Mechanomicrobiology: how bacteria sense and respond to forces

Yves F. Dufrêne[1,2] *and Alexandre Persat*[3]*

Abstract | Microorganisms have evolved to thrive in virtually any terrestrial and marine environment, exposing them to various mechanical cues mainly generated by fluid flow and pressure as well as surface contact. Cellular components enable bacteria to sense and respond to physical cues to optimize their function, ultimately improving bacterial fitness. Owing to newly developed biophysical techniques, we are now starting to appreciate the breadth of bacterial phenotypes influenced by mechanical inputs: adhesion, motility, biofilm formation and pathogenicity. In this Review, we discuss how microbiology and biophysics are converging to advance our understanding of the mechanobiology of microorganisms. We first review the various physical forces that bacteria experience in their natural environments and describe the structures that transmit these forces to a cell. We then discuss how forces can provide feedback to enhance adhesion and motility and how they can be transduced by dedicated cellular machinery to regulate diverse phenotypes. Finally, we provide a perspective on how mechanics influence biofilm spatial organization and homeostasis.

Forces
Interactions that change the motion or shape of a body.

Hydrostatic pressure
The pressure in a static fluid generated by gravity.

Shear forces
Forces that are applied tangentially to a body's surface, generally generated by flow.

Mechanical stress
A quantity that expresses the internal forces of a material.

[1]*Institute of Life Sciences, UCLouvain, Louvain-la-Neuve, Belgium.*

[2]*Walloon Excellence in Life Sciences and Biotechnology (WELBIO), Wavre, Belgium.*

[3]*Institute of Bioengineering and Global Health Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.*

**e-mail: alexandre.persat@epfl.ch*

https://doi.org/10.1038/s41579-019-0314-2

The mechanic's feel comes from a deep inner kinesthetic feeling for the elasticity of materials. Robert M. Pirsig, *Zen and the art of motorcycle maintenance.*

Environmental forces have key roles in the physiology of any living system, from the origins of life, when flow may have driven the division of protocells[1], to guiding the differentiation of stem cells[2]. Microorganisms are no exception: they experience forces most often imposed by their natural environment[3]. We know that microorganisms must resist these forces, but what is less clear is whether they actively sense and respond to mechanical cues. In short, do mechanical forces influence bacterial physiology?

Single bacteria experience contact forces as they associate with the abiotic surfaces of rocks, soil particles, ships or medical devices (FIG. 1a) or with biological surfaces such as epithelia, extracellular matrices, mucus, teeth and skin (FIG. 1b). These forces can be compressive (inward) as an attached cell lands and pushes itself onto the surface, or they can be tensile (outward) as a cell is pulled away from the surface. In the same manner, bacteria experience compression in deep-sea environments where hydrostatic pressure reaches hundreds of atmospheres or as they become engulfed by host cells during phagocytosis[4] (FIG. 2).

Additionally, microorganisms must contend with the flow of fluid in various environments: in vivo, as bacteria colonize the blood vasculature, urinary tract, intestine and airways, or in the abiotic environments of catheters, sedimenting marine snow, ships and riverbeds. Here, the resulting hydrodynamic forces are tangential to the surface, in the direction of the flow. A consequence of shear forces is the removal of surface-associated cells; bacteria have evolved adhesive structures to counteract shear forces and thus maintain their surface-attached state[5].

Mechanically, bacterial cells and their surface-exposed molecular and supramolecular structures experience external shear, tension or compression (FIG. 2). The cell envelope is subject to these forces. More specifically, the outer membrane in Gram-negative bacteria and peptidoglycan in Gram-positive bacteria potentially transmit mechanical stress to core structures such as the cytoplasmic membrane. Surface extensions such as flagella, pili or the capsule that mediate the physical interactions between the cell and a surface also bear external forces, being under tension or compression, or being deformed. How force is transmitted to a cell depends on the mechanical properties of these structures. The mechanical properties of peptidoglycan and the outer membrane control cell shape, growth and division, and protect the cell from chemical stresses, including mechanical stress induced by osmotic shock[6,7]. Similarly, flagella, pili, cell envelope-associated adhesins, the capsule and biofilm matrices have intrinsic mechanical characteristics, but how these experience mechanical stress is largely unknown[8,9]. Until now, their molecular structures and functions have been mostly studied in force-free environments (that is, at equilibrium),

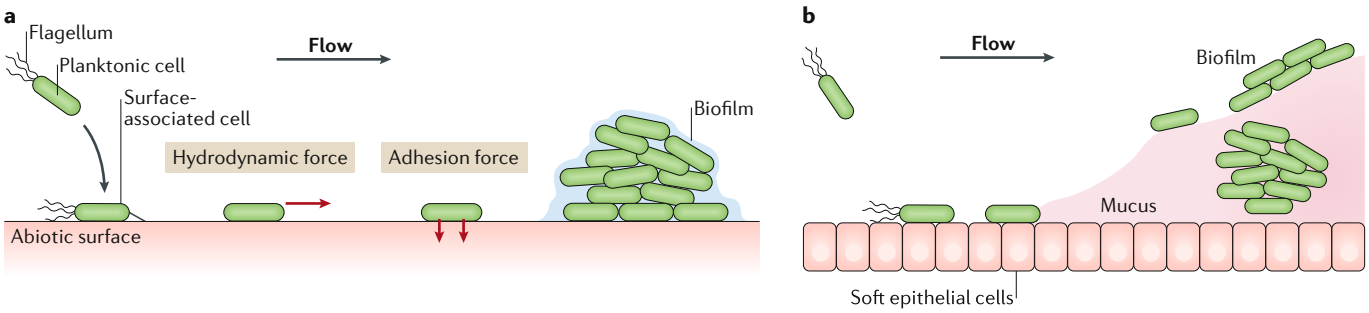

Fig. 1 | **Forces on bacteria.** Single cells experience forces in abiotic (**a**) and biological (**b**) environments as they colonize surfaces in flow. Surface attachment can generate adhesion force. Hydrodynamic forces, which are generated by fluid motion, are experienced by surface-attached cells. Bacteria interact with their surroundings via surface structures such as adhesins, motorized pili and flagella. During host colonization, bacteria can experience mechanical forces as they contact soft materials such as cells and tissues, including dynamic mucus layers, and encounter flow of blood, urine and gastrointestinal fluids.

a paradox when considering that they are inherently subject to unsteady mechanical influences and are therefore out of equilibrium.

The development of new methodologies that enable researchers to mechanically probe living systems at the organism, single-cell and molecular levels has been instrumental to the advances in studying eukaryotic mechanobiology. With these, we now have abundant evidence supporting the central role of mechanics in regulating eukaryotic cell function, including cell division, differentiation, immunity, development and motility. The knowledge gap in our understanding of how bacteria and archaea sense and respond to mechanical stress is largely explained by the size of cells and of their surface machinery, which complicates mechanical experimentation at such small scale.

In this Review, we focus on the following question: how do bacteria sense and respond to the mechanics of their environments? We first discuss how mechanical inputs feed back on adhesion and motility systems, increasing their efficiency in the face of external applied force loads. We subsequently describe how single cells can actively sense forces to regulate distant phenotypes, such as gene expression and pathogenicity, and specifically highlight the molecular mechanisms of mechanosensing and signal transduction. Finally, we expand our discussion of mechanoregulation to biofilms — the architecture of which is essentially guided by mechanics, yet little is known about how mechanics affect the physiology of biofilm-dwelling cells. In this Review, we aim to provide a fresh perspective on the signals that regulate bacterial behaviour and phenotypes, which should not be uniquely focused on the influence of chemical cues.

Mechanosensitive feedback

Motility mechanoswitching. Bacteria actively propel themselves by converting chemical energy into mechanical work. For example, to swim within the bulk of a fluid, single cells rotate their flagella. These helical filaments make use of the physics of fluids at the micrometre scale to convert their rotation into a translational motion of the cell body[10,11]. The interactions between molecular rotors and stators drive flagellum rotation and torque, which in turn exert a force on the surrounding fluid. The rotor, which is also known as the C-ring, is a cytoplasmic structure tightly coupled with the flagellum basal body to drive rotation[12]. Stators are stationary proteins (MotA and MotB in *Escherichia coli*) that are anchored in the cell envelope and interact with the rotor. Chemical energy provided by ion-motive forces drives conformational changes in stator components, which lead to spinning of the rotor.

Mechanical feedback on flagellar motorized activity enables many bacterial species to adapt their swimming behaviour to increases in fluid viscosity, increases in drag force at the vicinity of surfaces or in biofilms[12]. In *E. coli*, forces that oppose flagellum rotation activate a positive feedback mechanism that helps recover rotation rate (FIG. 3a). This effect was demonstrated by attaching micrometre beads to flagella using optical tweezers or by controlling torque on the motors of immobilized cells with rotating electric fields[13–15]. In response, the rotation frequency of the flagellum first drops, but recovers within 1–2 minutes[13,15]. At the same time, increasing torque promotes the polar localization of a fluorescently tagged MotB protein, which indicates stator assembly. Thus, increasing torque on the motor recruits additional stator units in a stepwise manner, thereby increasing rotation frequency. At the molecular level, the force decreases the dissociation rate of MotB from peptidoglycan, which leads to the accumulation of this protein near the rotor[15,16]. This force-induced decrease in dissociation rate is known as a catch-bond mechanism (see below). Furthermore, the flagellum structure itself responds to a mechanical load by adopting various polymorphisms[17]. In *Shewanella putrefaciens*, these polymorphic changes drive different swimming patterns that enable the bacteria to escape from traps or to move in high viscosity environments[18,19].

Positive feedbacks on motility also occur over longer timescales. *Pseudomonas aeruginosa* adapts flagellar torque upon changes in viscosity by swapping between stators that generate distinct magnitudes of torque: MotA and MotB are used in low-viscosity environments, whereas MotC and MotD engage to power swimming in high viscosity or at the surface of soft swarm agar plates, where the load applied on the flagellum motor is high[20,21]. Positive feedback on swarming motility has

Torque
An interaction causing a body to rotate.

Flagellum basal body
A group of proteins that anchors the flagellum to the cell body and includes the flagellum motor.

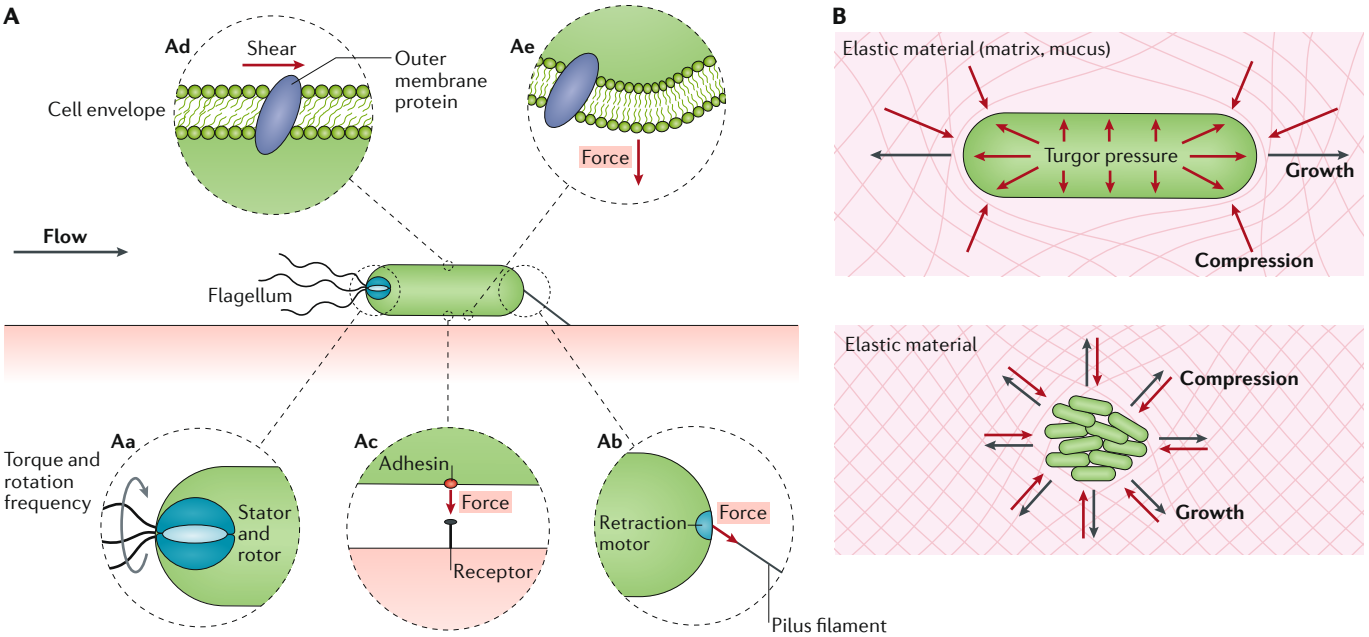

Fig. 2 | **Bacterial structures experiencing external forces. Aa** | The interactions between molecular rotors and stators drive flagellum rotation and torque. When single cells attach to a solid surface, steric or hydrodynamic interactions inhibit flagellum rotation, putting a load on the motor machinery. **Ab** | Retraction of surface-attached type IV pili generates a tension force in the filament, on its motor and on other structural components. **Ac** | Adhesins or sticky substances can transmit tension to the cell surface when experiencing a force load. **Ad**, **Ae** | Forces generated by flow or by the motility machinery can also bend, shear or stretch the cell envelope, for example, the lipid bilayer and outer-membrane proteins. **B** | Bacteria also experience mechanical forces as they grow within elastic materials, for example, in the biofilm matrix or in mucus. Growth driven by (internal) turgor pressure deforms and mechanically stresses the surrounding elastic material, compressing the cell envelope. Flagella, pili and adhesion components are also mechanically perturbed under these conditions (not shown).

been observed in various species, including in the pathogens *Vibrio parahaemolyticus* and *Proteus mirabilis* and the soil bacteria *Azospirillum spp*., all of which differentiate into highly flagellated swarmers upon surface contact[22–24]. These examples suggest that dedicated sensors activate transcription or assembly of flagellar components, leading to increased power output due to greater numbers of flagella.

