Surface sensing in Escherichia coli

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Chapter 1

How Bacteria Recognise and Respond to Surface Attachment

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This chapter (excluding the section 'Aim and Outline of this thesis') is under review at Microbiology and Molecular Biology Reviews.

Table of contents

Abstract

Bacterial biofilms can cause medical problems and issues in technical systems. While a large body of knowledge exists on the phenotypes of planktonic and of sessile cells in mature biofilms, our understanding of what happens when bacteria change from the planktonic to the sessile state is still very incomplete. Fundamental questions are unanswered: for instance, how do bacteria sense that they are close to, or in contact with, a surface, and what are the very initial cellular responses to surface contact. Here, we review the current knowledge on the signals that bacteria could perceive once they attach to a surface, the signal transduction systems that could be involved in sensing the surface contact, and the cellular responses that are triggered as a consequence to surface contact ultimately leading to biofilm formation. Finally, as the main obstacle in investigating the initial responses to surface contact has been the experimental difficulty to study the dynamic response of single cells upon surface attachment, we also review recent experimental approaches that could be employed to study bacterial surface sensing, which ultimately could lead to an improved understanding how biofilm formation could be prevented.

Introduction

Bacterial biofilms generate significant technological and therapeutic problems, ranging from increased fuel consumption of ships due to higher flow resistance [1, 2], via fouling of membranes in water treatment facilities [3] to serious medical problems. It is estimated that two-thirds of human infections involve biofilm formation, including infections of the urinary tract, lungs and ears, dental plaque and fouling of implants and contact lenses [4]. The increased resistance of bacteria in biofilms towards antimicrobial compounds and the host immune system constitutes a central issue in treatment of bacterial infections [5].

While the developmental steps leading to a mature biofilm are well-characterised [6–8], little is known about the very first step of biofilm development, i.e. how cells sense initial surface attachment, which eventually leads to phenotypic adjustments, involving substantial changes in gene expression [9–12], from the planktonic (suspended) to the sessile (surface-attached) state. The main reason for this limited knowledge likely lies in the challenge to experimentally investigate the dynamic response of cells during the transition between these two states.

Here, towards pointing out the existing gaps in our understanding of surface sensing, we review the current knowledge on how bacteria recognise and respond to surface attachment with a special emphasis on the model organism *Escherichia coli*. Specifically, we first review the signals a cell might perceive in close proximity to, or in contact with, a surface and describe the mechanisms that bacteria employ to perceive the presence of and attachment to a surface. Second, we report the initial downstream effects that are triggered in response to surface attachment. Third, as the limited understanding of surface sensing and of the very first steps of biofilm formation is likely connected with the fact that the respective processes are difficult to study, we highlight recent technological developments that might support future research on further elucidating the process of surface sensing. Closing these knowledge gaps will offer valuable insights on how to combat biofilm formation.

Surface sensing

Bacteria can adhere to a large variety of surfaces, including glass, metals, many different polymers, as well as to other cells (for a review, see [13]). In fact, it is practically impossible to develop a surface that cannot be colonized by bacteria, making it resistant towards biofouling, while at the same time being harmless to humans and the environment [2]. When a planktonic bacterial cell advances towards a surface from the bulk of a liquid, there are three different phenomena that might be sensed: (i) changes in physicochemical properties, (ii) attachment of cell appendages, and (iii) attachment of the cell body (Figure 1).

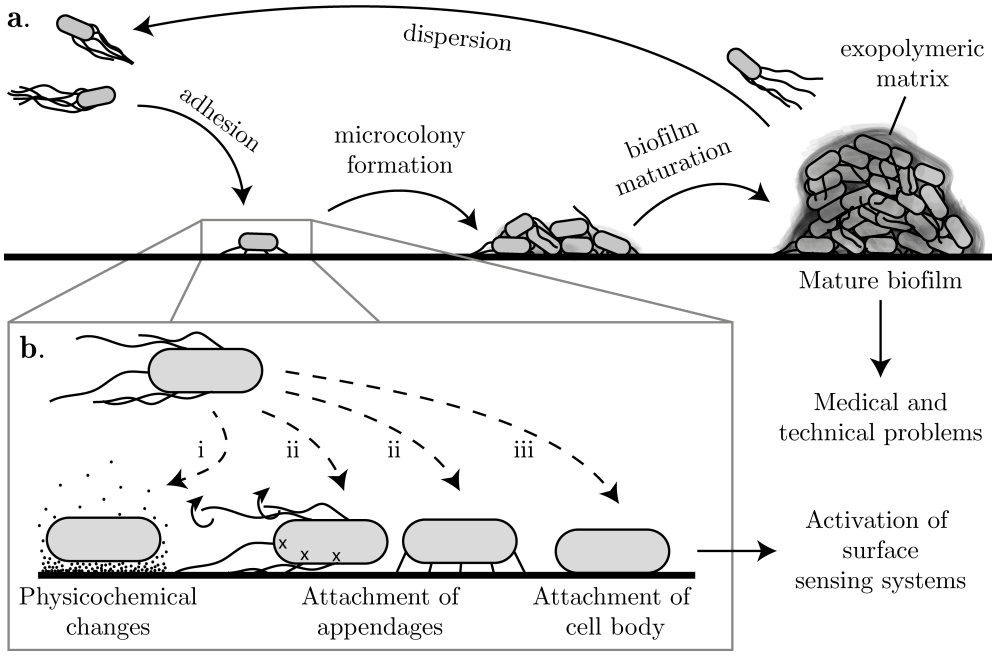

Figure 1: Schematic overview of biofilm formation and surface sensing. (**a**) Biofilm formation involves a number of stages. After initial adhesion, attached bacteria grow to form a microcolony, which gradually develops into a mature biofilm, from which new planktonic cells can disperse. (**b**) When a bacterium approaches and contacts the surface, it may sense (**i**) different physicochemical properties compared to the bulk liquid, (**ii**) attachment of cell appendages and (**iii**) envelope stress due to attachment of the cell body.

Physicochemical changes

The microenvironment close to the surface differs from the bulk liquid in terms of ionic strength, osmolarity, pH, and nutrient availability (for a review, see [14]). Since many surfaces are charged, counter-ions accumulate at the solid-liquid interface. In the case of a negatively charged surface, which is the most common, these counter-ions include protons, causing a lowered pH at the surface. It has been suggested that close to a glass surface, the pH may decrease by as much as two units [15]. Furthermore, organic molecules, present in the bulk liquid or secreted by bacteria, can be adsorbed onto surfaces and form a conditioning film (reviewed in [13]). Such films can consist of a mixture of (macro)molecules, including proteins, amino acids, lipids, and polysaccharides [16–18] nutrients that can be metabolised by attached bacteria [19, 20]. Sessile bacteria may therefore be able to grow, even when the nutrient concentration in the bulk liquid is insufficient to sustain growth of planktonic cells [21, 22].

To sense changes in pH, ionic strength, osmolarity, and nutrient availability, bacteria

typically employ two-component signal transduction, consisting of a membrane-bound histidine kinase, which senses the stimulus, and a cytoplasmic response regulator mediating the cellular response. As the physicochemical properties can vary also in the bulk liquid, the stimuli of respective systems are not specific for surfaces. By exemplarily reviewing signal transduction systems that sense these properties in the model organism *E. coli*, however, we show that many of these systems have downstream target genes linked to biofilm formation, which indicates that these systems could be part of the surface sensing machinery.

In *E. coli*, systems that sense physicochemical properties and have a role in biofilm formation are CpxAR, EnvZ/OmpR, RcsCDB and possibly BasSR. A multitude of conditions, including high osmolarity [23] as well as alkaline pH [24, 25] can induce the activity of the CpxAR two-component system [26]. Also the EnvZ/OmpR two-component system can sense both the osmolarity [27–29] and pH [24, 30–32]. The osmolarity is thought to be sensed via the intracellular potassium ion concentration, which increases in response to high extracellular osmolarity, and is a known inducing signal for EnvZ [33]. Indeed, it has been shown that the potassium concentration increases in surface-attached bacteria [9]. An upshift in osmolarity is also a known inducing cue for the RcsCDB phosphorelay [34, 35]. The BasSR two-component system, for which only the inducing signals iron [36] and zinc [37] are known in *E. coli*, is another signal transduction pathway that might sense physicochemical properties associated with the vicinity of surfaces: the *E. coli* BasSR may be pH-responsive, because in *Salmonella enterica* the homologous pathway has been shown to be activated by mild acidic conditions and the essential residues for pH sensing are conserved in *E. coli* [38]. Thus, there are multiple signal transduction systems in *E. coli* that respond to the same physicochemical properties. These systems have downstream biofilm-related targets (Table 1), with for instance motility being regulated by Cpx [39], EnvZ/OmpR [40] and Rcs [41]. The downstream effects are discussed in more detail below.

Alternatively, close to surfaces, bacteria could sense increased nutrient concentrations via changes in metabolism. The uptake of a compound may in some cases act as a signal that is transmitted by the respective transporter to a sensor protein (for a review, see [42]). If the adsorbed nutrients are metabolised, this will change the intracellular metabolic fluxes and may in turn affect gene expression [43, 44]. The metabolic state of the cell is also influenced by alterations in pH, as for instance a local drop in pH close to a negatively charged surface can directly affect the proton motive force [45].

Overall, the physicochemical properties in the vicinity of a surface may trigger a cellular response via two-component systems. However, because these properties can also vary in the bulk liquid, these stimuli are not specific for surfaces and are therefore unlikely to be the main cue indicating surface contact.

Attachment of cell appendages

In the following, we describe the processes that also occur when a cell approaches a surface, for instance the attachment of cell appendages to the surface, and we review how these processes can be sensed.

Flagella. When bacteria approach a surface, cell appendages might stick to it. Adhesion is supported by flagella, which due to their hydrophobic nature particularly adhere to hydrophobic surfaces [46–50]. Not only the presence of flagella, but also the ability to rotate them is important for adhesion, as mutants with non-functional flagella are impaired in biofilm formation and detach more readily compared to the wild-type [48, 51].

Once attached, flagella can provide signals to the cell indicating surface contact, which originate from hindered rotation (for a review, see [52]). It was recently shown that when flagellar rotation is blocked, either by mutations in flagellar motor genes or by addition of anti-flagellin antibodies, the DegS-DegU signal transduction pathway, controlling biofilm formation, is activated in *Bacillus subtilis* [53]. Furthermore, in *Vibrio parahaemolyticus*, an organism that has been long known to sense flagellar inhibition as a signal to initiate swarming [54], a transcriptomics study both on mutant strains defective in flagellar rotation and on wild-type cells treated with flagellum-inhibiting drugs, found a gene expression pattern similar to sessile cells [55]. Specifically, about half of the genes that were differentially expressed in surface-attached cells also had altered expression levels when rotation was impaired, suggesting that the flagella are a main surface sensor in this organism. The *E. coli* flagellum has also been shown to be sensitive towards mechanical forces: the number of stators, i.e. the force-generating protein complexes of the flagellar motor, increases within minutes when the load of rotation is increased by binding a microbead to truncated flagella [56–58].

How hindered flagellar rotation is sensed on the molecular level seems to vary between bacterial species and the mechanism has not been resolved in all cases. In *B. subtilis*, the interaction affinity of the flagellar motor and the cytoplasmic histidine kinase DegS may be affected by the rotation of the motor, such that halted rotation leads to activation of the DegS-DegU two-component system [53]. In *Caulobacter crescentus*, the diguanylate cyclase DgcB associates with the flagellar motor and starts synthesizing the second messenger molecule cyclic diguanylate (c-di-GMP) upon surface-induced halting of flagellar rotation, leading to rapid irreversible attachment [59]. A complex regulatory mechanism then reinforces the switch to the sessile phenotype by inhibition of motility [60]. In other cases, signal transduction of flagellar attachment might go via the flagellar stator-associated FliL protein [53]. In one study, FliL was shown to play an important role in sensing the presence of a surface, as respective deletion mutants of *Proteus mirabilis* and *E. coli* responded differently to soft agar surfaces than wild-type cells, in terms of motility and gene expression [61]. However, another study reported that FliL plays no significant role in mechanosensitivity of the *E. coli* flagellum, instead suggesting that higher torque in hindered flagella results in exposure of binding sites on the flagellar rotor [62]. Other

explanations for surface sensing by flagella are that, after rotation has stopped, a reduced ion flux through the flagellar motor may impact the membrane potential and energy state of the cell [53] or that blocked rotation could result in an increased torque of the motor, leading to perturbations in the cell envelope [63]. Thus, while flagella are known to contribute to the perception of surface contact in a range of bacterial species, they might employ different sensing mechanisms.

