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Microbial biofilms are shaped by the constant dialogue between biological and physical forces in the extracellular matrix

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SIGNIFICANCE STATEMENT

The biofilm matrix, with its diversity of extracellular polymeric substances (EPS), remains a poorly understood entity. It consists of a heterogeneous, multifunctional microenvironment that imparts a range of emergent properties to the biofilm, including social cooperation and resource sharing, adaptation to environmental changes, and resistance to harmful chemicals and antibiotics. Generally, studies of the biofilm matrix focus on the regulation of EPS gene expression and associated biofilm phenotypes (Flemming et al., 2022; Flemming & Wingender, 2010). For example, the differential regulation of exopolymers, which impart different mechanical properties, is an often-studied genetic marker for characterizing transitions between different stages of biofilm development (Chew, Kundukad, et al., 2014; Irie et al., 2012). New insights, however, suggest that the emergent properties of the matrix, which arise because of physical interactions between EPS molecules as well as those between EPS and bacterial cells, also play important roles in biofilm formation and organization (Liu et al., 2022; Rubinstein et al., 2012). For example, the secretion and accumulation of EPS components generate new physical forces, such as *osmotic stresses*, *bridging interactions*, and *depletion effects* within the crowded matrix (Liu et al., 2022). These forces alter the physical environment of the biofilm, affecting conformational and aggregational landscapes and dynamics, and thus functions, of matrix biopolymers. Together with the biological program, they stabilize extended structural, compositional, and morphological gradients in space and time, drive phase transitions, immobilize cells, and induce phase separation, creating spatial functional niches within the matrix (Worlitzer et al., 2022). Considering these insights, we highlight here the emerging perspective that understanding the competition and the collaboration between physical and biological factors is crucial for a more complete appreciation of biofilm formation, dynamics, organization, and function.

INTRODUCTION

Although single-celled, most bacteria acquire a multicellular lifestyle by their organization into collective, complex populations or communities, consisting of single or multiple species of microorganisms (Berlanga & Guerrero, 2016). This multicellular mode of life enables bacteria to develop social synergies such as communication, labor division, spatial arrangements, and metabolic cooperation (Elias & Banin, 2012). It also affords the assemblage and shared abilities to sense, respond, as well as adapt to cues, stresses, and perturbations from their microenvironment (Flemming et al., 2016). Taken together, these attributes enable bacterial communities to develop an organization that reflects an optimal survival strategy (Fux et al., 2005) and promotes their collective fitness (Elias & Banin, 2012)

In this regard, the biofilm lifestyle, in which heterogeneous aggregates of microorganisms become embedded within a three-dimensional matrix of self-secreted, extracellular polymeric substances (EPS), represents one of the most versatile forms of multicellularity (Costerton et al., 1995). The adoption of the biofilm lifestyle is mediated by the regulation of biofilm-specific genes in response to many different signals (Flemming et al., 2016). Common signals that trigger the lifestyle switch in bacteria include changes in temperature, pH, osmolality, nutrient availability, selected chemicals (e.g., antibiotics), and the presence of a surface (Morales & Kolter, 2014). These signals activate many core gene regulatory derived processes including (i) secretion of cell-density dependent quorum sensing molecules (e.g., cyclic-di-GMP) and (ii) modulation of genes responsible for cellular motility and the production of EPS (Fu et al., 2021; Mukherjee & Bassler, 2019). Together, these changes characterize the biological program for initiating biofilm formation.

However, the biological program alone does not fully determine the physical organization of the biofilm. This is because the very implementation of the biological program also leads to many emergent and significant physical mechanisms which also shape the biofilm (Flemming et al., 2016; Karimi et al., 2015). For example, EPS secretion crowds the extracellular surroundings with multicomponent mixtures of biopolymers containing different types of polysaccharides, proteins, lipids, and extracellular DNA (Ghosh et al., 2015). In this crowded macromolecular environment, bacterial cells become subject to new physical forces and interactions. Some prominent examples involve *excluded volume* (see Box 1) and steric interactions, entropic depletion forces, matrix-mediated attractive bridging interactions, and colloidal osmotic stresses (Ghosh et al., 2015; Worlitzer et al., 2022). Together, these emergent interactions (i) facilitate the creation of physical–chemical gradients, such as those of nutrients, oxygen, and pH; (ii) generate structural,

