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Critical Review

Extracellular Vesicles and Bacteriophages: New Directions in Environmental Biocolloid Research

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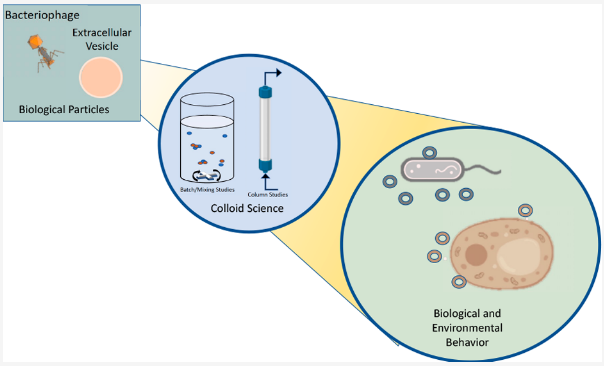
ABSTRACT: There is a long-standing appreciation among environmental engineers and scientists regarding the importance of biologically derived colloidal particles and their environmental fate. This interest has been recently renewed in considering bacteriophages and extracellular vesicles, which are each poised to offer engineers unique insights into fundamental aspects of environmental microbiology and novel approaches for engineering applications, including advances in wastewater treatment and sustainable agricultural practices. Challenges persist due to our limited understanding of interactions between these nanoscale particles with unique surface properties and their local environments. This review considers these biological particles through the lens of colloid science with attention given to their environmental impact and surface properties. We discuss methods developed for the study of inert (nonbiological) particle−particle interactions and the potential to use these to advance our understanding of the environmental fate and transport of extracellular vesicles and bacteriophages.



KEYWORDS: *extracellular vesicles, outer membrane vesicles, aggregation, deposition, environmental fate, bacteriophages, Smoluchowski, biocolloids*

1. INTRODUCTION

Diverse research arenas have revealed that human and environmental health are inextricably linked by particle−particle interactions, in particular for those involving biologically derived, nanoscale particles. These "bionanocolloids" are implicated in many activities at the human−environment interface, ranging from the spread of human, animal, and crop diseases to the influence of biological organic matter on particle flocculation in wastewater treatment.[1−5]

Colloids are defined as small particles (typically less than 1 μm) that remain as a stable (nonsettling) suspension when dispersed in a continuous medium,[6−8] while nanocolloids are specifically those existing at or below 100 nm. The term *bionanocolloid* encapsulates the domain of colloidal phenomena which involve analogous types of particle suspensions wherein at least one of those phases are biological in nature.[5] Bionanocolloids offer researchers unique insight into some of the most fundamental and valuable aspects of the environmental microbial community which underpin entire ecosystems, while also holding the potential to be engineered to positively contribute to human and environmental welfare. Two biological particles of current interest to a growing number of researchers are extracellular vesicles (or EVs) and bacteriophages (or phages) (Figure 1), both of which are possible vectors for genetic materials.

EVs are membrane-bounded nanoscale particles produced by cells in all domains of life.[10−12] While much research pertains to mammalian cell-derived EVs, a parallel interest exists in understanding more about EVs in microbial communities, specifically what roles they have in intercellular and interkingdom communication.[11,13−15] Spanning a variety of shapes, types, and host range specificities, bacteriophages are viruses which exclusively infect bacteria. As the most abundant known biological entity on the planet, many phages directly shape ecosystems through host cell lysis, acting as agents of nutrient recycling.[16,17]

EVs and phages are two biocolloids distinguished from other environmentally relevant particles primarily by their size, shape, functionality, and ubiquity. Many known phages and EVs have at least one characteristic dimension on the

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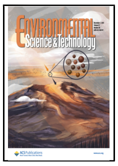

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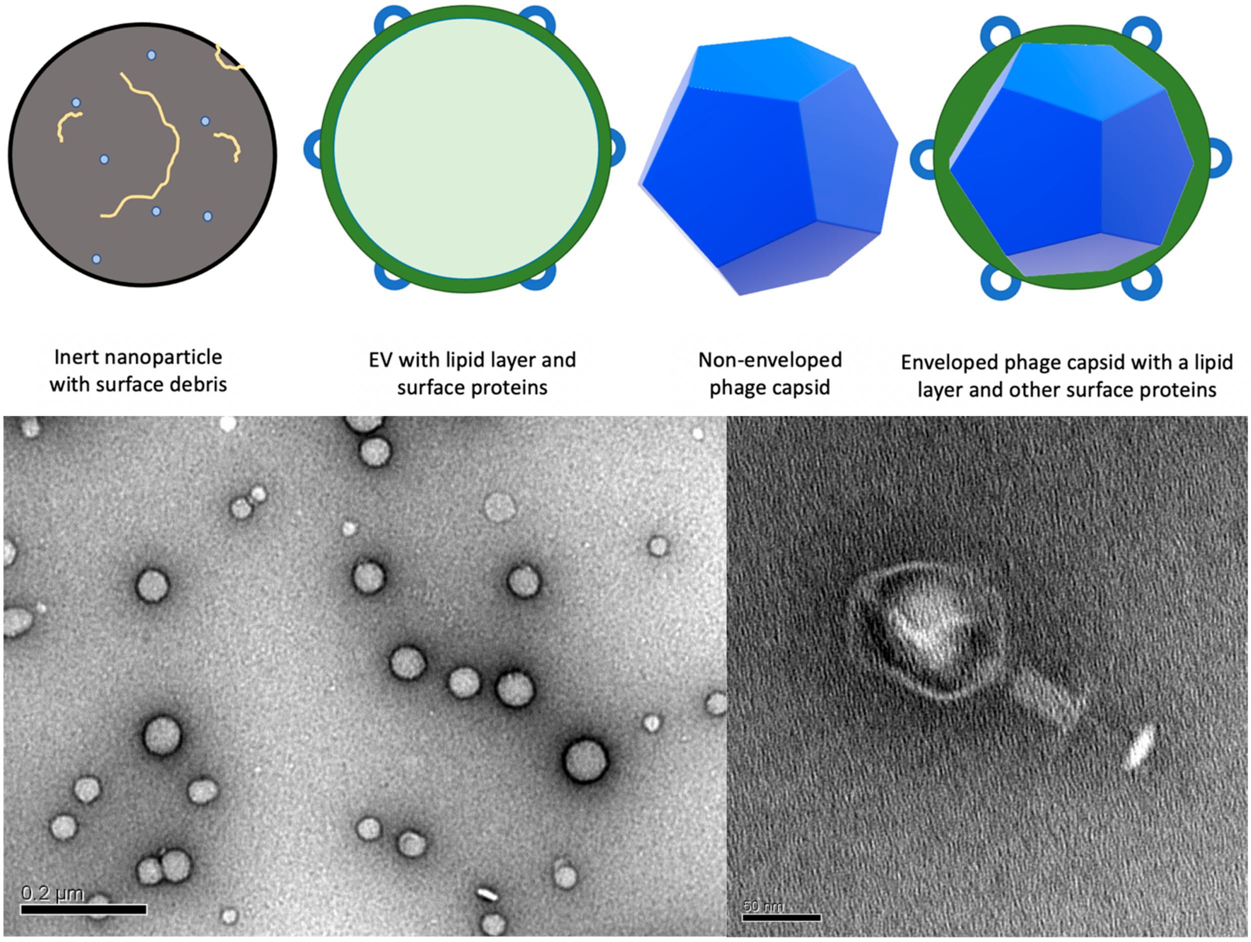

Figure 1. (Top) Images of nanoparticles discussed in this review; (bottom left) transmission electron micrographs of uranyl acetate negatively stained *Pseudomonas aeruginosa* OMVs purified by density gradient from log-phase broth cultures. Size bar: 200 nm; and (bottom right) T4 bacteriophage purified from *Escherichia coli*. Size bar: 50 nm. Images taken by Andrew Manning. See reference (Manning and Kuehn, 2011)[9] for detailed methods.

nanoscale. As a result, they exist on a scale that is well-suited for study through a colloidal lens, as many other scientific studies of particles of similar size have done to illuminative effect (discussed in the next section). Both types of particles have specific biological functionalities and are equipped with unique surface structures befitting these ends. For example, phages carry genetic cargo and are structurally able to attach to and insert their cargo into a specific host. Already, phages have been engineered to successfully target and inactivate microbes in wastewater treatment.[2] Moreover, Shen and Bradford (2021) demonstrated that the spiked surface elements of viruses reduce energetic barriers to attachment and detachment.[18] By comparison, EVs carry diverse cargo and possess specific surface properties that enable the preservation of genetic material, enzymes, nutrients, or compounds for improved transport that leads to interkingdom communication but generally with less target specificity.[15] One possible application of the engineering of EVs exists in the development of EVs designed to deliver vital nutrients to root systems, which could improve sustainable agricultural practices.[19] With these unique, evolutionarily optimized structural compositions, phages and EVs could provide researchers with a framework for engineering particles to perform specific functions.

The colloidal properties of EVs (ex. aggregation, deposition, colloidal stability) have received considerably less attention in the literature than their related characteristics of composition, origin, cargo, and biological activity.[20] While phages have been studied for decades regarding transport and retention in the environment,[21−25] the broader picture of phage colloidal properties remains incomplete. The lack of colloidal-focused studies for EVs and phages is somewhat surprising considering their qualification as colloidal particles and the fact that colloidal theory and methodology have already been applied to bacterial communities.[26,27] Previously, research into colloid behavior has largely focused on nonbiologically derived particles such as mineral soils, clays, micelles, and engineered particles related to environmental health and safety (nano-EHS).[28−31] This work has consistently revealed the importance of particle surface properties on their environmental fate and transport and just as importantly has provided researchers with means to model the fate and transport of a diverse range of particles using the same or similar techniques and modeling parameters (discussed more in the next section). Therefore, studying phages and EVs with a more explicit colloid-oriented approach could allow for an expansion of the application of colloid theory to additional biocolloidal particles. This also would challenge those who study EVs/phages to approach these biocolloids as such and would provide a more holistic perspective on the roles of these bioparticles in the natural environment than if they were only to be studied as isolated biological entities.

In this perspective, we consider the colloidal behavior of EVs and phages, especially with regard to their surface properties and their environmental activities. We also discuss how some of the same methods used to characterize the transport of inert nanoparticles could be applied to better understand EV and phage fate and transport.

2. COLLOIDS: THEORY AND PRACTICE

The environmental behavior of colloids can be considered through the physics of their transport and the chemistry of the surfaces they encounter.[32−35] For example, colloid aggregation and colloid deposition can both be formulated as a two-step process first involving the transport of colloids up to a surface: either another particle in the case of aggregation or an immobile surface in the case of deposition. Near-field interactions, largely related to the chemistry of the two approaching surfaces, then determines the probability of attachment.

Building on the work of Marian von Smoluchowski (1917),[36] expressions for the kinetics of aggregation can be written in terms of the contact frequency (β) between aggregating particles assumed to occur via multiple transport mechanisms, and attachment efficiency (α)[32] which considers near field interactions dominated by surface chemistry. The contact frequency refers to the rate at which surfaces make contact. Transport mechanisms by which particles come into contact, namely, Brownian motion, differential settling, and shear motion are assumed to be additive. Brownian motion increases as particle size decreases, and thus the contact frequency for smaller particles motion tends to be influenced by fluctuations in thermal energy, as reflected in Brownian motion. Contacts between larger particles (or aggregates of particles) are dominated by differential settling and shear flow (e.g., von Smoluchowski, 1917;[36] O'Melia, 1980;[32] Veerapaneni & Wiesner, 1996[37]). For aggregation, the product, $\alpha\beta$, summarizes the forces acting on particles in the near field, having the units of a second-order rate constant, and describes the overall two-step process of transport and attachment. Analogous to aggregation, deposition of colloids in a porous medium can be described as the product of contact frequency (η) and attachment (α) steps. η is the sum of the transport mechanisms of Brownian diffusion, gravity, and interception that determine the probability that particles will flow up to a single "collector" in the porous medium and contact the collector. Theory has been developed for both β and η but is limited by assumptions for the colloidal suspensions, such as assuming that particles are spherical.[38,39]

Whether a contact results in an attachment (α) is dependent upon the interacting particle's surface properties and chemistry. Furthermore, colloidal surface chemistry is the product of a variety of factors (e.g., surface moieties, surrounding media) and can be characterized in multiple ways (e.g., surface charge, hydrophobicity), the forces of which are classically described by Derjaguin, Landau, Verwey, Overbeek (DLVO) theory.[40,41] The attachment efficiency, α, is a lump parameter, usually experimentally derived, and traditionally interpreted as capturing the sum of surface interactions (although recent studies suggest that factors influencing η, such as fluid flow velocity, also affect α[42]). Similar to contact frequencies, theory for α such as DLVO or extended DLVO is constrained in application to highly idealized systems.[43−46] As a result, both α and β are often evaluated experimentally. Theoretically, the value of α should be identical for identical surfaces whether particle−surface interactions occur by aggregation or by deposition.