Pili. In addition to flagella, also pili (fimbriae) attach to surfaces and support biofilm development. Their importance is illustrated by a genome-wide study in *E. coli*, which revealed that loss of genes encoding type I pili had the most detrimental effect out of all single-gene deletions on the formation of biofilms [64]. The importance of type I pili for adhesion is also illustrated by the finding that attachment to a variety of abiotic surfaces can be greatly reduced by addition of mannose to the medium [46]. As the FimH subunit on the tip of type I pili is known to bind to mannose, which is also present on eukaryotic cells [65, 66], the decreased adhesion is likely due to a reduced interaction of the mannose-saturated pili with the surface [46, 65].

Also curli fimbriae stick to surfaces and are highly beneficial for adhesion [46, 47, 64, 67]. Increased production of curli through a point mutation in the ompR gene has been shown to enhance surface attachment, while mutations generating curli-deficient cells were found to result in a more than 50% reduction in biofilm formation [64, 67]. A wide range of pili are known to exist in various bacterial species, but also within single species generally multiple types of pili are expressed. For example, in addition to type I pili and curli, *E. coli* may also carry P-pili, type IV pili, and several others [68, 69].

Mediating stable adhesion to the surface is a main role of pili, but they may have other functions as well. In the case of type IV pili, that are present in many organisms, a continuous process of extension, eventual attachment, and retraction facilitates twitching motility on the surface [70, 71]. In *Pseudomonas aeruginosa*, another type of pilus, Cup, does not only mediate cell-surface interactions, but seems to also be involved in cell-cell aggregation [72, 73], underscoring the importance of pili also during subsequent stages of biofilm formation. In *Caulobacter crescentus*, one role of the polar Tad pili might be bringing the polar flagellum in close proximity to the surface, such that its rotation is hindered, which is used by the bacteria as a signal for surface attachment [59]. Countless other cell appendages exist, but listing them all is beyond the scope of this review, and the reader is referred to a comprehensive review [69]. Overall, bacteria use a wide range of pili mainly to mediate adhesion to surfaces, but also for a variety of other functions. Besides sensing surface contact via attached flagella, the attachment of pili is also known to be sensed. For instance, in *E. coli* attachment of pili was found in multiple studies to result in altered gene expression [74–76]. While the molecular mechanism here was not fully clear, for *Pseudomonas aeruginosa*, it has been suggested that the continuously extending and retracting type IV pili perceive tension when attached pili are retracted and that these forces may lead to depolymerisation of the pili and/or conformational changes in pilus subunits, which then enable the interaction between the major pilus subunit PilA and the sensor protein PilJ. As a result, a signalling pathway is activated that produces the second messenger cyclic AMP to initiate biofilm formation [77, 78]. In *C. crescentus*, a

different type of pilus, Tad, uses a comparable mechanism, as it also senses the inability to retract once it is attached to a surface and then possibly stimulates the diguanylate cyclase PleD, which signals via c-di-GMP, leading to synthesis of a holdfast structure that mediates adhesion [79]. Alternatively, pili-mediated attachment could be sensed as an accumulation of mislocalised pilus subunits in the periplasm of attached cells [80], known to induce the CpxAR two-component system in *E. coli* [81]. A second signalling system in *E. coli* that has been implicated in pili-mediated sensing of the surface is the BarA/UvrY two-component system: the transcription of *barA* is stimulated by P-pilus attachment in uropathogenic *E. coli* by a yet unknown mechanism [75]. For a recent review that covers surface sensing via type IV pili, see [82].

Mechanosensing of shear forces on bacteria that are attached via pili, may also induce biofilm formation. This has been shown in *P. aeruginosa* cells that were attached via type IV pili, where shear forces caused the production of c-di-GMP and subsequent biofilm initiation [83]. Similar results have been obtained in enterohemorrhagic *E. coli*, where increasing shear forces on attached cells led to increased induction of a pathogenicity island, although here the involvement of pili was less clear [84].

Thus, flagella and pili do not only facilitate adhesion to the surface, but also transmit signals that allow bacteria to respond to this adhesion. Surface sensing via cell appendages has been found in multiple species, indicating that it is a common mechanism to perceive surface contact in bacteria.

Cell body attachment

Next to the surface attachment via the cell appendages, also the whole cell body can attach to the surface. Here, adhesion to a surface is mainly mediated by Van der Waals, electrostatic and acid-base interactions between the bacterium and the surface (for reviews see [85–87]). Upon approaching a surface, a bacterium will initially be attracted by the long-range Van der Waals forces, but short-range repulsive electrostatic forces, for instance provided by the fact that most bacteria and material surfaces are both negatively charged [88], may subsequently prevent close contact to the surface. This phenomenon is the basis of the Derjaguin-Landau-Verwey-Overbeek theory, which has been indispensable in explaining cellular adhesion, although it is a highly simplified representation [89, 90]. Due to the oppositely oriented forces, the energy minimum may lie at a distance on the order of tens of nm from the surface (Figures 2a and 2b) [91]. Shielding of the charges on the surface by a conditioning film or high ionic strength of the medium can decrease the contribution of the electrostatic forces, allowing a smaller distance between cell and surface [86, 92]. Physical contact of the cell body with the surface during adhesion can furthermore be facilitated by the long O-antigen part of lipopolysaccharides (LPS), cell appendages or polar adhesion (Figure 2c) [46, 47, 87, 93–98].

Following the initial adhesion of the cell body, gradually the attachment becomes stronger over a time window of seconds to minutes, which is facilitated by rearrangements at the interface between cell envelope and surface that progressively maximize attractive interactions, e.g. by removal of interfacial water, protein

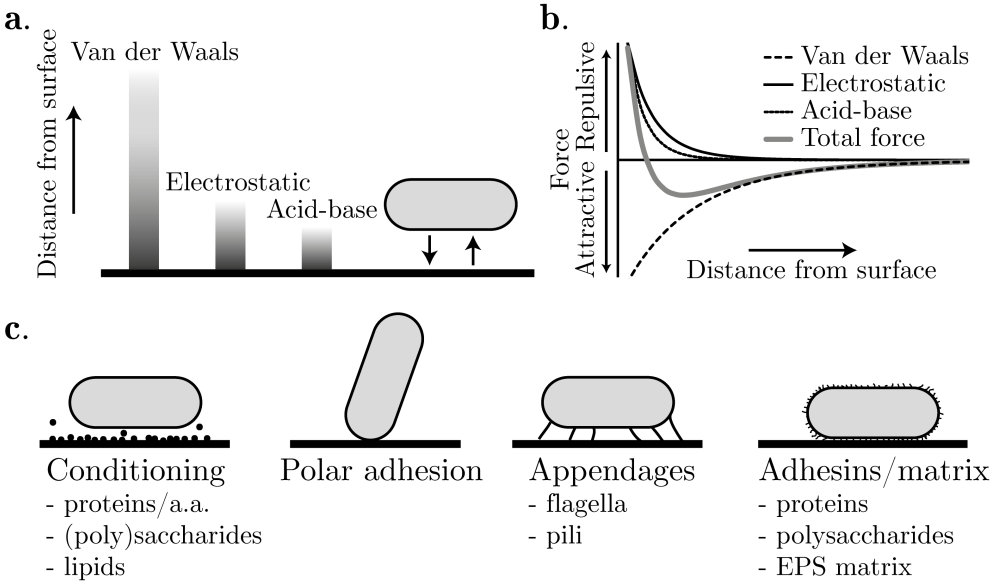

Figure 2: Summary of the forces involved in adhesion and strategies to overcome unfavourable surface interactions. (**a**) Schematic representation of forces acting on a bacterium when it approaches a surface, with the balance between attractive and repulsive forces keeping the bacterium at a small distance from the surface. (**b**) Adhesion forces as a function of distance from the surface. Van der Waals forces are attractive, electrostatic forces are generally repulsive and acid-base forces might be either. The total force reaches an energy minimum, which determines the separation of the bacterium from the surface [99]. (**c**) Strategies employed by bacteria to achieve a smaller separation from the surface than allowed by the initial energy minimum, which include (**i**) conditioning of the surface with e.g. proteins, amino acids, (poly)saccharides, and lipids, (**ii**) polar adhesion, (**iii**) attachment of appendages, and (**iv**) production of adhesive proteins and polysaccharides that are exposed on the cell surface.

conformational changes and an increase of favourable acid-base and hydrophobic interactions between cell and surface [99, 100]. Indeed, establishing initial stable attachment to the surface does not require biological activity, as the adhesion force of polystyrene particles to glass has also been found to strongly increase within minutes on a surface [101].

At this stage, the adhesion might already be considered irreversible, but could be reinforced through production of adhesins. Irreversible adhesion in fact does not require a large contact area between cell and surface. For instance, *Caulobacter crescentus* can irreversibly attach in a polar orientation due to the production of adhesins at the cell pole [59, 79]. Similar results have been found for *Asticcacaulis biprosthecum* and *Agrobacterium tumefaciens*, where also polar adhesins were produced within minutes following surface contact, which shows that rapid surface-

induced strengthening of polar adhesion is a common strategy in multiple genera [102]. However, other bacterial species preferentially assume a flat orientation on the surface. For example, in *Pseudomonas aeruginosa*, production of the Pel polysaccharide, a component of the exopolymeric matrix, stimulates a transition from polar adhesion to a flat orientation by increasing short-range attractive interactions [103]. *Pseudomonas fluorescens* uses a similar mechanism to transition to irreversible attachment, secreting the large adhesive protein LapA, which remains associated with the cell-surface, to assume a flat orientation [104]. Initial adhesion does not necessarily result in irreversible attachment within a short time span, as bacteria may first explore the surface by moving (or swarming) over it, using type IV pili or flagella [61, 105, 106]. Following irreversible adhesion, bacteria stick together mainly by production of an exopolymeric matrix and via their appendages [47], but also by synthesizing adhesins like Ag43 that promote aggregation of bacteria [107].

The importance of understanding adhesion forces is exemplified by the effect that combined adhesion forces between cell and surface have on the fate of the attached cell [108]. Weak adhesion forces (e.g. on polymer brush-coatings) can lead to the formation of unstable biofilms with aberrant structure and smaller thickness [109, 110]. Possibly, such weak interactions do not provide sufficient signal for the adhered bacteria to transition to subsequent steps in the development of the biofilm, such that they fail to form a robust matrix-enclosed structure. On the other hand, in the case of too strong adhesion forces, which bacteria especially encounter on positively charged surfaces, extensive envelope stress and loss of viability may occur [108, 111]. In the intermediate regime, corresponding to interactions between bacteria and many common materials, the forces are sufficiently strong to trigger biofilm formation, without affecting viability [108]. Generally, surfaces are not perfectly homogeneous, but instead contain patches with different properties, in terms of surface charge, hydrophobicity, roughness, and composition of the conditioning film [112]. This means that attached bacteria may experience different interactions with the same surface, which, together with the limited range of interbacterial communication, was proposed to be the cause of commonly observed heterogeneous micro-environments within biofilms where bacteria have distinct phenotypes [112]. Thus, understanding the forces that govern bacteria-surface interactions not only allows us to predict whether stable adhesion will occur, but might also explain emergent properties in mature biofilms.

Physical contact of the cell body with a surface is widely thought to give rise to envelope stress [82, 108, 113–119], which is often defined as the presence of unfolded proteins [81, 120] and lipopolysaccharides [121] in the periplasm. However, to the best of our knowledge, there is no direct experimental evidence for such surface-induced protein unfolding or LPS mislocalization. If a broader definition of envelope stress is used, including all perturbations of the extracytoplasmic space of the cell, such as altered membrane curvature [122, 123] and loss of membrane integrity [124], then adhesion of the cell body can indeed be seen as a cause of envelope stress. Specifically, there is evidence, obtained with three different experimental techniques, that adhesion causes deformations of the cell shape, and thus altered membrane curvature [125, 126], and compresses the thickness of the cell envelope near the site

of surface contact [110]. Such attachment-related membrane tension has also been proposed to cause mechanosensitive channels to open [119].