BOX 1 Terminology

1. Osmotic stresses originate from imbalances between extracellular and intracellular solute concentrations. They generate osmotic pressure gradients that induce water fluxes in the direction of higher osmotic pressure (Brocker et al., 2012).
2. Bridging interaction occurs when polymers adsorb simultaneously on more than one particle, bringing them together (Ghosh et al., 2015; Hogg, 2013).
3. Depletion effects are entropic forces that create effective attraction between larger colloidal particles due to the entropy maximization of the smaller particles in mixtures (Ghosh et al., 2015).
4. Excluded volume represents the volume that is inaccessible to other molecules in the system as a result of the presence of the first molecule (Gasser & Graham, 1995).
5. Motility-induced phase separation is a non-equilibrium phenomenon unique to energy-consuming active particles, in which larger clusters become sluggish in their movements and create co-existing low-density mobile and high-density sluggish phases (Be'er & Ariel, 2019; Cates & Tailleur, 2015).
6. Jamming is the physical process by which the viscosity of many mesoscopic materials, such as granular materials, glasses, foams, polymers, emulsions, and other complex fluids, increases with increasing particle density. A jamming transition leads to a non-equilibrium transition from a fluid-like to a solid-like state through a sudden arrest in their dynamics (Biroli, 2007).
7. Liquid–liquid phase separation (LLPS) is a process by which an aqueous (or an organic) phase separates into two- or more co-existing aqueous (or organic) phases. It may occur by associative interactions, in which solutes attract and separate the aqueous phase into solute-rich and solute-poor phases. It may also occur in a segregated manner, where dissimilar macromolecules divide the aqueous environment into co-existing phases (Guo et al., 2021).
8. Swarming is a collective mode of motion in which cells migrate rapidly over surfaces, forming dynamic patterns of whirls and jets. This review presents a physical point of view of swarming bacteria, with emphasis on the statistical properties of the swarm dynamics (Be'er & Ariel, 2019).
9. Kinetic trapping occurs when jamming or crowding kinetically traps the constituents and thereby precludes equilibration (Yan et al., 2016).

morphological, and topographical patterns, often extending over multiple length and timescales; and (iii) induce phase transitions, which produce the viscoelastic matrix, arrest cellular motility, and immobilize the biofilm. Thus, these physical factors, which arise due to the implementation of the biological program contribute non-trivially to shaping the biofilm organization, and endowing it with novel emergent properties and collective behaviours (Flemming et al., 2016).

The synergistic partnership between physical mechanisms and the biological program in determining the organization of biofilms is perhaps best exemplified by a recent observation of iterative feedback between biological and physical processes (Rubinstein et al., 2012). Here, the initiation of the biological program, highlighted by the accumulation of exopolysaccharides in the *Bacillus subtilis* matrix, gave rise to new physical forces. In particular, rising concentrations of exopolysaccharides in the biofilm creates an osmotic pressure gradient between the cell and the matrix. This in turn alters the biological program by inhibiting the expression of EPS genes. Thus, physical forces (i.e., osmotic stresses) arise as a consequence of a gene-regulated activity (i.e., the production of EPS components), and in turn suppress the very same gene regulatory program in a negative feedback loop. This iterative, biological-physical-biological, collaborative partnership illustrates one of the many intricate relationships between the biological program and physical interactions/mechanisms that emerge during the formation of biofilms.

Here, we highlight the perspective that a synergistic- and collaborative partnership, indeed a constant dialogue, between physical forces and the biological program determines the organization, dynamics, and ultimately the fate of the biofilm. We focus on the roles of the biofilm matrix, the biologically prompted secretion of which dynamically introduces new physical–chemical forces and interactions that enhance regulatory networks, enabling biofilm formation, growth, and organization. We consider three distinct classes of matrix-mediated physical processes, whose progression under non-equilibrium conditions play important roles in the formation, growth, and organization of the biofilm. These include: (1) *motility-induced phase separation* (see Box 1) and depletion interactions in facilitating the transition of bacterial swarms into biofilms; (2) *jamming* (see Box 1) and gelation in driving the formation of the glassy or viscoelastic EPS matrix; and (3) physical *liquid–liquid and liquid–solid phase separation* (see Box 1), in determining the spatial organization of the EPS components and generating compositional and thus functional niches within the otherwise unstructured EPS.

THE SWARMING TO BIOFILM TRANSITION

Many different microbial lifestyles (e.g., planktonic, dense colonies, active swarms) in diverse environments (e.g., bulk fluid, surface-attached bacteria) can switch to the biofilm mode of life (Worlitzer et al., 2022). These lifestyle swaps occur in response to environmental cues and involve the implementation of specific biological programs with changes in gene regulatory processes that alter cellular motility and EPS secretion. As discussed above, these outcomes inevitably introduce new physical forces and mechanisms (Flemming et al., 2016). Nonetheless, how the biological programs and physical forces interact in determining the biofilm fate are only beginning to be understood (Worlitzer et al., 2022).

