# Bacteria suit up with virus armor

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Particularly with the recent outbreak of coronavirus disease 2019 (COVID-19), viruses have a bad reputation these days. However, it is important to note that viruses come in many types and can infect not only humans but almost all life forms, and their infections are not always detrimental to the host. Consider viruses that infect bacteria—"bacteriophages," or "phages" for short. While most phages do cause the death of their bacterial host during propagation, it has been found that a subgroup, filamentous phages, cooperate with the host to provide "services," such as promoting virulence or optimizing the motility of the bacterium for better fitness (1–3). The relationship is akin to a facultative symbiosis by which two organisms live together and offer mutual benefits. A study by Tarafder et al. (4) reports a mechanism for this type of cooperation, in which the bacterium *Pseudomonas aeruginosa* can use a filamentous phage it harbors to create a barrier around itself that defends against antibiotics—like wearing special armor made of virus molecules (Fig. 1).

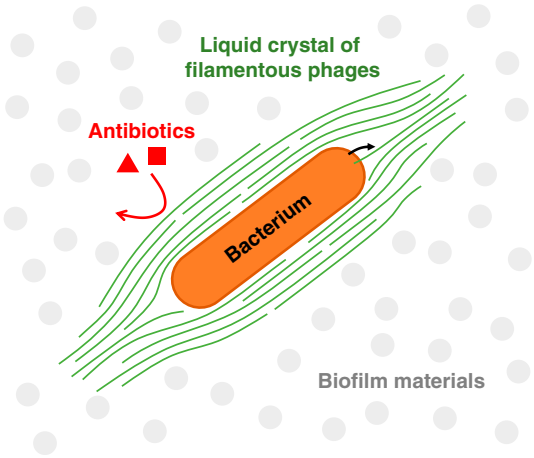


**Fig. 1. The bacterium *P. aeruginosa* produces filamentous phages in its biofilm to form liquid crystals encapsulating itself for antibiotic protection.**

Filamentous phages are characterized by their long and thin shape (~6 nm in diameter and ~800 to 4,000 nm in length). The filament is composed of a helically packed capsid protein shell surrounding an extended single-stranded DNA (ssDNA) genome. As they are produced by the host, filamentous phages are assembled at the bacterial cell envelope and exported through dedicated secretion machinery (5). This allows the phage to egress without killing the host, in contrast to most other phages which are assembled in the host cytoplasm and lyse the cell to exit. The unusual lifestyle of filamentous phages puts little burden on the host population and enables production of large amounts of phage (6).

It was previously observed that a filamentous phage named Pf4 is highly produced in biofilms of the opportunistic bacterial pathogen *P. aeruginosa* and, therefore, likely plays a role in this important virulence stage (7). Purified Pf4 phages were subsequently found to self-assemble into higher-order liquid crystalline droplets in conditions mimicking the bacterial biofilm environment, such as solutions containing biopolymers like polysaccharide alginate, and these phage droplets promoted antibiotic tolerance of *P. aeruginosa* in the mixture (8). This phage-mediated protection could partly explain the notorious antibiotic tolerance of *P. aeruginosa* (9). The mechanism of protection was hypothesized to be the large amount of negatively charged genomic ssDNA in the phage droplets sequestering positively charged antibiotics (8). Interestingly, however, the investigation by Tarafder et al. (4) has uncovered information pointing to a different mechanism.

Tarafder et al. (4) first used cryo-electron microscopy (cryo-EM) to resolve the atomic structure of Pf4 phage purified from *P. aeruginosa* biofilms and found that while the capsid proteins are tightly packed into the helical filamentous shell, its ssDNA genome is not held as tightly in the center. Further analysis of the cryo-EM images led them to discover that about one-third of the phage molecules imaged did not contain

