Supplemental Material for the manuscript

Charge, size distribution and hydrophobicity of viruses:
Effect of propagation and purification methods

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S1. Virus propagation procedures

S1.1 Virus propagation on double agar overlay

To propagate bacteriophages using double layer agar, fresh colonies of host bacteria were first inoculated into broth and incubated at 37 °C for ~ 2 h to reach log phase. Melted nutrient soft agar (8 mL, 0.5% w/v) was poured into each tube and kept at 48 °C. A suspension of host bacteria in log phase (300 µL) and diluted stock of bacteriophages, (200 µL, 5×10^5 phage/mL) were then inoculated into the soft agar sequentially. After inoculation, the agar was immediately overlaid on nutrient agar plates (1.5% w/v), which were then incubated at 37 °C overnight. To harvest viruses, 5 mL of broth was added on each plate and plates were placed again in 37 °C for 3 h. Then, to separate virus from soft agar and host bacteria, soft agar was scraped off the surface of agar plates, placed into a centrifuge tube and centrifuged at 8,000 g at 4 °C; after 15
min of centrifugation the supernatant was collected, filtered through a 0.22 µm filter and stored at 4 °C until purification.

S1.2 Virus propagation in broth

To propagate bacteriophages in broth, a fresh colony of host bacteria was inoculated into 10 mL broth one day prior to bacteriophage propagation and the suspension was incubated overnight at 37 °C. Next day, overnight culture was added into fresh broth and the mixture was shaken at 200 rpm at 37 °C. When optical density at 600 nm reached 0.1, bacteriophage stock was inoculated into bacterial culture with multiplicity of infection (MOI) of 0.1. The culture was further incubated at 37 °C with shaking (at 200 rpm for P22 and at 100 rpm for MS2) for another 8 h. Prior to virus harvest, chloroform was added to the culture. The culture was then rested for 10 to 15 min and transferred to a centrifuge tube. Bacterial debris were removed via centrifugation at 8,000 g for 10 min at 4 °C. The supernatant fluid containing the bacteriophage was filtered through a 0.22 µm filter and stored at 4 °C until purification.
S2 Virus purification procedures

S2.1 Virus purification by isopycnic CsCl density gradient centrifugation

Prior to purification by CsCl density gradient centrifugation, bacteriophage P22 suspension was concentrated by ultracentrifugation to ~ 4 mL. P22 phages were pelleted from the stock by centrifugation at 28,000 g for 90 min at 4 °C. After centrifugation, the supernatant was discarded. The pellet was resuspended in 4 mL of the pH 7.6 buffer (10 mM Tris, 10 mM MgCl$_2$; later in the text the buffer is denoted Tris-MgCl$_2$) by overnight nutation at 4 °C. The small size of MS2 virions makes pelleting this bacteriophage difficult; thus, MS2 stock suspension was concentrated to ~ 4 mL using Amicon Ultra-15 centrifugal filters.

CsCl solutions with different densities were prepared for isopycnic centrifugation. Each CsCl solution was layer-by-layer loaded into a tube with the highest density layer at the bottom and the lowest density layer at the top. CsCl solutions were then capped with a 25% sucrose solution. Density steps for MS2 were 1.50 g/cm$^3$ CsCl, 1.35 g/cm$^3$ CsCl, and 25% sucrose solution. For P22, density steps were 1.60 g/cm$^3$ CsCl, 1.40 g/cm$^3$ CsCl, and 25% sucrose solution [1, 2].

The resuspended phage was applied onto the 25% sucrose layer and a virus band was obtained via ultracentrifugation at 30,000 rpm at 18 °C for 3 h. The virus band was collected with a syringe by puncturing the tube. CsCl in the purified virus stock was then removed by dialysis against 1 mM NaCl (pH 5.5, unadjusted). The purified stock was filtered through 0.22 μm membrane and stored at 4 °C.

