

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Gels for the Propagation of Bacteriophages and the Characterization of Bacteriophage Assembly Intermediates

Philip Serwer

*The University of Texas Health Science Center, San Antonio,  
USA*

## 1. Introduction

Advances in biochemistry-based analysis of biotic systems depend on improved procedures for the sorting of the non-covalently joined macromolecular assemblies that are obtained by the expelling of cellular contents. The development of preparative ultracentrifugation, for example, made possible the discovery of both ribosomes and various intracellular organelles (reviewed in Alberts et al., 2002). Some biotic systems depend on assembly more than one might conclude at first (reviewed in Kurakin, 2007). Improving this biochemistry-based analysis is an ongoing process, given that (1) multi-molecular, assembly-derived mechanisms are not yet understood, including (especially) the mechanisms of biological motors (reviewed in Howard, 2009; Myong & Ha, 2010) and (2) one reasonably projects that components of current biotic systems have abiotic ancestors that were also non-covalently joined assemblies, the understanding of which is potentially essential for understanding the origins of life (Koonin, 2009; Serwer, 2011).

Separately, advances in analysis of environmental microbial systems depend on improved procedures for the propagation and sorting of individual microorganisms following their extraction from the environment. Improving microbial propagation/isolation/sorting remains an ongoing process (for example, Ferrari et al., 2008; Sait et al., 2002; Serwer et al., 2009), given that only a small fraction ( $< 0.01$ ) of environmental microbes have been propagated (Ferrari et al., 2008 and included references). I review here some advances in the use of agarose and agar gels for both (1) biochemistry-based sorting of macromolecular assemblies and (2) detection, propagation and sorting of unusual environmental viruses, with focus on bacterial viruses (bacteriophages), abbreviated phages.

### 1.1 Basics

Fractionation-based sorting and characterization of macromolecular assemblies is a strategy complementary to biochemical assay-based determining of the activities of single, unassembled macromolecules. The unassembled macromolecules include many that function when assembled with molecules of other types. The use of these two strategies is illustrated by the analysis of mechanisms of DNA replication. The proteins involved invariably include a single protein with DNA polymerase activity. In contrast to the original

thought, the process of DNA replication is so complex that numerous proteins of other types are also involved via a multi-molecular complex, usually called a replisome (reviews: Hamdan & Richardson, 2008; Langston et al., 2009). However, replisomes are difficult to isolate from cells and tend to dissociate during isolation. Thus, investigators usually replace sorting-based biochemistry with *in vitro* replisome assembly, followed by analysis of the transitions that occur *in vitro*. Nonetheless, if appropriate procedures can be developed, in theory, one might (1) isolate replisomes from a cell, (2) fractionate the replisomes by the extent of DNA replication and then (3) perform a biochemical/biophysical analysis of the replisome at each of the various stages of replication. Once this is done, a fluorescence-based signature can be developed for each state observed in the sorted replisomes. The signatures would provide a way to observe the progression of replisome-associated DNA replication *in vivo*.

To develop the tools needed for a sorting-based analysis of any biochemical system, we have focused on a multi-molecular, biotic system for which sorting has been relatively productive, because the multi-molecular complexes involved (1) are relatively stable, (2) are relatively uniform in surface characteristics and (3) can be fractionated by the extent of the biochemical process being analyzed. This system packages the double-stranded DNA of a phage after the phage DNA has been replicated in a phage-infected cell. For all studied double-stranded phages, DNA packaging is initiated by a protein capsid (procapsid) pre-assembled without interaction with DNA. This procapsid is called capsid I in the case of the related phages, T3 and T7. Capsid I converts to a larger, more phage-like capsid during DNA packaging (capsid II for T3/T7; Figure 1,a-b) and can be made to package DNA *in vitro*, after isolation of capsid I from a lysate of infected cells (reviews: Aksyuk & Rossmann, 2011; Catalano, 2000; Fujisawa, & Morita, 1997; Serwer, 2010). Subsequent steps in DNA packaging begin with capsid II and some are described in Section 1.2.

Returning to the topic of propagation-/isolation-based analysis of environmental phages, we have focused on developing and using procedures to propagate and isolate phages that are not isolated by conventional procedures. Our new procedures isolate phages that are unconventional in that they have one or more of the following characteristics: (1) propagation-associated aggregation, (2) unusually large size, (3) inactivation by dilution and (4) absence of sufficient propagation in liquid culture to produce cellular lysis in visible amount. The core procedure is based on initial isolation by incubation of soil samples in a medium-containing, dilute (0.1-0.2%) agarose overlay (Figure 2a; reviewed in Serwer et al., 2009). This procedure continues by platinum needle transfer only, as illustrated in Figures 2,b-d. As described in Section 5, the pores of the agar gels typically used for a phage plaque-supporting gel are too small for propagating some (maybe most) large or aggregating phages.

After presentation of some details of both DNA packaging and phage isolation in the remainder of Section 1, I will describe the studies of the gels and gel electrophoresis used for these studies. Understanding of these gels was (and presumably will be) essential to improving the use of them.

## 1.2 Some details

Past work on the sorting of infected cell-derived macromolecular assemblies has produced a hypothesis for the sequence of T3/T7 DNA packaging events. The solid arrows of Figure 1 indicate this proposed sequence, as derived from the fractionation-based sorting of particles

