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Min oscillations in bacteria as real-time reporter of environmental challenges at the single-cell level

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Min oscillations are a fascinating mechanism used by *Escherichia coli* to find their middle. Beyond their biological role, they provide a convenient and relatively unexplored method to monitor the effect of sublethal environmental challenges on bacterial physiology in real-time and at the single-cell level. In this review, we discuss the original papers that put forward the idea of using Min oscillations as a reporting tool to monitor the effect of extracellular cationic compounds, including antibiotics. More recent work from our laboratory explores this tool to follow bacterial response to other challenges such as weak mechanical interactions with nanomaterials or photodynamic treatment. We discuss the physiological meaning of the changes in Min oscillation period, likely related to membrane potential dynamics, as well as the benefits and limitations of using oscillations as a reporter in fluorescence microscopy. Overall, Min oscillations are a useful addition to the fluorescence microscopy toolbox in order to visualize stress responses in *E. coli*, and have the potential to provide full mechanistic understanding of the events that lead to bacterial cell death in different contexts.

1. Introduction

One of the most intriguing questions in microbiology is how bacteria find their middle in order to properly divide [1]. Decades of research have established that the Min protein system in *Escherichia coli*, which oscillates from pole to pole of the cell, constitutes a spatio-temporal regulatory mechanism for positioning the division machinery [1,2]. Unravelling the interplay between the components of the Min system has led to advances in understanding self-organized pattern formation *in vivo* and *in vitro* [3–5]. In addition to cell division, Min proteins have been reported to play a role in other bacterial functions such as motility, colonization and virulence [6]. Beyond its biological role, the oscillatory behaviour of the Min system has been used in fluorescence microscopy as a tool to report on the physiological state on *E. coli*, as it has been shown to be sensitive to a range of environmental challenges [7–12]. We review herein the use of Min oscillations as a single-cell reporter for sublethal treatment with antibiotics, temperature, mechanical interaction, or light-induced generation of reactive oxygen species (ROS). We discuss the physiological meaning of changes in Min oscillation behaviour, probably related to membrane potential dynamics [13], as well as the advantages and limitations of monitoring oscillations in quantitative fluorescence microscopy.

2. Biological role of the Min system

The spatial location of the bacterial division septum is of primary importance for the correct bacterial duplication [14,15]. In *E. coli*, the Min system is responsible for the placement at the midcell of the Z-ring [16], a supramolecular structure of

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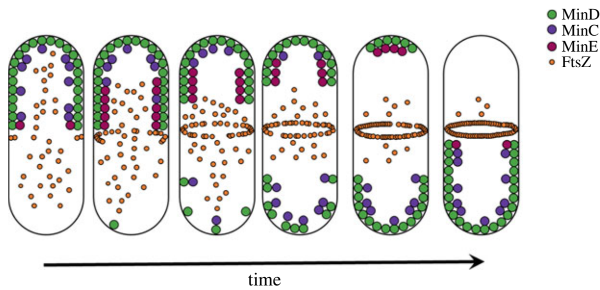

Figure 1. Interplay of the proteins that compose the Min system to drive the localization of the division machinery.

polymerized FtsZ that recruits other proteins forming the divisome, a complex that mediates cytokinesis [17,18]. The molecular interplay between the proteins in the Min system results in a tight control and precise location of the Z-ring formation [14].

