

Review Article

The role of filamentous matrix molecules in shaping the architecture and emergent properties of bacterial biofilms

Jan Böhning*, Abul K. Tarafder* and  Tanmay A.M. Bharat†

Structural Studies Division, MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, U.K.

Correspondence: Tanmay A.M. Bharat (tbharat@mrc-lmb.cam.ac.uk)



Numerous bacteria naturally occur within spatially organised, multicellular communities called biofilms. Moreover, most bacterial infections proceed with biofilm formation, posing major challenges to human health. Within biofilms, bacterial cells are embedded in a primarily self-produced extracellular matrix, which is a defining feature of all biofilms. The biofilm matrix is a complex, viscous mixture primarily composed of polymeric substances such as polysaccharides, filamentous protein fibres, and extracellular DNA. The structured arrangement of the matrix bestows bacteria with beneficial emergent properties that are not displayed by planktonic cells, conferring protection against physical and chemical stresses, including antibiotic treatment. However, a lack of multi-scale information at the molecular level has prevented a better understanding of this matrix and its properties. Here, we review recent progress on the molecular characterisation of filamentous biofilm matrix components and their three-dimensional spatial organisation within biofilms.

Introduction

Biofilms are multicellular ordered communities of bacteria that are the major mode of bacterial life found in nature [1–3]. Biofilms are organised, three-dimensional assemblies [4,5] that endow numerous survival advantages to constituent cells, including tolerance to antimicrobials, tolerance to extracellular physical stresses and protection from harmful external entities [6–9]. Biofilm formation begins when a planktonic cell attaches to an amenable surface [10], followed by growth and expansion into a colony, leading to biofilm maturation. During the maturation stage, bacterial cells secrete extracellular polymeric substances (EPS), including polysaccharides, filamentous proteins, extracellular DNA (eDNA) and other polymers forming an extracellular matrix (ECM) that surrounds bacterial cells and scaffolds the biofilm. The presence of an ECM is the defining characteristic and hallmark of all bacterial biofilms, and is responsible for many of the key properties exhibited by biofilms.

The biofilm matrix in many bacteria contains a wide-variety of polymeric components with spatial segregation and ordering [7]. Indeed, the three-dimensional architecture of the biofilm often includes pores, channels, and localised regions with differing physicochemical properties [11]. While different bacteria have differing molecular organisation within the ECM of biofilms, shared organisational principles have emerged from recent studies, because the components of the biofilm matrix must fulfil many defined functions. For instance, in the case of surface-attached biofilms, molecules in the ECM must provide adherence to the substrate. Furthermore, bacterial cells must adhere together to prevent unintended (and metabolically costly) disassembly of the biofilm due to random shear or mechanical stresses. Finally, EPS molecules within the ECM must protect bacterial cells from unwanted intrusion from predators (such as grazing protozoans), phages and chemical agents including antimicrobials. The protective function of the ECM is particularly relevant for biofilms formed during human infection [12], where biofilm formation can increase antibiotic tolerance of constituent bacteria by up to a 1000-fold [12].

*These authors contributed equally to this work.

†Tanmay Bharat was awarded the 2022 Colworth Medal.

Received: 18 September 2023

Revised: 29 January 2024

Accepted: 31 January 2024

Version of Record published:
15 February 2024

Several recent molecular biology, cell biology and microscopy studies have improved our understanding of how the ECM is arranged in biofilms. Recent structural studies have linked atomic detail of constituent molecules with imaging data, permitting a multi-scale understanding of the emergent properties of biofilms. While numerous polymeric molecules have been implicated in sculpting the biofilm architecture (Table 1), in this review, we focus on components of the ECM of bacterial biofilms where multi-scale data is available, allowing us to infer the molecular basis of architecture and the emergent properties displayed by biofilms. Using these illustrative cases, we discuss how EPS molecules within the ECM endow special traits to the biofilm. Understanding the collective effect of the ECM on constituent cells as well as entire biofilms will illuminate the core principles that shape biofilms and their emergent properties.

Proteinaceous components of the biofilm matrix

Functional amyloids in Gram-negative bacteria

A major component of many biofilms across the realms of life are proteinaceous fibres. Commonly, such fibres are formed from relatively small protein subunits secreted into the ECM, which then interact with each other to form larger-scale assemblies. Among the most well-known fibres in biofilms are so-called bacterial functional amyloids [13,17]. Functional bacterial amyloids were first discovered in *Escherichia coli* and were termed *curli*, after their winding appearance in electron micrographs [90]. Curli fibres make up a significant portion of the biofilm dry weight, being the major polymeric component of the biofilm matrix of *E. coli* and *Salmonella* spp., together with the polysaccharide cellulose [91]. Curli fibres appear to be widespread among Gram-negative bacteria [92,93].

As the name ‘functional amyloid’ suggests, curli fibres have a significant resemblance to human amyloids, as they also exhibit a so-called cross- β structure, where the filament is formed through β -sheet interactions between subunits stacked along the fibre axis [94]. Initially, the amyloid properties of curli fibres were puzzling as amyloid assembly was often considered non-functional and accidental in nature [13]. However, later studies found that many bacteria and eukaryotes have systems dedicated to the assembly of amyloids [95]. As a result of the β -sheet-rich architecture of amyloids, which produces exceptionally strong subunit interactions, amyloids have remarkable biophysical properties, including exceptional stability and resistance to protease treatment and denaturing agents [95,96]. In human amyloids, amyloidogenic subunits typically form a flat arrangement contributing one layer of β -strands to the amyloid fibre; however, how subunits arrange into an amyloid fold in bacteria had long been an open question. Recent structural work on curli fibres, combining electron cryomicroscopy (cryo-EM) and AlphaFold modelling, shows that the main amyloid-forming subunit, CsgA, forms an amyloid structure by assembling into a multi-turn β -helix which, upon polymerisation of the fibre, is continuously elongated by the addition of more subunits [92] (Figure 1). The mature CsgA monomer in *E. coli* contains five repeat regions that are highly similar to each other which results in a rise of ~ 23.75 Å per subunit within the amyloid fibre, in contrast with a rise of ~ 4.75 Å typically seen in human amyloids [94]. Other subunits contain variable numbers of repeats; for example, the CsgA subunit in *Pontibacter korlensis* has 15 full CsgA repeats (Figure 1). The hydrophobic surface of the fibres allows them to interact laterally to form enormous bundles, leading to a vast interacting network of fibres within the biofilm matrix.

Prior to assembly, the individual amyloidogenic subunit CsgA is disordered, requiring dedicated molecular machinery for controlled secretion and assembly in the biofilm matrix. Hence, *csgA* is encoded with several assembly factors in two operons (*csgBAC* and *csgDEFG*) [98], including factors that nucleate fibre assembly outside of the cell (CsgB) [99], prevent nucleation inside the cell (CsgC) [100], stabilise the unassembled subunits (CsgE) [101], form a translocation pore in the outer membrane (CsgG) [102], anchor fibres to the cell membrane (CsgF) [103,104], as well as positively regulate transcription of the fibre-forming subunits (CsgD) [105]. Curli fibres are extremely stable, resisting disassembly even with strong denaturing agents such as sodium dodecyl sulfate [13]. However, they are smaller and more flexible than many human amyloids characterised in neurodegenerative disease [92,106].

Since the initial discovery of curli, many functional bacterial amyloids have been characterised [107]. For example, biofilm fibres with similar amyloid properties were found in the Gram-negative Pseudomonads, where they were accordingly named *Functional amyloid in Pseudomonas* (Fap) [17,18]. Similar to curli, *fap* operons encode a main fibre-forming amyloidogenic subunit (FapC) [17], a potential nucleator or sub-stoichiometric fibre component (FapB) [17,18], a chaperone (FapA) [108], a protease (FapD) [109], a secretion pore (FapF) [109] and an accessory protein and minor fibre component that is also exported through the pore (FapE) [18]. Compared with CsgA, three larger repeat regions make up the amyloidogenic FapC subunit. Furthermore, Fap fibres are usually encoded in a single operon (*fapABCDEF*), a notable difference from the curli operons [17,18].

Table 1. Examples of polymeric molecules implicated in shaping biofilm architecture

Part 1 of 3

Molecule type	Name	Species	Structure and function	References
Proteins	Curli	<i>E. coli</i> , <i>Salmonella</i> spp., other Gram-negative bacteria	Functional amyloid with cross- β structure. Major proteinaceous component of biofilm ECM. Roles in structural maintenance of biofilm ECM, adhesion to surfaces, cell-cell adhesion, host cell adhesion and invasion.	[13–16]
	Fap	<i>P. aeruginosa</i>	Functional amyloid with predicted cross- β structure. Maintains structural integrity of biofilm ECM.	[17,18]
	PSM	<i>S. aureus</i>	Small, cytotoxic peptides that self-assemble into higher order amyloid-like fibres. PSM α 3 and PSM β 2 fibres formed of α -helical peptides stacked laterally to form a cross- α fold. PSM α 1 and PSM α 4 assemble into canonical cross- β amyloid fibres. Role in structural scaffolding of biofilm formation.	[19–21,22]
	TasA	<i>B. subtilis</i>	Globular β -sandwich domain protein polymerising into fibres by donor-strand exchange of β -strand with neighbouring subunit. Major proteinaceous component of biofilm ECM required for structural integrity. Fibres show propensity to bundle.	[23,24]
	CupA-D	<i>P. aeruginosa</i>	Classical CUP pilus — putative rod-like structure. Fibres formed through donor-strand exchange of donor β -strand between neighbouring subunits. Role in initial adhesion to surfaces and microcolony formation.	[25–27]
	Csu	<i>A. baumannii</i>	Archaic CUP pilus — forms highly elastic fibres with a linear zigzag arrangement of subunits. Fibres formed through donor-strand exchange of donor β -strand between neighbouring subunits. Capped with a hydrophobic tip subunit mediating adhesion to abiotic surfaces. A major virulence factor with roles in maintaining biofilm structure and attachment to abiotic surfaces.	[28–31]
	CupE	<i>P. aeruginosa</i>	Archaic CUP pilus — forms fibres with a linear zigzag arrangement of subunits. Homologous to Csu. Capped with a hydrophobic tip subunit. Stabilises 3D architecture of mature biofilms.	[32,33]
	Type IV pili	Many species	Polymeric hydrophobic proteinaceous fibres composed of repeating pilin subunits often capped with an adhesin subunit. Implicated in early stages of biofilm formation. Twitching motility involved in microcolony formation.	[3,34,35]
	FimA	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>S. marcescens</i>	Surface-attached type I Chaperone-Usher pilus. Forms 1–5 μ m long fibres. Fibres formed through donor-strand exchange of β -strands between neighbouring subunits. Pilus capped by an adhesive subunit that binds mannosylated glycoproteins. Instrumental in biofilm formation and critical for initial attachment to abiotic surfaces using a 'catch-bond' mechanism.	[36–39]
	ECP (<i>E. coli</i> common pili)	<i>E. coli</i>	Composed of multiple copies of 18 kDa EcpA subunit. Fibres formed through donor-strand exchange of an N-terminal β -strand between neighbouring subunits. Dual role in biofilm formation and host cell recognition. Fibres show tendency to aggregate into well-ordered bundles postulated to be important for biofilm formation.	[36,40,41]
	Tad (tight adherence pili)	<i>P. aeruginosa</i> , <i>C. crescentus</i> , <i>A. actinomycetemcomitans</i>	Subset of type IVb pili expressed in both Gram-negative and positive bacteria. Promote surface colonisation, cell-to-cell attachment and biofilm cohesion.	[36,42]
	TCP (Toxin co-regulated pilus)	<i>V. cholerae</i>	Receptor for CTX ϕ inovirus. Deletion of TCP disrupts microcolony formation and results in unstructured biofilms.	[36,43]
	Pf4	<i>P. aeruginosa</i>		[44–48]

Continued

Table 1. Examples of polymeric molecules implicated in shaping biofilm architecture