Force-induced motility feedback is well described in pathogens but is also found in species across various environmental niches, for example, in deep-sea environments. Flagellum-driven swimming motility is highly sensitive to changes in pressure. Deep-sea bacteria cope with this using pressure-dependent differentiation from planktonic to swarmer phenotypes. The swimming velocities of typical model organisms decrease with pressure and most are unable to swim at pressures above 50 MPa[25]. By contrast, piezophiles, which are bacteria that grow optimally at high pressure, adapt their flagellar machinery to efficiently swim in extreme mechanical environments. In fact, the deep-sea bacterium *Photobacterium profundum* swims faster under pressure and can maintain motility up to 170 MPa, more than a thousand times the atmospheric pressure[26]. *P. profundum* possesses two flagellar gene clusters: one encoding a polar flagellum and the other a lateral flagella system. Genes encoding lateral flagella and their corresponding proton-driven motors are upregulated under high pressure and in high viscosity media and are necessary to power swimming under these conditions. Upregulation of genes encoding the lateral flagella system depends on the presence of the polar flagellum, which does not function at high pressure. These observations suggest that single *P. profundum* cells mechanically sense local pressure and viscosity with their polar flagella, and respond by increasing the production of lateral flagella, thereby enabling swarming motility in high-pressure environments. Similarly, the piezophile *Shewanella piezotolerans* initiates swarming as pressure increases[27], which also indicates that the sessile lifestyle might be favoured over a planktonic one in the ocean depths.

Type IV pili (T4P) are motorized surface appendages that are a few micrometres long and that promote adhesion and motility on surfaces. Successive rounds of pili extensions and retractions enable single *P. aeruginosa*, *Acinetobacter baumannii* or *Xylella fastidiosa* cells to move on solid surfaces in a motility mode called twitching[28,29]. The molecular motors PilB and PilT hydrolyse ATP to respectively polymerize or depolymerize thousands of PilA pilin subunits within seconds[30]. Successive extensions and retractions involve swapping motors, but whether and how cells control this sequence had been unclear. Label-free visualization of *P. aeruginosa* T4P in live cells by interferometric scattering microscopy demonstrated that the motors coordinate their activity[31]. T4P remain extended at the cell surface until their tips touch a surface, an event that stimulates rapid retraction (FIG. 3b). This suggests that PilT responds to pilus surface

contact. The exact mechanism by which the mechanical signal is transmitted from the tip to the motors is unknown but is likely to involve force-induced changes in the conformation of the pilus along the filament[32,33].

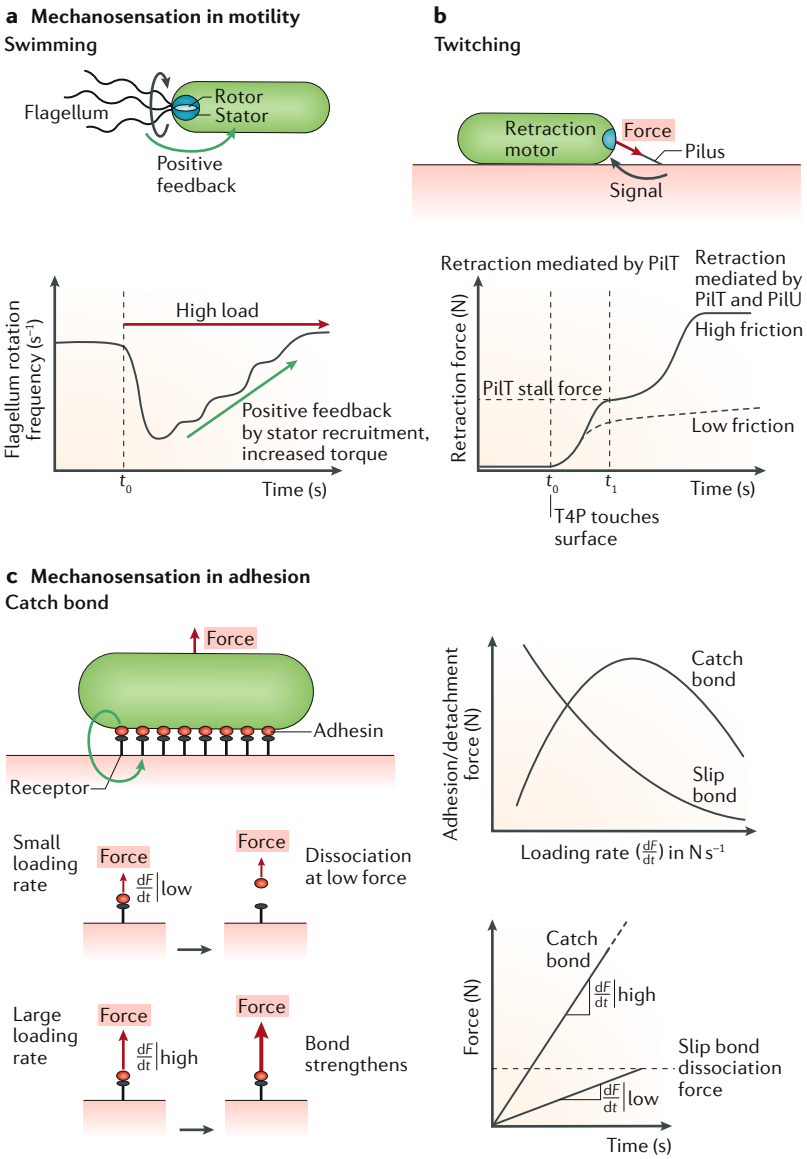

Fig. 3 | **Force-induced feedback on motility and adhesion. a** | Single cells swim through viscous fluids by rotating their flagella. Flagellar stators exert a torque on the helical filament. The power output of the machinery depends on the proton motive force, which drives flagellar rotation (not shown). Environmental changes, such as an increase in viscosity or proximity with a surface, increase the power burden on the stators, which adapt their rotation rate by recruiting additional torque-generating units. **b** | *Pseudomonas aeruginosa* type IV pili (T4P) control their dynamic activity by coordinating retraction with tip attachment. Contact of the T4P tip at time t_0 with a surface triggers retraction of the pilus. Retraction is mediated by the PilT molecular motor, which is a retraction ATPase that drives filament depolymerization to power motility in low-friction environments. In environments of increasing friction, a second retraction ATPase, PilU, can power filament depolymerization under high load, for example, at a time t_1 where PilT reaches a threshold force. PilU ultimately generates sufficient force to propel single cells. **c** | Catch bonds increase adhesion under strong force loads. Single adhesin–receptor interactions that experience a slowly applied tension force dissociate easily (at low bond dissociation force). When force is applied at a higher rate $\frac{dF}{dt}$, adhesion strength increases through the formation of catch bonds. By contrast, slip bond attachment strength always decreases with applied force.

We note that species whose pili are specialized in DNA uptake (*Vibrio cholerae*) and adhesion (*Caulobacter crescentus*) can frequently retract without sensing surfaces[34–36], which indicates that mechanical feedback on retraction evolved as a prerequisite for twitching motility.

Bacteria use T4P to move while maintaining a stable sessile lifestyle. *P. aeruginosa* use T4P at the surface of burn wounds[37] and airway epithelia during infections[38], and *X. fastidiosa* uses these pili when colonizing the vasculature of trees[39]. Flow and friction with the surface during movement increase force on the T4P machinery, for example, as *X. fastidiosa* twitches upstream[40]. Could cells adapt their retraction force in response, similarly to mechanosensory feedback on flagellar motors? A comparison of T4P dynamics and twitching motility in environments of increasing friction suggest that PilU, a second retraction ATPase, can power PilA depolymerization under high load, thereby generating sufficient force to propel single cells[31] (FIG. 3b). Consistent with this, retraction force measurements in *V. cholerae* show that PilT and PilU collectively generate larger force than PilT alone[41]. The mechanism of motor swapping is reminiscent of the reorganization of flagellar stators, highlighting a common 'gear-shifting' strategy in bacterial motility systems. We thus anticipate that mechanical feedback could have a role in the adaptation of other motility systems such as surface-specific gliding used by *Myxococcus xanthus*, during which cytoplasmic motors are mechanically coupled into focal adhesions to the solid surface to power displacement of the cell body[42].

Mechanical feedback in adhesion. Adhesion is a first key step to infections as many bacterial pathogens must attach to biomaterials and host tissues[5,43]. Microbial adhesion is also highly relevant in environmental and industrial microbiology as it leads to cell aggregation and biofilm formation. How do force loads on individual or multiple bonds influence adhesive functions? Due to single-cell and single-molecule technologies, the past years have witnessed substantial progress in our understanding of how microorganisms use and respond to force for cell adhesion.

Most proteins stretch by reversibly unfolding under the action of a force as a spring[44,45]. Because they must withstand stronger forces, some adhesins have unusual elastic properties. The soil bacterium *Pseudomonas fluorescens* sticks to various abiotic and biotic surfaces using the large adhesin protein LapA, a ~520 kDa protein that contains two structural features mediating adhesion: 37 N-terminal 100-amino-acid repeats and a C-terminal mechanosensitive von Willebrand factor type A. Under the force generated by an atomic force microscope (AFM), LapA displays remarkable elastic properties, with sequential force peaks reflecting unfolding of its multiple repeats[46]. Surface properties strongly affect the mechanical response of LapA, with sequential unfolding events being observed only on hydrophilic substrates. LapA thus provides *P. fluorescens* with multipurpose adhesion and thus enables the colonization of diverse environments. By contrast, some proteins are too stable to be easily unfolded. For instance, the *Staphylococcus aureus* surface protein SasG promotes strong cell–cell

adhesion during biofilm formation. SasG contains ~10 tandem repeats of G5 domains that are separated by E domain spacer regions. The force required to unfold individual G5 and E domains is strong, up to ~500 pN, compared with ~50–250 pN in most proteins, thus explaining how SasG–SasG bonds can resist physiological shear forces[47]. The high mechanical stability of SasG is likely to be related to its dynamic structure. Under high tension, the unfolding of SasG domains may expose extended conformations in which previously hidden domains become available for binding. The rod-like shape of the protein contributes to the formation of strong cell–cell bridges during biofilm formation while resisting shear forces.

Amyloid-forming sequences are found in a number of bacterial proteins, some of which might become stickier under tension. The fungal pathogen *Candida albicans* improves surface adhesion using force-sensitive surface agglutinin-like sequence (Als) proteins. Als proteins contain a threonine-rich region with a buried short amyloid-forming core sequence that becomes exposed under mechanical force. This induces the formation of cell surface nanodomains composed of laterally arranged Als proteins that promote homophilic cell–cell adhesion and biofilm formation[48]. By extension, tension force may also promote the aggregation of amyloid adhesins in *E. coli* and *Bacillus subtilis*[49,50].

Single-molecule force spectroscopy experiments have revealed that the mechanical behaviours of pili are distinct from those of globular proteins. *P. aeruginosa* T4P can resist up to 250 pN tension force, which implies that they can maintain adhesion under the ~100 pN retraction force[32]. In addition, force-displacement curves for T4P featured a constant force plateau, which indicates that they were elongated in a stable stretched state. Consistent with this, interferometric scattering microscopy imaging shows that T4P frequently attach flat against the surface when under tension, whereas relaxed pili only attach from their tip, which suggests that force increases adhesion along the filament length[31]. Hence, pili may respond to force by transitioning into an extended quaternary structure that exposes hidden residues capable of promoting adhesion[33].

