Review

‘Phase transitions’ in bacteria – From structural transitions in free living bacteria to phenotypic transitions in bacteria within biofilms

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Abstract

Phase transitions are common in inanimate systems and have been studied extensively in natural sciences. Less explored are the rich transitions that take place at the micro- and nano-scales in biological systems. In conventional phase transitions, large-scale properties of the media change discontinuously in response to continuous changes in external conditions. Such changes play a significant role in the dynamic behaviours of organisms. In this review, we focus on some transitions in both free-living and biofilms of bacteria. Particular attention is paid to the transitions in the flagellar motors and filaments of free-living bacteria, in cellular gene expression during the biofilm growth, in the biofilm morphology transitions during biofilm expansion, and in the cell motion pattern transitions during the biofilm formation. We analyse the dynamic characteristics and biophysical mechanisms of these phase transition phenomena and point out the parallels between these transitions and conventional phase transitions. We also discuss the applications of some theoretical and numerical methods, established for conventional phase transitions in inanimate systems, in bacterial biofilms.

Keywords: Phase transitions in bacteria; Flagellar structure; Biofilm; Phenotype; Morphology

1. Introduction

1.1. Phase and phase transitions

Phase transition phenomena have been studied for over a century [1–4]. In inanimate systems, a phase transition is characterised by the appearance, or disappearance, of order on small, sometimes molecular, scales. Traditional examples are the transitions from gas to liquid and solid with decreasing temperature, corresponding to the appearances of
order in the spatial correlation functions. Other examples include the occurrence of directional correlations in magnetic systems and liquid crystals and the structural change of graphite into diamond under pressure. Phase transitions often lead to changes in macroscopic properties, such as viscosity in the gas-to-liquid transition, elastic moduli in the liquid-to-solid transition, magnetisation in the paramagnetic-to-ferromagnetic transition, and optical properties in the thermotropic-to-nematic transition in liquid crystals. In these examples, the order emerging at the molecular scale corresponds to a structural symmetry or invariance. However, the smallest scale may be sometimes much larger, such as in suspensions of interacting colloids, which can be either disordered or ordered, depending on its density. We will refer to the smallest scale in such systems as the micro-scale even though it may in fact be much larger than microns. Such a micro-structural spatial rearrangement may result in changes in the macroscopic physical properties.

Recent research in biophysics raises a pertinent question: can the concept of phase transition be extended to characterise, understand, and model biological systems? If yes, this could lead to progress on a number of important issues, including how do phase transition phenomena arise in living organisms, what roles they play in the life process, the dynamic mechanisms driving them, their macro-scale effect and purpose, etc. These issues are important because most biological systems rely on a specific internal structure, into which they self-organise to perform specific functions. Self-organisation is, by definition, generation of structures of specific order, albeit not always the simple symmetry-based orders that occur in inanimate matter. Straightforward examples are the functions of all the different systems in the human body, from cell sorting during embryo formation to tissue self-organisation [5–8]. These spatial structures can change in response to external stimuli, modifying functionality and behaviour. Examples abound in nature: pulling a B-DNA by 70 pN transforms its structure into an S-DNA, which is twice as long and of different functionality [9–14]. Torsion transforms DNA from a denatured state to a P-DNA structure, which interacts better with some proteins [9]. Stretching proteins causes a transition from folded to unfolded states, which inspired the design of better glues [9–14]. By injecting its own DNA into Escherichia coli (E. Coli), the virus bacteriophage T4 changes its tail sheath structure [15–19].

The parallels between such transitions and conventional phase transitions suggest that insight gained from the latter and traditional methods from physics may be applied to biological systems. However, this raises further questions. Most biological systems can transition into a functional state from another state (whether functional or not), each of which having its own order and structure. This means that there is a huge number of possible structural states. Can each such state be regarded as a distinct phase? If yes, then the transition from a disordered, or disorganised state, to an ordered functional one is a phase transition. But, if so, how can one apply insight from traditional phase transitions to understand or characterise better biological systems? If the answer is no, then why not and what exactly distinguishes transitions in self-organising biological systems from traditional phase transitions? Can some such transitions be regarded as phase transitions while others cannot?

Many traditional phase transitions occur in what is known as Hamiltonian systems, in which energy and thermodynamic potentials are well defined. In such systems, the phases are equilibrium states, which are minima in the thermodynamic potentials. These potentials are functions of macroscopic variables, such as temperature, pressure, density, and external fields. A typical example of such potentials, the Helmholtz free energy, is sketched in Fig. 1, as a function of one variable, say, the density \( \phi \) of the system. The two minima represent two distinct phases of different energies, and a circle represents the state of the system. Raising the temperature, for example, increases the effect of entropy and reduces the barrier between the phases, until at some critical temperature, the system makes a transition from phase A (on the left on the red curve) to phase B (on the right on the black curve).

Non-Hamiltonian inanimate systems also display a wide range of transitions and processes, through processes of self-organisation into non-random static structures or steady states with unique characteristics [8]. Such states can also be classified and characterised usefully by phase diagrams and, in some limited cases, can be even described by models similar to conventional statistical mechanics [20–22].

In contrast, biological systems are active and inherently out of thermal equilibrium. In this sense, they resemble many self-organising, out-of-equilibrium inanimate systems. However, the main difference lies in their ability to convert external resources into energy that drives essential processes. Given external resources and stimuli, most biological systems can settle into a normal steady-state operation mode. Active matter, including both biological and non-biological systems that are able to convert external resources into motion, can also settle into a rich variety of steady states. Many works in the literature on these out-of-equilibrium systems have used successfully the concept of phase transformations [15,23–25].
In this review, we will address the above questions related to phase transitions in living bacterial organisms, including structural transitions in both bacterial flagellar filaments and flagellar motors. These transitions allow a cell to switch between the motions of swimming and tumbling. We also describe the transitions that occur in the formation of bacterial biofilms, including phenotypic transitions, morphological transitions, and cell motion pattern evolutions in *Bacillus Subtilis* (*B. Subtilis*) biofilms. We discuss how these transitions proceed and affect the bacterial behaviour. The similarities and differences between these and traditional phase transitions will be analysed. Finally, we propose ways to gain insight into biological transitions by using tools developed for phase transitions and self-organisation in inanimate systems.

### 1.2. Bacterium and its flagella

Bacteria are one of the first life forms that appeared on Earth. Measuring a few microns in size, these one-cell organisms come in a variety of shapes, including spheres, rods, and spirals. Flagella, first observed by Ehrenberg in 1838, is a typical structure that drives the locomotion of bacteria [26]. Flagella are membrane-embedded filamentous organelles, which the bacteria rotate to drive themselves in aqueous environments or over semi-solid media. The energy required to generate the flagellar rotation comes from the electrochemical gradient between the cell membrane due to flux of protons and ions. The rotation itself is generated by a motor at the base of the flagellum, which is helically shaped and, therefore, acts as a propeller. The flagellum consists of three major substructures: the filament, the hook (a flexible coupling universal joint), and the basal body (consisting of a reversible rotary motor embedded in the cell wall). The filament is a hollow tube, 20 nm in diameter and 15 – 20 µm long, which usually consists of thousands of copies of a single protein called flagellin, as shown in Fig. 2 [27–30].

We focus here on the types of bacteria whose motion is driven by flagella. A flagellum is shaped as a left-handed helix that, when rotating counter-clockwise (CCW), propels the cell through the fluid. When one or more flagella separate from the flagellar bundle and change conformation to form a right-handed helix [31], as shown in Fig. 3, the bacterium stops ‘swimming’ and tumbles. This transition is induced by the flagellar motors embedded in the cell wall and the cytoplasmic membrane. The motors act similarly to man-made ones, with the rotation driven by protons or ions crossing the cellular membrane. Bacterial swimming is possible when all the motors rotate in the CCW direction, while tumbling takes place when one or more motors start rotating in the clockwise (CW) direction.

### 1.3. Biofilm

The number of bacteria in the world is staggering. For example, a typical gram of soil contains 40 million bacteria and a millilitre of fresh water normally contains about a million bacterial cells. These group together for better survival and more than 99% of bacteria in the natural world live in biofilm communities. This way they can fight better other cells and survive environmental threats.

*B. Subtilis* bacteria can be either free-living individually or part of a biofilm attached to a surface. Free-living bacteria, also known as planktonic, can attach either reversibly or irreversibly to a surface [32]. The latter involves...
strengthening of bacteria-surface adhesion and production of extracellular polymeric substances (EPS), composed primarily of polysaccharides [33] and proteins [34,35]. The EPS forms the scaffolding for the biofilm, supporting its mechanical integrity. The biofilm life cycle comes a full circle when a biofilm disperses with individual bacteria leaving [36,37], as illustrated in Fig. 4. Biofilm-based bacteria exhibit surprisingly intricate social behaviour patterns, both cooperative and competitive, made possible by their sophisticated genetic networks and cell biology. To facilitate this, the biofilm’s organisation is driven by complex physical, chemical, and biological mechanisms. Consequently, to fully understand the properties of biofilms requires an interdisciplinary approach, integrating microbiology with physics, mechanics, materials science, and chemistry. In the following, we use tools borrowed from physics to discuss transitions and phase transition phenomena in both free-living and biofilm-based bacterial cells, as well as how these phenomena shape bacterial behaviours.