Experimentally derived values for α are primarily evaluated using *column studies (deposition)* (see for example Elimelech & O'Melia, 1990[47]). Column experiments track the change in particle concentration across a well-characterized, uniform porous medium or packed bed of collector particles, allowing for estimates of the collection efficiency to be applied.[35,47] Column studies thus allow for the description of attachment efficiencies for particles of one type with a surface of a second type represented by the porous medium. Some researchers have used this or similar methods to determine values for more mass-based particle partitioning coefficients, such as K_d,[48−50] while others use similar methods to determine more population-based particle attachment coefficients, such as α.[31] Alternatively, *batch mixing studies*, which primarily consider heteroaggregation,[51] have been utilized to determine α between one type of particle and a second "background" particle type. These mixing studies are better adapted for systems that are not suitable for column studies due to difficulties associated with certain packing materials (ex. soil) or from low analyte quantities, such as the attachment of engineered nanoparticles (NPs) to particles in slurries, or to biological particles such as algae. Indeed, a novel batch mixing for determining nanoparticle affinity was developed to analyze particular soil systems.[52] Additionally, other methods have been developed by determining α including impinging jet experiments,[53−55] quartz crystal microbalance,[42,56,57] and machine learning.[58]

With these concepts in mind, we consider the surface properties and colloidal behavior of EVs and phages and discuss how these experimental and theoretical modeling methods could be useful in framing a greater understanding of their fate and transport.

3. EXTRACELLULAR VESICLES AS FUNCTIONAL COLLOIDS IN THE ENVIRONMENT

In the natural environment, EVs perform many roles that are specifically dictated by their fate and transport. These properties are influenced by aggregation and deposition, which are directly determined by their surface properties. In this section, we explore both the roles of EVs in the environment and the relevant surface properties critical for these roles. We will focus on EVs produced by bacteria and fungi because of their prevalence in the environment. Some pertinent studies of EVs from mammalian cells are mentioned as well, particularly as these relate to the colloidal properties of biological nanoparticles.

3.1. Physical Characteristics of EVs.

In the environment, EVs are diffusible particles that travel to locations distal to the originating cell (Figure 2). As biocolloidal particles, this journey can be framed in the context of colloid transport and attachment (α). In particular, clear connections between colloidal surface composition and α oblige an examination of the current understanding of EV surfaces to identify specific factors (e.g., composition, surface moieties, charge) impacting their attachment efficiency.

EVs typically are composed of similar categories of primary components (e.g., proteins, lipids, nucleic acids, metabolites, and carbohydrates), but significant differences in specific surface-exposed moieties depend on the species and conditions

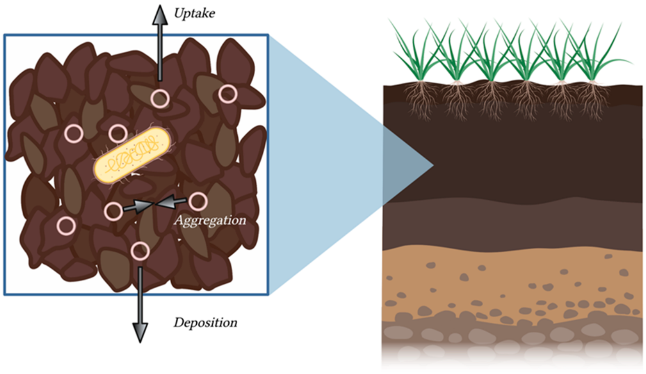

Figure 2. EVs from microbes can have a variety of possible fate outcomes in the natural environment. For example, in soil systems, EVs can deposit on soil particles, aggregate with other EVs or colloidal particles, or be taken up by plants, among other possible fates. Created with BioRender.com.

under which the vesicles are produced.[20,59] The variety of biological, "biogenesis" pathways by which vesicles are produced by different cell types informs the distinct surface properties of the resulting vesicles which are notably distinct from the cells (Figure 3). Their surface properties, in turn, inform their transport properties as colloids. Thus, it is critical to understand the biogenesis mechanisms of EVs for different types of organisms.

EVs from Gram-negative bacteria that bud from the outer membrane and encapsulate soluble periplasmic content are referred to as outer membrane vesicles or OMVs.[60,61] OMVs are typically between 20 and 400 nm in size.[11] The composition of OMVs closely reflects that of the outer membrane and periplasm of the parent cells, although they are not identical.[62−64] The function of OMV-associated proteins varies greatly, including virulence factors, porins, structural proteins, enzymes, and stress response proteins.[62,65] Not all outer membrane proteins (OMPs) were shown to be sorted into OMVs, implying a sorting system of proteins into vesicles for bacteria that is an important topic under investigation.[62,66] Orench-Rivera and Kuehn (2021) demonstrated that OMPs were differentially packaged into EVs under standard, as well as oxidative stress, culture conditions, and that protein sorting into OMVs is a function of the protein's ability to tether to the cell envelope.[66] Previous studies have distinguished surface-bound proteins from intravesicular proteins,[67−71] but having deeper knowledge of the protein content of EVs could help to make predictions as to their roles in the overall surface chemistry of OMVs. As with the protein content, the lipid profile of OMVs reflects that of the parent cell's outer membrane,[64] although in-depth studies have shown that the relative composition of lipids in OMVs differs somewhat from that of the outer membrane.[72,73] Also, previous work demonstrates that the relative composition of OMVs are species-dependent.[70,74] Furthermore, one research group even found that OMVs contain lipids that are not detected in the outer membrane.[75] Notably, recent studies have demonstrated an alternative mechanism by which EVs can be formed from the reassembled fragments of explosive cell lysis.[76] While this subcategory of EVs is relatively unexplored, the mechanism of formation suggests potential differences in surface properties.

One significant distinguishing surface feature that is unique to Gram-negative bacteria is the presence of lipopolysaccharides (LPSs). LPSs are comprised of long chains of repeating sugars that are covalently bonded to lipids.[77] Bacteria express a variety of LPS subtypes with regard to both the type of saccharide chain and the lipid constituents, and these have been shown to be selectively sorted into OMVs.[73,74,78] From a surface chemistry perspective, LPS's negative charge from phosphate groups helps to provide stability for OMVs.[40,41,77] In addition, due to the size of these molecules, LPSs could influence surface interactions by steric hindrances. Indeed, exposed LPS epitopes are used as a means to isolate OMVs from a complex mixture.[79] These steric interactions are also demonstrated in LPSs' role in adhesion during biofilm formation.[64] Intriguingly, data support a role for OMVs in modifying cellular LPS composition during biofilm formation, as the release of OMVs caused a significant increase in cell surface hydrophobicity and an increase in biofilm growth.[80]

Only recently have EVs from Gram-positive bacteria, termed cytoplasmic membrane vesicles (CMVs), begun to be isolated and characterized, and consequently less is known about their biogenesis and surface properties. This research was long hindered by the belief that vesicles would not be able to bud, much less pass through the thick wall of peptidoglycan surrounding the cells. Recently, studies have reported not only CMV composition, but also the genetic, biochemical, and stress-induced control of their production.[81−85] Proteomic analyses of CMVs revealed integral membrane polypeptides whose external loops would impact the surface characteristics of the CMVs. Such studies further report that the majority of proteins associated with CMVs are cytoplasmic, implying that the majority of Gram-positive EV proteins are in fact intralumenal.[86] However, extrapolating from reports characterizing OMVs,[70,71] it is possible that some soluble CMV proteins that are assumed to be lumenal could actually be found externally associated with the surface of the vesicles.

Another surface component to consider for Gram-positive bacteria is teichoic acids. Generally, teichoic acids are glycopolymers composed of repeating phosphodiester-linked polyol units.[87] Teichoic acids can be classified as either wall teichoic acids (WTAs), which are covalently embedded in the peptidoglycan cell wall, or lipoteichoic acids (LTAs), which are anchored to the cell membrane by means of a glycolipid.[87] While WTAs have not yet been found in Gram-positive bacterial EVs, LTA has been shown to be present on the surfaces of CMVs.[88] This observation has interesting implications for current debates regarding the presence of peptidoglycan (PG) on the surface of CMVs.[84,87,89,90] As a parallel to LPSs for OMVs, PG would seemingly be the most dominant and most external component of CMVs. However, the lack of detected WTAs on the surface of CMVs suggests that some regions of PG, or at least some PG-bound material, is excluded from CMVs.

An additional physical characteristic of bacterial EVs to consider is vesicle shape. Thus, far, EVs from bacteria have been shown to be primarily spherical, but also in tubes and chains.[11] Previous work regarding colloid retention in packed columns, quartz crystal microbalance systems, and theoretical models demonstrates that particle shape greatly affects the transport of colloidal particles and must be considered.[91−93] The effect of shape on EV transport has yet to be explored.

To date, EVs have been isolated from at least 11 different species of fungi, with most of them being from yeast.[94,95] EVs from fungi are believed to be produced intracellularly by multivesicular bodies mediated by endosomal sorting complex required for transport (ESCRT) protein complexes and then