Among the many different microbial lifestyle switches, that of the conversion of an active swarm into a biofilm is particularly interesting, as it involves a drastic transition between opposing and mutually exclusive phenotypes. During this lifestyle switch, an active swarm, which reflects a collective motility state characterized by dynamic patterns, is converted into a sessile, biofilm mode of life (Srinivasan et al., 2019; Verstraeten et al., 2008; Worlitzer et al., 2022). This transition also highlights two opposing scenarios that underscore the complex hierarchy and the sequence of interactions between the biological program and the emergent physical forces (Figure 1).

In the *crowding-first scenario*, it has been suggested that the transition begins with a physical change. According to this view, a non-equilibrium physical process, unique to self-propelled active particles and termed motility-induced phase separation (MIPS), (Cates & Tailleur, 2015) seeds early events. Here, in the dynamic patterns of the active swarm, fluctuations in cell densities can occur spontaneously and randomly. These fluctuations transiently produce small high-density clusters in parts of the swarm in which cells movement slows due to enhanced molecular crowding. These cells accumulate, further increasing the crowding, which further decreases subsequent motion (Be'er & Ariel, 2019). Thus, a positive feedback loop–slowing, accumulating, slowing–thereby drives phase separation and generates two co-existing phases: a low-density phase of *swarming* cells and high-density clusters of jamming (see Box 1) and immobilizing cells.

These high-density clusters of jammed cells are then proposed to initiate the biological program that produces EPS and restricts mobility, thus driving the transition from an active swarm to an immobile biofilm (Grobas et al., 2021; Srinivasan et al., 2019). Such transitions in the bacterial lifestyle and biofilm matrix phases have recently been observed during *B. subtilis* biofilm formation, and are suggested to be driven by physical interactions between swarming cells (Grobas et al., 2021).

The alternative EPS-first scenario regards the biological programs as the primary event driving the lifestyle switch. In this scenario, secreted EPS components, a key part of the biological program, surround the bacterial cells. In this crowded extracellular space, the EPS components act as small depletants