Usually, cellular responses to envelope stress are studied after invoking the stress by chemical means (e.g. treatment with compounds that insert into or disrupt the lipid bilayer [122, 127]), or by genetic modifications (e.g. overexpression of membrane proteins [120] or mutations in the synthesis pathways of lipopolysaccharides or phospholipids [128–130]). Since a number of signalling systems that respond to such laboratory-induced envelope stress regulate biofilm-associated genes, it is plausible that surface-induced envelope stress upon physical contact of the cell body may be a way to sense adhesion. While most of the following envelope stress response systems are described with a focus on *E. coli*, it should be noted that in many cases homologous systems exist in other species.

Besides the above mentioned physicochemical parameters and attachment of pili, envelope stress can activate the CpxAR two-component system. Activation can happen when there are defects in LPS assembly [131, 132]. Also, unfolded proteins can induce the system, by binding to the auxiliary regulator CpxP, which lifts its inhibition of the histidine kinase CpxA [133–136]. Once bound to an unfolded protein, the direct interaction of CpxP with CpxA is released and CpxP is proteolysed [137]. It has also been suggested that, in response to physical contact with a surface, the Cpx system gets activated via the outer membrane lipoprotein NlpE [113]. Its tertiary structure might predispose NlpE to unfolding by membrane perturbations, leading to activation of the kinase functionality of the inner membrane protein CpxA [114]. If this model is correct, then it exemplifies how envelope stress can be exploited by the cell as a signal for surface attachment. However, recent findings indicate that the CpxAR system is not activated by NlpE-mediated surface sensing, as the previously reported results could not be reproduced, neither by employing the original population-level assay, nor by a novel single-cell experimental approach [138].

Envelope stress can also activate the RcsCDB phosphorelay. The activity of this system was found to be altered upon deletion of genes involved in lipopolysaccharide synthesis [128, 139], mutation or overexpression of genes encoding envelope-localised proteins [140, 141], and upon membrane damage caused by the action of antimicrobial peptides [127]. When *E. coli* is grown on a solid surface, the expression of genes controlled by the Rcs phosphorelay has been shown to increase rapidly, but the underlying mechanism has not been solved [115]. Also in *P. mirabilis*, it was found that the Rcs system is responsive to surface contact, but, contrary to what was found in *E. coli*, surfaces inhibit the Rcs system in *P. mirabilis* [118]. Likely, the outer membrane lipoprotein RcsF is involved in surface sensing. RcsF is inserted into the outer membrane via the major subunit of the -barrel assembly machinery BamA and forms complexes with several abundant -barrel proteins (Figure 3) [142, 143]. Here, part of RcsF is exposed on the cell surface, which was proposed to enable RcsF to sense perturbations of the lipopolysaccharide layer [142]. Upon envelope stress, BamA may fail to generate the complexes between RcsF and other outer membrane proteins, thereby enabling RcsF to activate the Rcs phosphorelay via the inner membrane receptor IgaA [143]. This model is in agreement with findings that

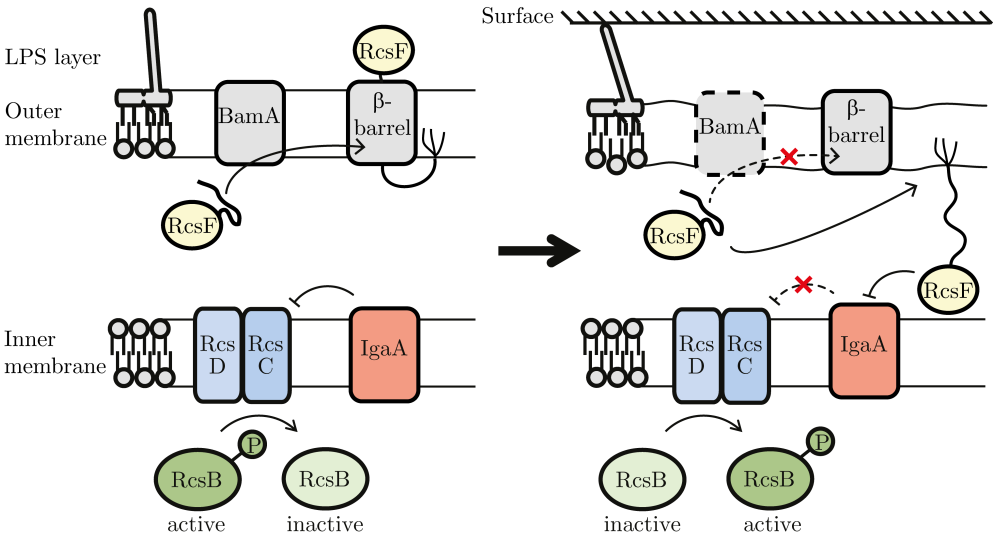

Figure 3: Proposed method of activation of the Rcs system via the outer membrane sensor RcsF. Left: Under non-inducing conditions, the lipoprotein RcsF is tethered to the outer membrane and threads through the lumen of β-barrel proteins [142, 143] (the two studies reported opposite orientations of RcsF in the membrane). In this state, RcsF is unable to contact its inner membrane receptor IgaA, and can therefore not activate the Rcs system, i.e. the dephosphorylation activity of RcsC predominates. Right: By a yet unclear mechanism, envelope stress can interfere with the ability of periplasmic chaperones or BamA to localize RcsF to its regular location and thereby enables it to activate the Rcs system [143].

inner membrane anchored RcsF or truncated forms that localise to the periplasm, constitutively activate the Rcs phosphorelay [127]. A recent study found that it is crucial for activation of the pathway that RcsF has the correct length to span the periplasm [144]. Possibly, the activation of the Rcs system on a surface might be facilitated by small deformations in the cell envelope that affect the distance between the membranes.

Three other systems in *E. coli* that seem to be responsive to envelope stresses, although without any clear involvement in surface sensing or role in biofilm formation, are the sigma factor E [121, 145], the phage shock protein (PSP) response system [146–149] and the BaeSR two-component system [150, 151].

Also in *P. aeruginosa*, surface-induced envelope stress could be a trigger for biofilm initiation. Specifically, contact with a surface can lead to the activation of the Wsp pathway [152, 153]. It has been proposed that deformation of the cell envelope is the signal that is sensed by this pathway, as the WspA protein forms weakly interacting clusters in the inner membrane that might be affected by mechanical forces originating from bacteria-surface interactions [154]. Activation of this system leads to the phosphorylation of the diguanylate cyclase WspR and, in turn, the produced c-di-GMP inhibits the transcription factor FleQ [155]. Thereby, activation of the Wsp

system on a surface represses flagella biosynthesis genes and induces the production of biofilm matrix components.

Recently it was shown that mechanical forces on surface-attached cells could, besides causing envelope stress, induce voltage and calcium transients in *E. coli* [156]. The changes in calcium concentration were found to result in alterations in protein levels. The mechanisms by which these calcium transients are generated and regulate gene expression remain unsolved, but may prove to be an interesting novel sensing system for bacteria attached via the cell body.

In summary, attachment of the cell body can give rise to envelope deformations and maybe other forms of envelope stress. These perturbations are sensed by the Rcs system in *E. coli* and *P. mirabilis*, and the Wsp system in *P. aeruginosa*. It is very plausible that other bacteria also recognise surface-induced envelope deformations as a signal for adhesion.

Downstream effects of the potential surface sensing pathways

In the following, we outline how the above mentioned systems that may sense the proximity to or contact with a surface could induce the changes necessary to switch from a mobile to a sessile lifestyle. Here, we will focus on how the potential surface sensing systems specifically affect motility and adhesion, as key phenotypic changes for the lifestyle switch.

Central role of c-di-GMP

Several of the above sensing systems ultimately lead to increased cyclic-diguanylate (c-di-GMP) levels, which plays a central role in signal transduction that leads to the switch to the sessile phenotype. At high concentrations, c-di-GMP leads to enhanced synthesis of pili and matrix and reduced motility, and thereby plays an important role in the switch between mobile and biofilm lifestyles (for reviews see [168, 169]). Regulation of biofilm initiation via c-di-GMP is highly conserved in the bacterial kingdom, and species that do not synthesize this compound, e.g. *Staphylococcus aureus*, use similar second messengers, e.g. c-di-AMP, to fulfill the same functions [170]. The *E. coli* genome contains 29 genes that are proposed to synthesize or degrade c-di-GMP [171], a number of them known to be induced by the above described potential surface sensing pathways. For instance, the CpxAR system upregulates the expression of the diguanylate cyclase *dgcZ* (*ydeH*) [39, 172]. Furthermore, the production of a number of diguanylate cyclases is regulated by the carbon storage protein CsrA [158], whose activity is indirectly controlled by the BarA/UvrY two-component system via regulation of the expression of the small RNAs CsrB and CsrC [159, 160]. Thus, c-di-GMP links the activation of some potential surface sensing systems to biofilm initiation.

Sensing system	**Adhesion-related signals**	**Biofilm-related targets**
BaeSR	Envelope stress [151, 157]	-
BarA-UvrY	Attached pili [75]	Motility [158–160], pili [161], matrix [162]
BasSR	pH [38]	Curli [163], pili [163]
CpxAR	Osmolarity [23, 164], pH [24, 25], pili subunits [81], envelope stress [134]	Motility [39], curli [165]
EnvZ-OmpR	Osmolarity [27–29], pH [24, 30–32]	Motility [40], curli [165]
PSP	Envelope stress [149]	Motility [149]
RcsCDB	Osmolarity [34, 35], envelope stress [127, 128, 139, 141]	Motility [41], curli [165], pili [115], Ag43 [115], matrix [166]
σ^E	Envelope stress [121, 145, 167]	-
Flagella	Hindered rotation [56–58]	Unknown in *E. coli*

Table 1: An overview of the sensing systems in *E. coli* that can sense properties associated with surface proximity or attachment. Only the inducing signals that may be relevant for surface sensing, as described in the section 'Surface sensing', are shown. Also, the downstream targets are limited to those that are related to biofilm formation and described in the section 'Downstream effects of the potential surface sensing pathways'.

Downregulation of motility

Some of the above mentioned surface sensing systems downregulate motility, which is no longer needed for sessile cells in a biofilm. Although the biosynthesis and rotation of flagella are decreased on a surface, there are generally microenvironments within biofilms where flagella are still expressed to mediate cell-cell and cell-surface interactions [173]. With constitutive expression of the flagellar regulator FlhDC, biofilm formation by *E. coli* is significantly impaired, indicating the importance of their timely regulation [174]. The above outlined, putative surface sensing pathways CpxAR [39], RcsCDB [41], EnvZ/OmpR [40] and BarA/UvrY [158–160] are all involved in controlling flagellar expression and activity, for instance by repressing the flagellar regulator *flhDC* [175] or by increasing the production of c-di-GMP [168], which activates YcgR, which in turn binds to and slows down the rotation of the *E. coli* flagella [176]. For a review about two-component-system-based regulation of motility, see [177].

Control over adhesive appendages

Some of the surface sensing systems exert control over expression of adhesive appendages. For switching to the sessile lifestyle, it is essential to control attachment to the surface and other cells, which can be mediated in part by the synthesis of pili and curli. A number of the above mentioned pathways are involved in expression of curli (for a review see [165]). Activators are the two-component systems EnvZ/OmpR and BasSR, while the CpxAR and RcsCDB systems have been found to repress the curli genes [165]. Probably connected to the loss of curli, deletion of the gene encoding the response regulator OmpR has been found to result in complete loss of adhesion [67], although other studies only reported a moderate reduction in biofilm formation [64, 174]. The genes for curli synthesis are encoded by two operons in *E. coli*, *csgBAC* and *csgDEFG*, both of which are dependent on the general stress response sigma factor σ^S [178]. The *csgD* gene encodes a transcription factor that plays a major role in reduction of motility and is considered as a master regulator of the switch to the sessile phenotype [179].