2. Phase transition phenomena

2.1. Non-equilibrium phase transition in the flagellar motor switching between CCW and CW rotations

Rotary motors, which are ubiquitous in man-made machinery, also exist in biological systems. Only flagellated bacteria are known to incorporate this mechanism in their locomotive strategy. Their motion is driven by rotary bacterial flagellar motors that spin long filaments, which sprout from the cell body [38–42]. Bacterial flagellar motors are distributed across the cellular surface, each comprising several transmembrane rings that connect to the bacterium’s flagellar filament by a flexible hook. These rings are approximately 45 nm in diameter and contain about 25 different proteins, shown in Figs. 5a and b. The flagellar motors of E. Coli and Salmonella, which consist of a rotor and a dozen stator units, is powered by an electrochemical potential of protons across the cytoplasmic membrane, known as a proton motive force [43]. Marine Vibrio and extremely alkalophilic Bacillus utilise instead sodium motive force as the energy source to drive flagellar motor rotation, shown schematically in Fig. 5c.

The motors can rotate either CCW or CW and they alternate between the two. Experiments [52] have shown that regulation of the flagellar motor rotation direction is inherent to the three flagella-driven motility species: Salmonella, B. Subtilis, and E. Coli. The regulation is achieved by modifying the cellular metabolism, which changes the proton-
or ion-motive potential, and is independent of the viscosity load, i.e., the resistance to rotation [52]. The higher the motor rotation speed the longer the flagella rotate in the CW direction. The motility threshold in *B. Subtilis* is about 30 mV and the threshold for switching rotation direction is about 45 mV. By measuring the relative durations of the CW and CCW states as a function of motor speed, an analogue of a thermal-equilibrium two-state activation diagram has been constructed in [52], reproduced in Fig. 6. Specifically, reducing the protonmotive force reduces the motor speed, $\phi$, which leads to lowering of the barrier for the CW $\rightarrow$ CCW transition. This lowering can be interpreted as the Arrhenius law for the rate of chemical reactions. This measurement made possible to estimate the changes in the relative state free energies, $\delta F_{CW}(\phi)$ and $\delta F_{CCW}(\phi)$, as functions of the motor speed and the exponential increase of the activation rate as it changes from $\phi$ to $\phi-\delta\phi$ [52,53] (see Fig. 6). The signalling regulating the motor speed was shown to a signalling protein, CheY-P, with lower levels of CheY-P promoting CCW rotation and vice versa [54–57].

A recent study [58] found two different molecular architectures of the flagellar motors in *B. Burgdorferi* during CCW and CW rotations, which are triggered by the protein CheY3-P binding, as shown in Fig. 7. In the absence of bound CheY3-P, the FlIG2 proteins, which are protein components in the C-ring, interact with the inner part of the stator complex, which gives rise to the CCW state. CheY3-P induces conformational changes in the C-ring, resulting in altered interactions between the stator and the C-ring, causing in turn a switch to CW rotation. The CheY3-P
protein interacts directly with the FlgM protein, which is part of the C-ring, thereby inducing a major remodelling of the switch protein FlgG2. The back-and-forth conformational changes of FlgG2 cause the switch complex to interact with opposite sides of the rotating torque generator and it is this interaction that facilitates the switch between CW and CCW rotations, as sketched in Fig. 8.

In most conventional models, the switch is regarded as an equilibrium of a two-state system (Fig. 6), with the PMF driving a conformational change in the motor by changing the concentration of the binding CheY-P. In turn, this changes the free energies of the two states [52,59,60]. Similar thermal-equilibrium models include the classical Monod–Wyman–Changeux allostery model [61–63] and the two-state Ising model [64] with nearest-neighbour interactions. Korobkova et al. [65] found that the CW and CCW intervals could be described by a gamma distribution, suggesting the existence of hidden Markov steps preceding each motor switch, which indicates a non-equilibrium switching process of the bacterial motor, as well as in other large cooperative molecular systems. Tu proposed a
Fig. 5. Organisation of the flagellar motor. A. Electron micrograph of flagella purified from Salmonella. The flagellum consists of a basal body acting as a rotary motor, a hook acting as a universal joint, and a filament acting as a molecular screw. B. CryoEM image of Salmonella basal body [44]. C. A sketch of the bacterial hook basal-body architecture: on the left is the hook basal-body complex of the Salmonella H⁺-driven motor and on the right is that of the Vibrio Na⁺-driven motor. The schematic model is drawn based on the electron microscopy (EM) analyses of the Salmonella and Vibrio motors. S. enterica consists of the cytoplasmic (C), membrane/supra-membrane (MS), peptidoglycan (P), lipopolysaccharide (L) rings, as well as a rod. The C, MS, P, and L rings are located in the cytoplasm, the cytoplasmic membrane, peptidoglycan (PG) layer, and the outer membrane, respectively. The rod is practically a driveshaft composed of three proximal rod proteins: FlgB, FlgC, and FlgF, and a distal rod protein (FlgG), and it traverses the periplasmic space [45–47]. The MS–C ring complex is a reversible rotor. FliG, FliM, and FliN form the C ring on the cytoplasmic face of the MS ring, which is formed by a single flagellar protein, FliF [48–50]. Flg1 and FlgH assemble around the rod in the PG layer and outer membrane, respectively, forming the LP ring complex that functions as a molecular bushing. The abbreviations in the figure are: IM - inner membrane, OM - outer membrane, and PG - peptidoglycan layer [51].

dissipative allosteric model to describe a non-equilibrium bacterial flagellar motor switch, e.g., involving a dwell-time distribution functions of the CW and CCW states [66]. However, different experiments in clarifying the basic mechanisms have found different CCW and CW interval distributions, with exponential forms [67–69] or the gamma distributions in others [65,70]. The latter observations suggest self-organised steady states, casting doubt on thermal equilibrium-like approaches.

Wang et al. [71] investigated motor switching under various load conditions by addressing different proton motive forces and varying the number of torque-generating stators, which significantly affect the motor torque. They demonstrated that the interval distributions change from exponential to non-exponential shape as the motor torque is varied from zero to a high value. This supports the viewpoint that motor switching is facilitated by a torque-dependent
Fig. 6. A thermodynamic model of the flagellar motor isomerisation. The motor fluctuates spontaneously between the CCW and CW states at rates proportional to $e^{-(\delta F_{CW}(\phi) - \delta F_{CCW}(\phi))/kT}$. As the proton motive force drops from its normal value $\phi$ to $\phi - \delta \phi$, the activation energy $\delta F_{CW}(\phi)$ increases by an amount proportional to $\delta$ while the activation energy $\delta F_{CCW}(\phi)$ decreases by a similar amount. Consequently, the probabilities of the transition CCW $\rightarrow$ CW and of its reverse, CW $\rightarrow$ CCW depend exponentially on $\delta$ and are anti-correlated.

Fig. 7. The molecular architectures of the flagellar motors. The molecular architectures of the flagellar motors with and without the protein CheY3-P. (a) A medial cross-section of the flagellar motor structure without CheY3-P. (b) A model of the C-ring unit shown in (a). FliM and FliN have a stoichiometry of 1:3, and the C-terminal of FliM (FliMC) together with three FliN units form a spiral at the base of the C-ring. (c) Interactions between the bell-shaped stator complex and the C-ring. The charged residues (Lys275, Arg292, Glu299, and Asp300 in red) in FliG2C interact with the inner part of the stator complex. (d) A different view of five C-ring units connected at the based on the C-ring. (e) A medial cross-section of the flagellar motor structure in the presence of CheY3-P. (f) A model of the C-ring unit with CheY3-P binding on the N-terminal of FliM (FliMN). (g) The charged residues (Lys275, Arg292, Glu299, and Asp300 in red) in FliG2C interact with outer part of the stator complex. (h) A different view of five C-ring units occupied by five CheY3-P proteins [58].

This non-equilibrium dynamic mechanism. This idea led to the development of the equilibrium conformation spread model by Duke and Bray [64], based on two states with different free energies – one active and one inactive (see Fig. 9). This modified model describes well how the motor’s response curve (CW bias versus CheY-P level) shifts to lower CheY-P concentration as the motor torque increases, thereby increasing the motor sensitivity to CheY-P and leading to a mechanism of force sensing, as shown in Fig. 10.
Fig. 8. Model of the rotational switching mechanism. (a, b) Interactions of the stator with FliG2 in the C-ring during CCW rotation. In the default state, in the absence of bound CheY3-P, the FliG2 proteins interact with the inner part of the stator complex (in yellow). The influx of protons through the stator channel causes the cytoplasmic subunits of each stator complex to spin CW. Consequently, the C-ring (blue) is forced to spin CCW. (c) A zoomed-in view of the interaction between the C-ring and the stator complex. (d) A perpendicular view to (c) shows that four C-ring units are connected by FliM/FliN interactions. (e, f) CheY3-P induces conformational changes in the C-ring, leading to altered interactions between the stator and C-ring, thereby causing a switch to CW rotation. The binding of CheY3-P to FliM on the exterior surface of the C-ring, triggers a shift (g) and a tilt (h) of FliG2, giving rise to an interaction of FliG2C with the outer part of the cytoplasmic domain of the stator complex (g). Because the cytoplasmic domain of the stator always spins CW, the C-ring is forced to spin CW (e). During the rotational switch, the spiral ring structure, formed by FliM and FliN, acts as a base that holds the C-ring structure together (d, h) [58].

2.2. Cooperative stator assembly induces discontinuous transition in bacterial flagellar motor rotation

The potential threshold for the rotation in B. Subtilis is approximately 30 mV. This is lower than the switching threshold of 45 mV between CCW and CW rotation in the flagellar motor, but higher than the potential corresponding to the thermal fluctuations of protons at room temperature $k_B T = 25$ mV, with $k_B$ being Boltzmann’s constant. Thus, in addition to CW/CCW switching direction, there is another non-equilibrium transition [52,72,73] between two modes of torque regulation: a quasi-continuous stepwise and a discontinuous jump, both summarised in Fig. 11.