The Min (or MinCDE) system consists of three proteins, MinC, MinD and MinE, with highly dynamic interactions (figure 1) [3,4,19]. MinD is a monomeric ATPase that cooperatively self-assembles on the cytoplasmic side of the inner bacterial membrane upon ATP binding, interacting with membrane phospholipids in the presence of Mg^{2+} and covering one cell pole up to the midcell [20]. MinD harbours a membrane-targeting sequence (an amphipathic helix at the C-terminus) with a weak affinity, and therefore only supports membrane binding in the presence of more than one copy [4,21,22]. At a threshold concentration of membrane-bound MinD, MinC is recruited. MinC is then displaced by MinE, as they compete for the same binding site in MinD [20,23]. Upon interaction with MinD, MinE changes conformation and also binds to the membrane [24,25]. MinE stimulates the ATPase activity of MinD by inducing a conformational change in a region involved in ATP binding [20], resulting in the monomerization and detachment of MinD from the bacterial inner membrane at one of the cell poles, and increasing the cytoplasmic concentration of MinD and MinC. Meanwhile the process starts at the opposite pole, and this reaction–diffusion mechanism of membrane attachment and detachment is responsible for the apparent pole-to-pole oscillations [26]. MinC, which is an inhibitor of FtsZ assembly, does not participate in the reaction–diffusion mechanism and can be considered an effector of the system [4]. The interactions between MinD/E proteins and the differences in their diffusion rates creates an intracellular gradient of soluble MinD and MinC that inhibits the polymerization of the Z-ring at the cell poles and enables its formation at the cell centre [27,28].

While the Min system has been extensively studied, the exact interaction mechanisms are not yet fully understood at the structural level. We point the reader to comprehensive reviews that discuss in detail the interplay between its components, and how their self-organization leads to pattern formation [1–4,15,29,30]. Moreover, other studies have suggested that the Min system participates in other cellular processes beyond cell division such as motility, colonization, virulence and RNA decay [6].

3. Min oscillations as reporter of environmental challenges

Fluorescent protein fusions with MinD and time-lapse microscopy have been used extensively to monitor its oscillation

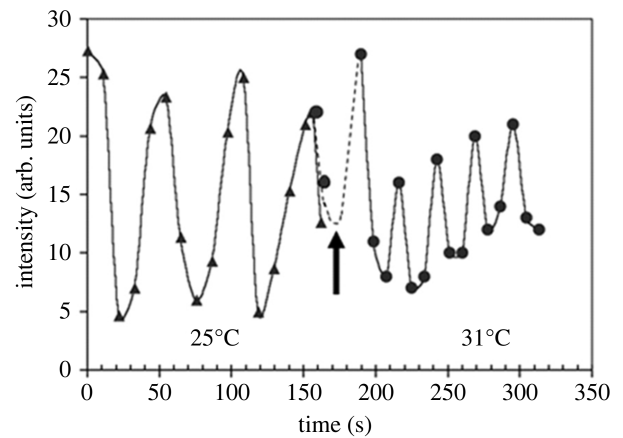

Figure 2. Temperature dependence of Min oscillations in *E. coli*. A 6°C jump rapidly changes the oscillation period from 51 s to about 26 s. Adapted with permission from Touhami *et al.* [11].

dynamics [31], although Min proteins are sensitive to fusions and it is important to verify that their function is not impaired [32]. The oscillation period depends on several parameters, and ranges between 40 and 120 s [4]. There are some inherent features that contribute to this variability, such as the type of strain, or MinD/MinE ratios [31]. However, it has been shown that external factors such as temperature or sublethal doses of antibiotic compounds also affect the oscillation period. On that basis, the period of Min oscillations was proposed as a reporter of bacterial physiological state. In this section, we describe the effect on oscillations by a range of different environmental challenges.

3.1. Temperature

The temperature dependence of the Min system oscillation period was an early report of the effect of external factors on Min dynamics [11]. The study was motivated by the observation that the growth rate in *E. coli* increases four-fold between 21°C and 37°C according to an Arrhenius law. It was found that Min system oscillations follow the same model and are four times faster at 40°C than at 20°C. The authors also reported that the response to temperature changes is very fast, and a 6°C jump decreases the oscillation period from 51 s to 26 s within a few seconds (figure 2). The molecular origin of this phenomenon was suggested to be related to temperature-dependent rates of ATP hydrolysis, followed by nucleotide exchange, which govern the interactions between proteins in the Min system or with other cellular components, and were previously shown to be the rate-limiting factors for Min oscillation [33]. A subsequent model based on experimental data provided additional evidence to support this hypothesis as an explanation for the observed temperature dependence [34].