The production of pili has been shown to be regulated by the second messenger c-di-GMP in a pathogenic *E. coli* strain [180]. The BarA/UvrY two-component system also plays a role in the expression of pili; in a *uvrY* deletion strain fewer cells express pili, while the opposite is true for a strain missing the *csrA* gene, whose product is negatively regulated by BarA/UvrY [161]. However, since CsrA regulates the expression of a number of diguanylate cyclases [158], the effect of the BarA/UvrY system on pili expression could also be indirect, via altered levels of c-di-GMP. Furthermore, the two-component system BasSR was found to directly control expression of pilus genes [163]. Biosynthesis of the CupD fimbriae of *P. aeruginosa*, which play an important role in biofilm formation in more virulent strains, is regulated by the Rcs system [181]. As described above, this system is highly responsive to surface contact in *E. coli* and *P. mirabilis* [115, 118], however, it has not been tested for surface sensing in *P. aeruginosa*.

Production of the exopolymeric matrix

Another important aspect of the sessile lifestyle is the synthesis of the exopolymeric matrix, supporting bacteria to stick together and shielding them to some extent from influences from outside, both physical and chemical. The RcsCDB system positively regulates the expression of the *wca* (also called *cps*) genes, which are responsible for the production of the polysaccharide colanic acid [166]. Colanic acid is essential for the development of the three-dimensional biofilm structure [182]. The BarA/UvrY system can increase the production of another polysaccharide, poly-β-1,6-N-acetyl-D-glucosamine (PGA), via inhibition of CsrA [162]. PGA is important for sessile *E. coli* cells, as indicated by the finding that enzymatic hydrolysis of this compound greatly reduces the ability to form biofilms [183, 184].

Experimental developments for studying surface sensing

Investigation of surface sensing and the corresponding initial responses is complicated due to several inherent and experimental challenges. First, cells simultaneously encounter multiple changes once they approach a surface, i.e. variation in pH, osmolarity, nutrient availability, forces on the flagella and pili, and potentially envelope stress, which makes it difficult to trace the response to a single stimulus. Also, biofilm-related genes are not solely regulated by surface-induced stimuli. For instance, it has been found that pH affects motility and the ability of planktonic *E. coli* to adhere, as exemplified by the presence of fewer and shorter flagella and more pili when cells are cultured in non-neutral pH [185]. Thus, identifying the molecular mechanisms activating surface-induced systems requires either 'isolation' of the phenomena that can be sensed at a surface or requires solving of an intertwined, multivariate problem.

Second, the different potential surface sensing systems overlap in both their activating signals and downstream functions. For instance, the activity of the EnvZ/OmpR system depends on at least three other systems implicated in surface sensing: CpxAR regulates transcription of *ompR* itself [186], both CpxAR and σ^E control transcription of *mzrA* [39, 187], which in turn influences the activity of the EnvZ/OmpR system [188], and there appears to be crosstalk between histidine kinase BarA and response regulator OmpR [189]. Also for the downstream targets there is overlap, as both CpxAR and EnvZ/OmpR regulate the expression of the membrane proteins TppB, OmpC and OmpF [39, 190, 191] and both RcsCDB and EnvZ/OmpR may control the colanic acid synthesis genes [192]. Similar overlaps in inputs and outputs exist also for other proposed surface sensing systems. Thus, together the systems form an entangled network that obscures investigation of individual pathways.

Third, a more practical limitation with respect to the study of initial surface contact is that most current experimental approaches require relatively large numbers of cells. However, if the initial responses to spontaneous cellular adhesion to surfaces are studied, generally only very few cells will be attached to the surface initially. Additionally, not all cells get in contact with the surface at the same time, implying that in the earliest stages of surface attachment there will be significant heterogeneity within the population.

The low number of attached cells and heterogeneity in the population, could be tackled with single-cell methods. In one study, it was demonstrated that incubating bacteria with microbeads to which they can adhere, followed by flow cytometric analysis of this mixture, allows for the observation of both planktonic and sessile cells at the same time (Figure 4a) [193]. Using this method, it was shown that there is a rapid decrease in respiration when *E. coli* cells adhere to polystyrene microbeads [194].

Moreover, recent improvements of RNA-seq techniques towards single cell sensitivity [195] will enable transcriptome analyses on the single attached cell level. For transcriptomic profiling of individual bacterial cells, single-cell RNA-seq is still plagued

by a number of problems, i.e. handling of individual cells, low amounts of mRNA and the absence of polyadenylated tails [196]. Microfluidics-based platforms for isolation of DNA or mRNA from single cells in micro-chambers exist, that also allow for microscopic observation prior to cell lysis (e.g. Fluidigm C1). While such devices are generally aimed at research on eukaryotic cells, they have been successfully applied also to a bacterial study [197]. If adhesion in the micro-chambers could be well-controlled, for instance by application of surface coatings, and the time point of initial surface contact of each cell would be known from microscopic observation, then the transcriptional response to adhesion could be studied in bacteria with single-cell sensitivity.

For transcriptomics of surface-attached cells, instead of RNA-seq, a recent adaptation to single-molecule fluorescence in situ hybridization (smFISH) could be employed, which greatly increased the throughput and number of detectable transcripts. This technique, called multiplexed error-robust FISH (MERFISH), allows for the detection and quantification of hundreds to thousands of individual mRNA species in single-cells [198, 199]. As MERFISH uses fixed cells that are immobilized to a cover glass, it should be possible to get transcriptomic data of individual cells at several time points after adhesion, by varying the time between surface attachment and fixation. While mainly designed for eukaryotic cells, MERFISH has been successfully applied in an *E. coli* study [200]. Also in the field of proteomics, there are experimental developments that may in the future prove promising for the analysis of single surface-attached cells, with the sensitivity now approaching the level of single mammalian cells [201, 202].

To study the adhesion strength of single cells to surfaces, atomic force microscopy (AFM) is a well-established method [203]. Briefly, in AFM the deflection of a cantilever is detected while a sharp tip connected to the cantilever interacts with a surface. From the deflection and spring constant of the cantilever, the force of interaction can then be calculated. By binding a single bacterium to the tip, its interaction with a variety of surfaces can be determined, or alternatively, the tip can be modified with different surface properties (i.e. by attaching a microbead) and sample a surface containing a confluent layer of attached cells [204–206]. Surface-induced cellular responses that can be detected by AFM are limited to those that affect the interaction strength with the surface, such as the regulation of cell appendages (Figure 4b). With AFM it has been found that *Shewanella oneidensis* produced an iron reductase that affected its adhesion to an iron mineral surface within 30 minutes of surface contact [207]. While this response was probably not due to physical contact sensing per se, but rather due to the chemical signal iron, AFM should be a suitable technique to study surface sensing. Indeed, AFM was used to show that attachment of *Staphylococcus aureus* led to higher abundance of an adhesin on the cell surface [208]. AFM has also been used to study surface-induced cell envelope deformation in single bacteria [125].

Ultimately, microscopic techniques might be the method of choice to observe changes upon surface contact. Nearly all currently used techniques for microscopic time-lapse analyses, such as immobilization of cells under an agar pad or binding of cells to a glass slide (Figure 4c; for a comparison of methods, see e.g. [213]), are in fact techniques to investigate cells while they are attached to a surface. In contrast, only

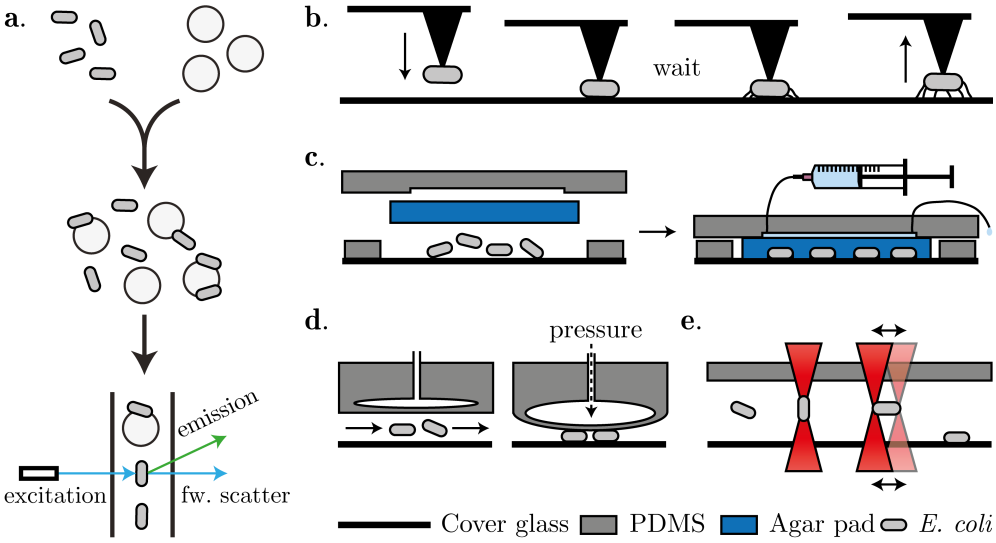

Figure 4: Experimental approaches for the study of surface sensing. (**a**) Mixing of bacteria with microbeads leads to a mixed population, in which both planktonic and sessile cells are present. Subsequent measurements with flow cytometry will therefore allow for a comparison of both phenotypes in the same experiment, such that surface-specific induction can be fully isolated from any potentially confounding effects [193]. (**b**) Atomic force microscopy could be employed to measure changes in adhesion forces. Shown here is a bacterium that is brought into contact with a surface for an extended time, which may induce production of adhesins or appendages, followed by retraction of the cantilever and measurement of the required force. (**c**) A typical microfluidic setup for continuous microscopic observation of sessile cells. Pictured here is the immobilization of bacteria under an agar pad. However, with a surface coating such as polylysine, the bacteria can also be immobilized directly to the glass without the need for an agar pad, in which case the flow of medium is directly over the cells. This kind of experimental approach is not suitable for obtaining quantitative fluorescence data for planktonic cells. (**d**) A specially designed microfluidic setup that allows for invoking temporary surface contact of bacteria in a flow channel, by reversibly collapsing the channel [209, 210]. (**e**) Side view of a microfluidic channel, showing a focussed infrared laser beam ("optical tweezers") that enables the manipulation of individual bacteria. Once trapped, the bacteria can be moved freely through a microfluidic device. Oscillating the laser trap gives control over the orientation of the bacteria, such that well-focussed images of planktonic cells can be obtained [211, 212].

very few microscopic techniques allow for tracking single planktonic cells over time (e.g. [214], where single bacteria are hydrodynamically trapped at the junction of two flow channels, or [215], which uses phase-contrast microscopy to follow the position of freely-moving bacteria over time in 3D, for which the Z-position is determined from the diffraction pattern of out-of-focus cells). Generally, microscopic methods for planktonic cells are either very low-throughput or not suitable for quantitative fluorescence measurements, as freely moving cells will rarely be located perfectly within the focal plane.

Although microscopic investigation of planktonic cells is problematic, as it is hindered by lack of focus, it is possible to enforce a temporary surface contact and immediately image the cells. Planktonic cells can be forced into contact with the surface by reversibly collapsing the flow channel in a specially designed PDMS-based microfluidic device (Figure 4d) [209, 210]. This setup should also enable the observation of the response to attachment, by collapsing the channel for an extended time.

The possibility to handle individual cells during continued microscopic observation, such that surface attachment could be induced in a controlled manner, would facilitate investigations of the initial response to surface contact. Here, optical tweezers, an instrumentation that uses focussed light to hold objects, might offer exciting possibilities, specifically the manipulation of single cells in a microfluidic setup (Figure 4e) [216, 217]. Use of an oscillating optical trap allows for imaging of rod-shaped cells with the long cell axis along the focal plane [211]. Optimization of this approach enabled stable holding of bacteria for tens of minutes without affecting their viability [212], such that planktonic cells could be investigated under the microscope and subsequently be brought into contact with the surface in a controlled and dynamic manner to observe their initial response. Apart from controlling attachment, it is conceivable that with two tweezers forces could be applied to two different points on the cell surface, which might induce tension in the membrane and therefore enable controlled generation of envelope stress. So far, however, optical tweezers have been applied to investigate the effect of spatial organisation in a multispecies biofilm [218] and to inhibit rotation of *E. coli* flagella [56], but not to investigate the cellular response to induced surface contact. Combined with high-resolution microscopy and fluorescence microscopy techniques, such as fluorescence resonance energy transfer (FRET) [219], optical tweezers might ultimately allow for the investigation of conformational changes and protein-protein interactions that eventually are responsible for surface sensing.