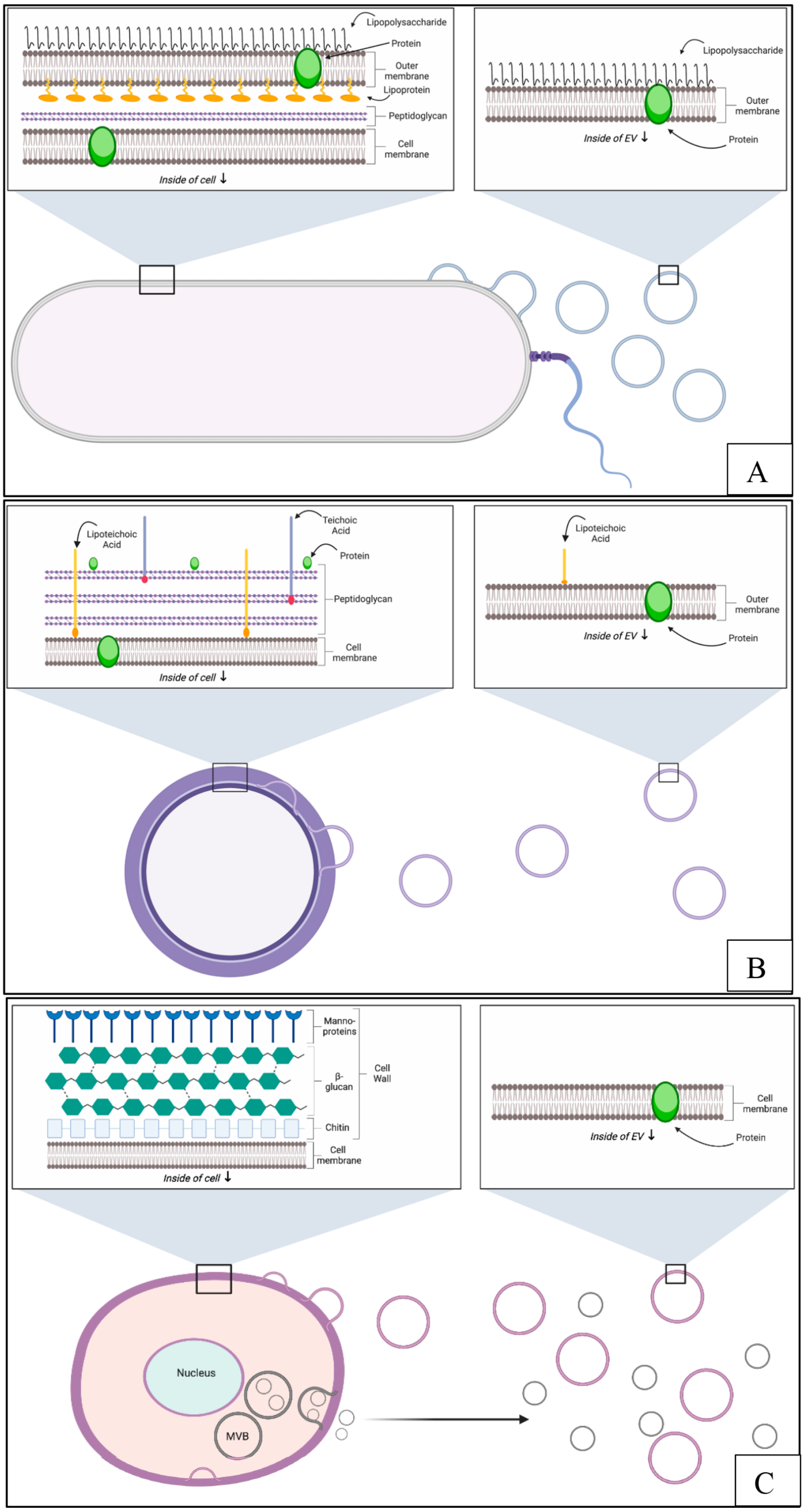

Figure 3. Schematic revealing differences and similarities between cellular and EV surfaces. (a) Gram-negative bacteria produce OMVs from the blebbing of their outer membrane, resulting in the presence of outer membrane components, including LPSs, on the surface of the EVs; (b) Gram-positive bacterial CMVs originate from the cytoplasmic membrane and must traverse the thick peptidoglycan cell wall. LTAs, but not WTAs, are known to be present in CMVs in addition to other cell membrane components; and (c) yeast EVs are produced both from the cell membrane fusion of intracellularly produced MVBs and from the outward blebbing of the cell membrane. Proteins and lipids present on the surface of yeast EVs would distinguish these biogenesis routes. Created with BioRender.com.

secreted into the extracellular space.[96−102] Because of their production mechanism, the proteome of EVs closely mirrors that of the cytosolic content of whole fungal cells, but with membrane proteins tending to be present in higher quantities relative to cytosolic proteins compared to the corresponding ratios in parent cells. Again, the identity of proteins present on the surfaces of EVs has yet to be explored. A universal biomarker for fungal EVs, such as CD9 or CD63 for EVs derived from mammalian cells, has yet to be identified. However, Bleackley et al. (2019) noted 12 proteins were in common between four different fungal strains, with all of them annotated as surface proteins.[103] Lipid composition has also been explored for fungal EVs and indicates a high degree of variability. For example, for EVs from *Cryptococcus neoformans* and *Candida albicans*, the primary lipids found were sterols and glucosylceramide, whereas for *Histoplasma capsulatum*, phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine were the more abundant species.[95,104,105] Their distinct lipid content points to a variety of potential surface chemistries among EVs of various fungal origins.

3.2. Colloidal Characteristics of EVs. Overall, the colloidal characteristics, including zeta (ζ)-potential, aggregation potential, and attachment efficiency (α), of EVs are dictated by their surface composition, which ultimately depends on the organism producing them. These compositional differences originate from the various biogenesis pathways for EVs, leading to an expected wide range of outcomes regarding the fate of these vesicles. An example of this may be seen in Rogers et al., who examines the ζ-potential of EVs from a yeast (*Saccharomyces cerevisiae*), a Gram-positive bacterium (*Staphylococcus aureus*), and a Gram-negative bacterium (*Pseudomonas fluorescens*). Produced by different biogenesis pathways from different cell types, these three types of EVs were shown to possess a different magnitude of surface charge and to respond differently to various environmental conditions.[106] The only commonality found between these EVs was a sensitivity to changes in pH. Similar experiments were performed by Midekessa et al. for human carcinoma JAr cells, which demonstrated that pH, ionic strength, and ionic composition influence ζ-potential.[107] Moreover, it is interesting to consider that because of various biogenesis routes, the surface chemistry of vesicles can contribute to colloidal characteristics that are significantly different from that of their parent cells. Rogers et al. also examined this, demonstrating that there is no common relationship between the ζ-potential of EVs for three different organisms relative to their respective parent cells.

Gnopo et al. demonstrated that the fusion and aggregation potential of EVs from *Escherichia coli* is particularly sensitive to acidic conditions as well as elevated ionic strength.[108] In comparing wild-type *E. coli* EVs to those from a modified "ClearColi" strain that is missing the repeating polysaccharide of LPS, the authors note that intact LPS may be shielding EVs from charges with a 2-fold effect. First, the presence of LPS reduces electrostatic repulsion, allowing aggregation more readily; second, if the charges on the EV bilayer are exposed (that would otherwise be shielded by LPSs with a long polysaccharide chain), EV fusion may be more favorable. In addition, the interaction energies between two vesicles were modeled from DLVO theory, although these predictions did not always mirror the experimental results. Describing an analogous colloidal process, Rogers et al. examined the deposition of EVs from *S. cerevisiae*, *S. aureus*, and *P. fluorescens* by means of column studies.[109] EVs from *S. cerevisiae* were transported as DLVO theory would predict, showing increased deposition with increasing ionic strength. Furthermore, *S. cerevisiae* EV deposition was decreased in the presence of humic acid, as predicted, due to steric stabilization. However, *P. fluorescens* EVs did not deposit according to predictions from DLVO theory, nor did they deposit less when in the presence of humic acid. Similarly to Gnopo et al., the authors suggest that LPSs on the surface of *P. fluorescens* EVs may be shielding the EVs from increased ion concentrations but also may be interacting with humic acid in such a way that deposition is increased. From these deposition experiments, the attachment efficiency (α) was determined and utilized to predict the distance EVs could travel using a clean bed filtration model. For a homogeneous porous media, this model indicates that EVs could be transported up to 15 m. Information and models such as these would allow for the prediction of vesicle interactions in the environment, both with each other and with other surfaces. In turn, more sound predictions for the fate of EVs and their respective ecosystems would be possible.

Outside of conventional studies of colloidal properties, many reports have investigated EV properties that may complement future research on EVs as colloids. For example, multiple studies have examined the specific interactions of ligands driving the adherence of microbial EVs to mammalian cell surfaces.[60,61,71,110,111] Also, EVs from neutrophilic granulocytes were shown to disrupt bacterial cell growth by aggregating to the surfaces of the cells.[112] Another study revealed that EVs from plants aggregate near the surface of infection from fungi.[113] Yet another report demonstrates the capacity for mammalian EVs to adhere to polypropylene plastic in storage, sometimes exceeding losses of 50% due to adhesion to the plastic surfaces.[114] While the specific mechanisms of ligand−receptor based attachment have often been detailed, plastic adherence mechanisms were not directly investigated. This observation of the various colloidal properties of EVs aligns well with the range of functions that EVs perform in the natural environment; if EVs must perform multiple different functions in the environment, it follows that they must have some diversity in surface composition to achieve these goals. Thus, the versatility of EV functionality points to the value of understanding the range of colloidal properties of all types of EVs in more detail.

3.3. Functions of EVs as Colloids in the Environment. Thus far, EVs have been identified widely in organisms that are relevant in the natural environment, primarily in terrestrial and aquatic systems. Consider first EVs in agriculture. For instance, Samuel et al. and An et al. discuss fungal−plant interactions: how sometimes vesicular structures aid in the dissemination of fungal pathogens but also how similar structures can induce immune responses to combat the growth of harmful fungi on plants.[113,115] More recently, McMillan et al. demonstrated that OMVs from *P. syringae* and *P. fluorescens* can elicit protective plant immune responses.[3] Other studies demonstrated a similar phenomena for *Xanthomonas campestris* and *X. oryzae* OMVs, which inhibited the growth of *Arabidopsis thaliana*.[116,117] Other nonplant associated bacteria *P. aeruginosa, S. aureus*, and enterotoxigenic *E. coli* also induced plant immune responses, albeit to different extents.[3] McMillan et al. and Bahar et al. further suggest that OMV-associated protein may not be the primary category of molecules that stimulates a response preventing plant infection. In another study, enzymes contained in OMVs from *P. putida* have been shown to help to degrade lignin-derived aromatic compounds from plants. Katsir and Bahar and Rudnicka et al. review the relationship between OMVs and plants more thoroughly elsewhere.[118,119]

Besides plant−microbe interactions in agricultural systems, attention has been directed toward understanding other roles for EVs in soil and sediment. EVs from both Gram-negative (*Mycobacterium tuberculosis*) and Gram-positive (*Dietzia* sp. DQ12-45-1b) bacteria have been shown to contain siderophores that aid in the acquisition of iron, which is an essential nutrient for both bacterial and plant health.[120,121] Even without the presence of siderophores, OMVs from *P. aeruginosa* have been shown to be involved in the iron acquisition process.[122] In addition to transporting cellular materials, some vesicles have been shown to shuttle electrons between cells. *Shewanella oneidensis*, a common Gram-negative bacterium found in aquatic sediment, has been shown to generate nanowire vesicles that can transport electrons between cells.[123] More recently, it was shown that OMVs from *Geobacter sulfurreducens*, another Gram-negative bacterium that lives in anaerobic soils and sediments, contain cytochromes that mediate electron transfer between cells.[124] Furthermore, *G. sulfurreducens* could promote this electron transfer at a rate 1.73 times higher than that of *S. oneidensis*. This reflects a potential range of efficiencies for microbial energy transfer in soil.

EVs in aquatic environments have been primarily studied in seawater. Biller et al. first reported on EVs in seawater from species of the cyanobacteria *Prochlorococcus* and *Synechococcus*.[125] This report demonstrated both that EVs are produced by common marine cyanobacteria, and that EVs can be found in seawater at concentrations between the 10^5 and 10^6 EVs per mL. In addition, initial characterizations revealed DNA contained within EVs from the studies microbes. In a subsequent study, Biller et al. demonstrated that this packaging of DNA in marine microbes is not homogeneous between EVs for five different evaluated strains.[126] Since this initial report, more recent reports have suggested that EVs from seawater may be involved in the uptake of iron and phosphorus, the mitigation of toxic reactive oxygen species, the formation of biofilms, and the catalysis of extracellular biochemical reactions.[127,128]

Molecules present as cargo associated with EVs may be potentially persistent and able to travel longer distances than molecules not associated with EVs. A study of EVs in feces showed that vesicles can help to preserve RNA from degradation.[129] This would be environmentally relevant with the land application of solids but could extend to other instances of the delivery of genetic material. Similarly, other reports have noted that the association of extracellular nucleic acids was protected from nuclease degradation when associated with EVs.[130,131] We note that this phenomenon was also demonstrated for various strains of *Thermus thermophilus,* which is a Gram-negative bacterium that is commonly found in high temperature climates in thermal springs. Thus, extracellular DNA can be preserved in high temperature environments when associated with EVs. This claim is confirmed by Soler et al. (2008), albeit with different species of bacteria.[132] At the other environmental extreme, researchers have characterized secreted vesicles from bacteria that grow in Antarctic environments. In particular, EVs from *Shewanella livingstonensis* contained proteins related to nutrient processing and transport, implying some role in the parent cell's survival.[133] Furthermore, depending on the temperature, the protein composition, morphology, and secreted quantity of these vesicles changed, highlighting the adaptability of cellular communities as they relate to their corresponding vesicles. With this persistence, cargo from EVs could potentially scale trophic transfer chains. This trend has already been identified for other persistent nanomaterials.[134] Moreover, multiple reports discuss the ability of EVs to transport cargo long distances, ranging from a few micrometers in cell culture to tens of meters in the natural environment, implying that organically produced membrane vesicles may be created to persist in environments in order to travel long distances to their target location.[109,135,136] Investigating the persistence of EVs could reveal unique discoveries regarding their purposes in the environment. More broadly, through studying the colloidal properties of EVs in the various environmental systems in which they exist, we can better understand the processes by which they disperse through these systems.