The latter transition is directly related to the structural changes in the stator. As mentioned in the previous section, each stator is a protein complex and contains about ten torque-generating protein units anchored to the cell wall at the perimeter of the rotor. Inspired by a direct observation of steps in ATP-driven molecular motors, Sowa, Rowe [78] managed to measure steps in the flagellar motors and demonstrated the existence of a stepping motion of a Na$^+$-driven chimeric flagellar motor in E. coli at low sodium-motive force and with controlled expression of a small number of stators. They found 26 steps per revolution in the forward direction and 35 in the backward direction. Significantly, these steps occurred in the absence of the flagellar switching protein CheY, suggesting that the differences between the free energies of steps are small.

Ito et al. [79] showed that the steps are hardly affected by changes in the stator-rotor interactions, but that they are sensitive to the stator-stator interaction (Fig. 11a and 11b) and, hence, that it is the latter that drives the stator assembly (Fig. 11c). In other words, in the quasi-continuous step-wise mode the torque in each step is a direct function of the number of bonds between the stators. When there is no bond (the depleted state) there is only one large step from the lowest to the highest torque, resembling a discontinuous torque change. Moreover, the latter transition is accompanied by a hysteresis loop (Fig. 12). This loop ensures a controlled response, securing against over-sensitivity to small
Fig. 9. The free energy diagram of the states of a subunit of the flagellar switch in the equilibrium conformation spread model. 

a: Free energy diagram of the four states of a subunit of the flagellar switch in the equilibrium conformation spread model. Each subunit can be in either CCW or CW states, and has a single binding site for CheY-P. The free energy changes, associated with CheY-P binding, are $E_L + E_A$ and $E_L - E_A$ for the CCW and CW states, respectively. 

b: Detailed balance of the transition rates is satisfied between each pair of states in the equilibrium model. 

c: The addition of the motor torque increases the free energy gap between the CW and CCW states, but takes the process out of equilibrium. 

d: A sketch of the broken detailed balance between CW and CCW states, which results in a cyclic net rate flux (dashed line) [71].

Fig. 10. The motor’s response curve, using the non-equilibrium conformation spread model. The motor’s response curve (CW bias versus CheY-P level) at zero and high loads, simulated using the non-equilibrium conformation spread model [71].
Fig. 11. The Bacterial Flagellar Motor’s structure and its kinematics. A. A sketch of the Bacterial Flagellar Motor’s complex structure. The motor’s size is about 45 nm and it comprises a rotor (light brown) and several stators, which generate torque (purple). The torque is generated at the interface with the rotor and is driven by the free energy released by ion currents through ion channels in the stators. The stators are attached to the peptidoglycan layer at the top of the rotor and exchange energy with its bottom [74–77]. B. The coupling and decoupling of the stators with the motor. These processes are modulated by the load on the rotor and the ion flux through the ion channels. C. A sketch of the inter-stator interaction that drives the dynamics of the stator-assembly.

thermal fluctuations. This mechanism also ensures that the rotor does not rotate during the flagellar-motor assembly. These observations reveal a sophisticated torque regulation.

Further experiments focused on the stator-rotor interaction by studying the stator dynamics under various conditions. Nord, Gachon [80] measured the kinetics of arrival and departure of the stator units in individual motors under varying load and found that the stators assemble at high load and disperse at low load. They also showed that the rate of stator association is independent of the fluid viscosity, while the rate of stator dissociation is viscosity-dependent. Wadhwa, Phillips [81] found that no disassembly of stator units took place at high torque and that all of the stator units were released when the motor was spun at near-zero torque speed. Reverting to high loads led to recruiting stator units and increased the motor speed in the above step-wise manner. Shi, Ma [82] measured the time that motors stay at a constant speed (dwell time) during a particular step at high load at steady state. They observed that the dwell time distribution for any number of stators can be described by two exponentials,

\[ P_N (t) = Ae^{-at} + Be^{-bt} \]
Fig. 12. The motor torque changes in response to the load. The motor torque, which is the sum of the mean torques per stator (∼105 pN nm), changes in response to the load. A. The free energy minimum, calculated numerically with stator-stator interaction. The discontinuous jump in the torque is the fingerprint of a first-order transition. The arrows sketch the direction of the hysteretic process. B. Sketch of the free energy with a metastable state for zero torque and stable state for the large torques. Different graphs correspond to different loads. C. The free energy minimum, calculated numerically without stator-stator interaction. Here the transition is continuous, reminiscent of a second order transition. D. Sketch of the free energy profile in the absence of stator-stator interaction. There is only one stable state.

with $b < a$ the slow-decaying term. While plausibly exponential at long times, the experimental data is quite scattered. Considering only the stator-rotor interactions, they suggested the existence of a hidden state to explain this form. However, they calculate that such a hidden state would lead to a multi-exponential form and, to reconcile with the measurements, they concluded that the decay rate from the hidden state is much larger than the other rates, which overwhelms the distribution and reduces it to two exponentials.

Ito et al. [79] showed that the steps are hardly affected by changes in the stator-rotor interactions, which led Shi et al. [82] to ignore the more dominant effect of the stator-stator interaction. It is the latter that gives rise to the discontinuous transitions, which in turn acts as a binary switch between zero and large torques in response to increasing load.

2.3. Polymorphic transitions in bacterial flagellar filaments

Another component of the bacterial flagellum is the long helical filament, which is a hollow tube 20 nm in diameter and 15-20 µm long. It comprises thousands of copies of the protein flagellin (Fig. 2) [27,29,30,83]. When bacteria swim, the flagella are bundled and in a normal form of a left-handed coil. When the bundle disperses, the bacteria tumble, which leads to a change in the swimming direction. This is accompanied by a structural transition of some of
the filaments to a right-handed either semi-coiled or curly forms, as shown in Fig. 3. In total, there are 12 different forms of flagellar filaments with varying diameters and pitch lengths, all of which are sketched in Fig. 13D.

2.3.1. The molecular structure

The flagellar filament contains over 4000 subunits in each helical turn. The large number of protein molecules forming one turn of a helix necessitates a regular and very precise intermolecular arrangement. The first model describing the arrangement of these subunits was developed in 1965, based on electron micrographs of negatively-stained filaments [84]. In that model, a filament consists of eight longitudinal strands, each made of spherical subunits of diameter of about 50 Å. O’Brien and Bennett [85] found that the flagellar filament is actually a tubular bundle of 11 protofilaments, each of which an axial array of subunits. Askura et al. [86–88] proposed in the 1970s a protofilaments model comprising of two helical states of slightly different repeat distances (Fig. 13A). They argued that it is a mixture of these states that gives rise to the 12 different forms of the filament [86–88]. Samatey et al. [29] and Yamashita et al. [89] determined the repeat lengths of the two protofilament: 52.7 Å and 51.9 Å. They also found that the L-type protofilament tilts to the left hand by 3.4±1.0°, while the R-type tilts to the right hand by 7.8±0.8° (see Fig. 14B).
The differences in the lengths and the tilted angles between the protofilaments lead to the helical formation of the filament. The bending curvature of the filament, $\alpha$, and the twist angle, $\varphi$, are shown in Fig. 13C.

It is interesting to note that the slight difference of only 0.8 Å between the two pitches is responsible for the relative complexity of flagellar filaments through different combinations of the two protofilament states, shown in Fig. 13D. When the motor reverses, the reversal is almost instantaneous, causing the flagella to experience a shear stress that leads to a slight sliding shift between neighbouring protofilaments. This causes several protofilaments to switch into states of shorter repeat length and the helix changes its coiling direction from left to right. The bundle of the flagellar filaments then disperses and the loss of balance leads to tumbling.

2.3.2. Atomic structure

Yonekura, Maki-Yonekura [90] determined the full atomic structure of the R-type filaments from electron cryomicroscopy. Their image data allowed to construct a density map to a resolution of 4 Å, showing an $\alpha$-helical backbone with several large side chains. The flagellin resembles the letter with a vertical and horizontal dimensions 140 Å and 110 Å, respectively. It consists of four domains connected in series: D0, D1, D2 and D3. These are arranged from the inside of the filament outwards, as shown in Fig. 14. The ribbon diagram of the Cα backbone in Fig. 14a shows the chain folding, and the distribution of hydrophobic side chains. Fig. 14b shows the hydrophobic cores that define the domains D0, D1, D2a, D2b, and D3.

A flagellar filament is organised hierarchically and looks like a hollow cylinder. Flagellin proteins are arranged on top of one another into a protofilament and 11 protofilaments are lined up side by side to form the hollow cylinder. An end-on view of the filament from the flagellum distal end (Fig. 15a) exhibits a concentric double-tubular structure, made of D0 and D1 domains in the densely-packed filament core. Side views from outside and inside the filament (Figs. 15b, 15c), shows D0 and D1 domains interacting closely with internal subunit, both axially and radially. The repeat distance along the axial direction is 51.8 Å, which is the same as the shorter repeat distance of the flagellar protofilament. To study the switching mechanism between L- and R-type repeat, Samatey et al. [29] carried out computer simulations of the local conformational switch in the atomic model of F41. In the simulations, the top subunit of a three-subunit protofilament model was fixed and the bottom subunit was pulled gradually in the axial direction. The aim was to investigate the response of the middle subunit. They found that a discontinuous change in

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Fig. 14. The flagellin’s molecular structure, its Cα backbone, the hydrophobic side-chains, and its structural data. Inset: The flagellin molecule’s domains: D0, D1, D2, D3 and the spoke region, labelled S. The full structural details have been detailed in [29].

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the conformation of the β-hairpin in domain D1 is responsible for the two-state switching that produces the distinct repeat distances.

