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Review

Modeling tailed bacteriophage adsorption: Insight into mechanisms

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ABSTRACT

The process of a bacteriophage attaching to its host cell is a combination of physical diffusion, biochemical surface interactions, and reaction-induced conformational changes in receptor proteins. Local variations in the physico-chemical properties of the medium, the phage's mode of action, and the physiology of the host cell also all influence adsorption kinetics. These characteristics can affect a specific phage's binding capabilities and the susceptibility of the host cell to phage attack. Despite the complexity of this process, describing adsorption kinetics of a population of bacteriophages binding to a culture of cells has been accomplished with relatively simple equations governed by the laws of mass-action. Many permutations and modifications to the basic set of reactions have been suggested through the years. While no single solution emerges as a universal answer, this review provides the fundamentals of current phage adsorption modeling and will guide researchers in the selection of valid, appropriate models.

Contents

Introduction

Bacteriophages have fallen in and out of favor among researchers since their discovery almost a century ago (d'Herelle, 1917; Twort, 1915). Euphoria over the existence of a natural prophylactic agent that could prevent and cure bacterial infections gave rise to snake-oil salesmen peddling bacteriophages as a solution to nearly everything from gallstones to herpes (a virus) (Harper et al., 2014). Inadequate understanding of phage biology led to many unsuccessful attempts at using phage therapy to treat bacterial infections in humans and animals (Pirnay et al., 2011; Sulakvelidze et al., 2001). By the late 1920s, the discovery of penicillin, an indiscriminate weapon against gram-positive pathogens, quenched whatever residual enthusiasm for phage therapy may have remained in the majority of the scientific world (Pirnay et al., 2012), save for countries of the Eastern Bloc.

Bacteriophages found new life in other circles though; in fact, much of our understanding of modern genetics is owed to studies involving bacteriophages (Ptashne, 2004). Even now, modern genetic engineering and synthetic biology techniques make heavy use of bacteriophage promoters, polymerases, and genes as tools to achieve recombinant or novel biological systems. Where would we be today without the temperature sensitive promoters of phage λ or the hyper-expression levels of the T7 promoter?

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Today, bacteriophages are used in situations far beyond what the original practitioners envisioned: vehicles of drug delivery (Dickerson et al., 2005; Lee et al., 2009; Tao et al., 2013a, 2013b), highly specific biological sensors (Guntupalli et al., 2012; Huang et al., 2008; Monk et al., 2010; Tawil et al., 2012) – particularly the luciferase-expressing reporter phages (Loessner et al., 1996; Schofield et al., 2009), viral-based electronics (Dang et al., 2011), and nanotechnology (Petrenko and Smith, 2011). But the original vision has also made a resurgence. Bacteriophages are being used as bio-control agents in agriculture and food processing applications (Adriaenssens et al., 2012; Fox, 2000; Fujiwara et al., 2011; Guenther et al., 2012; Jones et al., 2007; Loc Carrillo et al., 2005; Park and Nakai, 2003; Schnabel and Jones, 2001) and their use as anti-bacterial agents in the treatment of humans and animals is once again *au goût du jour* (Merabishvili et al., 2009; Pirnay et al., 2011, 2012; Rhoads et al., 2009; Verbeken et al., 2012; Wright et al., 2009).

While advances in modern imaging techniques such as Cryo-TEM have enabled visualization of the mechanism of bacteriophage infection at the nanometer scale (Hu et al., 2013), a comprehensive explanation of bacteriophage adsorption kinetics has not been reported in the literature, mostly due to the diversity of mechanisms exploited by different phages (Guerrero-Ferreira et al., 2011; Hu et al., 2013). In this review, we examine how researchers have dealt with modeling the often unintuitive nature of adsorption kinetics. We give some history around the progression of scientific understanding of bacteriophage adsorption and highlight the remarkably prescient hypotheses the early phage researchers used to explain the mechanics of phage adsorption. Next, we summarize the major types of adsorption models used to describe phage population dynamics in bacterial cultures. Finally, we comment on the approach to selecting an adsorption model most suitable for a specific virus–host system.

The adsorption paradox

One of the early topics of debate surrounding bacteriophage adsorption was how to explain the paradoxical notion that nearly every collision between a virus particle and host cell leads to irreversible attachment.