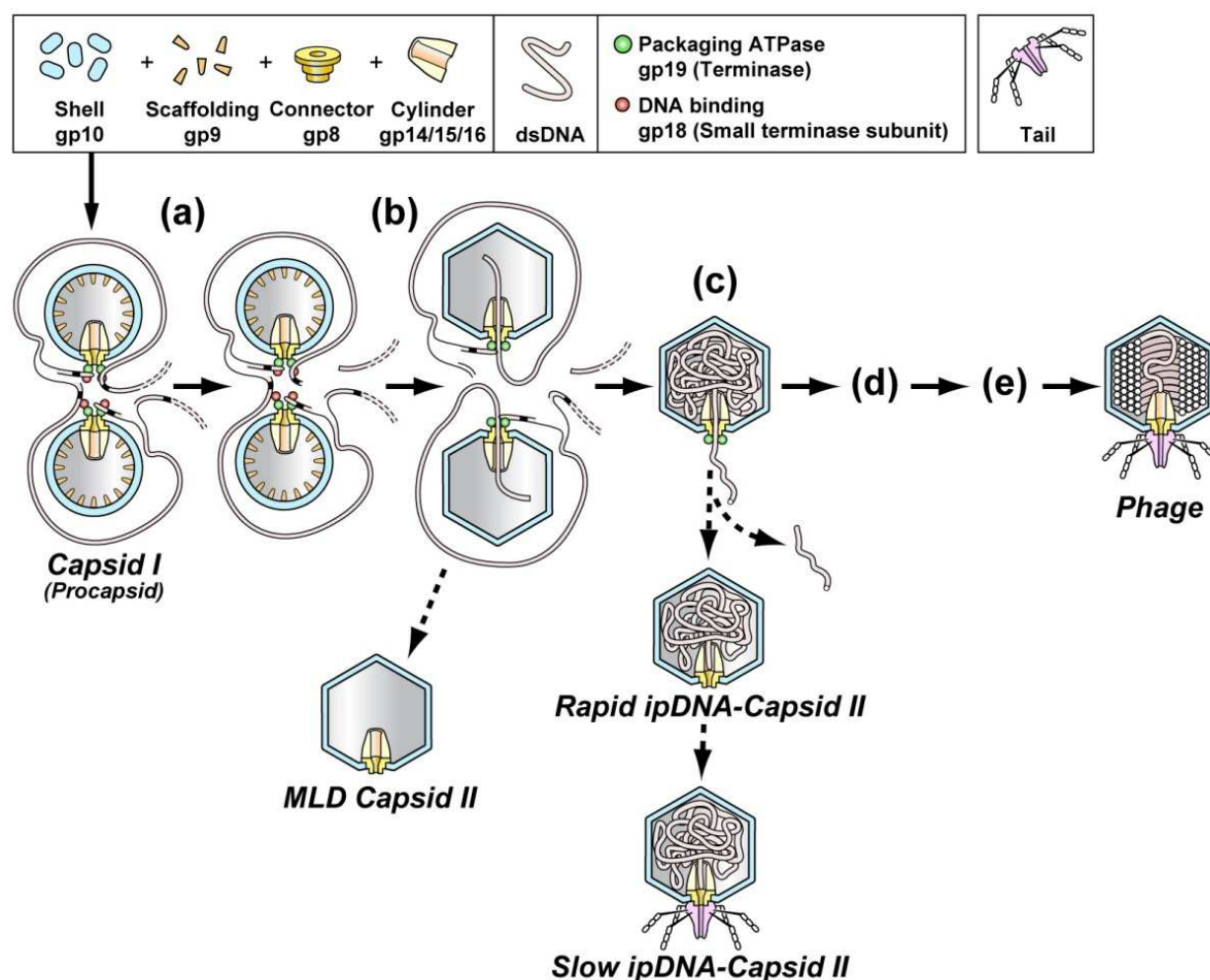


Fig. 1. Bacteriophage T3/T7 DNA packaging. The solid arrows connect intermediates that have been deduced from observed intermediates. Dashed arrows indicate the observed intermediates.

produced during packaging and found in lysates of infected cells. These latter particles will be called intermediates whether or not altered during fractionation. The isolated, fractionated intermediates include incompletely packaged DNA (ipDNA)-containing capsids (ipDNA-capsids). The ipDNA-capsids were originally sorted by ipDNA length via buoyant density-based ultracentrifugal fractionation (Fang et al., 2008). The ipDNA-capsids were then identified and the capsid further characterized by agarose gel electrophoresis (AGE) of intact particles (Fang et al., 2008), as further described in Section 3. A dashed arrow in Figure 1 connects a fractionated and characterized ipDNA-capsid to an intermediate proposed to exist during DNA packaging *in vivo*. The detection of ipDNA-capsid II by AGE was simplified by the fact that the AGE-migration of ipDNA-capsid II is independent of the length of ipDNA. In general, the migration of any particle during AGE depends only on the characteristics of the particle's surface, not on what is packaged inside. This point is discussed in more detail in Section 3.

The use of advanced procedures of AGE revealed that the protein shell of at least some ipDNA-capsid II does eventually undergo changes as ipDNA becomes longer. These changes produce intermediates at (d) and (e) in Figure 1. In brief, the changes at (d) and (e)

suggest that the T3/T7 DNA packaging motor has two cycles, the second of which changes the capsid's shell and acts as a back-up cycle when the first stalls. The details (Serwer et al., 2010; Serwer & Wright, 2011) are not reviewed here because they are complex enough to be distracting to the main objectives.

## 2. Gel-forming polymers

### 2.1 Basics

Gels have spaces through which molecules migrate either by thermal motion or by response to an external potential gradient. The potential gradient is typically, but not necessarily, electrical. Agar gels, although initially (and still: Rasmussen & Morrissey, 2007) used as a supporting matrix for food, were subsequently found to be similarly useful as a supporting matrix for bacterial colonies. The bacterial colonies typically grew on the surface, but use of dilute (0.4%) agar in the presence of a complex medium was found to permit *Salmonella typhimurium* to swim through the gel. This swimming was used to assay transfer of genes needed for motility (Stocker et al., 1953). Thus, the pore size of the medium-containing 0.4% agar gel, while not precisely defined by these studies, could be estimated to be at least as large as the width of the bacteria, assumed to be  $\sim 500$  nm. Smaller pores would not have allowed the bacteria to migrate to the interior of the gel.