Pili from Gram-positive bacteria, which are less well characterized, have different assembly mechanisms and mechanical properties compared with pili from Gram-negative bacteria[51]. One example is the pilus of the probiotic strain *Lactobacillus rhamnosus* GG, which enables binding to intestinal epithelia and aggregation[52]. Stretching experiments on single cells revealed that *L. rhamnosus* GG pili exhibit two distinct mechanical responses depending on force: at low force, a zipper-like adhesion was observed involving multiple SpaC pilin subunits distributed along the pilus length; at large force, a nanospring behaviour (that is, pilin proteins do not unfold), enabling pili to resist high loads[53]. The nanospring behaviour is consistent with stretching experiments performed on single pilin subunits in the Gram-positive pathogen *Streptococcus pyogenes*: pilins cannot be unfolded even by large mechanical forces, meaning that they are completely inextensible[54]. In contrast to Gram-negative bacteria, pili from Gram-positive bacteria form by covalent polymerization and are stabilized by internal isopeptide bonds, which results in extreme stiffness and resistance to force[55]. These studies demonstrate that pili from Gram-negative and Gram-positive bacteria display fascinating force-dependent adhesion and that force must be accounted for when exploring their functions.

A remarkable, counterintuitive trait of some adhesins is their ability to strengthen adhesion under tension (FIG. 3c). The function of adhesins can be physically and quantitatively described by their unbinding strength, that is, the force at which they will detach. Typically, adhesion strength between a single adhesin and its ligand decreases with applied force (BOX 1), a behaviour termed slip bond. Counterintuitively, some adhesins tend to strengthen when experiencing a tensile load, a phenomenon referred to as catch bond[56,57].

The adhesion of the type I pilus tip protein FimH of *E. coli* is the prototypical example of catch bonds[58]. FimH specifically binds mannose residues on glycoproteins that decorate the surfaces of epithelial cells. During infection of the urinary tract, FimH–mannose catch bonds stabilize the attachment of uropathogenic *E. coli* (UPEC) to the bladder and urinary tract by improving adhesion in the presence of flow. The affinity between FimH and mannose is strengthened with increasing mechanical force[59]. A force-induced allosteric conformational change in FimH switches the adhesin to a state of high affinity for its mannose ligand[60].

In a similar manner, recent AFM studies showed that force promotes the adhesion of *S. aureus* and *Staphylococcus epidermidis*, which are common and devastating nosocomial pathogens, providing single bacteria the ability to resist increases in shear stress during colonization[61]. Their adhesins SdrG, ClfA and ClfB bind fibrinogen and loricrin ligands via three separate subdomains with forces in the nano-Newton range. These forces are equivalent to that of a covalent bond, which makes them the strongest receptor–ligand bonds measured to date, surpassing the biotin–streptavidin bond by an order of magnitude[62,63]. These extreme adhesion forces result from the high affinity 'dock, lock and latch' binding mechanism, which involves dynamic conformational changes that lead to highly stable complexes[64,65]. Steered molecular dynamics simulations combined with AFM revealed that the extreme mechanical stability of these interactions originates at the molecular level from an intricate hydrogen-bond network between the ligand peptide backbone and the adhesin[62]. Such ultra-high binding forces are in stark contrast to the values measured by standard bulk techniques, which demonstrates that single-molecule forces measured out of equilibrium and binding constants measured at equilibrium do not correlate[66]. Understanding these non-equilibrium properties can provide insights into the development of new therapeutic strategies, for example, the development of adhesin-binding antagonists[67,68].

From force signal to cellular response

Above, we described how forces directly feed back to motility and adhesion machineries. Next, we focus on how dedicated sensors can transduce mechanical input into a

cellular response, activating other cellular machinery and eliciting distinct phenotypes. These mechanotransduction systems most often involve a mechanosensor and relay components such as two-component systems and second messenger molecules[69,70] (FIG. 4).

Mechanotransduction in P. aeruginosa. In *P. aeruginosa*, surface contact and flow stimulate multiple behavioural changes at different timescales — within seconds after the transition from planktonic to sessile states or days during the process of infection and biofilm formation. A clear link has been established between mechanical input generated by surface contact and the production of the second messenger molecules cyclic AMP (cAMP) and cyclic di-GMP (c-di-GMP), two ubiquitous signalling molecules that enable cells to control multiple phenotypic changes in response to specific environmental inputs.

Within the few seconds following surface contact, single *P. aeruginosa* cells deploy T4P, improving adhesion and initiating surface motility[71]. This response depends on the flagellar stator proteins MotA and MotB and the c-di-GMP-binding protein FimW. Thus, the stator MotA–MotB could function as a mechanosensor for surface attachment, although T4P deployment does not depend on the flagellum itself (FIG. 4a). In addition, the phosphodiesterase Pch, which degrades c-di-GMP, localizes at the flagellated cell pole, driving asymmetric distribution of the second messenger between cell progeny. This differentially affects the activity of FimW, which is only active at the non-flagellated pole. Daughter cells subsequently possess distinct adhesive properties, separating them into sessile and planktonic states. This asymmetry accelerates the dispersion process and thus enables rapid colonization and infection of host cells.

Box 1 | **Adhesion, fluid and solid mechanics**

The force and deformation fields in liquid and solid materials obey constitutive laws, the governing equations of which can be solved computationally and sometimes analytically. Granted a basic knowledge of mechanics and some simplifications, it is possible to obtain reasonable estimates for these forces. We provide some practical tools and definitions that may help the reader to build physical, quantitative insights for relevant microscale fluid and solid mechanics in the bacterial world.

Adhesion

Adhesion forces are a result of the physicochemical interactions between the cell surface and its attachment substrate[5,159]. The adhesion strength for a single cell or a single receptor–ligand interaction is generally defined as the tension force at which it will detach (in units of N). When attachment involves multiple receptor–ligand interactions, adhesion strength can be quantified as the adhesion force per unit of contact area (in units of Nm^{-2} or Pa, analogous to a pressure). Whether it is specific or not, adhesion can be viewed as a process whereby a cell receptor binds to a surface-anchored ligand. Detachment thus depends on the dissociation of a bond under force F. By invoking the Arrhenius equation, the dissociation constant can be calculated under load $k_{off} = k^0_{off} e^{F\delta/k_bT}$ (also known as Bell's equation; where k^0_{off} is the force-free dissociation rate constant, δ is a characteristic length scale, generally determined empirically but which can be viewed as a bond extension, k_b is Boltzmann's constant and T is the temperature)[170]. Although more accurate models have been used to describe experimental data, Bell's equation provides a quantitative glimpse at how force influences receptor–ligand dissociation kinetics and highlights an important intrinsic force scale of the adhesive bond k_bT/δ. Typically, the strength of receptor–ligand bonds is in the range of 50 pN to 250 pN[44].

Fluid mechanics

How do cells physically experience moving fluids? In flow, single cells and biofilms experience a hydrodynamic force that is a result of shear stress. In virtually any flow, a fluid is at rest at the surface of solid boundaries, including the ones of bacterial cells, a phenomenon also known as 'no-slip'. This boundary condition strongly affects the motion of a fluid near surfaces by generating strong velocity gradients, quantified by the shear rate: $\gamma = \frac{\partial v}{\partial y}$ (where v is the fluid velocity and y is a coordinate perpendicular to the surface). The shear stress τ quantifies how hard it is to move the fluid near the surface and thus depends on its dynamic viscosity μ and the shear rate at the surface $\gamma_{surface}$: $\tau = \mu \gamma_{surface}$. Shear stress is a force density, so that the hydrodynamic force generated by a uniform flow on a cell of surface area A is $F = \tau A$. In a typical microfluidic or flow chamber experiment, flow conditions yield shear stresses of 0.01 Pa to 10 Pa, which generate forces on a single 1 μm² cell of approximately 0.01 pN to 10 pN. For more complex, non-uniform flows, shear stress must be integrated over the surface of the object, similarly to the calculation of the drag force of a sphere (or a cell) of radius a swimming at velocity V, also known as Stoke's drag: $F_D = 6\pi \mu Va$. A bacterium swimming at 10 μm/s^{-1} experiences a hydrodynamic force of approximately 0.1 pN, which is relatively low and explains the low energetic requirement on flagellar motors. Finally, we note that microorganisms living in strong-flow environments do not necessarily experience strong hydrodynamic forces. For example, turbulent oceanic flows influence the transport of cells and molecules[171] but bacteria associated with suspended particulate matter do not experience strong shear as they move with the flow.

Solid mechanics

Solid materials deform when subject to forces, a property known as elasticity. Bacteria and proteins can be viewed as elastic materials: they deform under the influence of forces (a change in protein conformation is a deformation). Mechanosensitive machinery such as proteins, protein assemblies or membranes modulate their function and ultimately generate biochemical signals in response to these deformations. The force can be self-generated (by pili and flagella) or applied externally (flow), but mechanosensing always requires the generation of a force.

To understand the relationships between force and deformations, consider an object of uniform cross-sectional area A and length L subject to an end-to-end force F extending the object by a length ΔL. We define the strain ε as the relative deformation of this object: $\varepsilon = \Delta L/L$. The stress is defined as the force per unit area across the solid object: $\sigma = F/A$. According to Hooke's law, there is a linear relationship between stress and strain, $\sigma = \varepsilon E$, reminiscent of the relationship between force and the extension of a spring. The Young's modulus E has units of Pa (force per unit area) and is essentially a measurement of the stiffness of a material. Very soft tissues, such as the brain or some biofilm matrix components, have a Young's modulus below 1 kPa, whereas very stiff biomaterials such as the bacterial cell wall have stiffness in the order of megapascals[172,173]. From Hooke's law, a ~10 MPa stiff, 1 μm spherical bacterium experiencing a 10 pN physiological force would deform by about 1 pm (smaller than the scale of an atom), whereas a 1 kPa stiff, 1 μm cube of biofilm matrix subject to the same force would deform by 10 nm (the typical size of a protein). Under the 10 kPa internal stress generated by bacterial growth[118], the matrix would experience a 10% strain.

To model the deformations of more complex and heterogeneous materials, the stress field can be computed using constitutive equations. However, a simplified framework using a decomposition in spring elements can help construct physical and quantitative models for forces and deformations. This decomposition has enabled biophysicists to rapidly generate models for polymers, biofilms, mammalian cells and cell sheets, plasma membranes, and single proteins[174–176].

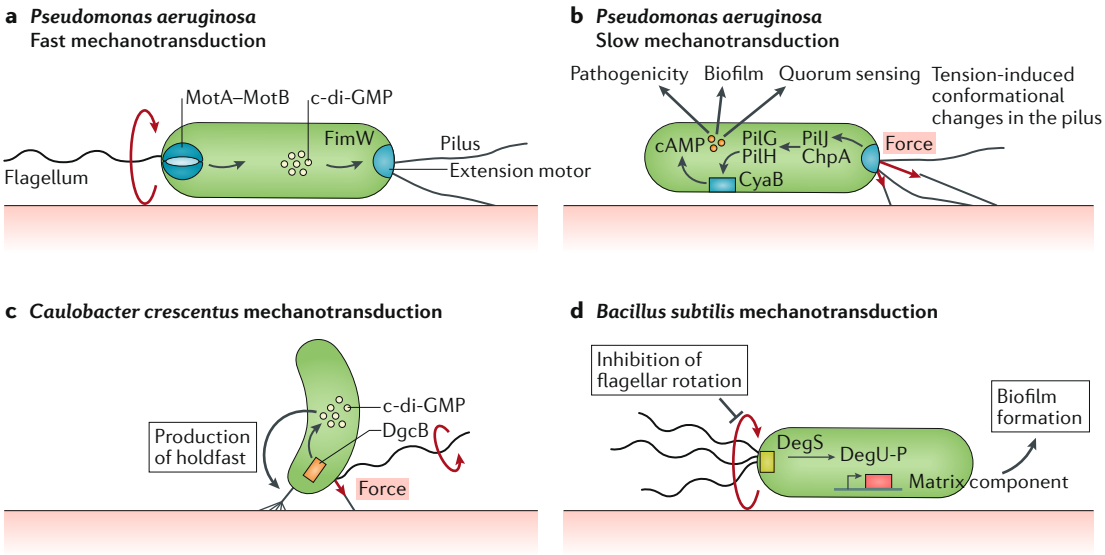

Fig. 4 | **Bacteria use mechanotransduction to regulate various phenotypes in response to forces.** Force-sensing components such as type IV pili (T4P) and flagella transmit mechanical signals to sensing components. Information can be relayed over short timescales by various chemosensory systems. **a** | For example, in *Pseudomonas aeruginosa*, the flagellum stator (MotA–MotB) responds to surface contact by inducing an increase in the levels of cyclic di-GMP (c-di-GMP). The c-di-GMP-binding protein FimW binds c-di-GMP, activating the deployment of T4P to promote adhesion and initiating surface motility. **b** | Over longer timescales, surface-attached *P. aeruginosa* increase their intracellular levels of cyclic AMP (cAMP). Increased cAMP levels stimulate the transcription of genes, including genes encoding secretion systems, components of the T4P and regulators of quorum sensing, while downregulating flagellar genes to promote pathogenicity and biofilm formation. Surface contact-induced cAMP production depends on the Chp chemotaxis-like system. Chp is activated by its methyl-accepting chemotaxis protein PilJ. The major pilin subunit PilA interacts with PilJ in vitro, which suggests that T4P directly activates the Chp system, potentially through tension-induced conformational changes. This input signal controls autophosphorylation of the histidine kinase ChpA, transferring phosphoryl groups to two response regulators, PilG and PilH, which ultimately control the activity of the adenylate cyclase CyaB. **c** | In *Caulobacter crescentus*, surface contact stimulates the production of a sticky polar holdfast, irreversibly committing a cell to surface attachment. Mechanical force at the pilus or the flagellum motor activates the diguanylate cyclase DgcB to increase intracellular c-di-GMP levels, which promotes the biogenesis of the holdfast. **d** | In *Bacillus subtilis*, inhibition of flagellar rotation activates the two-component system DegS–DegU. Phosphorylated DegU (DegU-P) initiates the transcription of the genes encoding poly-γ-DL-glutamic acid, which is a biofilm matrix component.