Studies on phage adsorption have revealed that in the early minutes of adsorption the interactions between the phage particle (P) and bacterium (B) can often be described by the simplified reaction

$$B + P \rightarrow I \tag{1}$$

where I is the irreversibly adsorbed phage–bacterium complex. Many studies have demonstrated that this mechanism obeys a first order observed rate of reaction where the concentration of the host as an available binding entity remains constant (Delbruck, 1940; Krueger, 1931; Schlesinger, 1932). In this case, the virus concentration decreases exponentially and the rate of adsorption can be described by the rate function

$$r_{ads} = kBP \tag{2}$$

where k is the adsorption rate constant, B is the bacterial concentration and P is the free phage concentration or phage titer. Note that if B is assumed to be constant in Eq. (2), the reaction rate reduces from a 2nd order reaction rate (two variables: B and P) to a pseudo 1st order reaction rate (where P is the only variable, k and B remain constant). Experiments on the adsorption of phage to living and heat killed *Staphylococcus aureus* in excess bacterial concentrations led Krueger to propose the following pseudo 1st order model to describe the decrease of free phage concentration over time (Krueger, 1931):

$$\frac{dP}{dt} = -kBP \tag{3}$$

As long as the ratio of phage to bacteria was low enough to assume an unchanged available bacterial surface area during adsorption, Krueger concluded that B could be assumed constant. Schlesinger (1932) and, later, Delbruck (1940) applied the coagulation theory of von Smoluchowski (1917) to phage adsorption, treating the bacterium and phage particle as two molecules interacting in space. According to this theory, if all collisions between phage and bacteria lead to irreversible attachment, the maximum value of k is given by

$$k = 4\pi r D \tag{4}$$

where r is the radius of a sphere "equivalent" to the bacterium (r in this context is not to be confused with the adsorption rate of Eq. (3)) and D is the diffusion coefficient of the phage particle. The maximum rate constant predicted by this theory is on the same order of magnitude as k-values determined experimentally (Delbruck, 1940; Schwartz, 1976), implying that nearly every collision between phage and bacteria leads to irreversible adsorption. How this is possible when the binding sites of both phage and bacteria constitute only a small fraction of their respective surface areas has long been a topic of debate.

The mechanism of phage attachment

Delbruck (1940) postulated that greater than predicted rate constants under optimal growth conditions of the host could be due to larger cell sizes and increased cell motility. However, high adsorption rates were still recorded for experiments completed on heat-killed bacteria (Krueger, 1931) or on stationary phase cultures (Gallet et al., 2012; Storms et al., 2010, 2012). Furthermore, calculations have shown that the influence of cell motility on adsorption rate is insignificant (Berg and Purcell, 1977). A more comprehensive explanation for phage adsorption that focused on the individual interactions between the virus particle and the cell surface was offered by Anderson (1949). Observing that (1) interactions between phage and bacterium are highly specific, (2) nearly every collision leads to irreversible attachment in undisturbed media, and (3) almost no collisions lead to irreversible attachment in violently agitated media, Anderson hypothesized that small protruding elements located on the virus particle are the first point of contact between phage and cell. These small elements would have higher rates of Brownian motions relative to the larger bacterial surface and therefore result in many collisions while the phage particle diffuses over the cell. If one of these collisions results in the proper orientation of the element with the receptor, it would lead to a "steric fitting of the elements and the formation of a weak bond between virus and host" (Anderson, 1949). This bond would be weak enough that intense agitation could break it, but strong enough to keep the virus–host complex together until irreversible attachment in undisturbed media.

In a comprehensive study of phage λ, Schwartz (1976) demonstrated that Anderson's proposed mechanism provides an adequate description of the mechanics of phage attachment. Adsorption rate is proportional to not only collision frequency, but also the probability that the appropriate interactions between virus and host occur within the average collision time. Using Einstein's equation of Brownian movement, Schwartz estimated that the λ particle will spend on average 5×10^{-3} s close enough to the cell receptor during each collision, but that only 1.6×10^{-3} s of the collision time will see the phage tail oriented in the right position for meaningful interactions. Then, applying the classical kinetic theory of gases to the ligand–membrane interactions on the cell surface and making some simplifying assumptions, Schwartz derived an equation describing the probability that a phage will react with a receptor during the effective collision. For maltose-grown *Escherichia coli* cells with a λ phage receptor density of ~630 mol μm^{-2},

there is a 99.96% chance that the phage will interact with the receptor (Schwartz, 1976). Schwartz' elegant derivation provides a rigorous explanation for the adsorption mechanism originally proposed by Anderson. However, it should be noted that in cases where the receptor density is low on the cell surface – for example the LamB receptor on glucose-grown *E. coli* cells has a density of only 30 molecules/cell (Schwartz, 1976), the probability of meaningful virus–host interaction during each collision is much lower. For a detailed review of the interactions between phage λ and LamB, see Chatterjee and Rothenberg (2012).