Part 2 of 3

Molecule type	Name	Species	Structure and function	References
			Filamentous (pro)phage integrated into the <i>P. aeruginosa</i> genome — expression highly up-regulated on switch to biofilm lifestyle. Linked to an increase in <i>P. aeruginosa</i> pathogenicity. Capsid composed of interdigitated array of α -helical coat protein subunits surrounding a ssDNA genome. Pf4 phage filaments phase-separate into liquid crystalline structures termed tactoids. Increased antibiotic tolerance due to Pf4 tactoids encapsulating cells forming a diffusion barrier. Contributes to biofilm stability and resistance to desiccation. Acts as an immune decoy inhibiting clearance of <i>P. aeruginosa</i> .	
	Bap	<i>S. aureus</i>	Cell surface-attached high molecular mass multi-domain proteins. N-terminal dumbbell-shaped domain is released after proteolytic cleavage and forms insoluble amyloid-like fibres through liquid-liquid phase separation in response to pH and Ca^{2+} . Link environmental stimuli to formation of the biofilm ECM.	[49,50]
	Ag43, AIDA, tibia	<i>E. coli</i>	Members of the self-associating autotransporter (SAAT) family of proteins. Outer membrane anchored proteins that are independently transported across the membrane (type V secretion). Composed of a surface localised passenger domain and a C-terminal translocation domain which forms a β -barrel pore in outer membrane to transport passenger domain. Mediate bacterial aggregation by close range homotypic/heterotypic inter-cell association between SAATs. Ag43 promotes biofilm formation on abiotic surfaces. Autotransporters are widespread throughout Gram-negative bacteria with roles in host cell invasion and bacterial aggregation, for example Pertactin in <i>B. pertussis</i> and Hap in <i>H. influenzae</i> .	[51–53]
	P1 adhesin (Ag I/II)	<i>S. mutans</i>	Multi-functional cell-wall anchored adhesin. Predicted long α -helical stalk capped with a β -rich globular head with a putative carbohydrate binding trench. Binds to ECM and host surfaces - Interacts with salivary agglutinin (SAG) complex, collagen, and fibronectin.	[54]
	CdrA	<i>P. aeruginosa</i>	Cell surface adhesin. Interacts with the ECM polysaccharide Psl. Filamentous ‘periscope’ protein comprised of tandem repeats extending from the cell surface with a sugar binding tip domain. Promotes cohesion between cells within biofilms.	[55–57]
	LecB	<i>P. aeruginosa</i>	Homo-tetrameric lectin associated with the cell surface. Interacts with the ECM polysaccharide Psl. Increases retention of cells and ECM within biofilms.	[58,59]
	LapA	<i>P. fluorescens</i>	Large (~520 kDa) cell surface adhesin. Initial attachment to abiotic surfaces, promotes biofilm formation.	[60,61]
	Bap1	<i>V. cholerae</i>	Cell surface adhesin. Interacts with the ECM polysaccharide VPS. Multi-domain protein with β -propeller domain mediating VPS binding. Cross-links cells to the ECM matrix. ‘Double-sided tape’ mechanism with binding to VPS and the external environment. Mediates adhesion to abiotic surfaces.	[62–64]
	RbmA	<i>V. cholerae</i>	Consists of tandem fibronectin type III domains with a surface groove lined with positive charges — putative substrate binding site. Interacts with the ECM polysaccharide VPS. Acts as a flexible tether linking cells and the ECM. Crucial for ordering cells into clusters within the biofilm. Maintains 3D architecture of the biofilm.	[62,65–67]
	RbmC	<i>V. cholerae</i>		[63,64,68]

Continued

Table 1. Examples of polymeric molecules implicated in shaping biofilm architecture

Part 3 of 3

Molecule type	Name	Species	Structure and function	References
	BslA	<i>B. subtilis</i>	Cell surface adhesin. Interacts with the ECM polysaccharide VPS. Multi-domain protein with similar domain architecture to Bap1 with additional β -prism and $\beta\gamma$ -crystallin domains that bind to mucins. Cross-links cells to the ECM matrix. ‘Double-sided tape’ mechanism with binding to VPS and the external environment. Role in adhesion to abiotic surfaces.	
			Hydrophobin that forms a hydrophobic layer on the surface of biofilms — protects against environmental stresses. Small protein with an immunoglobulin-like fold and an extremely hydrophobic cap region.	[69,70]
Polysaccharides	PIA	<i>S. aureus</i> and <i>S. epidermidis</i>	Cationic, partially deacetylated <i>N</i> -acetylglucosamine homopolymer. Major component of the ECM, producing a fibrous net capturing cells and building up biofilm mass.	[70,71]
	Psl	<i>P. aeruginosa</i>	Mannose and galactose-rich neutral polysaccharide. Binds to cell surface attached adhesins CdrA/LecB. Implicated in initial attachment, cohesion between cells and microcolony formation.	[55,57,58,72,73]
	Pel	<i>P. aeruginosa</i>	Cationic polysaccharide comprised of galactosamine and <i>N</i> -acetyl-galactosamine. Mediates cell-cell contacts, adherence to surfaces and protection against antibiotics. Interacts with eDNA at sites of adhesion. Possible role in cross-linking host biopolymers and role in host colonisation.	[74–77]
	Alginate	<i>P. aeruginosa</i>	Comprised of β -D-mannuronic acid and α -L-guluronic acid. Replaces Psl and Pel in mucoid strains.	[72,78]
	EPS	<i>B. subtilis</i>	Major and essential component of biofilm ECM. Deletion greatly reduces biofilm size. Interacts with eDNA — responsible for 3D architecture of biofilm.	[79–81]
	VPS	<i>V. cholerae</i>	Major ECM polysaccharide comprised of glucose, galactose, <i>N</i> -acetylglucosamine and mannose. Interact with adhesins RmbA, RbmC and Bap1. Required for biofilm development and maintenance of 3D architecture.	[62,64,82]
	Cellulose	Many species	Major ECM polysaccharide comprised of β -1,4-linked D-Glucose. Cellulose fibres can bundle into ribbons that scaffold biofilm growth and 3D architecture. Can be decorated with phosphoethanolamine, allowing cellulose to form nanocomposites with amyloid fibres.	[36,83]
Nucleic acids	eDNA	All species	Ubiquitous in all biofilm ECMs. Crucial for biofilm stability. Cross-stranded structure resembling Holliday junctions. Transitions between low-energy B-form to high-energy Z-form during biofilm maturation. Triggers amyloid-like fibre formation of PSM by acting as a nucleator of subunit polymerisation in <i>S. aureus</i> . Implicated in binding to curli fibres and Pel polysaccharide.	[50,77,84–89]

This list includes the proposed function for each respective molecule and is not exhaustive.

While Fap fibres were originally discovered in the environmental strain UK4 from non-pathogenic *Pseudomonas fluorescens*, they are also encoded in pathogenic species including *Pseudomonas aeruginosa*. Since biofilms formed by *P. aeruginosa* are a major health threat [110], compounds targeting biofilm matrix fibres like curli or Fap may be an avenue for treatment of such infections. Future research will shed light on the function and spatial organisation of functional bacterial amyloids in Gram-negative bacterial biofilms.

Biofilm matrix fibres in Gram-positive bacteria

Many Gram-positive bacteria scaffold their biofilms using protein fibres that share functional similarity with bacterial amyloids discussed above. The biofilm matrix in the Gram-positive model organism *Bacillus subtilis* consists of


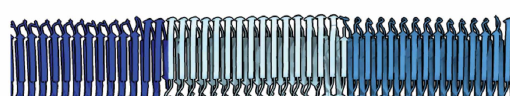

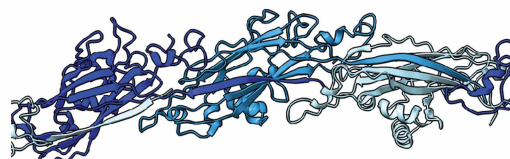

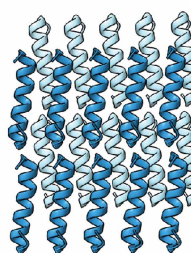
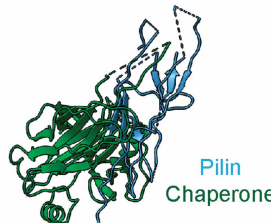
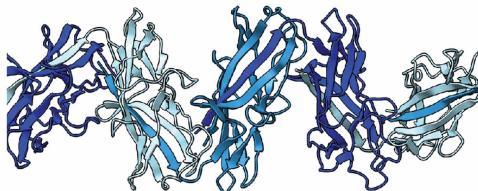
Fibre structure		Assembly mechanism
curli fibres (<i>P. korlensis</i>)		
Monomer (8C50)	Fibre (8C50)	Nucleation precipitation
		
TasA fibres (<i>B. subtilis</i>)		
Monomer (5OF1)	Fibre (8AUR)	Donor-strand exchange
		
PSMα3 (<i>S. aureus</i>)		
Monomer (5I55)	Fibre (5I55)	Cross-α aggregation
		
CsuA/B (<i>A. baumannii</i>)		
Chaperone-bound monomer (5D6H)	Fibre (7ZL4)	Chaperone-assisted donor-strand exchange
		

Figure 1. Structural features of biofilm matrix fibres.

Monomeric and fibre forms are shown for each example: curli fibres composed of CsgA subunits (structural model from AlphaFold2 and cryo-EM [92], PDB ID 8C50), TasA (monomer PDB ID 5OF1 [97], fibre PDB ID 8AUR [24]), putative PSMα3 fibres as predicted from its crystal structure [20] (PDB ID 5I55), and the Csu pilus (monomer PDB ID 5D6H [30], fibre PDB ID 7ZL4 [31]).

three main components: exopolysaccharides that promote cohesion [111], the BslA protein that forms a hydrophobic layer on the surface of the biofilm [112,113], and TasA matrix fibres that scaffold the biofilm [23,114]. TasA fibres consist of a 26 kDa monomeric subunit that is encoded together with a minor fibre component, TapA, and a putative signal peptidase (SipW) [115]. TasA fibres were initially thought to be amyloid-like, and hence structurally similar to curli and Fap fibres expressed by Gram-negative bacteria [23]. Supporting this hypothesis, TasA fibres exhibit a range of characteristics consistent with amyloid character, including high β-sheet content, protease

resistance, and affinity to amyloid-specific antibodies and dyes [23]. Later studies provided evidence contrasting this assessment, suggesting fibres instead consist of globular subunits positioned in a head-to-tail arrangement [116]. Recent cryo-EM studies revealed that TasA fibres indeed are not amyloid-like, but are donor strand-exchanged, meaning each globular β -sandwich domain subunit extends an N-terminal strand into the next subunit to complete its fold [24] (Figure 1). This fibre arrangement is reminiscent of Gram-negative Chaperone-Usher Pathway (CUP) pili [117], although TasA fibres appear to have evolved independently [24]. TasA fibres assemble from soluble TasA subunits secreted into the biofilm matrix [23,97], requiring only the sub-stoichiometric action of TapA, a minor component that provides the first donor strand to trigger fibre assembly [118]. Once assembled within the biofilm matrix, TasA fibres then congregate into larger bundles [24,119,120]. This process is spontaneous and does not appear to depend on strong chemical interaction [24], although future studies will be required to determine further details of the underlying mechanisms at play. Once assembled, TasA bundles provide stability to the biofilm and are crucial for maintaining the delicate, crinkly three-dimensional architecture that is typical of *B. subtilis* biofilms [23]. While TasA fibres have predominantly been characterised in *Bacillus* species, TasA-like systems were found to be phylogenetically wide-spread, existing even in archaea living in extreme environments [121], underlining the ubiquity of this donor-strand exchange system in promoting fibre and biofilm stability. A study on an archaeal TasA-like system in the hyperthermophilic archaeon *Pyrobaculum calidifontis* suggests that biofilm formation is promoted by the formation of antiparallel bundles, with fibres extending from the surface of one cell to interact with fibres extending from other cells, thus physically tethering cells together [121]. It is plausible that this scenario may also be at play in *B. subtilis*, although neither the directionality of bundle interactions nor potential molecular interactions with the cell wall of *B. subtilis* have thus far been resolved [24].

Phenol-soluble modulins in Gram-positive bacteria

Phenol-soluble modulins (PSMs) are important virulence factors in the human pathogen *Staphylococcus aureus* [122,123]. Compared with the larger protein subunits forming the previously described functional amyloid-like fibres in Gram-negative bacteria, PSMs are small peptides with a distinct propensity to self-assemble into higher-order fibres. Three major operons of PSMs have been described: *psmA* encoding four peptides (PSM α 1–4) that are each ~20–25 amino acids in length; *psm β* encoding PSM β 1–2, which are ~44 residues in length, as well as δ -toxin, which is ~22 residues in length and encoded within the RNA effector molecule of a quorum sensing system [124]. Individual, monomeric PSM peptides have been described to be biologically active without polymerising into higher-order structures, inducing host cell lysis and modulating host immune responses [122]. However, the most remarkable property of PSM peptides is their propensity to assemble into fibres with amyloid-like properties that scaffold biofilm formation [19]. The first described X-ray structure of such a fibre reconstituted *in vitro* showed an intriguing departure from typical structural motifs of amyloids containing a cross- β structure: Despite the fibres staining positive for Thioflavin T, a conventional marker for cross- β amyloids, high-resolution structures of PSMs revealed that they actually contain α -helical peptides that stack laterally to form a filament, resulting in a ‘cross- α ’ fold [20] that had not previously been seen in naturally occurring peptides (Figure 1). Through later cryo-EM structure determination, the cross- α structure was confirmed for both PSM α 3 and PSM β 2, with individual filaments shown to self-assemble into polymorphic tube-shaped structures [21]. Oddly, other PSM α peptides (PSM α 1 and PSM α 4) were found to assemble into canonical cross- β amyloid fibres and sequence alterations could be used to modulate cytotoxic properties and fibre-forming propensities [125].

Many different amyloidogenic subunits exist in *S. aureus*, polymerising into a variety of fibres. While the reason behind harbouring such an arsenal of fibre-forming subunits is not fully clear, one study has shown that expressing a variety of different PSMs results in cross-talk between them and accelerates the assembly of the peptides into fibres, improving the efficiency of biofilm formation [22]. The factors that influence the assembly and structure of fibres formed by PSMs are not fully elucidated and remain an active area of research.

Adhesive pili in the biofilm matrix of Gram-negative bacteria

Adhesive pili play important roles in biofilm formation. A primary function of pili is to anchor cells to a surface or other substrates, a crucial step in the early stages of biofilm formation. The role of type IV pili in this step, including in twitching motility and surface adhesion, is well-described in literature; however, their function is thought to be limited to early stages of biofilm formation and is therefore not discussed here, but is discussed in several authoritative reviews [3,34,35]. The so-called CUP pili, on the other hand, have a crucial role in stabilising mature biofilms of Gram-negative bacteria. In *P. aeruginosa*, up to five different CUP pili

exist that can be classified into two types: one type, termed classical CUP pili [126], have roles in initial adhesion and the formation of microcolonies (CupA–D) [25–27], while the second type, termed ‘archaic CUP pilus’, is prevalent in mature biofilms (CupE), where it stabilises the three-dimensional architecture of the biofilm [32]. Archaic CUP pili are widely expressed and are often major components of the ECM, reported for example in *Acinetobacter baumannii*, where the archaic Csu pilus is a major virulence factor [28]. Interestingly, in addition to maintaining biofilm architecture, the archaic Csu pilus also appears to help bacteria persist on plastic surfaces in hospital environments, and thus aid its spread as a hospital-acquired pathogen [29].