Transitions can be induced in two ways: environmental chemical changes and mechanical forces. Placing flagella of wild type bacteria on a chemically designed substrate containing p-fluorophenylalanine, the filaments turn curly [91], as shown in Fig. 16A. A careful procedure can be used to free flagella from the bacterial cells, monomerise them and then reconstitute and purify them to have the same characteristics [92]. Such purified flagella can be de-polymerised completely into monomeric flagellin under a number of treatments: heating or exposure to acid, such
as urea and guanidine-HCl. Copolymerisation can then be re-induced [93,94] at certain chemical conditions, such as using 3.0 mg protein/ml and 0.15 M NaCl in the solutions containing the flagella. Changing the ratios of monomeric and fractionated polymeric flagella in solutions, was shown to give rise to copolymerisation of flagella of distinctly different polymorphic forms. Hotani [94] copolymerised two different flagellins and obtained mixed-type filaments, as shown in Fig. 16B. Kamiya and Asakura [95,96] showed transitions under PH changes in Salmonella flagella, which underwent helical reversible transformations both in acidic and alkaline environments (Fig. 16C and 16D). Matsuura et al. [97] studied transformation of straight flagella in mutant E. Coli (Fig. 16E) and found that straight and transformed flagella co-existed within some pH ranges. However, no stable forms other than the two were found.

The polymorphic transition can be induced chemically, e.g., either by changing the pH, or by using mutant E. Coli bacteria. The mutants have straight flagella owing to either substitutions of some amino acid in the flagellin or by a mutation in the structural gene of the flagellin in the non-motile E. Coli strain W3623 ha-177. All these methods control the specific growth of the protofilaments, resulting in various polymorphic flagellar forms [98].

There are also several ways to induce structural changes by mechanical forces. Macnab and Ornston [99] demonstrated that polymorphism emerges when mechanical torsion is applied by the motor. At steady state, such a torque is counter-acted by the medium’s viscous torque, with the resulting torque applying torsion to the filament. The polymorphic transition induced in the flagellin is to a helical structure that is more tightly wound than the normal one. This is a major structural change that leads to motor reversal, which in turn produces tumbling. Thus, this polymorphic transition plays a key role in the locomotion mechanism. Turner et al. [31] developed a simple procedure for labelling fluorescently cells and filaments, which made possible recording their motion in real time. Using this method to study E. Coli cells, they showed that polymorphic transformations occurred in a specific sequence: from normal to semi-coiled to curly1 (Figs. 3b and 3c).

Hotani’s experiments on isolated flagella demonstrated the validity of the idea of Macnab [100]. Hotani developed a technique for studying the response of flagellar filaments to mechanical torque in vitro. Detached normal flagellar filaments were tethered to a microscope slide and viscous liquid was made to flow past them. This flow produced a transformation of the filament at the fixed end into a right-handed helical form. Two different patterns of behaviour were found: filaments attached to the slide by only a small length at one end appeared to rotate in a clockwise direction, while filaments attached to the slide by longer lengths displayed a more complex behaviour. A cyclic transition between normal and semi-coiled forms is illustrated in Fig. 17a, and cyclic transition between normal and curly forms is illustrated in Fig. 17b.

In these experiments, the transition to the right-handed helix spreads away from the attached end until eventually a reverse transformation back to the normal structure takes place at the attached end. Then the right-handed section moves relatively quickly down the filament and ‘escapes’ from the free end. In a way, this behaviour resembles the one shown in Fig. 3c, except that the torque is provided by viscous drag of a moving fluid rather than the rotation of the motor. Fig. 18 shows the stages in this repeating three-part pattern of transitions: initiation, growth, and travel. By estimating the accumulated tension at the attached end, Hotani obtained an estimate of the accumulated torque. His conclusion was that the torque is responsible for the initiation of both right- and left-handed helices, but that it plays no role in the growth and travel phase. The critical torque for initiating normal to semi-coiled transition was about $-11 \times 10^{-19}$ Nm and the torque for the reverse transition from semi-coiled to normal was $4 \times 10^{-19}$ Nm. Darnton and Berg [101] showed that the transition of the flagellar filament from normal to hyperextended depends on the rate of loading. They pulled at filaments using oscillatory pulling-releasing force cycles. Rapid pulling during one cycle at 0.4 μm/s causes the filament to behave as a simple elastic, hysteresis-free, spring in 9 of 19 cycles without any polymorphic transition. In the other 10 cycles, a polymorphic transition was observed. Reducing the pulling rate to 0.04 μm/s gave rise to a transition on each cycle.

### 2.3.3. Mechanical construction

Calladine et al. [86,88,98,103] modelled the polymorphic mechanism of the filament by assuming two states for the subunit conformation. The two states correspond to the sub-units connecting at either point $\alpha$ or point $\beta$ in the top left inset of Fig. 19. Connecting at point $\alpha$ generates an L-type protofilament and connecting at point $\beta$ generates an R-type (see Fig. 14). Different sequences of these connections lead to different configurations of the flagella. Fig. 19 depicts 11 nearly vertical lines having 12 distinct packing states of vertical ‘strands’ of connections, labelled $f_0$, $f_1$, $f_2$, $f_3$, $f_4$, $f_5$, $f_6$, $f_7$, $f_8$, $f_9$, $f_{10}$, $f_{11}$.
Fig. 16. Polymorphic transitions in bacterial flagellar filament in response to chemical changes. A. (a). Normal-type *Salmonella* flagellar filaments, produced by the wild type SJ25 strain. (b). Curling of the same flagellar filaments, produced by the curly mutant SJ30 strain. (c). The straight mutant SJ814 produces straight flagellar filaments. The shown scale is 1 µm and it applies to all three images. B. Different stable waveform conformations of the flagellin copolymers. Shown are: two kinds of normally flagellated strains SJ670 (i) and SJ25 (n), a curly flagellar mutant SJ30 (c), and a straight flagellar mutant SJ814 (s). The waveforms were characterised by the contour length contained in one period, L, and the amplitude, h. Homogeneous filaments of i- and n-flagellins usually exhibit a long period waveform while the s-flagellin is straight. Mixing the i- and n-flagellins with s, the values of both L and h in the copolymers (i+s) and (n+s) decrease with increasing concentration of s-monomers. The change, however, is not gradual but takes place via a number of morphological transitions. B. Top panel: Five stable conformations of the copolymers (i+s). (a). Type I filament in a mixture 1:s=1:0; (b). type II filament in a mixture 1:s=9:1; (c), type III filaments in a mixture 1:s=1:3; (d), type IV filament contained in 1:9; (e), a straight filament in a mixture 1:s=0:1. The scale, 1 µm, is shown in B(e). B. Bottom panel: the mixture of the type II and type I filaments, at magnification 500x. C. Dark-field light micrographs of i-flagella in acidic environment at magnification 600x. (a), Normal form observed at pH 7.0; (b), coiled form at pH 4.7; (c), curly form at pH 4.1. The flagella were suspended in a solution of 0.1 M NaCl, 1 mM acetic acid, and 1 mM sodium acetate. The suspension’s pH was varied by adding either 1 M HCl or 1 M NaOH. D. Dark-field light micrographs of i-flagella in an alkaline environment at magnification 600x. (a) Normal form at pH 7.0, (b) coiled at pH 11.0, (c) Curly I at pH 12.0, (d) curly II at pH 12.5. E. The non-motile strain W3623 ha-177 of *E. Coli* produces straight flagella as a result of a mutation in the structural gene for the flagellin. Shown are dark-field light micrographs of flagella from mutant *E. Coli* suspended in 0.1 M NaCl solutions at magnification 500x at: (a) pH6.0, (b) pH 8.0. [91–97].
Fig. 17. A. The cyclic response of a flagellar filament, attached to a glass surface at its top portion. Successive snapshots (from left to right at intervals of 5/9 s) of the filament’s cyclic conformation changes between normal and semi-coiled, as a viscous fluid flows from top to bottom. B. The cyclic transformation between normal and curly forms in the same set-up as in A, with successive frames from left to right, taking in total 36 s.

Hasegawa et al. [104] extended the above model by explaining the roles that the R-type and the L-type protofilaments play in determining the positions of the connecting points, as illustrated in Fig. 20. Their model differs from that of Calladine et al., in which the subunit has elastic deformation with identical bonding energy at different connection points. They name this quasi-equivalence. In contrast, the subunit in Hasegawa et al. involves elastic deformations with different bonding energies at different connecting points, which they named non-equivalence. In this model, the mechanical energy is minimised when short protofilaments are next to short protofilaments and long protofilaments are next to long protofilaments [86].