In a more general analysis of chemoreception by bacterial cells, Berg and Purcell (1977) demonstrated that once the initial contact occurs between cell and molecule (phage, in this instance) subsequent collisions are bound to follow based on the geometry of the surfaces and physical properties of the environment. Consequently, even when the probability of a meaningful interaction between the phage and host is low, the phage is more than likely to diffuse back to the cell surface multiple times before drifting away from the cell entirely. They conclude that for an average cell of 1 μm in length with surface receptors of radius 1 nm, a receptor density beyond roughly 1000 receptors per cell offers only marginal improvement in sorption capability. This prediction was experimentally validated by the work of Schwartz (1976), who observed little increase in the adsorption rate constant of bacteriophage λ after receptor surface densities reached 1000 molecules per cell.

A common conceptual visualization of phage adsorption is the reduction of dimensionality theory (Adam and Delbruck, 1968). According to this model, three-dimensional diffusion to the cell surface is followed by a two-dimensional 'random walk' along the cell membrane in search of the appropriate receptor. The authors suggested that this is more efficient than three-dimensional diffusion alone. Regardless, as Berg and Purcell (1977) pointed out, finding the cell in three-dimensional space is the major roadblock to adsorption; once it has found the cell, the phage would brush up against the surface numerous times in a quasi-two dimensional diffusion with similar effect to the prediction of reduction of dimensionality.

In fact, modern studies of phage adsorption have validated most of the hypotheses of these early phage researchers. In order to prevent diffusion away from the cell surface before finding its appropriate receptor, tail fibers of both phages T4 (Goldberg et al., 1994) and T7 (Hu et al., 2013) have evolved to 'walk' the bacterial surface using reversible interactions weak enough to allow one or two fibers to detach at any given time but strong and numerous enough to prevent all from desorbing. Similarly, it has been suggested that siphoviridae phages λ and SPP1 contain adhesion modules on their tails that play a similar role in adsorption (Spinelli et al., 2014). Detailed visualization and modeling of phage λ particles attaching to the cell surface receptors has been beautifully described by Rothenberg et al. (2011). Comprehensive studies of SPP1 attachment to *Bacillus subtilis* (Baptista et al., 2008) and lactococcal phage p2 infection (Bebeacua et al., 2013) offer clear evidence that phages infecting gram positive bacteria also employ strategies to 'walk' the bacterial surface in search of the receptor binding site. Amazingly, the modern microscopy and analytical techniques used in these studies have cemented the adsorption mechanism proposed by phage luminary Anderson (1949) as a robust description of the physiological adsorption process for a diverse selection of tailed bacteriophages.

Kinetic models of phage adsorption

While the physiological adsorption process of an individual phage consists of numerous small-scale reactions and complicated steps, modeling large-scale adsorption of a phage population can be done with surprisingly simple kinetic models. The one-step mechanism of Krueger (1931) has proven reliable at high cell concentrations over short time periods and has been widely used (Delbruck, 1940; Guerrero-Ferreira et al., 2011; Hadas et al., 1997; Puck et al., 1951; Schlesinger, 1932; Zarybnicky et al., 1980). But once a certain fraction of the phage population adsorbs, there is usually a dramatic deceleration in adsorption rate, which cannot be adequately explained with first-order kinetics alone (Eq. (3)). Various approaches have been proposed to account for this.

Two-step adsorption models

Typical bacteriophage adsorption curves are shown in Fig. 1. The curves represent three general cases of adsorption models. The simplest model, Case 1, is the 1st order model. It exhibits a logarithmic decrease in free phage concentration over time. Although shown for a period of 60 min in Fig. 1, a simple exponential decay of the free phage concentration often does not describe the data sufficiently well after the first few minutes of adsorption (*i.e.* Cases 2 and 3). The curves of Cases 2 and 3 represent two different approaches to modeling adsorption kinetics, but two common features: an initial rapid rate of decay followed by much slower kinetics. Phage researchers have suggested a variety of ways to account for this kinetic behavior.

Krueger (1931) believed the plateauing adsorption curve represented an equilibrium between attached and free phages. But once irreversibly adsorbed, phages cannot desorb and thus a simple equilibrium is an inadequate description (Delbruck, 1940). Working with T-series phages, Puck et al. (1951) determined that the first step in phage attachment is reversible and governed by electrostatic bonds between virus and host. However, desorption was only observed under unfavorable binding conditions. Stent and Wollman (1952) also suggested a two-step mechanism and proposed three possible theories to explain the sometimes conflicting observations reported by the early phage studies: the activity–inactivity model, the competitive model, and the sequential model.