Conclusion

In this review, we provided an overview of the current knowledge of surface sensing mechanisms and the very initial steps of biofilm formation. The phenomena that can occur when a cell approaches a surface (i.e. physicochemical changes, attachment of surface appendages, envelope stress) are mostly well characterised. Also, the global phenotypic changes that cells undergo when switching from planktonic to sessile

lifestyle are known. However, much less is understood about how contact with a surface is perceived and how the actual biofilm initiation is regulated. Thus, the complete picture of the switch from planktonic to sessile lifestyle remains elusive.

For most of the discussed sensing systems, even those that are extensively investigated, involvement in surface sensing has not been confirmed and the precise molecular mechanisms are still unknown. For example, upon attachment to a surface, does the cell envelope slightly compress, allowing outer membrane-localized RcsF to span the periplasm to transduce the signal to the inner membrane, or does attachment-induced envelope stress prevent insertion of RcsF into the outer membrane, thereby facilitating interaction with its inner membrane receptor IgaA? How does *E. coli* sense the attachment of its flagella and pili and does the former regulate gene expression? Further, for many pathways that have a biofilm-related downstream effect, the primary stimulus of surface sensing remains unsolved.

Another key question is why *E. coli* has multiple pathways that may sense adhesion, and how these systems are interconnected with each other. The decision to switch to a sessile lifestyle has important implications for the fate of the cell. Therefore, the presence and usage of multiple sensing systems, each responding to different inputs, likely ensures that the adaptation to the sessile lifestyle is only initiated if all conditions indicating surface attachment are met. However, the advantage of sensing the same input via multiple systems is difficult to understand (e.g. osmolarity can be sensed by EnvZ/OmpR, CpxAR, and RcsCDB). Are these seemingly redundant sensors all activated under the same conditions or do they respond differently to specific surface properties? It has been proposed that the EnvZ/OmpR, CpxAR, and RcsCDB systems form a combinatorial sensor, enabling a cell to distinguish between different inputs by the ratio of induction of these pathways [24]. Even though this has not been shown in relation to surface sensing, such combinatorial sensing could be relevant in this case as well.

Until now, the multiple and simultaneous changes occurring when a cell approaches a surface, the similar inputs for multiple surface-related sensing systems and the overlap in their target genes tremendously obscures a systems-level picture of the first steps in the initiation of biofilm formation. However, novel single-cell technologies could generate valuable insights into time-dependent cellular responses after surface contact. Improved understanding of surface sensing will greatly contribute to better prevention of biofilm formation.

Acknowledgments

We would like to thank Hannah Schramke for valuable feedback on the manuscript and Bert Poolman for helpful discussions. This work was financed by the Netherlands Organisation for Scientific Research (NWO) through a VIDI grant to MH (project number 864.11.001). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The authors declare no conflict of interest.

Aim of this thesis

In this thesis, we sought to identify the surface sensing systems used by *E. coli*, characterise them and discern the hypothesised connection to persistence, while also contributing to the development of experimental tools for the manipulation of single cells under the microscope.

Outline of this thesis

In the first chapter of this thesis, a thorough overview of cell-surface interactions, potential mechanisms for surface sensing and initial steps of biofilm formation is provided, with a strong emphasis on surface sensing by *Escherichia coli*, but also covering other species when knowledge in *E. coli* is scarce. In Chapter 2, we set out to investigate the previously reported surface sensing system CpxAR, only to find that this two-component system is unresponsive to surface contact. Our findings challenge one of the milestone papers about surface sensing. In Chapter 3, we established the possibilities and limits for the use of optical tweezers in microbiological studies. Specifically, we tested several configurations of stationary and oscillating optical traps to stably hold *E. coli* cells, and found that these cells can be trapped for at least 30 min. Therefore, optical trapping could become an enabling tool for future single-cell manipulation studies, with great potential for surface sensing investigations. In the fourth chapter, eight candidate surface sensing systems of *E. coli* were tested for a response to adhesion and only the Rcs system was found to sense surface contact. We found that surface-mediated activation of the Rcs system plays a role in persistence, as it allows dormant cells to resume growth. Hence, our results implicate Rcs in biofilm initiation and persistence, both important virulence phenotypes. Finally, in Chapter 5, we investigated how *E. coli* copes with toxic concentrations of copper. Here, we found that CpxR plays a major role in the defence against copper, even though activation of this response regulator by copper happens only via cross-talk with other pathways.

References

[1] Schultz MP, Bendick JA, Holm ER, Hertel WM (2011) Economic impact of biofouling on a naval surface ship. *Biofouling* 27(1):87–98.

[2] Callow JA, Callow ME (2011) Trends in the development of environmentally friendly fouling-resistant marine coatings. *Nature communications* 2:244.

[3] Subramani A, Hoek EM (2010) Biofilm formation, cleaning, re-formation on polyamide composite membranes. *Desalination* 257:73–79.

[4] Potera C (1999) Forging a Link Between Biofilms and Disease. *Science* 283(5409):1837–1839.

[5] Mah TFC, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. *Trends in microbiology* 9(1):34–39.

[6] Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-scott HM (1995) Microbial biofilms. *Annual review of microbiology* 49:711–745.

[7] Monds RD, O'Toole GA (2009) The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends in microbiology* 17(2):73–87.

[8] Laverty G, Gorman SP, Gilmore BF (2014) Biomolecular Mechanisms of *Pseudomonas aeruginosa* and *Escherichia coli* Biofilm Formation. *Pathogens* 3(3):596–632.

[9] Prigent-Combaret C, Vidal O, Dorel C, Lejeune P (1999) Abiotic Surface Sensing and Biofilm-Dependent Regulation of Gene Expression in *Escherichia coli*. *Journal of bacteriology* 181(19):5993–6002.

[10] Kuchma SL, O'Toole GA (2000) Surface-induced and biofilm-induced changes in gene expression. *Current opinion in biotechnology* 11:429–433.

[11] Beloin C, et al. (2004) Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. *Molecular Microbiology* 51(3):659–674.

[12] Domka J, Lee J, Bansal T, Wood TK (2007) Temporal gene-expression in *Escherichia coli* K-12 biofilms. *Environmental Microbiology* 9(2):332–346.

[13] Tuson HH, Weibel DB (2013) Bacteria-surface interactions. *Soft Matter* 9:4368–4380.

[14] Goodman AE, Marshall KC (1995) Genetic responses of bacteria at surfaces in *Microbial biofilms*, eds. Costerton JW, Lappin-Scott HM. (Cambridge University Press, Cambridge), pp. 80–98.

[15] Hong Y, Brown DG (2010) Alteration of bacterial surface electrostatic potential and pH upon adhesion to a solid surface and impacts to cellular bioenergetics. *Biotechnology and Bioengineering* 105(5):965–972.

[16] Loeb GI, Neihof RA (1975) Marine Conditioning Films in *Applied Chemistry at Protein Interfaces*, ed. Baier RE. (American Chemical Society, Washington D.C.) Vol. 145, pp. 319–335.

[17] Taylor GT, et al. (1997) Influence of surface properties on accumulation of conditioning films and marine bacteria on substrata exposed to oligotrophic waters. *Biofouling: The Journal of Bioadhesion and Biofilm Research* 11(1):31–57.

[18] Garg A, Jain A, Bhosle NB (2009) Chemical characterization of a marine conditioning film. *International Biodeterioration and Biodegradation* 63(1):7–11.

[19] Marshall KC (1988) Adhesion and growth of bacteria at surfaces in oligotrophic habitats. *Canadian Journal of Microbiology* 34(4):503–506.

[20] Samuelsson MO, Kirchman DL (1990) Degradation of adsorbed protein by attached bacteria in relationship to surface hydrophobicity. *Applied and Environmental Microbiology* 56(12):3643–3648.

[21] Zobell CE (1943) The effect of solid surfaces upon bacterial activity. *Journal of bacteriology* 46(1):39–56.

[22] Kjelleberg S, Humphrey BA, Marshall KC (1982) Effect of interfaces on small, starved marine bacteria. *Applied and Environmental Microbiology* 43(5):1166–1172.

[23] Jubelin G, et al. (2005) CpxR/OmpR Interplay Regulates Curli Gene Expression in Response to Osmolarity in *Escherichia coli*. *Journal of bacteriology* 187(6):2038–2049.

[24] Clarke EJ, Voigt CA (2011) Characterization of combinatorial patterns generated by multiple two-component sensors in *E. coli* that respond to many stimuli. *Biotechnology and Bioengineering* 108(3):666–675.

[25] Danese PN, Silhavy TJ (1998) CpxP, a stress-combative member of the Cpx regulon. *Journal of Bacteriology* 180(4):831–839.

[26] Raivio TL (2014) Everything old is new again: An update on current research on the Cpx envelope stress response. *Biochimica et Biophysica Acta* 1843:1529–1541.

[27] Hall MN, Silhavy TJ (1981) The *ompB* locus and the regulation of the major outer membrane porin proteins of *Escherichia coli* K12. *Journal of molecular biology* 146:23–43.

[28] Mizuno T, Mizushima S (1990) Signal transduction and gene regulation through the phosphorylation of two regulatory components: the molecular basis for the osmotic regulation of the porin genes. *Molecular microbiology* 4(7):1077–1082.

[29] Pratt LA, Hsing W, Gibson KE, Silhavy TJ (1996) From acids to *osmZ*: multiple factors influence synthesis of the OmpF and OmpC porins in *Escherichia coli*. *Molecular microbiology* 20(5):911–917.

[30] Heyde M, Portalier R (1987) Regulation of major outer membrane porin proteins of *Escherichia coli* K12 by pH. *Molecular & general genetics* 208:511–517.

[31] Thomas AD, Booth IR (1992) The regulation of expression of the porin gene *ompC* by acid pH. *Journal of general microbiology* 138(9):1829–1835.

[32] Sato M, et al. (2000) Expression of Outer Membrane Proteins in *Escherichia coli* Growing at Acid pH. *Applied and Environmental Microbiology* 66(3):943–947.

[33] Jung K, Hamann K, Revermann A (2001) K^+ Stimulates Specifically the Autokinase Activity of Purified and Reconstituted EnvZ of *Escherichia coli*. *Journal of Biological Chemistry* 276(44):40896–40902.

[34] Sledjeski DD, Gottesman S (1996) Osmotic shock induction of capsule synthesis in *Escherichia coli* K-12. *Journal of bacteriology* 178(4):1204–1206.

[35] Francez-Charlot A, Castanié-Cornet MP, Gutierrez C, Cam K (2005) Osmotic Regulation of the *Escherichia coli bdm* (Biofilm-Dependent Modulation) Gene by the RcsCDB His-Asp Phosphorelay. *Journal of bacteriology* 187(11):3873–3877.

[36] Hagiwara D, Yamashino T, Mizuno T (2004) A Genome-Wide View of the *Escherichia coli* BasS-BasR Two-component System Implicated in Iron-responses. *Bioscience, Biotechnology and Biochemistry* 68(8):1758–1767.

[37] Lee LJ, Barrett JA, Poole RK (2005) Genome-Wide Transcriptional Response of Chemostat-Cultured *Escherichia coli* to Zinc. *Journal of bacteriology* 187(3):1124–1134.

[38] Perez JC, Groisman EA (2007) Acid pH activation of the PmrA/PmrB two-component regulatory system of *Salmonella enterica*. *Molecular Microbiology* 63(1):283–293.

[39] Raivio TL, Leblanc SKD, Price NL (2013) The *Escherichia coli* Cpx envelope stress response regulates genes of diverse function that impact antibiotic resistance and membrane integrity. *Journal of bacteriology* 195(12):2755–2767.

[40] Shin S, Park C (1995) Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. *Journal of bacteriology* 177(16):4696–4702.

[41] Francez-Charlot A, et al. (2003) RcsCDB His-Asp phosphorelay system negatively regulates the *flhDC* operon in *Escherichia coli*. *Molecular Microbiology* 49(3):823–832.