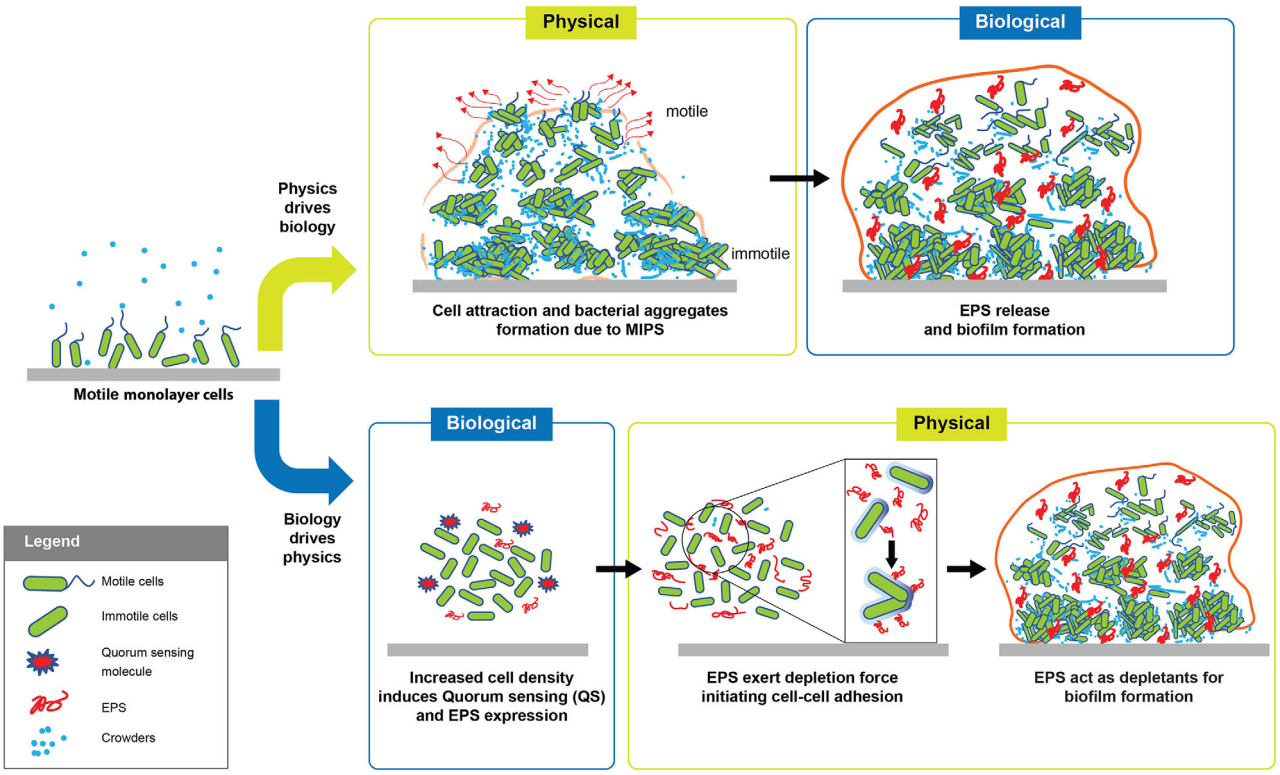

FIGURE 1 Biofilm formation from motile monolayer cells via two mechanisms. Upper boxes, physics drives biology. A non-equilibrium biophysical process, motility-induced phase-separation (MIPS) driven by fluctuations in cell densities produces larger aggregates of jammed cells at the bottom layer that are immotile in contrast to highly motile smaller aggregates at the top (red arrows). The high-density clusters then initiate the biological program of swarm to biofilm formation by EPS release. Lower boxes, biology drives physics. Quorum sensing (QS) and the expression of EPS are first activated by increased cell density. The expressed EPS acts as depletants and drives cell–cell adhesion, biofilm formation and expansion. The expanded insert depicts the decrease in excluded volume (the shaded area around cells) with cluster formation.

and introduce new physical forces (see Box 2). Specifically, as non-adhering molecules in the matrix, they engage in depletion interaction with the bacterial cells. Here, the depletants push bacterial cells to cluster together and undergo phase separation to maximize their own translational entropy (see Box 1). A recent computer simulation confirms this scenario in the context of the swarm-to-biofilm transition. It suggests that the presence of nonadsorbing EPS can lead to the spontaneous aggregation of active bacterial cells through the depletion force, thereby generating nonequilibrium emergent patterns of phase-separation in the bacterial colony (Ghosh et al., 2015).

In summary, the two scenarios above illustrate two processes by which physical interactions and the biological program can interact to guide biofilm creation and organization.

THE JAMMING MATRIX TRANSITION DURING THE ADOPTION OF THE BIOFILM LIFESTYLE

A key step in the adoption of the biofilm mode of life is a bacterial microenvironment transformation into a gel-

BOX 2 EPS-first phenomenon

The EPS-first phenomenon was recognized by Asakura and Oosawa (Asakura & Oosawa, 1954; Asakura & Oosawa, 1958).

They reasoned that, because the center-of-mass of the depletants cannot approach the larger cells beyond its own radius, a corona of excluded-volume surrounds each of the larger bacterial particles. When large particles approach one another, at distances smaller than their individual excluded-volumes, their coronas begin to overlap, effectively increasing the total space accessible to the center-of-mass of the smaller depletant particle. As a consequence, the depletant entropy increases and the overall free energy of the system decreases. The net result is an osmotic pressure imbalance arising from the difference in the concentration of small depletants, which acts to push the larger particles together (Yodh et al., 2001), giving rise to the depletion force.

like state, immobilizing bacteria and producing a consolidated community. This transformation is enabled by a component of the biological program (Wolska et al., 2016), which triggers EPS secretion and macromolecular crowding of the bacterial environments (Figure 2). As discussed above, these events introduce new physical forces that drive significant material changes to the system. In addition to the depletion interactions, which aggregate and phase-separate bacterial cells (see above), the crowding of EPS components also densifies the matrix due to their high molecular weights and elevated local concentrations, thereby creating conditions for macromolecular jamming. Here, beyond a threshold concentration of macromolecules, the dynamics are abruptly arrested, kinetically trapping (see Box 1) the system into a fixed state. This non-equilibrium phase transition then converts the bacterial environment into a dense, gel-like matrix, thus completing the biofilm formation.

At the molecular level, matrix gelation can occur through a variety of different pathways. A number of disparate mechanisms for this process have been identified including physical entanglements, hydrogen or ionic bond interactions, and intermediate supra-structure formation (Dumitriu, 2004; Ganesan et al., 2013; Ganesan et al., 2016; Kundukad et al., 2017). Below, we highlight two prominent pathways that facilitate EPS gelation, one dominated by physical interactions, and the other involving molecule-specific information transfer.