Over longer timescales (minutes to hours), surface-attached *P. aeruginosa* increase their intracellular levels of cAMP[72] (FIG. 4b). Increased cAMP levels stimulate the transcription of hundreds of genes via the transcription factor Vfr (virulence factor regulator), including genes encoding type II and III secretion systems, components of the T4P and regulators of quorum sensing, while downregulating flagellar genes, which demonstrates that contact with host cells is a key event in the regulation of pathogenicity[73,74]. Surface-induced stimulation of cAMP depends on T4P and its motors as well as on the stiffness of the material substrate, which indicates that cells kinaesthetically sense the surface. Surface contact-induced cAMP production also depends on a chemotaxis-like system called Chp, the activating signal of which had remained elusive until recently. As in the canonical chemotaxis system, Chp activity depends on stimulation of its methyl-accepting chemotaxis protein PilJ. The major pilin subunit PilA interacts with PilJ in vitro, which suggests that T4P directly activates the Chp system, potentially through tension-induced conformational changes. This input signal controls autophosphorylation of the histidine kinase ChpA, transferring phosphoryl groups to two response regulators, PilG and PilH, which ultimately control the activity of the adenylate cyclase CyaB[75,76]. The Pil–Chp system and cAMP promote surface attachment of single cells over longer timescales, which is crucial for the subsequent formation of biofilms[77]. Furthermore, oscillations in cAMP levels following surface detachment provides cells with a memory for attachment over many generations, which enables prompt surface recolonization by formerly attached cells. It is unclear why *P. aeruginosa* uses such a complex system to regulate transcriptional responses over long timescales, but we anticipate that Chp controls other behaviours on shorter timescales. The Pil–Chp system is conserved among several pathogens and environmental species, which suggests that it has been repurposed to function during surface adaptation in various contexts, probably to regulate diverse phenotypes[75].

Surface contact also stimulates the production of c-di-GMP over long timescales. Increasing the levels of c-di-GMP is crucial in the development of biofilms, as this second messenger controls the production of extracellular polymeric substance (EPS) matrix components.

Holdfast
An extremely sticky substance secreted at the poles of many prosthecate bacteria that enables irreversible attachment.

Consistent with this, c-di-GMP levels depend on the magnitude of fluid shear and the adhesion strength of single cells to a surface[78]. Cells that lack T4P components, including the minor pilin protein PilY1, do not respond to surface contact or shear and maintain planktonic levels of c-di-GMP[79]. At the molecular level, PilY1 interacts with the T4P alignment complex PilMNOP to activate the diguanylate cyclase SadC, thus increasing the production of c-di-GMP[79]. PilY1 has homology to the mechanosensitive von Willebrand factor and is positioned in T4P and/or at the outer membrane, making it an attractive candidate mechanosensor. PilY1 is also a key element in the surface-activated virulence against amoebae[80]. In addition to regulating the production of matrix components, increased c-di-GMP levels affect flagellar function by disengaging the high-torque stator complex MotC–MotD in favour of MotA–MotB[14,81], hence repressing swarming motility. MotC itself interacts with SadC to stimulate c-di-GMP production, now providing negative feedback on swarming motility[82]. Moreover, the chemotaxis-like Wsp system provides an additional input for regulation of c-di-GMP levels during long-term surface contact, but whether and how Wsp senses mechanical input remains unclear. Overall, it is clear that mechanosensing contributes to the regulation of intracellular c-di-GMP levels, but the molecular mechanisms of sensing and transduction must be rigorously elucidated. The large number of sensing systems that control c-di-GMP levels suggest that multiple mechanical (for example, surface and shear sensing) and chemical inputs (for example, nitric oxide) contribute to its regulation[83].

Finally, microfluidics experiments showed that fluid flow also tunes the expression of other genes in *P. aeruginosa* in a force-independent manner[84]. In flow, single cells upregulate the *fro* operon, the function of which remains unknown. The magnitude of the response is a function of the shear rate but not shear stress, that is, single cells respond to fluid velocity rather than the force it generates. Accordingly, the expression levels of *fro* do not depend on any known mechanosensors.

Mechanotransduction via pili and flagella. In *Shewanella oneidensis*, a metal-reducing marine bacterium, surface contact controls phenotypes as fundamental as growth rate. In particular, the precision of cell-size homeostasis depends on the attachment state of a bacterium. The distribution of growth rates in populations of surface-attached cells is tight in comparison with that of planktonic cells. Surface-attached mutants that lack T4P or flagella have planktonic-like growth rate distributions, showing that these structures mediate mechanosensing[85].

In the stalked bacteria *C. crescentus* and *Asticcacaulis biprosthecum* as well as in the plant pathogen *Agrobacterium tumefaciens*, surface contact stimulates the production of sticky polar adhesins, which enables single cells to rapidly establish a nearly irreversible attachment to solid substrates[86]. In *C. crescentus*, the flagellar motor and pili collectively mediate this response, which indicates their potential roles as mechanosensors[34,87]. In a similar manner to *P. aeruginosa*, intracellular levels of c-di-GMP rapidly increase upon surface contact (FIG. 4c). The diguanylate cyclase DgcB transduces the mechanical signal into a cellular response that accelerates the production of the holdfast and inhibits flagellum rotation[86–88]. This enables single planktonic cells to rapidly establish a sessile lifestyle and sessile mother cells to promote the formation of biofilms[87,89]. *C. crescentus* and *P. aeruginosa* display a conserved strategy for mechanotransduction that leverages motorized machinery (pili and flagella), transducing components (two-component systems, adenylate and diguanylate cyclases) and second messenger molecules (cAMP and c-di-GMP). By contrast, *B. subtilis* responds to surface attachment independently of second messengers using the two-component system DegS–DegU. Inhibition of flagellum rotation activates the histidine kinase DegS, which activates the response regulator DegU to promote the transcription of genes encoding matrix exopolysaccharides[90] (FIG. 4d).

Mechanotransduction via the cell envelope. The attachment of enteropathogenic *E. coli* to intestinal host cells promotes the expression of virulence factors encoded in the locus of enterocyte effacement[91]. Upregulation of these factors is further exacerbated in flow, reinforcing the hypothesis of mechanosensation. Ler, a major regulator of virulence in enteropathogenic *E. coli*, participates in transducing the mechanical signal into a pathogenic output. The NlpE–Cpx system, which regulates protein folding in the periplasm, is a candidate mechanosensor localized on the outer membrane. Surface contact activates NlpE–Cpx, initiating the transcription of Ler by the transcription factor LrhA[92,93]. However, recent experiments measuring the response to surface contact at the single-cell level mitigate this hypothesis, contradicting the possibility that NlpE–Cpx functions as a surface mechanosensor[94].

Nevertheless, the NlpE–Cpx system illustrates the possibility that membrane-associated machines sense mechanical stimulation. Consistent with this, monitoring the fluorescence of a genetically encoded calcium biosensor in *E. coli* during mechanical compression showed that force increases calcium influx induced by membrane voltage depolarization[95]. Such mechanically induced changes in ion flux are reminiscent of mechanosensitive ion channel gating stimulated by tension of their supporting membrane[96,97]. As for mechanoresponse, the authors found that the levels of several *E. coli* proteins increase, including the small RNA-binding protein Hfq, which is a regulator of virulence in many pathogens[95]. The corresponding mechanosensitive components have not been identified; however, intuitively, mechanosensitive ion channels localized in the inner membrane could be activated by compression through deformations of the cell wall.

In the depths of oceans, microorganisms must cope with the extreme mechanics generated by high hydrostatic pressure. Piezophiles can grow, and thrive, at depths where pressure reaches up to 1,000 atm (REF.[4]). During transitions from low-pressure to high-pressure growth conditions, these deep-sea microorganisms undergo vast phenotypic changes not limited to motility as discussed above. As pressure increases, their outer

membrane becomes more rigid. In response, piezophiles adapt their lipid composition to reduce membrane fluidity[98] and also change their membrane protein composition. For example, *P. profundum* upregulates the outer membrane porin OmpH and downregulates OmpL, thereby improving their molecular uptake in the nutrient-deprived deep sea[99,100]. ToxR–ToxS, two inner membrane proteins that regulate virulence in response to various physicochemical signals in *V. cholerae*, control the pressure-dependent expression of these porins in *P. profundum*, thus functioning as a pressure sensor[101]. Consistent with a role in mechanical sensing, the activation of ToxR–ToxS depends on the fluidity of the outer membrane[101]. Although these pressure-sensitive phenotypes seem to be unusual, they might represent a vast and overlooked aspect of bacterial physiology given the large bacterial and archaeal populations colonizing subsurface environments[102–104]. Understanding the mechanobiology of these cryptic organisms could help us better understand the origins of life, which probably arose at high pressure[105].

Biofilm mechanobiology

Biofilm mechanics. In the wild, bacteria are commonly found as dense, cohesive communities associated with surfaces, called biofilms[106,107]. Biofilms form in any type of environment, including in rivers and streams, where they have crucial roles in biogeochemical fluxes[108,109], in our intestine, where they contribute to host metabolism and immunity[110,111], or within biological tissues and on medical devices, where they can cause antibiotic-resilient chronic infections[112]. The self-secreted EPS matrix is a hallmark of biofilms. The matrix is composed of polysaccharides, proteins, DNA and other cellular debris that maintain cohesion between cells and with a surface, while protecting the community against chemical, biological and mechanical stressors[113]. The matrix is both viscous (flows like a fluid) and elastic (deforms like a solid). Biofilms are active matter systems[114]: bacterial growth generates mechanical stress within the biofilm by deforming the elastic matrix (FIG. 2b), strongly influencing its morphology and the orientation of single cells in the community[115–117]. The magnitude of the mechanical stresses generated within biofilms is not exactly known. *E. coli* colonies growing in confinement generate ~10 kPa stress on the material surrounding them[118], whereas yeast colonies can generate stress in the magnitude of megapascals[119], providing us with some estimate of internal mechanical stresses. In principle, the viscoelastic nature of biofilms, in particular their ability to 'flow' over long timescales, enables the dissipation and relaxation of mechanical stress generated by growth or by external forces such as shear stress[120,121]. Knowledge of the behaviour of the matrix material is necessary to develop a holistic understanding of biofilm development. Although mechanical properties can be measured with standard and advanced techniques[121,122], understanding their relevance in the context of biofilm formation and maintenance is complex as matrix composition is heterogeneous within and between biofilms and across environmental conditions.

External and self-generated forces vastly contribute to development and morphogenesis of multicellular organisms, ultimately being vital[123]. Similar mechanical phenomena take place during the development of biofilms, also having roles in their morphogenesis and spatial architecture[116,117,124]; however, their functional consequences remain unclear. In nature, biofilms exhibit strong genotypic and phenotypic heterogeneity[125,126]. The relative positioning of distinct cells determines how they interact and therefore has a strong influence on the behaviour and fitness of individual cells, particularly in a biofilm in which cells are separated by less than a micrometer[127,128]. For example, spatial organization affects how single cells experience signalling molecules, such as quorum-sensing autoinducers, nutrients or antimicrobials[129], and also influences the emergent properties of multicellular structures such as iridescence or the formation of flocks and fruiting bodies[130,131]. In multispecies biofilms, this arrangement also leads to variations in interspecies social interactions, ultimately governing whether strains compete or cooperate[128]. Beyond affecting the transport of signalling and nutrient molecules[129,132], mechanical forces generated by flow or contact can deform or damage the matrix and carry single cells to or away from biofilms, thereby influencing cellular arrangements.