S2.2 Virus purification by PEG precipitation

PEG was added into the bacteriophage stock with gentle stirring to reach the final concentration of 10% (w/v), followed by addition of NaCl with the final concentration of 0.5 M. Upon addition of PEG and NaCl, the virus stock became turbid. The virus stock was stored in the dark at 4 °C with gentle stirring overnight. The stock was then centrifuged at 10,000 g for 30 min at 4 °C. The supernatant was discarded and the pellet was resuspended via overnight nutation with 1 mM NaCl (pH 5.5, unadjusted) which was filtered through a 0.22 μm pore size membrane prior to use. The solution with the resuspended virus was treated by adding chloroform (1:1 v/v) to
remove remaining PEG. Upon addition of chloroform, resuspension was vigorously vortexed for 30 s and then centrifuged at 1,700 g for 30 min. The virus-containing aqueous fraction above the white layer, assumed to be made up of impurities, was carefully aspirated into tube as stock without disrupting the white layer. Finally, the virus stock was dialyzed against 1 mM NaCl (pH 5.5, unadjusted), filtered through 0.22 μm membrane and stored at 4 °C.

S2.3 Virus purification by centrifugal diafiltration

**Table S1:** Average hydrodynamic diameter, polydispersity index and half width at half maximum for MS2 in storage solution recorded for the six different preparations evaluated in this study.

In the case of multiple peaks in the particle size distribution, half width at half maximum is calculated based on the peak of the highest intensity.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Average size, ( \bar{d}_{h} ), nm</th>
<th>Polydispersity index, PDI</th>
<th>Half width at half maximum, HWHM(^a), nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>“MS2-agar-CsCl”</td>
<td>32.2 ± 0.1</td>
<td>0.19</td>
<td>15.4 ± 1.0</td>
</tr>
<tr>
<td>“MS2-agar-PEG”</td>
<td>32.3 ± 0.1</td>
<td>0.17</td>
<td>21.5 ± 0.8</td>
</tr>
<tr>
<td>“MS2-agar-DF”</td>
<td>70.4 ± 0.3</td>
<td>0.48</td>
<td>34.0 ± 4.4</td>
</tr>
<tr>
<td>“MS2-broth-CsCl”</td>
<td>26.9 ± 0.1</td>
<td>0.02</td>
<td>9.2 ± 0.4</td>
</tr>
<tr>
<td>“MS2-broth-PEG”</td>
<td>27.6 ± 0.1</td>
<td>0.05</td>
<td>10.7 ± 0.4</td>
</tr>
<tr>
<td>“MS2-broth-DF”</td>
<td>29.6 ± 0.2</td>
<td>0.14</td>
<td>15.9 ± 1.0</td>
</tr>
</tbody>
</table>

The bacteriophage stock was loaded into Amicon Ultra-15 centrifugal filters (molecular weight cutoff 100 kDa) and centrifuged at 1,500 g to bring the concentrate volume to ~0.5 mL in each tube. Pre-filtered 1 mM NaCl solution (pH 5.5, unadjusted) was then added to the concentrate to fill up the centrifuge tube and the suspension was centrifuged again at 1,500 g until 0.5 mL sample remained in the concentrate. This washing step was repeated at least 20 times to remove broth remnants and to complete storage solution exchange. Finally, the concentrate
with viruses was collected and filtered through 0.22 μm membrane and stored at 4 °C as the purified stock.

**Figure S2:** Particle size distributions of agar control (virus free) samples purified by a) PEG precipitation and b) centrifugal diafiltration.
Figure S3: Electrophoretic mobility as a function of pH for MS2 propagated on double agar overlay and agar control (virus free) samples with two purification methods: a) PEG precipitation and b) centrifugal diafiltration.
Figure S4: Average hydrodynamic diameter as function of pH for MS2 propagated by two different methods - in broth and on double agar overlay - and purified by a) CsCl density gradient centrifugation, b) PEG precipitation and c) centrifugal diafiltration. Empty and filled symbols correspond to measurements in 1mM NaCl and in Tris-MgCl₂ buffer, respectively.
Figure S5: Average hydrodynamic diameter as function of pH for broth-propagated MS2 (a) and P22 (b) purified by CsCl density gradient centrifugation, PEG precipitation and centrifugal diafiltration. Empty and filled symbols correspond to measurements in 1mM NaCl and in Tris-MgCl₂ buffer, respectively.
References
