While we have selected virus particles with a range of architectures that are relatively common in nature, it is possible that other virus particles nucleate ice more or less effectively. In particular, the specific virus types we have studied here are from
330 the terrestrial and freshwater aquatic environment; the isolation and testing of a range of marine viruses presents an important next step in quantifying the importance of viral ice nucleators. More work needs to be done to understand what drives viral ice nucleation, whether it would be dependent on virus structure/morphology, host, or some other factor.

This study shows the potential role viruses play as atmospheric INPs in certain environments. The ubiquity of viruses in the atmosphere implies they could serve as a baseline of INPs in situations where other, better-known atmospheric INPs are absent
335 in any meaningful quantity. However, our estimates for the upper limit of virus INPs suggest they do not play a meaningful



role in terrestrial environment, but may contribute to the INP population in marine environments. More work needs to be done to understand both why viruses nucleate ice and what role they play in both regional and global atmospheric ice nucleation.

Acknowledgements

340 Helin Veskiväli and Emeline Vidal are thanked for technical assistance. We thank Dr. Leonard Mindich for providing bacteriophages Phi12, Phi13, Phi2954 and *P. syringae* bacterial strain. Professor Ben Fane is thanked for providing PhiX174 bacteriophage. The use of the facilities and expertise of the Instruct-HiLIFE Biocomplex unit, member of Biocenter Finland and Instruct-FI, is gratefully acknowledged. N.S.A. wants to acknowledge the Academy of Finland Postdoctoral Grant 309570 and the Scientific Advisory Board for Defence grant VN/627/2020-PLM-9. We thank the European Research Council (648661
345 MarineIce, and 713664 CryoProtect) and the Natural Environment Research Council (NE/T00648X/1, M-Phase) for funding.

Data availability

The data associated with this paper are openly available from the University of Leeds Data Repository <https://doi.org/XXXXX>.

350 Competing interests

The authors declare no competing interests.

Author Contributions

M.A., N.S.A., S.S., A.H. and Z.B. performed the experiments. S.S. designed and performed bioinformatic analyses. D.B.
355 developed the NC purification protocol. M.A., N.S.A., B.M., and D.B. designed the experiments. All authors participated in data analysis and interpretation of results. N.S.A., J.D., J.R., B.M., and D.B. supervised and supported the project. The manuscript was written by M.A., N.S.A., S.S., B.M., D.B. All authors reviewed and approved the manuscript.

References

360 Abrescia, Nicola G.A., Dennis H. Bamford, Jonathan M. Grimes, and David I. Stuart. 2012. "Structure Unifies the Viral Universe." *Annual Review of Biochemistry* 81 (1): 795–822. <https://doi.org/10.1146/annurev-biochem-060910-095130>.



- Araujo, Gabriel Guarany de, Fabio Rodrigues, Fabio Luiz Teixeira Gonçalves, and Douglas Galante. 2019. “Survival and Ice Nucleation Activity of *Pseudomonas Syringae* Strains Exposed to Simulated High-Altitude Atmospheric Conditions.” *Scientific Reports* 9 (1): 1–11. <https://doi.org/10.1038/s41598-019-44283-3>.
- 365 Atanasova, Nina S., Tatiana A. Demina, Andrius Buivydas, Dennis H. Bamford, and Hanna M. Oksanen. 2015. “Archaeal Viruses Multiply: Temporal Screening in a Solar Saltern.” *Viruses* 7 (4): 1902–26. <https://doi.org/10.3390/v7041902>.
- Atanasova, Nina S., Elina Roine, Aharon Oren, Dennis H. Bamford, and Hanna M. Oksanen. 2012. “Global Network of Specific Virus-Host Interactions in Hypersaline Environments.” *Environmental Microbiology* 14 (2): 426–40. <https://doi.org/10.1111/j.1462-2920.2011.02603.x>.
- 370 Bailey, Timothy L., Mikael Bodén, Fabian A. Buske, Martin Frith, Charles E. Grant, Luca Clementi, Jingyuan Ren, Wilfred W. Li, and William S. Noble. 2009. “MEME SUITE: Tools for Motif Discovery and Searching.” *Nucleic Acids Research*. <https://doi.org/10.1093/nar/gkp335>.
- Bamford, Dennis H. 2003. “Do Viruses Form Lineages across Different Domains of Life?” *Research in Microbiology*. Elsevier Masson SAS. [https://doi.org/10.1016/S0923-2508\(03\)00065-2](https://doi.org/10.1016/S0923-2508(03)00065-2).
- 375 Bamford, Dennis H., Päivi M. Ojala, Mikko Frilander, Laura Walin, and Jaana K.H. Bamford. 1995. “Isolation, Purification, and Function of Assembly Intermediates and Subviral Particles of Bacteriophages PRD1 and $\Sigma 6$.” *Methods in Molecular Genetics* 6 (C): 455–74. [https://doi.org/10.1016/S1067-2389\(06\)80028-2](https://doi.org/10.1016/S1067-2389(06)80028-2).
- Bath, Carolyn, Tania Cukalac, Kate Porter, and Michael L. Dyal-Smith. 2006. “His1 and His2 Are Distantly Related, Spindle-Shaped Haloviruses Belonging to the Novel Virus Group, Salterprovirus.” *Virology* 350 (1): 228–39. <https://doi.org/10.1016/j.virol.2006.02.005>.
- 380 Bradford, Marion M. 1976. “A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding.” *Analytical Biochemistry* 72 (1–2): 248–54. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- Burrows, S. M., C. Hoose, U. Pöschl, and M. G. Lawrence. 2013. “Ice Nuclei in Marine Air: Biogenic Particles or Dust?” *Atmospheric Chemistry and Physics* 13 (1): 245–67. <https://doi.org/10.5194/acp-13-245-2013>.
- 385 Cai, Lanlan, Bo B. Jørgensen, Curtis A. Suttle, Maoqiu He, Barry A. Cragg, Nianzhi Jiao, and Rui Zhang. 2019. “Active and Diverse Viruses Persist in the Deep Sub-Sea-floor Sediments over Thousands of Years.” *ISME Journal* 13 (7): 1857–64. <https://doi.org/10.1038/s41396-019-0397-9>.
- Cascajo-Castresana, María, Robert O. David, Maiara A. Iriarte-Alonso, Alexander M. Bittner, and Claudia Marcolli. 2020. “Protein Aggregates Nucleate Ice: The Example of Apoferritin.” *Atmospheric Chemistry and Physics* 20 (6): 3291–3315. <https://doi.org/10.5194/acp-20-3291-2020>.