[42] Tetsch L, Jung K (2009) The regulatory interplay between membrane-integrated sensors and transport proteins in bacteria. *Molecular Microbiology* 73(6):982–991.

[43] Kotte O, Zaugg JB, Heinemann M (2010) Bacterial adaptation through distributed sensing of metabolic fluxes. *Molecular systems biology* 6:355.

[44] Kochanowski K, et al. (2013) Functioning of a metabolic flux sensor in *Escherichia coli*. *Proceedings of the National Academy of Sciences* 110(3):1130–1135.

[45] Hong Y, Brown DG (2009) Variation in bacterial ATP level and proton motive force due to adhesion to a solid surface. *Applied and environmental microbiology* 75(8):2346–2353.

[46] Pratt LA, Kolter R (1998) Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Molecular microbiology* 30(2):285–293.

[47] van Houdt R, Michiels CW (2005) Role of bacterial cell surface structures in *Escherichia coli* biofilm formation. *Research in microbiology* 156:626–633.

[48] Wood TK, González Barrios AF, Herzberg M, Lee J (2006) Motility influences biofilm architecture in *Escherichia coli*. *Applied Microbiology and Biotechnology* 72(2):361–367.

[49] Friedlander RS, Vogel N, Aizenberg J (2015) Role of Flagella in Adhesion of *Escherichia coli* to Abiotic Surfaces. *Langmuir* 31:6137–6144.

[50] Bruzaud J, et al. (2015) Flagella but not type IV pili are involved in the initial adhesion of *Pseudomonas aeruginosa* PAO1 to hydrophobic or superhydrophobic surfaces. *Colloids and Surfaces B: Biointerfaces* 131:59–66.

[51] Yoshihara A, et al. (2015) Estimation of the adhesive force distribution for the flagellar adhesion of *Escherichia coli* on a glass surface. *Colloids and Surfaces B: Biointerfaces* 131:67–72.

[52] Belas R (2014) Biofilms, flagella, and mechanosensing of surfaces by bacteria. *Trends in microbiology* 22(9):517–527.

[53] Cairns LS, Marlow VL, Bissett E, Ostrowski A, Stanley-Wall NR (2013) A mechanical signal transmitted by the flagellum controls signalling in *Bacillus subtilis*. *Molecular microbiology* 90(1):6–21.

[54] McCarter L, Hilmen M, Silverman M (1988) Flagellar dynamometer controls swarmer cell differentiation of *V. parahaemolyticus*. *Cell* 54:345–351.

[55] Gode-Potratz CJ, Kustusch RJ, Breheny PJ, Weiss DS, McCarter LL (2011) Surface sensing in *Vibrio parahaemolyticus* triggers a programme of gene expression that promotes colonization and virulence. *Molecular microbiology* 79(1):240–263.

[56] Lele PP, Hosu BG, Berg HC (2013) Dynamics of mechanosensing in the bacterial flagellar motor. *Proceedings of the National Academy of Sciences* 110(29):11839–11844.

[57] Tipping MJ, Delalez NJ, Lim R, Berry RM, Armitage JP (2013) Load-dependent assembly of the bacterial flagellar motor. *mBio* 4(4):e00551–13.

[58] Nord AL, et al. (2017) Catch bond drives stator mechanosensitivity in the bacterial flagellar motor. *Proceedings of the National Academy of Sciences* 114(49):12952–12957.

[59] Hug I, Deshpande S, Sprecher KS, Pfohl T, Jenal U (2017) Second messenger-mediated tactile response by a bacterial rotary motor. *Science* 358:531–534.

[60] Nesper J, et al. (2017) Cyclic di-GMP differentially tunes a bacterial flagellar motor through a novel class of CheY-like regulators. *eLife* 6:e28842.

[61] Lee YY, Belas R (2015) Loss of FliL Alters *Proteus mirabilis* Surface Sensing and Temperature-Dependent Swarming. *Journal of Bacteriology* 197(1):159–173.

[62] Chawla R, Ford KM, Lele PP (2017) Torque, but not FliL, regulates mechanosensitive flagellar motor-function. *Scientific Reports* 7:5565.

[63] Belas R (2013) When the swimming gets tough, the tough form a biofilm. *Molecular microbiology* 90(1):1–5.

[64] Niba ETE, Naka Y, Nagase M, Mori H, Kitakawa M (2007) A Genome-wide Approach to Identify the Genes Involved in Biofilm Formation in *E. coli*. *DNA Research* 14(6):237–246.

[65] Old DC (1972) Inhibition of the Interaction Between Fimbrial Haemagglutinins and Erythrocytes by D-Mannose and Other Carbohydrates. *Journal of general microbiology* 71(1):149–157.

[66] Ofek I, Mirelman D, Sharon N (1977) Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. *Nature* 265:623–625.

[67] Vidal O, et al. (1998) Isolation of an *Escherichia coli* K-12 Mutant Strain Able To Form Biofilms on Inert Surfaces: Involvement of a New *ompR* Allele That Increases Curli Expression. *Journal of bacteriology* 180(9):2442–2449.

[68] Wurpel DJ, Beatson SA, Totsika M, Petty NK, Schembri MA (2013) Chaperone-Usher Fimbriae of *Escherichia coli*. *PLoS ONE* 8(1):e52835.

[69] Berne C, Ducret A, Hardy GG, Brun YV (2015) Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. *Microbiology Spectrum* 3(4):MB–0018–2015.

[70] Skerker JM, Berg HC (2001) Direct observation of extension and retraction of type IV pili. *Proceedings of the National Academy of Sciences* 98(12):6901–6904.

[71] Xicohtencatl-Cortes J, et al. (2009) The type 4 pili of enterohemorrhagic *Escherichia coli* O157:H7 are multipurpose structures with pathogenic attributes. *Journal of Bacteriology* 191(1):411–421.

[72] D'Argenio DA, Calfee MW, Rainey PB, Pesci EC (2002) Autolysis and autoaggregation in *Pseudomonas aeruginosa* colony morphology mutants. *Journal of Bacteriology* 184(23):6481–6489.

[73] Ruer S, Stender S, Filloux A, de Bentzmann S (2007) Assembly of fimbrial structures in *Pseudomonas aeruginosa*: Functionality and specificity of chaperone-usher machineries. *Journal of Bacteriology* 189(9):3547–3555.

[74] Bhomkar P, Materi W, Semenchenko V, Wishart DS (2010) Transcriptional Response Of *E. coli* Upon FimH-mediated Fimbrial Adhesion. *Gene Regulation and Systems Biology* 4:1–17.

[75] Zhang JP, Normark S (1996) Induction of gene expression in *Escherichia coli* after pilus-mediated adherence. *Science* 273:1234–1236.

[76] Otto K, Norbeck J, Larsson T, Karlsson KA, Hermansson M (2001) Adhesion of type 1-fimbriated *Escherichia coli* to abiotic surfaces leads to altered composition of outer membrane proteins. *Journal of Bacteriology* 183(8):2445–2453.

[77] Persat A, Inclan YF, Engel JN, Stone HA, Gitai Z (2015) Type IV pili mechanochemically regulate virulence factors in *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences* 112(24):7563–7568.

[78] Inclan YF, et al. (2016) A scaffold protein connects type IV pili with the Chp chemosensory system to mediate activation of virulence signaling in *Pseudomonas aeruginosa*. *Molecular Microbiology* 101(4):590–605.

[79] Ellison CK, et al. (2017) Obstruction of pilus retraction stimulates bacterial surface sensing. *Science* 358:535–538.

[80] Mulvey MA, et al. (1998) Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* 282:1494–1497.

[81] Jones CH, Danese PN, Pinkner JS, Silhavy TJ, Hultgren SJ (1997) The chaperone-assisted membrane release and folding pathway is sensed by two signal transduction systems. *EMBO Journal* 16(21):6394–6406.

[82] O'Toole GA, Wong GCL (2016) Sensational biofilms: Surface sensing in bacteria. *Current Opinion in Microbiology* 30:139–146.

[83] Rodesney CA, et al. (2017) Mechanosensing of shear by *Pseudomonas aeruginosa* leads to increased levels of the cyclic-di-GMP signal initiating biofilm development. *Proceedings of the National Academy of Sciences* 114(23):5906–5911.

[84] Alsharif G, et al. (2015) Host attachment and fluid shear are integrated into a mechanical signal regulating virulence in *Escherichia coli* O157:H7. *Proceedings of the National Academy of Sciences* 112(17):5503–5508.

[85] Hermansson M (1999) The DLVO theory in microbial adhesion. *Colloids and Surfaces B: Biointerfaces* 14:105–119.

[86] Renner LD, Weibel DB (2011) Physicochemical regulation of biofilm formation. *MRS bulletin* 36(5):347–355.

[87] Berne C, Ellison CK, Ducret A, Brun YV (2018) Bacterial adhesion at the single-cell level. *Nature Reviews Microbiology* 16:616–627.

[88] Jucker BA, Harms H, Zehnder AJB (1996) Adhesion of the Positively Charged Bacterium *Stenotrophomonas* (*Xanthomonas*) *maltophilia* 70401 to glass and teflon. *Journal of Bacteriology* 178(18):5472–5479.

[89] Derjaguin B, Landau L (1941) Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. *Acta Physicochimica U.R.S.S.* 14:633–662.

[90] Verwey EJW, Overbeek JTG (1948) Theory of the stability of lyophobic colloids: the interaction of sol particles having an electric double layer. (Elsevier Inc.).

[91] Simoni SF, Harms H, Bosma TNP, Zehnder AJB (1998) Population heterogeneity affects transport of bacteria through sand columns at low flow rates. *Environmental Science and Technology* 32:2100–2105.

[92] Marshall KC, Stout R, Mitchell R (1971) Mechanism of the Initial Events in the Sorption of Marine Bacteria to Surfaces. *Journal of General Microbiology* 68:337–348.

[93] Prigent-Combaret C, et al. (2000) Developmental pathway for biofilm formation in curli-producing *Escherichia coli* strains: role of flagella, curli and colanic acid. *Environmental microbiology* 2(4):450–464.

[94] Chao Y, Zhang T (2011) Probing roles of lipopolysaccharide, type 1 fimbria, and colanic acid in the attachment of *Escherichia coli* strains on inert surfaces. *Langmuir* 27:11545–11553.

[95] Jucker BA, Harms H, Hug SJ, Zehnder A (1997) Adsorption of bacterial surface polysaccharides on mineral oxides is mediated by hydrogen bonds. *Colloids and Surfaces B: Biointerfaces* 9:331–343.

[96] DeBenedictis EP, Liu J, Keten S (2016) Adhesion mechanisms of curli subunit CsgA to abiotic surfaces. *Science Advances* 2:e1600998.

[97] Feldner J, Bredt W, Kahane I (1983) Influence of cell shape and surface charge on attachment of *Mycoplasma pneumoniae* to glass surfaces. *Journal of bacteriology* 153(1):1–5.

[98] Jones JF, et al. (2003) Oriented Adhesion of *Escherichia coli* to Polystyrene Particles. *Applied and Environmental Microbiology* 69(11):6515–6519.

[99] Busscher HJ, Norde W, Sharma PK, van der Mei HC (2010) Interfacial rearrangement in initial microbial adhesion to surfaces. *Current Opinion in Colloid & Interface Science* 15:510–517.

[100] Boks NP, Busscher HJ, van der Mei HC, Norde W (2008) Bond-Strengthening in Staphylococcal Adhesion to Hydrophilic and Hydrophobic Surfaces Using Atomic Force Microscopy. *Langmuir* 24(22):12990–12994.

[101] Meinders JM, Busscher HJ (1993) Influence of ionic strength and shear rate on the desorption of polystyrene particles from a glass collector as studied in a parallel-plate flow chamber. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 80:279–285.

[102] Li G, et al. (2012) Surface contact stimulates the just-in-time deployment of bacterial adhesins. *Molecular Microbiology* 83(1):41–51.

[103] Cooley BJ, et al. (2013) The extracellular polysaccharide Pel makes the attachment of *P. aeruginosa* to surfaces symmetric and short-ranged. *Soft Matter* 9(14):3871–3876.

[104] Hinsa SM, Espinosa-Urgel M, Ramos JL, O'Toole GA (2003) Transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas fluorescens* WCS365 requires an ABC transporter and a large secreted protein. *Molecular Microbiology* 49(4):905–918.