All CUP pili are assembled from small (12–20 kDa) subunits through a donor-strand exchange mechanism [117,127], similar to TasA fibres discussed above. Prior to assembly, each pilin subunit consists of an incomplete Ig-like β -sandwich fold that lacks its final β -strand, but contains an extra N-terminal β -strand [127]. Upon assembly, this additional strand (designated as the donor strand) inserts into the subsequent subunit, completing its fold [127]. Classical CUP pili form tubular structures, where a single subunit assembles into a helical, hollow, rod-like architecture [128,129]. The archaic type of CUP pili, however, significantly differs in their architecture: While the subunits themselves are similar to those of the classical type [30], archaic CUP pili consist of a linear, zigzag-arrangement of pilin subunits (Figure 1) capped by a hydrophobic adhesive subunit at the pilus tip [29,31]. This adhesive tip likely binds to components of the biofilm ECM, however, the identity of substrates for archaic CUP pili is currently unknown. Some archaic CUP pili were shown to display remarkable elastic properties in optical tweezer experiments, where the archaic CUP pilus Csu from *A. baumannii* could extend up to twice its length [31]. This was made possible by breaking some of its subunit interactions, essentially acting as an elastic molecular rubber band [31]. Once the applied force ceased, the pilus coiled back up into a zigzag shape by re-forming subunit interactions [31]. A similar zigzag pilus architecture was reported for the archaic CUP pilus CupE from *P. aeruginosa* [33], a phylogenetically related bacterium, suggesting pilus elasticity might be a conserved feature. It remains to be seen how exactly archaic CUP pili help maintaining biofilm architecture, since molecular details of pilus arrangement and binding in biofilms is not yet described. The indiscriminate hydrophobic nature of the adhesin [29] means a variety of hydrophobic biofilm components or cell surfaces could be substrates, resulting in a cross-linking effect to aid cohesion of the biofilm matrix. Since the biofilm matrix is not easily amenable to conventional interaction assays, such as pull-downs or cross-linking mass spectrometry, *in situ* molecular imaging techniques such as electron cryotomography (cryo-ET) may prove to be particularly useful in determining such interactions within the biofilm ECM [130].

Bacteriophage liquid crystalline droplets in biofilms

Classically, bacteriophages are considered to be detrimental to bacterial cell survival. While many bacteriophages indeed kill their host during their replication cycles, some phages are continuously secreted from the host membrane, without lysing the host, allowing both to coexist [131]. Going one step further, akin to endogenous retroviruses in the human genome [132], some bacteriophages have even integrated into the host genome (called prophages), forming a symbiotic relationship with their bacterial host and providing it with survival advantages in biofilms [44,133]. A prominent example of this is Pf4, a prophage encoded in the *P. aeruginosa* genome, which enhances *P. aeruginosa* survival in harsh environments [133]. Pf4 is a filamentous, rod-shaped bacteriophage of the *Inoviridae* family [134], and as a prophage, its expression is tightly controlled by *P. aeruginosa* cells. Upon switching from planktonic to biofilm lifecycle, genes encoding Pf4 bacteriophage are massively up-regulated [135], secreting thousands of phage copies into the extracellular biofilm matrix. Intriguingly, the presence of Pf4 was shown to increase the pathogenicity of *P. aeruginosa*, correlating with increased morbidity in patients, and is prevalent in clinical isolates [45,136]. Upon host infection by *P. aeruginosa*, the presence of large amounts of phage subverts the host immune system into triggering an anti-viral rather than antibacterial immune response, increasing *P. aeruginosa* virulence [137]. Pf4 phage also promote biofilm stability by self-assembling into higher-order structures within the biofilm matrix [44,46]. In the presence of polysaccharides that are naturally present in the biofilm matrix such as alginate, Pf4 spontaneously phase-separates into spindle-shaped liquid crystalline droplets termed tactoids [44,46]. These Pf4 droplets are birefringent and individual phage filaments show mobility within the droplet, both characteristic of a nematic liquid crystalline phase [44,46]. Light microscopy and cryo-ET revealed that Pf4 liquid crystalline droplets encapsulate bacterial cells, thus physically shielding them from antibiotics [46]. The formation of Pf4 liquid crystalline droplets does not occur through chemical interactions between phage [47], which have negatively charged surfaces. Instead, phage interact with each other through depletion attraction (discussed later), an entropically driven attractive force that arises between large particles in the presence of depletants, such as other biopolymers in the biofilm matrix, which are excluded from the volume in the process [138]. Due to the

abundance of Pf4 in the ECM, Pf4 liquid crystalline droplets have even been proposed to organise the entire biofilm into a liquid crystalline structure [48], contributing to biofilm stability and ability to withstand desiccation [139]. An emergent property bestowed by Pf4 liquid crystalline droplets is antibiotic tolerance of *P. aeruginosa*. Bacterial cells can be encapsulated by Pf4 droplets, resulting in protection by the formation of a barrier preventing antibiotic from reaching the cell [46,47]. The formation of liquid crystalline droplets and their interactions with bacteria depend on biophysical properties of the liquid crystalline droplet, determined by phase shape and packing, rather than specific biochemical interactions between the *P. aeruginosa* cell surface and phage capsid [47].

Inoviral prophages are pervasive across prokaryotic biomes [140], and such prophages increase bacterial pathogenicity in other Gram-negative bacteria. Examples include MDAΦ in *Neisseria meningitidis*, which promotes colonisation of epithelial cells and invasion of the blood-brain barrier, and CTXΦ in *Vibrio cholerae*, which increases bacterial virulence, amongst others [133,141]. In addition to Pf4, *P. aeruginosa* also encodes several other prophages [142]. Although the role and mechanism of action of these prophages remain underexplored, it is possible that given their biophysical similarities, they act in an analogous manner to Pf4 as a structural and protective component in biofilms. The fact that filaments can interact with the bacterial cell in a protective manner may provide a rational for why filaments are preferred components of the biofilm matrix and formed by most biofilm-forming bacteria.

Polysaccharide organisation in the biofilm ECM

Extracellular polysaccharides (exopolysaccharides) are a key component of the biofilm ECM and have been implicated in cell-cell cohesion and intra-matrix interactions [55,143–145]. *P. aeruginosa* is known to express at least three major exopolysaccharides, namely Psl, Pel and alginate, in a strain-dependent manner, with all having important roles in biofilm development [72,146]. Psl, a mannose- and galactose-rich neutral exopolysaccharide, is involved in initial surface attachment, cell-cell attachment and microcolony formation [73]. Using Psl-specific labelled lectins, Psl was shown to associate with bacterial cells with a distinctive helical pattern upon cell adhesion to surfaces [73]. During biofilm maturation, Psl localises to the periphery of microcolonies, resulting in a Psl-free cavity at the centre of the biofilm, which may act as a pool of free cells for dispersal from the biofilm [73]. Psl has been implicated in promoting cell-cell cohesion within the biofilm [147–149], and interaction with the cell surface adhesin CdrA is essential to this phenotype [56]. CdrA is a monomeric cell surface ‘periscope’ protein forming a filamentous arrangement comprised of tandem repeat domains extending from the cell surface with a tip domain that interacts with Psl [150]. Thus, CdrA cross-links the cell with the matrix Psl, resulting in cohesion between cells [55–57]. Nanobodies targeting this interaction by blocking the adhesive domain of CdrA disrupt the biofilm and render it more susceptible to antibiotic treatment [55]. Equally, a monoclonal antibody targeting Psl was found effective in mouse models [151] and employed in clinical trials [152], underlining not only the importance of the CdrA:Psl interaction [151], but also the importance of polysaccharides in the ECM in maintaining biofilms. Apart from CdrA, a different cell surface-tethered lectin LecB has also been implicated in interaction with Psl to stabilise the biofilm matrix [58], presumably also by cross-linking Psl with the cell-surface [58,153].

Pel is a major cationic exopolysaccharide in *P. aeruginosa* biofilms that consists of galactosamine and *N*-acetyl-galactosamine moieties [74]. Pel mediates cell-cell contacts, adherence to cell surfaces and protection against aminoglycoside antibiotics [75]. It localises to the periphery of biofilms in the same manner as Psl, but is also seen at sites of attachment to surfaces [76]. Due to its net positive charge, Pel can cross-link eDNA at the site of surface adhesion in biofilms through electrostatic interactions, suggesting that Pel/eDNA interactions could be an important structural feature in early biofilm development. Interestingly, Pel could also cross-link polymers such as mucins and hyaluronan, which are secreted by the host during infection, suggesting that exopolysaccharides could play specific roles in enabling host colonisation [77].

A third type of polysaccharide, alginate, is well-characterised for its role in *P. aeruginosa* infection of hosts. Upon entering the lung environment, biofilms often adopt a mucoid, alginate-rich phenotype resulting from a mutation in the *mucA* gene leading to overexpression of this polymer [78]. Alginate consists of β-D-mannuronic acid and α-L-guluronic acid moieties, and alginate typically replaces Psl and Pel in mucoid strains [72]. As alginate is highly expressed during lung infections by *P. aeruginosa* in cystic fibrosis patients, alginate assembly and integrity has been suggested as a possible target for biofilm therapeutics [154–156].

Exopolysaccharides also play important roles in the biofilm matrix of many other bacterial species. In *B. subtilis*, the exopolysaccharide EPS is a major component of the biofilm matrix, and is essential for biofilm

formation under most conditions [79]. Deletion of EPS results in a greatly reduced biofilm size, and deletion of both EPS and TasA results in a complete ablation of biofilm formation [80]. *B. subtilis* EPS interacts with eDNA in the biofilm matrix and this interaction is required for the complex three-dimensional structure and spatial heterogeneity of the biofilm [81]. The interaction of EPS with eDNA predominantly occurs in the initial stages of biofilm development, although EPS alone prevails in mature biofilms [81].

Another model organism for biofilm formation is *V. cholerae*, where the ECM is particularly enriched in polysaccharides when compared with other bacteria [82]. In *V. cholerae* biofilms, the exopolysaccharide VPS (*V*ibrio *p*olysaccharide) is estimated to comprise up to 50% of the biofilm matrix mass [82]. VPS is secreted from bacteria after initial attachment of cells and VPS expression is maintained during biofilm maturation [62]. VPS is required for all stages of biofilm formation, including development and maintenance of the three-dimensional architecture of *V. cholerae* biofilms. VPS also plays a role in effective colonisation and infection in disease models [82,157,158]. Similar to the Pseudomonads, *Vibrio* also employs adhesins that directly connect cells with exopolysaccharides. Interestingly, two adhesins — Bap1 (*B*iofilm-*a*ssociated *p*rotein 1) and its homologue RbmC (*R*ugosity and *b*iofilm *m*odulator C) directly bind to VPS [63], thus resulting in a cross-linking of cells with the matrix similar to CdrA in *P. aeruginosa*. Indeed, in super-resolution imaging of *Vibrio* biofilms, these two adhesins were found to co-localise with VPS [62]. Bap1 is a multi-domain protein consisting of a β -propeller that mediates binding to VPS and a β -prism domain that binds to N-glycans [64]. RbmC contains the same overall domain arrangement but with an additional β -prism domain as well as a $\beta\gamma$ -crystallin domain that is mucin-binding [64]. Recent literature suggests that both proteins can be considered ‘double-sided tape’, meaning they can interact with both VPS and the outside environment [64]. The functional difference between the two adhesins lies in the type of environment they interact with: RbmC was found to preferentially cause adhesion to biotic surfaces through its mucin- and N-glycan-binding domains, while Bap1 preferentially causes adhesion to abiotic surfaces and lipids through a loop within its β -propeller domain, and shows preferential expression near the biofilm-surface interface [64]. A third protein component of the ECM in *V. cholerae*, RbmA, is crucial for ordering cells into clusters within the biofilm [62,65,66]. RbmA consists of tandem fibronectin type III domains that can directly bind to VPS and, through a previously described structural switch [66], assemble into multimers that influence the biofilm architecture of *Vibrio* through an unknown mechanism.