From the practical point of view, the strong temperature dependence of this phenomenon introduces a large variability in the oscillation periods when experiments are performed at (varying) ambient temperatures, and therefore temperature control should be used to achieve best reproducibility.

3.2. Polycations and cationic antimicrobial compounds

Another early study focused on using Min oscillations as an intracellular reporter to study the bacterial response to divalent

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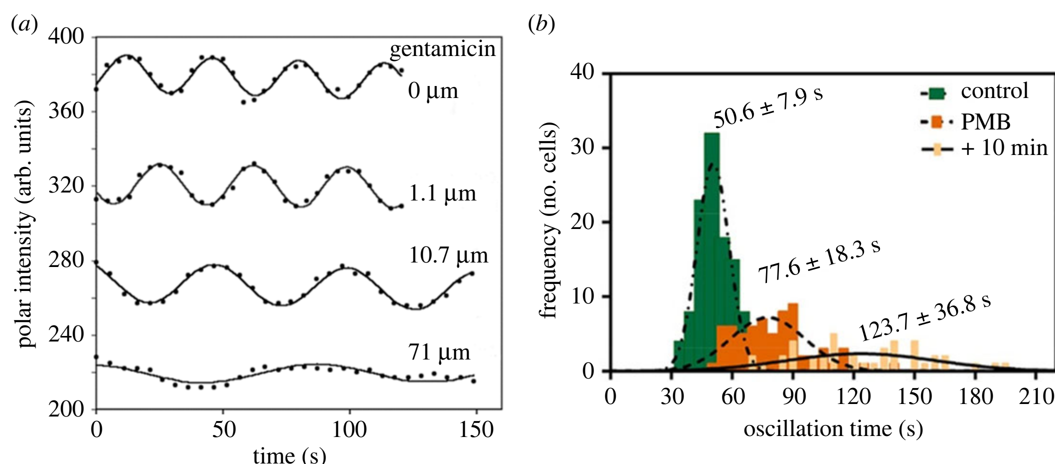

Figure 3. (*a*) Effect of the antibiotic gentamicin on the Min oscillation period of a single *E. coli* PB103 cell (from reference [10]). (*b*) Histograms showing the increase in pole-to-pole oscillation period induced by addition and 10 min incubation with polymyxin B (PMB) in *E. coli* DH10β cells (from Ortega *et al.* [8]).

cations, such as Ca^{2+} and Mg^{2+}, which are necessary for basic functions, as well as to polycationic antimicrobial compounds [10]. At a time when genetically encoded cation sensors were still in their infancy, this strategy provided an interesting possibility to follow the effect of polycation cytoplasmic penetration in real time and at the single cell level.

Increasing concentrations of Ca^{2+}, Mg^{2+}, the antimicrobial peptide protamine and the aminoglycoside gentamicin increased the average Min oscillation period in a concentration-dependent manner (figure 3*a*). Interestingly, the effect was transient for divalent cations, even with constant extracellular concentration, consistent with homeostasis of intracellular Ca^{2+}. On the other hand, the increase of the oscillation period was irreversible for the antimicrobial compounds. Moreover, moderate concentrations of Ca^{2+} or Mg^{2+} reduced the effect of the antimicrobial agents on Min oscillations, suggesting that they prevented the latter from entering the cell [10].