The physical interaction pathway relies on concentration-dependent entanglements and chemical cross-linking (Kim et al., 2013; Zhu et al., 2008). As the concentration of the matrix biopolymers crosses a threshold entanglement concentration, matrix polymers begin to intermingle with one another, forming physical entanglements (Dumitriu, 2004; Ganesan et al., 2013; Ganesan et al., 2016). In addition, specific functional groups of matrix polymers may also form chemical cross-links (with other matrix biopolymers, bacteria, or ions) through localized hydrogen bonding (e.g., —OH mediated), ionic (e.g., Ca^{2+} mediated), or hydrophobic interactions (e.g., CH_2 mediated) (Edens, 2005; Limoli et al., 2015). For example, the cationic exopolysaccharides, Pel and Psl, crosslink with eDNA in *P. aeruginosa* biofilms to form entanglements, (Jennings et al., 2015; Wang et al., 2015) whereas polysaccharide intercellular adhesin (PIA) in *Staphylococcus epidermis* biofilms self-assembles by associative interactions rather than entanglements, as PIA concentration in *S. epidermis* biofilms is far less than the entanglement concentration (Ganesan et al., 2016).

From a mechanical point of view, it is important to note that the entanglements and crosslinks impart the EPS matrix with different properties. Physical entanglements allow the matrix to transmit, distribute, and share any mechanical forces (e.g., tension) it experiences. Whereas crosslinks serve to prevent disentangling under mechanical stresses. Thus, differential expression of the polysaccharides, Pel and Psl renders *P. aeruginosa* biofilms either softer or stiffer respectively, enabling for different functional outcomes (Chew et al., 2014; Kundukad et al., 2016).

For the molecule-specific information transfer matrix gelation pathway, some molecules (e.g., eDNA and certain polysaccharides) of the biofilm matrix can adopt higher order structures that are important in their abilities to form gels (Stokke, 2019; Tako, 2015; Wilking et al., 2011). Here, the essential information needed to execute matrix gelation is coded in the design of the molecular structure itself. In other words, gelation through this pathway is pre-programmed, and regulated internally by molecule-specific information that is highly prescriptive. In this regard, the pathway resembles the biological program. This pathway is perhaps most prominently expressed by the higher-order organization of eDNA in the EPS matrix. Biofilm matrix eDNA forms highly specific supra-structures. Two major examples include G-quadruplex (Seviour et al., 2021) and Holliday junctions (Devaraj et al., 2019), both of which facilitate matrix gelation (Seviour et al., 2021).

LIQUID–LIQUID PHASE SEPARATION AS A MECHANISM FOR REGULATING EXTRACELLULAR PROCESSES IN BIOFILMS

The biofilm matrix is a crowded environment with high concentrations of large macromolecules, including exopolysaccharides, eDNA, and proteins. Under these conditions, the matrix constituents experience (i) excluded-volume interactions (arising from the inaccessible space pre-occupied by neighbouring molecules), which reduce the translational mobilities (or diffusion); (ii) steric repulsions, and (iii) short-range depletion attractions, all of which have important consequences as discussed above.

Here, we consider another significant influence of the crowded molecular environment of the biofilm matrix, namely its effect on the phase behaviour of the EPS matrix itself. Molecules of the matrix inevitably engage in a variety of intermolecular interactions, both associative and segregative, which act to separate the matrix into complex emulsion consisting of co-existing phases through the thermodynamic tendencies of liquid–liquid or liquid–solid phase separation (LLPS or LSPS).

Indeed, these behaviours are reverberating across much of the discipline of eukaryotic cell biology. A recent pioneering study (Li et al., 2012) used a cell-free, in vitro assay to demonstrate interactions between many different polymers (including proteins and RNA) through multivalent associations that give rise to liquid–liquid phase separation, as characterized by