Stream biofilms are under constant influence of fluid flow, which strongly affects the composition as well as the spatial and dynamic distribution of different species[109]. The forces generated by flow on planktonic and surface-associated communities affect the complexity of stream biofilms by directly modulating the rate of encounter of new clones with the surface[133]. Additionally, biofilms and suspended aggregates grown in laminar and turbulent flows from the same initial planktonic inoculum have drastically different community compositions[134]. These differences are likely to be caused by the heterogeneity in adhesive and motility properties of the different species present in the planktonic inoculum, highlighting the importance of fluid flow and physical interactions with surfaces in establishing the cell–cell interaction network in environmental biofilms.

Modelling these ecosystems in defined microfluidic environments can help identify the physical mechanisms involved in structuring biofilm communities. At the single-cell scale, fluid flow strongly influences lineage distribution in *C. crescentus*[135]. Growth of *C. crescentus* biofilms at controlled shear rates demonstrated that contiguous cells have a high probability of originating from distinct lineages in weak flows, whereas lineages strongly segregate in strong flow. This flow-induced segregation depends on the ability of single planktonic cells to swim in the bulk fluid and invade existing biofilms. An advection–diffusion theoretical framework inspired from mass transport phenomena helps describe the relationship between flow and lineage mixing: single planktonic cells diffuse by Brownian-like swimming trajectories and are directionally transported by flow. The relative contributions of these two modes of transport influence mixing, wherein a low flow promotes swimming-dependent mixing and a strong flow favours the local attachment of progeny[89]. Accordingly,

Iridescence
An optical effect where light reflecting on a surface generates rainbow-like colours by interference.

simulations predict that the joint contributions of flow and adhesion strengths has a strong impact on spatial organization, which was also verified experimentally in the early stages of *V. cholerae* biofilm formation[136]. Genotypic biofilm mixing also occurs in complex and irregular flows: strains of *P. aeruginosa* that do not produce a matrix can invade existing biofilms of matrix producers, thereby inducing lineage heterogeneity in the biofilm[137]. Invasion occurs when biofilms clog parts of the fluidic network, generating regions of zero fluid velocity that enable non-producing cells to attach to the existing matrix without being washed away. In another example, extensional flow around chitin particles enables matrix-depleted filamenting strains of *V. cholerae* to associate with a surface[138] — biofilms form rapidly via entanglement of filamentous cells, which comes with the trade-off of permitting invasion by competitors. We note that these last two phenomena would not take place in more regular flow profiles: cheating cells would be removed from existing biofilms and filamentous cells would not form biofilms.

Forces within biofilms. Self-generated internal forces contribute to the organization and morphogenesis of biofilms. A recent study combining single-cell level imaging with a theoretical framework inspired from the physics of liquid crystals highlighted the importance of matrix mechanical properties and flow in shaping the spatial organization of *V. cholerae* biofilms[139]. These insightful measurements were then used to computationally predict biofilm growth behaviour, which revealed that physical cell–cell interactions within *V. cholerae* biofilms are largely regulated by the production of the matrix protein RbmA. RbmA controls matrix mechanical cohesion since the biofilms of *rbmA* mutants are permeable to invading cells[140]. External conditions can influence internal matrix mechanics: the secretion of polymeric components by biofilm dwelling cells generates an osmotic pressure difference with the external environment that drives colony expansion and influences biofilm morphology[141,142]. This pressure difference also influences spatial organization and resistance to invaders: high osmotic pressure tends to expand the matrix, permitting the penetration of invaders, whereas low osmotic pressure compacts the matrix, thereby preserving clonality[143].

Biofilms form at mucosal surfaces in vivo, where they are exposed to mechanical forces imposed by their hosts[111,144]. These surfaces, which include host cell surfaces or eukaryotic extracellular matrix (ECM) components, are generally soft and sometimes dynamic. We know very little about how biofilms form in this context, in particular at the surface of mucus, which is a hydrogel layer composed of glycosylated proteins secreted by the epithelium to provide a physical barrier against invading microorganisms[145]. In vivo visualization of fixed samples showed that the mucus layer separates host epithelia from intestinal biofilms[146] or traps *P. aeruginosa* biofilms in the sputum of patients with cystic fibrosis[147]. Reproducing the mechanical and dynamic properties of native mucus in the laboratory is complex, but careful in vitro experiments showed that adhesion to mucins is an important component of host–bacteria and inter-bacterial interactions[145,148,149]. Although we still lack a dynamic view of how mucus secretion shapes the spatial organization of bacterial communities at the epithelium[127,150], agent-based simulations help predict the influence of mucus production on the development of multispecies biofilms[151]. These simulations predict that the balance between mucus secretion from epithelial cells and bacterial adhesion to mucus strongly affects the spatial organization and social interactions between species: low mucus-secretion rates promote colonization by mucin-adhering cells, whereas rapid secretion promotes colonization of strains that have a lower binding affinity for mucus.

Mucins
Highly glycosylated proteins that can form a gel layer at epithelial surfaces upon exocytosis or decorate the surface of mammalian cells.

Mechanosensing in biofilms. In eukaryotes, ECM components such as collagen and fibrinogen are important structural elements that provide a solid substrate on which single or multiple cells can organize and grow[152]. The ECM additionally provides mechanical cues that guide cellular decision making[153,154]. Mammalian cells can sense and respond to the mechanical and chemical properties of the ECM, and phenotypic differences, such as morphologies, have been observed for the same cell types on or within the ECM (that is, in 2D or 3D, respectively)[155]. During the development and growth of multicellular organisms, self-generated internal forces not only passively guide morphogenesis but also function as mechanical inputs that regulate the cellular processes important for morphogenesis[156]. Hydrodynamic forces also have a role in development and in intercellular communication through the intermediary of mechanosensory systems[157,158]. By analogy, one could ask whether mechanosensing and mechanotransduction could have active roles in bacterial multicellular organization within biofilms.

The primary function of the biofilm EPS matrix is to mechanically maintain cohesion between bacterial cells. We could thus hypothesize that the mechanical properties of the EPS matrix, which ultimately permit cohesion, directly regulate the deployment of cellular factors that maintain biofilm integrity; this could include feedback on EPS production or degradation. Cells locally sensing a decrease in elasticity could increase the production of matrix components, stiffening their surroundings. Conversely, cells sensing a high stiffness could secrete matrix-degrading enzymes, locally reducing matrix elasticity to permit growth. Could single cells mechanically sense matrix properties in a biofilm? Our knowledge of EPS regulation has mainly focused on the contributions of chemical inputs, including quorum sensing and nutrient limitation[159]. Mechanosensitive systems help initiate biofilm formation, for example in *P. aeruginosa*, in which surface-induced production of c-di-GMP promotes EPS production[22,78,160]. Interestingly, *P. aeruginosa* senses one of its main matrix components, the polysaccharide Psl, which, in turn, stimulates the production of c-di-GMP, thereby providing a positive feedback loop for matrix production[161]. Whether cells sense Psl mechanically is unknown but constitutes an interesting hypothesis as Psl is the main contributor of matrix elasticity and thus controls biofilm stiffness[162,163]. Flagellum-dependent

Box 2 | **New instrumentation for mechanomicrobiology**

The exploration of mechanomicrobiology is inherently a multiscale problem, where piconewton to micronewton forces act within the nanometre scale of single proteins to the centimetre scale of multicellular structures. As such, it requires instrumentation that spans these force and length scales to enable the control and measurement of forces on single molecules, single cells and biofilms. Various force-measurement techniques are already available but unfortunately not entirely democratized. Optical tweezers, magnetic tweezers and atomic force microscopy (AFM) enable the mechanical characterization and stimulation of single proteins or cells[177,178]. These techniques largely differ according to their force sensitivity and spatiotemporal resolution. Forces are applied to molecules and cells either through mechanical force transducers, such as in AFM, or via external fields (hydrodynamic, magnetic or photon fields), thus acting on molecules from a distance. By using optical and magnetic tweezers, researchers can probe molecules and cells in solution, thereby shedding new light onto the forces experienced or generated by cells, appendages and molecules[179,180]. One limitation of optical tweezers is that the sample may be subject to heating or photodamage. Magnetic tweezers are well suited for manipulations in complex environments such as the interior of cells. AFM enables researchers to sense, apply and locate forces on cells at high resolution (~10 nm) and with the widest range of forces, from 5 pN to 100 nN[44,181]. One current challenge for single-molecule force-spectroscopy techniques is to increase throughput, which would enable mechanical interrogation on large scales, for example, of mutant libraries. As a complement to in vitro and in vivo single-molecule force experiments, in silico approaches using molecular dynamics simulations have recently become a powerful addition to understanding the molecular basis of molecular adhesion and mechanics[62].

Traction force microscopy and micropillars enable spatially resolved measurements of force generated by cells or groups of cells. These are based on the deformation of soft materials on which cells exert force. With the knowledge of the mechanical properties of the substrate, the stress within the material and the forces that generated it can be reconstructed. These have been successfully applied to bacterial systems to measure pili retraction forces[182] and the force generated by groups of growing or migrating cells[183,184]. The sensitivity of these techniques is limited by microscope resolution and material stiffness. At the biofilm level, rheology techniques have been applied to measure biofilm matrix viscous and elastic moduli, including in situ microrheology, which tracks mean square displacement of beads associated with extracellular polymeric substances, and classic rheology on purified matrix components[121]. Force measurements within biofilms remain scarce, but novel imaging techniques have generated insights into the stress distribution within *Pseudomonas aeruginosa* biofilms[185]. Microfluidics is also a powerful and simple tool to generate controlled shear stress on single cells or on cell communities[186,187]. We anticipate that technologies developed for the field of eukaryotic mechanobiology rooted in materials science[188], chemistry[189] and soft robotics[190,191] will be adapted and democratized for mechanomicrobiology studies.

mechanosensation in *B. subtilis* is a more concrete example: it stimulates the production of the matrix component poly-γ-DL-glutamic acid upon surface contact[90]. We postulate that, in addition, EPS secreted by the same cell or others can inhibit flagellum rotation as demonstrated in *Salmonella enterica* subsp. *enterica* serovar Typhimurium[164]. Finally, in UPEC, the self-generated mechanical stress on multicellular structures promotes the formation of biofilms (FIG. 2B)[118]. Confining and crowding UPEC within microfluidic chambers models growth conditions within intracellular pods at the uroepithelium. Under pressure, UPEC increases the production of EPS components by upregulating *rpoH*, which encodes a sigma factor involved in the heat-shock response. Whether the pressure generated by internal stress during biofilm growth eludes a similar response in single biofilm-dwelling bacteria is unknown. In summary, mechanoregulation of matrix production could generate positive feedback to maintain appropriate local stiffness or viscosity.

Conclusions and perspectives

In this Review, we have highlighted multiple examples of mechanically modulated bacterial behaviours and the associated sensory mechanisms, including mechanosensory feedback mechanisms that affect motility systems and adhesion. Furthermore, force-sensitive machineries can interface with transducing components and thus broaden the range of mechanoresponses, which enable mechanoregulation of diverse phenotypes such as pathogenicity and biofilm formation. Demonstrating that force alone modulates the functions of proteins, protein assemblies or cells is not a simple task. It requires an ensemble of physical and biological evidence only made possible by the combination of advanced instrumentation, ecologically relevant in vitro experiments and physical theory. Therefore, the role of mechanics in regulating the phenotypes of various microorganisms may be underestimated. The catch bond behaviours of FimH and ClfA are examples of force-dependent bacterial phenotypes that have been elucidated by combinations of molecular, cellular, biophysical, computational and microbiological techniques. In parallel, mechanosensation is relatively well understood in eukaryotes. For example, the characterization of Piezo1 and Piezo2, two mechanosensors that control many force-dependent cellular processes, such as the sense of touch[165], recently culminated with high resolution structures of their relaxed and mechanically stressed conformations 10 years after their discovery[166–168]. The field of mechanomicrobiology will benefit from these studies but methodologies must be adapted to the smaller scale of microorganisms (BOX 2).

We also pointed out that mechanics influences bacterial biology on evolutionary timescales by affecting the spatial organization of surface-associated populations. The relative position of microorganisms in dense communities dictates their interaction network within and between clonal groups. Recent experimental and computational investigations have shown that flow can transport cells and deform the matrix, influencing attachment patterns and shaping the morphology of biofilms and the position of matrix-embedded cells. Further studies are needed to determine the contributions of forces in comparison with social interactions in governing the heterogeneity and stability of biofilms. The host-associated gut microbiota is a particularly fascinating but complex example of a dense community showing heterogeneity at many scales and under the concerted influence of flow, peristaltic motion of the intestinal epithelium and mucus secretion[127,150]. Finally, most explorations of biofilm mechanobiology have focused on pathogens and model organisms but, to fully understand the biophysical rules guiding the formation of multicellular structures, we must expand our view to less-characterized systems, including archaea[169].

Do bacteria possess the 'mechanic's feel'? Bacteria push and pull on soft and stiff materials, they possess machineries enabling them to probe forces and deformations, and modulate the way they interact with these materials in response — thus, indeed, it seems that bacteria possess "a deep inner kinesthetic feeling".