2.3.4. Continuum theoretical models

A number of continuum models for different chiral states exist. Goldstein et al. [105] and Coombs et al. [106] proposed a bi-stable energy function to describe the transitions between the two helical states phases observed in Hotani’s experiment. Srigiriraju and Powers [107] and Srikanth et al. [108] proposed a continuum model for the polymorphism of the filament, based on molecular switches and an elastic mismatch strain between the inner and outer cores of the flagellum. Speier et al. [109] and Vogel and Stark [110] proposed a coarse-grained model based on
an elastic network of rigid bodies and used Ising Hamiltonian to represent the two states of the flagellin protein. Wada and Netz [111] introduced a bi-stable helical filament model that accounts for different elastic monomeric states by a discrete Ising-like spin variable along the arclength. Using a hybrid of Brownian dynamics Monte–Carlo simulations, they showed that filament phase transition depends on the pulling rate. Wang et al. [102,112–116] used the time dependent Ginzburg–Landau continuum model to analyse Hotani’s experiment [100]. They modelled the free energy, \( F \), as a function of the twist, \( \tau \), and the curvature, \( k \), both per unit length, 

\[
F = -a \tau^2 + b \tau^4 + dk^4.
\]

This free energy has two minima for the normal and semi-coiled states, shown in Fig. 19b for \( a = d = 8.2 \times 10^{-25}\) and \( b = 1 \times 10^{-25}\). The strain gradient energy density in the \( \tau-k \) plane is 

\[
G = g_1 \left( \frac{\partial k}{\partial s} \right)^2 + g_2 \left( \frac{\partial \tau}{\partial s} \right)^2 + g_3 \frac{\partial k}{\partial s} \frac{\partial \tau}{\partial s}
\]

in which \( g_1, g_2, \) and \( g_3 \) are material-dependent coefficients that are functions of the interface thickness. Assuming an inextensible 20 \( \mu\)m-long filament and \( g_1 = g_2 = g_3 = g \), they used these equations within a Finite Element Method to simulate by the transition of the filament from an initial normal state, N, to a semi-coiled state, SC. Their results are presented in Fig. 21. Specifically, they simulated different aspects of the filament’s transition and showed the effects of the rotation angle loading rate, the bending deflection loading rate, and the magnitude of the energy barrier, on the transition. The equilibrium twists of the N and SC states are approximately 0 rad/\( \mu \)m and 4 rad/\( \mu \)m, and their equilibrium curvatures are approximately 0 rad/\( \mu \)m and 1.2 rad/\( \mu \)m, respectively. The torque-twist and bending-curvature curves of a filament at the loading end are shown in Figs. 21a and 21b. After an initial elastic deformation of the N phase, the torque drops to a constant value that does not change with increasing twist, Fig. 21a. Similarly, the bending moment drops to a constant value that does not change with increasing curvature, Fig. 21b. These constant values correspond to a nucleation of the SC phase and its growth along the filament. During this growth the N and SC phases coexist, which can be observed in Figs. 21c and 21f. This plateau is the fingerprint of a first-order phase transition, e.g., the temperature plateau during water boiling at atmospheric pressure. As soon as the SC phase boundary reaches the end of the filament, the torque and bending decrease momentarily, which corresponds to the disappearance of the interface between the phases. Further loading then leads to elastic deformation of the remaining SC phase.

The nucleation and growth of the SC state along the filament upon increase of the rotation angle and the bending deflection, can be clearly observed in Figs. 21e and 21f, respectively. When the two states coexist, they are separated by a transition zone of about 1 \( \mu \)m. Loading-unloading cycles of the rotation angle and bending deflection are shown in Figs. 21c and 21d. Both torque and bending drop down in the loading curve (corresponding to phase nucleation,
Fig. 19. The relations between the sub-unit structure and the filament helical phases. (a) and (b): Alternative arrangements of the outer part of the subunit. The upper left corner of each subunit has two distinct bonding sites, to which the adjacent subunit can connect. All twelve possible surface packing patterns are shown, designated \( f_i \), with \( i = 0 \rightarrow 11 \) the number of longitudinal strands. The patterns are generated by subunit connecting to two distinct bonding sites, shown in the top left inset. The strands marked \( \bullet \) are slightly shorter than the others, causing the filament to curve such that their conformation minimises strain energy. Tilting of longitudinal columns, such as the left-hand tilt in f10, twists the filament. (c): The curvature, \( \kappa \), against the twist, \( \tau \), corresponding to the structures in (b). The curvature and twist of the ideal waveforms are denoted by open circles, \( o \), and also correspond to the patterns in (b). Included are: data from dark-field light microscopy [95] of the waveforms of filaments constructed from \( i \)-flagellin (\( \triangle \)), \( n \)-- flagellin (\( \triangledown \)), and \( e \)-flagellin (\( \bullet \)); data from [85] on the twist of straight filaments built by \( s \)-flagellin (\( \blacksquare \)); and data on waveform IV (\( \blacksquare \)) from [91,93] (shown in Fig. 17). (d) The shapes of the 12 ideal waveforms, corresponding to (b) and (c), drawn for a contour length of 4 \( \text{mm} \). (e) Conventional names of the waveforms. The absence of name indicates theoretically predicted waveforms that have not been observed yet [88].

with the arrow down) and jump up in the unloading curve (corresponding to phase disappearance, with the arrow up). The formation of a hysteresis-like loop by the loading and unloading curves suggests that energy is dissipated during the nucleation and disappearance stages of the process, most likely in the form of elastic vibrations and heat.

The twist and curvature profiles, as the rotation angle and the bending deflection are applied, are shown in Figs. 21g and 21h, respectively. As the loading is reversed (unloading) the SC phase shrinks. When this phase shrinks to roughly twice the transition zone thickness, the SC phase suddenly disappears, which takes place during the unloading steps 10 \( \text{µm} \rightarrow 5 \text{µm} \rightarrow 0 \text{µm} \), and the filament reverts to the N state. The value of \( g \) has to be adjusted to \( g = 0.08 \text{µm} \) to obtain a quantitative agreement with the thickness of the transition zone and the twist profile in Fig. 21i. But fitting
Traditionally, \( \delta t \) denotes the disappearance of torsion by rapid increase. Phenotypic twists may be one aspect of a bacterial transition. The development of the twist profile with time was simulated at small time steps of \( \delta t = 0.5 \) s and it is shown in Fig. 21k. Both states coexist initially, each in its own equilibrium profile configuration (black line \( t = 0 \) in Fig. 22k). The twists of the two coexisting phases are at the equilibrium values: 0 rad/\( \mu m \) and 4 rad/\( \mu m \), respectively. The total torsion angle of the filament is 24 rad. It can be observed in Fig. 21k that, when a step torsion loading is applied at \( \delta t = 0.02 \) s (red line), the twist increases instantaneously at both phases.

Fig. 21 shows that a rapid increase in the twist angle loading quenches the configuration and suppresses the transition. Initially, the two phases coexist in their equilibrium twist values, 0 rad/\( \mu m \) and 4 rad/\( \mu m \), respectively. A rapid increase of the total twist angle to 24 rad at \( t = 0 \), gives rise to no significant change in the twist profile along the filament. The SC phase does not grow because the loading time is much smaller than the response time required for the transition to proceed. This is analogous to quenching in conventional phase transitions, e.g., in metals.

### 2.4. Phenotypic switching in bacteria

Prokhnevsky et al. [117] defined bacterial development as a “series of stable and meta-stable changes in the form and function of a cell, where those changes are part of normal life cycle of the cell”. Bacterial growth has been traditionally viewed as the result of symmetrical cell division into genetically identical siblings. However, it has been known for a while now that phenotypic variation is a widespread phenomenon in the isogenic populations. The phenotypic variation has been attracting much attention because of its relevance for cellular differentiation and its implications for the treatment of bacterial infections [118]. Phenotypic switching is one of the mechanisms by which
Fig. 21. The mechanical loading-deformation characteristics of a filament. The deformation characteristics of a filament, of length 20 mm, under different types of loading. (a) Torque-twist curve of a filament under proportional loading, i.e., a load increase of the rotation angle and bending deflection by the same ratio. (b) Bending-curvature curve of a filament under proportional loading. (c) The hysteretic torque-twist curve of proportional loading and unloading of rotation angle and bending deflection. (d) The hysteretic bending-curvature curve of proportional loading and unloading. (e) The twist profile, as a function of position along the filament, L, for g = 1 mm (see text) under proportional loading. (f) Curvature profile for g = 1 mm under the same proportional loading; (g) Twist profile of a filament under the same proportional unloading; (h) Curvature profile of a filament under the same proportional unloading. (i) Simulated twist profile for g = 0.08 mm at different torsion loadings – in contrast to (e), a sharp interface is obtained. (j) Torque–twist response of bacterial filament under slow and fast twist loading. (k) Evolution of the twist distribution of an initial two-phase coexistent state after a slow torsion loading, carried out over a duration that is much longer than the material characteristic time $t_\text{c}$. Here, $\delta t = 0.5$ s. (l) Torsion loading, applied to an initial two-phase coexistent state on a time scale much shorter than the material characteristic time. The evolution of the twist distribution is no different than in an elastic filament. Here, $\delta t = 0.02$ s.
Fig. 21. (continued)
bacteria survive in ever changing environmental conditions and it can be induced by either inheritance or epigenetics. This mechanism is not limited to bacteria. One of the oldest examples of phenotypic switching was discovered by Zioberg in Linaria vulgaris (Fig. 22) [119]. The morphology of the flower shape of this phenotype is completely different from the more common form of five petals united to form a corolla tube (Fig. 22A). In contrast, the petal of the phenotype found by Zioberg form five spurs with a distinct radial symmetry (Fig. 22B).

Heritable variation of phenotypic switching is often caused by random mutations in the DNA sequence, which have a beneficial impact on reproductive success. Depending on their fitness effect and the population size and structure, the frequency of genotypes changes over time determines the evolution process [120]. The first attempts to catalogue and document phenotypic variation began at 17th century with John Ray, who classified groups depending on their similarities, and termed as species any group that shares characteristics, which distinguish them from another group [121]. By realising that such characteristics must be stably transmitted from parents to their offspring, he was one of the first to recognise the connection between inheritance and inter-species variation [122]. For Darwin, variation between individuals is the basis of the evolutionary process: “Owing to this struggle of life, any variation, however slight and from whatever cause proceeding, if it be in any degree profitable to an individual of any species..., if useful, is preserved” [123]. At about the same time, Mendel found phenotypic variations in plants, pointing out that the particular inheritance is determined by independent particles [124]. Johannsen coined in the 19th century the terms ‘genes’, ‘phenotypes’, for observable characteristics of individuals, and ‘genotype’ for underlying genetic determinants [13]. De Vries defined mutations as any changes in observable characters that deviate from those found in other individuals of the same species [125].