Taken together, these studies from different bacteria have shown that polysaccharides play a crucial role in the structuring of most, if not all, biofilms, and that interactions of polysaccharides with the cell surface, be it by binding to surface proteins or sorption to the cell, are essential for spatial organisation of the biofilm ECM.

eDNA organisation in the biofilm ECM

Alongside proteinaceous material and polysaccharides, eDNA is another major component of the biofilm ECM [84]. eDNA is ubiquitous in the biofilm ECM from all bacterial species, and is critical for biofilm ECM stability [85]. Within the biofilm ECM, eDNA forms a cross-stranded structure stabilised by the DNABII family of proteins, which structurally resemble Holliday junctions [86,159]. Degradation of eDNA by nucleases disrupts initial biofilm formation, but does not affect mature biofilms [160,161]. A recent study provided an answer as to why this is the case: Biofilm eDNA was shown to transition from B-form to Z-form DNA during biofilm maturation [87], going from a right-handed low-energy conformation that is nuclease-sensitive to a left-handed high-energy conformation that is resistant to nucleases [162,163]. Indeed, the equilibrium between Z-form and B-form plays an important role in determining the rigidity of the biofilm [87].

eDNA also plays an important role in triggering amyloid-like fibre formation in the biofilm ECM by acting as a nucleator of subunit polymerisation, decreasing the concentration at which phase separation and fibre formation of amyloid-like proteins occur [88]. Direct interaction of eDNA with *Salmonella* curli fibre has been observed, which protects the eDNA from nuclease degradation [89]. Hence, eDNA and amyloid fibres show a mutualistic relationship in the biofilm ECM, with eDNA promoting fibre formation and phase separation, which in turn protects the eDNA from degradation.

eDNA in the biofilm ECM has been implicated in antibiotic tolerance with the highly negatively charged DNA proposed to sequester positively charged antibiotics such as aminoglycosides [44,48]. eDNA in the ECM can provide the ideal environment for liquid crystalline droplets formation, as such droplets form in the presence of high molecular mass DNA [44,48]. The multiple roles of eDNA in phase separation, amyloid-like fibre formation and sequestration of molecules shows the crucial importance of eDNA in the biofilm ECM.

Mechanisms of biofilm formation revealed by multi-scale studies of EPS molecules

Bacterial cells within biofilms show significant levels of spatio-temporal organisation and patterning [164]. Bacterial biofilm architecture results from a complex interplay of physical, chemical, and biological mechanisms, with the ECM playing a central role [165–168]. We propose that there are two organisational principles that could explain cohesion of bacterial cells in biofilms (Figure 2). The first mechanism, termed bridging, is based on cross-linking or sorption of cells with polysaccharides in the ECM, tethering cells together and causing their aggregation. The second mechanism is based on depletion attraction, causing the formation of tightly packed structures in the presence of biopolymer [164,171–173].

Bridging occurs when a polymer interacts with multiple bacterial cells, causing the cells to be spontaneously pulled together [171,174]. Bridging can involve more than one chemical component, and may for example be

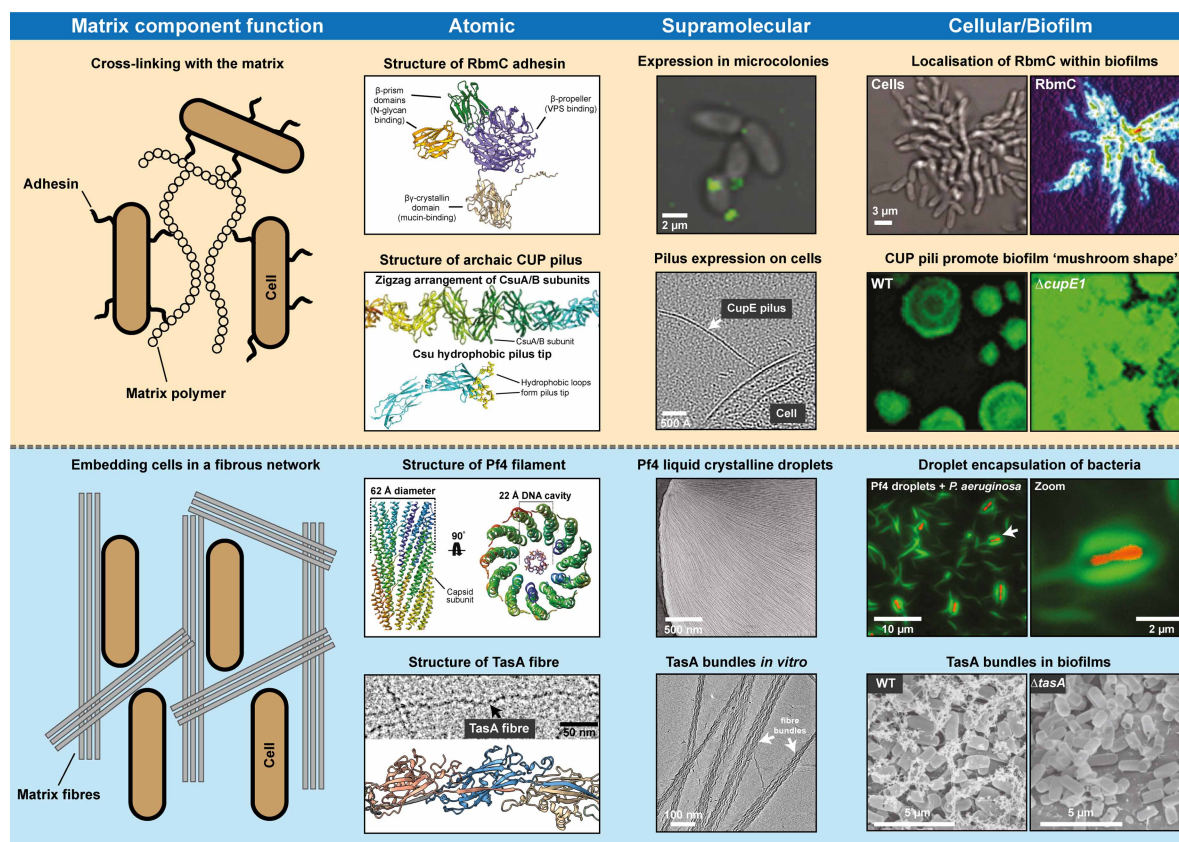


Figure 2. Multi-scale studies elucidate the interactions of cells with the extracellular matrix that give rise to the complex three-dimensional architecture found in biofilms.

First column (from left): General principles of cell-matrix interactions: Many of the fibrous components in biofilms either cross-link cells with matrix polysaccharides or DNA (upper) or form bundles in which cells are embedded (lower). A mixture of chemical and entropic effects may contribute to both mechanisms. Second column: Atomic models of biofilm fibres produced in recent years, including the *V. cholerae* RbmC adhesin AlphaFold prediction [169,170] annotated by domain function as described previously [64], an archaic CUP pilus from *A. baumannii* [29,31], Pf4 phage [46], and TasA fibres [24]. Third column: Localisation of RbmC expression in *V. cholerae* microcolonies as shown by fluorescence microscopy (from reference [62]; reprinted with permission from AAAS), CupE pilus extending from a *P. aeruginosa* cell visualised by electron cryotomography [33], and formation of bundles by Pf4 [46] and TasA [24]. Fourth column: RbmC expression in *V. cholerae* biofilms (from reference [62]; reprinted with permission from AAAS), fluorescent *P. aeruginosa* biofilms showcasing a distinct mushroom-shaped 3D architecture that depends on the expression of CupE pili (reprinted with permission from reference [32]), encapsulation of *P. aeruginosa* cells by Pf4 liquid crystalline droplets [46], and SEM imaging of *B. subtilis* biofilms with and without *tasA* [120].

mediated by a proteinaceous adhesin binding to a polysaccharide, as seen in the CdrA/Psl system of *P. aeruginosa* [55,56] and RbmC/VPS system of *V. cholerae* [63].

Depletion attraction is an entropic principle that causes alignment of objects of similar shape in the presence of high levels of non-adsorbing polymers. Spontaneous movement causes bacterial cells to come closer together, as this will exclude the polymer, allowing higher degrees of freedom for biopolymer movement and increasing the entropy of the system [44,164]. Depletion attraction can induce interactions between objects of similar shapes, including cells, but also between fibrous matrix components, and between cells and fibrous matrix components. For example, depletion attraction is the driving force of liquid crystalline droplet formation by Pf4 phages in the *P. aeruginosa* biofilm matrix, where phages spontaneously align in the presence of polysaccharide polymer, which is excluded from the droplet in the process [47]. This process could also contribute to the formation of higher-order assemblies by other biofilm fibres, such as TasA and curli. Given the abundance of fibre bundles and polysaccharides in many bacterial systems, it is plausible that entropic forces such as depletion attraction play a significant role in producing cohesion and order in many other biofilms, most of which contain polysaccharides that could act as a depletant.

Previous studies have shown that the environment can have significant effects in producing order within the biofilm [5,175–179]. While it is not completely understood how they drive such changes, these environmental factors can substantially modulate the three-dimensional shape and properties of biofilms. For example, in *V. cholerae* biofilms embedded in hydrogels, but not on flat surfaces, cells align into bipolar structures reminiscent of nematic phase liquid crystalline droplets [179]. This cell ordering was proposed to occur due to biomechanical stress transmission across the biofilm mediated by ECM biopolymers such as VPS [179]. At the same time, it was shown in *V. cholerae* biofilms that mechanical interactions between cells and matrix components are of significant importance in imparting order in the biofilm [180]. Generally, a combination of physical (entropy-driven) and chemical (adhesion) effects are most likely responsible for maintaining the three-dimensional organisation of the biofilm, and it will take focused further research to accurately assess the contributions of such factors in shaping bacterial biofilms.

Conclusion

The three-dimensional architecture of biofilms arises from complex interactions of ECM components and bacterial cells. This matrix has long been imperfectly understood, because on one hand, the structure and spatial organisation of many of the components was poorly characterised, and on the other hand, the interactions between components were unclear. Many fibre components of the biofilm matrix have now been structurally characterised and first principles of the molecular organisation of the biofilm matrix are being revealed. Recent studies have led to the realisation that a complex interplay of chemical interactions and physical principles gives rise to the exceptional emergent properties of the biofilms.

Bacterial biofilms are a major cause of human disease, accounting for 65–80% of all chronic infections [181,182]. Biofilms are notoriously difficult to treat as the ECM provides an effective barrier to many antimicrobials, making them up to 1000-fold more tolerant to antibiotic treatment than planktonic bacteria [183], leading to poor bacterial eradication and chronic infection [76]. Employing delivery mechanisms that circumvent the ability of the biofilm matrix to reduce antibiotic uptake should allow greater penetration of antimicrobials into biofilms. For this goal, further insights into the interactions between the matrix components and the physicochemical microenvironments arising through them are urgently needed. This fundamental knowledge will help improve our understanding of how bacteria protect themselves against antibiotics, aiding our understanding of multicellular bacterial life and the development of better treatments for bacterial infections.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Open Access

Open access for this article was enabled by the participation of MRC Laboratory of Molecular Biology in an all-inclusive *Read & Publish* agreement with Portland Press and the Biochemical Society under a transformative agreement with JISC.

CRedit Author Contribution

Tanmay Bharat: Conceptualization, Resources, Supervision, Funding acquisition, Writing — original draft, Project administration, Writing — review and editing. **Jan Böhning:** Conceptualization, Visualization, Writing — original draft, Writing — review and editing. **Abul K. Tarafder:** Conceptualization, Visualization, Writing — original draft, Writing — review and editing.

Acknowledgements

We would like to thank Kazune Tamura for advice on the initial plan of the manuscript, and Freya Bull as well as Zhexin (Eric) Wang for critical reading of the manuscript. T. A. M. B. would like to thank UKRI MRC (Programme MC_UP_1201/31), the EPSRC (Grant EP/V026623/1), the European Molecular Biology Organisation, the Leverhulme Trust and the Lister Institute for Preventative Medicine for support. For the purpose of open access, the MRC Laboratory of Molecular Biology has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising.

Abbreviations

cryo-EM, electron cryomicroscopy; cryo-ET, electron cryotomography; CUP, Chaperone-Usher Pathway; ECM, extracellular matrix; eDNA, extracellular DNA; EPS, extracellular polymeric substances; PSM, Phenol-soluble modulin; VPS, Vibrio polysaccharide.