- Charles E. Grant, Timothy L. Bailey, and William Stafford. 2011. “FIMO: Scanning for Occurrences of a given Motif.” *Bioinformatics* 27 (7): 1017–18. <https://doi.org/10.1093/bioinformatics/btr064>.
- Conen, Franz, Emiliano Stopelli, and Lukas Zimmermann. 2016. “Clues That Decaying Leaves Enrich Arctic Air with Ice Nucleating Particles.” *Atmospheric Environment* 129 (March): 91–94. <https://doi.org/10.1016/j.atmosenv.2016.01.027>.
- 395 Creamean, J. M., J. N. Cross, R. Pickart, L. McRaven, P. Lin, A. Pacini, R. Hanlon, et al. 2019. “Ice Nucleating Particles Carried From Below a Phytoplankton Bloom to the Arctic Atmosphere.” *Geophysical Research Letters* 46 (14): 8572–81. <https://doi.org/10.1029/2019GL083039>.
- Creamean, Jessie M, Rachel M Kirpes, Kerri A Pratt, Nicholas J Spada, Maximilian Maahn, Gijs De Boer, Russell C Schnell, and Swarup China. 2018. “Marine and Terrestrial Influences on Ice Nucleating Particles during Continuous Springtime Measurements in an Arctic Oilfield Location.” *Atmos. Chem. Phys* 18: 18023–42. <https://doi.org/10.5194/acp-18-18023-2018>.
- 400 Demina, Tatiana A., Maija K. Pietilä, Julija Svirskaitė, Janne J. Ravantti, Nina S. Atanasova, Dennis H. Bamford, and Hanna M. Oksanen. 2016. “Archaeal Haloarcula Californiae Icosahedral Virus 1 Highlights Conserved Elements in Icosahedral Membrane-Containing DNA Viruses from Extreme Environments.” *MBio* 7 (4). <https://doi.org/10.1128/mBio.00699-16>.
- 405 DeMott, P. J., A. J. Prenni, X. Liu, S. M. Kreidenweis, M. D. Petters, C. H. Twohy, M. S. Richardson, T. Eidhammer, and D. C. Rogers. 2010. “Predicting Global Atmospheric Ice Nuclei Distributions and Their Impacts on Climate.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (25): 11217–22. <https://doi.org/10.1073/pnas.0910818107>.
- DeMott, Paul J, Thomas C J Hill, Christina S McCluskey, Kimberly A Prather, Douglas B Collins, Ryan C Sullivan, Matthew J Ruppel, et al. 2016. “Sea Spray Aerosol as a Unique Source of Ice Nucleating Particles.” *Proceedings of the National Academy of Sciences of the United States of America* 113 (21): 5797–5803. <https://doi.org/10.1073/pnas.1514034112>.
- 410 Dreischmeier, Katharina, Carsten Budke, Lars Wiehemeier, Tilman Kottke, and Thomas Koop. 2017. “Boreal Pollen Contain Ice-Nucleating as Well as Ice-Binding ‘antifreeze’ Polysaccharides.” *Scientific Reports* 7 (1): 1–13. <https://doi.org/10.1038/srep41890>.
- Du, Rui, Pengrui Du, Zedong Lu, Weishan Ren, Zongmin Liang, Saisai Qin, Ziming Li, Yaling Wang, and Pingqing Fu. 2017. “Evidence for a Missing Source of Efficient Ice Nuclei.” *Scientific Reports* 7 (1): 1–8. <https://doi.org/10.1038/srep39673>.
- 415 Eskelin, Katri, and Minna M. Poranen. 2018. “Controlled Disassembly and Purification of Functional Viral Subassemblies Using Asymmetrical Flow Field-Flow Fractionation (AF4).” *Viruses* 10 (11). <https://doi.org/10.3390/v10110579>.
- Eskelin, Katri, Minna M. Poranen, and Hanna M. Oksanen. 2019. “Asymmetrical Flow Field-Flow Fractionation on Virus and Virus-like Particle Applications.” *Microorganisms*. MDPI AG. <https://doi.org/10.3390/microorganisms7110555>.