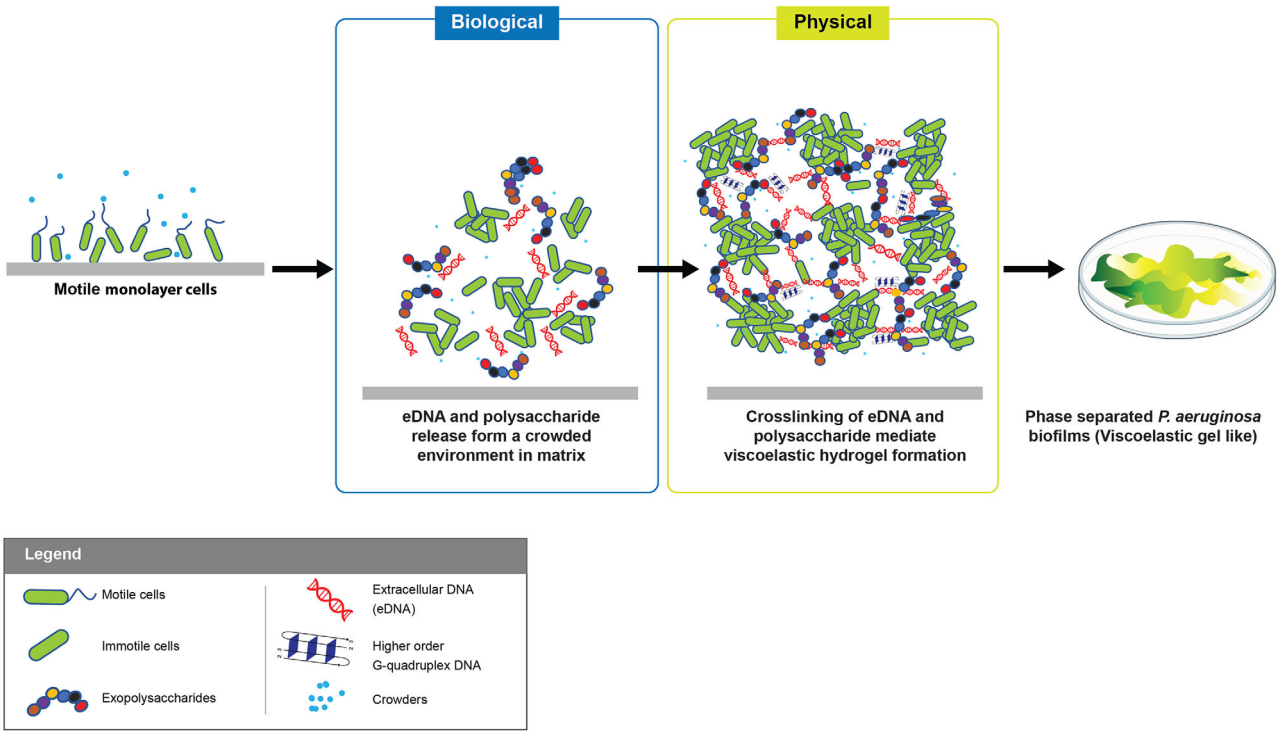

FIGURE 2 The gelation process in a biofilm system through both biological and physical processes. The first step shows the motile monolayer cells loosely attached to a surface. The second step depicts the biological program by activation of EPS expression, illustrated here by extracellular DNA (eDNA) and polysaccharide. Next, above a certain entanglement concentration, polymers crosslink, as shown by the crosslinking of eDNA and polysaccharide, aided by a crowded environment and intermolecular interactions. This results in an abrupt arrest of molecular and cellular mobilities, which convert the environment into a dense, gel-like matrix, as shown in the illustration of a phase separated viscoelastic *Pseudomonas aeruginosa* biofilm.

micrometre-scale liquid-like droplets in aqueous solution. Another study (Patel et al., 2015) demonstrated that in vitro, the prion-like FUS protein, mutations of which are associated with amyotrophic lateral sclerosis (ALS) disease, also produces micrometre-scale liquid-like droplets. Since these early observations, a large number of disparate cases confirm crowding-induced cytosolic phase separation. Some examples include protein-RNA droplets, such as (i) Cajal bodies (Handwerger et al., 2005) in the nucleus, which play a role in RNA metabolism; (ii) cytoplasmic P-granules in *Caenorhabditis elegans*, (Brangwynne et al., 2009) which are implicated in germline formation; and (iii) cytoplasmic nucleoli (Brangwynne et al., 2011), which serve as a site for ribosome synthesis. In these and other cases, while the number of molecules present in droplets is generally large, only a handful are thought to be needed to induce LLPS (Brangwynne et al., 2015; Hyman et al., 2014; Li et al., 2012). A common property shared by these LLPS-inducing molecules appears to be the presence of low-complexity, repeat sequences, producing intrinsically disordered regions (IDRs) (Dyson & Wright, 2005; Hofmann et al., 2012). Indeed, a recent series of studies suggest that the presence of IDRs may be a requirement, and even an evolutionarily conserved factor, for inducing protein-mediated LLPS in the cellular context (Brodsky et al., 2020; Hsu et al., 2021).