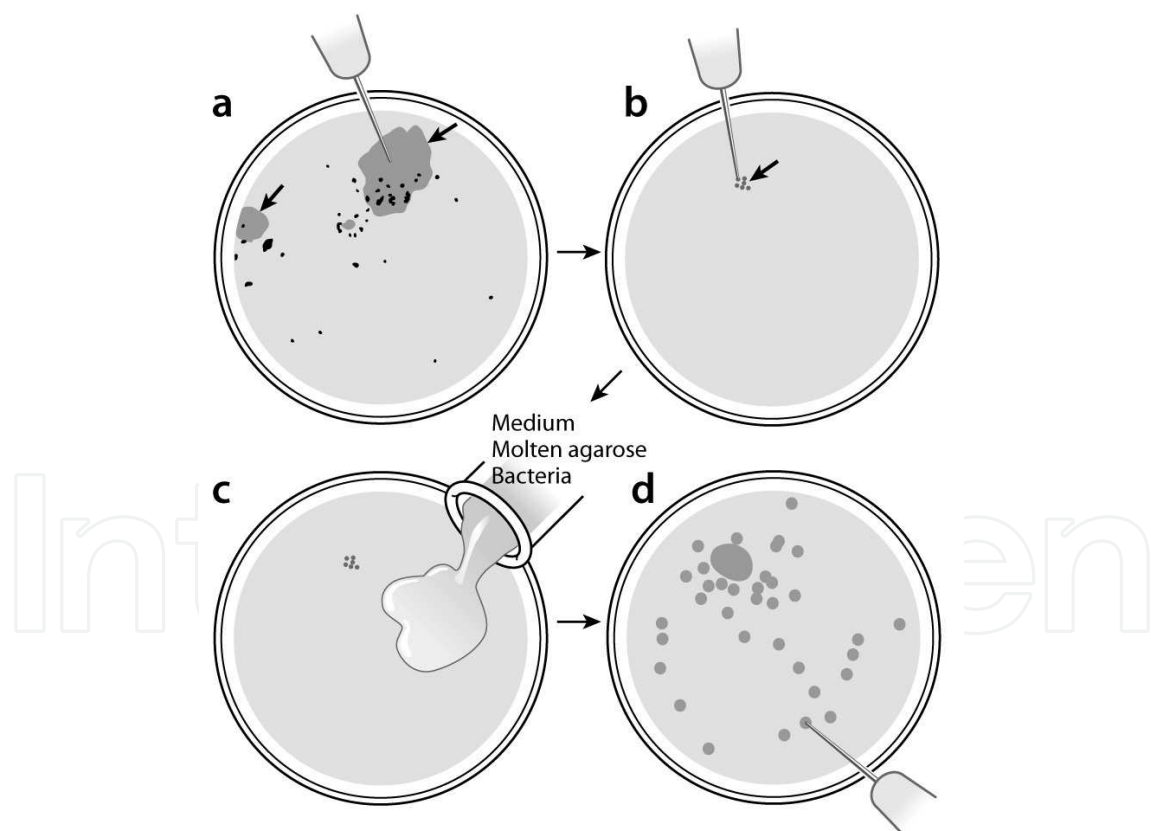


Fig. 2. Phage isolation. (a) Post-incubation initial plate with soil (irregular black objects) embedded in a dilute agarose overlay and phage-induced zones of clearing (arrows) in a host lawn. (b) Needle transfer via stabs (arrow) to the bottom agar of a new Petri plate. (c) Pouring of a new overlay. (d) Post-incubation secondary plate with single phage plaques one of which is being used for cloning. Further details are reviewed in Serwer et al. (2009)



The cause of the relatively large pores of some polysaccharide gels is the lateral aggregation of the polysaccharide polymer to form multi-chain “pillars” that provide gel strength while forming a mesh with relatively large pores. The existence of the pillars is qualitatively confirmed by simply observing the light scattering of a gel. Agarose gels are typically turbid because of the pillars (Rees, 1972). In contrast, polyacrylamide gels of the same total concentration and conventional cross-linker concentrations are not turbid (Chen and Chrambach, 1979). The turbidity of an agarose gel decreases as (1) the temperature of gelation and buffer ionic strength decrease, and (2) the agarose molecular weight increases (Griess et al, 1993; Griess et al., 1998; Serwer & Griess, 1999), with an associated decrease in the radius of the effective pore ( $P_E$ ). If one adds consideration of the agarose source-, purification- and derivatization-dependence of  $P_E$  (Griess et al., 1989; Griess et al., 1998), one can only conclude that  $P_E$ -dependent results from different studies cannot be compared quantitatively unless one is willing to tolerate the likelihood of  $P_E$  errors of at least 100%. In general, quantitative comparisons should be performed with internal standards.

## 2.2 Some details

The gels to be discussed here are cast by cooling solutions of either agar or agarose that had been dissolved by boiling. Agar is a  $\beta$ -linked alternating co-polymer of two sugars; negatively charged groups are attached in variable amount to the sugars. Agar is obtained from red seaweed. Agarose is a sub-fraction of agar that has a relatively low density of charged groups (reviewed in Rees, 1972). The extent of residual charge is often used to name agarose preparations via the field-induced flow of buffer that gel-attached charged groups cause (electro-osmosis, abbreviated EEO; Griess et al., 1989). The minimum agarose concentration for gel formation varies somewhat with agarose EEO, and agarose chain length but can be as low as 0.03% for a high-strength agarose, when the gel is supported at its sides by embedding in a more concentrated gel (Serwer et al., 1988).

If one extrapolates previous determinations of  $P_E$  to 0.03% agarose, one finds that micron-sized particles can enter agarose gels. If entry into the gel is to be driven by a potential gradient, the entry will become limited by trapping of a micron-sized particle in the pores that are relatively small, if the potential gradient is too high in magnitude. The trapping occurs because of the relatively low thermal motion of particles this large (Serwer et al., 1988; Serwer & Griess, 1998). Nonetheless, by use of an electrical potential gradient relatively low in magnitude (0.5 V/cm; 2.0 V/cm is too high), intact (alive) cells of the bacterium, *Escherichia coli*, have been subjected to agarose gel electrophoresis and fractionated by length (Serwer et al., 1988).

On the other hand, one lowers  $P_E$  by raising agarose gel concentration, a process that is assisted, if necessary, by lowering the average agarose chain length and, therefore, reducing viscosity (Griess et al., 1993). The studies reported below have not been limited by difficulties in attaining any  $P_E$  needed.

## 3. Gel electrophoresis

### 3.1 Electrophoretic principles and some of their applications

Fractionation by gel electrophoresis depends on two characteristics of a roughly spherical particle being fractionated. The first characteristic is the average, per area, of the particle's

surface electrical charge that is not counter ion-neutralized ( $\sigma$ ). The force produced by application of an electrical potential is proportional to  $\sigma$  (Shaw, 1969; Stellwagen et al., 2003). Therefore, the terminal velocity ( $v$ ) induced by an electrical potential is also proportional to  $\sigma$ . The magnitudes of  $\sigma$  and  $v$  typically decrease as the concentration of counter ions increases, because of the increase in surface charge neutralization, as described by the Debye-Hückel theory (Bull, 1971). The ionic strength of electrophoresis is kept relatively low both to increase the magnitude of  $v$  and to lower the heat produced during electrophoresis. A result is that adding any salt to an electrophoresis buffer reduces the force on the particle and, therefore,  $v$ .

This effect causes band spreading when a sample is in a relatively high ionic strength solution and electrophoresis is to be conducted at lower ionic strength. The relatively high ionic strength of the sample, coupled with diffusion of sample ions into the electrophoresis buffer, will cause band spreading because the leading edge of the sample will initially have a  $v$  higher in magnitude than  $v$  of the rest of the sample. Because  $v$  is usually (not always) proportional to the electrical potential gradient ( $E$ ), I will sometimes refer to the  $v/E$  ratio, rather than  $v$ . The  $v/E$  ratio is also called the electrophoretic mobility ( $\mu$ ).

This band spreading becomes important when, to avoid loss of particles during dialysis, one wants perform AGE of particles that are in concentrated (2-4 M) solutions of cesium chloride. This situation arises after preparative fractionation by ultracentrifugation in a cesium chloride density gradient. Band spreading is avoidable, however, if AGE is performed with the gel submerged beneath the electrophoresis buffer (submerged gel electrophoresis). Submerged gel electrophoresis is a standard procedure with which I assume that the reader is familiar. After loading samples for submerged gel AGE, one avoids sample salt-induced band spreading by waiting for 1.0-1.5 hours before starting electrophoresis. In this time, salt ions dialyze into the electrophoresis buffer. This procedure was based on the previous observation that dialysis of 2-4 M cesium chloride from 0.5 inch dialysis tubing is complete by 30 minutes, as judged by measuring the refractive index of the cesium chloride solution after removing it from the dialysis tubing (unpublished data).