- 420 Fröhlich-Nowoisky, J., T. C. J. Hill, B. G. Pummer, P. Yordanova, G. D. Franc, and U. Pöschl. 2015. “Ice Nucleation Activity in the Widespread Soil Fungus *Mortierella Alpina*.” *Biogeosciences* 12 (4): 1057–71. <https://doi.org/10.5194/bg-12-1057-2015>.
- Green, Robert L., and Gareth J. Warren. 1985. “Physical and Functional Repetition in a Bacterial Ice Nucleation Gene.” *Nature* 317 (6038): 645–48. <https://doi.org/10.1038/317645a0>.
- 425 Grythe, H, J Ström, R Krejci, P Quinn, and A Stohl. 2014. “Atmospheric Chemistry and Physics A Review of Sea-Spray Aerosol Source Functions Using a Large Global Set of Sea Salt Aerosol Concentration Measurements.” *Atmos. Chem. Phys* 14: 1277–97. <https://doi.org/10.5194/acp-14-1277-2014>.
- Gurian-Sherman, Douglas, and Steven E. Lindow. 1993. “Bacterial Ice Nucleation: Significance and Molecular Basis.” *The FASEB Journal* 7 (14): 1338–43. <https://doi.org/10.1096/fasebj.7.14.8224607>.
- 430 Hill, Tom C J, Paul J Demott, Yutaka Tobo, Janine Fröhlich-Nowoisky, Bruce F Moffett, Gary D Franc, and Sonia M Kreidenweis. 2016. “Sources of Organic Ice Nucleating Particles in Soils.” *Atmos. Chem. Phys* 16: 7195–7211. <https://doi.org/10.5194/acp-16-7195-2016>.
- Hoose, C., J. E. Kristjánsson, and S. M. Burrows. 2010. “How Important Is Biological Ice Nucleation in Clouds on a Global Scale?” *Environmental Research Letters* 5 (2). <https://doi.org/10.1088/1748-9326/5/2/024009>.
- 435 Hoose, C., and O. Möhler. 2012. “Heterogeneous Ice Nucleation on Atmospheric Aerosols: A Review of Results from Laboratory Experiments.” *Atmospheric Chemistry and Physics* 12 (20): 9817–54. <https://doi.org/10.5194/acp-12-9817-2012>.
- Irish, Victoria E., Pablo Elizondo, Jessie Chen, Cédric Chou, Joannie Charette, Martine Lizotte, Luis A. Ladino, et al. 2017. “Ice-Nucleating Particles in Canadian Arctic Sea-Surface Microlayer and Bulk Seawater.” *Atmospheric Chemistry and Physics* 17 (17): 10583–95. <https://doi.org/10.5194/acp-17-10583-2017>.
- 440 Irish, Victoria E., Sarah J. Hanna, Yu Xi, Matthew Boyer, Elena Polishchuk, Mohamed Ahmed, Jessie Chen, et al. 2019. “Revisiting Properties and Concentrations of Ice-Nucleating Particles in the Sea Surface Microlayer and Bulk Seawater in the Canadian Arctic during Summer.” *Atmospheric Chemistry and Physics* 19 (11): 7775–87. <https://doi.org/10.5194/acp-19-7775-2019>.
- Junge, K, and B D Swanson. 2008. “High-Resolution Ice Nucleation Spectra of Sea-Ice Bacteria: Implications for Cloud
445 Formation and Life in Frozen Environments.” *Biogeosciences*. Vol. 5. www.biogeosciences.net/5/865/2008/.
- Kajava, Andrey V., and Steven E. Lindow. 1993. “A Model of the Three-Dimensional Structure of Ice Nucleation Proteins.” *Journal of Molecular Biology*. <https://doi.org/10.1006/jmbi.1993.1424>.



- 450 Kanji, Zamin A., Luis A. Ladino, Heike Wex, Yvonne Boose, Monika Burkert-Kohn, Daniel J. Cziczo, Martina Krämer, et al. 2017. “Overview of Ice Nucleating Particles.” *Meteorological Monographs* 58 (January): 1.1-1.33. <https://doi.org/10.1175/AMSMONOGRAPHS-D-16-0006.1>.
- Lampi, Mirka, Hanna M. Oksanen, Florian Meier, Evelin Moldenhauer, Minna M. Poranen, Dennis H. Bamford, and Katri Eskelin. 2018. “Asymmetrical Flow Field-Flow Fractionation in Purification of an Enveloped Bacteriophage $\Phi 6$.” *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 1095 (September): 251–57. <https://doi.org/10.1016/j.jchromb.2018.07.008>.
- 455 Lindow’, Steven E, Deane C Army, and Christen D Upper. 1982. “Bacterial Ice Nucleation: A Factor in Frost Injury to Plants’.” *Plant Physiol.* Vol. 70. www.plantphysiol.org.
- McCluskey, C. S., T. C. J. Hill, R. S. Humphries, A. M. Rauker, S. Moreau, P. G. Strutton, S. D. Chambers, et al. 2018. “Observations of Ice Nucleating Particles Over Southern Ocean Waters.” *Geophysical Research Letters* 45 (21): 11,989–11,997. <https://doi.org/10.1029/2018GL079981>.
- 460 McCluskey, Christina S., Jurgita Ovadnevaite, Matteo Rinaldi, James Atkinson, Franco Belosi, Darius Ceburnis, Salvatore Marullo, et al. 2018. “Marine and Terrestrial Organic Ice-Nucleating Particles in Pristine Marine to Continentally Influenced Northeast Atlantic Air Masses.” *Journal of Geophysical Research: Atmospheres* 123 (11): 6196–6212. <https://doi.org/10.1029/2017JD028033>.
- Morris, C. E., D. G. Georgakopoulos, and D. C. Sands. 2004. “Ice Nucleation Active Bacteria and Their Potential Role in
465 Precipitation.” *Journal de Physique IV (Proceedings)* 121 (December): 87–103. <https://doi.org/10.1051/jp4:2004121004>.
- Morris, Cindy E., Caroline L. Monteil, and Odile Berge. 2013a. “The Life History of *Pseudomonas Syringae*: Linking Agriculture to Earth System Processes.” *Annual Review of Phytopathology* 51 (August): 85–104. <https://doi.org/10.1146/annurev-phyto-082712-102402>.
- . 2013b. “The Life History of *Pseudomonas Syringae*: Linking Agriculture to Earth System Processes .” *Annual
470 Review of Phytopathology* 51 (1): 85–104. <https://doi.org/10.1146/annurev-phyto-082712-102402>.
- Murray, B. J., D. O’Sullivan, J. D. Atkinson, and M. E. Webb. 2012. “Ice Nucleation by Particles Immersed in Supercooled Cloud Droplets.” *Chemical Society Reviews* 41 (19): 6519. <https://doi.org/10.1039/c2cs35200a>.
- Nuttall, Stewart D., and Michael L. Dyal Smith. 1993. “HF1 and HF2: Novel Bacteriophages of Halophilic Archaea.” *Virology* 197 (2): 678–84. <https://doi.org/10.1006/viro.1993.1643>.
- 475 O’Sullivan, D., M. P. Adams, M. D. Tarn, A. D. Harrison, J. Vergara-Temprado, G. C. E. Porter, M. A. Holden, et al. 2018. “Contributions of Biogenic Material to the Atmospheric Ice-Nucleating Particle Population in North Western Europe.” *Scientific Reports* 8 (1): 13821. <https://doi.org/10.1038/s41598-018-31981-7>.