3.4. Methodological Challenges for EVs in the Environment. Notable concerns have been raised regarding the methods for detection, isolation, and characterization of EVs *in situ*, e.g., in environmental matrices. One such topic is the distinction of EVs from viruses (phage). Past reports have demonstrated interactions between these two biocolloids. For instance, EVs prevent phage infection of bacteria[9,137] or serve as collectors for phage,[138] whereas phage can induce the increased production of EVs.[139,140] Due to similarities in physical−chemical properties, however, EVs and phages can be difficult to distinguish. An example of this difficulty is highlighted in studies of seawater. Soler et al. (2015) mentioned in their commentary on Biller et al. (2014) that there is a possibility that EVs containing genetic material in seawater could actually be viruses and vice versa, which would impact reported concentrations of both.[141] This claim was at least partially inspired by a previous study by Soler et al. (2008), which demonstrated that EVs associated with extracellular DNA could be confused for viruses when evaluated using fluorescence microscopy.[132] Forterre et al. (2013) expanded upon this claim by adding that other gene transfer agents or cell debris could also be mistaken as viruses or EVs when studied using these same fluorescence microscopy methods.[142] Biller et al. (2017) responded to this criticism by indicating that the method that was being used to detect viruses (epifluorescence microscopy-based particle counting) was only minutely influenced by the presence of EVs.[126] In this report, methods were adjusted to include a chloroform pretreatment step, so that EVs could be distinguished from viruses and revealed a minimal impact on virus quantification (less than an order of magnitude). Building on this work, another report detailed a method in interferometry to likewise distinguish between virus particles and EVs in river water.[143] The primary findings from this study show that EVs could represent up to 28−58% of biotic nanoparticles, compared to 42−72% of particles being viruses. This demonstrates a wide range of possible natural EV concentrations. Further, fluctuations in concentration likely depend on time and space, making it challenging to accurately quantify EVs in environmental matrices and to verify these results in different systems. Beyond quantitative challenges, this report also argued that it is difficult to distinguish between EVs and viruses in form and function. Differentiating between the sources of viral DNA and EV DNA, or visually between EVs, tailless viruses, and other biologically derived colloidal particles, for example, are among the many challenges that work in this field must address. This does not even account for the numerous inert particles that exist in different environmental systems that could also interfere with EV isolation. Methodological challenges such as these dictate labor-intensive, low-throughput experiments which slow critical characterization studies.

Another challenge to studying specific impacts and interactions of microbial EVs in their natural environment is the diversity of EVs that are found in the environment. Several studies describe EVs produced by a variety of plants, including grape, lemon, grapefruit, ginger, and carrots.[144−146] A variety of EVs from multitudes of organisms in the natural environment confound the assignment of specific functionalities to particular EVs. Indeed, EV preparations isolated from environmental samples would likely contain materials from all present organisms. Therefore, both careful detection methods and interpretation are needed to study heterogeneous EV functions *in situ*.

In comparison to land-based and aquatic systems, air stands out as the most neglected compartment of the environment with regard to EV research. Some reports have linked particulate matter exposure to vesicle release in the body,[147] while others have identified EV-associated LPSs to contribute to the proliferation of asthma,[148−150] However, few have investigated EVs in the air in environmental contexts. The lone exception to this lack of research relates to EVs in indoor dust. Qin et al. identified concentrations in dust samples up to 10^{11} EVs per gram of dust, which also contained antibiotic resistance gene subtypes.[151] Another report found that dust specifically contained EVs from Gram-negative bacteria, leading to inflammation from LPSs.[152] While other forms of nanomaterials have been analyzed in the air, measurement of and characterization of EVs in air will require considerable adaption of existing methodologies to ensure the preservation of EVs, as well as a detectable quantity.[153] Moreover, no studies exist for EVs at environmentally relevant interfaces, such as in soil pore water or for inert particle interactions in water or air. Accounting for these factors could allow for EV transport modeling similar to existing models for environmentally relevant nanomaterials.

Although the methods for isolating EVs from environmental samples are underdeveloped, these methods must be generated to understand the roles of both EVs and viruses [phages] separately and collectively in the environment. Protocols adopted from other fields may prove useful. Most methods for isolating and characterizing EVs from complex environments have largely come from the medical field, and biological fluids or tissues.[154] However, environmental samples are relatively dilute and are composed of highly complex and uncharacterized media, with much ecological diversity and chemical variety. As such, borrowing methods from nanoEHS may actually be more appropriate, such as the aforementioned techniques for determining the attachment efficiency of colloidal particles. Extensive research has been performed on nanomaterials (NMs) in a variety of environmental matrices. While some methods may not translate well, since protocols for largely inorganic NMs may not work for organic EVs, methods for the surface characterization of EVs as colloidal particles could be adapted for vesicles. Some studies have already explored these properties of EVs,[106−109] but this should be studied more widely, exploring a more extensive range of organisms' EVs in different environmental conditions.

In sum, EVs are ubiquitous in the terrestrial and aquatic natural environment, with functions spanning from host immune modulation to shuttling electrons between cells. EVs have been documented to have the potential for persistence and long distance transport too. Still, many knowledge gaps exist, especially relating to EVs at the interfaces between organisms or phases, or in the air. Examining these new frontiers will be enabled by improved methods for isolating and characterizing EVs in the natural environment and may be assisted by the convergence of other fields where these methods may already be available.

4. BACTERIOPHAGES AS FUNCTIONAL COLLOIDS IN THE ENVIRONMENT

In this section, we consider phages from a colloid-centric perspective in order to raise awareness of the kinds of environmental contexts where these characteristics might play an important role. We will begin with a brief outline of some of the ways phages directly influence their local environment and then shift into exploring their specific surface characteristics.

4.1. Functions of Phages in the Environment.

Phages impact the health of environments largely through the destruction of their hosts as the last step in their propagation cycle. This characteristic of phage−host interactions directly impacts environmental health by both altering the distribution of microbial populations and by enabling nutrient mobilization. Following attachment, phages inject genetic cargo into the host where they replicate, the host eventually lyses, and the newly formed phages are released. Briefly, two of the most common replication cycles are the lytic and the lysogenic. The most noticeable difference between the lytic and lysogenic cycle is that phages which undergo the lysogenic pathway (termed *temperate phages*) integrate genetic material into the bacterial chromosome where it then becomes what is termed a *prophage*. Lysis occurs sometime later once the phage genes are excised from the chromosome and phage replication begins similarly to the lytic.[155]

The lysis of the host also impacts the distribution of nutrients, especially in aquatic systems, by preventing portions of dissolved organic matter (DOM) from exiting the local environment by moving up trophic levels. This redirection of nutrients from higher trophic level organisms is referred to as the "viral shunt" (Figure 4). Much of the DOM in the ocean does not pass through to higher trophic levels but is confined within the bacterial-viral loop where DOM is recycled from

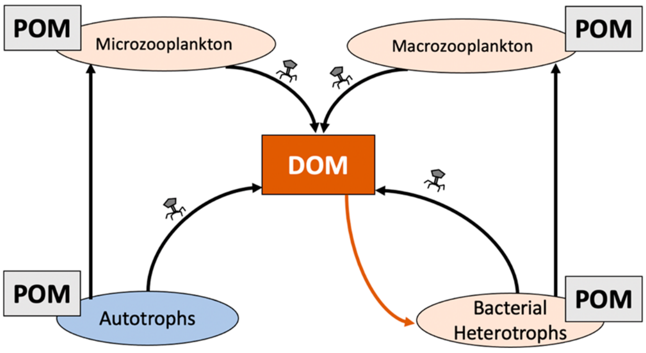

Figure 4. A diagram showing the viral shunt. The arrows show the movement of dissolved organic matter (DOM) and particulate organic matter (POM) in the environment.

bacteria to viruses, then back to DOM for bacterial respiration. However, inefficiencies reduce the amount of recycled DOM over time.[16] In the absence of other DOM generating processes this would potentially result in the depletion of DOM and strain the survival of heterotrophic bacteria. Particulate organic matter (POM) in the form of bacteria and plankton, however, can be transformed into new DOM via phage-induced lysis. The lysis of autotrophs, such as bacteria and grazing plankton, diverts some of this POM into DOM, making it available for heterotrophic bacteria.[156] While nutrient cycling in some environmental contexts involves additional processes, such as the biological pump in ocean systems, the viral shunt is significant for maintaining the necessary balance between the available forms of carbon in microbial communities.

Apart from host lysis, phages also shape microbial communities through horizontal gene transfer, or the sharing of genetic information to an organism that is not a direct descendent of the parent organism. One example of this is the sharing of photosynthetic genes between phages and their hosts, ultimately contributing to the diversity of both the microbial and phage communities.[157] Generally, this phenomenon is more easily seen in the activity of temperate phages, forming prophages within the host, and thus directly influencing the evolution of successive generations to come.[158] A more contemporary example showing the importance of this aspect of phage behavior is the development of antibiotic resistant microbes. It has been shown that phages carrying antibiotic resistance genes could disseminate those resistance genes to microbes within its host range by the same or similar gene transfer mechanism.[159] This means that phages, on one hand, show promise as a therapeutic for antibiotic resistant diseases, yet also function as vectors for antibiotic resistance in the environment.

4.2. Phage as Environmental Colloids. With knowledge of how phages influence their local environment in mind, we can consider one of the more potentially challenging aspects of studying phage aggregation behavior: their morphology. This is because how particles aggregate is a function of their ability to come into surface contact. However, in some instances, especially when dealing with particles whose morphologies are far from the theoretically idealized sphere engineers and scientists enjoy, their geometries can potentially hinder or bolster their attachment with each other or with other particles.[160] Whereas EVs generally can be confidently construed as spheres, phages are more geometrically diverse.[161] This is especially true when considering the tailed phages, since tail orientation can directly influence a phage's ability to bind to a host surface and even how they may approach the surface of nonhost particles.[162] A TEM image of a tailed bacteriophage is provided in Figure 1.