Although submerged gel AGE of nucleic acids is almost always done without attempting to control pH gradients, this absence of control is not a good idea when proteins are the samples. Without a counter-measure, a pH gradient is unavoidable because hydrogen gas is released at the cathode, thereby raising the pH, and oxygen gas is released at the anode, thereby lowering the pH. Proteins titrate much more than nucleic acids in the pH range of the pH gradient generated during submerged gel AGE. The result of this protein titration is likely to be disastrous. Informally, I have been told of failures of AGE and, in some cases, pH gradients would probably have caused failure even if other aspects were in order.

The most efficient way to prevent a pH gradient with a submerged agarose gel is to circulate the electrophoresis buffer from one buffer tank to the other. The buffer flows back to the source tank across the surface of the submerged gel. One can also reduce the pH gradient by reducing the height of the buffer, but not so much that the cross-sectional area of the buffer starts to fluctuate. To avoid buffer circulation-induced washing of the sample out of sample wells, the circulation is started after the electrophoresis. The details of timing and circulation speed are empirically determined for each system. We circulate at  $\sim 100$  ml/minute, beginning at 30 minutes after the start of electrophoresis at 1 V/cm, with a phosphate buffer, pH 7.4 and a buffer height of about 0.8 cm.

### 3.2 Other factors that determine procedure

Any particle can be fractionated by AGE if the particle (1) is electrically charged in the buffer used, (2) is small enough to fit into the pores of the gel and is not electrophoretically trapped, (3) does not adhere to the gel and (4) is not damaged or dissociated by the process of electrophoresis. Particle-gel adherence and particle dissociation are the most likely causes of failure if the aspects from the previous section are in order. Particle-gel adherence is generally the case when particles either are found either to be broadly distributed near the origin or to form a sharp band at the origin edge of the agarose gel.

Responses to particle-gel adherence include changing the composition of the gel, overloading the binding sites by increasing particle concentration (Serwer & Hayes, 1982) and proteolytic cleavage of the gel-binding region of the particle (Serwer et al., 1982). Responses to dissociation include cross-linking, which is necessary in the case of microtubules, for example. Cross-linked microtubules do migrate during AGE, but dissociate if not cross-linked (Serwer et al., 1989).

### 3.3 Sieving during AGE

As solid, spherical particles migrate through a gel, they experience both hydrodynamic and steric effects of the presence of fibers that form the gel. If a sphere is almost as large as the effective pore of the gel, motion will be restricted to the point that the particle hardly moves. As the particle becomes smaller, the “sieving” effect of the fibers decreases and the particle undergoes more rapid motion. Eventually, while never zero, the sieving effect becomes so small that it changes almost imperceptibly with a percentage change in particle radius that caused a large change in sieving for larger spheres. So, to increase the sieving-based resolution by radius of a spherical particle, one decreases  $P_E$  (increases gel concentration), but stops before the pores are so small that the particles do not migrate. One pays for the increase in sieving-based resolution with an increase in the time of fractionation. A quantitative analysis of these effects for spheres is in Griess et al. (1989).

The effect of particle shape on gel sieving has been investigated for rod-shaped viruses. Without discussing the quantitative details, the lessons learned are the following. (1) Sieving effects do not discriminate a rod from a sphere when the  $0.5 \times \text{rod length} / P_E$  ratio is below  $\sim 1$  (Griess et al., 1990). (2) For a rod-shaped particle with length in this range, the effective radius that best describes the gel electrophoretic sieving is determined by assuming that the rod has a surface area (in contrast to either a length or a volume) equal to that of a sphere that exhibits the same sieving (Griess et al., 1990). (3) At smaller  $P_E$  values, a rod (unlike a sphere) has a gel electrophoretic  $\mu$  that increases in magnitude as the magnitude of  $E$  increases. This effect can be exploited to help identify rod-shaped particles after AGE (Serwer et al., 1995).

### 3.4 One-dimensional gel electrophoresis

A frequent application of gel electrophoresis is sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis (SDSPAGE) of proteins (Studier, 2000). This procedure starts with boiling of the proteins in the presence of SDS, a negatively charged ionic detergent. The SDS binds to the proteins and produces a surface that has a  $\sigma$  that is assumed be the same for all proteins, based on empirical measurements of SDS binding (Reynolds & Tanford, 1970). Thus, even though both  $\sigma$  and sieving determine  $\mu$ , the assumed uniformity of  $\sigma$  makes



possible the interpretation of SDS-PAGE patterns via sieving only. That is why SDS-PAGE, even though one-dimensional, is useful for estimating the molecular weight of a protein.

Uniformity of  $\sigma$  is usually also assumed during the AGE of DNA and RNA. This assumption is based on the uniformity of the phosphate backbone and remains accurate until end-effects occur as double-stranded DNA fragments are shortened (Stellwagen et al., 2003). Thus, DNA fractionations are usually interpreted via sieving, without considering possible changes in  $\sigma$ . The sieving effects are complicated by flexibility and, in some cases, by either branched or circular conformation (Åkerman, & Cole, 2002; Brewer, & Fangman, 1991). Conformation-dependent effects on ion binding and, therefore,  $\sigma$ , also occur for unusually bent DNA molecules (Stellwagen et al., 2005). Both circular DNA and DNA bound to solid objects undergo elevated  $E$ -induced trapping effects. These trapping effects are the basis for pulsed field-based separations (Åkerman, & Cole, 2002; Gauthier & Slater, 2003, for example) that are outside of the area of this review.

One electrophoretic direction (dimension) is usually used for both SDS-PAGE and nucleic acid gel electrophoresis (1d-AGE in the case of agarose gels). However, the electrophoretic profile does not have a unique interpretation, if both  $\sigma$  and particle dimension vary among particles subjected to 1d-AGE. To achieve a unique interpretation, based on both  $\sigma$  and particle dimensions (effective radius for a sphere), a second dimension of electrophoresis is added.

### 3.5 Separate analysis of $\sigma$ and effective radius: a second dimension

To separately measure both the effective radius ( $R_E$ ) and the  $\sigma$  of roughly spherical particles fractionated by AGE, one must add a second dimension (2d-AGE). Figure 3 illustrates a 2d-AGE procedure whereby one performs the first dimensional electrophoresis in a relatively

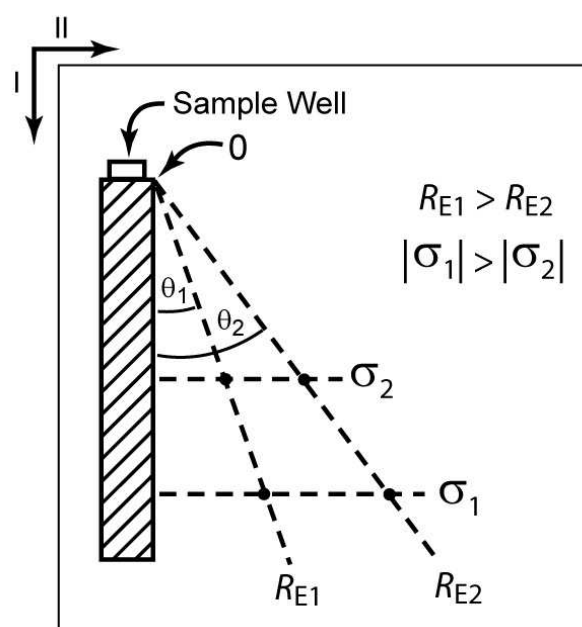


Fig. 3. Illustration of 2d-AGE. A sample is layered in the sample well and subjected to electrophoresis (arrow I indicates direction) through a dilute first dimension gel (diagonal bars) and, then, at a right angle (arrow II indicates direction) through a more concentrated second dimension gel.

dilute agarose gel so that  $\mu$  is determined primarily by  $\sigma$ . The dilute, first dimensional gel is stabilized by embedding it in a more concentrated gel used for the second dimension. To perform the second dimensional electrophoresis, the field/gel angle is rotated by  $90^\circ$  and electrophoresis is repeated with a second dimensional gel that is much more concentrated than the first dimensional gel.