- O'Sullivan, D., B. J. Murray, J. F. Ross, T. F. Whale, H. C. Price, J. D. Atkinson, N. S. Umo, and M. E. Webb. 2015. "The Relevance of Nanoscale Biological Fragments for Ice Nucleation in Clouds." *Scientific Reports* 5 (January): 8082.
480 <https://doi.org/10.1038/srep08082>.
- Olkkonen, Vesa M., and Dennis H. Bamford. 1989. "Quantitation of the Adsorption and Penetration Stages of Bacteriophage Φ 6 Infection." *Virology* 171 (1): 229–38. [https://doi.org/10.1016/0042-6822\(89\)90530-8](https://doi.org/10.1016/0042-6822(89)90530-8).
- Pandey, Ravindra, Kota Usui, Ruth A. Livingstone, Sean A. Fischer, Jim Pfaendtner, Ellen H.G. Backus, Yuki Nagata, et al. 2016. "Ice-Nucleating Bacteria Control the Order and Dynamics of Interfacial Water." *Science Advances* 2 (4): e1501630.
485 <https://doi.org/10.1126/sciadv.1501630>.
- Pietila, M. K., N. S. Atanasova, V. Manole, L. Liljeroos, S. J. Butcher, H. M. Oksanen, and D. H. Bamford. 2012. "Virion Architecture Unifies Globally Distributed Pleolipoviruses Infecting Halophilic Archaea." *Journal of Virology* 86 (9): 5067–79. <https://doi.org/10.1128/jvi.06915-11>.
- Pietilä, Maija K., Elina Roine, Lars Paulin, Nisse Kalkkinen, and Dennis H. Bamford. 2009. "An SsDNA Virus Infecting
490 Archaea: A New Lineage of Viruses with a Membrane Envelope." *Molecular Microbiology* 72 (2): 307–19.
<https://doi.org/10.1111/j.1365-2958.2009.06642.x>.
- Porter, Grace C. E., Sebastien N. F. Sikora, Michael P. Adams, Ulrike Proske, Alexander D. Harrison, Mark D. Tarn, Ian M. Brooks, and Benjamin J. Murray. 2020. "Resolving the Size of Ice-Nucleating Particles with a Balloon Deployable Aerosol Sampler: The SHARK." *Atmospheric Measurement Techniques* 13 (6): 2905–21. <https://doi.org/10.5194/amt-13-2905-2020>.
- 495 Pouleur, Stephan, Claude Richard, Jean-guy Martin, and Hani Antoun. 1992. *Ice Nucleation Activity in Fusarium Acuminatum and Fusarium Avenaceumt*. Vol. 58. American Society for Microbiology. <https://doi.org/10.1128/aem.58.9.2960-2964.1992>.
- Pratt, Kerri A., Paul J. Demott, Jeffrey R. French, Zhien Wang, Douglas L. Westphal, Andrew J. Heymsfield, Cynthia H. Twohy, Anthony J. Prenni, and Kimberly A. Prather. 2009. "In Situ Detection of Biological Particles in Cloud Ice-Crystals." *Nature Geoscience* 2 (6): 398–401. <https://doi.org/10.1038/NGEO521>.
- 500 Prussin, Aaron J., Ellen B. Garcia, and Linsey C. Marr. 2015. "Total Concentrations of Virus and Bacteria in Indoor and Outdoor Air." *Environmental Science and Technology Letters* 2 (4): 84–88. <https://doi.org/10.1021/acs.estlett.5b00050>.
- Pummer, B. G., C. Budke, S. Augustin-Bauditz, D. Niedermeier, L. Felgitsch, C. J. Kampf, R. G. Huber, et al. 2015. "Ice Nucleation by Water-Soluble Macromolecules." *Atmospheric Chemistry and Physics* 15 (8): 4077–91. <https://doi.org/10.5194/acp-15-4077-2015>.
- 505 Pummer, B G, H Bauer, J Bernardi, S Bleicher, and H Grothe. 2012. "Suspendable Macromolecules Are Responsible for Ice Nucleation Activity of Birch and Conifer Pollen." *Atmos. Chem. Phys* 12. <https://doi.org/10.5194/acp-12-2541-2012>.