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the ssDNA, showing that the ssDNA genome is not required for maintaining the structure of the capsid shell, and suggesting that the net negative charge of the phage population is less than previously thought. To examine the potential role of phage ssDNA in bacterial antibiotic protection, Tarafder et al. (4) used a chemical treatment to remove the ssDNA from all of the purified Pf4 phages and, intriguingly, observed that the resulting empty capsid shells (called "Pf4 ghosts") still formed the same liquid crystalline droplets and still provided antibiotic protection to the bacteria. These findings suggest that it is likely not the negatively charged ssDNA but the capsid filaments themselves that can protect the bacterium. To further understand this protective mechanism, they next used cryo-electron tomography (cryo-ET) to inspect the three-dimensional ultrastructure of frozen–hydrated Pf4 phage liquid crystalline droplets, resolving individual Pf4 phage filaments. They found that in each droplet all of the phage filaments were aligned parallel to one another, together forming a spindle structure. Interestingly, even with this constrained orientation in the spindle, phage assembly was highly dynamic. When Tarafder et al. (4) used real-time optical imaging with a focused laser to photobleach one part of a fluorescently labeled phage spindle, recovery of the local fluorescence signal happened within seconds, suggesting a fluid-like nature. Further fluorescence imaging of Pf4 liquid crystalline droplets mixed with *P. aeruginosa* bacteria revealed that the majority of the bacterial cells were individually encased in phage droplets, and that unencapsulated cells were much more easily killed by antibiotics. These observations suggested that the Pf4 phage spindle uses its fluid-like property to engulf *P. aeruginosa* cells to provide protection from antibiotics. Since both native Pf4 and Pf4 ghosts showed the same activity and effect, Tarafder et al. (4) conclude that the basis of the antibiotic protection is mainly the physical occlusion provided by the filamentous spindle around the bacterium.

As mentioned by Tarafder et al. (4), the spontaneous formation of phage liquid crystals in the presence of bacterial biofilm components may be explained by a theoretical entropy-driven effect known as the "depletion interaction" (10–12). In suspension systems, this effect has been proposed to drive like particles to flocculate, and therefore to phase-separate from dissimilar ones. Simply put, in a mixture of long Pf4 phage filaments and small alginate biopolymers, when the space between two phage filaments becomes small enough that alginate molecules cannot fit in between, the two phage filaments will be pushed together by the entropic force from the surrounding osmotic pressure. Because of the phage's elongated shape and stiffness, this effect will quickly propagate along the length of the two phage filaments, like zipping up a zipper. As more phage filaments are pushed together by this effect, the orientational ordering among them will give rise to the phage spindle. Moreover, since both the Pf4 phage and *P. aeruginosa* bacterium are rod-shaped, despite the difference in width it is conceivable that the same entropic-driven effect could promote a similar association along their length and result in the bacterium's encapsulation by the phage spindle. This is supported by Tarafder et al.'s (4) images showing that the rod-shaped bacterial cells were aligned with individual phage filaments during interaction (by cryo-ET imaging) as well as with phage spindles after encapsulation (by optical imaging). The depletion interaction effect has been proposed to occur even in the absence of any direct interactions, such as electrostatic forces, between particles. The particles feel each other's presence and adjust their spatial arrangement simply due to the fact that two molecules cannot occupy the same space at the same time. To test whether the bacterium-engulfing behavior is driven more by physical than by chemical properties, Tarafder et al. (4) replaced the bacterial cells with colloidal gold rods of corresponding size and shape and, indeed, observed the same encapsulation behavior. This largely physics-driven process might offer versatility, enabling protection by different filamentous phages of various rod-shaped bacteria with diverse surface chemistries, as well as in varied environments where the bacteria may establish biofilms in different hosts or stages of infection.

**It is increasingly clear that filamentous phages have an amazing variety of impacts on the physiology of their bacterial hosts, and understanding the detailed mechanisms of these interactions could potentially enable further biotechnological applications involving both phages and bacteria or help us fight against the phages' pathogenic bacterial hosts such as *P. aeruginosa*.**

Due to the simplicity of filamentous phages and their ease of production and modification, they have been successfully utilized in several biotechnological applications, including phage display (a powerful method for finding peptides and antibodies of a desired specificity, which was awarded the 2018 Nobel Prize in Chemistry) (13), precision nanomedicine (14), assembly of nanostructures (15), and synthesis of biosensors (16). It is increasingly clear that filamentous phages have an amazing variety of impacts on the physiology of their bacterial hosts, and understanding the detailed mechanisms of these interactions could potentially enable further biotechnological applications involving both phages and bacteria or help us fight against the phages' pathogenic bacterial hosts such as *P. aeruginosa*. As new filamentous phages, and their effects, are continuously being discovered (3, 17), the field is now filled with extraordinary potential.

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