Yet, a phenotype needs not originate only in a nucleotide sequence, as an outcome of a myriad of complex interactions between the organism and the environment. Since the beginning of the 20th century, it has been accepted that the same genotype can produce different phenotypes, depending on the environment that the organism is experiencing during the development, a phenomenon known as phenotypic plasticity [126]. Moreover, phenotypic switching can also occur, namely, the phenotype of an organism switching between generations at a certain rate. This can happen either because of the stochastic nature of the underlying developmental mechanisms or it can be induced by environmental cues, such as temporal fluctuations in pressure [127], molecular noise in metabolic enzyme expression, positive feedback loops, and asymmetric partitioning of cellular components during cell division [128]. The concept of epigenetic landscape was introduced by C. Waddington to explain phenotypic plasticity and its possible contribution to evolution [129]. In modern terms, epigenetic inheritance comprises many different mechanisms that modulate gene expression and cause production of different phenotypes. Intriguingly, some of which can be inherited seemingly in-

Fig. 22. Pelorism in Linaria vulgaris. (A) Common form of flower shape in Linaria. The petals form a corolla tube with a distinct dorsoventral asymmetry. (B) Peloric flower shape: the petals form five spurs with a distinct radial symmetry [120].
dependently of the underlying DNA sequence. In particular, phenotypic switching is one of the mechanisms by which bacteria adapt relatively rapidly to ever-changing environmental conditions, speeding up microbial evolution [130].

2.5. Lifestyle switch from free-living bacteria to biofilms

Bacteria constantly face a multitude of chemical and physical stresses associated with external environments and host-specific niches. To survive, they have evolved molecular mechanisms for altering their lifestyles in response to changes in environmental conditions. For example, free-living bacteria can switch their lifestyle to undergo development to form matrix-encased aggregates called biofilms on different abiotic and biotic surfaces [131]. We define the ability to shift from a planktonic lifestyle to a multicellular community as a ‘lifestyle switch’, which is observed in a majority of chronic bacterial infections [132]. The regulation of lifestyle switches in bacterial pathogens is important to enable successful pathogenesis.

Bacteria often exist in organised communities - biofilms – consisting of cells and EP [133], illustrated in Fig. 4. Biofilm formation is an efficient microbial strategy to persist in harsh environments. The advantages of bacteria within a biofilm relative to their planktonic counterparts are several: increased antimicrobial resistance, protection against predation, dehydration, and phagocytosis [134]. Biofilms are the result of free-living bacteria attaching irreversibly to either an abiotic or a biotic surface, as well as of bacterial growth and division to form a mature biofilm. During the growth of a biofilm, bacteria adopt a biofilm-specific phenotype that is radically different from that expressed in the corresponding planktonic cells [135]. Biofilms are a heterogeneous aggregation of microbial phenotypes [136, 137] that live together as a community, coordinating group activities, such as circulation, dispersion, and aggregate movement [138–142]. The different phenotypes rely on one another and interact cooperatively toward the success of the entire community [143].

2.6. Phenotype switching in biofilms

Phenotype switching in biofilms has been observed in a wide range of bacterial species, including both Gram-negative and Gram-positive bacteria [144], which differ significantly in the structure and thickness of their cell walls [145]. Several studies have demonstrated that genetic variants arise in biofilms through mutation or recombination. These variants have been detected primarily as changes in the colony morphology of subpopulations of cells following biofilm growth. Following the growth of the parent strain in biofilms, small fractions of isolated strains exhibit colony morphologies that differ distinctly from the parent. Examples include the small rough or wrinkly variants of *Pseudomonas aeruginosa* [136,146], the small non-mucoid variants of *Streptococcus pneumonia* [147] and the rugose colony type of *Vibrio cholera* [148]. Because variants can constitute 10% or more of a biofilm population within a few days of being formed from a homogenous inoculum [136,149], it is unlikely that mutation processes alone are responsible for the accumulation of variants. Mutation and genetic rearrangements probably generate variants that are then enhanced through natural selection in the local biofilm environment. In a mixed-species biofilm, genetic changes during biofilm development can lead to the evolution of species interactions [150].

Stochastic gene expression may also lead to phenotype variation. In contrast to changes in DNA sequences, some subpopulations of cells may express genes in a stochastic manner, rather than in response to a particular environmental cue, and therefore differently from the larger population. Baty et al. [151,152] studied the expression of a chitinase gene (chiA), in a marine *Pseudoalteromonas* species, in young biofilms that were attached to chitin and silicone films. They observed that individual cells that expressed strongly chiA could be found adjacent to cells that did not express chiA. They and suggested that this differential expression reflects a strategy of ‘division of labour’ by bacterial subpopulations. The very thinness of those biofilms meant that cells exhibiting differential activity were very close to one another, which makes it very likely that they experienced almost identical chemical environments. This then suggests, again, that gene expression in an originally clonal population is driven by a bistable mechanism. Bistability might indeed explain the occurrence of subsets of bacterial cells, called persister cells, in biofilms [153–155]. Persister cells are proposed to be a subpopulation of cells that have an enhanced resistance to antibiotics. Using a microfluidics approach, this idea was substantiated for a clonal population of *E. Coli* at the single-cell level [156]. This was demonstrated by first noting that cells experiencing nearly identical environmental conditions divided at different rates [156,157] and then that the much slower-dividing or non-dividing cells survived antibiotic treatment. When the antibiotic treatment was removed those cells switched to rapid growth. There is currently active research to identify the genetic basis of persistence [158–161].
B. Subtilis practises a different strategy forming, biofilms through a series of phenotype switches, with three main phenotypes comprising 90% of all cell types. This species is one of the most intensively studied Gram-positive bacteria, being a good model organism to study biofilm formation [162]. B. Subtilis is useful for treatments for gastric and urinary tract diseases [163], for improving agricultural yields, as well as an ingredient in the production of a Japanese delicacy known as 'natto' [164]. Its three main phenotypes during biofilm growth are: motile cells, cells that produce extracellular matrix, and cells that differentiate into spores. The motile cells have flagella that enable them to swim in solution or to swarm along wet surfaces [165,166]. Matrix-producing cells produce a mixture of amyloid proteins and exopolysaccharides that hold the community together [34]. Spores are metabolically inactive, resistant to heat and antibiotics, and can germinate under certain favourable conditions [167,168]. These three phenotypes are different in their gene expressions and together they account for nearly all of the cells [169]. Additional cell states include competent cells, which are able to take up extracellular DNA from their environment [170,171], and cannibalistic cells, which can kill their sister cells by secreting toxins and utilise them as a nutrient [172]. It is the process of phenotypic differentiation that allows the bacterial colony to optimise its resources and stay resistant to harsh environmental condition.

Many of the details of the genetic circuits controlling these different cell states are understood at the single-cell level [34]. Conventional techniques for monitoring phenotypes in growing B. Subtilis biofilms include flow cytometry and fluorescent microscopy, using bacterial strains with fluorescent reporters for one or two phenotypes [34,173–178]. By tagging fluorescently one or two cell types at a time [34,179,180] it is possible to understand fully biofilm evolution. Wang et al. [181] developed a non-destructive method to analyse bacterial biofilm growth of a triple-labelled B. Subtilis strain, using optical transmission and fluorescence microscopy. This method made it possible to observe the spatial and temporal distribution of cell states, as well as that of low-fluorescent or non-fluorescent materials. The latter consists of the extracellular matrix and/or cells that are either dead or have decreased fluorescent expression levels [181], as shown in Figs. 23 and 24.

2.7. Wrinkle pattern evolution in B. Subtilis biofilms

Soft tissues in organisms tend to lose stability as a result of environmental changes and external incentives, which gives rise to a continuous evolution of surface wrinkling and morphology as the colony grows. This phenomenon has been observed both in vivo and in vitro. Additionally, different cell phenotypes can produce different wrinkle geometries, examples of which are the convoluted bowel [182] and cerebral cortex [183]. It is the heterogeneous growth of cell populations in organisms which dictates the evolution of their surface morphology and structure. There are well known examples from different growth phenomena: the edge of a leaf grows faster than the centre, generating residual stresses that can cause waviness at the edge [184,185]; the decay (or atrophy or lesions) of fruits and flowers drives to formation of irregular morphology [186–188]. Similar mechanisms affect the morphology of bacterial biofilms. The formation mechanism and physiological function of the biofilm morphology have been explored extensively in recent years. Experimental observations demonstrate that a network of folds can be generated in biofilms, creating water channels that facilitate material exchange between the internal parts of the biofilm and the environment [189,190]. Such a channel is shown in Fig. 25A. An important determinant of the complex morphology and structure of biofilms is cell differentiations into different phenotypes, which results in a heterogeneous population [191,192]. The non-uniform cell proliferation induces local stress gradients in biofilms, giving rise to mechanical instabilities that affect significantly surface wrinkling and interface delamination [193,194]. Another important morphology-driving mechanism is the withering and death of cells in the central area of the biofilm. When such cells stop functioning the stress around them is released to an extent. This leads to formation of irregular wrinkles around the centre of the biofilm [195], as shown in Figs. 25B–25D. Evolution and wrinkle formation can induce morphological transitions that, in turn, can shape further the biofilm dynamically changing morphology. The morphological transition is an irreversible phase transition [196–199] in that it leaves behind a fixed morphology that cannot be changed.