- Qiao, Xueying, Yang Sun, Jian Qiao, Fabiana Di Sanzo, and Leonard Mindich. 2010. “Characterization of Φ 2954, a Newly Isolated Bacteriophage Containing Three DsRNA Genomic Segments.” *BMC Microbiology* 10. <https://doi.org/10.1186/1471-2180-10-55>.
- 510 Rangel-Alvarado, Rodrigo Benjamin, Yevgen Nazarenko, and Parisa A. Ariya. 2015. “Snow-Borne Nanosized Particles: Abundance, Distribution, Composition, and Significance in Ice Nucleation Processes.” *Journal of Geophysical Research: Atmospheres* 120 (22): 11,760–11,774. <https://doi.org/10.1002/2015JD023773>.
- Rastelli, Eugenio, Cinzia Corinaldesi, Antonio Dell’Anno, Marco Lo Martire, Silvestro Greco, Maria Cristina Facchini, Matteo Rinaldi, Colin O’Dowd, Darius Ceburnis, and Roberto Danovaro. 2017. “Transfer of Labile Organic Matter and Microbes
515 from the Ocean Surface to the Marine Aerosol: An Experimental Approach.” *Scientific Reports* 7 (1): 11475. <https://doi.org/10.1038/s41598-017-10563-z>.
- Reche, Isabel, Gaetano D’Orta, Natalie Mladenov, Danielle M. Winget, and Curtis A. Suttle. 2018. “Deposition Rates of Viruses and Bacteria above the Atmospheric Boundary Layer.” *The ISME Journal* 12 (4): 1154–62. <https://doi.org/10.1038/s41396-017-0042-4>.
- 520 Sanchez-Marroquin, A., O. Arnalds, K. J. Baustian-Dorsi, J. Browse, P. Dagsson-Waldhauserova, A. D. Harrison, E. C. Maters, et al. 2020. “Iceland Is an Episodic Source of Atmospheric Ice-Nucleating Particles Relevant for Mixed-Phase Clouds.” *Science Advances* 6 (26): eaba8137. <https://doi.org/10.1126/sciadv.aba8137>.
- Šantl-Temkiv, Tina, Branko Sikoparija, Teruya Maki, Federico Carotenuto, Pierre Amato, Maosheng Yao, Cindy E. Morris, et al. 2019. “Bioaerosol Field Measurements: Challenges and Perspectives in Outdoor Studies.” *Aerosol Science and
525 Technology*. Taylor and Francis Inc. <https://doi.org/10.1080/02786826.2019.1676395>.
- Saren, Ari Matti, Janne J. Ravanti, Stacy D. Benson, Roger M. Burnett, Lars Paulin, Dennis H. Bamford, and Jaana K.H. Bamford. 2005. “A Snapshot of Viral Evolution from Genome Analysis of the Tectiviridae Family.” *Journal of Molecular Biology* 350 (3): 427–40. <https://doi.org/10.1016/j.jmb.2005.04.059>.
- Schnell, R. C., Gabor Vali, R. C. Schnell, and Gabor Vali. 1976. “Biogenic Ice Nuclei: Part I. Terrestrial and Marine Sources.”
530 [Http://Dx.Doi.Org/10.1175/1520-0469\(1976\)033<1554:BINPIT>2.0.CO;2](http://Dx.Doi.Org/10.1175/1520-0469(1976)033<1554:BINPIT>2.0.CO;2), August. [https://doi.org/10.1175/1520-0469\(1976\)033<1554:BINPIT>2.0.CO;2](https://doi.org/10.1175/1520-0469(1976)033<1554:BINPIT>2.0.CO;2).
- Schnell, R C, G Vali, and R C Schnell’. 1975. “Freezing Nuclei in Marine Waters.” <https://doi.org/10.3402/tellusa.v27i3.9911>.
- Srinivasiah, Sharath, Jaysheel Bhavsar, Kanika Thapar, Mark Liles, Tom Schoenfeld, and K. Eric Wommack. 2008. “Phages across the Biosphere: Contrasts of Viruses in Soil and Aquatic Environments.” *Research in Microbiology* 159 (5): 349–57.
535 <https://doi.org/10.1016/j.resmic.2008.04.010>.
- Suttle, Curtis A. 2005. “Viruses in the Sea.” *Nature*. Nature Publishing Group. <https://doi.org/10.1038/nature04160>.