In the activity–inactivity model, the phage oscillates between an active and inactive state. This reversible equilibrium is

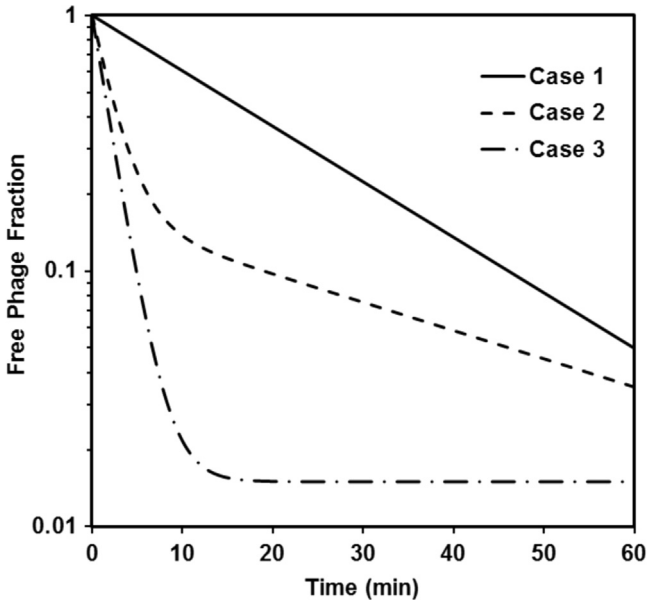

Fig. 1. Representative adsorption curves. Phage adsorption kinetic data is generally reported as a decrease in the free phage titer, here normalized with respect to the initial phage titer. Each case represents general characteristics of three commonly used phage models: 1st order model (Eq. (3), Case 1), sequential model (Eq. (6), Case 2) and the adsorption efficiency model (Eq. (7), Case 3).

temperature-dependent and is demonstrated with the following scheme:

$$P_I \leftrightarrow P_A + B \rightarrow I \quad (4)$$

where P_I and P_A represent the inactive and active states of the virus, respectively. This model was developed based on observations that some phages require the presence of specific organic co-factors to adsorb in synthetic media (Stent and Wollman, 1952).

The competitive model, or alternative collision theory, assumes two competing reactions, one reversible and one irreversible, can occur between the virus and host. The reversible reactions do not lead to virus attachment and consequently, the virus particle must detach before binding irreversibly in the correct orientation. This model is summarized by the following scheme:

$$P + B \leftrightarrow R \quad (5A)$$

$$P + B \rightarrow I \quad (5B)$$

where R represents the reversibly attached phage.

The sequential model, or surface reaction theory, proposes a two-step process where a reversible step must precede the irreversible attachment

$$P + B \leftrightarrow R \rightarrow I \quad (6)$$

Although 60 years have passed since these models were first proposed, a consensus has not emerged on which is the most appropriate. A recent study of T4 adsorption kinetics over short time scales concluded that the sequential model, modified to account for bacterial growth, was the most appropriate (Zonenstein et al., 2010). But others have pointed out that it is probably not feasible to propose a single "model embracing the whole spectrum of existing bacterial viruses and their hosts" (Rakhuba et al., 2010). The reality is that selecting the most appropriate model for a particular system depends on the phage, the environmental conditions, and the application.

In their studies on phages T1 and T2, Garen and Puck (1951) and Puck (1953) favoured the sequential model. They found that the reversible step was temperature-independent and that the second, irreversible step followed swiftly from the first. This model has been applied to numerous other phage species including λ (Moldovan et al., 2007; Schwartz, 1976), T4 (Zonenstein et al., 2010), T5 (Zarybnicky et al., 1980), and the *B. subtilis* phage PBS Z (Steensma, 1981, 1982) among others. The appeal of the sequential model is that it contains easily determined constants, making the model easily adjustable for various adsorption conditions. However, this does not necessarily equate to model robustness. For an in depth study of the sequential model, including an analytical solution to the system of reaction rate equations and empirical determination of reaction rate constants, see the work of Moldovan et al. (2007). Case 2 (Fig. 1) is a representative adsorption curve described by the sequential model.