Rich morphological patterns were also found during the growth of triple fluorescent labelled B. Subtilis strains on MSgg agar substrate over 20 days [200–202]. The patterns included branches, labyrinthine networks, radial ridges, concentric rings and more. Similar results, including further morphologies were reported in [203] and are shown in Fig. 26A. Interesting are the morphological transitions of the wrinkle pattern during the growth. It underwent five successive evolutions from the biofilm centre to its edge: chaotic concentric rings (I), labyrinthine networks (II), radial ridges (III), branches (IV), and burgeons (V). The dynamics giving rise to these morphologies are varied. The wrinkle patterns I and II were shown to be caused by cell death in the centre of the biofilm, while the branching in IV (Fig. 26B)
resulted directly from the wrinkle morphology in III and can be regarded as inherited from it. Similarly, the evolution into the complexity of the branching in V was a result of the patterning in IV. An example, shown in Fig. 26C, is of a wrinkle dividing into more than three branches. Beyond visual observations, measurements of wrinkle thickness and width in different regions showed systematic trends: radial wrinkles in III were the thickest and widest, with these decreasing steadily with the transitions into stages IV and V. This decrease was accompanied by an increase in the density of wrinkles, as shown in Figs. 26D and 26E.

Analysis in [203] of the phenotype distributions through the fluorescent intensity also revealed systematic patterns. Spores population outnumbered motile and matrix-producing cells in regions III and IV, but spores were in the minority in region V (Fig. 26G). The spores population was positively correlated with surface complexity (Fig. 26F), which the latter defined as the ratio of the biofilm’s surface area, in contact with the air, to the projection of this area onto the plane.

A noticeable distinctive boundary between the radial ridge pattern and the dendritic pattern [203] has been attributed to interface instability, in which protrusions in a growing surface are prone to grow even faster, generating fingering phenomena, often leading to fractal patterns [204–206]. In the biofilm, the interface’s instability resulted from slightly anisotropic local growth generating accumulation of residual stresses within the biofilm [207]. This is in contrast to other pattern evolution dynamics in the biofilm, where the evolution is gradual through a region. The evolution across boundaries is causal - from the radial ridge pattern to the dendritic pattern and then to the burgeon pattern. A similar pattern can be seen in bifurcated branches and lungs. Intriguingly, each biofilm pattern corresponds to different cell differentiation and growth rate. Specifically, when the growth state and internal stress of the phenotype
Fig. 24. Biofilm characteristics, averaged over the azimuthal angle, as functions of the distance from the centre, \( r \), and time, \( t \). A. The height of the biofilm as a whole, deduced from the optical density. B. The difference between the heights deduced from the optical density measurements and from fluorescent intensities of multiple channels, presumably because of excessive low-fluorescent and/or non-fluorescent materials. C. The phenotype majority and fluorescence spatial distribution in the biofilm. D–F. The height of the biofilm in the regions populated by the different phenotypes: motile, matrix producing, and sporulating cells. The full black line in A, D, E, and F indicates the size of the biofilm as a function of time. The dashed black line shows the points in time and space where the substrate has been colonised for 5 hours.

Fig. 25. The wrinkles in biofilm structure correlate with concentrations of dead cells. A. A water channel formed under a biofilm wrinkle. B. Sketch of the biofilm as viewed from the microscope for the fluorescence and bright-field imaging of a biofilm (in grey). Dashed lines indicate the direction of observation. C. Cross-section of an artificially coloured fluorescence image of a 30 h-old biofilm wrinkle. The PttsAZ-CFP is in grey and the cell death reporter Sytox is in green. D. A transmission electron microscope image of dead cells (black arrow) within the section of a biofilm wrinkle taken within the white box in C.
reach a certain threshold, the morphology undergoes a transition. These transitions determine the interface evolution of the biofilm [208,209].

The morphology is also very sensitive to environmental conditions, including nutrient concentration and temperature. Analyses to determine the effects of these factors, as well as of the type of mutant, eps or flagellar, on the structural patterns, were carried out in [203]. Increasing agar concentration from 1.5 wt% to 2.0 wt%, was found to cause earlier formation of radial ridge wrinkles and branches, and a significant increase in biofilm surface complexity. Increasing the agar concentration further to 2.5 wt% revealed a domino effect: while not increasing the surface complexity (see Figs. 27A and 27C), this made the substrate harder. This hardening reduced the rate of water and nutrients absorption in cells, thereby accelerating the differentiation of matrix-producing cells into spores. The increase in spores population then increased rapidly the surface area, giving rise to wrinkles.
Fig. 27. Surface morphologies of 14 days old WT and mutant B. Subtilis biofilms in several environments. A. Surface morphologies of 14 days old WT B. Subtilis biofilms, grown on substrates with agar concentration of 1.5 wt%, 2.0 wt%, and 2.5 wt%, respectively. B. Surface morphologies of eps-mutant, flagellar mutant, and WT at the given temperatures. C-D. Average surface complexities of biofilms in A. and B. All scale bars are 3 mm.

The structural patterning was also found to depend strongly on the species: eps mutant B. Subtilis strains failed to form biofilms, as shown in Fig. 27B, while biofilm morphologies generated by wild-type B. Subtilis and flagellar mutants exhibited similar surface complexities, as shown in Fig. 27D.

The temperature-dependence of the biofilm morphology was studied in [203] by culturing biofilms at two different temperatures, 23 °C and 32 °C. A typical difference is shown in Fig. 27B - the patterning is more distinct and tighter at the higher temperature.

2.8. Collective cell motion patterns evolving in B. Subtilis biofilms

Biofilms fit well into the definition of complex systems [210,211] because their evolution is strongly constrained by the large number of cells in the biofilm and by the need to exchange chemical information both among cells and be-
between cells and the environment [212, 213]. Indeed, many complex systems, made of elementary units, self-organise into distinct patterns. Examples abound in nature: snowflakes, desert dunes, diffusion-limited aggregates, viscous fingering, and many more. The global patterning is an emerging property of seemingly random but cooperative dynamics on the element-scale. The cooperativity is dictated by the inter-element interactions, which can be widely varied [214–218]. In biofilms, these interactions are governed by the aforementioned constraints, which determine cell proliferation, mechanical interactions, and information exchange [219, 220].

During the growth of *B. Subtilis* biofilm, to cope with diverse environments, cells differentiate into the different phenotypes - motile cells, matrix-producing cells, and spores [34, 221]. These phenotypes differ in their adhesion, motility, and shape characteristics, and their inter-phenotype interactions govern the emergent diverse biofilm structures [222, 223]. In a recent study [224], it was found that motile cells tend to be compact within few hours of the initial inoculation before matrix-producing cells appear. The matrix-producing cells then form elongated structures that interfere with tendency of motile cells to form rounded ones. This is a structure-mediated interaction between the motile and matrix-producing cells, forcing the former to grow within the boundaries forced by the latter. This process is illustrated in Fig. 28. After ten hours of inoculation, some colonies tend to be compact and only have motile cells, seen in Fig. 28a Blocks B and C (in red), while others appear more oriented (Fig. 28a Blocks A and D) because of the directional influence of the chain-like structure of the matrix-producing cells (in green) on the colony’s expansion.

To quantify the structural analysis, we must consider the energetic barriers to growth in different directions, as different cell types deal with these barriers differently. The energetics of the growth dynamics depend on the cell types, sizes, numbers, and the inter-cellular interactions. The energy barrier to overcome during growth can be written in the form

\[
E = \sum_{i=1}^{n} \left( \int_{0}^{d_i} F_i \, dl + \int_{0}^{\theta_i} M_i \, d\theta \right),
\]

in which the sum is over all the *n* cells, *F*<sub>*i*</sub> is the frictional resistance to the *i*th cell when it deviates from the centre of the population, *d*<sub>*i*</sub> is the cell’s distance from the centre of the colony, *θ*<sub>*i*</sub> is the angle between the long axis of the *i*th
cell and $d_i$, and $M_i$ is the resistance moment to the $i$th cell being deflected from this angle. For a uniform circle with no angular preference,

$$E_{\text{circle}} = \int_0^R \sigma r^2 2\pi r dr = \frac{2\pi R^3 \sigma}{3}. \tag{2}$$

There are several ways to characterise a colony’s shape. We define the mean cell distance as the average distance between the cell positions and the colony’s centre, $|r_{\text{cell}} - r_0|$, measured in either pixels or µm,

$$\bar{r} = \frac{\sum_{\text{cells}} |r_{\text{cell}} - r_0| a}{\text{Colony area}}, \tag{3}$$

in which $a$ is an individual cell’s area and the colony’s centre is defined as

$$r_0 = \frac{\sum_{\text{cells}} r_{\text{cell}}}{\text{Number of cells}}. \tag{4}$$

Neglecting buckling out of the plane, the shapes range from the most compact one - the circle - to the most stretched one - the line. For these extremes the mean distances are:

$$\bar{r}_{\text{circle}} = \frac{1}{\pi R^2} \int_0^R r 2\pi r dr = \frac{2}{3} R \tag{5a}$$

$$\bar{r}_{\text{line}} = \frac{2}{L} \int_0^{L/2} r dr = \frac{1}{4} L, \tag{5b}$$

in which $R$ and $L$ are the circle’s radius and the line’s length, respectively. For illustration, consider a linear and a circular single-phenotype colonies, each of $N = 2^{t/\tau}$ cells, with $t$ time and $\tau$ the mean division period. The areas of both colonies are $Na$. Their mean distances are

$$\bar{r}_{\text{circle}} = \frac{2}{3} \sqrt[3]{\frac{a}{\pi} 2^{t/2\tau}} = \frac{2}{3} \sqrt[3]{\frac{aN}{\pi}} \tag{6a}$$

$$\bar{r}_{\text{line}} = \frac{\sqrt{a}}{4} 2^{t/\tau} = \frac{\sqrt{a}}{4} N. \tag{6b}$$