In this regard, it is notable that many biofilm matrices contain proteins that have low-complexity sequences or tandem repeats that form IDRs. Some major examples include biofilm-associated protein (Bap) in *Staphylococcus aureus*, (Cucarella et al., 2001; Taglialegna et al., 2016) enterococcal surface protein (Esp) in *Enterococcus faecalis*, (Lasa & Penadés, 2006; Taglialegna et al., 2020) and curli in *Escherichia coli* (Hammer et al., 2012; Shu et al., 2012; Van Gerven et al., 2015). These proteins are thought to have structural roles in facilitating colonization of inert surfaces, binding to host proteins, and inducing EPS gelation during the formative stages of the biofilm. The proteins achieve this by exploiting the conformational flexibility (i.e., plasticity) needed to transition into conformational states (i.e., ß-sheet structure (Fong & Yildiz, 2015)) that drive their self-assembly into amyloid-like fibres.

Based on the considerations above, we suggest that the IDR-containing functional bacterial amyloids also promote LLPS in the molecularly crowded, extracellular context of the EPS matrix (André & Spruijt, 2020; Babinchak & Surewicz, 2020a).

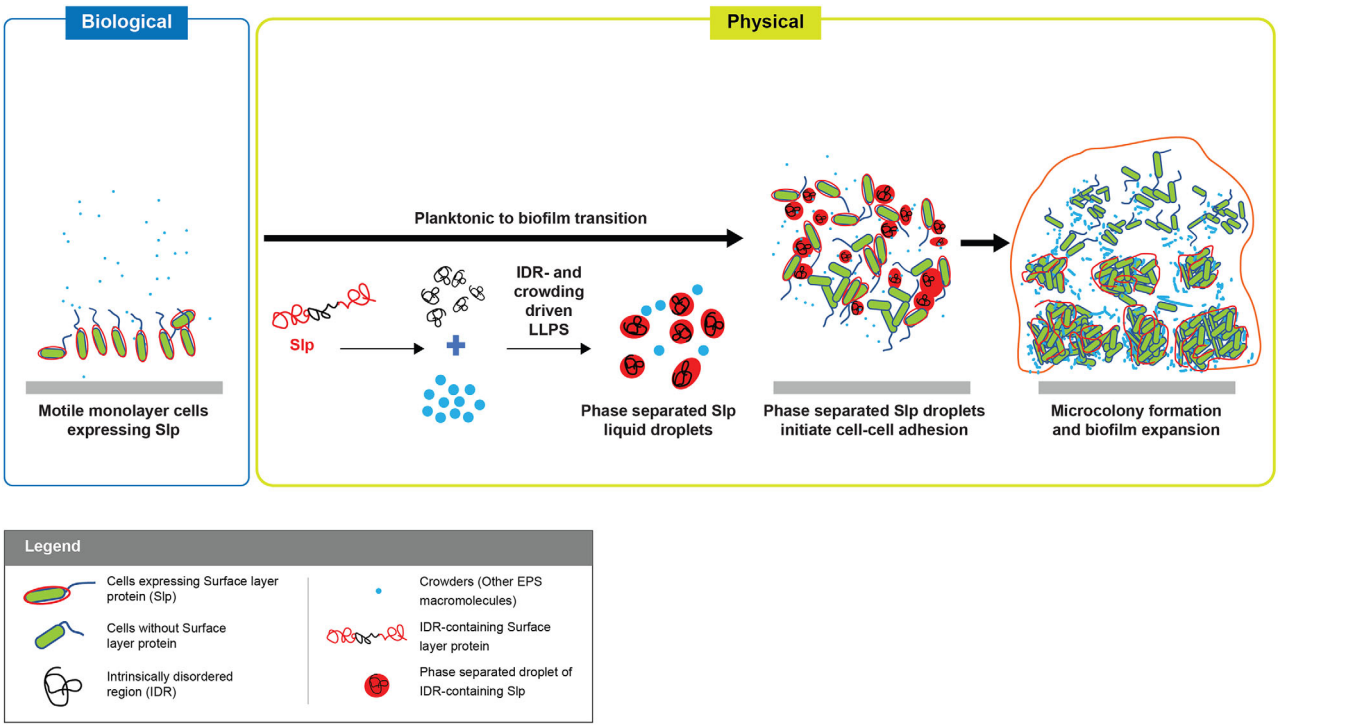

FIGURE 3 The overall scheme of planktonic to biofilm transition of bacterial cells expressing surface layer protein. Matrix proteins containing low complexity sequences and IDR (e.g., anammox biofilms) are susceptible to undergoing liquid–liquid phase separation in the crowded milieu, initiating cell–cell adhesion and subsequent biofilm formation.