A number of researchers have commented on the shortfalls of the sequential model. Unsatisfied with the agreement between experimental observations and the predictions of the sequential or competitive model, Christensen (1965) combined the concepts of the two to form the modified sequential model

$$R_b \leftrightarrow P + B \leftrightarrow R_a \rightarrow I \quad (7)$$

where R_b and R_a represent 'bad' and 'good' reversible complexes, respectively. Only a 'good' reversible complex can lead to irreversible adsorption. While Christensen worked with phage T1, this type of model has been used with other bacterial viruses as well, including *Lactobacillus* phage PL-1 (Watanabe et al., 1982) and mycoplasma virus L3 (Haberer and Maniloff, 1982).

Biphasic models

While the existence of the reversible step in phage adsorption is generally accepted as a necessary step in the process, the adsorption process does not always behave as predicted by the above models. None of the models discussed above (Eqs. (1), (4)–(7)) account for the inherent heterogeneity of the phage population. Phage heterogeneity, where a fraction of the population exhibits markedly slower adsorption rates or fails to adsorb at all (Fig. 1, Case 3), was first reported by Schlesinger (1932) as the residual fraction. The residual fraction consists of phages that apparently have a physiological defect hindering their adsorption capabilities, possibly in the organelles of attachment (Gallet et al., 2012; Steensma, 1982; Storms and Sauvageau, 2014). Confirmation that the residual fraction represents a form of heterogeneity in the population, and is not the result of an equilibrium, has been achieved by isolating residual phages and mixing them with fresh bacterial cells. The adsorption kinetics observed are significantly slower than those of the original phage stock, and in some cases no adsorption is observed (Delbruck, 1940; Gallet et al., 2012; Steensma, 1982; Storms and Sauvageau, 2014). The diversity of phages found to display heterogeneity suggests that it may be a universal phenomenon.

Models that do not account for the heterogeneity of the phage population risk mistaking heterogeneity for equilibrium, or not accounting for non-adsorbing phages. An important study on the adsorption of phage PBS Z demonstrated that while the sequential model predicted that 30% of the phages were still reversibly attached after 1 h of incubation, electron micrographs showed over 99% of the phages were in a contracted state on the cell surface, implying irreversible adsorption (Steensma, 1981). A similar disconnect between the number of reversible complexes predicted by the sequential model and those observed experimentally was reported in a study on phage T6 (Storms et al., 2012). When looking only at the change in free phage concentration during adsorption, this important observation can go unnoticed. For example, one could fit the sequential model (Eq. (6)) to the adsorption curve represented by Case 3 (Fig. 1) by adjusting the reaction rate constants such that the second, irreversible step had extremely slow kinetics. However, this would indicate that the initial decrease in phage titer was due to the formation of reversible complexes and that the formation of infected cells was nearly nonexistent – a physical phenomenon that would render the cell unsusceptible to phage attack for all intents and purposes. To properly account for the heterogeneity of the phage population, some have suggested biphasic modeling approaches. Garen (1954) and Christensen (1965) both acknowledged the existence of 'slow adsorbers' in their phage populations. Christensen dealt with the situation by sometimes using a different set of rate constants in his model (Eq. (7)) for these phages, an approach that has been adopted by other researchers (Watanabe et al., 1982). Steensma (1982) tried using two different rate constants to describe the two distinct subpopulations of the phage, both adsorbing with first-order kinetics (Eq. (1)). He found a better fit was obtained by assuming a fraction of the phage population was unable to adsorb at all (1% in his case). Using this assumption in combination with the sequential model provided the best fit to his data; but no experimental confirmation has been provided to validate this approach.

The practice of modeling a fraction of the phage population as non-adsorbing was studied in detail by Storms et al. (2010, 2012) and Storms and Sauvageau (2014). The authors assumed the forward reaction in the reversible step was highly favored over the reverse reaction, and that the irreversible step followed quickly after reversible attachment. With these assumptions, the sequential model (Eq. (6)) can be simplified to the first-order model Eq. (1). However, in this model, the heterogeneity observed in phage populations is taken into account by the introduction of an additional term, the adsorption efficiency ε. This term, determined experimentally, is defined as the fraction of the phage population able to adsorb to a host cell over the course of the experiment

(essentially the fast adsorbing subpopulation). This term can be used to modify Eq. (2) so that only the effective phage titer is considered during adsorption, as shown below:

$$\frac{dP}{dt} = -kB[P - P_0(1-\varepsilon)] \quad (8)$$

where P_0 is the initial phage titer. The term $P_0(1-\varepsilon)$ is a constant representing phages unable to adsorb to a host (note that $(1-\varepsilon)$ represents the free phage fraction in an adsorption experiment). Consequently, the entire term in brackets on the right side of Eq. (8) describes the effective phage titer – those phages within the population capable of adsorbing to a cell. Case 3 (Fig. 1) is a representative adsorption curve generated using this model. The adsorption efficiency model proved robust enough to describe the adsorption of numerous T-series strains representing the three major tailed-phage families, and gave a more accurate representation of the concentration of reversible complexes observed experimentally than the sequential model (Storms et al., 2012).