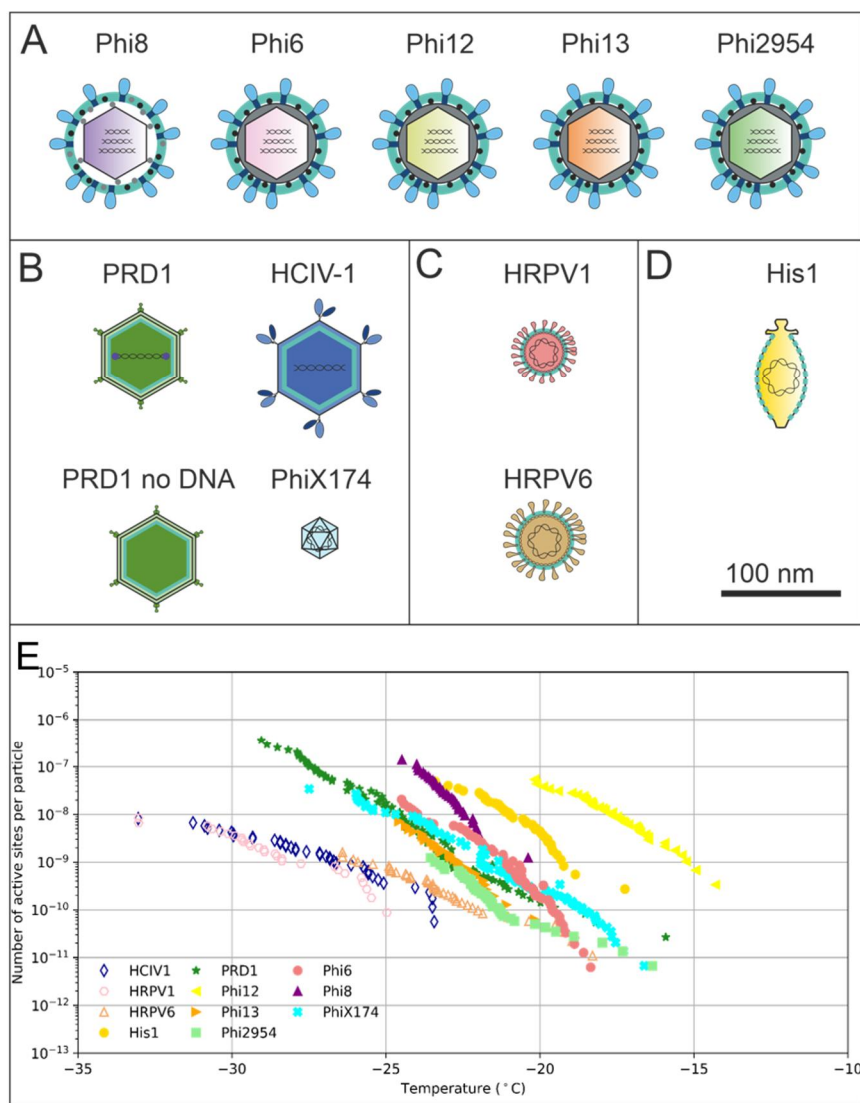
- . 2007. “Marine Viruses - Major Players in the Global Ecosystem.” *Nature Reviews Microbiology* 5 (10): 801–12. <https://doi.org/10.1038/nrmicro1750>.
- 540 Tan, Ivy, Trude Storelvmo, and Mark D Zelinka. 2016. “Observational Constraints on Mixed-Phase Clouds Imply Higher Climate Sensitivity.” *Science (New York, N.Y.)* 352 (6282): 224–27. <https://doi.org/10.1126/science.aad5300>.
- Timothy L. Bailey, and William Stafford Noble. 2003. “Searching for Statistically Significant Regulatory Modules.” *Bioinformatics* 19 (2): 16–25. <https://doi.org/10.1093/bioinformatics/btg1054>.
- 545 Tobo, Yutaka, Kouji Adachi, Paul J. DeMott, Thomas C.J. Hill, Douglas S. Hamilton, Natalie M. Mahowald, Naoko Nagatsuka, et al. 2019. “Glacially Sourced Dust as a Potentially Significant Source of Ice Nucleating Particles.” *Nature Geoscience* 12 (April). <https://doi.org/10.1038/s41561-019-0314-x>.
- Tschitschko, Bernhard, Timothy J. Williams, Michelle A. Allen, David Páez-Espino, Nikos Kyrpides, Ling Zhong, Mark J. Raftery, and Ricardo Cavicchioli. 2015. “Antarctic Archaea-Virus Interactions: Metaproteome-Led Analysis of Invasion, Evasion and Adaptation.” *ISME Journal* 9 (9): 2094–2107. <https://doi.org/10.1038/ismej.2015.110>.
- 550 Vali, G., M. Christensen, R. W. Fresh, E. L. Galyan, L. R. Maki, R. C. Schnell, G. Vali, et al. 1976. “Biogenic Ice Nuclei. Part II: Bacterial Sources.” [Http://Dx.Doi.Org/10.1175/1520-0469\(1976\)033<1565:BINPIB>2.0.CO;2](http://Dx.Doi.Org/10.1175/1520-0469(1976)033<1565:BINPIB>2.0.CO;2), August. [https://doi.org/10.1175/1520-0469\(1976\)033<1565:BINPIB>2.0.CO;2](https://doi.org/10.1175/1520-0469(1976)033<1565:BINPIB>2.0.CO;2).
- Vergara-Temprado, Jesús, Annette K Miltenberger, Kalli Furtado, Daniel P Grosvenor, Ben J Shipway, Adrian A Hill, Jonathan M Wilkinson, Paul R Field, Benjamin J Murray, and Ken S Carslaw. 2018. “Strong Control of Southern Ocean Cloud Reflectivity by Ice-Nucleating Particles.” *Proceedings of the National Academy of Sciences of the United States of America* 555 115 (11): 2687–92. <https://doi.org/10.1073/pnas.1721627115>.
- Vergara-Temprado, Jesús, Benjamin J. Murray, Theodore W. Wilson, Daniel O’Sullivan, Jo Browse, Kirsty J. Pringle, Karin Ardon-Dryer, et al. 2017. “Contribution of Feldspar and Marine Organic Aerosols to Global Ice Nucleating Particle Concentrations.” *Atmospheric Chemistry and Physics* 17 (5): 3637–58. <https://doi.org/10.5194/acp-17-3637-2017>.
- 560 Wang, Xiaofei, Camille M. Sultana, Jonathan Trueblood, Thomas C.J. Hill, Francesca Malfatti, Christopher Lee, Olga Laskina, et al. 2015. “Microbial Control of Sea Spray Aerosol Composition: A Tale of Two Blooms.” *ACS Central Science* 1 (3): 124–31. <https://doi.org/10.1021/acscentsci.5b00148>.
- Whale, T. F., B. J. Murray, D. O’Sullivan, T. W. Wilson, N. S. Umo, K. J. Baustian, J. D. Atkinson, D. A. Workneh, and G. J. Morris. 2015. “A Technique for Quantifying Heterogeneous Ice Nucleation in Microlitre Supercooled Water Droplets.” *Atmospheric Measurement Techniques* 8 (6): 2437–47. <https://doi.org/10.5194/amt-8-2437-2015>.
- 565 Whitman, William B., David C. Coleman, and William J. Wiebe. 1998. “Prokaryotes: The Unseen Majority.” *Proceedings of the National Academy of Sciences of the United States of America*. <https://doi.org/10.1073/pnas.95.12.6578>.