Published online: 20 January 2020

1. Zhu, T. F. & Szostak, J. W. Coupled growth and division of model protocell membranes. *J. Am. Chem. Soc.* **131**, 5705–5713 (2009).
2. Dupont, S. et al. Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179–183 (2011).
3. Persat, A. et al. The mechanical world of bacteria. *Cell* **161**, 988–997 (2015).
4. Bartlett, D. H. Pressure effects on in vivo microbial processes. *Biochim. Biophys. Acta* **1595**, 367–381 (2002).
5. Berne, C., Ellison, C. K., Ducret, A. & Brun, Y. V. Bacterial adhesion at the single-cell level. *Nat. Rev. Microbiol.* **16**, 616–627 (2018).
6. Auer, G. K. & Weibel, D. B. Bacterial cell mechanics. *Biochemistry* **56**, 3710–3724 (2017).
7. Rojas, E. R. et al. The outer membrane is an essential load-bearing element in Gram-negative bacteria. *Nature* **559**, 617–621 (2018).
8. Chen, Y., Norde, W., van der Mei, H. C. & Busscher, H. J. Bacterial cell surface deformation under external loading. *MBio* https://doi.org/10.1128/mBio.00378-12 (2012).
9. Li, J. et al. Residence-time dependent cell wall deformation of different *Staphylococcus aureus* strains on gold measured using surface-enhanced-fluorescence. *Soft Matter* **10**, 7638–7646 (2014).
10. Lauga, E. & Powers, T. R. The hydrodynamics of swimming microorganisms. *Rep. Prog. Phys.* **72**, 096601 (2009).
A thorough review on the fluid mechanics involved in swimming motility.
11. Purcell, E. M. Life at low Reynolds number. *Am. J. Phys.* **45**, 3–11 (1977).
12. Subramanian, S. & Kearns, D. B. Functional regulators of bacterial flagella. *Annu. Rev. Microbiol.* **73**, 225–246 (2019).
13. Lele, P. P., Hosu, B. G. & Berg, H. C. Dynamics of mechanosensing in the bacterial flagellar motor. *Proc. Natl Acad. Sci. USA* **110**, 11839–11844 (2013).
This paper demonstrates how the *E. coli* flagellum motor adapts its rotation rate upon changes in torque load by recruiting stator units.
14. Tipping, M. J., Delalez, N. J., Lim, R., Berry, R. M. & Armitage, J. P. Load-dependent assembly of the bacterial flagellar motor. *MBio* https://doi.org/10.1128/mBio.00551-13 (2013).
This paper shows that increasing loads on the flagellum motor increase torque generation by recruiting stator units.
15. Wadhwa, N., Phillips, R. & Berg, H. C. Torque-dependent remodeling of the bacterial flagellar motor. *Proc. Natl Acad. Sci. USA* **116**, 11764–11769 (2019).
This study combines biophysical experimentation and theory to understand how load changes drive the assembly/disassembly of the flagellum motor.
16. Nord, A. L. et al. Catch bond drives stator mechanosensitivity in the bacterial flagellar motor. *Proc. Natl Acad. Sci. USA* **114**, 12952–12957 (2017).
A demonstration that a catch bond mechanism drives the torque-dependent recruitment of additional flagellum stators.
17. Darnton, N. C. & Berg, H. C. Force-extension measurements on bacterial flagella: triggering polymorphic transformations. *Biophys. J.* **92**, 2230–2236 (2007).
18. Kühn, M. J., Schmidt, F. K., Eckhardt, B. & Thormann, K. M. Bacteria exploit a polymorphic instability of the flagellar filament to escape from traps. *Proc. Natl Acad. Sci. USA* **114**, 6340–6345 (2017).
19. Kühn, M. J. et al. Spatial arrangement of several flagellins within bacterial flagella improves motility in different environments. *Nat. Commun.* **9**, 5369 (2018).
20. Toutain, C. M., Zegans, M. E. & O'Toole, G. A. Evidence for two flagellar stators and their role in the motility of *Pseudomonas aeruginosa*. *J. Bacteriol.* **187**, 771–777 (2005).
21. Doyle, T. B., Hawkins, A. C. & McCarter, L. L. The complex flagellar torque generator of *Pseudomonas aeruginosa*. *J. Bacteriol.* **186**, 6341–6350 (2004).
22. Belas, R. Biofilms, flagella, and mechanosensing of surfaces by bacteria. *Trends Microbiol.* **22**, 517–527 (2014).
23. Alexandre, G., Rohr, R. & Bally, R. A phase variant of *Azospirillum lipoferum* lacks a polar flagellum and constitutively expresses mechanosensing lateral flagella. *Appl. Environ. Microbiol.* **65**, 4701–4704 (1999).
24. Khammas, K. M., Ageron, E., Grimont, P. A. D. & Kaiser, P. *Azospirillum irakense* sp. nov., a nitrogen-fixing bacterium associated with rice roots and rhizosphere soil. *Res. Microbiol.* **140**, 679–693 (1989).
25. Meganathan, R. & Marquis, R. E. Loss of bacterial motility under pressure. *Nature* **246**, 525–527 (1973).
26. Eloe, E. A., Lauro, F. M., Vogel, R. F. & Bartlett, D. H. The deep-sea bacterium *Photobacterium profundum* ss9 utilizes separate flagellar systems for swimming and swarming under high-pressure conditions. *Appl. Environ. Microbiol.* **74**, 6298–6305 (2008).
27. Wang, F. et al. Environmental adaptation: genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium *Shewanella piezotolerans* WP3. *PLoS One* **3**, e1937 (2008).
28. Mattick, J. S. Type IV pili and twitching motility. *Annu. Rev. Microbiol.* **56**, 289–314 (2002).
29. Denise, R., Abby, S. S. & Rocha, E. P. C. Diversification of the type IV filament superfamily into machines for adhesion, protein secretion, DNA uptake, and motility. *PLoS Biol.* **17**, e3000390 (2019).
30. Burrows, L. L. *Pseudomonas aeruginosa* twitching motility: type IV pili in action. *Annu. Rev. Microbiol.* **66**, 493–520 (2012).
31. Talà, L., Fineberg, A., Kukura, P. & Persat, A. *Pseudomonas aeruginosa* orchestrates twitching motility by sequential control of type IV pili movements. *Nat. Microbiol.* **4**, 774–780 (2019).
A demonstration that *P. aeruginosa* coordinate T4P retraction with contact of their tip with a surface.
32. Beaussart, A. et al. Nanoscale adhesion forces of *Pseudomonas aeruginosa* type IV pili. *ACS Nano* **8**, 10723–10733 (2014).
This paper suggests that stretching with AFM can induce conformational changes in T4P.
33. Biais, N., Higashi, D. L., Brujic, J., So, M. & Sheetz, M. P. Force-dependent polymorphism in type IV pili reveals hidden epitopes. *Proc. Natl Acad. Sci. USA* **107**, 11358–11363 (2010).
34. Ellison, C. K. et al. Obstruction of pilus retraction stimulates bacterial surface sensing. *Science* **358**, 535–538 (2017).
This paper demonstrates that *C. crescentus* pili mechanosense surfaces to regulate deployment of polar adhesins.
35. Ellison, C. K. et al. Retraction of DNA-bound type IV competence pili initiates DNA uptake during natural transformation in *Vibrio cholerae*. *Nat. Microbiol.* **3**, 773–780 (2018).
36. Adams, D. W., Stutzmann, S., Stoudmann, C. & Blokesch, M. DNA-uptake pili of *Vibrio cholerae* are required for chitin colonization and capable of kin recognition via sequence-specific self-interaction. *Nat. Microbiol.* **4**, 1545–1557 (2019).
37. Turner, K. H., Everett, J., Trivedi, U., Rumbaugh, K. P. & Whiteley, M. Requirements for *Pseudomonas aeruginosa* acute burn and chronic surgical wound infection. *PLoS Genet.* **10**, e1004518 (2014).
38. Kang, P. J. et al. Identification of *Pseudomonas aeruginosa* genes required for epithelial cell injury. *Mol. Microbiol.* **24**, 1249–1262 (1997).
39. Fuente, L. D. L. et al. Assessing adhesion forces of type I and type IV Pili of *Xylella fastidiosa* bacteria by use of a microfluidic flow chamber. *Appl. Environ. Microbiol.* **73**, 2690–2696 (2007).
40. Meng, Y. et al. Upstream migration of *Xylella fastidiosa* via pilus-driven twitching motility. *J. Bacteriol.* **187**, 5560–5567 (2005).
41. Chlebek, J. L. et al. PilT and PilU are homohexameric ATPases that coordinate to retract type IVa pili. *PLoS Genet.* **15**, e1008448 (2019).
42. Faure, L. M. et al. The mechanism of force transmission at bacterial focal adhesion complexes. *Nature* **539**, 530–535 (2016).
43. Pizarro-Cerdá, J. & Cossart, P. Bacterial adhesion and entry into host cells. *Cell* **124**, 715–727 (2006).
44. Müller, D. J., Helenius, J., Alsteens, D. & Dufrêne, Y. F. Force probing surfaces of living cells to molecular resolution. *Nat. Chem. Biol.* **5**, 383–390 (2009).
45. Marszalek, P. E. & Dufrêne, Y. F. Stretching single polysaccharides and proteins using atomic force microscopy. *Chem. Soc. Rev.* **41**, 3523–3534 (2012).
46. El-Kirat-Chatel, S., Beaussart, A., Boyd, C. D., O'Toole, G. A. & Dufrêne, Y. F. Single-cell and single-molecule analysis deciphers the localization, adhesion, and mechanics of the biofilm adhesin LapA. *ACS Chem. Biol.* **9**, 485–494 (2014).
47. Formosa-Dague, C., Speziale, P., Foster, T. J., Geoghegan, J. A. & Dufrêne, Y. F. Zinc-dependent mechanical properties of *Staphylococcus aureus* biofilm-forming surface protein SasG. *Proc. Natl Acad. Sci. USA* **113**, 410–415 (2016).
48. Alsteens, D., Garcia, M. C., Lipke, P. N. & Dufrêne, Y. F. Force-induced formation and propagation of adhesion nanodomains in living fungal cells. *Proc. Natl Acad. Sci. USA* **107**, 20744–20749 (2010).
49. Larsen, P. et al. Amyloid adhesins are abundant in natural biofilms. *Environ. Microbiol.* **9**, 3077–3090 (2007).
50. Romero, D., Aguilar, C., Losick, R. & Kolter, R. Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc. Natl Acad. Sci. USA* **107**, 2230–2234 (2010).
51. Mandlik, A., Swierczynski, A., Das, A. & Ton-That, H. Pili in Gram-positive bacteria: assembly, involvement in colonization and biofilm development. *Trends Microbiol.* **16**, 33–40 (2008).
52. Kankainen, M. et al. Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human-mucus binding protein. *Proc. Natl Acad. Sci. USA* **106**, 17193–17198 (2009).
53. Tripathi, P. et al. Adhesion and nanomechanics of pili from the probiotic *Lactobacillus rhamnosus* GG. *ACS Nano* **7**, 3685–3697 (2013).
54. Alegre-Cebollada, J., Badilla, C. L. & Fernández, J. M. Isopeptide bonds block the mechanical extension of pili in pathogenic *Streptococcus pyogenes*. *J. Biol. Chem.* **285**, 11235–11242 (2010).
55. Kang, H. J., Coulibaly, F., Clow, F., Proft, T. & Baker, E. N. Stabilizing isopeptide bonds revealed in gram-positive bacterial pilus structure. *Science* **318**, 1625–1628 (2007).
This paper demonstrates that isopeptide covalent bonds promote the extreme stability of pili from Gram-positive bacteria.
56. Thomas, W. Catch bonds in adhesion. *Annu. Rev. Biomed. Eng.* **10**, 39–57 (2008).
57. Thomas, W. E., Vogel, V. & Sokurenko, E. Biophysics of catch bonds. *Annu. Rev. Biophys.* **37**, 399–416 (2008).
58. Thomas, W. E., Trintchina, E., Forero, M., Vogel, V. & Sokurenko, E. V. Bacterial adhesion to target cells enhanced by shear force. *Cell* **109**, 913–923 (2002).
Demonstrates that flow increases the binding strength of the FimH *E. coli* adhesin.
59. Yakovenko, O. et al. FimH forms catch bonds that are enhanced by mechanical force due to allosteric regulation. *J. Biol. Chem.* **283**, 11596–11605 (2008).
60. Le Trong, I. et al. Structural basis for mechanical force regulation of the adhesin FimH via finger trap-like β sheet twisting. *Cell* **141**, 645–655 (2010).
61. Kerrigan, S. W. et al. Molecular basis for *Staphylococcus aureus*-mediated platelet aggregate formation under arterial shear in vitro. *Arterioscler. Thromb. Vasc. Biol.* **28**, 335–340 (2008).
62. Milles, L. F., Schulten, K., Gaub, H. E. & Bernardi, R. C. Molecular mechanism of extreme mechanostability in a pathogen adhesin. *Science* **359**, 1527–1533 (2018).
This paper identifies the structural rearrangement in the SdrG adhesin that drives its ability to withstand nanonewton-range forces.
63. Herman, P. et al. The binding force of the staphylococcal adhesin SdrG is remarkably strong. *Mol. Microbiol.* **93**, 356–368 (2014).
64. Herman-Bausier, P. et al. *Staphylococcus aureus* clumping factor A is a force-sensitive molecular switch that activates bacterial adhesion. *Proc. Natl Acad. Sci. USA* **115**, 5564–5569 (2018).
65. Vitry, P. et al. Force-induced strengthening of the interaction between *Staphylococcus aureus* clumping factor B and loricrin. *MBio* **8**, e01748-17 (2017).
66. McDevitt, D. et al. Characterization of the interaction between the *Staphylococcus aureus* clumping factor (ClfA) and fibrinogen. *Eur. J. Biochem.* **247**, 416–424 (1997).
67. Jarvis, C. et al. Antivirulence isoquinolone mannosides: optimization of the biaryl aglycone for FimH lectin binding affinity and efficacy in the treatment of chronic UTI. *ChemMedChem* **11**, 367–373 (2016).
68. Spaulding, C. N. et al. Selective depletion of uropathogenic *E. coli* from the gut by a FimH antagonist. *Nature* **546**, 528–532 (2017).
69. Persat, A. Bacterial mechanotransduction. *Curr. Opin. Microbiol.* **36**, 1–6 (2017).
70. Gordon, V. D. & Wang, L. Bacterial mechanosensing: the force will be with you, always. *J. Cell Sci.* **132**, jcs227694 (2019).
71. Laventie, B.-J. et al. A surface-induced asymmetric program promotes tissue colonization by *Pseudomonas aeruginosa*. *Cell Host Microbe* **25**, 140–152.e6 (2019).
This paper shows that levels of c-di-GMP rapidly increase upon *P. aeruginosa* surface contact, promoting the immediate deployment of T4P.