Selecting the appropriate adsorption model

Knowing the correct model for a specific phage–host system *a priori* is often not possible. In almost all cases, experimental data must be gathered and compared with various model predictions before deciding on the most suitable approach. Examples of experimental data for different phage–host systems that fit well into the common modeling structures is presented in Fig. 2. Each data set is best described by one of the three modeling approaches shown in Fig. 1. The adsorption data for residual T4 phage believed to be delinquent in its tail fiber structure exhibits the typical 1st order kinetics (Case 1). Contrast this with the fast and efficient adsorption kinetics displayed by a classic wild-type T4 population – exemplary of the adsorption efficiency model (Case 3). Finally, bacteriophage λ adsorption data displays the distinct two-step decay pattern best described by the sequential model (Case 2).

There are also some general trends to consider that can facilitate the selection process. The activity–inactivity model – used alone or in combination with the adsorption efficiency model – is best suited for phages requiring a specific organic co-factor for adsorption. For example, certain strains of T4 require the presence of L-tryptophan for adsorption. The tryptophan molecules interact with the long tail fibers of the virus to position the fibers extending outward, away from the phage tail, the orientation required for efficient adsorption (Kellenberger et al., 1965). The adsorption efficiency model is most appropriate when the assumptions of population heterogeneity and a strongly favored forward reaction in the reversible step of adsorption are met. Tailed phages that use long side-tail fibers to reversibly attach to the host cell generally meet this requirement quite well (Storms et al., 2010, 2012) due to the mechanism of adsorption (Goldberg et al., 1994). Yet suboptimal media composition, pH or temperature can sometimes make this assumption invalid. Certain phage morphologies may also influence adsorption kinetics. Studies on phage λ suggest that it adsorbs according to the mechanism described by the adsorption efficiency model only if it possesses more than one long side-tail fiber (Storms et al., 2012). Most laboratory strains of phage λ contain only a single tail fiber extending from the baseplate (Hendrix and Duda, 1992) which are better described by the sequential model (Moldovan et al., 2007; Schwartz, 1976; Storms et al., 2012). When the assumption of a strong forward reaction in the reversible step cannot be met, the sequential model or modified sequential model is appropriate. However, ignoring the reality of phage heterogeneity in these models may lead to an incomplete picture of the adsorption process. In many practical applications involving bacteriophages, such as modeling phage amplification in a bioreactor or in the human body, the trade-off between model complexity and accuracy become very important. Generally, the simple first-order adsorption reaction rate constant (from Eq. (1)) will suffice as an indication of attachment rate, but we recommend including the adsorption efficiency as well for increased model accuracy with minimal added complexity.

When conducting adsorption studies to gather kinetic data on a particular phage–host system, we caution the researcher to carefully consider the experimental set-up of their adsorption protocols. While the details are beyond the scope of this review, we refer the interested reader to studies correctly employing the distinct protocols used to differentiate among total adsorbed phage, reversibly adsorbed phage, and irreversibly adsorbed phage that will be present in the population (Baptista et al., 2008; Storms et al., 2012; Adams, 1959).

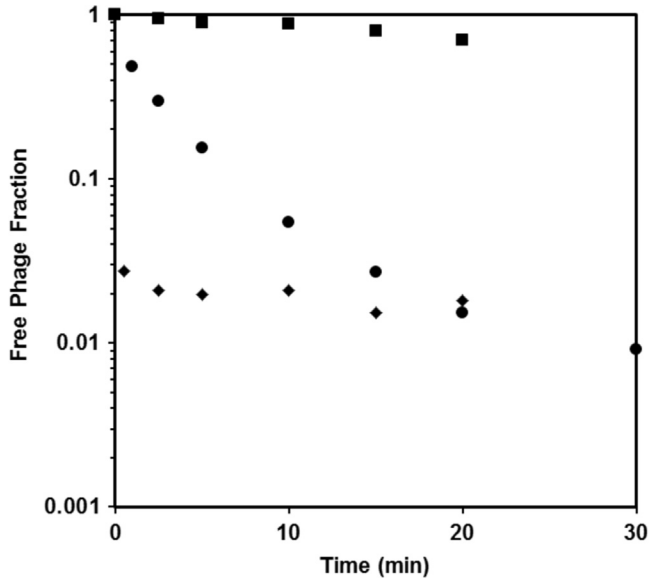

Fig. 2. Representative adsorption data. Three different phage populations adsorbing to *Escherichia coli* are shown. Each data set is best described by one of the adsorption models shown in Fig. 1: 1st order model (residual phage T4, squares), two-step sequential model (phage λ, circles), and the adsorption efficiency model (wild-type phage T4, diamonds).