The situation is slightly more complicated in multi-phenotype colonies, both because each phenotype has a different growth pattern and because the phenotypes divide preferentially at different stages of the colony’s evolution.
In the growth of *B. Subtilis* biofilms, the time-dependence of the cell numbers and the mean distances are shown in Figs. 29b and 29c, respectively. The growth pattern of block B differs from the other three, most pronouncedly between \( t = 200 \) min and \( t = 400 \) min, when the colony grows but the mean distance hardly changes. This is a result of the separation into two sub-colonies in this block, which affects directly the mean distance. This establishes that the mean distance is a useful descriptor of the shape. Beyond \( t = 450 \) min, the growth rates are similar in all the blocks, as is reflected by the shapes shown in Fig. 29a, with all the colonies being roughly compact. It is noteworthy that at this stage there are fewer cells in block B (Fig. 29b) than in the other blocks and that the cell number ratios, e.g., \( N_A/N_B \), increases faster than the ratio \( \bar{r}_A/\bar{r}_B \). This is because the mean distance grows as \( \sqrt{N} \) in compact clusters, as eq. (6a) shows. The increase with time of cell number ratios is the result of the emergence of matrix-producing cells, which tend to form elongated structures and bias the expansion towards the directional and line-like. The transition from a directional growth to compact is best seen in block A around \( t = 150 \) min, when the mean distance’s growth changes from being proportional to \( N \) to being proportional to \( \sqrt{N} \). This transition is also clearly observed in the time-dependence of the energy barrier, shown in Fig. 29.

The large number of cells of each phenotype in the biofilm and their seemingly random movements [225] makes it impractical to describe the dynamics on the cell-scale. ‘Macroscopic’ dynamic models of colony shape evolutions are then more useful. To bridge between the cell and the colony scales, Wolfram [226] adopted a methodology that is common in analysing inanimate complex systems. The first step is to generalise key characteristics of single-cell movement, as follows:

(i) The driving force for the cell movement is its elongation and proliferation.
(ii) After division, the orientation of the long axis of the daughter cell is correlated strongly with that of the mother cell, as illustrated in Fig. 30a. This correlation is higher in matrix-producing cells than in motile cells, which is the cause for the former forming elongated structures. However, too high correlations, resulting in almost exact orientations, lead to breakage of cell bonds.
(iii) Adjacent non-mother-daughter cells can be either parallel or antiparallel, as illustrated in Fig. 30b.

The compactness of the colony at the late stage of the colony’s evolution is determined to a large extent by the interplay between characteristics (ii) and (iii).

Using this methodology to analyse experimental images, Olufati-Saber [227] and Reynolds [228] obtained relations between key characteristics of single-cell movement and colony shapes. They also constructed a multi-agent model, which was implemented in a numerical simulation, in which cells were modelled as single particles (agents). Simulating agents that behave only as motile cells, i.e., with a predetermined angle between the orientations of mothers and daughters, indeed gave rise to compact colonies. Simulating colonies of the two phenotypes, the motile cells arrays changed from compact to expansion along the chain-like directions of matrix-producing cells. The experimental observations suggested that colony growth along the chain direction lasts several hours after the appearance of matrix-
producing cells. However, as the matrix-producing cells proliferate, the colony reverts to the compact structure. This is because of two effects. One is that too long chain structures are unstable and tend to break or bend significantly. Another is that matrix-producing cells ‘nucleate’ in a number of locations, with chain directions oriented randomly in each location. These then interfere with one another as the chains elongate, disrupting the directional correlations.

3. Concluding remarks

A number of experimental measurements and computational techniques have been developed to investigate the complex cooperative behaviour of bacteria [229–231]. Despite the great progresses described above, there is still no fundamental theoretical and predictive modelling of the dynamics of biofilm evolution. One difficulty is the difficulty to obtain reliable data on local parameters [232] and one way around this problem is to adopt a more physics-based approach and shift the focus from the local to the larger-scale picture by modelling ‘coarse-grained’ properties and behaviour. It is only on larger length-scales and longer time-scales that the self-organisation [233] of these systems become apparent. Introduced in 1987, self-organised criticality is a pattern of behaviour that many dynamical out-of-equilibrium systems share. By definition, self-organised states are of low-entropy, in contrast to entropy-maximising equilibrium systems. The ordered phase in such systems is often quantified by an ‘order parameter’ and the entropy is a measure of the system’s disorder. In spite of the contrast in the entropic behaviour, the characteristic approach to self-organised criticality is similar to the way equilibrium systems approach second-order phase transitions and critical points. Additionally, phase transitions in inanimate system are characterised by ‘collective behaviour’ [234], in which the behaviour of far-away micro-scale units is correlated in spite of the fact that the interactions are short-range on the unit scale. Such a collective behaviour, correlated over distances larger than a few bacteria, is also prevalent in biofilms. This similarity, combined with the large body of existing knowledge on behaviour in the vicinity of critical points, can be used to develop a new generation of predictive models for the evolving morphology of biofilms. Moreover, many inanimate physical systems, of widely different microscale properties, approach a phase transition along the same route, often with an identical divergence of the correlation length. Such systems are said to be in the same ‘universality class’. This means that the details of the evolving morphology are blurred out and only coarse-grained parameters are important to model the process of the growth of the correlation length. It is then possible that the modelling of such phenomena in inanimate systems, of which there are many examples, could be a promising way towards predictive modelling in bacterial biofilms. In particular, existing models of potential systems in the same universality class could be adapted. Nevertheless, unlike physical systems, biological ones may have many states (or ‘phases’), each of which characterisable by a specific order parameter, such as network activity, large-scale low expression genes, and a range of structural descriptors [235,236]. Examples of the latter include curvature and twist to describe transitions of the bacterial flagellar filament. Order parameters need not be scalar – they could be vectors or tensors. Indeed, tensors are often used to describe structures quantitatively [20,237]. Conceptually, modelling many-phase systems is no different than few-phase ones, but the behaviour would most likely be richer and more complex, not least because of the different order parameters of different phases.

Adaptation of physical models of phase transitions to emergent phenomena in bacterial biofilm has started to attract attention. Several examples are the following. You et al. [238] demonstrated that a mono-to-multi-layer transition in growing bacterial colonies is driven by a competition between growth-induced in-plane active stresses and vertical restoring forces resulting from cell-substrate interactions. The transition sets in when individual cells become unstable to rotations. Modelling cell division as a Poisson process and defining the Poisson process rate as an order parameter, they showed that the transition from monolayers to multilayered structures can be described as a continuous phase transition. Depletion-induced phase separations have also been observed in biofilms, both on solid substrates and in fluid environments [239–245]. The order parameter Q6 can be used to distinguish between liquid-like disorder, in which Q6=0, and solid-like order, in which Q6=1 [246]. Ghosh et al. [247] showed that the typical value of Q6 increases significantly with depletion. This was then explained as the result of mechanically driven depletion inducing cell ordering as the biofilms grow on the solid substrates. Zhai et al. [248] discovered recently that the long-range propagation of electrical signals in biofilms of B. Subtilis can be modelled by an extended version of the classical percolation theory [249]. While in the standard theory, sites are on or off independent from one another, they found that the probability of a cell to signal is correlated with its nearest neighbours. They then extended the classical model to describe correlated percolation of signalling, driven cell division, phenotypic inheritance, and cell displacement. The order parameter, which they define, is then the difference between the probability of a cell to be on when the cell
above it is on and when the cell above it is off. The observation of these correlations encouraged Zhai et al. to use nearest-neighbour interacting spin models to describe the signal propagation phenomenon, a direction that would be interesting to continue exploring. Dell’Arciprete et al. [250] used the physics-based formalism of liquid crystals to model the structural evolution of growing two-dimensional colonies of rod-shaped E. Coli. They focus on ‘topological charges’ that appear during the growth, which are points where the directional orientation order parameter is singular. Using analytical tools commonly employed in analyses of phase transitions, they show that the colonies self-organise into a scale-invariant structure. This places those structures in a universality class that is different from conventional active nematic liquid crystals. The main significance of that work is not as much in understanding the particular phenomenon they studied but rather in the attempt to describe the large-scale dynamics with continuum dynamic equations that are characteristic to many fields in physics, from the atomic to the cosmological scales. Hartmann et al. [251] combined experimental and theoretical analyses to show that the emergence of local nematic order in growing V. Cholerae biofilms can be captured by an effective cell–cell interaction potential, thus translating molecular mechanisms into force parameters. Although the potential they used is unlike any other in conventional physical systems, it appears to describe reasonably well the global behaviour of a liquid-crystalline-like colony of about 10,000 individual bacteria.

These examples suggest that bridging between the cell-scale and coarse-grained continuum descriptions could benefit greatly the understanding global behaviour of biofilms. In particular, this can lead to advances in modelling transitions between different morphological and growth states. The quest for such a bridging is the main approach in physics, where the default modelling of most physical systems is often in the continuum. Turning to physics-based models in general, and to descriptions of phase transitions in particular, to gain insight into large-scale dynamics and self-organisation of biofilms, is a promising emerging approach. Nevertheless, going down this route should be done with caution. This is because physics models often comprise simplified versions of the real systems and the skills of a physicist are to reduce a system to the smallest number of significant mechanisms and variables. While this approach has been extremely useful in modelling inanimate systems, biofilms and active matter are quite complex and often cannot be reduced to a few-parameters modelling. The reason is that active matter can adapt to changing circumstances and environments by changing the behaviour both on the intra-cellular level, as well as manipulate inter-cellular interactions. While this makes the dynamics of richer, it also means that the models need also be adaptable and more versatile than in conventional physics.

CRediT authorship contribution statement

The authors contributed equally to all aspects of the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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