References

- Costerton, J.W., Cheng, K.J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M. et al. (1987) Bacterial biofilms in nature and disease. *Annu. Rev. Microbiol.* **41**, 435–464 <https://doi.org/10.1146/annurev.mi.41.100187.002251>
- O'Toole, G., Kaplan, H.B. and Kolter, R. (2000) Biofilm formation as microbial development. *Annu. Rev. Microbiol.* **54**, 49–79 <https://doi.org/10.1146/annurev.micro.54.1.49>
- Stoodley, P., Sauer, K., Davies, D.G. and Costerton, J.W. (2002) Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* **56**, 187–209 <https://doi.org/10.1146/annurev.micro.56.012302.160705>
- Nadell, C.D., Drescher, K. and Foster, K.R. (2016) Spatial structure, cooperation and competition in biofilms. *Nat. Rev. Microbiol.* **14**, 589–600 <https://doi.org/10.1038/nrmicro.2016.84>
- Qin, B., Fei, C., Bridges, A.A., Mashruwala, A.A., Stone, H.A., Wingreen, N.S. et al. (2020) Cell position fates and collective fountain flow in bacterial biofilms revealed by light-sheet microscopy. *Science* **369**, 71–77 <https://doi.org/10.1126/science.abb8501>
- Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A. and Kjelleberg, S. (2016) Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* **14**, 563–575 <https://doi.org/10.1038/nrmicro.2016.94>
- Flemming, H.C., Neu, T.R. and Wozniak, D.J. (2007) The EPS matrix: the “house of biofilm cells”. *J. Bacteriol.* **189**, 7945–7947 <https://doi.org/10.1128/JB.00858-07>
- Højby, N., Bjarnsholt, T., Givskov, M., Molin, S. and Ciofu, O. (2010) Antibiotic resistance of bacterial biofilms. *Int. J. Antimicrob. Agents* **35**, 322–332 <https://doi.org/10.1016/j.ijantimicag.2009.12.011>
- Hall-Stoodley, L. and Stoodley, P. (2009) Evolving concepts in biofilm infections. *Cell Microbiol.* **11**, 1034–1043 <https://doi.org/10.1111/j.1462-5822.2009.01323.x>
- O'Toole, G.A. and Kolter, R. (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol.* **30**, 295–304 <https://doi.org/10.1046/j.1365-2958.1998.01062.x>
- Guzmán-Soto, I., McTiernan, C., Gonzalez-Gomez, M., Ross, A., Gupta, K., Suuronen, E.J. et al. (2021) Mimicking biofilm formation and development: recent progress in in vitro and in vivo biofilm models. *iScience* **24**, 102443 <https://doi.org/10.1016/j.isci.2021.102443>
- Ceri, H., Olson, M.E., Stremick, C., Read, R.R., Morck, D. and Buret, A. (1999) The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J. Clin. Microbiol.* **37**, 1771–1776 <https://doi.org/10.1128/JCM.37.6.1771-1776.1999>
- Chapman, M.R., Robinson, L.S., Pinkner, J.S., Roth, R., Heuser, J., Hammar, M. et al. (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* **295**, 851–855 <https://doi.org/10.1126/science.1067484>
- Barnhart, M.M. and Chapman, M.R. (2006) Curli biogenesis and function. *Annu. Rev. Microbiol.* **60**, 131–147 <https://doi.org/10.1146/annurev.micro.60.080805.142106>
- Kikuchi, T., Mizunoe, Y., Takade, A., Naito, S. and Yoshida, S. (2005) Curli fibers are required for development of biofilm architecture in *Escherichia coli* K-12 and enhance bacterial adherence to human uroepithelial cells. *Microbiol. Immunol.* **49**, 875–884 <https://doi.org/10.1111/j.1348-0421.2005.tb03678.x>
- Gophna, U., Barlev, M., Seijffers, R., Oelschlager, T.A., Hacker, J. and Ron, E.Z. (2001) Curli fibers mediate internalization of *Escherichia coli* by eukaryotic cells. *Infect. Immun.* **69**, 2659–2665 <https://doi.org/10.1128/IAI.69.4.2659-2665.2001>
- Dueholm, M.S., Petersen, S.V., Sønderkær, M., Larsen, P., Christiansen, G., Hein, K.L. et al. (2010) Functional amyloid in *Pseudomonas*. *Mol. Microbiol.* **77**, 1009–1020 <https://doi.org/10.1111/j.1365-2958.2010.07269.x>
- Dueholm, M.S., Søndergaard, M.T., Nilsson, M., Christiansen, G., Stensballe, A., Overgaard, M.T. et al. (2013) Expression of Fap amyloids in *Pseudomonas aeruginosa*, *P. fluorescens*, and *P. putida* results in aggregation and increased biofilm formation. *Microbiologyopen* **2**, 365–382 <https://doi.org/10.1002/mbo3.81>
- Schwartz, K., Syed, A.K., Stephenson, R.E., Rickard, A.H. and Boles, B.R. (2012) Functional amyloids composed of phenol soluble modulins stabilize *Staphylococcus aureus* biofilms. *PLoS Pathog.* **8**, e1002744 <https://doi.org/10.1371/journal.ppat.1002744>

- 20 Tayeb-Fligelman, E., Tabachnikov, O., Moshe, A., Goldshmidt-Tran, O., Sawaya, M.R., Coquelle, N. et al. (2017) The cytotoxic *Staphylococcus aureus* PSM α 3 reveals a cross- α amyloid-like fibril. *Science* **355**, 831–833 <https://doi.org/10.1126/science.aaf4901>
- 21 Kreutzberger, M.A.B., Wang, S., Beltran, L.C., Tuachi, A., Zuo, X., Egelman, E.H. et al. (2022) Phenol-soluble modulins PSM α 3 and PSM β 2 form nanotubes that are cross- α amyloids. *Proc. Natl Acad. Sci. U.S.A.* **119**, e2121586119 <https://doi.org/10.1073/pnas.2121586119>
- 22 Zaman, M. and Andreasen, M. (2020) Cross-talk between individual phenol-soluble modulins in *Staphylococcus aureus* biofilm enables rapid and efficient amyloid formation. *Elife* **9**, e59776 <https://doi.org/10.7554/eLife.59776>
- 23 Romero, D., Aguilar, C., Losick, R. and Kolter, R. (2010) Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc. Natl Acad. Sci. U.S.A.* **107**, 2230–2234 <https://doi.org/10.1073/pnas.0910560107>
- 24 Böhning, J., Ghayeb, M., Pedebos, C., Abbas, D.K., Khalid, S., Chai, L. et al. (2022) Donor-strand exchange drives assembly of the TasA scaffold in *Bacillus subtilis* biofilms. *Nat. Commun.* **13**, 7082 <https://doi.org/10.1038/s41467-022-34700-z>
- 25 Vallet, I., Olson, J.W., Lory, S., Lazdunski, A. and Filloux, A. (2001) The chaperone/usher pathways of *Pseudomonas aeruginosa*: identification of fimbrial gene clusters (cup) and their involvement in biofilm formation. *Proc. Natl Acad. Sci. U.S.A.* **98**, 6911–6916 <https://doi.org/10.1073/pnas.111551898>
- 26 Ruer, S., Stender, S., Filloux, A. and de Bentzmann, S. (2007) Assembly of fimbrial structures in *Pseudomonas aeruginosa*: functionality and specificity of chaperone-usher machineries. *J. Bacteriol.* **189**, 3547–3555 <https://doi.org/10.1128/JB.00093-07>
- 27 Mikkelsen, H., Ball, G., Giraud, C. and Filloux, A. (2009) Expression of *Pseudomonas aeruginosa* CupD fimbrial genes is antagonistically controlled by RcsB and the EAL-containing PvrR response regulators. *PLoS One* **4**, e6018 <https://doi.org/10.1371/journal.pone.0006018>
- 28 Ahmad, I., Nadeem, A., Mushtaq, F., Zlatkov, N., Shahzad, M., Zavialov, A.V. et al. (2023) Csu pili dependent biofilm formation and virulence of *Acinetobacter baumannii*. *NPJ Biofilms Microbiomes* **9**, 101 <https://doi.org/10.1038/s41522-023-00465-6>
- 29 Pakharukova, N., Tuittila, M., Paavilainen, S., Malmi, H., Parilova, O., Teneberg, S. et al. (2018) Structural basis for *Acinetobacter baumannii* biofilm formation. *Proc. Natl Acad. Sci. U.S.A.* **115**, 5558–5563 <https://doi.org/10.1073/pnas.1800961115>
- 30 Pakharukova, N., Garnett, J.A., Tuittila, M., Paavilainen, S., Diallo, M., Xu, Y. et al. (2015) Structural insight into archaic and alternative chaperone-usher pathways reveals a novel mechanism of pilus biogenesis. *PLoS Pathog.* **11**, e1005269 <https://doi.org/10.1371/journal.ppat.1005269>
- 31 Pakharukova, N., Malmi, H., Tuittila, M., Dahlberg, T., Ghosal, D., Chang, Y.W. et al. (2022) Archaic chaperone-usher pili self-secrete into superelastic zigzag springs. *Nature* **609**, 335–340 <https://doi.org/10.1038/s41586-022-05095-0>
- 32 Giraud, C., Bernard, C.S., Calderon, V., Yang, L., Filloux, A., Molin, S. et al. (2011) The PprA-PprB two-component system activates CupE, the first non-archetypal *Pseudomonas aeruginosa* chaperone-usher pathway system assembling fimbriae. *Environ. Microbiol.* **13**, 666–683 <https://doi.org/10.1111/j.1462-2920.2010.02372.x>
- 33 Böhning, J., Döbelstein, A.W., Sulkowski, N., Eilers, K., von Kögelen, A., Tarafder, A.K. et al. (2023) Architecture of the biofilm-associated archaic Chaperone-Usher pilus CupE from *Pseudomonas aeruginosa*. *PLoS Pathog.* **19**, e1011177 <https://doi.org/10.1371/journal.ppat.1011177>
- 34 Klausen, M., Heydorn, A., Ragas, P., Lambertsen, L., Aaes-Jorgensen, A., Molin, S. et al. (2003) Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. *Mol. Microbiol.* **48**, 1511–1524 <https://doi.org/10.1046/j.1365-2958.2003.03525.x>
- 35 Ellison, C.K., Whitfield, G.B. and Brun, Y.V. (2022) Type IV pili: dynamic bacterial nanomachines. *FEMS Microbiol. Rev.* **46**, fuab053 <https://doi.org/10.1093/femsre/fuab053>
- 36 Berne, C., Ducret, A., Hardy, G.G. and Brun, Y.V. (2015) Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. *Microbiol. Spectr.* **3** <https://doi.org/10.1128/microbiolspec.MB-0018-2015>
- 37 Jin, X. and Marshall, J.S. (2020) Mechanics of biofilms formed of bacteria with fimbriae appendages. *PLoS One* **15**, e0243280 <https://doi.org/10.1371/journal.pone.0243280>
- 38 Schembri, M.A. and Klemm, P. (2001) Biofilm formation in a hydrodynamic environment by novel fimh variants and ramifications for virulence. *Infect. Immun.* **69**, 1322–1328 <https://doi.org/10.1128/IAI.69.3.1322-1328.2001>
- 39 Busch, A. and Waksman, G. (2012) Chaperone-usher pathways: diversity and pilus assembly mechanism. *Philos. Trans. R. Soc. Lond B Biol. Sci.* **367**, 1112–1122 <https://doi.org/10.1098/rstb.2011.0206>
- 40 Garnett, J.A., Martinez-Santos, V.I., Saldana, Z., Pape, T., Hawthorne, W., Chan, J. et al. (2012) Structural insights into the biogenesis and biofilm formation by the *Escherichia coli* common pilus. *Proc. Natl Acad. Sci. U.S.A.* **109**, 3950–3955 <https://doi.org/10.1073/pnas.1106733109>
- 41 Rendón, M.A., Saldana, Z., Erdem, A.L., Monteiro-Neto, V., Vazquez, A., Kaper, J.B. et al. (2007) Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization. *Proc. Natl Acad. Sci. U.S.A.* **104**, 10637–10642 <https://doi.org/10.1073/pnas.0704104104>
- 42 Sangermani, M., Hug, I., Sauter, N., Pfohl, T. and Jenal, U. (2019) Tad pili play a dynamic role in *Caulobacter crescentus* surface colonization. *mBio* **10**, e01237-19 <https://doi.org/10.1128/mBio.01237-19>
- 43 Reguera, G. and Kolter, R. (2005) Virulence and the environment: a novel role for *Vibrio cholerae* toxin-coregulated pili in biofilm formation on chitin. *J. Bacteriol.* **187**, 3551–3555 <https://doi.org/10.1128/JB.187.10.3551-3555.2005>
- 44 Secor, P.R., Sweere, J.M., Michaels, L.A., Malkovskiy, A.V., Lazzareschi, D., Katznelson, E. et al. (2015) Filamentous bacteriophage promote biofilm assembly and function. *Cell Host Microbe* **18**, 549–559 <https://doi.org/10.1016/j.chom.2015.10.013>
- 45 Secor, P.R., Burgener, E.B., Kinnersley, M., Jennings, L.K., Roman-Cruz, V., Popescu, M. et al. (2020) Pf bacteriophage and their impact on *Pseudomonas* virulence, mammalian immunity, and chronic infections. *Front. Immunol.* **11**, 244 <https://doi.org/10.3389/fimmu.2020.00244>
- 46 Tarafder, A.K., von Kögelen, A., Mellul, A.J., Schulze, U., Aarts, D. and Bharat, T.A.M. (2020) Phage liquid crystalline droplets form occlusive sheaths that encapsulate and protect infectious rod-shaped bacteria. *Proc. Natl Acad. Sci. U.S.A.* **117**, 4724–4731 <https://doi.org/10.1073/pnas.1917726117>
- 47 Böhning, J., Graham, M., Letham, S.C., Davis, L.K., Schulze, U., Stansfeld, P.J. et al. (2023) Biophysical basis of filamentous phage tactoid-mediated antibiotic tolerance in *P. aeruginosa*. *Nat. Commun.* **14**, 8429 <https://doi.org/10.1038/s41467-023-44160-8>
- 48 Secor, P.R., Jennings, L.K., Michaels, L.A., Sweere, J.M., Singh, P.K., Parks, W.C. et al. (2016) Biofilm assembly becomes crystal clear - filamentous bacteriophage organize the *Pseudomonas aeruginosa* biofilm matrix into a liquid crystal. *Microb. Cell* **3**, 49–52 <https://doi.org/10.15698/mic2016.01.475>
- 49 Ma, J., Cheng, X., Xu, Z., Zhang, Y., Valle, J., Fan, S. et al. (2021) Structural mechanism for modulation of functional amyloid and biofilm formation by *Staphylococcal* Bap protein switch. *EMBO J.* **40**, e107500 <https://doi.org/10.15252/emj.202107500>