Adsorption modeling in phage amplification

Accurate predictions of phage amplification are important when designing large scale phage-production processes for downstream applications, when using phages as antibacterial control agents in fermentation, (*e.g.* Bertozzi Silva and Sauvageau, 2014; Sauvageau and Cooper, 2010; Worley-Morse et al., 2015), or when designing dosage levels for phage therapy treatments (Levin and Bull, 1996; Payne and Jansen, 2001; Weld et al., 2004). The phage replication cycle has generally been modeled using a variation of the classic Lotka–Volterra predator–prey relationship first described by Levin et al. (1977). The model assumes three trophic levels: (1) primary resources (*e.g.* sugar), (2) first order consumers or prey (bacteria), and (3) predators (phage). The time variation in the concentrations of resource, bacteria, infected bacteria, and phage are related through a set of differential equations that incorporate growth-associated parameters (*e.g.* cell growth rate, latent period, burst size, adsorption rate). This approach has yielded relatively accurate predictions of phage proliferation in chemostats and batch reactors (Levin et al., 1977; Worley-Morse et al., 2015), but has been less successful in modeling *in vivo* phage therapy studies (Weld et al., 2004). For a detailed review of general approaches for modeling phage growth, see Stopar and Abedon (2008).

To avoid rapidly escalating the complexity of the predator–prey differential equations, most models of the phage amplification process approximate adsorption using a single variable (k) that acts as an all-encompassing adsorption rate (Levin and Bull, 1996; Levin et al., 1977; Payne and Jansen, 2001). Consequently, the adsorption step in amplification models is almost always simplified to the single, first order reaction (Eqs. (1) and (2)). Adding additional complexity to the adsorption model has been generally thought to either render the predator–prey differential equations too difficult to solve or to not improve the model enough to justify its inclusion.

One modeling approach that may offer improvements significant enough to justify its use is a bimodal model. The adsorption efficiency model is one such model, where adsorption is assumed to proceed according to Eq. (1), but with the addition of the adsorption efficiency to account for inherent heterogeneity in the phage population (Storms et al., 2010, 2012). Adsorption efficiency is a term that has been used somewhat ambiguously in phage literature, but it is not uncommon to see it reported during virulence characterization studies to indicate what percentage of the phage population adsorbed to a host over a given timeframe (Chaudhry et al., 2014; Plaut et al., 2014; Vandersteegen et al., 2013; Wong et al., 2014). In addition, studies have demonstrated that adsorption efficiency can significantly influence the outcome of a phage infection in a batch reactor (Storms et al., 2010), and thus could do the same in other systems. We have proposed a phage infection model that accounts for adsorption efficiency (Bertozzi Silva, 2013) and used it to describe the virus–host interactions of *Lactobacillus plantarum* bacteriophages (Bertozzi Silva, 2013). The adsorption efficiency is assumed to be a constant in the model, independent of the host cell physiological state; an observation noted in numerous adsorption studies (Golec et al., 2014; Storms et al., 2010, 2014).

Another possible improvement to adsorption approximations in models of phage amplification is to assume a distribution of adsorption rates. Santos et al. (2014) modeled the population dynamics of a lytic *Salmonella* phage using the classic predator–prey relationship of Levin et al., but were not able to obtain acceptable agreement between model and experimental data. Troubleshooting with various modifications, they found modeling the adsorption rate as a distribution function, dependent on the host cell growth rate, yielded the best model fit to the data. However, the model approximates the adsorption rate to stationary phase cells as zero without experimental justification. Moreover, no explanation is given as to why modifying other parameters (burst size, lysis time) did not have a significant impact on the model prediction. While it is certainly likely that adsorption rate is a function of host cell physiology, caution should be exercised when using the model proposed by Santos et al. There is a great body of evidence demonstrating high rates of phage adsorption to stationary phase cells (Gallet et al., 2012; Golec et al., 2014; Storms et al., 2010, 2012, 2014). A safer approach is found in the model of Weitz and Dushoff (2008), where phage-induced mortality is linked more generally to the host reproduction rate. Consequently, a number of factors such as reduced adsorption, reduced burst size, cell wall thickening, and an increase in non-viable infections may reduce the phage 'carrying capacity' of the host in stationary phase (Weitz and Dushoff, 2008).