[105] Gibiansky ML, et al. (2010) Bacteria Use Type IV Pili to Walk Upright and Detach from Surfaces. *Science* 330:197–197.

[106] Mobley HLT, Belas R (1995) Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract. *Trends in Microbiology* 3(7):280–284.

[107] van der Woude MW, Henderson IR (2008) Regulation and function of Ag43 (*flu*). *Annual review of microbiology* 62:153–169.

[108] Busscher HJ, van der Mei HC (2012) How do bacteria know they are on a surface and regulate their response to an adhering state? *PLoS Pathogens* 8(1):e1002440.

[109] Nejadnik MR, van der Mei HC, Norde W, Busscher HJ (2008) Bacterial adhesion and growth on a polymer brush-coating. *Biomaterials* 29:4117–4121.

[110] Gu J, Valdevit A, Chou TM, Libera M (2017) Substrate effects on cell-envelope deformation during early-stage *Staphylococcus aureus* biofilm formation. *Soft Matter* 13:2967–2976.

[111] Liu Y, Strauss J, Camesano TA (2008) Adhesion forces between *Staphylococcus* epidermidis and surfaces bearing self-assembled monolayers in the presence of model proteins. *Biomaterials* 29:4374–4382.

[112] Ren Y, et al. (2018) Emergent heterogeneous micro-environments in biofilms: substratum surface heterogeneity and bacterial adhesion force-sensing. *FEMS Microbiology Reviews* 42(3):259–272.

[113] Otto K, Silhavy TJ (2002) Surface sensing and adhesion of *Escherichia coli* controlled by the Cpx-signaling pathway. *Proceedings of the National Academy of Sciences* 99(4):2287–2292.

[114] Hirano Y, Hossain MM, Takeda K, Tokuda H, Miki K (2007) Structural Studies of the Cpx Pathway Activator NlpE on the Outer Membrane of *Escherichia coli*. *Structure* 15:963–976.

[115] Ferrières L, Clarke DJ (2003) The RcsC sensor kinase is required for normal biofilm formation in *Escherichia coli* K-12 and controls the expression of a regulon in response to growth on a solid surface. *Molecular Microbiology* 50(5):1665–1682.

[116] Lejeune P (2003) Contamination of abiotic surfaces: what a colonizing bacterium sees and how to blur it. *Trends in Microbiology* 11(4):179–184.

[117] Dorel C (2010) Manipulating bacterial cell fate: key role of surface-sensing and signal transduction in *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, ed. Méndez-Vilas A. (Formatex, Badajoz) Vol. 2, pp. 791–800.

[118] Morgenstein RM, Rather PN (2012) Role of the Umo proteins and the Rcs phosphorelay in the swarming motility of the wild type and an O-antigen (*waaL*) mutant of *Proteus mirabilis*. *Journal of Bacteriology* 194(3):669–676.

[119] Harapanahalli AK, Younes JA, Allan E, van der Mei HC, Busscher HJ (2015) Chemical Signals and Mechanosensing in Bacterial Responses to Their Environment. *PLoS Pathogens* 11(8):e1005057.

[120] DiGiuseppe PA, Silhavy TJ (2003) Signal Detection and Target Gene Induction by the CpxRA Two-Component System. *Journal of Bacteriology* 185(8):2432–2440.

[121] Lima S, Guo MS, Chaba R, Gross CA, Sauer RT (2013) Dual molecular signals mediate the bacterial response to outer-membrane stress. *Science* 340(6134):837–841.

[122] Conter A, Sturny R, Gutierrez C, Cam K (2002) The RcsCB His-Asp phosphorelay system is essential to overcome chlorpromazine-induced stress in *Escherichia coli*. *Journal of Bacteriology* 184(10):2850–2853.

[123] Mcdonald C, Jovanovic G, Ces O, Buck M (2015) Membrane Stored Curvature Elastic Stress Modulates Recruitment of Maintenance Proteins PspA and Vipp1. *mBio* 6(5):e01188–15.

[124] Guilvout I, Chami M, Engel A, Pugsley AP, Bayan N (2006) Bacterial outer membrane secretin PulD assembles and inserts into the inner membrane in the absence of its pilotin. *The EMBO journal* 25(22):5241–5249.

[125] Chen Y, Harapanahalli AK, Busscher HJ, Norde W, van der Mei HC (2014) Nanoscale Cell Wall Deformation Impacts Long-Range Bacterial Adhesion Forces on Surfaces. *Applied and Environmental Microbiology* 80(2):637–643.

[126] Harapanahalli AK, Chen Y, Li J, Busscher HJ, van der Mei HC (2015) Influence of adhesion force on *icaA* and *cidA* gene expression and production of matrix components in *Staphylococcus aureus* biofilms. *Applied and Environmental Microbiology* 81(10):3369–3378.

[127] Farris C, Sanowar S, Bader MW, Pfuetzner R, Miller SI (2010) Antimicrobial peptides activate the Rcs regulon through the outer membrane lipoprotein RcsF. *Journal of bacteriology* 192(19):4894–4903.

[128] Parker CT, et al. (1992) Role of the *rfaG* and *rfaP* genes in determining the lipopolysaccharide core structure and cell surface properties of *Escherichia coli* K-12. *Journal of bacteriology* 174(8):2525–2538.

[129] Mileykovskaya E, Dowhan W (1997) The Cpx two-component signal transduction pathway is activated in *Escherichia coli* mutant strains lacking phosphatidylethanolamine. *Journal of Bacteriology* 179(4):1029–1034.

[130] Keller R, et al. (2015) The *Escherichia coli* Envelope Stress Sensor CpxA Responds to Changes in Lipid Bilayer Properties. *Biochemistry* 54(23):3670–3676.

[131] Klein G, Lindner B, Brabetz W, Brade H, Raina S (2009) *Escherichia coli* K-12 suppressor-free mutants lacking early glycosyltransferases and late acyltransferases. Minimal lipopolysaccharide structure and induction of envelope stress response. *Journal of Biological Chemistry* 284(23):15369–15389.

[132] Klein G, Kobylak N, Lindner B, Stupak A, Raina S (2014) Assembly of lipopolysaccharide in *Escherichia coli* requires the essential LapB heat shock protein. *Journal of Biological Chemistry* 289(21):14829–14853.

[133] Raivio TL, Popkin DL, Silhavy TJ (1999) The Cpx Envelope Stress Response Is Controlled by Amplification and Feedback Inhibition. *Journal of bacteriology* 181(17):5263–5272.

[134] Vogt SL, Raivio TL (2012) Just scratching the surface: an expanding view of the Cpx envelope stress response. *FEMS microbiology letters* 326(1):2–11.

[135] Zhou X, et al. (2011) Structural basis for two-component system inhibition and pilus sensing by the auxiliary CpxP protein. *Journal of Biological Chemistry* 286(11):9805–9814.

[136] Tschauner K, Hörnschemeyer P, Müller VS, Hunke S (2014) Dynamic interaction between the CpxA sensor kinase and the periplasmic accessory protein CpxP mediates signal recognition in *E. coli*. *PloS one* 9(9):e107383.

[137] Isaac DD, Pinkner JS, Hultgren SJ, Silhavy TJ (2005) The extracytoplasmic adaptor protein CpxP is degraded with substrate by DegP. *Proceedings of the National Academy of Sciences* 102(49):17775–17779.

[138] Kimkes TEP, Heinemann M (2018) Reassessing the role of the *Escherichia coli* CpxAR system in sensing surface contact. *PLoS ONE* 13(11):e0207181.

[139] Majdalani N, Heck M, Stout V, Gottesman S (2005) Role of RcsF in Signaling to the Rcs Phosphorelay Pathway in *Escherichia coli*. *Journal of bacteriology* 187(19):6770–6778.

[140] Majdalani N, Gottesman S (2006) The Rcs phosphorelay: a complex signal transduction system. *Annual review of microbiology* 59:379–405.

[141] Chen MH, et al. (2001) Characterization of the RcsC -> YojN -> RcsB Phosphorelay Signaling Pathway Involved in Capsular Synthesis in *Escherichia coli*. *Bioscience, Biotechnology and Biochemistry* 65(10):2364–2367.

[142] Konovalova A, Perlman DH, Cowles CE, Silhavy TJ (2014) Transmembrane domain of surface-exposed outer membrane lipoprotein RcsF is threaded through the lumen of β -barrel proteins. *Proceedings of the National Academy of Sciences* 111(41):E4350–E4358.

[143] Cho SH, et al. (2014) Detecting envelope stress by monitoring beta-barrel assembly. *Cell* 159(7):1652–1664.

[144] Asmar AT, et al. (2017) Communication across the bacterial cell envelope depends on the size of the periplasm. *PLoS Biology* 15(12):e2004303.

[145] Mecsas J, Rouviere PE, Erickson JW, Donohue TJ, Gross CA (1993) The activity of sigma E, an *Escherichia coli* heat-inducible sigma-factor, is modulated by expression of outer membrane proteins. *Genes & Development* 7:2618–2628.

[146] Brissette JL, Weiner L, Ripmaster TL, Model P (1991) Characterization and Sequence of the *Escherichia coli* stress-induced *psp* operon. *Journal of molecular biology* 220:35–48.

[147] Jovanovic G, Weiner L, Model P (1996) Identification, Nucleotide Sequence, and Characterization of PspF, the Transcriptional Activator of the *Escherichia coli* Stress-Induced *psp* Operon. *Journal of bacteriology* 178(7):1936–1945.

[148] Lloyd LJ, et al. (2004) Identification of a new member of the phage shock protein response in *Escherichia coli*, the phage shock protein G (PspG). *The Journal of biological chemistry* 279(53):55707–55714.

[149] Jovanovic G, Lloyd LJ, Stumpf MPH, Mayhew AJ, Buck M (2006) Induction and function of the phage shock protein extracytoplasmic stress response in *Escherichia coli*. *The Journal of biological chemistry* 281(30):21147–21161.

[150] Nagasawa S, Ishige K, Mizuno T (1993) Novel Members of the Two-Component Signal Transduction Genes in *Escherichia coli*. *Journal of Biochemistry* 114(3):350–357.

[151] Raffa RG, Raivio TL (2002) A third envelope stress signal transduction pathway in *Escherichia coli*. *Molecular microbiology* 45(6):1599–1611.

[152] Güvener ZT, Harwood CS (2007) Subcellular location characteristics of the *Pseudomonas aeruginosa* GGDEF protein, WspR, indicate that it produces cyclic-di-GMP in response to growth on surfaces. *Molecular Microbiology* 66(6):1459–1473.

[153] Francis VI, Stevenson EC, Porter SL (2017) Two-component systems required for virulence in *Pseudomonas aeruginosa*. *FEMS Microbiology Letters* 364:fnx104.

[154] O'Connor JR, Kuwada NJ, Huangyutitham V, Wiggins PA, Harwood CS (2012) Surface sensing and lateral subcellular localization of WspA, the receptor in a chemosensory-like system leading to c-di-GMP production. *Molecular Microbiology* 86(3):720–729.

[155] Hickman JW, Harwood CS (2008) Identification of FleQ from *Pseudomonas aeruginosa* as a c-di-GMP-responsive transcription factor. *Molecular Microbiology* 69(2):376–389.

[156] Bruni GN, Weekley RA, Dodd BJT, Kralj JM (2017) Voltage-gated calcium flux mediates *Escherichia coli* mechanosensation. *Proceedings of the National Academy of Sciences* 114(35):9445–9450.

[157] Leblanc SKD, Oates CW, Raivio TL (2011) Characterization of the induction and cellular role of the BaeSR two-component envelope stress response of *Escherichia coli*. *Journal of bacteriology* 193(13):3367–3375.

[158] Jonas K, et al. (2008) The RNA binding protein CsrA controls cyclic di-GMP metabolism by directly regulating the expression of GGDEF proteins. *Molecular microbiology* 70(1):236–257.

[159] Suzuki K, et al. (2002) Regulatory Circuitry of the CsrA/CsrB and BarA/UvrY Systems of *Escherichia coli*. *Journal of bacteriology* 184(18):5130–5140.

[160] Weilbacher T, et al. (2003) A novel sRNA component of the carbon storage regulatory system of *Escherichia coli*. *Molecular microbiology* 48(3):657–670.