Bacteriophages contained within the order of *Caudoviricetes* are distinct for being tailed and nonenveloped, meaning they possess a protruding tail structure necessary for host attachment and lack an additional lipid envelope around the capsid (note: *capsid* is not interchangeable with *envelope*).[163−165] Nonenveloped phages have generally been more extensively characterized than enveloped phages in aggregation studies.[166] In terms of environmental research, this is largely because nonenveloped phage capsids better withstand harsh environmental conditions (such as heat or acidity) than lipid envelopes which surround the capsids of enveloped phages.[167] The composition of the lipid envelope is a function of the host membrane, as the benefit of the envelope is to better allow the phage to reincorporate with another host to replicate and also evade immune responses.[168] Laurinavicius et al. (2004) found that in the case of phi6, a *P. syringae* phage, the lipids and proteins comprising the envelope are obtained from the cytoplasmic membrane.[169] As one would suspect, the surface charge of enveloped viruses is determined primarily by the chemistry of the envelope proteins (which would also include viral proteins) and lipids. Still, some research suggests that even despite the presence of an envelope, certain similar behavioral patterns can be seen between enveloped and nonenveloped phages. As a case example, Katz et al. (2018), studying enveloped phage heteroaggregation, and Syngouna and Chrysikopoulos (2019), studying a nonenveloped phage, both highlight the importance of hydrophobic interactions in phage heteroaggregation in their respective systems.[166,170]

Shifting now from considering classes of phage morphologies and structures as such, we can begin to consider more specifically how phage aggregation can be construed in a more classical sense. The first component of the phage propagation cycle, host attachment, is vital. Here, an analogy between phage-host and particle−particle attachment is particularly evident, and approaches typically used to studying particle aggregation, especially those proposed by Smoluchowski (1917), are very informative.[36] This analogy has been known since the 1930s when researchers, such as Kruger (1931), Schlesinger (1932), and Delbrück (1940), considered phage-host attachment as a process which could be described by Schmolochowski's diffusion equations.[171−173] Most of these early models make the assumption that nearly all phage-host collisions result in an attachment event (or using more recent aggregation-like terms, $\alpha = 1$). This assumption seems valid for some cases, based on work conducted by Schwartz (1976), who studied the rates of λ-phage attachment to *E. coli* K12 under various divalent ion concentrations.[162] Hicks et al. (2020) investigated the phage-host attachment of phages to their host using a Smoluchowskian approach found that the theoretical approach to calculating β[32,36] alongside an assumption of an α of 1 allowed for the creation of a fairly predictive model for phage-induced bacterial inactivation.[174]

Phage−host and even phage−phage or phage−particle attachment (and virus attachment, more broadly) have been shown to depend upon several of the same factors which influence inert particle−particle attachment (factors discussed in the first section),[5] even though outcomes could be more difficult to predict and the assumption that $\alpha = 1$ cannot be taken for granted. Phages, whose environmental transport has been relatively better characterized than EVs, do not always follow the same theoretical patterns of nonbiological nanoEHS particles. This is seen most clearly in the way some phages remain dispersed under conditions of near physiological ionic strength while aggregating at lower ionic strengths.[175−177] This is contrary to classical DLVO theory, which predicts that aggregation is encouraged under increasing ionic strength conditions.[178] Szermer-Olearnik et al. (2017) suggests that this unique aspect of phage aggregation likely stems from an evolutionary mechanism whereby phages aggregate under harsh conditions as a means of protection in nonphysiological environments.[179]

Even though there may be some exceptions to how phages respond to changes in their ionic environment than what is accounted for in more classic considerations of DLVO, there is still a need to better characterize their surface charge and often approximated by ζ-potential. This is because determining a particle's ζ-potential is still one of the most insightful methods for predicting its stability.[180] Even the most well characterized phage, T4, has only had its ζ-potential determined by a few researchers. In a review, Michen & Graule (2010) amassed a table showing the isoelectric points of several phage and other viruses. In their review, phage T4, arguably the most well characterized phage, has only had its ζ-potential determined a few times since the 1940s.[181] Hicks (2022) also reported T4 ζ-potential values in a study of the attachment and breakup of phage-T4 and kaolinite heteroaggregation, publishing one of the few sets of experimentally determined α values and breakup constants for phage−particle heteroaggregation.[182] That study also showed that the influence of ionic strength on phage-kaolinite particle aggregation does not follow the trends of classical DLVO theory, concluding with other researchers that the extended DLVO forces ought to be explored more thoroughly to better predict the attachment behaviors of phages with inert environmental particles.

5. DISCUSSION

Theoretical and practical approaches to studying inert particle fate and transport in the environment have relevance to understanding the behavior of biological particles such as EVs and phage. The affinity of particles for surfaces as described by the parameter α has been shown to describe much concerning particle fate in complex environmental systems.[31,52,134] Robust methods for acquiring α through *in vitro* experiments have been developed already and have been used for a variety of colloidal particles. Though the surface of EVs and some phages, especially those possessing a lipid envelope, will likely have similarly determinative surface chemistries (such as the protonation/deprotonation of outer proteins or hydrophobic interactions), little can be said specifically about how to best approach modeling their fate when considering them only as isolated biological particles, rather than as members of a diverse colloidal spectrum which could be studied using similar methods. Expanding the bank of bioparticles that are evaluated as colloids will help to paint a more complete picture of the interactions among diverse species found in natural ecosystems. However, these models cannot be truly validated until methods are developed to study EVs and phages in more natural systems.

While it would be ideal to directly probe EV and phage surface interactions *in situ*, in their native environments, the methodology necessary for this type of experimentation is not yet available. The detection, isolation, and characterization of such biocolloids taken from the natural environment are underdeveloped. Moreover, some concerns arise in misidentifying EVs as viruses (e.g., phages) or other micro- or nanoscale biocolloids. Even during the early stages of EV research, EVs were even referred to as "virus-like particles".[183,184] Both biocolloids are complex and can vary compositionally within a population.

Another challenge is the dearth of knowledge regarding EV and phage surface properties. This is especially true for EVs, whose surfaces differ for each type of EV being considered due at least in part to the different biogenesis pathways of EVs. For a relatively small number of specific phages, more information is known of their surface chemistry, and this has clarified the role their chemistry plays in their biological and environmental interactions (as in the case T4 mentioned prior). However, as enveloped phages consist of specific cellular membrane components combined with specific phage proteins, it would not be surprising if phage surfaces also will be discovered to be very diverse. Experimentally, investigating the changes in surface charge (i.e., ζ- potential) of EVs and phages in different environmental conditions would begin to generate a more comprehensive perspective on the surface chemistry of a suspension of these bionanoparticles.

In considering the experimental methods presented above in section 2 (the use of batch and/or column methods), the method one chooses with which to characterize EV and phage attachment to background particles may best be based on the consideration of the natural environmental context. For example, when considering how to best characterize the attachment efficiency of a bacteriophage to a background particle in an aquatic suspension, it would seem most appropriate to take the more novel *batch* experimental approach since such an experimental design would better represent an aquatic suspension.[182] Likewise, as has been touched on in this review, the more commonly used column approach is ideal for characterizing attachment to collector particles in possible soil columns. Hence, while it may still be too early to conclude what minute changes in surface properties might have on a phage or EV surface potential and attachment efficiency, the emergence of two different experimental approaches to attachment characterization allows researchers more freedom to design meaningful transport experiments and models. As an example, Syngouna and Chrysikopoulus (2013), in a phage-based column example, studied phage and clay recovery from pore-water column experiments under varied fluid velocities.[25]

The next step forward for intersecting biocolloid and traditional colloid research will be to characterize the sensitivity of more diverse classes and types of EV and phage particles in response to changes in background ionic strength and pH. Ideally, this extended range of studied EVs and phages would include more wild type strains of organisms (as opposed to lab strains), as well as environmental (rather than mammalian host-associated) microbes, to obtain a more complete picture of the possible surface properties of these biocolloidal particles. This expansion is vital for developing a theoretical framework which could be as valuable to the study of environmnental biocolloids, as DLVO theory is generally helpful for inert environmental colloidal particles. From there, researchers can make discretionary decisions concerning what type of environmental system to study and from there which of the two leading experimental techniques for characterizing particle attachment would be more beneficial for answering questions and addressing hypotheses. Eventually, more robust models of EV and phage transport can be constructed and lend insight into the possible fates that these particles may have in the environment. By adopting some of the methods put forth in this discussion, we will contribute both to our understanding of the mechanisms guiding EV and phage transport and to predicting the roles EVs and phages play in natural systems.

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Notes

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■ REFERENCES

(1) Goodridge, L. D. Bacteriophage biocontrol of plant pathogens: fact or fiction? *Trends Biotechnol.* **2004**, *22*, 384−385.

(2) Martins, A. M.; Pagilla, K.; Heijnen, J. J.; van Loosdrecht, M. C. Filamentous bulking sludge—a critical review. *Water research* **2004**, *38*, 793−817.

(3) McMillan, H. M.; Zebell, S. G.; Ristaino, J. B.; Dong, X.; Kuehn, M. J. Protective plant immune responses are elicited by bacterial outer membrane vesicles. *Cell Reports* **2021**, *34*, 108645.

(4) Syngouna, V. I.; Chrysikopoulos, C. V. Transport of biocolloids in water saturated columns packed with sand: Effect of grain size and pore water velocity. *Journal of Contaminant Hydrology* **2011**, *126*, 301−314.

(5) Wang, H.; Adeleye, A. S.; Huang, Y.; Li, F.; Keller, A. A. Heteroaggregation of nanoparticles with biocolloids and geocolloids. *Advances in colloid and interface Science* **2015**, *226*, 24−36.

(6) Lyklema, J. *Fundamentals of Interface and Colloid Science: Soft Colloids*; Elsevier, 2005; Vol. *5*.

(7) Hunter, R. J. *Foundations of Colloid Science*; Clarendon: Oxford, 1989; pp 992−1052, Vol. *II*.

(8) Derjaguin, B.; Dukhin, S.; Matijevic, E. Nonequilibrium double layer and electrokinetic phenomena. *Surface and Colloid Science* **1974**, *7*, 273−335.

(9) Manning, A. J.; Kuehn, M. J. Contribution of bacterial outer membrane vesicles to innate bacterial defense. *BMC Microbiology* **2011**, *11*, 258.

(10) Gill, S.; Catchpole, R.; Forterre, P. Extracellular membrane vesicles in the three domains of life and beyond. *FEMS microbiology reviews* **2019**, *43*, 273−303.

(11) Toyofuku, M.; Nomura, N.; Eberl, L. Types and origins of bacterial membrane vesicles. *Nature Reviews Microbiology* **2019**, *17*, 13−24.

(12) Yanez-Mo, M.; Siljander, P. R.-M.; Andreu, Z.; Bedina Zavec, A.; Borras, F. E.; Buzas, E. I.; Buzas, K.; Casal, E.; Cappello, F.; Carvalho, J.; Colas, E.; Cordeiro-da Silva, A.; Fais, S.; Falcon-Perez, J. M.; Ghobrial, I. M.; Giebel, B.; Gimona, M.; Graner, M.; Gursel, I.; Gursel, M.; Heegaard, N. H. H.; Hendrix, A.; Kierulf, P.; Kokubun, K.; Kosanovic, M.; Kralj-Iglic, V.; Kramer-Albers, E.-M.; Laitinen, S.; Lasser, C.; Lener, T.; Ligeti, E.; Line, A.; Lipps, G.; Llorente, A.; Lotvall, J.; Mancek-Keber, M.; Marcilla, A.; Mittelbrunn, M.; Nazarenko, I.; Nolte-'t Hoen, E. N. M.; Nyman, T. A.; O'Driscoll, L.; Olivan, M.; Oliveira, C.; Pallinger, E.; del Portillo, H. A.; Reventos, J.; Rigau, M.; Rohde, E.; Sammar, M.; Sanchez-Madrid, F.; Santarem, N.; Schallmoser, K.; Stampe Ostenfeld, M.; Stoorvogel, W.; Stukelj, R.; Van der Grein, S. G.; Helena Vasconcelos, M.; Wauben, M. H. M.; De Wever, O. Biological properties of extracellular vesicles and their physiological functions. *Journal of Extracellular Vesicles* **2015**, *4*, No. 27066.

(13) Schatz, D.; Vardi, A. Extracellular vesicles—new players in cell−cell communication in aquatic environments. *Curr. Opin. Microbiol.* **2018**, *43*, 148−154.

(14) Kaparakis-Liaskos, M.; Kufer, T. A. *Bacterial Membrane Vesicles*; Springer Nature: Cham, 2020.

(15) McMillan, H. M.; et al. Microbial vesicle-mediated communication: convergence to understand interactions within and between domains of life. *Environmental Science: Processes & Impacts* **2021**, *23*, 664−677.

(16) Fuhrman, J.; Suttle, C. Viruses in marine planktonic systems. *Oceanography* **1993**, *6*, 51−63.

(17) Hendrix, R. W. Bacteriophages: evolution of the majority. *Theoretical population biology* **2002**, *61*, 471−480.

(18) Shen, C.; Bradford, S. A. Why are viruses spiked? *Msphere* **2021**, *6*, .

(19) Cai, Q.; et al. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science* **2018**, *360*, 1126−1129.

(20) Orench-Rivera, N.; Kuehn, M. J. Environmentally controlled bacterial vesicle-mediated export. *Cellular microbiology* **2016**, *18*, 1525−1536.