Slower Min oscillations were also observed upon treatment with polymyxin B (PMB), a cationic peptide with antimicrobial activity that is specific towards Gram-negative bacteria and permeabilizes both the outer and the cytoplasmic membranes [35,36]. Addition of PMB slowed down Min oscillations and increased cell-to-cell variability in the oscillation period (figure 3*b*) [7,8]. By contrast to gentamicin and protamine, the effect was independent of concentration, suggesting that ionic effects dominate over structure-specific mechanisms above a certain threshold of PMB concentration [7]. Unexpectedly, treatment with the related compound polymyxyn B nonapeptide (PMBN) which lacks a fatty acid present in PMB and only disrupts the outer membrane, speeds up oscillations [7]. This observation was unusual, since the entire Min protein system exists in the cytoplasmic space of the cell. Moreover, this is to the best of our knowledge the only example of an extrinsic factor, besides increasing temperature, which shortens the Min oscillation period.

The increase in the Min oscillation period observed for polycations was used to report the effect of poly L-lysine (PLL) [12], a cationic polymer with well-established antimicrobial action but that is commonly used to immobilize bacterial cells for microscopy experiments. The effect of different PLL preparations on bacteria was compared by following their Min oscillation period. This study highlighted that oscillations in bacteria immobilized on thick PLL coatings slowed down significantly, and therefore these substrates have a significant effect on bacterial physiology and should be avoided. On the other hand, in milder immobilization conditions pole-to-pole oscillations were only moderately slowed down and stable for hours. This constitutes a convenient method to optimize bacterial immobilization conditions for live-cell microscopy experiments [8,9].

3.3. Effect of reactive oxygen species: phototoxicity and photodynamic treatment

Photosensitized formation of ROS, such as singlet oxygen, superoxide anion, hydrogen peroxide or hydroxyl radical, is part of the most common mechanism of fluorophore-induced phototoxicity in fluorescence microscopy experiments. Early studies found that cumulative light excitation slowed down GFP-MinD oscillations by about 10 s [10], presumably due to light-induced generation of ROS by GFP [37] or buffer components [38], and therefore providing a sensitive tool to monitor phototoxicity in bacteria.

The interaction between bacteria and ROS is also important in the context of photodynamic inactivation (PDI), a strategy that has emerged as a response to the increasing threat of multidrug resistance [39]. PDI combines light, a photosensitizer and molecular oxygen in order to generate cytotoxic ROS that oxidize lipids, proteins, DNA and other biomolecules. While PDI is an increasingly popular method, questions remain about its mechanism and the inflicted functional changes [40,41]. In this context, our laboratory has recently explored the effect of sublethal photodynamic treatment of *E. coli* on MinD oscillations [8], since phototoxicity was suggested to affect the oscillation period as described above. We found that irradiation of bacteria in the presence of the photosensitizer methylene blue disrupts the MinD oscillation pattern depending on its concentration. This method is sensitive enough to distinguish the effect of the three photosensitizer concentrations tested in irradiation conditions (figure 4). While not discussed in the original paper, an interesting observation is that, in contrast to the phototoxic effects observed in cumulative irradiation of *E. coli* expressing GFP-MinD, which slow down the oscillation [10], photodynamic treatment results in an abrupt interruption, reflecting divergent physiological consequences of ROS. Similarly to the effects on Min oscillations of the antibiotics PMB and PMBN described in the