Such an outcome would enable IDR-containing proteins to seed biofilm matrix formation. While this concept is only beginning to be explored in the biofilm context, there is a precedent for this with amyloids in other settings. Amyloid fibre formation, which leads to amyloid plaques in the brain and subsequently neurodegenerative disease, is also preceded by protein condensation into liquid droplets (Babinchak & Surewicz, 2020b; Kanaan et al., 2020). Neurodegenerative disease-causing proteins contain IDR and undergo LLPS to form liquid droplets under crowded condition (Kanaan et al., 2020). Protein liquid droplets subsequently convert to amyloid fibres (Martinelli et al., 2019; Ren et al., 2022). Furthermore, continuous aggregation of amyloid fibres in vitro produces biogels (Wang et al., 2019). It is thus plausible that analogous IDR-containing exoproteins in biofilms may also transition through intermediate phases, and contribute directly to establish rheologically distinct localized regions that seed biofilm formation or support biofilm maturation.

An S-layer protein, otherwise known to form paracrystalline structures around cell envelopes, was recently found to be a major EPS biopolymer in an anaerobic ammonium oxidation (anammox) biofilm (Wong et al., 2020). This protein also contains IDRs, and undergoes LLPS to produce liquid droplets under crowded conditions. These liquid droplets could wet and fuse cells, supporting the aforementioned mechanism for LLPS in promoting initial cell–cell adhesion and microcolony formation (Seviour et al., 2020) (Figure 3). In addition, the S-layer protein also displays a predominately ß-sheet secondary structure. We hypothesize, that due to IDRs, and post translational mechanisms (e.g., glycosylation) single extracellular proteins could potentially transition through multiple states depending on life-cycle, to effectively achieve multiple outcomes. For the S-layer protein, this could include secretion through the cell membrane, formation of paracrystalline structures on the cell envelope, and transport through the extracellular matrix, and potentially yield the gel-forming constituent of anammox biofilm matrix. While it is unclear how the S-layer protein transitions through these structures, the observations illustrate the need to focus on protein dynamics and phase transitions, rather than a single stage of the transition continuum, in order to resolve the role of extracellular proteins in biofilm biophysics and formation.

CONCLUDING REMARKS AND FUTURE DIRECTION

In this perspective, we summarize two relatively well studied biofilm-inducing physical forces, i.e. motility-induced phase separation in shaping bacterial swarms into biofilms, and the collective jamming-gelation-glass transition in shaping the EPS matrix. In the former, we highlight the collaborative nature between the inherent

biological program and the emerging physical forces, in which one mechanism precedes the other, and vice versa. The latter, which is initiated by biological programs, operates via different physical mechanisms, which ultimately lead to a molecularly jammed gel-like matrix. Here, we highlight the notion that the information necessary for the transition is encoded, not only in the primary sequence of the biopolymers but also in the higher-order structures, for instance G-quadruplex eDNA and kinetically trapped folding intermediates for proteins. Further, drawing parallels between physical–chemical properties of recently studied bacterial extracellular proteins (Bap, Esp, curli and Slp) and more well-known examples (amyloids and others of eukaryotic origin), we propose a plausible third physical force, namely liquid–liquid or liquid–solid phase separation as a driver for EPS matrix formation. Here, the crowded biofilm matrix serves as a conducive environment for large macromolecules (exopolysaccharides, eDNA and proteins) to phase separate into heterogeneous microenvironments. Additional efforts to delineate the various physical forces and biological cues will allow us to further decrypt the transition into complex biofilm architectures, and to predict the emergent behaviours of dominant EPS matrix biopolymers. Collectively, they should enable a better understanding of the biofilm matrix in terms of phase transition arising from cell-associated, cell-EPS-associated, and EPS-EPS-associated physical interactions, which in a constant dialogue with the biology program shapes the microbial world in a complex, subtle, but essential manner.

AUTHOR CONTRIBUTIONS

Lan Li Wong: Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Sudarsan Mugunthan:** Formal analysis (equal); writing – original draft (equal); writing – review and editing (equal). **Binu Kundukad:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **James Chin Shing Ho:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Scott Rice:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Jamie Hinks:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Thomas Seviour:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Atul Parikh:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Staffan Kjelleberg:** Conceptualization (equal); funding acquisition (equal); project administration (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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