The key to the 2d-AGE-based analysis of  $R_E$  is that the percentage change in  $\mu$  is independent of  $\sigma$ , when one compares  $\mu$  in the second dimension with  $\mu$  in the first, as first empirically confirmed in Serwer et al. (1986). Geometrically, this relationship implies that all particles of any given  $R_E$  are on one line (called a size line) that extends from the effective origin of electrophoresis (O in Figure 3) through the center of the band formed by a particle. As the angle ( $\theta$ ) between this line and the direction of the first electrophoresis decreases,  $R_E$  increases, as illustrated in Figure 3. The value of  $\sigma$  is proportional to the distance migrated in the first dimension, as illustrated in Figure 3. With the use of standards of size known by small-angle x-ray scattering (Serwer et al., 1986; Serwer et al., 1989), differences in  $R_E$  as small as 0.5% have been resolved by using  $P_E$  values close to the  $R_E$ 's of the particles analyzed (Casjens et al., 1992).

An advantage of 2d-AGE is that patterns can be interpreted for particles heterogeneous in either  $\sigma$  or  $R_E$  (or both). This aspect was originally demonstrated for vaccine conjugates heterogeneous in  $R_E$  (Serwer & Hayes, 1986) and has been developed in quantitative detail, given that these conjugates are of high utility (Tietz, 2007, 2009). More recently, the use of 2d-AGE with particles heterogeneous in both  $\sigma$  and  $R_E$  has been used to detect ipDNA-capsids of new type. These new ipDNA-capsids are at positions (d) and (e) in the pathway of Figure 1. Details are in Serwer et al. (2010) and Serwer and Wright (2011).

#### 4. Use of 1d-AGE to determine the kinetics of assembly *in vivo*

Major advantages of AGE are (1) the efficiency of fractionation of multiple samples and (2) the efficiency and accuracy of the quantification, via either autoradiography or fluorography, of the amount of radioisotope in each of several fractionated and detected particles. Thus, one can observe the kinetics of the passage of radiolabel through various intermediates, for the purpose of both determining the order of intermediate appearance and analyzing the mechanism of assembly and associated function. The efficiency makes this analysis possible not only for the wild type process, but also for the same process as it occurs for a mutant, with the 1d-AGE typically performed in a single agarose slab gel (see Serwer & Watson, 1982).

##### 4.1 An example of information previously obtained

This strategy was previously used for determining the effects on the assembly of phage T7 capsid I of removing the protein (called the connector or portal protein; Figure 1) that connects the gp10-containing shell with the tail of the mature phage. T7 proteins are labeled by gp, followed by gene number, as reviewed in Pajunen et al. (2002); comparable genes in T3 and T7 are given the same number. The T3 and T7 connectors are 12-mers of gp8. Although gp8 was in a position that suggested a role in nucleating shell assembly (Figure 1), genetic removal of gp8 had no detectable effect on the initial kinetics of capsid I assembly. That is to say, gp8 is not part of the nucleus for shell assembly. However, capsid I assembly

terminated prematurely in the absence of gp8 (Serwer & Watson, 1982). In these experiments, we analyzed completely unfractionated lysates of T7-infected *E. coli* by 1d-AGE.

Connector-independent shell assembly nucleation was subsequently also observed for phage P22, by use of rate zonal centrifugation in a sucrose gradient, rather than 1d-AGE, to assay for procapsids (Bazinet & King, 1988). Phages P22 and T7 are basically unrelated, but both have icosahedral shells with a triangulation number of 7 (P22: Chang et al., 2006; T7: Fang et al., 2008). Thus, the connector apparently has evolved after the shell. I note that the nucleus for shell assembly, whatever it is (a proposal is in Serwer, 1987), is likely to have at least 6 independent components, which implies a 6<sup>th</sup> order nucleation reaction, at least. The formation of a nucleus will, therefore, have a very high dependence on effective capsid protein concentration and, therefore, on excluded volume. Therefore, studies of *in vitro* shell assembly must be performed under conditions that mimic *in vivo* assembly, if any interpretation of what happens *in vivo* is intended. This *in vivo*-first priority cannot logically be reversed.

Finally, I note that herpes simplex virus also has a connector and that the herpes simplex virus connector (portal) is also not the nucleus for shell assembly, based on experiments similar in concept to those performed for phages (Newcomb et al., 2005). The work on phages preceded the work on herpes simplex virus by about 20 years largely because of the relative simplicity and speed of propagating and performing genetics with phages. Combining the simplicity and speed of work on phages with the simplicity and speed of AGE is a powerful addition to genetics.

#### 4.2 Utilizing chromatographical effects during electrophoresis

Although the basics of 1d-AGE and 2d-AGE are well defined and standardized, the transformations of macromolecular assemblies have complex determinants and are generally unpredictable. Thus, 1d-AGE and 2d-AGE analysis of macromolecular assemblies should be interpreted with as little bias as possible. The data are primary and have sometimes been surprising, as illustrated in the previous section. The following, additional surprise occurred while we were determining the kinetics of T7 capsid I assembly by 1d-AGE. We found that some of the assembled, radiolabeled capsid protein appeared only near the origin of the agarose gels. This observation appeared, at first, to be a liability in that one could not initially characterize this material. However, the apparent liability rapidly became an asset when we discovered that these particles were capsid-like, as found by digestion with protease. Protease digestion converted these “agarose gel adherent” particles to particles that migrated as capsid II. One can only anticipate that non-standardized responses, such as this one, will be needed for most, if not all, comparable analyses of the biochemistry of multi-molecular complexes.