72. Persat, A., Inclan, Y. F., Engel, J. N., Stone, H. A. & Gitai, Z. Type IV pili mechanochemically regulate virulence factors in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA* **112**, 7563–7568 (2015).
A demonstration of the ability of *P. aeruginosa* to mechanically sense surfaces with T4P and the Chp system to regulate cAMP levels.
73. Wolfgang, M. C., Lee, V. T., Gilmore, M. E. & Lory, S. Coordinate regulation of bacterial virulence genes by a novel adenylate cyclase-dependent signaling pathway. *Dev. Cell* **4**, 253–263 (2003).
74. Chuang, S. K., Vrla, G. D., Fröhlich, K. S. & Gitai, Z. Surface association sensitizes *Pseudomonas aeruginosa* to quorum sensing. *Nat. Commun.* **10**, 4118 (2019).
75. Inclan, Y. F. et al. A scaffold protein connects type IV pili with the Chp chemosensory system to mediate activation of virulence signaling in *Pseudomonas aeruginosa*. *Mol. Microbiol.* **101**, 590–605 (2016).
76. Bertrand, J. J., West, J. T. & Engel, J. N. Genetic analysis of the regulation of type IV pilus function by the Chp chemosensory system of *Pseudomonas aeruginosa*. *J. Bacteriol.* **192**, 994–1010 (2010).
77. Lee, C. K. et al. Multigenerational memory and adaptive adhesion in early bacterial biofilm communities. *Proc. Natl Acad. Sci. USA* **115**, 4471–4476 (2018).
78. Rodesney, C. A. et al. Mechanosensing of shear by *Pseudomonas aeruginosa* leads to increased levels of the cyclic-di-GMP signal initiating biofilm development. *Proc. Natl Acad. Sci. USA* **114**, 5906–5911 (2017).
This paper shows that shear forces promote the production of c-di-GMP in *P. aeruginosa*.
79. Luo, Y. et al. A hierarchical cascade of second messengers regulates *Pseudomonas aeruginosa* surface behaviors. *MBio* https://doi.org/10.1128/mBio.02456-14 (2015).
This paper demonstrates that *P. aeruginosa* use distinct mechanosensitive machineries to successively regulate cAMP and c-di-GMP levels.
80. Siryaporn, A., Kuchma, S. L., O'Toole, G. A. & Gitai, Z. Surface attachment induces *Pseudomonas aeruginosa* virulence. *Proc. Natl Acad. Sci. USA* **111**, 16860–16865 (2014).
81. Kuchma, S. L. et al. Cyclic Di-GMP-mediated repression of swarming motility by *Pseudomonas aeruginosa* PA14 requires the MotAB stator. *J. Bacteriol.* **197**, 420–430 (2015).
82. Baker, A. E. et al. Flagellar stators stimulate c-di-GMP production by *Pseudomonas aeruginosa*. *J. Bacteriol.* **201**, e00741–18 (2019).
83. Yan, J. et al. Bow-tie signaling in c-di-GMP: machine learning in a simple biochemical network. *PLoS Comput. Biol.* **13**, e1005677 (2017).
84. Sanfilippo, J. E. et al. Microfluidic-based transcriptomics reveal force-independent bacterial rheosensing. *Nat. Microbiol.* **4**, 1274–1281 (2019).
85. Lee, C. K. et al. Evolution of cell size homeostasis and growth rate diversity during initial surface colonization of *Shewanella oneidensis*. *ACS Nano* **10**, 9183–9192 (2016).
86. Li, G. et al. Surface contact stimulates the just-in-time deployment of bacterial adhesins. *Mol. Microbiol.* **83**, 41–51 (2012).
87. Hug, I., Deshpande, S., Sprecher, K. S., Pfohl, T. & Jenal, U. Second messenger-mediated tactile response by a bacterial rotary motor. *Science* **358**, 531–534 (2017).
This paper shows that the flagellum motor of *C. crescentus* acts as a mechanosensor to regulate the deployment of its polar adhesin.
88. Nesper, J. et al. Cyclic di-GMP differentially tunes a bacterial flagellar motor through a novel class of CheY-like regulators. *eLife* **6**, e28842 (2017).
89. Persat, A., Stone, H. A. & Gitai, Z. The curved shape of *Caulobacter crescentus* enhances surface colonization in flow. *Nat. Commun.* **5**, 3824 (2014).
90. Cairns, L. S., Marlow, V. L., Bissett, E., Ostrowski, A. & Stanley-Wall, N. R. A mechanical signal transmitted by the flagellum controls signalling in *Bacillus subtilis*. *Mol. Microbiol.* **90**, 6–21 (2013).
91. Alsharif, G. et al. Host attachment and fluid shear are integrated into a mechanical signal regulating virulence in *Escherichia coli* O157:H7. *Proc. Natl Acad. Sci. USA* **112**, 5503–5508 (2015).
A demonstration that host cell contact and flow induce virulence of enterohemorrhagic *E. coli*.
92. Otto, K. & Silhavy, T. J. Surface sensing and adhesion of *Escherichia coli* controlled by the Cpx-signaling pathway. *Proc. Natl Acad. Sci. USA* **99**, 2287–2292 (2002).
93. Shimizu, T., Ichimura, K. & Noda, M. The surface sensor NlpE of enterohemorrhagic *Escherichia coli* contributes to regulation of the type III secretion system and flagella by the Cpx response to adhesion. *Infect. Immun.* **84**, 537–549 (2015).
94. Kimkes, T. E. P. & Heinemann, M. Reassessing the role of the *Escherichia coli* CpxAR system in sensing surface contact. *PLoS One* **13**, e0207181 (2018).
95. Bruni, G. N., Weekley, R. A., Dodd, B. J. T. & Kralj, J. M. Voltage-gated calcium flux mediates *Escherichia coli* mechanosensation. *Proc. Natl Acad. Sci. USA* **114**, 9445–9450 (2017).
This paper shows that mechanical compression activates membrane-bound ion channels in *E. coli*.
96. Haswell, E. S., Phillips, R. & Rees, D. C. Mechanosensitive channels: what can they do and how do they do it? *Structure* **19**, 1356–1369 (2011).
97. Perozo, E., Kloda, A., Cortes, D. M. & Martinac, B. Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating. *Nat. Struct. Biol.* **9**, 696–703 (2002).
98. Abe, F. Dynamic structural changes in microbial membranes in response to high hydrostatic pressure analyzed using time-resolved fluorescence anisotropy measurement. *Biophys. Chem.* **183**, 3–8 (2013).
99. Bartlett, D., Wright, M., Yayanos, A. A. & Silverman, M. Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium. *Nature* **342**, 572–574 (1989).
100. Myka, K. K. et al. in *Microbial Ecology of Extreme Environments* (eds Chenard, C. & Lauro, F. M.) 51–80 (Springer, 2017).
101. Welch, T. J. & Bartlett, D. H. Identification of a regulatory protein required for pressure-responsive gene expression in the deep-sea bacterium *Photobacterium* species strain SS9. *Mol. Microbiol.* **27**, 977–985 (1998).
102. Bar-On, Y. M., Phillips, R. & Milo, R. The biomass distribution on earth. *Proc. Natl Acad. Sci. USA* **115**, 6506–6511 (2018).
103. Whitman, W. B., Coleman, D. C. & Wiebe, W. J. Prokaryotes: the unseen majority. *Proc. Natl Acad. Sci. USA* **95**, 6578–6583 (1998).
104. Flemming, H.-C. & Wuertz, S. Bacteria and archaea on earth and their abundance in biofilms. *Nat. Rev. Microbiol.* **17**, 247–260 (2019).
105. Daniel, I., Oger, P. & Winter, R. Origins of life and biochemistry under high-pressure conditions. *Chem. Soc. Rev.* **35**, 858–875 (2006).
106. Flemming, H.-C. et al. Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* **14**, 563–575 (2016).
107. Hall-Stoodley, L., Costerton, J. W. & Stoodley, P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.* **2**, 95–108 (2004).
108. Battin, T. J., Kaplan, L. A., Denis Newbold, J. & Hansen, C. M. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* **426**, 439–442 (2003).
109. Battin, T. J., Besemer, K., Bengtsson, M. M., Romani, A. M. & Packmann, A. I. The ecology and biogeochemistry of stream biofilms. *Nat. Rev. Microbiol.* **14**, 251–263 (2016).
110. Hooper, L. V. & Gordon, J. I. Commensal host-bacterial relationships in the gut. *Science* **292**, 1115–1118 (2001).
111. De Weirdt, R. & Van de Wiele, T. Micromanagement in the gut: microenvironmental factors govern colon mucosal biofilm structure and functionality. *NPJ Biofilms Microbiomes* **1**, 15026 (2015).
112. Mah, T. F. & O'Toole, G. A. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol.* **9**, 34–39 (2001).
113. Flemming, H.-C. & Wingender, J. The biofilm matrix. *Nat. Rev. Microbiol.* **8**, 623–633 (2010).
114. Marchetti, M. C. et al. Hydrodynamics of soft active matter. *Rev. Mod. Phys.* **85**, 1143–1189 (2013).
115. Douarche, C., Allain, J.-M. & Raspaud, E. *Bacillus subtilis* bacteria generate an internal mechanical force within a biofilm. *Biophys. J.* **109**, 2195–2202 (2015).
116. Beroz, F. et al. Verticalization of bacterial biofilms. *Nat. Phys.* **14**, 954–960 (2018).
117. Yan, J. et al. Mechanical instability and interfacial energy drive biofilm morphogenesis. *eLife*. **8**, e43920 (2019).
118. Chu, E. K., Kilic, O., Cho, H., Groisman, A. & Levchenko, A. Self-induced mechanical stress can trigger biofilm formation in uropathogenic *Escherichia coli*. *Nat. Commun.* **9**, 4087 (2018).
This paper shows that growth-generated mechanical compression can induce the formation of biofilms.
119. Delarue, M. et al. Self-driven jamming in growing microbial populations. *Nat. Phys.* **12**, 762–766 (2016).
120. Ewoldt, R. H., Hosoi, A. E. & McKinley, G. H. Nonlinear viscoelastic biomaterials: meaningful characterization and engineering inspiration. *Integr. Comp. Biol.* **49**, 40–50 (2009).
121. Boudarel, H., Mathias, J.-D., Blaysat, B. & Grédiac, M. Towards standardized mechanical characterization of microbial biofilms: analysis and critical review. *NPJ Biofilms Microbiomes* **4**, 17 (2018).
122. Even, C. et al. Recent advances in studying single bacteria and biofilm mechanics. *Adv. Colloid Interface Sci.* **247**, 573–588 (2017).
123. Heisenberg, C.-P. & Bellaïche, Y. Forces in tissue morphogenesis and patterning. *Cell* **153**, 948–962 (2013).
124. Rivera-Yoshida, N., Arias Del Angel, J. A. & Benítez, M. Microbial multicellular development: mechanical forces in action. *Curr. Opin. Genet. Dev.* **51**, 37–45 (2018).
125. Stewart, P. S. & Franklin, M. J. Physiological heterogeneity in biofilms. *Nat. Rev. Microbiol.* **6**, 199–210 (2008).
126. Earle, K. A. et al. Quantitative imaging of gut microbiota spatial organization. *Cell Host Microbe* **18**, 478–488 (2015).
127. Tropini, C., Earle, K. A., Huang, K. C. & Sonnenburg, J. L. The gut microbiome: connecting spatial organization to function. *Cell Host Microbe* **21**, 433–442 (2017).
128. Nadell, C. D., Drescher, K. & Foster, K. R. Spatial structure, cooperation and competition in biofilms. *Nat. Rev. Microbiol.* **14**, 589–600 (2016).
129. Mukherjee, S. & Bassler, B. L. Bacterial quorum sensing in complex and dynamically changing environments. *Nat. Rev. Microbiol.* **17**, 371–382 (2019).
130. Liu, G. et al. Self-driven phase transitions drive *Myxococcus xanthus* fruiting body formation. *Phys. Rev. Lett.* **122**, 248102 (2019).
131. Kientz, B. et al. Glitter-like iridescence within the bacteroidetes especially cellulophaga spp.: optical properties and correlation with gliding motility. *PLoS One* **7**, e52900 (2012).
132. Drescher, K., Nadell, C. D., Stone, H. A., Wingreen, N. S. & Bassler, B. L. Solutions to the public goods dilemma in bacterial biofilms. *Curr. Biol.* **24**, 50–55 (2014).
133. Besemer, K. et al. Biophysical controls on community succession in stream biofilms. *Appl. Environ. Microbiol.* **73**, 4966–4974 (2007).
134. Niederdorfer, R., Peter, H. & Battin, T. J. Attached biofilms and suspended aggregates are distinct microbial lifestyles emanating from differing hydraulics. *Nat. Microbiol.* **1**, 16178 (2016).
135. Rossy, T., Nadell, C. D. & Persat, A. Cellular advective-diffusion drives the emergence of bacterial surface colonization patterns and heterogeneity. *Nat. Commun.* **10**, 2471 (2019).
This paper shows that fluid flow influences biofilm morphology and spatial organization.
136. Martínez-Garcīa, R., Nadell, C. D., Hartmann, R., Drescher, K. & Bonachela, J. A. Cell adhesion and fluid flow jointly initiate genotype spatial distribution in biofilms. *PLoS Comput. Biol.* **14**, e1006094 (2018).
137. Nadell, C. D., Ricaurte, D., Yan, J., Drescher, K. & Bassler, B. L. Flow environment and matrix structure interact to determine spatial competition in *Pseudomonas aeruginosa* biofilms. *eLife* **6**, e21855 (2017).
138. Wucher, B. R. et al. *Vibrio cholerae* filamentation promotes chitin surface attachment at the expense of competition in biofilms. *Proc. Natl Acad. Sci. USA* **116**, 14216–14221 (2019).
139. Hartmann, R. et al. Emergence of three-dimensional order and structure in growing biofilms. *Nat. Phys.* **15**, 251–256 (2019).
This paper shows that mechanical cell–cell interactions are the dominant contributor of spatial organization in biofilms.
140. Nadell, C. D., Drescher, K., Wingreen, N. S. & Bassler, B. L. Extracellular matrix structure governs invasion resistance in bacterial biofilms. *ISME J.* **9**, 1700–1709 (2015).
141. Seminara, A. et al. Osmotic spreading of *Bacillus subtilis* biofilms driven by an extracellular matrix. *Proc. Natl Acad. Sci. USA* **109**, 1116–1121 (2012).
142. Srinivasan, S., Kaplan, C. N. & Mahadevan, L. A multiphase theory for spreading microbial swarms and films. *eLife* **8**, e42697 (2019).
143. Yan, J., Nadell, C. D., Stone, H. A., Wingreen, N. S. & Bassler, B. L. Extracellular-matrix-mediated osmotic pressure drives *Vibrio cholerae* biofilm expansion and cheater exclusion. *Nat. Commun.* **8**, 327 (2017).