[161] Mitra A, Palaniyandi S, Herren CD, Zhu X, Mukhopadhyay S (2013) Pleiotropic roles of *uvrY* on biofilm formation, motility and virulence in uropathogenic *Escherichia coli* CFT073. *PloS one* 8(2):e55492.

[162] Wang X, et al. (2005) CsrA post-transcriptionally represses *pgaABCD*, responsible for synthesis of a biofilm polysaccharide adhesin of *Escherichia coli*. *Molecular microbiology* 56(6):1648–1663.

[163] Ogasawara H, Shinohara S, Yamamoto K, Ishihama A (2012) Novel regulation targets of the metal-response BasS-BasR two-component system of *Escherichia coli*. *Microbiology* 158:1482–1492.

[164] Prigent-Combaret C, et al. (2001) Complex Regulatory Network Controls Initial Adhesion and Biofilm Formation in *Escherichia coli* via Regulation of the *csgD* Gene. *Journal of bacteriology* 183(24):7213–7223.

[165] Evans ML, Chapman MR (2014) Curli biogenesis: Order out of disorder. *Biochimica et Biophysica Acta* 1843:1551–1558.

[166] Gottesman S, Trisler P, Torres-Cabassa A (1985) Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12: characterization of three regulatory genes. *Journal of bacteriology* 162(3):1111–1119.

[167] Walsh NP, Alba BM, Bose B, Gross CA, Sauer RT (2003) OMP peptide signals initiate the envelope-stress response by activating DegS protease via relief of inhibition mediated by its PDZ domain. *Cell* 113:61–71.

[168] Wolfe AJ, Visick KL (2008) Get the message out: cyclic-di-GMP regulates multiple levels of flagellum-based motility. *Journal of bacteriology* 190(2):463–475.

[169] Jenal U, Reinders A, Lori C (2017) Cyclic di-GMP: second messenger extraordinaire. *Nature Reviews Microbiology* 15:271–284.

[170] Corrigan RM, Abbott JC, Burhenne H, Kaever V, Gründling A (2011) c-di-AMP is a new second messenger in *Staphylococcus aureus* with a role in controlling cell size and envelope stress. *PLoS Pathogens* 7(9):e1002217.

[171] Weber H, Pesavento C, Possling A, Tischendorf G, Hengge R (2006) Cyclic-di-GMP-mediated signalling within the sigma-S network of *Escherichia coli*. *Molecular Microbiology* 62(4):1014–1034.

[172] Lacanna E, Bigosch C, Kaever V, Boehm A, Becker A (2016) Evidence for *Escherichia coli* Diguanylate Cyclase DgcZ Interlinking Surface Sensing and Adhesion via Multiple Regulatory Routes. *Journal of Bacteriology* 198(18):2524–2535.

[173] Serra DO, Richter AM, Klauck G, Mika F, Hengge R (2013) Microanatomy at cellular resolution and spatial order of physiological differentiation in a bacterial biofilm. *MBio* 4(2):e00103–13.

[174] Prüß BM, et al. (2010) Environmental and genetic factors that contribute to *Escherichia coli* K-12 biofilm formation. *Archives of microbiology* 192:715–728.

[175] Soutourina OA, Bertin PN (2003) Regulation cascade of flagellar expression in Gram-negative bacteria. *FEMS Microbiology Reviews* 27(4):505–523.

[176] Boehm A, et al. (2010) Second Messenger-Mediated Adjustment of Bacterial Swimming Velocity. *Cell* 141(1):107–116.

[177] Prüß BM (2017) Involvement of two-component signaling on bacterial motility and biofilm development. *Journal of Bacteriology* 199(18):e00259–17.

[178] Hammar M, Arnqvist A, Bian Z, Olsén A, Normark S (1995) Expression of two *csg* operons is required for production of fibronectin- and congo red-binding curli polymers in *Escherichia coli* K-12. *Molecular microbiology* 18(4):661–670.

[179] Ogasawara H, Yamamoto K, Ishihama A (2011) Role of the biofilm master regulator CsgD in cross-regulation between biofilm formation and flagellar synthesis. *Journal of bacteriology* 193(10):2587–2597.

[180] Claret L, et al. (2007) The Flagellar Sigma Factor FliA Regulates Adhesion and Invasion of Crohn Disease-associated *Escherichia coli* via a Cyclic Dimeric GMP-dependent Pathway. *Journal of biological chemistry* 282(46):33275–33283.

[181] Mikkelsen H, Ball G, Giraud C, Filloux A (2009) Expression of *Pseudomonas aeruginosa* CupD fimbrial genes is antagonistically controlled by RcsB and the EAL-containing PvrR response regulators. *PLoS ONE* 4(6):e6018.

[182] Danese PN, Pratt LA, Kolter R (2000) Exopolysaccharide Production Is Required for Development of *Escherichia coli* K-12 Biofilm Architecture. *Journal of Bacteriology* 182(12):3593–3596.

[183] Wang X, Preston III JF, Romeo T (2004) The *pgaABCD* locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. *Journal of bacteriology* 186(9):2724–2734.

[184] Itoh Y, Wang X, Hinnebusch BJ, Preston III JF, Romeo T (2005) Depolymerization of beta-1,6-N-Acetyl-D-Glucosamine Disrupts the Integrity of Diverse Bacterial Biofilms. *Journal of bacteriology* 187(1):382–387.

[185] Chang KC, Cheng SJ, Chen YC, Huang HR, Liou J (2013) Nanoscopic analysis on pH induced morphological changes of flagella in *Escherichia coli*. *Journal of Microbiology, Immunology and Infection* 46:405–412.

[186] Dudin O, Geiselmann J, Ogasawara H, Ishihama A, Lacour S (2014) Repression of flagellar genes in exponential phase by CsgD and CpxR, two crucial modulators of *Escherichia coli* biofilm formation. *Journal of Bacteriology* 196(3):707–715.

[187] Dartigalongue C, Missiakas D, Raina S (2001) Characterization of the *Escherichia coli* sigma(E) regulon. *The Journal of biological chemistry* 276(24):20866–20875.

[188] Gerken H, Charlson ES, Cicirelli EM, Kenney LJ, Misra R (2009) MzrA: a novel modulator of the EnvZ/OmpR two-component regulon. *Molecular microbiology* 72(6):1408–1422.

[189] Nagasawa S, Tokishita S, Aiba H, Mizuno T (1992) A novel sensor-regulator protein that belongs to the homologous family of signal-transduction proteins involved in adaptive responses in *Escherichia coli*. *Molecular microbiology* 6(6):799–807.

[190] Batchelor E, Walthers D, Kenney LJ, Goulian M (2005) The *Escherichia coli* CpxA-CpxR Envelope Stress Response System Regulates Expression of the Porins OmpF and OmpC. *Journal of bacteriology* 187(16):5723–5731.

[191] Goh EB, Siino DF, Igo MM (2004) The *Escherichia coli tppB* (*ydgR*) gene represents a new class of OmpR-regulated genes. *Journal of Bacteriology* 186(12):4019–4024.

[192] Hagiwara D, et al. (2003) Genome-Wide Analyses Revealing a Signaling Network of the RcsC-YojN-RcsB Phosphorelay System in *Escherichia coli*. *Journal of bacteriology* 185(19):5735–5746.

[193] Beloin C, Houry A, Froment M, Ghigo JM, Henry N (2008) A short-time scale colloidal system reveals early bacterial adhesion dynamics. *PLoS Biology* 6(7):e167.

[194] Geng J, Beloin C, Ghigo JM, Henry N (2014) Bacteria Hold Their Breath upon Surface Contact as Shown in a Strain of *Escherichia coli*, Using Dispersed Surfaces and Flow Cytometry Analysis. *PloS one* 9(7):e102049.

[195] Wu AR, et al. (2014) Quantitative assessment of single-cell RNA-sequencing methods. *Nature methods* 11(1):41–46.

[196] Zhang Y, Gao J, Huang Y, Wang J (2018) Recent Developments in Single-Cell RNA-Seq of Microorganisms. *Biophysical Journal* 115(2):173–180.

[197] Yu FB, et al. (2017) Microfluidic-based mini-metagenomics enables discovery of novel microbial lineages from complex environmental samples. *eLife* 6:e26580.

[198] Chen KH, Boettiger AN, Moffitt JR, Wang S, Zhuang X (2015) Spatially resolved, highly multiplexed RNA profiling in single cells. *Science* 348(6233):aaa6090.

[199] Moffitt JR, et al. (2016) High-throughput single-cell gene-expression profiling with multiplexed error-robust fluorescence in situ hybridization. *Proceedings of the National Academy of Sciences* 113(39):11046–11051.

[200] Moffitt JR, Pandey S, Boettiger AN, Wang S, Zhuang X (2016) Spatial organization shapes the turnover of a bacterial transcriptome. *eLife* 5:e13065.

[201] Budnik B, Levy E, Harmange G, Slavov N (2018) Mass-spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation. *Genome Biology* 19(161):1–12.

[202] Swaminathan J, et al. (2018) Highly parallel single-molecule identification of proteins in zeptomole-scale mixtures. *Nature Biotechnology* 36:1076–1082.

[203] Camesano TA, Liu Y, Datta M (2007) Measuring bacterial adhesion at environmental interfaces with single-cell and single-molecule techniques. *Advances in Water Resources* 30:1470–1491.

[204] Razatos A, Ong YL, Sharma MM, Georgiou G (1998) Evaluating the interaction of bacteria with biomaterials using atomic force microscopy. *Journal of Biomaterials Science, Polymer Edition* 9(12):1361–1373.

[205] Razatos A, Ong YL, Sharma MM, Georgiou G (1998) Molecular determinants of bacterial adhesion monitored by atomic force microscopy. *Proceedings of the National Academy of Sciences* 95:11059–11064.

[206] Ong YL, Razatos A, Georgiou G, Sharma MM (1999) Adhesion Forces between *E. coli* Bacteria and Biomaterial Surfaces. *Langmuir* 15(8):2719–2725.

[207] Lower SK, Hochella MF, Beveridge TJ (2001) Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and α-FeOOH. *Science* 292:1360–1363.

[208] Lower SK, et al. (2010) A tactile response in *Staphylococcus aureus*. *Biophysical Journal* 99(9):2803–2811.

[209] Okumus B, et al. (2016) Mechanical slowing-down of cytoplasmic diffusion allows in vivo counting of proteins in individual cells. *Nature Communications* 7:11641.

[210] Okumus B, et al. (2018) Single-cell microscopy of suspension cultures using a microfluidics-assisted cell screening platform. *Nature Protocols* 13(1):170–194.

[211] Carmon G, Feingold M (2011) Controlled alignment of bacterial cells with oscillating optical tweezers. *Journal of Nanophotonics* 5:051803.

[212] Zhang Z, Kimkes TEP, Heinemann M (2018) Manipulating rod-shaped bacteria with optical tweezers. *Submitted*.

[213] Louise Meyer R, et al. (2010) Immobilisation of living bacteria for AFM imaging under physiological conditions. *Ultramicroscopy* 110(11):1349–1357.

[214] Johnson-Chavarria EM, Agrawal U, Tanyeri M, Kuhlman TE, Schroeder CM (2014) Automated single cell microbioreactor for monitoring intracellular dynamics and cell growth in free solution. *Lab on a chip* 14(1):1–10.

[215] Taute KM, Gude S, Tans SJ, Shimizu TS (2015) High-throughput 3D tracking of bacteria on a standard phase contrast microscope. *Nature communications* 6:8776.

[216] Ashkin A, Dziedzic JM (1989) Optical trapping and manipulation of single living cells using infra-red laser beams. *Berichte der Bunsengesellschaft für physikalische Chemie* 93:254–260.

[217] Zhang H, Liu KK (2008) Optical tweezers for single cells. *Journal of the Royal Society, Interface* 5:671–690.

[218] Hutchison JB, et al. (2014) Single-cell control of initial spatial structure in biofilm development using laser trapping. *Langmuir* 30(15):4522–4530.

[219] Kentner D, Sourjik V (2010) Use of fluorescence microscopy to study intracellular signaling in bacteria. *Annual review of microbiology* 64:373–390.