- 50 Taglialegna, A., Navarro, S., Ventura, S., Garnett, J.A., Matthews, S., Penades, J.R. et al. (2016) Staphylococcal Bap proteins build amyloid scaffold biofilm matrices in response to environmental signals. *PLoS Pathog.* **12**, e1005711 <https://doi.org/10.1371/journal.ppat.1005711>
- 51 Klemm, P., Vejborg, R.M. and Sherlock, O. (2006) Self-associating autotransporters, SAATs: functional and structural similarities. *Int. J. Med. Microbiol.* **296**, 187–195 <https://doi.org/10.1016/j.jimm.2005.10.002>
- 52 Klemm, P., Hjerrild, L., Gjermansen, M. and Schembri, M.A. (2004) Structure-function analysis of the self-recognizing Antigen 43 autotransporter protein from *Escherichia coli*. *Mol. Microbiol.* **51**, 283–296 <https://doi.org/10.1046/j.1365-2958.2003.03833.x>
- 53 Wells, T.J., Tree, J.J., Ulett, G.C. and Schembri, M.A. (2007) Autotransporter proteins: novel targets at the bacterial cell surface. *FEMS Microbiol. Lett.* **274**, 163–172 <https://doi.org/10.1111/j.1574-6968.2007.00833.x>
- 54 Sullan, R.M., Li, J.K., Crowley, P.J., Brady, L.J. and Dufrene, Y.F. (2015) Binding forces of Streptococcus mutans P1 adhesin. *ACS Nano* **9**, 1448–1460 <https://doi.org/10.1021/nn5058886>
- 55 Melia, C.E., Bolla, J.R., Katharios-Lanwermyer, S., Mihaylov, D.B., Hoffmann, P.C., Huo, J. et al. (2021) Architecture of cell-cell junctions in situ reveals a mechanism for bacterial biofilm inhibition. *Proc. Natl Acad. Sci. U.S.A.* **118**, e2109940118 <https://doi.org/10.1073/pnas.2109940118>
- 56 Borlee, B.R., Goldman, A.D., Murakami, K., Samudrala, R., Wozniak, D.J. and Parsek, M.R. (2010) *Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. *Mol. Microbiol.* **75**, 827–842 <https://doi.org/10.1111/j.1365-2958.2009.06991.x>
- 57 Reichhardt, C., Jacobs, H.M., Matwichuk, M., Wong, C., Wozniak, D.J. and Parsek, M.R. (2020) The versatile *Pseudomonas aeruginosa* biofilm matrix protein CdrA promotes aggregation through different extracellular exopolysaccharide interactions. *J. Bacteriol.* **202**, e00216–20 <https://doi.org/10.1128/JB.00216-20>
- 58 Passos da Silva, D., Matwichuk, M.L., Townsend, D.O., Reichhardt, C., Lamba, D., Wozniak, D.J. et al. (2019) The *Pseudomonas aeruginosa* lectin LecB binds to the exopolysaccharide Psl and stabilizes the biofilm matrix. *Nat. Commun.* **10**, 2183 <https://doi.org/10.1038/s41467-019-10201-4>
- 59 Tielker, D., Hacker, S., Loris, R., Strathmann, M., Wingender, J., Wilhelm, S. et al. (2005) *Pseudomonas aeruginosa* lectin LecB is located in the outer membrane and is involved in biofilm formation. *Microbiology* **151**, 1313–1323 <https://doi.org/10.1099/mic.0.27701-0>
- 60 Boyd, C.D., Smith, T.J., El-Kirat-Chatel, S., Newell, P.D., Dufrene, Y.F. and O'Toole, G.A. (2014) Structural features of the *Pseudomonas fluorescens* biofilm adhesin LapA required for LapG-dependent cleavage, biofilm formation, and cell surface localization. *J. Bacteriol.* **196**, 2775–2788 <https://doi.org/10.1128/JB.01629-14>
- 61 Newell, P.D., Boyd, C.D., Sondermann, H. and O'Toole, G.A. (2011) A c-di-GMP effector system controls cell adhesion by inside-out signaling and surface protein cleavage. *PLoS Biol.* **9**, e1000587 <https://doi.org/10.1371/journal.pbio.1000587>
- 62 Berk, V., Fong, J.C., Dempsey, G.T., Develioglou, O.N., Zhuang, X., Liphardt, J. et al. (2012) Molecular architecture and assembly principles of *Vibrio cholerae* biofilms. *Science* **337**, 236–239 <https://doi.org/10.1126/science.1222981>
- 63 Fong, J.C. and Yildiz, F.H. (2007) The *rbmBCDEF* gene cluster modulates development of rugose colony morphology and biofilm formation in *Vibrio cholerae*. *J. Bacteriol.* **189**, 2319–2330 <https://doi.org/10.1128/JB.01569-06>
- 64 Huang, X., Nero, T., Weerasekera, R., Matej, K.H., Hinbest, A., Jiang, Z. et al. (2023) *Vibrio cholerae* biofilms use modular adhesins with glycan-targeting and nonspecific surface binding domains for colonization. *Nat. Commun.* **14**, 2104 <https://doi.org/10.1038/s41467-023-37660-0>
- 65 Fong, J.C., Karplus, K., Schoolnik, G.K. and Yildiz, F.H. (2006) Identification and characterization of RbmA, a novel protein required for the development of rugose colony morphology and biofilm structure in *Vibrio cholerae*. *J. Bacteriol.* **188**, 1049–1059 <https://doi.org/10.1128/JB.188.3.1049-1059.2006>
- 66 Fong, J.C., Rogers, A., Michael, A.K., Parsley, N.C., Cornell, W.-C., Lin, Y.-C. et al. (2017) Structural dynamics of RbmA governs plasticity of *Vibrio cholerae* biofilms. *Elife* **6**, e26163 <https://doi.org/10.7554/eLife.26163>
- 67 Giglio, K.M., Fong, J.C., Yildiz, F.H. and Sondermann, H. (2013) Structural basis for biofilm formation via the *Vibrio cholerae* matrix protein RbmA. *J. Bacteriol.* **195**, 3277–3286 <https://doi.org/10.1128/JB.00374-13>
- 68 Fong, J.N. and Yildiz, F.H. (2015) Biofilm matrix proteins. In *Microbial Biofilms* (Ghannoum, M., Parsek, M., Whiteley, M. and Mukherjee, P.K.), pp. 201–222, Wiley
- 69 Hogley, L., Ostrowski, A., Rao, F.V., Bromley, K.M., Porter, M., Prescott, A.R. et al. (2013) BslA is a self-assembling bacterial hydrophobin that coats the *Bacillus subtilis* biofilm. *Proc. Natl Acad. Sci. U.S.A.* **110**, 13600–13605 <https://doi.org/10.1073/pnas.1306390110>
- 70 Schommer, N.N., Christner, M., Hentschke, M., Ruckdeschel, K., Apfelbacher, M. and Rohde, H. (2011) *Staphylococcus epidermidis* uses distinct mechanisms of biofilm formation to interfere with phagocytosis and activation of mouse macrophage-like cells 774A.1. *Infect. Immun.* **79**, 2267–2276 <https://doi.org/10.1128/IAI.01142-10>
- 71 Nguyen, H.T.T., Nguyen, T.H. and Otto, M. (2020) The staphylococcal exopolysaccharide PIA - biosynthesis and role in biofilm formation, colonization, and infection. *Comput. Struct. Biotechnol. J.* **18**, 3324–3334 <https://doi.org/10.1016/j.csbj.2020.10.027>
- 72 Franklin, M.J., Nivens, D.E., Weadge, J.T. and Howell, P.L. (2011) Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, Pel, and Psl. *Front. Microbiol.* **2**, 167 <https://doi.org/10.3389/fmicb.2011.00167>
- 73 Ma, L., Conover, M., Lu, H., Parsek, M.R., Bayles, K. and Wozniak, D.J. (2009) Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. *PLoS Pathog.* **5**, e1000354 <https://doi.org/10.1371/journal.ppat.1000354>
- 74 Le Mauff, F., Razvi, E., Reichhardt, C., Sivarajah, P., Parsek, M.R., Howell, P.L. et al. (2022) The Pel polysaccharide is predominantly composed of a dimeric repeat of α -1,4 linked galactosamine and N-acetylgalactosamine. *Commun. Biol.* **5**, 502 <https://doi.org/10.1038/s42003-022-03453-2>
- 75 Balducci, E., Papi, F., Capiabbi, D.E. and Del Bino, L. (2023) Polysaccharides' structures and functions in biofilm architecture of antimicrobial-resistant (AMR) pathogens. *Int. J. Mol. Sci.* **24**, 4030 <https://doi.org/10.3390/jms24044030>
- 76 Colvin, K.M., Irie, Y., Tart, C.S., Urbano, R., Whitney, J.C., Ryder, C. et al. (2012) The Pel and Psl polysaccharides provide *Pseudomonas aeruginosa* structural redundancy within the biofilm matrix. *Environ. Microbiol.* **14**, 1913–1928 <https://doi.org/10.1111/j.1462-2920.2011.02657.x>
- 77 Jennings, L.K., Storek, K.M., Ledvina, H.E., Coulon, C., Marmont, L.S., Sadovskaya, I. et al. (2015) Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the *Pseudomonas aeruginosa* biofilm matrix. *Proc. Natl Acad. Sci. U.S.A.* **112**, 11353–11358 <https://doi.org/10.1073/pnas.1503058112>
- 78 Marvig, R.L., Sommer, L.M., Molin, S. and Johansen, H.K. (2015) Convergent evolution and adaptation of *Pseudomonas aeruginosa* within patients with cystic fibrosis. *Nat. Genet.* **47**, 57–64 <https://doi.org/10.1038/ng.3148>