(21) Zhang, D.; Zabarankin, M.; Prigiobbe, V. Modeling salinity-dependent transport of viruses in porous media. *Advances in Water Resources* **2019**, *127*, 252−263.

(22) Zhang, W.; et al. Transport of Escherichia coli phage through saturated porous media considering managed aquifer recharge. *Environmental Science and Pollution Research* **2018**, *25*, 6497−6513.

(23) Xu, S.; et al. Mutually facilitated co-transport of two different viruses through reactive porous media. *Water Res.* **2017**, *123*, 40−48.

(24) Knappett, P. S.; Emelko, M. B.; Zhuang, J.; McKay, L. D. Transport and retention of a bacteriophage and microspheres in saturated, angular porous media: Effects of ionic strength and grain size. *Water Res.* **2008**, *42*, 4368−4378.

(25) Syngouna, V. I.; Chrysikopoulos, C. V. Cotransport of clay colloids and viruses in water saturated porous media. *Colloids Surf., A* **2013**, *416*, 56−65.

(26) Norde, W.; Lyklema, J. Protein adsorption and bacterial adhesion to solid surfaces: a colloid-chemical approach. *Colloids and surfaces* **1989**, *38*, 1−13.

(27) Trivedi, R. R.; Maeda, R.; Abbott, N. L.; Spagnolie, S. E.; Weibel, D. B. Bacterial transport of colloids in liquid crystalline environments. *Soft Matter* **2015**, *11*, 8404−8408.

(28) Jones, M.-C.; Leroux, J.-C. Polymeric micelles—a new generation of colloidal drug carriers. *Eur. J. Pharm. Biopharm.* **1999**, *48*, 101−111.

(29) Sen, T. K.; Khilar, K. C. Review on subsurface colloids and colloid-associated contaminant transport in saturated porous media. *Advances in Colloid and Interface Science* **2006**, *119*, 71−96.

(30) Molnar, I. L.; Johnson, W. P.; Gerhard, J. I.; Willson, C. S.; O'carroll, D. M. Predicting colloid transport through saturated porous media: A critical review. *Water Resour. Res.* **2015**, *51*, 6804−6845.

(31) Geitner, N. K.; O'Brien, N. J.; Turner, A. A.; Cummins, E. J.; Wiesner, M. R. Measuring Nanoparticle Attachment Efficiency in Complex Systems. *Environ. Sci. Technol.* **2017**, *51*, 13288−13294.

(32) O'Melia, C. R. ES&T features: Aquasols: The behavior of small particles in aquatic systems. *Environ. Sci. Technol.* **1980**, *14*, 1052−1060.

(33) Hotze, E. M.; Phenrat, T.; Lowry, G. V. Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment. *Journal of environmental quality* **2010**, *39*, 1909−1924.

(34) Alimi, O. S.; Farner Budarz, J.; Hernandez, L. M.; Tufenkji, N. Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. *Environ. Sci. Technol.* **2018**, *52*, 1704−1724.

(35) Petosa, A. R.; Jaisi, D. P.; Quevedo, I. R.; Elimelech, M.; Tufenkji, N. Aggregation and Deposition of Engineered Nanomaterials in Aquatic Environments: Role of Physicochemical Interactions. *Environ. Sci. Technol.* **2010**, *44*, 6532−6549.

(36) von Smoluchowski, M. Outline of the coagulation kinetics of colloidal solutions. *Kolloid-Z.* **1917**, *21*, 98−104.

(37) Veerapaneni, S.; Wiesner, M. R. Hydrodynamics of fractal aggregates with radially varying permeability. *J. Colloid Interface Sci.* **1996**, *177*, 45−57.

(38) Rajagopalan, R.; Tien, C. Single collector analysis of collection mechanisms in water filtration. *Canadian Journal of Chemical Engineering* **1977**, *55*, 246−255.

(39) Tufenkji, N.; Elimelech, M. Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. *Environ. Sci. Technol.* **2004**, *38*, 529−536.

(40) Derjaguin, B.; Landau, L. Theory of the Stability of Strongly Charged Lyophobic Sols and of the Adhesion of Strongly Charged-Particles in Solutions of Electrolytes. *Prog. Surf. Sci.* **1993**, *43*, 30−59.

(41) Verwey, E. J. W.; Overbeek, J. T. G.; Nes, K. v. *Theory of the Stability of Lyophobic Colloids; the Interaction of Sol Particles Having an Electric Double Layer*; Elsevier Pub. Co., 1948.

(42) Kim, C.; Pennell, K. D.; Fortner, J. D. Delineating the relationship between nanoparticle attachment efficiency and fluid flow velocity. *Environ. Sci. Technol.* **2020**, *54*, 13992−13999.

(43) Tufenkji, N.; Elimelech, M. Breakdown of colloid filtration theory: Role of the secondary energy minimum and surface charge heterogeneities. *Langmuir* **2005**, *21*, 841−852.

(44) Hermansson, M. The DLVO theory in microbial adhesion. *Colloids Surf., B* **1999**, *14*, 105−119.

(45) Van Oss, C. Hydrophobicity of biosurfaces—origin, quantitative determination and interaction energies. *Colloids Surf., B* **1995**, *5*, 91−110.

(46) Elimelech, M.; O'Melia, C. R. Effect of particle size on collision efficiency in the deposition of Brownian particles with electrostatic energy barriers. *langmuir* **1990**, *6*, 1153−1163.

(47) Elimelech, M.; O'Melia, C. R. Kinetics of deposition of colloidal particles in porous media. *Environ. Sci. Technol.* **1990**, *24*, 1528−1536.

(48) Treumann, S.; Torkzaban, S.; Bradford, S. A.; Visalakshan, R. M.; Page, D. An explanation for differences in the process of colloid adsorption in batch and column studies. *Journal of contaminant hydrology* **2014**, *164*, 219−229.

(49) Shen, C.; et al. Observed equilibrium partition and second-order kinetic interaction of quantum dot nanoparticles in saturated porous media. *Journal of Contaminant Hydrology* **2021**, *240*, 103799.

(50) Buddemeier, R. W.; Hunt, J. R. Transport of colloidal contaminants in groundwater: radionuclide migration at the Nevada Test Site. *Applied geochemistry* **1988**, *3*, 535−548.

(51) Barton, L. E.; Therezien, M.; Auffan, M.; Bottero, J. Y.; Wiesner, M. R. Theory and Methodology for Determining Nanoparticle Affinity for Heteroaggregation in Environmental Matrices Using Batch Measurements. *Environmental Engineering Science* **2014**, *31*, 421−427.

(52) Turner, A. A.; Rogers, N. M.; Geitner, N. K.; Wiesner, M. R. Nanoparticle affinity for natural soils: a functional assay for determining particle attachment efficiency in complex systems. *Environmental Science: Nano* **2020**, *7*, 1719−1729.

(53) Johnson, W. P.; Tong, M. Observed and simulated fluid drag effects on colloid deposition in the presence of an energy barrier in an impinging jet system. *Environ. Sci. Technol.* **2006**, *40*, 5015−5021.

(54) Tong, M.; Johnson, W. P. Excess colloid retention in porous media as a function of colloid size, fluid velocity, and grain angularity. *Environ. Sci. Technol.* **2006**, *40*, 7725−7731.

(55) Ron, C. A.; VanNess, K.; Rasmuson, A.; Johnson, W. P. How nanoscale surface heterogeneity impacts transport of nano-to micro-particles on surfaces under unfavorable attachment conditions. *Environmental Science: Nano* **2019**, *6*, 1921−1931.

(56) Liu, X.; Chen, G.; Su, C. Influence of collector surface composition and water chemistry on the deposition of cerium dioxide nanoparticles: QCM-D and column experiment approaches. *Environ. Sci. Technol.* **2012**, *46*, 6681−6688.

(57) Chen, K. L.; Elimelech, M. Aggregation and deposition kinetics of fullerene (C60) nanoparticles. *Langmuir* **2006**, *22*, 10994−11001.

(58) Gomez-Flores, A.; Bradford, S. A.; Cai, L.; Urík, M.; Kim, H. Prediction of attachment efficiency using machine learning on a comprehensive database and its validation. *Water Res.* **2023**, *229*, 119429.

(59) Oliveira, D. L.; et al. Characterization of yeast extracellular vesicles: evidence for the participation of different pathways of cellular traffic in vesicle biogenesis. *PloS one* **2010**, *5*, e11113.

(60) Kulp, A.; Kuehn, M. J. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. *Annual review of microbiology* **2010**, *64*, 163−184.

(61) Schwechheimer, C.; Kuehn, M. J. Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. *Nature reviews microbiology* **2015**, *13*, 605−619.

(62) Lee, E. Y.; et al. Global proteomic profiling of native outer membrane vesicles derived from Escherichia coli. *Proteomics* **2007**, *7*, 3143−3153.

(63) Wensink, J.; Witholt, B. Outer-membrane vesicles released by normally growing Escherichia coli contain very little lipoprotein. *European journal of biochemistry* **1981**, *116*, 331−335.

(64) Kulkarni, H. M.; Jagannadham, M. V. Biogenesis and multifaceted roles of outer membrane vesicles from Gram-negative bacteria. *Microbiology* **2014**, *160*, 2109−2121.

(65) Lee, J.; Kim, O. Y.; Gho, Y. S. Proteomic profiling of Gram-negative bacterial outer membrane vesicles: Current perspectives. *Proteomics Clinical Apps* **2016**, *10*, 897−909.

(66) Orench-Rivera, N.; Kuehn, M. J. Differential packaging into outer membrane vesicles upon oxidative stress reveals a general mechanism for cargo selectivity. *Frontiers in Microbiology* **2021**, *12*.

(67) Bauman, S. J.; Kuehn, M. J. Pseudomonas aeruginosa vesicles associate with and are internalized by human lung epithelial cells. *BMC Microbiology* **2009**, *9*, 26.

(68) Esoda, C. N.; Kuehn, M. J. Pseudomonas aeruginosa leucine aminopeptidase influences early biofilm composition and structure via vesicle-associated antibiofilm activity. *MBio* **2019**, *10*, e02548−02519.

(69) Horstman, A. L.; Bauman, S. J.; Kuehn, M. J. Lipopolysaccharide 3-deoxy-D-manno-octulosonic acid (Kdo) core determines bacterial association of secreted toxins. *J. Biol. Chem.* **2004**, *279*, 8070−8075.

(70) Horstman, A. L.; Kuehn, M. J. Enterotoxigenic Escherichia coli secretes active heat-labile enterotoxin via outer membrane vesicles. *J. Biol. Chem.* **2000**, *275*, 12489−12496.

(71) Horstman, A. L.; Kuehn, M. J. Bacterial surface association of heat-labile enterotoxin through lipopolysaccharide after secretion via the general secretory pathway. *J. Biol. Chem.* **2002**, *277*, 32538–32545.

(72) Tashiro, Y.; et al. Characterization of phospholipids in membrane vesicles derived from Pseudomonas aeruginosa. *Bioscience, biotechnology, and biochemistry* **2011**, *75*, 605–607.

(73) Bonnington, K. E.; Kuehn, M. J. Outer membrane vesicle production facilitates LPS remodeling and outer membrane maintenance in Salmonella during environmental transitions. *MBio* **2016**, *7*, e01532–01516.

(74) Chowdhury, C.; Jagannadham, M. V. Virulence factors are released in association with outer membrane vesicles of Pseudomonas syringae pv. tomato T1 during normal growth. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* **2013**, *1834*, 231–239.

(75) Kato, S.; Kowashi, Y.; Demuth, D. R. Outer membrane-like vesicles secreted by Actinobacillus actinomycetemcomitans are enriched in leukotoxin. *Microbial pathogenesis* **2002**, *32*, 1–13.

(76) Baeza, N.; Delgado, L.; Comas, J.; Mercade, E. Phage-mediated explosive cell lysis induces the formation of a different type of O-IMV in Shewanella vesiculosa M7T. *Frontiers in Microbiology* **2021**, *12*, 713669.