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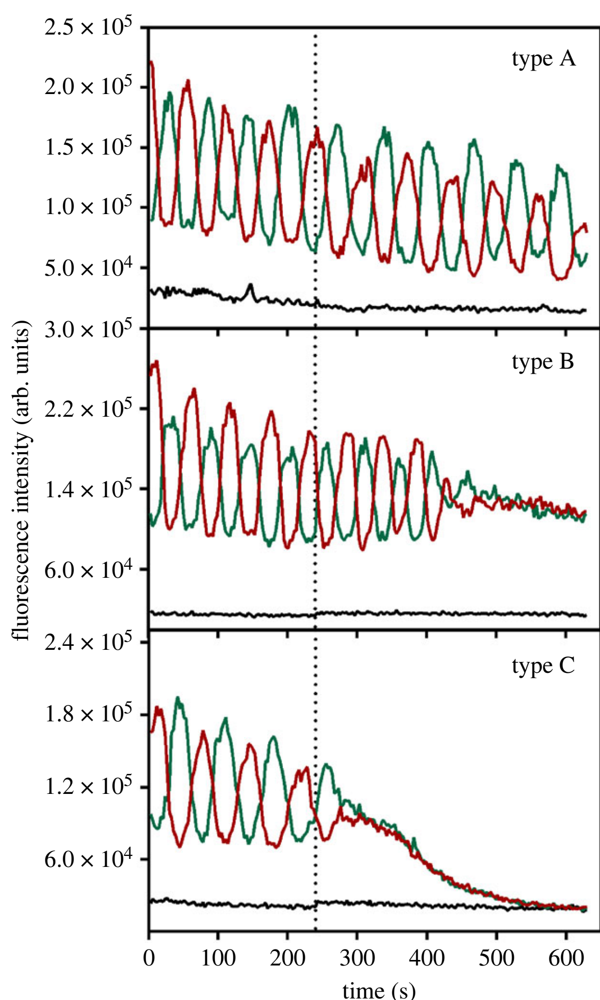

Figure 4. The MinD oscillation pattern in individual *E. coli* DH10*β* cells is sensitive to photodynamic treatment with increasing concentrations of the photosensitizer methylene blue (MB). At 0.5 µM MB, 41% and 59% of the cells show a type A and B pattern, respectively. At 1 µM MB, type B behaviour increases to 92%. At 2 µM MB all studied cells show a type C pattern, which corresponds to major cell membrane damage and leaching out of GFP-MinD. The black curves correspond to the fluorescence background values. The dotted line shows the start of red-light illumination. From Ortega *et al*. [8].

previous section, it is likely that the difference stems from the location of the photosensitizer: cytoplasmic for GFP-MinD and outside the cytoplasm for methylene blue. The sensitivity of the Min oscillation pattern to sublethal ROS challenges from both PDI and fluorophore-induced phototoxicity is therefore a useful reporting tool for mechanistic studies of the consequences of photosensitized ROS formation in bacteria.

3.4. Mechanical interaction

Min oscillations have also been used to monitor the effect of mechanical damage on the physiological response of bacteria, in the context of understanding the interaction between bacteria and ‘mechano-bactericidal’ nanomaterials [9]. Experiments were first performed by nanoindenting an *E. coli* cell with the tip of an atomic force microscope (AFM) and simultaneously monitoring its Min oscillations by fluorescence microscopy. Figure 5*a* shows the oscillatory behaviour of the Min system in an individual bacterium expressing GFP-MinD. Before puncture, the oscillation period is about 63 s. After one indentation with a low force of 5 nN (dotted line), below the rupture threshold of the cell wall of about 20 nN [9], the oscillation slows down only slightly. However, indentation with a force above the threshold (45 nN, solid line), which typically leads to cell wall damage and positive propidium iodide staining, results in the abrupt halt of the oscillation. Interestingly, the repeated application of low forces of 5 nN also results in the halt of Min oscillations (figure 5*b*), but no propidium iodide staining, which we interpreted as bacteria being compromised by a fatigue effect [9], i.e. stress responses that lead to high levels of ROS [43,44], impaired metabolic activity [45] or altered genomic or proteomic profiles [46]. Thus, these changes may eventually lead to cell death with no direct cell wall rupture. The observed fatigue effect is consistent with the suggestion that the antibacterial properties of high aspect ratio colloidal nanoparticles such as single-walled carbon nanotubes (SWCNTs) may stem from the accumulative action of many low force collisions [47]. This study also highlighted that cell wall integrity markers do not provide a complete picture of bacterial viability, and that using Min oscillation as a reporter for bacterial physiological state is a useful complement to study the events that lead to cell death.