- 79 Branda, S.S., González-Pastor, J.E., Dervyn, E., Ehrlich, S.D., Losick, R. and Kolter, R. (2004) Genes involved in formation of structured multicellular communities by *Bacillus subtilis*. *J. Bacteriol.* **186**, 3970–3979 <https://doi.org/10.1128/JB.186.12.3970-3979.2004>
- 80 Branda, S.S., Chu, F., Kearns, D.B., Losick, R. and Kolter, R. (2006) A major protein component of the *Bacillus subtilis* biofilm matrix. *Mol. Microbiol.* **59**, 1229–1238 <https://doi.org/10.1111/j.1365-2958.2005.05020.x>
- 81 Peng, N., Cai, P., Mortimer, M., Wu, Y., Gao, C. and Huang, Q. (2020) The exopolysaccharide-eDNA interaction modulates 3D architecture of *Bacillus subtilis* biofilm. *BMC Microbiol.* **20**, 115 <https://doi.org/10.1186/s12866-020-01789-5>
- 82 Fong, J.C.N., Syed, K.A., Klose, K.E. and Yildiz, F.H. (2010) Role of *Vibrio* polysaccharide (vps) genes in VPS production, biofilm formation and *Vibrio cholerae* pathogenesis. *Microbiology* **156**, 2757–2769 <https://doi.org/10.1099/mic.0.040196-0>
- 83 Serra, D.O., Richter, A.M. and Hengge, R. (2013) Cellulose as an architectural element in spatially structured *Escherichia coli* biofilms. *J. Bacteriol.* **195**, 5540–5554 <https://doi.org/10.1128/JB.00946-13>
- 84 Whitchurch, C.B., Tolker-Nielsen, T., Ragas, P.C. and Mattick, J.S. (2002) Extracellular DNA required for bacterial biofilm formation. *Science* **295**, 1487 <https://doi.org/10.1126/science.295.5559.1487>
- 85 Campoccia, D., Montanaro, L. and Arciola, C.R. (2021) Extracellular DNA (eDNA). A major ubiquitous element of the bacterial biofilm architecture. *Int. J. Mol. Sci.* **22**, 9100 <https://doi.org/10.3390/ijms22169100>
- 86 Devaraj, A., Buzzo, J.R., Mashburn-Warren, L., Gloag, E.S., Novotny, L.A., Stoodley, P. et al. (2019) The extracellular DNA lattice of bacterial biofilms is structurally related to Holliday junction recombination intermediates. *Proc. Natl Acad. Sci. U.S.A.* **116**, 25068–25077 <https://doi.org/10.1073/pnas.1909017116>
- 87 Buzzo, J.R., Devaraj, A., Gloag, E.S., Jurcisek, J.A., Robledo-Avila, F., Kesler, T. et al. (2021) Z-form extracellular DNA is a structural component of the bacterial biofilm matrix. *Cell* **184**, 5740–5758.e17 <https://doi.org/10.1016/j.cell.2021.10.010>
- 88 Taglialegna, A., Lasa, I. and Valle, J. (2016) Amyloid structures as biofilm matrix scaffolds. *J. Bacteriol.* **198**, 2579–2588 <https://doi.org/10.1128/JB.00122-16>
- 89 Gallo, P.M., Rapsinski, G.J., Wilson, R.P., Oppong, G.O., Sriram, U., Goulian, M. et al. (2015) Amyloid-DNA composites of bacterial biofilms stimulate autoimmunity. *Immunity* **42**, 1171–1184 <https://doi.org/10.1016/j.immuni.2015.06.002>
- 90 Olsén, A., Jonsson, A. and Normark, S. (1989) Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. *Nature* **338**, 652–655 <https://doi.org/10.1038/338652a0>
- 91 Austin, J.W., Sanders, G., Kay, W.W. and Collinson, S.K. (1998) Thin aggregative fimbriae enhance *Salmonella enteritidis* biofilm formation. *FEMS Microbiol. Lett.* **162**, 295–301 <https://doi.org/10.1111/j.1574-6968.1998.tb13012.x>
- 92 Sleutel, M., Pradhan, B., Volkov, A.N. and Remaut, H. (2023) Structural analysis and architectural principles of the bacterial amyloid curli. *Nat. Commun.* **14**, 2822 <https://doi.org/10.1038/s41467-023-38204-2>
- 93 Dueholm, M.S., Albertsen, M., Otzen, D. and Nielsen, P.H. (2012) Curli functional amyloid systems are phylogenetically widespread and display large diversity in operon and protein structure. *PLoS One* **7**, e51274 <https://doi.org/10.1371/journal.pone.0051274>
- 94 Eisenberg, D. and Jucker, M. (2012) The amyloid state of proteins in human diseases. *Cell* **148**, 1188–1203 <https://doi.org/10.1016/j.cell.2012.02.022>
- 95 Pham, C.L., Kwan, A.H. and Sunde, M. (2014) Functional amyloid: widespread in Nature, diverse in purpose. *Essays Biochem.* **56**, 207–219 <https://doi.org/10.1042/bse0560207>
- 96 Fowler, D.M., Koulou, A.V., Balch, W.E. and Kelly, J.W. (2007) Functional amyloid – from bacteria to humans. *Trends Biochem. Sci.* **32**, 217–224 <https://doi.org/10.1016/j.tibs.2007.03.003>
- 97 Diehl, A., Roske, Y., Ball, L., Chowdhury, A., Hiller, M., Molière, N. et al. (2018) Structural changes of TasA in biofilm formation of *Bacillus subtilis*. *Proc. Natl Acad. Sci. U.S.A.* **115**, 3237–3242 <https://doi.org/10.1073/pnas.1718102115>
- 98 Hammar, M., Amqvist, A., Bian, Z., Olsén, A. and Normark, S. (1995) Expression of two csg operons is required for production of fibronectin- and congo red-binding curli polymers in *Escherichia coli* K-12. *Mol. Microbiol.* **18**, 661–670 https://doi.org/10.1111/j.1365-2958.1995.mmi_18040661.x
- 99 Hammer, N.D., Schmidt, J.C. and Chapman, M.R. (2007) The curli nucleator protein, CsgB, contains an amyloidogenic domain that directs CsgA polymerization. *Proc. Natl Acad. Sci. U.S.A.* **104**, 12494–12499 <https://doi.org/10.1073/pnas.0703310104>
- 100 Evans, M.L., Chorell, E., Taylor, J.D., Åden, J., Götheson, A., Li, F. et al. (2015) The bacterial curli system possesses a potent and selective inhibitor of amyloid formation. *Mol. Cell* **57**, 445–455 <https://doi.org/10.1016/j.molcel.2014.12.025>
- 101 Nenninger, A.A., Robinson, L.S., Hammer, N.D., Epstein, E.A., Badtke, M.P., Hultgren, S.J. et al. (2011) CsgE is a curli secretion specificity factor that prevents amyloid fibre aggregation. *Mol. Microbiol.* **81**, 486–499 <https://doi.org/10.1111/j.1365-2958.2011.07706.x>
- 102 Robinson, L.S., Ashman, E.M., Hultgren, S.J. and Chapman, M.R. (2006) Secretion of curli fibre subunits is mediated by the outer membrane-localized CsgG protein. *Mol. Microbiol.* **59**, 870–881 <https://doi.org/10.1111/j.1365-2958.2005.04997.x>
- 103 Yan, Z., Yin, M., Chen, J. and Li, X. (2020) Assembly and substrate recognition of curli biogenesis system. *Nat. Commun.* **11**, 241 <https://doi.org/10.1038/s41467-019-14145-7>
- 104 Nenninger, A.A., Robinson, L.S. and Hultgren, S.J. (2009) Localized and efficient curli nucleation requires the chaperone-like amyloid assembly protein CsgF. *Proc. Natl Acad. Sci. U.S.A.* **106**, 900–905 <https://doi.org/10.1073/pnas.0812143106>
- 105 Brombacher, E., Baratto, A., Dorel, C. and Landini, P. (2006) Gene expression regulation by the Curli activator CsgD protein: modulation of cellulose biosynthesis and control of negative determinants for microbial adhesion. *J. Bacteriol.* **188**, 2027–2037 <https://doi.org/10.1128/JB.188.6.2027-2037.2006>
- 106 Scheres, S.H., Zhang, W., Falcon, B. and Goedert, M. (2020) Cryo-EM structures of tau filaments. *Curr. Opin. Struct. Biol.* **64**, 17–25 <https://doi.org/10.1016/j.sbi.2020.05.011>
- 107 Balistreri, A., Goetzler, E. and Chapman, M. (2020) Functional amyloids are the rule rather than the exception in cellular biology. *Microorganisms* **8**, 1951 <https://doi.org/10.3390/microorganisms8121951>
- 108 Rasmussen, H.O., Kumar, A., Shin, B., Stylianou, F., Sewell, L., Xu, Y. et al. (2023) FapA is an intrinsically disordered chaperone for *Pseudomonas* functional amyloid FapC. *J. Mol. Biol.* **435**, 167878 <https://doi.org/10.1016/j.jmb.2022.167878>
- 109 Rouse, S.L., Hawthorne, W.J., Berry, J.L., Chorev, D.S., Ionescu, S.A., Lambert, S. et al. (2017) A new class of hybrid secretion system is employed in *Pseudomonas* amyloid biogenesis. *Nat. Commun.* **8**, 263 <https://doi.org/10.1038/s41467-017-00361-6>
- 110 Murray, C.J.L., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A. et al. (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **399**, 629–655 [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)

- 111 Marvasi, M., Visscher, P.T. and Martinez, L.C. (2010) Exopolymeric substances (EPS) from *Bacillus subtilis*: polymers and genes encoding their synthesis. *FEMS Microbiol. Lett.* **313**, 1–9 <https://doi.org/10.1111/j.1574-6968.2010.02085.x>
- 112 Kobayashi, K. and Iwano, M. (2012) BslA(YuaB) forms a hydrophobic layer on the surface of *Bacillus subtilis* biofilms. *Mol. Microbiol.* **85**, 51–66 <https://doi.org/10.1111/j.1365-2958.2012.08094.x>
- 113 Holey, L., Ostrowski, A., Rao, F.V., Bromley, K.M., Porter, M., Prescott, A.R. et al. (2015) BslA is a self-assembling bacterial hydrophobin that coats the *Bacillus subtilis* biofilm (vol 110, pg 13600, 2013). *Proc. Natl Acad. Sci. U.S.A.* **112**, E5371–E5375 <https://doi.org/10.1073/pnas.1516042112>
- 114 Arnaouteli, S., Bamford, N.C., Stanley-Wall, N.R. and Kovács, Á.T. (2021) *Bacillus subtilis* biofilm formation and social interactions. *Nat. Rev. Microbiol.* **19**, 600–614 <https://doi.org/10.1038/s41579-021-00540-9>
- 115 Stöver, A.G. and Driks, A. (1999) Secretion, localization, and antibacterial activity of TasA, a *Bacillus subtilis* spore-associated protein. *J. Bacteriol.* **181**, 1664–1672 <https://doi.org/10.1128/JB.181.5.1664-1672.1999>
- 116 Erskine, E., Morris, R.J., Schor, M., Earl, C., Gillespie, R.M.C., Bromley, K.M. et al. (2018) Formation of functional, non-amyloidogenic fibres by recombinant *Bacillus subtilis* TasA. *Mol. Microbiol.* **110**, 897–913 <https://doi.org/10.1111/mmi.13985>
- 117 Waksman, G. and Hultgren, S.J. (2009) Structural biology of the chaperone-usher pathway of pilus biogenesis. *Nat. Rev. Microbiol.* **7**, 765–774 <https://doi.org/10.1038/nrmicro2220>
- 118 Roske, Y., Lindemann, F., Diehl, A., Cremer, N., Higman, V.A., Schlegel, B. et al. (2023) TapA acts as specific chaperone in TasA filament formation by strand complementation. *Proc. Natl Acad. Sci. U.S.A.* **120**, e2217070120 <https://doi.org/10.1073/pnas.2217070120>
- 119 Ghraeyeb, M., Hayet, S., Lester-Zer, N., Levi-Kalishman, Y. and Chai, L. (2021) Fibrillar polymorphism of the bacterial extracellular matrix protein TasA. *Microorganisms* **9**, 529 <https://doi.org/10.3390/microorganisms9030529>
- 120 Cámara-Almirón, J., Navarro, Y., Díaz-Martínez, L., Magno-Pérez-Bryan, M.C., Molina-Santiago, C., Pearson, J.R. et al. (2020) Dual functionality of the amyloid protein TasA in *Bacillus* physiology and fitness on the phylloplane. *Nat. Commun.* **11**, 1859 <https://doi.org/10.1038/s41467-020-15758-z>
- 121 Wang, F., Cvirkaitė-Krupovic, V., Krupovic, M. and Egelman, E.H. (2022) Archaeal bundling pili of *Pyrobaculum calidifontis* reveal similarities between archaeal and bacterial biofilms. *Proc. Natl Acad. Sci. U.S.A.* **119**, e2207037119 <https://doi.org/10.1073/pnas.2207037119>
- 122 Peschel, A. and Otto, M. (2013) Phenol-soluble modulins and staphylococcal infection. *Nat. Rev. Microbiol.* **11**, 667–673 <https://doi.org/10.1038/nrmicro3110>
- 123 Wang, R., Braughton, K.R., Kretschmer, D., Bach, T.H., Queck, S.Y., Li, M. et al. (2007) Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat. Med.* **13**, 1510–1514 <https://doi.org/10.1038/nm1656>
- 124 Tam, K. and Torres, V.J. (2019) *Staphylococcus aureus* secreted toxins and extracellular enzymes. *Microbiol. Spectr.* **7** <https://doi.org/10.1128/microbiolspec.GPP3-0039-2018>
- 125 Salinas, N., Colletier, J.P., Moshe, A. and Landau, M. (2018) Extreme amyloid polymorphism in *Staphylococcus aureus* virulent PSM α peptides. *Nat. Commun.* **9**, 3512 <https://doi.org/10.1038/s41467-018-05490-0>
- 126 Nuccio, S.P. and Bäuml, A.J. (2007) Evolution of the chaperone/usher assembly pathway: fimbrial classification goes Greek. *Microbiol. Mol. Biol. Rev.* **71**, 551–575 <https://doi.org/10.1128/MMBR.00014-07>
- 127 Remaut, H., Rose, R.J., Hannan, T.J., Hultgren, S.J., Radford, S.E., Ashcroft, A.E. et al. (2006) Donor-strand exchange in chaperone-assisted pilus assembly proceeds through a concerted β strand displacement mechanism. *Mol. Cell* **22**, 831–842 <https://doi.org/10.1016/j.molcel.2006.05.033>
- 128 Hospenthal, M.K., Redzej, A., Dodson, K., Ukleja, M., Frenz, B., Rodrigues, C. et al. (2016) Structure of a chaperone-usher pilus reveals the molecular basis of rod uncoiling. *Cell* **164**, 269–278 <https://doi.org/10.1016/j.cell.2015.11.049>
- 129 Hospenthal, M.K., Zyla, D., Costa, T.R.D., Redzej, A., Giese, C., Lillington, J. et al. (2017) The cryoelectron microscopy structure of the type 1 chaperone-usher pilus rod. *Structure* **25**, 1829–1838.e4 <https://doi.org/10.1016/j.str.2017.10.004>
- 130 Khanna, K. and Villa, E. (2022) Revealing bacterial cell biology using cryo-electron tomography. *Curr. Opin. Struct. Biol.* **75**, 102419 <https://doi.org/10.1016/j.sbi.2022.102419>
- 131 Hay, I.D. and Lithgow, T. (2019) Filamentous phages: masters of a microbial sharing economy. *EMBO Rep.* **20**, e47427 <https://doi.org/10.15252/embr.201847427>
- 132 Lesbats, P., Engelman, A.N. and Cherepanov, P. (2016) Retroviral DNA integration. *Chem. Rev.* **116**, 12730–12757 <https://doi.org/10.1021/acs.chemrev.6b00125>
- 133 Bille, E., Meyer, J., Jamet, A., Euphrasie, D., Barnier, J.P., Brissac, T. et al. (2017) A virulence-associated filamentous bacteriophage of *Neisseria meningitidis* increases host-cell colonisation. *PLoS Pathog.* **13**, e1006495 <https://doi.org/10.1371/journal.ppat.1006495>
- 134 Marvin, D.A., Symmons, M.F. and Straus, S.K. (2014) Structure and assembly of filamentous bacteriophages. *Prog. Biophys. Mol. Biol.* **114**, 80–122 <https://doi.org/10.1016/j.pbiomolbio.2014.02.003>
- 135 Whiteley, M., Banger, M.G., Bumgarner, R.E., Parsek, M.R., Teitzel, G.M., Lory, S. et al. (2001) Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature* **413**, 860–864 <https://doi.org/10.1038/35101627>
- 136 Burgener, E.B., Sweere, J.M., Bach, M.S., Secor, P.R., Haddock, N., Jennings, L.K. et al. (2019) Filamentous bacteriophages are associated with chronic *Pseudomonas* lung infections and antibiotic resistance in cystic fibrosis. *Sci. Transl. Med.* **11**, eaau9748 <https://doi.org/10.1126/scitranslmed.aau9748>
- 137 Sweere, J.M., Van Belleghem, J.D., Ishak, H., Bach, M.S., Popescu, M., Sunkari, V. et al. (2019) Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science* **363**, eaat9691 <https://doi.org/10.1126/science.aat9691>
- 138 Lekkerkerker, H.N. and Tuinier, R. (2011) Depletion interaction. In *Colloids and the Depletion Interaction*, pp. 57–108, Springer, Dordrecht
- 139 Rice, S.A., Tan, C.H., Mikkelsen, P.J., Kung, V., Woo, J., Tay, M. et al. (2009) The biofilm life cycle and virulence of *Pseudomonas aeruginosa* are dependent on a filamentous prophage. *ISME J.* **3**, 271–282 <https://doi.org/10.1038/ismej.2008.109>
- 140 Roux, S., Krupovic, M., Daly, R.A., Borges, A.L., Nayfach, S., Schulz, F. et al. (2019) Cryptic inoviruses revealed as pervasive in bacteria and archaea across Earth's biomes. *Nat. Microbiol.* **4**, 1895–1906 <https://doi.org/10.1038/s41564-019-0510-x>
- 141 Davis, B.M., Moyer, K.E., Boyd, E.F. and Waldor, M.K. (2000) CTX prophages in classical biotype *Vibrio cholerae*: functional phage genes but dysfunctional phage genomes. *J. Bacteriol.* **182**, 6992–6998 <https://doi.org/10.1128/JB.182.24.6992-6998.2000>
- 142 Johnson, G., Banerjee, S. and Putonti, C. (2022) Diversity of *Pseudomonas aeruginosa* temperate phages. *mSphere* **7**, e0101521 <https://doi.org/10.1128/msphere.01015-21>