- Whon, Tae Woong, Min-Soo Kim, Seong Woon Roh, Na-Ri Shin, Hae-Won Lee, and Jin-Woo Bae. 2012. “Metagenomic Characterization of Airborne Viral DNA Diversity in the Near-Surface Atmosphere.” *Journal of Virology* 86 (15): 8221–31. <https://doi.org/10.1128/JVI.00293-12>.
- 570 Wilson, Sandra L., Paul Grogan, and Virginia K. Walker. 2012. “Prospecting for Ice Association: Characterization of Freeze-Thaw Selected Enrichment Cultures from Latitudinally Distant Soils.” *Canadian Journal of Microbiology* 58 (4): 402–12. <https://doi.org/10.1139/W2012-010>.
- Wilson, Theodore W., Luis A. Ladino, Peter A. Alpert, Mark N. Breckels, Ian M. Brooks, Jo Browse, Susannah M. Burrows, et al. 2015. “A Marine Biogenic Source of Atmospheric Ice-Nucleating Particles.” *Nature* 525 (7568): 234–38.
- 575 <https://doi.org/10.1038/nature14986>.



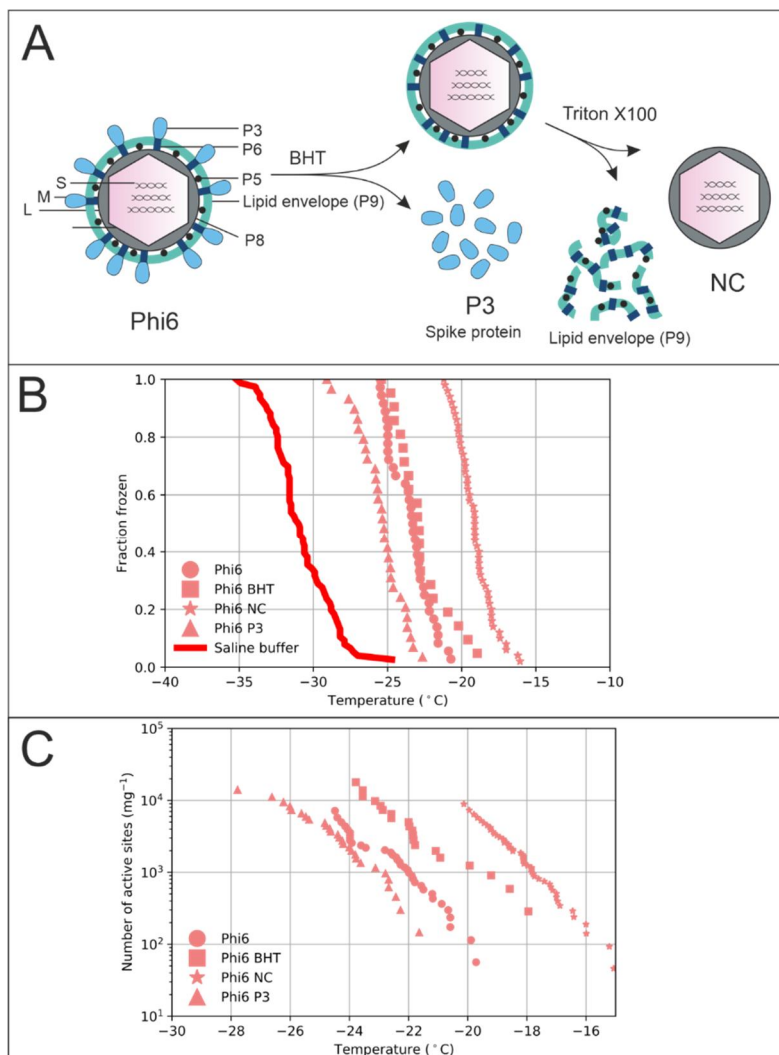
Figures and Tables



580

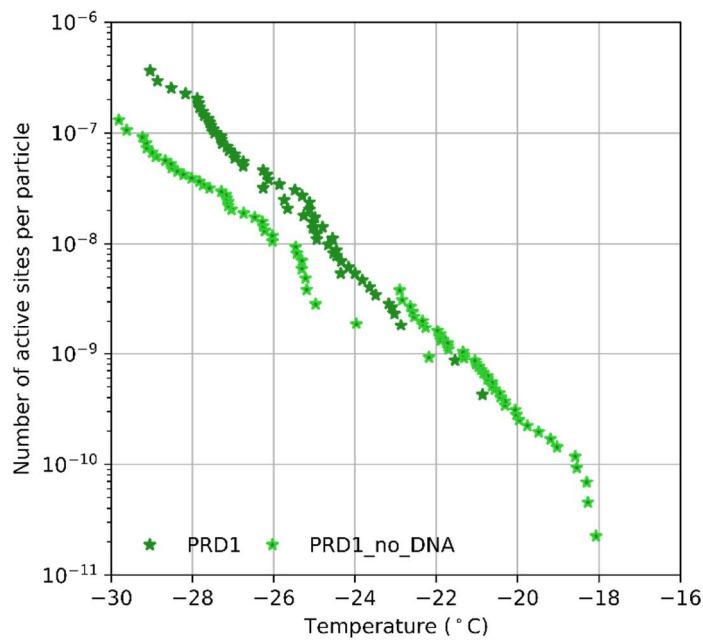
Figure 1. Graphical representation of the virus particles used in the ice nucleation study and their ice nucleating ability. A) Enveloped icosahedral viruses. B) Icosahedral viruses. C) Pleomorphic viruses. D) Lemon-shaped virus. E) Ice nucleation activity plots (expressed as n_n), where hollow marker indicate limit of detection (LoD) measurements in which the freezing temperatures were consistent with the virus free saline buffer control. Virus particles are to scale according to the 100 nm scale bar. Temperature values have been corrected for freezing point depression of NaCl.

585

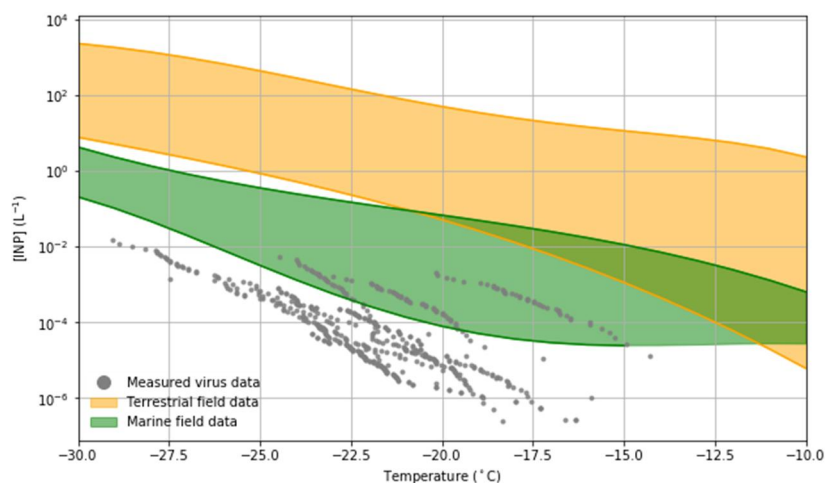


590

Figure 2. Ice nucleation activity of the subviral particles of Phi6 virus. A) Biochemical dissociation of Phi6 virion. Small genome fragment is marked as S, medium genome fragment as M and large genome fragment as L; P3 is spike protein, P5 is lytic enzyme, P6 is membrane fusion protein, P8 is outer capsid lattice protein and P9 is major envelope protein; BHT means butylated hydroxyl toluene; NC is nucleocapsid. B) Fraction frozen curves for Phi6 and its sub-viral components. These values have not been corrected for freezing point depression due to NaCl. C) The INA (expressed as active sites per unit mass, n_m) of Phi6 and its sub-viral components normalized to the mass of particle per volume of suspension. Phi6 BHT in graphs B and C refers to spikeless enveloped icosahedral structure. Temperature values have been corrected for freezing point depression of NaCl.