A subsequent study has investigated how Min oscillations in *E. coli* respond to weak mechanical perturbations in a more realistic scenario of interactions between bacteria and mechano-bactericidal nanomaterials [48]. To that end, nanostructured topographies and ‘nanodarts’, exemplified by flowing SWCNTs, were used to inflict a physiological response on bacteria. In both cases, Min oscillations slowed down, and it was estimated that the contact time at which the initial stages of bacterial death occur is in the order of a few tens of minutes (figure 6).

4. What are Min oscillations reporting?

While initial reports were unclear about the specific effect(s) of external factors on the oscillation period of the Min system [10,12], it was later shown that MinD oscillations in *E. coli*, as well as its localization in *B. subtilis*, are affected by the ionophore carbonyl cyanide m-chlorophenylhydrazone [13]. This observation strongly suggests that MinD behaviour and, in turn, cell division, is strongly dependent on membrane potential. On the basis of this discovery, MinD mislocalization has been used as an indirect indicator or proxy for membrane depolarization [49–55]. There are caveats to this method, namely the fact that reduced levels of ATP may also lead to altered MinD function and localization, as MinD is an ATP-binding protein [55]. Moreover, delocalization of MinD may be also an indirect consequence of the effect of membrane potential changes on the localization of MreB, an actin homologue involved in cell wall synthesis [55]. It has been shown that MreB delocalization results in the perturbation of lipid homeostasis, which in turn affects the localization of other membrane proteins like MinD [56]. While these potential limitations need to be considered, the challenges in probing membrane potential dynamics with fluorescent dyes, especially dye exclusion by the outer membrane in Gram-negative bacteria [55,57–59], makes Min oscillations a tool well worth exploring. Membrane potential dynamics is essential in complex bacterial behaviour such as motility, antibiotic resistance, environmental sensing or

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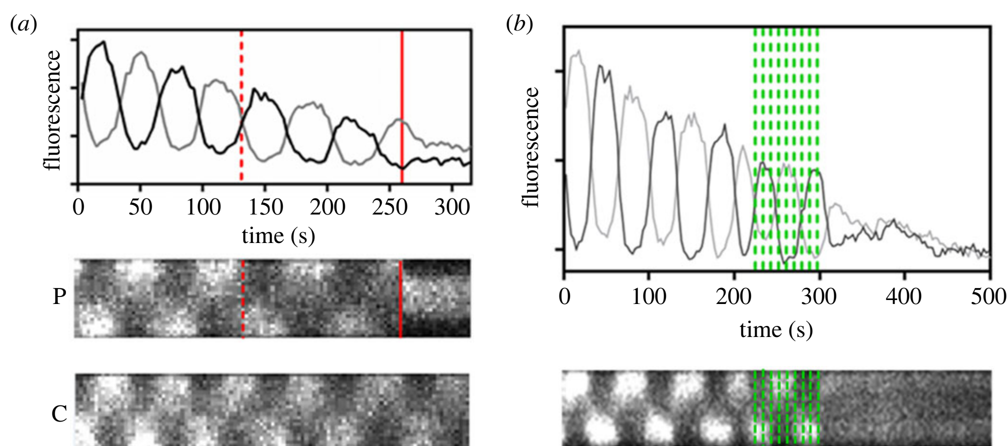

Figure 5. Simultaneous AFM nanoindentation and fluorescence imaging of Min oscillations in *E. coli* DH10β. (*a*) Pole-to-pole oscillations of GFP-MinD upon indentation of an individual bacterium with a low force (5 nN, dotted lines) and above critical damage of the cell wall (45 nN, solid lines). Black and gray curves correspond to the average fluorescence intensity of each pole. P and C show kymographs of the punctured and a control (nonindented) bacterium, respectively. The dimension of the *y*-axis in both kymographs is about 2.5 μm. The gradual decrease in fluorescence intensity in all panels is due to GFP photobleaching. (*b*) Pole-to-pole oscillations of GFP-MinD upon repeated indentation (5 nN for each green line). The dimension of the *y*-axis in the kymograph below is about 3 μm [9,42].