(77) Wassenaar, T. M.; Zimmermann, K. Lipopolysaccharides in food, food supplements, and probiotics: should we be worried? *European Journal of Microbiology and Immunology* **2018**, *8*, 63–69.

(78) Li, Z.; Clarke, A. J.; Beveridge, T. J. A major autolysin of Pseudomonas aeruginosa: subcellular distribution, potential role in cell growth and division and secretion in surface membrane vesicles. *Journal of bacteriology* **1996**, *178*, 2479–2488.

(79) Alves, N. J.; Turner, K. B.; DiVito, K. A.; Daniele, M. A.; Walper, S. A. Affinity purification of bacterial outer membrane vesicles (OMVs) utilizing a His-tag mutant. *Research in microbiology* **2017**, *168*, 139–146.

(80) Baumgarten, T.; et al. Membrane vesicle formation as a multiple-stress response mechanism enhances Pseudomonas putida DOT-T1E cell surface hydrophobicity and biofilm formation. *Applied and environmental microbiology* **2012**, *78*, 6217–6224.

(81) Lee, J.; et al. Staphylococcus aureus extracellular vesicles carry biologically active β-lactamase. *Antimicrob. Agents Chemother.* **2013**, *57*, 2589–2595.

(82) Palacios, A.; et al. Biogenesis and function of extracellular vesicles in gram-positive bacteria, mycobacteria, and fungi. *Bacterial Membrane Vesicles: Biogenesis, Functions and Applications* **2020**, 47–74.

(83) Rath, P. Genetic regulation of vesiculogenesis and immunomodulation in Mycobacterium tuberculosis. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, E4790–E4797.

(84) Toyofuku, M.; Carcamo-Oyarce, G.; Yamamoto, T.; Eisenstein, F.; Hsiao, C.-C.; Kurosawa, M.; Gademann, K.; Pilhofer, M.; Nomura, N.; Eberl, L. Prophage-triggered membrane vesicle formation through peptidoglycan damage in Bacillus subtilis. *Nat. Commun.* **2017**, *8*, 481.

(85) White, D. W., Elliott, S. R., Odean, E., Bemis, L. T.; Tischler, A. D. Mycobacterium tuberculosis Pst/SenX3-RegX3 regulates membrane vesicle production independently of ESX-5 activity. *MBio* **2018**, *9*, .

(86) Lee, E. Y.; et al. Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles. *Proteomics* **2009**, *9*, 5425–5436.

(87) Brown, L.; Wolf, J. M.; Prados-Rosales, R.; Casadevall, A. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. *Nature Reviews Microbiology* **2015**, *13*, 620–630.

(88) Shiraishi, T.; et al. Lipoteichoic acids are embedded in cell walls during logarithmic phase, but exposed on membrane vesicles in Lactobacillus gasseri JCM 1131T. *Beneficial Microbes* **2018**, *9*, 653–662.

(89) Toyofuku, M.; Schild, S.; Kaparakis-Liaskos, M.; Eberl, L. Composition and functions of bacterial membrane vesicles. *Nature Reviews Microbiology* **2023**, *21*, 415.

(90) Bose, S.; Aggarwal, S.; Singh, D. V.; Acharya, N. Extracellular vesicles: An emerging platform in gram-positive bacteria. *Microbial Cell* **2020**, *7*, 312.

(91) Li, K.; Ma, H. Rotation and retention dynamics of rod-shaped colloids with surface charge heterogeneity in sphere-in-cell porous media model. *Langmuir* **2019**, *35*, 5471–5483.

(92) Seymour, M. B.; Chen, G.; Su, C.; Li, Y. Transport and retention of colloids in porous media: does shape really matter? *Environ. Sci. Technol.* **2013**, *47*, 8391–8398.

(93) Ma, H.; et al. Coupled influences of particle shape, surface property and flow hydrodynamics on rod-shaped colloid transport in porous media. *J. Colloid Interface Sci.* **2020**, *577*, 471–480.

(94) Bielska, E.; May, R. C. Extracellular vesicles of human pathogenic fungi. *Curr. Opin. Microbiol.* **2019**, *52*, 90–99.

(95) Rodrigues, M. L.; et al. Vesicular polysaccharide export in Cryptococcus neoformans is a eukaryotic solution to the problem of fungal trans-cell wall transport. *Eukaryotic cell* **2007**, *6*, 48–59.

(96) Henne, W. M.; Buchkovich, N. J.; Emr, S. D. The ESCRT pathway. *Developmental cell* **2011**, *21*, 77–91.

(97) Nosanchuk, J. D.; Nimrichter, L.; Casadevall, A.; Rodrigues, M. L. A role for vesicular transport of macromolecules across cell walls in fungal pathogenesis. *Communicative & integrative biology* **2008**, *1*, 37–39.

(98) Oliveira, D. L.; Nakayasu, E. S.; Joffe, L. S.; Guimaraes, A. J.; Sobreira, T. J. P.; Nosanchuk, J. D.; Cordero, R. J. B.; Frases, S.; Casadevall, A.; Almeida, I. C.; Nimrichter, L.; Rodrigues, M. L. Characterization of yeast extracellular vesicles: evidence for the participation of different pathways of cellular traffic in vesicle biogenesis. *PloS One* **2010**, *5*, e11113

(99) Rodrigues, M. L.; Djordjevic, J. T. Unravelling secretion in Cryptococcus neoformans: more than one way to skin a cat. *Mycopathologia* **2012**, *173*, 407–418.

(100) Rodrigues, M. L.; et al. Extracellular vesicles produced by Cryptococcus neoformans contain protein components associated with virulence. *Eukaryotic cell* **2008**, *7*, 58–67.

(101) Vallejo, M. C.; et al. Vesicle and vesicle-free extracellular proteome of Paracoccidioides brasiliensis: comparative analysis with other pathogenic fungi. *J. Proteome Res.* **2012**, *11*, 1676–1685.

(102) Wolf, J. M.; Casadevall, A. Challenges posed by extracellular vesicles from eukaryotic microbes. *Curr. Opin. Microbiol.* **2014**, *22*, 73–78.

(103) Bleackley, M. R.; Dawson, C. S.; Anderson, M. A. Fungal extracellular vesicles with a focus on proteomic analysis. *Proteomics* **2019**, *19*, 1800232.

(104) Albuquerque, P. C.; et al. Vesicular transport in Histoplasma capsulatum: an effective mechanism for trans-cell wall transfer of proteins and lipids in ascomycetes. *Cellular microbiology* **2008**, *10*, 1695–1710.

(105) Vargas, G.; et al. Compositional and immunobiological analyses of extracellular vesicles released by C andida albicans. *Cellular microbiology* **2015**, *17*, 389–407.

(106) Rogers, N. M.; et al. Comparative electrokinetic properties of extracellular vesicles produced by yeast and bacteria. *Colloids Surf., B* **2023**, *225*, 113249.

(107) Midekessa, G.; et al. Zeta potential of extracellular vesicles: toward understanding the attributes that determine colloidal stability. *Acs Omega* **2020**, *5*, 16701–16710.

(108) Gnopo, Y. M.; et al. Induced fusion and aggregation of bacterial outer membrane vesicles: Experimental and theoretical analysis. *J. Colloid Interface Sci.* **2020**, *578*, 522–532.

(109) Rogers, N. M. K.; Hicks, E.; Kan, C.; Martin, E.; Gao, L.; Limso, C.; Hendren, C. O.; Kuehn, M.; Wiesner, M. R. Characterizing the Transport and Surface Affinity of Extracellular Vesicles Isolated from Yeast and Bacteria in Well-Characterized Porous Media. *Environ. Sci. Technol.* **2023**, *57*, 13182.

(110) Shoberg, R. J.; Thomas, D. D. Specific adherence of Borrelia burgdorferi extracellular vesicles to human endothelial cells in culture. *Infection and immunity* **1993**, *61*, 3892–3900.

(111) Kesty, N. C.; Kuehn, M. J. Incorporation of heterologous outer membrane and periplasmic proteins into Escherichia coli outer membrane vesicles. *J. Biol. Chem.* **2004**, *279*, 2069−2076.

(112) Timár, C. I.; Lőrincz, Á. M.; Ligeti, E. Changing world of neutrophils. *Pflügers Archiv-European Journal of Physiology* **2013**, *465*, 1521−1533.

(113) An, Q. L.; Ehlers, K.; Kogel, K. H.; van Bel, A. J. E.; Huckelhoven, R. Multivesicular compartments proliferate in susceptible and resistant MLA12-barley leaves in response to infection by the biotrophic powdery mildew fungus. *New Phytologist* **2006**, *172*, 563−576.

(114) Evtushenko, E. G.; Bagrov, D. V.; Lazarev, V. N.; Livshits, M. A.; Khomyakova, E. Adsorption of extracellular vesicles onto the tube walls during storage in solution. *PloS one* **2020**, *15*, No. e0243738.

(115) Samuel, M.; Bleackley, M.; Anderson, M.; Mathivanan, S. Extracellular vesicles including exosomes in cross kingdom regulation: a viewpoint from plant-fungal interactions. *Front Plant Sci.* **2015**, *6*, 766.

(116) Chalupowicz, L.; Mordukhovich, G.; Assoline, N.; Katsir, L.; Sela, N.; Bahar, O. Bacterial outer membrane vesicles induce a transcriptional shift in arabidopsis towards immune system activation leading to suppression of pathogen growth in planta. *Journal of Extracellular Vesicles* **2023**, *12*, 12285.

(117) Bahar, O.; et al. Bacterial outer membrane vesicles induce plant immune responses. *Mol. Plant-Microbe Interact.* **2016**, *29*, 374−384.

(118) Katsir, L.; Bahar, O. Bacterial outer membrane vesicles at the plant−pathogen interface. *PLoS pathogens* **2017**, *13*, e1006306.

(119) Rudnicka, M.; et al. Outer Membrane Vesicles as Mediators of Plant−Bacterial Interactions. *Frontiers in Microbiology* **2022**, *13*, 902181.

(120) Prados-Rosales, R.; et al. Role for Mycobacterium tuberculosis membrane vesicles in iron acquisition. *Journal of bacteriology* **2014**, *196*, 1250−1256.

(121) Wang, M.; Nie, Y.; Wu, X. L. Membrane vesicles from a Dietzia bacterium containing multiple cargoes and their roles in iron delivery. *Environmental Microbiology* **2021**, *23*, 1009−1019.

(122) Lin, J.; Zhang, W.; Cheng, J.; Yang, X.; Zhu, K.; Wang, Y.; Wei, G.; Qian, P.-Y.; Luo, Z.-Q.; Shen, X. A Pseudomonas T6SS effector recruits PQS-containing outer membrane vesicles for iron acquisition. *Nat. Commun.* **2017**, *8*, 14888.

(123) Pirbadian, S.; et al. Shewanella oneidensis MR-1 nanowires are outer membrane and periplasmic extensions of the extracellular electron transport components. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 12883−12888.

(124) Liu, X.; Jing, X.; Ye, Y.; Zhan, J.; Ye, J.; Zhou, S. Bacterial Vesicles Mediate Extracellular Electron Transfer. *Environmental Science & Technology Letters* **2020**, *7*, 27.

(125) Biller, S. J.; et al. Bacterial vesicles in marine ecosystems. *Science* **2014**, *343*, 183−186.

(126) Biller, S. J.; et al. Membrane vesicles in sea water: heterogeneous DNA content and implications for viral abundance estimates. *ISME J.* **2017**, *11*, 394−404.

(127) Biller, S. J.et al. Environmental and taxonomic drivers of bacterial extracellular vesicle production in marine ecosystems. *bioRxiv* **2022**.

(128) Fadeev, E.; Carpaneto Bastos, C.; Hennenfeind, J. H; Biller, S. J; Sher, D.; Wietz, M.; Herndl, G. J Characterization of membrane vesicles in Alteromonas macleodii indicates potential roles in their copiotrophic lifestyle. *Microlife* **2023**, *4*, uqac025.