595 **Figure 3: The number of active sites per particle (n_n) for PRD1 with and without DNA. Temperature values have been corrected for freezing point depression of NaCl.**



600 **Figure 4.** Estimated viral INP concentration based on measured ice-nucleating ability of virus particles and upper limit literature values of viral particle concentrations in the atmosphere compared to measured INP concentrations in both terrestrial (orange) and marine/polar (green) environments. Table S4 shows a list of the studies from which the data to create the field measurement envelopes were obtained. Temperature values have been corrected for freezing point depression of NaCl.



Table 1. Potential IN proteins, their location, function and INM-coverage.

Virus name	IN-protein candidates	Protein function	Location in virus	IN-motif coverage (%)	Protein number	ID
Phi6	P3	Adsorption to host cells, attachment to type IV pilus	spikes	18	NP_620351.1	
	P1	Major inner capsid protein, RNA replication and transcription	capsid	20	NP_620348.1	
Phi12	P3c	Putative attachment protein	host spikes	16	NP_690834.1	
	P9	Membrane protein	external lipid membrane	28	NP_690828.1	
Phi13	P3b	Putative attachment protein	host spikes	31	NP_690814.1	
	P4	Hexameric NTPase	packaging 5-fold vertices of the procapsid	21	NP_690818.1	
Phi2954	P3	Host attachment protein	spikes	18	YP_002600769.1	
PhiX174	P11	Minor spike protein	spikes	29	NP_040713.1	
HCIV1	Putative protein VP18	Putative minor capsid protein	unknown hypothetically capsid	24	YP_009272867.1	
	VP3	Capsid protein	capsid	31	YP_009272848.1	
HRPV6	ORF2	Unknown	unknown hypothetically capsid	33	YP_005454286.1	
	ORF7	Integral component of the membrane	external lipid membrane	47	YP_005454291.1	



	ORF8	ATP-binding, type ATPase	AAA-	unknown hypothetically capsid	16	YP_005454292.1
PRD1	P7	Transglycosylase		lipid membrane	18	YP_009639979.1
	P14	DNA delivery		lipid membrane	31	YP_009639980.1

605