- 143 Stewart, P.S. and Costerton, J.W. (2001) Antibiotic resistance of bacteria in biofilms. *Lancet* **358**, 135–138 [https://doi.org/10.1016/S0140-6736\(01\)05321-1](https://doi.org/10.1016/S0140-6736(01)05321-1)
- 144 Hentzer, M., Teitzel, G.M., Balzer, G.J., Heydorn, A., Molin, S., Givskov, M. et al. (2001) Alginate overproduction affects *Pseudomonas aeruginosa* biofilm structure and function. *J. Bacteriol.* **183**, 5395–5401 <https://doi.org/10.1128/JB.183.18.5395-5401.2001>
- 145 Harrison, J.J., Ceri, H., Stremick, C.A. and Turner, R.J. (2004) Biofilm susceptibility to metal toxicity. *Environ. Microbiol.* **6**, 1220–1227 <https://doi.org/10.1111/j.1462-2920.2004.00656.x>
- 146 Mann, E.E. and Wozniak, D.J. (2012) *Pseudomonas* biofilm matrix composition and niche biology. *FEMS Microbiol. Rev.* **36**, 893–916 <https://doi.org/10.1111/j.1574-6976.2011.00322.x>
- 147 Jackson, K.D., Starkey, M., Kremer, S., Parsek, M.R. and Wozniak, D.J. (2004) Identification of psl, a locus encoding a potential exopolysaccharide that is essential for *Pseudomonas aeruginosa* PAO1 biofilm formation. *J. Bacteriol.* **186**, 4466–4475 <https://doi.org/10.1128/JB.186.14.4466-4475.2004>
- 148 Friedman, L. and Kolter, R. (2004) Two genetic loci produce distinct carbohydrate-rich structural components of the *Pseudomonas aeruginosa* biofilm matrix. *J. Bacteriol.* **186**, 4457–4465 <https://doi.org/10.1128/JB.186.14.4457-4465.2004>
- 149 Ma, L., Jackson, K.D., Landry, R.M., Parsek, M.R. and Wozniak, D.J. (2006) Analysis of *Pseudomonas aeruginosa* conditional psl variants reveals roles for the psl polysaccharide in adhesion and maintaining biofilm structure postattachment. *J. Bacteriol.* **188**, 8213–8221 <https://doi.org/10.1128/JB.01202-06>
- 150 Whelan, F., Lafita, A., Gilbert, J., Degut, C., Griffiths, S.C., Jenkins, H.T. et al. (2021) Periscope Proteins are variable-length regulators of bacterial cell surface interactions. *Proc. Natl Acad. Sci. U.S.A.* **118**, e2101349118 <https://doi.org/10.1073/pnas.2101349118>
- 151 DiGiandomenico, A., Warrenner, P., Hamilton, M., Guillard, S., Ravn, P., Minter, R. et al. (2012) Identification of broadly protective human antibodies to *Pseudomonas aeruginosa* exopolysaccharide Psl by phenotypic screening. *J. Exp. Med.* **209**, 1273–1287 <https://doi.org/10.1084/jem.20120033>
- 152 Ali, S.O., Yu, X.Q., Robbie, G.J., Wu, Y., Shoemaker, K., Yu, L. et al. (2019) Phase 1 study of MEDI3902, an investigational anti-*Pseudomonas aeruginosa* PcrV and Psl bispecific human monoclonal antibody, in healthy adults. *Clin. Microbiol. Infect.* **25**, 629.e1–629.e6 <https://doi.org/10.1016/j.cmi.2018.08.004>
- 153 Funken, H., Bartels, K.M., Wilhelm, S., Brocker, M., Bott, M., Bains, M. et al. (2012) Specific association of lectin LecB with the surface of *Pseudomonas aeruginosa*: role of outer membrane protein OprF. *PLoS One* **7**, e46857 <https://doi.org/10.1371/journal.pone.0046857>
- 154 Blanco-Cabra, N., Paetzold, B., Ferrar, T., Mazzolini, R., Torrents, E., Serrano, L. et al. (2020) Characterization of different alginate lyases for dissolving *Pseudomonas aeruginosa* biofilms. *Sci. Rep.* **10**, 9390 <https://doi.org/10.1038/s41598-020-66293-2>
- 155 Powell, L.C., Pritchard, M.F., Ferguson, E.L., Powell, K.A., Patel, S.U., Rye, P.D. et al. (2018) Targeted disruption of the extracellular polymeric network of *Pseudomonas aeruginosa* biofilms by alginate oligosaccharides. *NPJ Biofilms Microbiomes* **4**, 13 <https://doi.org/10.1038/s41522-018-0056-3>
- 156 Patel, K.K., Tripathi, M., Pandey, N., Agrawal, A.K., Gade, S., Anjum, M.M. et al. (2019) Alginate lyase immobilized chitosan nanoparticles of ciprofloxacin for the improved antimicrobial activity against the biofilm associated mucoid *P. aeruginosa* infection in cystic fibrosis. *Int. J. Pharm.* **563**, 30–42 <https://doi.org/10.1016/j.ijpharm.2019.03.051>
- 157 Kamruzzaman, M., Udden, S.M., Cameron, D.E., Calderwood, S.B., Nair, G.B., Mekalanos, J.J. et al. (2010) Quorum-regulated biofilms enhance the development of conditionally viable, environmental *Vibrio cholerae*. *Proc. Natl Acad. Sci. U.S.A.* **107**, 1588–1593 <https://doi.org/10.1073/pnas.0913404107>
- 158 Purdy, A.E. and Watnick, P.I. (2011) Spatially selective colonization of the arthropod intestine through activation of *Vibrio cholerae* biofilm formation. *Proc. Natl Acad. Sci. U.S.A.* **108**, 19737–19742 <https://doi.org/10.1073/pnas.1111530108>
- 159 Devaraj, A., Buzzo, J., Rocco, C.J., Bakaletz, L.O. and Goodman, S.D. (2018) The DNABII family of proteins is comprised of the only nucleoid associated proteins required for nontypeable *Haemophilus influenzae* biofilm structure. *Microbiologyopen* **7**, e00563 <https://doi.org/10.1002/mbo3.563>
- 160 Frederiksen, B., Pressler, T., Hansen, A., Koch, C. and Høiby, N. (2006) Effect of aerosolized rhDNase (Pulmozyme®) on pulmonary colonization in patients with cystic fibrosis. *Acta Paediatr.* **95**, 1070–1074 <https://doi.org/10.1080/08035250600752466>
- 161 Gunn, J.S., Bakaletz, L.O. and Wozniak, D.J. (2016) What's on the outside matters: the role of the extracellular polymeric substance of Gram-negative biofilms in evading host immunity and as a target for therapeutic intervention. *J. Biol. Chem.* **291**, 12538–12546 <https://doi.org/10.1074/jbc.R115.707547>
- 162 Bezanilla, M., Drake, B., Nudler, E., Kashlev, M., Hansma, P.K. and Hansma, H.G. (1994) Motion and enzymatic degradation of DNA in the atomic force microscope. *Biophys. J.* **67**, 2454 [https://doi.org/10.1016/S0006-3495\(94\)80733-7](https://doi.org/10.1016/S0006-3495(94)80733-7)
- 163 Ramesh, N. and Brahmachari, S.K. (1989) Structural alteration from non-B to B-form could reflect DNase I hypersensitivity. *J. Biomol. Struct. Dyn.* **6**, 899–906 <https://doi.org/10.1080/07391102.1989.10506521>
- 164 Ghosh, P., Mondal, J., Ben-Jacob, E. and Levine, H. (2015) Mechanically-driven phase separation in a growing bacterial colony. *Proc. Natl Acad. Sci. U.S.A.* **112**, E2166–E2173 <https://doi.org/10.1073/pnas.1504948112>
- 165 Pratt, L.A. and Kolter, R. (1998) Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Mol. Microbiol.* **30**, 285–293 <https://doi.org/10.1046/j.1365-2958.1998.01061.x>
- 166 Cates, M.E., Marenduzzo, D., Pagonabarraga, I. and Tailleur, J. (2010) Arrested phase separation in reproducing bacteria creates a generic route to pattern formation. *Proc. Natl Acad. Sci. U.S.A.* **107**, 11715–11720 <https://doi.org/10.1073/pnas.1001994107>
- 167 Liu, C., Fu, X., Liu, L., Ren, X., Chau, C.K., Li, S. et al. (2011) Sequential establishment of stripe patterns in an expanding cell population. *Science* **334**, 238–241 <https://doi.org/10.1126/science.1209042>
- 168 McBride, M.J. (2001) Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. *Annu. Rev. Microbiol.* **55**, 49–75 <https://doi.org/10.1146/annurev.micro.55.1.49>
- 169 Jumper, J. and Hassabis, D. (2022) Protein structure predictions to atomic accuracy with AlphaFold. *Nat. Methods* **19**, 11–12 <https://doi.org/10.1038/s41592-021-01362-6>
- 170 Varadi, M., Anyango, S., Deshpande, M., Nair, S., Natassia, C., Yordanova, G. et al. (2022) AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Res.* **50**, D439–D444 <https://doi.org/10.1093/nar/gkab1061>
- 171 Harris, R.H. and Mitchell, R. (1973) The role of polymers in microbial aggregation. *Annu. Rev. Microbiol.* **27**, 27–50 <https://doi.org/10.1146/annurev.mi.27.100173.000331>

- 172 Neu, B. and Meiselman, H.J. (2002) Depletion-mediated red blood cell aggregation in polymer solutions. *Biophys. J.* **83**, 2482–2490 [https://doi.org/10.1016/S0006-3495\(02\)75259-4](https://doi.org/10.1016/S0006-3495(02)75259-4)
- 173 Dorken, G., Ferguson, G.P., French, C.E. and Poon, W.C. (2012) Aggregation by depletion attraction in cultures of bacteria producing exopolysaccharide. *J. R. Soc. Interface* **9**, 3490–3502 <https://doi.org/10.1098/rsif.2012.0498>
- 174 Tenney, M.W. and Stumm, W. (1965) Chemical flocculation of microorganisms in biological waste treatment. *J. Water Pollut. Control Fed.* **37**, 1370–1388 <https://www.jstor.org/stable/25035388>
- 175 Fei, C., Mao, S., Yan, J., Alert, R., Stone, H.A., Bassler, B.L. et al. (2020) Nonuniform growth and surface friction determine bacterial biofilm morphology on soft substrates. *Proc. Natl Acad. Sci. U.S.A.* **117**, 7622–7632 <https://doi.org/10.1073/pnas.1919607117>
- 176 Beroz, F., Yan, J., Sabass, B., Stone, H.A., Bassler, B.L., Wingreen, N.S. et al. (2018) Verticalization of bacterial biofilms. *Nat. Phys.* **14**, 954–960 <https://doi.org/10.1038/s41567-018-0170-4>
- 177 Pearce, P., Song, B., Skinner, D.J., Mok, R., Hartmann, R., Singh, P.K. et al. (2019) Flow-induced symmetry breaking in growing bacterial biofilms. *Phys. Rev. Lett.* **123**, 258101 <https://doi.org/10.1103/PhysRevLett.123.258101>
- 178 You, Z., Pearce, D.J.G. and Giomi, L. (2021) Confinement-induced self-organization in growing bacterial colonies. *Sci. Adv.* **7**, eabc8685 <https://doi.org/10.1126/sciadv.abc8685>
- 179 Zhang, Q., Li, J., Nijjer, J., Lu, H., Kothari, M., Alert, R. et al. (2021) Morphogenesis and cell ordering in confined bacterial biofilms. *Proc. Natl Acad. Sci. U.S.A.* **118**, e2107107118 <https://doi.org/10.1073/pnas.2107107118>
- 180 Hartmann, R., Singh, P.K., Pearce, P., Mok, R., Song, B., Díaz-Pascual, F. et al. (2019) Emergence of three-dimensional order and structure in growing biofilms. *Nat. Phys.* **15**, 251–256 <https://doi.org/10.1038/s41567-018-0356-9>
- 181 Høiby, N., Bjarnsholt, T., Moser, C., Bassi, G.L., Coenye, T., Donelli, G. et al. (2015) ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. *Clin. Microbiol. Infect.* **21**, S1–S25 <https://doi.org/10.1016/j.cmi.2014.10.024>
- 182 Ciofu, O., Rojo-Molinero, E., Macia, M.D. and Oliver, A. (2017) Antibiotic treatment of biofilm infections. *APMIS* **125**, 304–319 <https://doi.org/10.1111/apm.12673>
- 183 Lewis, K. (2005) Persister cells and the riddle of biofilm survival. *Biochemistry* **70**, 267–274 <https://doi.org/10.1007/s10541-005-0111-6>