Knowing that the T7 agarose-adherent particles were capsid-like, we determined the kinetics of their formation in the presence and absence of the gp8 connector. The results were the following (Serwer & Watson, 1982). (1) In the presence of the gp8 connector, the agarose-adherent particles first increased in amount and then decreased, i.e., the agarose-adherent particles behaved as though either they or, more likely, related capsid I-like *in vivo* particles (that decayed to agarose adherent particles), were intermediates in the assembly of

capsid I. (2) In the absence of the gp8 connector, the agarose-adherent particles increased in amount progressively, before and after the assembly of capsid I was terminated, as though the agarose-adherent particles had now become an end product of abortive assembly. These observations supported the previous conclusion that the gp8 connector did not nucleate shell assembly. In addition, the apparent conversion from intermediate to abortive end product in the absence of the gp8 connector was interpreted by the assumption that the connector was necessary for correction of errors of shell assembly. A role of the connector in assembly error correction explains why mis-assembled shells continued to accumulate in the absence, but not in the presence, of the connector (Serwer et al., 1982; Serwer & Watson, 1982). Apparently, analysis of *in vivo* procapsid assembly has not subsequently advanced past this point (recent review: Aksyuk & Rossmann, 2011).

To give some idea of the uniqueness of the data that can be obtained by AGE, I mention that (unpublished) efforts to purify the agarose adherent particles in large amount failed because these particles are lost as aggregates when the scale of the lysates was increased. We made this observation by mixing radiolabeled particles (from a small lysate) with relatively large lysates; the radiolabeled particles had been partially purified by rate zonal sucrose gradient centrifugation. That is to say, 1d-AGE (with and without protease digestion) is, thus far, the only way to identify these particles.

## 5. Propagation of large phages: a use of $P_E$ values

Recent surprises in microbiology include the discovery of “giant” eukaryotic, double-stranded DNA viruses with genomes larger than 1 million base pairs (reviews: Claverie et al., 2009; Colson & Raoult, 2010). These giant viruses, originally thought to be cells, really are viruses based on the packaging of the genome in an icosahedral capsid shell assembled from subunits that have sequence similarity with the major shell protein of other viruses, including *Paramecium Bursaria Chlorella Virus 1* (Azza et al., 2009). The first of these viruses, *Acanthamoebae polyphaga* Mimivirus, has shell-associated spikes that extend to an outer radius of 373 nm (Klose et al., 2010). This large radius is presumably the reason that no plaque assay was initially reported and, to my knowledge, has still not been reported. Based on previous determinations of  $P_E$ , a plaque assay should be possible for these viruses, as described in detail in Section 5.1, below.

The work on gel concentration-dependence of the formation of plaques by relatively large viruses began with the largest known phage, called phage G (for giant). Phage G makes only very small plaques in the traditional plaque supporting, 0.4-0.7% agar gels. However, the dimensions of phage G were roughly the same as the  $P_E$  of the plaque-supporting agar gels. When relatively dilute agarose gels are used to propagate phage G, this phage was found to make large plaques that, when confluent, produce an overlay that is an excellent preparative source of phage G (Serwer et al., 2009).

### 5.1 $P_E$ values

The considerations of Sections 2.1 and 2.2 already suggest that  $P_E$  values of plaque-supporting gels can be made high enough to form plaques of Mimivirus and related giant viruses. The culmination of a series of sieving-based measurements of  $P_E$  produced the following equation that describes the relationship between  $P_E$  (in nm) and the percentage,  $\underline{A}$ , of



the LE (low EEO) agarose usually used; gels were formed at  $\sim 25^\circ\text{C}$  in buffer that contained 0.025 M sodium phosphate, pH 7.4, 0.001 M  $\text{MgCl}_2$  (Griess et al., 1989).  $P_E = 148\bar{A}^{-0.87}$ . This relationship predicts that LE agarose gels with  $\bar{A}$  below about 0.34% are dilute enough for plaque formation by mimivirus; a concentration  $\sim 2\times$  lower is appropriate for an initial test. This relationship also predicts that a 0.25%LE agarose gel is the most concentrated LE agarose gel with a  $P_E$  value large enough so that mobile *Salmonella* cells, assumed to have a diameter of about 500 nm, migrate through the gel. This  $\bar{A}$  value is lower than the 0.4% used by Stocker et al. (1953) for bacterial migration, as discussed in Section 2.1. Higher EEO agarose preparations, which should better mimic the agar used in Stocker et al. (1953), form gels with an even lower maximal  $\bar{A}$  for migration of bacteria (Griess et al., 1989). Thus, the medium present in the 0.4% agar gels of Stocker et al. (1953) appears to have caused an increase in  $P_E$ .

As discussed in Section 2.2, the value of  $P_E$  increases as the temperature of gelation increases, as judged by both sieving during gel electrophoresis and electron microscopy of thin sections. We have applied this principle to the propagation of a phage that is both large (shell radius  $\sim 50$  nm; tail length  $\sim 486$  nm) and aggregating and found that, indeed, plaque size increases as the temperature of gelation increases for the plaque-supporting gel (Serwer et al., 2009). That is to say, (1) the electrophoretic sieving and the apparent sieving during plaque formation move, as expected, in the same direction with change in  $P_E$  and (2) a plaque assay should be a possibility for Mimivirus, unless a trapping effect is encountered.

In the case of Mimivirus, however, the possibility of gravitational field-induced arrest of motion exists. If 1g sedimentation causes trapping of Mimivirus in gels, then buoying Mimivirus should make plaque formation possible in appropriately dilute agarose, plaque-supporting gels.

## 5.2 Gel-supported propagation of new phages: large and aggregating phages

Evidence exists that the viruses thus far isolated and propagated are not any more than 1% and probably much less of the total in the environment. This evidence includes the sequences of environmental viral RNA and DNA obtained without propagating the viruses involved (metagenomics: reviewed in Casas & Rohwer, 2007). We have used dilute agarose gel propagation to isolate several phages that cannot be propagated in any other way, including propagation in traditional agar gels and liquid enrichment culture. Several of these phages undergo extensive aggregation during plaque formation (Serwer & Wang, 2005; Serwer et al., 2009), which suggests that these phages would not be detected by metagenomics, because of loss during procedures (filtration, low speed centrifugation, for example) that are used to remove bacteria.

Virus aggregation is a well-known phenomenon, potentially important to new frontiers in virology. Historically, virus aggregation was important because of its potential (occasionally realized) to inhibit antibody neutralization of several eukaryotic viruses (Wallis, C. & Melnick, 1967). The need for revised procedures in the isolation of some, including aggregating, phages suggests that revised procedures will also be needed for the isolation of some not-yet-isolated eukaryotic viruses. As is usually the case, the advances needed are most rapidly explored with phages.



## 6. Acknowledgments

Recent work in the author's laboratory was supported by the Welch Foundation (AQ-764).

## 7. In Memoriam

Dr. Gary A. Griess made large contributions to our current knowledge gel electrophoresis, as apparent from the attached manuscript. These contributions were in several areas, perhaps most notably in the areas of the structure and sieving of gels. Gary died from complications of cancer on April 28, 2008. He had received an undergraduate degree in physics from MIT in 1962 and a PhD in biophysical chemistry from the University of Massachusetts Amherst (Advisor, John F. Brandts) in 1970. By 1985, Gary and I had established a collaboration that began with work on the biophysical characterization of phages. We soon developed a focus on the structure and sieving of gels. Gary provided essential computational, biophysical and experimental aspects of this work, much of which depended on his creativity and ingenuity. He was also very generous with his assistance to others in all laboratories of our department. We all miss him. This manuscript is dedicated to Gary.