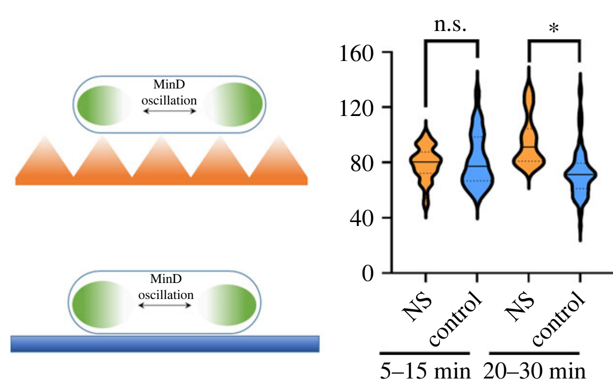

Figure 6. Impact of polymer nanotopographies on bacterial physiology. Violin plot showing the distribution of MinD oscillation data at 2 different time ranges on the nanostructured topography (NS, orange) compared to a flat surface (control, blue). Solid line represents mean value, dashed lines represent 1st and 3rd quartile (*$p < 0.05$). Adapted from Viela *et al.* [48].

electrical communication in biofilms, and is also key to understand the mechanism of action of antimicrobials [55,57,60,61].

5. Oscillations as a reporting tool in fluorescence microscopy

Min oscillations in *E. coli* are a classic example of collective dynamic behaviour of interacting components in a cell [62,63]. Spatio-temporal oscillations of this system provide the bacterium with information about its geometry, and thus showcase how large-scale properties of a cell can be assessed by processes at the molecular level [63]. Because of its relative simplicity, the Min system has been the subject of many theoretical models that recapitulate the oscillatory behaviour *in vivo* as well as in self-organized protein patterns on surfaces [62,64].

From the purely practical point of view of a live-cell fluorescence microscopy experiment, using oscillations as reporter of a biological parameter has advantages and limitations. The latter include slow data acquisition (ideally 2–3 full oscillation periods), which may be further limited by photobleaching or the long-term effect of immobilization. However, there are advantages to monitoring oscillations as opposed to changes in fluorescence intensity of a reporter. These advantages are similar to those provided by fluorescence lifetime imaging from the quantitative point of view, namely an independence of heterogeneities in the sample and imaging system, as well as in the fluorophore concentration [65]. This is particularly interesting for experiments in microfluidic systems or on non-uniform surfaces such as nanofabricated topographies mentioned above [48].

6. Conclusion and outlook

Min oscillations are a fascinating mechanism used by bacteria to find their middle. Beyond their biological role(s), they provide a relatively simple method to monitor the effect of environmental challenges on bacterial physiology in real time and at the single-cell level. In this review, we have discussed the original papers that put forward the idea of using Min oscillations as a reporting tool, at a time when the choice for fluorescent sensors was limited. More recently, our laboratory has been interested in reviving and expanding this method to understand the effect of other challenges on bacteria such as mechanical interaction or ROS. Further work is needed to establish more clearly the link between changes in the oscillation pattern and membrane potential dynamics, including quantification of the latter. However, the limitations in the use of membrane potential dyes in Gram-negative bacteria, due to their exclusion by the outer membrane, are a major motivation to further explore alternative methods to study and quantify changes in membrane potential.

In conclusion, Min oscillations are a useful addition to the toolbox of fluorescence microscopy methods to visualize stress responses in bacteria at the single-cell level and in real time [66,67]. In combination with other fluorescent reporters, it has the potential to provide full mechanistic understanding of the events that lead to bacterial cell death in different contexts.

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Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. This article has no additional data.

Authors' contributions. I.V.O.: formal analysis, investigation, writing—review and editing; F.V.: funding acquisition, supervision, writing—review and editing; C.F.: conceptualization, funding acquisition, supervision, writing—original draft.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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