Recent work by Bull et al. (2014) suggests that the situation is more muddled still. Although it is unlikely that adsorption rates go to zero when cells are in stationary phase, Bull suggests that a combination of genetic and non-genetic factors may affect the observed adsorption rate in a typical adsorption curve. As such, the curve does not represent a specific adsorption rate for the virus, but rather an average value based on the unique adsorption rate of each host cell. Such factors as receptor density on the cell surface, cell size, and motility will influence the adsorption rate of the virus and give the cell a specific adsorption phenotype. Phenotypic variations in adsorption rate within the culture could be caused by a combination of induced changes in gene expression, intrinsic variations within the population, and dynamic reactions such as self-regulation in response to the presence of phage proteins (Bull et al., 2014). Using the assumption of intrinsic phenotypic variations within the culture, Bull proposes a model where the bacteria population could exist in two distinct states: one susceptible to phage infection and the other resistant. While this model would not significantly impact the typical adsorption curve, it does significantly impact the population dynamics of phages and bacteria during infection (Bull et al., 2014). Such a mechanism could be used to explain some unexpected observations reported in the literature such as the large variation in efficiency of plating observed for individual colonies derived from the same batch culture (Bull et al., 2014) or the rise of mostly sensitive bacteria after a phage attack (Levin et al., 2013). We concur with Bull's analysis and recently proposed a model for phage amplification that deals with the constant flux between susceptible and non-susceptible cells as a reversible reaction (Bertozzi Silva, 2013).

Detailed experimental evidence that heterogeneous gene expression levels confer resistance to phage attack exists in the literature for at least one phage–host system (Chapman-McQuiston and Wu, 2008a, 2008b). In their meticulous study, Chapman-McQuiston and Wu observed that the naturally stochastic gene expression of the LamB protein in *E. coli* cells – the receptor binding site for phage λ – results in a broad, nearly continuous distribution of receptor densities on the cell surface. Accordingly, some cells within the population exhibit an insensitivity to phage attack due to phenotypic heterogeneity (little to no LamB expression). Importantly, experimental evidence strongly supports the conclusion that phenotypic switching from high LamB expression states to low LamB expression states exists within the population over the course the phage infection studies performed by the authors. While this study provides evidence of continuous spectrum of phage sensitivity levels rather than a binary distribution, the nature of the distribution may be species dependent. Additional studies in this area are needed to more fully understand the impact of heterogeneous cell populations on phage adsorption.

Adsorption modeling in plaque growth

One application where the two-step sequential model has found important use is in modeling plaque growth on a soft agar lawn. Plaque enlargement can be thought of as a reaction-diffusion mechanism where diffusion – the spread of phages – increases plaque diameter and infection – reaction – increases phage numbers within the plaque. The majority of the infections will occur at the interface between the plaque and the bacterial lawn (Krone and Abedon, 2008). Models of plaque growth give an approximation for the wavefront velocity, or the rate of plaque increase. While simple models have been proposed that rely only on a single, overall rate of phage diffusion and the phage latent period (Koch, 1964), the most comprehensive models are the reaction-diffusion models put forth by Yin and McCaskill (1992) and You and Yin (1999) and Ortega-Cejas et al. (2004). In both instances adsorption is modeled as a two-step reaction following the scheme of the sequential model as presented in Eq. (6). Note that additional factors influence plaque growth aside from diffusion and adsorption rates. Burst size, lysis time, and host cell growth rate will also play an important role in plaque formation and wavefront velocity. For a detailed discussion on the factors influencing plaque growth and how they are treated

by the various models in the literature, see the reviews by Krone and Abedon (2008) and Abedon and Culler (2007).

Conclusion

A look back at the studies of early phage researchers shows many of their conclusions regarding the phage attachment process were generally insightful, if not precise. More modern studies have corroborated the premises of the originally proposed mechanism: reversible interactions between specific elements of the phage and host lead to an irreversible attachment. While the two-step process of phage adsorption has been described using a variety of reaction kinetics equations, the simplest description in its original form (1st order kinetics) remains, if not the most accurate, one of the most widely used today. A single, robust equation suitable for all phage–host systems interacting in all environments will probably never be found, but adjusting the fundamental two-step process to account for various physico-chemical properties of the medium, physiological characteristics of the cell, and the binding mechanism of the phage can provide highly accurate predictions of adsorption dynamics with relatively simple models.

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