(129) Koga, Y.; et al. Exosome can prevent RNase from degrading microRNA in feces. *Journal of Gastrointestinal Oncology* **2011**, *2*, 215.

(130) Rodriguez, B. V.; Kuehn, M. J. Staphylococcus aureus secretes immunomodulatory RNA and DNA via membrane vesicles. *Sci. Rep.* **2020**, *10*, 1−22.

(131) Blesa, A.; Berenguer, J. Contribution of vesicle-protected extracellular DNA to horizontal gene transfer in Thermus spp. *Int. Microbiol.* **2015**, *18*, 177−187.

(132) Soler, N.; Marguet, E.; Verbavatz, J.-M.; Forterre, P. Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order Thermococcales. *Research in Microbiology* **2008**, *159*, 390−399.

(133) Frias, A.; Manresa, A.; de Oliveira, E.; López-Iglesias, C.; Mercade, E. Membrane vesicles: a common feature in the extracellular matter of cold-adapted Antarctic bacteria. *Microbial ecology* **2010**, *59*, 476−486.

(134) Geitner, N. K.; Marinakos, S. M.; Guo, C.; O'Brien, N.; Wiesner, M. R. Nanoparticle surface affinity as a predictor of trophic transfer. *Environ. Sci. Technol.* **2016**, *50*, 6663−6669.

(135) Bomberger, J. M.; et al. Long-distance delivery of bacterial virulence factors by Pseudomonas aeruginosa outer membrane vesicles. *PLoS pathogens* **2009**, *5*, e1000382.

(136) Bos, J.; Cisneros, L. H.; Mazel, D. Real-time tracking of bacterial membrane vesicles reveals enhanced membrane traffic upon antibiotic exposure. *Science Advances* **2021**, *7*, eabd1033.

(137) Reyes-Robles, T.; Dillard, R. S.; Cairns, L. S.; Silva-Valenzuela, C. A.; Housman, M.; Ali, A.; Wright, E. R.; Camilli, A. Vibrio cholerae outer membrane vesicles inhibit bacteriophage infection. *Journal of Bacteriology* **2018**, *200*, .

(138) Kharina, A.; et al. Temperate bacteriophages collected by outer membrane vesicles in Komagataeibacter intermedius. *Journal of basic microbiology* **2015**, *55*, 509−513.

(139) Mandal, P. K.; Ballerin, G.; Nolan, L. M.; Petty, N. K.; Whitchurch, C. B. Bacteriophage infection of Escherichia coli leads to the formation of membrane vesicles via both explosive cell lysis and membrane blebbing. *Microbiology* **2021**, *167*.

(140) Yasuda, M.; Yamamoto, T.; Nagakubo, T.; Morinaga, K.; Obana, N.; Nomura, N.; Toyofuku, M. Phage genes induce quorum sensing signal release through membrane vesicle formation. *Microbes and Environments* **2022**, *37*, ME21067.

(141) Soler, N.; Krupovic, M.; Marguet, E.; Forterre, P. Membrane vesicles in natural environments: a major challenge in viral ecology. *ISME Journal* **2015**, *9*, 793−796.

(142) Forterre, P.; Soler, N.; Krupovic, M.; Marguet, E.; Ackermann, H.-W. Fake virus particles generated by fluorescence microscopy. *Trends in microbiology* **2013**, *21*, 1−5.

(143) Roose-Amsaleg, C.; et al. Utilization of interferometric light microscopy for the rapid analysis of virus abundance in a river. *Research in microbiology* **2017**, *168*, 413−418.

(144) Rutter, B. D.; Innes, R. W. Extracellular Vesicles Isolated from the Leaf Apoplast Carry Stress-Response Proteins. *Plant Physiol* **2017**, *173*, 728−741.

(145) Rome, S. Biological properties of plant-derived extracellular vesicles. *Food & function* **2019**, *10*, 529−538.

(146) Zhang, M.; Viennois, E.; Xu, C.; Merlin, D. Plant derived edible nanoparticles as a new therapeutic approach against diseases. *Tissue barriers* **2016**, *4*, e1134415.

(147) Bonzini, M.; et al. Short-term particulate matter exposure induces extracellular vesicle release in overweight subjects. *Environmental research* **2017**, *155*, 228−234.

(148) Dutkiewicz, J. Pantoea agglomerans: a mysterious bacterium of evil and good. P. 2. Deleterious effects: Dust-borne endotoxins and allergens-focus on grain dust, other agricultural dusts and wood dust. *Annals of Agricultural and Environmental Medicine* **2016**, *23*, 6.

(149) Alashkar Alhamwe, B.; et al. Extracellular vesicles and asthma—More than just a co-existence. *International journal of molecular sciences* **2021**, *22*, 4984.

(150) Yang, J.; Shin, T.-S.; Kim, J. S.; Jee, Y.-K.; Kim, Y.-K. A new horizon of precision medicine: combination of the microbiome and extracellular vesicles. *Experimental & molecular medicine* **2022**, *54*, 466−482.

(151) Qin, Y.; et al. Widespread of potential pathogen-derived extracellular vesicles carrying antibiotic resistance genes in indoor dust. *Environ. Sci. Technol.* **2022**, *56*, 5653−5663.

(152) Kim, Y. S.; et al. Extracellular vesicles, especially derived from Gram-negative bacteria, in indoor dust induce neutrophilic pulmonary

inflammation associated with both Th1 and Th17 cell responses. *Clinical & Experimental Allergy* **2013**, *43*, 443−454.

(153) Wiesner, M. R.; et al. Decreasing Uncertainties in Assessing Environmental Exposure, Risk, and Ecological Implications of Nanomaterials. *Environ. Sci. Technol.* **2009**, *43*, 6458−6462.

(154) Gandham, S. Technologies and standardization in research on extracellular vesicles. *Trends Biotechnol.* **2020**, *38*, 1066.

(155) Clokie, M. R.; Millard, A. D.; Letarov, A. V.; Heaphy, S. Phages in nature. *Bacteriophage* **2011**, *1*, 31−45.

(156) Wilhelm, S. W.; Suttle, C. A. Viruses and nutrient cycles in the sea: viruses play critical roles in the structure and function of aquatic food webs. *Bioscience* **1999**, *49*, 781−788.

(157) Lindell, D.; et al. Transfer of photosynthesis genes to and from Prochlorococcus viruses. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 11013−11018.

(158) Canchaya, C.; Fournous, G.; Chibani-Chennoufi, S.; Dillmann, M.-L.; Brüssow, H. Phage as agents of lateral gene transfer. *Curr. Opin. Microbiol.* **2003**, *6*, 417−424.

(159) Gomez-Gomez, C.; Blanco-Picazo, P.; Brown-Jaque, M.; Quiros, P.; Rodriguez-Rubio, L.; Cerda-Cuellar, M.; Muniesa, M. Infectious phage particles packaging antibiotic resistance genes found in meat products and chicken feces. *Sci. Rep.* **2019**, *9*, 13281.

(160) Han, M.; Lawler, D. F. The (relative) insignificance of G in flocculation. *Journal-American Water Works Association* **1992**, *84*, 79−91.

(161) Ratajczak, M. Z.; Ratajczak, J. Extracellular microvesicles/exosomes: discovery, disbelief, acceptance, and the future? *Leukemia* **2020**, *34*, 3126−3135.

(162) Schwartz, M. The adsorption of coliphage lambda to its host: effect of variations in the surface density of receptor and in phage-receptor affinity. *Journal of molecular biology* **1976**, *103*, 521−536.

(163) Kuhn, J. H. Virus taxonomy. *Encyclopedia of Virology* **2021**, *28*, 28.

(164) Fenner, F.; Maurin, J. The classification and nomenclature of viruses. *Arch. Virol.* **1976**, *51*, 141−149.

(165) Gibbs, A. J.; Harrison, B.; Watson, D.; Wildy, P. What's in a virus name? *Nature* **1966**, *209*, 450−454.

(166) Katz, A.; et al. Heteroaggregation of an enveloped bacteriophage with colloidal sediments and effect on virus viability. *Sci. Total Environ.* **2018**, *637*, 104−111.

(167) Mäntynen, S.; Sundberg, L.-R.; Oksanen, H. M.; Poranen, M. M. Half a century of research on membrane-containing bacteriophages: bringing new concepts to modern virology. *Viruses* **2019**, *11*, 76.

(168) Drulis-Kawa, Z.; Majkowska-Skrobek, G.; Maciejewska, B.; Delattre, A.-S.; Lavigne, R. Learning from bacteriophages-advantages and limitations of phage and phage-encoded protein applications. *Current Protein and Peptide Science* **2012**, *13*, 699−722.

(169) Laurinavičius, S.; Käkelä, R.; Bamford, D. H.; Somerharju, P. The origin of phospholipids of the enveloped bacteriophage phi6. *Virology* **2004**, *326*, 182−190.

(170) Syngouna, V. I.; Chrysikopoulos, C. V. Bacteriophage MS2 and titanium dioxide heteroaggregation: Effects of ambient light and the presence of quartz sand. *Colloids Surf., B* **2019**, *180*, 281−288.

(171) Krueger, A. P. The sorption of bacteriophage by living and dead susceptible bacteria: I. Equilibrium conditions. *J. Gen. Physiol.* **1931**, *14*, 493−516.

(172) Schlesinger, M. Über die Bindung des Bakteriophagen an homologe Bakterien: I. Mitteilung: Die Unterscheidung von Gruppen verschiedener Bindungsaffinität innerhalb der Bakteriophagen desselben Lysats. Die Frage der Reversibilität oder Irreversibilität der Bindung. *Zeitschrift für Hygiene und Infektionskrankheiten* **1932**, *114*, 136−148.

(173) Delbrück, M. Adsorption of bacteriophage under various physiological conditions of the host. *J. Gen. Physiol.* **1940**, *23*, 631.

(174) Hicks, E.; Wiesner, M. R.; Gunsch, C. K. Modeling bacteriophage-induced inactivation of Escherichia coli utilizing particle aggregation kinetics. *Water Res.* **2020**, *171*, 115438.

(175) Dunlap, R.; Brown, E.; Barry, D. Determination of the viral particle content of influenza vaccines by electron microscopy. *Journal of Biological Standardization* **1975**, *3*, 281−289.

(176) Floyd, R.; Sharp, D. Aggregation of poliovirus and reovirus by dilution in water. *Applied and environmental microbiology* **1977**, *33*, 159−167.

(177) Lark, K. G.; Adams, M. H. The stability of phages as a function of the ionic environment. In *Cold Spring Harbor Symposia on Quantitative Biology*; Cold Spring Harbor Laboratory Press. 1953, *18*, 171−183.

(178) Israelachvili, J.; Ninham, B. Intermolecular forces—the long and short of it. *J. Colloid Interface Sci.* **1977**, *58*, 14−25.

(179) Szermer-Olearnik, B.; Drab, M.; Makosa, M.; Zembala, M.; Barbasz, J.; Dabrowska, K.; Boratynski, J. Aggregation/dispersion transitions of T4 phage triggered by environmental ion availability. *J. Nanobiotechnol.* **2017**, *15*, 1−15.

(180) Hunter, R. J. *Introduction to Modern Colloid Science*; Oxford University Press, 1993.

(181) Michen, B.; Graule, T. Isoelectric points of viruses. *Journal of applied microbiology* **2010**, *109*, 388−397.

(182) Hicks, E.; Wiesner, M. R. Exploring the design implications of bacteriophages in mixed suspensions by considering attachment and break-up. *Water Res.* **2022**, *216*, 118303.

(183) Haguenau, F. *Viruslike Particles As Observed with the Electron Microscope. Ultrastructure of Animal Viruses and Bacteriophages: An Atlas*; Academic Press, Inc: New York City, 1973; pp 391−397.

(184) Seman, G.; Dmochowski, L. Electron microscope observation of viruslike particles in comedocarcinoma of the human breast. *Cancer* **1973**, *32*, 822−829.