144. Bjarnsholt, T. et al. The in vivo biofilm. *Trends Microbiol.* **21**, 466–474 (2013).
145. Wagner, C. E., Wheeler, K. M. & Ribbeck, K. Mucins and their role in shaping the functions of mucus barriers. *Annu. Rev. Cell Dev. Biol.* **34**, 189–215 (2018).
146. Johansson, M. E. V. et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc. Natl Acad. Sci. USA* **105**, 15064–15069 (2008).
147. DePas, W. H. et al. Exposing the three-dimensional biogeography and metabolic states of pathogens in cystic fibrosis sputum via hydrogel embedding, clearing, and rRNA labeling. *MBio* **7**, e00796–16, https://doi.org/10.1128/mBio.00796-16 (2016).
148. Caldara, M. et al. Mucin biopolymers prevent bacterial aggregation by retaining cells in the free-swimming state. *Curr. Biol.* **22**, 2325–2330 (2012).
149. Frenkel, E. S. & Ribbeck, K. Salivary mucins promote the coexistence of competing oral bacterial species. *ISME J.* **11**, 1286–1290 (2017).
150. Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* **14**, 20–32 (2016).
151. McLoughlin, K., Schluter, J., Rakoff-Nahoum, S., Smith, A. L. & Foster, K. R. Host selection of microbiota via differential adhesion. *Cell Host Microbe* **19**, 550–559 (2016).
This paper provides computational predictions of the dynamics and spatial organisation of mucus-associated biofilms.
152. Frantz, C., Stewart, K. M. & Weaver, V. M. The extracellular matrix at a glance. *J. Cell Sci.* **123**, 4195–4200 (2010).
153. Plotnikov, S. V., Pasapera, A. M., Sabass, B. & Waterman, C. M. Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. *Cell* **151**, 1513–1527 (2012).
154. Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677–689 (2006).
155. Baker, B. M. & Chen, C. S. Deconstructing the third dimension – how 3D culture microenvironments alter cellular cues. *J. Cell Sci.* **125**, 3015–3024 (2012).
156. Ladoux, B. & Mège, R.-M. Mechanobiology of collective cell behaviours. *Nat. Rev. Mol. Cell Biol.* **18**, 743–757 (2017).
157. Ranade, S. S. et al. Piezo1, a mechanically activated ion channel, is required for vascular development in mice. *Proc. Natl Acad. Sci. USA* **111**, 10347–10352 (2014).
158. Mathijssen, A. J. T. M., Culver, J., Bhamla, M. S. & Prakash, M. Collective intercellular communication through ultra-fast hydrodynamic trigger waves. *Nature* **571**, 560–564 (2019).
159. Renner, L. D. & Weibel, D. B. Physicochemical regulation of biofilm formation. *MRS Bull.* **36**, 347–355 (2011).
160. O'Toole, G. A. & Wong, G. C. Sensational biofilms: surface sensing in bacteria. *Curr. Opin. Microbiol.* **30**, 139–146 (2016).
161. Irie, Y. et al. Self-produced exopolysaccharide is a signal that stimulates biofilm formation in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA* **109**, 20632–20636 (2012).
162. Kovach, K. et al. Evolutionary adaptations of biofilms infecting cystic fibrosis lungs promote mechanical toughness by adjusting polysaccharide production. *NPJ Biofilms Microbiomes* **3**, 1–9 (2017).
163. Chew, S. C. et al. Dynamic remodeling of microbial biofilms by functionally distinct exopolysaccharides. *MBio* https://doi.org/10.1128/mBio.01536-14 (2014).
164. Zorraquino, V. et al. Coordinated cyclic-di-GMP repression of *Salmonella* motility through YcgR and cellulose. *J. Bacteriol.* **195**, 417–428 (2013).
165. Murthy, S. E., Dubin, A. E. & Patapoutian, A. Piezos thrive under pressure: mechanically activated ion channels in health and disease. *Nat. Rev. Mol. Cell Biol.* **18**, 771–783 (2017).
166. Coste, B. et al. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* **330**, 55–60 (2010).
167. Wang, L. et al. Structure and mechanogating of the mammalian tactile channel PIEZO2. *Nature* **573**, 225–229 (2019).
168. Lin, Y.-C. et al. Force-induced conformational changes in PIEZO1. *Nature* **573**, 230–234 (2019).
169. Orell, A., Fröls, S. & Albers, S.-V. Archaeal biofilms: the great unexplored. *Annu. Rev. Microbiol.* **67**, 337–354 (2013).
170. Bell, G. I. Models for the specific adhesion of cells to cells. *Science* **200**, 618–627 (1978).
171. Stocker, R. Marine microbes see a sea of gradients. *Science* **338**, 628–633 (2012).
172. Deng, Y., Sun, M. & Shaevitz, J. W. Direct measurement of cell wall stress stiffening and turgor pressure in live bacterial cells. *Phys. Rev. Lett.* **107**, 158101 (2011).
173. Swift, J. et al. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* **341**, 1240104 (2013).
174. Gennes, P.-G. de & Gennes, P. P.-G. *Scaling Concepts in Polymer Physics* (Cornell University Press, 1979).
175. Phillips, R., Kondev, J. & Theriot, J. *Physical Biology of the Cell* (Garland Science, 2013).
176. Jacobs, C. R., Huang, H. & Kwon, R. Y. *Introduction to Cell Mechanics and Mechanobiology* (Garland Science, 2012).
177. Moffitt, J. R., Chemla, Y. R., Smith, S. B. & Bustamante, C. Recent advances in optical tweezers. *Annu. Rev. Biochem.* **77**, 205–228 (2008).
178. Neuman, K. C. & Nagy, A. Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy. *Nat. Methods* **5**, 491–505 (2008).
179. Merz, A. J., So, M. & Sheetz, M. P. Pilus retraction powers bacterial twitching motility. *Nature* **407**, 98–102 (2000).
180. Maier, B., Potter, L., So, M., Seifert, H. S. & Sheetz, M. P. Single pilus motor forces exceed 100 pN. *Proc. Natl Acad. Sci. USA* **99**, 16012–16017 (2002).
181. Xiao, J. & Dufrêne, Y. F. Optical and force nanoscopy in microbiology. *Nat. Microbiol.* **1**, 16186 (2016).
182. Biais, N., Higashi, D., So, M. & Ladoux, B. Techniques to measure pilus retraction forces. *Methods Mol. Biol.* **799**, 197–216 (2012).
183. Sabass, B., Koch, M. D., Liu, G., Stone, H. A. & Shaevitz, J. W. Force generation by groups of migrating bacteria. *Proc. Natl Acad. Sci. USA* **114**, 7266–7271 (2017).
184. Duvernoy, M.-C. et al. Asymmetric adhesion of rod-shaped bacteria controls microcolony morphogenesis. *Nat. Commun.* **9**, 1120 (2018).
185. Karampatzakis, A. et al. Probing the internal micromechanical properties of *Pseudomonas aeruginosa* biofilms by Brillouin imaging. *NPJ Biofilms Microbiomes* **3**, 20 (2017).
186. Rusconi, R., Garren, M. & Stocker, R. Microfluidics expanding the frontiers of microbial ecology. *Annu. Rev. Biophys.* **43**, 65–91 (2014).
187. Son, K., Brumley, D. R. & Stocker, R. Live from under the lens: exploring microbial motility with dynamic imaging and microfluidics. *Nat. Rev. Microbiol.* **13**, 761–775 (2015).
188. Lewis, K. J. R. & Anseth, K. S. Hydrogel scaffolds to study cell biology in four dimensions. *MRS Bull.* **38**, 260–268 (2013).
189. Colom, A. et al. A fluorescent membrane tension probe. *Nat. Chem.* **10**, 1118–1125 (2018).
190. Özkale, B. et al. Modular soft robotic microdevices for dexterous biomanipulation. *Lab. Chip* **19**, 778–788 (2019).
191. Mongera, A. et al. A fluid-to-solid jamming transition underlies vertebrate body axis elongation. *Nature* **561**, 401–405 (2018).

Acknowledgements
The authors would like to thank N. Biais and members of the Persat laboratory for useful discussions and feedback. A.P. is supported by the Swiss National Science Foundation Projects Grant 31003A_169377, the Gabriella Giorgi-Cavaglieri Foundation, the Gebert Rüf Stiftung and the Fondation Beytout. Work at UCLouvain was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement n°693630), the FNRS-WELBIO (grant n°WELBIO-CR-2015A-05), the National Fund for Scientific Research (FNRS), and the Research Department of the Communauté française de Belgique (Concerted Research Action).

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Microbiology thanks U. Jenal, D. Limoli and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.