## 8. References

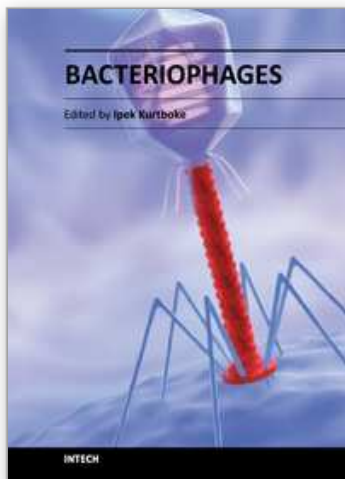
- Aksyuk, A.A. & Rossmann, M.G. (2011) Bacteriophage assembly. *Viruses* 3, 172–203.
- Åkerman, B. & Cole, K.D. (2002) Electrophoretic capture of circular DNA in gels. *Electrophoresis* 23, 2549–2561.
- Alberts, B., Johnson, A., Lewis, J., Raff, M., Keith Roberts, K. & Peter Walter, P. (2002) *Molecular Biology of the Cell*, 4th edition, Garland Science, New York.
- Azza, S., Cambillau, C., Didier, R., & Suzan-Monti, M. (2009) Revised Mimivirus major capsid protein sequence reveals intron-containing gene structure and extra domain. *BMC Molecular Biology* 10, 39.
- Bazinet, C., and King, J. (1988). Initiation of p22 procapsid assembly *in vivo*. *Journal of Molecular Biology* 202, 77–86.
- Brewer, B.J. & Fangman, W.L. (1991) Mapping replication origins in yeast chromosomes. *Bioessays* 13, 317–322.
- Bull, H.B. (1971) *An Introduction to Physical Biochemistry*. F.A. Davis, Philadelphia, Chapter, 3.
- Casas, V. & Rohwer, F. (2007) Phage metagenomics. *Methods in Enzymology* 421, 259–268.
- Casjens, S., Wyckoff, E., Hayden, M., Sampson, L., Eppler, K., Randall, S., Moreno, E.T. & Serwer, P. (1992) Bacteriophage P22 portal protein is part of the gauge that regulates packing density of intravirion DNA. *Journal of Molecular Biology* 1992, 224, 1055–1074.
- Catalano, C.E. (2000) The terminase enzyme from bacteriophage lambda: a DNA-packaging machine. *Cellular and Molecular Life Sciences* 57, 128–148.
- Chang, J., Weigele, P., King, J., Chiu, W. & Jiang, W. (2006) Cryo-EM asymmetric reconstruction of bacteriophage P22 reveals organization of its DNA packaging and infecting machinery. *Structure* 14, 1073–1082.
- Chen, B. & Chrambach, A. (1979) Estimation of polymerization efficiency in the formation of polyacrylamide gel, using continuous optical scanning during polymerization. *Journal of Biochemical and Biophysical Methods* 1, 105–116.

- Claverie, J.M., Abergel, C. & Ogata, H. (2009) *Current Topics in Microbiology and Immunology* 328, 89-121.
- Colson, P. & Raoult, D. (2010) Gene repertoire of amoeba-associated giant viruses. *Intervirology* 53, 330-343.
- Fang, P.A., Wright, E.T., Weintraub, S.T., Hakala, K., Wu, W., Serwer, P. & Jiang, W. (2008) Visualization of bacteriophage T3 capsids with DNA incompletely packaged *in vivo*. *Journal of Molecular Biology* 384, 1384-1399.
- Ferrari, B.C., Winsley, T., Gillings, M. & Binnerup, S. (2008). Cultivating previously uncultured soil bacteria using a soil substrate membrane system. *Nature Protocols* 3, 1261-1269.
- Fujisawa, H. & Morita, M. (1997) Phage DNA packaging. *Genes Cells* 2, 537-545.
- Gauthier, M.G. & Slater, G.W. (2003) An exactly solvable Ogston model of gel electrophoresis: X. Application to high-field separation techniques. *Electrophoresis* 24, 441-451.
- Griess, G.A., Edwards, D.M., Dumais, M., Harris, R.A., Renn, D.W. & Serwer, P. (1993) Heterogeneity of the pores of polysaccharide gels: Dependence on the molecular weight and derivatization of the polysaccharide. *Journal of Structural Biology* 111, 39-47.
- Griess, G.A., Guiseley, K.B., Miller, M.M., Harris, R.A. & Serwer, P. (1998) The formation of small-pore gels by an electrically charged agarose derivative. *Journal of Structural Biology* 123, 134-142.
- Griess, G.A., Moreno, E.T., Easom, R.A. & Serwer, P. (1989) The sieving of spheres during agarose gel electrophoresis: quantitation and modeling. *Biopolymers* 28, 1475-1484.
- Griess, G.A., Moreno, E.T., Herrmann, R. & Serwer, P. (1990) The sieving of rod-shaped viruses during agarose gel electrophoresis. I. Comparison with the sieving of spheres. *Biopolymers* 29, 1277-1287.
- Hamdan, S.M. & Richardson, C.C. (2008). Motors, switches, and contacts in the replisome. *Annual Review of Biochemistry* 78, 205-243.
- Howard, J. (2009) Motor proteins as nanomachines: The roles of thermal fluctuations in generating force and motion. *Séminaire Poincaré* 12, 33-44.
- Klose, T., Kuznetsov, Y.G., Xiao, C., Sun S., McPherson, A. & Rossmann, M.G. (2010) The three-dimensional structure of Mimivirus. *Intervirology* 53, 268-273.
- Koonin, E.V. (2009) On the origin of cells and viruses: Primordial virus world scenario. *Annals of the New York Academy of Sciences* 1178, 47-64.
- Kurakin, A. (2007) Self-organization *versus* watchmaker: Ambiguity of molecular recognition and design charts of cellular circuitry. *Journal of Molecular Recognition* 20, 205-214.
- Langston, L.D., Indiani, C. & O'Donnell, M. (2009) Whither the replisome: emerging perspectives on the dynamic nature of the DNA replication machinery. *Cell Cycle* 8, 2686-2691.
- Myong, S. & Ha, T. (2010) Stepwise translocation of nucleic acid motors. *Current Opinion in Structural Biology* 20, 121-127.
- Newcomb, W.W., Homa, F.L. & Brown, J.C. (2005) Involvement of the portal at an early step in herpes simplex virus capsid assembly. *Journal of Virology* 79, 10540-10546.

- Pajunen, M.I., Elizondo, M.R., Skurnik, M., Kieleczawa, J. & Molineux, I.J. (2002) Complete nucleotide sequence and likely recombinatorial origin of bacteriophage T3. *Journal of Molecular Biology* 319, 1115-1132.
- Rasmussen, R.S. and Morrissey, M.T. (2007) Marine biotechnology for production of food ingredients. *Advances in Food and Nutrition Research* 52, 237-292.
- Rees, D.A. (1972) Shapely polysaccharides. The eighth Colworth medal lecture. *Biochemical Journal* 126, 257-273.
- Reynolds, J.A. & Tanford, C. (1970) Binding of dodecyl sulfate to proteins at high binding ratios. Possible implications for the state of proteins in biological membranes. *Proceedings of the National Academy of Sciences of the United States of America*, 66, 1002-1007.
- Sait, M., Hugenholtz, P. & Janssen, P.H. (2002) Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. *Environmental Microbiology* 4, 654-666.
- Serwer, P. (1987) The mechanism for producing two symmetries at the head-tail junction of bacteriophages: a hypothesis. *Journal of Theoretical Biology* 127, 155-161.
- Serwer, P. (2010) A hypothesis for bacteriophage DNA packaging motors. *Viruses* 2, 1821-1843.
- Serwer, P. (2011) Proposed ancestors of phage nucleic acid packaging motors (and cells). *Viruses* 3, 1249-1280.
- Serwer, P. & Griess, G.A. (1998) Adaptation of pulsed-field gel electrophoresis for the improved fractionation of spheres. *Analytica Chimica Acta* 372, 299-306.
- Serwer, P. & Griess, G.A. (1999) Advances in the separation of bacteriophages and related particles. *Journal of Chromatography B* 722, 179-190.
- Serwer, P. & Hayes, S.J. (1982) Agarose gel electrophoresis of bacteriophages and related particles. I. Avoidance of binding to the gel and recognizing of particles with packaged DNA. *Electrophoresis* 3, 76-80.
- Serwer, P. & Hayes, S.J. (1986) Two-dimensional agarose gel electrophoresis. In: (Dunn, M.J., Ed.) *Electrophoresis '86*, VCH, Weinheim, pp. 243-251.
- Serwer, P. & Wang, H. (2005) Single-particle light microscopy of bacteriophages. *Journal of Nanoscience and Nanotechnology* 5, 2014-2028.
- Serwer, P. & Watson, R.H. (1982) Function of an internal bacteriophage T7 core during assembly of a T7 procapsid. *Journal of Virology* 42, 595-601.
- Serwer, P. & Wright, E.T. (2011) Agarose gel electrophoresis reveals structural fluidity of a phage T3 DNA Packaging intermediate. *Electrophoresis*, 33, 352-365.
- Serwer, P., Easom, R.A., Hayes, S.J. & Olson, M.S. (1989) Rapid detection and characterization of multimolecular cellular constituents by two-dimensional agarose gel electrophoresis. *Trends in Biochemical Sciences* 14, 4-7.
- Serwer, P., Hayes, S.J. & Griess, G.A. (1986) Determination of a particle's radius by two-dimensional agarose gel electrophoresis. *Analytical Biochemistry* 152, 339-345.
- Serwer, P., Hayes, S.J., Thomas, J.A., Demeler, B. & Hardies, S.C. (2009) Isolation of large and aggregating bacteriophages. *Methods in Molecular Biology* 1, 55-66.
- Serwer, P., Khan, S.A. & Griess, G.A. (1995) Non-denaturing gel electrophoresis of biological nanoparticles: viruses *Journal of Chromatography A* 698, 251-261.

- Serwer, P., Moreno, E.T. & Griess, G.A. (1988) Agarose gel electrophoresis of particles larger than 100 nm: Fractionation of intact *Escherichia coli*. In: (Schafer-Nielson, C., Ed.) *Electrophoresis '88*, VCH, Weinheim, pp. 216-227.
- Serwer, P., Watson, R.H. & Hayes, S.J. (1982) Detection and characterization of agarose-binding, capsid-like particles produced during assembly of a bacteriophage T7 procapsid. *Journal of Virology* 42, 583-594.
- Serwer, P., Wright, E.T., Hakala, K., Weintraub, S.T., Su, M. & Jiang, W. (2010) DNA packaging-associated hyper-capsid expansion of bacteriophage T3. *Journal of Molecular Biology* 397, 361-374.
- Shaw, D.J. (1969) *Electrophoresis*, Academic Press, London, p. 4-26.
- Stellwagen, E., Lu, Y. & Stellwagen, N.C. (2003) Unified description of electrophoresis and diffusion for DNA and other polyions. *Biochemistry* 42, 11745-11750.
- Stellwagen, E., Lu, Y. and Stellwagen, N.C. (2005) Curved DNA molecules migrate anomalously slowly in free solution. *Nucleic Acids Research* 33, 4425-4432.
- Stocker, B.A.D., Zinder, N.D., & Lederberg, J. (1953) Transduction of flagellar characters in *Salmonella*. *Journal of General Microbiology* 9, 410-433.
- Studier, F.W. (2000) Slab-gel electrophoresis. *Trends in Biochemical Sciences* 25, 588-590.
- Tietz, D. (2007) Computer-assisted 2-D agarose electrophoresis of *Haemophilus influenzae* type B meningitis vaccines and analysis of polydisperse particle populations in the size range of viruses: a review. *Electrophoresis* 28, 512-524.
- Tietz, D. (2009) An innovative method for quality control of conjugated *Haemophilus influenzae* vaccines: A short review of two-dimensional nanoparticle electrophoresis. *Journal of Chromatography A* 1216, 9082-9033.
- Wallis, C. & Melnick, J.L. (1967) Virus aggregation as the cause of the non-neutralizable persistent fraction. *Journal of Virology* 1, 478-488.

IntechOpen



## **Bacteriophages**

Edited by Dr. Ipek Kurtboke

ISBN 978-953-51-0272-4

Hard cover, 256 pages

**Publisher** InTech

**Published online** 14, March, 2012

**Published in print edition** March, 2012

Bacteriophages have received attention as biological control agents since their discovery and recently their value as tools has been further emphasized in many different fields of microbiology. Particularly, in drug design and development programs, phage and prophage genomics provide the field with new insights.

Bacteriophages reveals information on the organisms ranging from their biology to their applications in agriculture and medicine. Contributors address a variety of topics capturing information on advancing technologies in the field. The book starts with the biology and classification of bacteriophages with subsequent chapters addressing phage infections in industrial processes and their use as therapeutic or biocontrol agents. Microbiologists, biotechnologists, agricultural, biomedical and sanitary engineers will find Bacteriophages invaluable as a solid resource and reference book.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Philip Serwer (2012). Gels for the Propagation of Bacteriophages and the Characterization of Bacteriophage Assembly Intermediates, Bacteriophages, Dr. Ipek Kurtboke (Ed.), ISBN: 978-953-51-0272-4, InTech, Available from: <http://www.intechopen.com/books/bacteriophages/gels-for-the-propagation-of-bacteriophages-and-the-characterization-of-bacteriophage-assembly-interm>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821



© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen