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Surface waves of Min-proteins

Elisabeth Fischer-Friedrich¹, Romain Nguyen van yen^{1,2} and Karsten Kruse^{1,3}

¹ Max-Planck Institute for the Physics of Complex Systems, Nöthnitzer Strasse 38, 01187 Dresden, Germany

² Ecole normale supérieure, Département de physique, 24, rue Lhomond, 75005 Paris, France
 ³ Theoretische Physik, Universität des Saarlandes, Postfach 151150, 66041 Saarbrücken, Germany (Present address)

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Abstract

In the bacterium *Escherichia coli*, the Min-proteins show pronounced pole-to-pole oscillations. They are functional for suppressing cell division at the cell ends, leaving the center as the only possible site for division. Analyzing different models of Min-protein dynamics in a bacterial geometry, we find waves on the cytoplasmic membrane. Interestingly, the surface wave solutions of different models belong to different symmetry classes. We suggest that experiments on Min-protein surface waves *in vitro* are helpful in distinguishing between different classes of models of Min-protein dynamics.

1. Introduction

Cells are able to generate complex spatio-temporal patterns. Very often they appear in the form of waves. Examples are intracellular calcium waves [1], the beating patterns of sperm flagella [2], or Min oscillations in the bacterium Escherichia Many of these patterns can be understood by coli [3]. self-organization of cellular components [4]. First physical approaches to these patterns were based on reaction-diffusion systems, which were introduced in a biological context by Turing [5]. The above-mentioned calcium waves provide a good example for which a description in terms of a reactiondiffusion system has been successful [6]. In other cases, however, the mechanism underlying self-organization turned out to be different from reaction-diffusion. The beating of flagella, for example, is caused by spontaneous mechanical oscillations of motor proteins [7, 8]. For many other systems, the mechanism has not yet been clearly identified.

An example for the latter is given by the Min oscillations in *E. coli* [9]. There, the proteins MinC, MinD and MinE help to select the center of the rod-shaped bacterium as the location of division. By periodically shifting from one cell pole to the other, these proteins inhibit division at the poles, leaving the center as the only possible site. An increasing body of theoretical works aims at elucidating the mechanism generating the oscillations [10–19]. While there is a broad consensus that the oscillations arise through self-organization, the precise underlying mechanism remains elusive. The models that have been suggested so far can roughly be grouped into two classes. One group belongs to the class of reaction– diffusion systems, although with the peculiar feature that the total protein number is conserved. The models forming the other group are based on the idea that self-organization relies essentially on aggregation of proteins bound to the cytoplasmic membrane.

On a molecular level, the oscillations are generated by the proteins MinD and MinE [20]. MinD is an ATPase, which has a high affinity for the cytoplasmic membrane when ATP is bound, but a low affinity otherwise [21]. As to MinE, it is able to increase the rate of ATP hydrolysis by MinD when bound to MinD. This preferentially occurs when MinD is attached to the membrane, which leads to the following cycle of MinD and MinE exchange between the cytoplasmic membrane and the cytoplasm: MinD binds ATP and associates with the membrane. Subsequently, MinE binds to membranebound MinD and induces ATP hydrolysis. Consequently, MinD as well as MinE detach from the membrane. These basic steps are incorporated in all descriptions of the Min dynamics. However, this exchange dynamics together with diffusive transport of MinD and MinE does not suffice to generate oscillations [10–12]. In cooperative attachment (CA) models, which belong to the class of reaction-diffusion systems, selforganization is caused by cooperative effects between the Minproteins in the course of membrane binding and unbinding in addition to those mentioned before [10, 11, 13, 14, 16-19]. Mobility of membrane-bound proteins is dispensable,



Figure 1. Chiral solution of the dynamic equations (1)–(4). (*a*) Concentration of membrane-bound MinD, $c_d + c_{de}$. The black line on the bacterium indicates an iso-concentration curve with $c_d + c_{de} = 295 \ \mu\text{m}^{-2}$. In addition to the pole-to-pole oscillations, the distribution turns around the long axis. A solution with the opposite sense of rotation coexists with the one presented here. (*b*) Location of the maximum MinD concentration on the red circle indicated on the top (distribution at t = 0 s) for the solution presented in (*a*). Color code as in (*a*). Parameters are $L = 3.2 \ \mu\text{m}$, $R = 0.68 \ \mu\text{m}$, $D = 1300 \ \mu\text{m}^{-3}$, $\mathcal{E} = 500 \ \mu\text{m}^{-3}$, $\omega_D = 8.4 \times 10^{-5} \ \mu\text{m}^3 \text{ s}^{-1}$, $\omega_{de} = 0.04 \ \text{s}^{-1}$, $\omega_E = 3.4 \times 10^{-4} \ \mu\text{m}^3 \text{ s}^{-1}$, $k_1 = 16.6 \times 10^{-6} \ \mu\text{m}^6 \text{ s}^{-1}$, $k_2 = 26.6 \times 10^{-7} \ \mu\text{m}^8 \text{ s}^{-1}$, $\bar{k}_1 = -k_1$, $\bar{k}_2 = 10^{-4}k_2$, $D_D = D_E = 15.4 \ \mu\text{m}^2 \text{ s}^{-1}$, $D_d = 0.2 \ \mu\text{m}^2 \text{ s}^{-1}$, $c_{\text{max}} = 500 \ \mu\text{m}^{-2}$. D and \mathcal{E} are the total numbers of MinD and MinE in the cell divided by the cell volume.

although it does not necessarily destroy oscillations [19]. By contrast, in aggregation current (AC) models, the Min-proteins self-organize into an oscillatory state by attractive interactions between proteins already bound to the membrane.

So far, there are no experiments that would allow one to exclude any of these mechanisms. Instead, the introduction of both additional cooperative effects during binding as well as an aggregation current can be justified by experimental data. First of all, the existence of aggregates of MinD bound to the membrane was demonstrated in vivo and in vitro [21, 22]. In both cases helices are formed, but the corresponding pitches differ largely. Supporting the hypothesis of an aggregation current, in vitro experiments involving a non-hydrolyzable ATP-analog suggested a two-step mechanism for the formation of MinD helices [21]. This mechanism involves as a first step the binding of MinD to the membrane and as a second step the clustering of MinD. Further support for an important role of attractive interactions between MinD bound to the membrane results from a yeast two-hybrid assay [23]. This study revealed significantly stronger interactions between two MinD proteins when both are bound to the membrane as compared to situations in which at least one member is not bound. The existence of cooperative effects during MinD binding to the membrane is indicated by in vitro experiments revealing that binding of MinD to phospholipid membranes deviates from simple Langmuir isotherms [24] and can be described by a Hill function with a Hill coefficient 2 [25]. Note, however, that these data can be explained by assuming that after binding of ATP, cytoplasmic MinD dimerizes before attaching to the membrane as has been suggested in [26]. This cooperative effect is not sufficient to generate an instability of the homogenous protein distribution, though.

Analysis of the different models has until now largely focused on solutions that are rotationally symmetric with respect to the bacterial long axis. In this work, we investigate the aggregation current model introduced in [15] in a bacterial We find solutions that are not invariant with geometry. respect to rotations around the bacterial long axis. Instead, superimposed on the pole-to-pole oscillations, we find a traveling wave on the inner bacterial surface moving around the circumference. Solutions breaking rotational symmetry can also be found in the most studied cooperative attachment model [13] when solved in a bacterial geometry. They can be either standing waves or traveling waves on the membrane along the bacterial long axis and are thus qualitatively different from the waves observed in the AC model. We went on to solve the models in a geometry corresponding to a flat membrane immersed in a buffer containing MinD, MinE and ATP. Again, we find traveling waves on the membrane surface when using the AC model. By contrast, we find for the CA model that it always relaxes into a homogenous stationary state. We suggest that such an experiment can be used to distinguish between AC and CA as being the essential cause of the Min oscillations in vivo.

2. Results and discussion

2.1. Min-dynamics in a bacterial geometry

Consider the dynamics of Min-proteins in a bacterial geometry as described by the aggregation current (AC) model introduced in [15]. The bacterial geometry is approximated by a cylindrical domain with hemispherical caps at the cylinder ends, see figure 1(a). The state of the system is given in terms of the MinD and MinE distributions. The distributions of MinD and MinE in the cytoplasm are described by the fields c_D and c_E , that are defined in the interior of the domain. The distributions of MinD and MinDE complexes bound to the membrane are given by the fields c_d and c_{de} , that are defined on the two-dimensional domain boundary. The dynamic equations for the densities capture the exchange of MinD and MinE between the cytoplasm and the membrane as well as the transport of membrane-bound MinD molecules, which are subject to mutually attractive interactions. Explicitly, we have

$$\partial_t c_D = -\omega_D (c_{\max} - c_d - c_{de}) c_D \delta(\mathcal{S}) + \omega_{de} c_{de} \delta(\mathcal{S}) + D_D \Delta c_D$$
(1)

$$\partial_t c_E = \omega_{de} c_{de} \delta(\mathcal{S}) - \omega_E c_d c_E \delta(\mathcal{S}) + D_E \Delta c_E \tag{2}$$

$$\partial_t c_d = \omega_D (c_{\max} - c_d - c_{de}) c_D - \omega_E c_d c_E - \nabla \cdot \mathbf{j}_d \tag{3}$$

$$\partial_t c_{de} = -\omega_{de} c_{de} + \omega_E c_d c_E. \tag{4}$$

Here, c_{max} is the maximal density of MinD on the membrane and the parameters ω_D , ω_E and ω_{de} determine the rates of MinD- and MinE-binding to the membrane and of MinDEunbinding from the membrane. Binding is restricted to particles in the vicinity of the cytoplasmic membrane by $\delta(S)$, where S is the surface of the model bacterium. The diffusion constants of cytoplasmic MinD and MinE are D_D and D_E , respectively. The operator ∇ denotes the gradient operator on the surface S, while Δ is the Laplace operator in three dimensions.

The two-dimensional current \mathbf{j}_d , which describes transport of MinD bound to the membrane, has the form of a Cahn– Hilliard current [27]:

$$\mathbf{j}_d = -D_d \nabla c_d + c_d (c_{\max} - c_d - c_{de}) [k_1 \nabla c_d + k_2 \nabla \triangle c_d + \bar{k}_1 \nabla c_{de} + \bar{k}_2 \nabla \triangle c_{de}].$$
(5)

In this expression, \triangle is the Laplace operator on the surface S, D_d is the diffusion constant of MinD on the membrane and the coefficients k_1 and $k_2 > 0$ are parameters characterizing the interactions between MinD molecules. If $k_1 > 0$ then this interaction is attractive. Possible modifications of MinD-MinD interactions due to the binding of MinE to MinD are taken into account by the parameters \bar{k}_1 and \bar{k}_2 . For simplicity, a possible MinDE current is neglected. Furthermore, the equations do not incorporate a possible MinD dimerization before binding to the membrane [26]. Adding corresponding terms does not significantly alter the dynamics generated by the model for the parameters used. This can be understood by noting that the large diffusion constants of cytoplasmic MinD and MinE, $D_D \approx 15 \ \mu \text{m}^2 \text{ s}^{-1}$ and $D_E \approx 10 \ \mu \text{m}^2 \text{ s}^{-1}$ [28], for cells a few micrometers in size, result in an almost uniform spatial distribution of cytoplasmic MinD. To a large extent, the effect of MinD dimerization prior to binding to the membrane can thus be accounted for by an appropriate value of the parameter ω_D .

In the following, we will consider c_{max} , k_1 and k_2 to be phenomenological parameters. In principle, though, their values are related to microscopic parameters [15, 29, 30]. A simple guess for the maximal density of membrane-bound MinD is $c_{\text{max}} = 1/(\text{lateral size of a MinD molecule})$, which leads to $c_{\text{max}} = 10^4 \ \mu \text{m}^{-2}$. However, there is evidence that MinD binds only to specific lipids in the membrane reducing this value [25]. We will use a value of $c_{\text{max}} = 500 \ \mu \text{m}^{-2}$ to produce oscillations that are compatible with experimental data. Furthermore, k_1 and k_2 give a characteristic length scale $r = (k_2/k_1)^{1/2}$ which determines the scale of the pattern generated by this mechanism. It can therefore not be equal to the bare interaction range of the MinD–MinD interaction which should be on the order of a few nanometers.

Assuming that the distributions are invariant with respect to rotations around the cylinder axis, an analysis of the dynamic equations in one spatial dimension is appropriate. In that case, equations (1)–(4) generate solutions which present the essential features of the Min oscillations [15]⁴. The helical arrangement of MinD can, of course, not be captured in this case.

We numerically integrate the dynamic equations (1)–(4)in the bacterial geometry described above with total length L and radius R ⁵. For sufficiently small values of k_1 , the stationary spatially uniform distribution of Min-proteins is stable against small perturbations. If k_1 exceeds a critical value, depending on parameter values, either stationary or oscillating spatially heterogeneous solutions are found. A subset of these solutions is invariant with respect to rotations around the system's long axis. They confirm the results of the one-dimensional analysis [15]. In addition, we find solutions breaking rotational invariance when the cell radius is increased beyond the wild-type radius of *E. coli*. A typical example is presented in figure 1(a), where snapshots of the total MinDdistribution, $c_d + c_{de}$, are presented for several points in time. Pole-to-pole oscillations are clearly visible⁶. Superimposed on these are traveling waves on the membrane surface that circle around the cell's long axis. The existence of chiral waves is independent of the system length, as long as the system displays oscillations.

In figure 1(b), we show for different times the position and amplitude of the maximum of the total MinD distribution, $c_d + c_{de}$, on a path encircling the cell surface. It reveals that the rotation velocity of the traveling wave is not constant. The time needed for one full turn is in general incommensurate with the period of the pole-to-pole oscillations. Note, furthermore, that these distributions break chiral symmetry. The handedness of the solution is determined spontaneously by the initial condition. A solution mirror-symmetric to that presented here coexists for the same parameter values. The distribution of membrane-bound MinE, c_{de} , is similar to that of MinD, see figure 2.

Figure 3(*a*) presents a phase diagram of the system as a function of the dimensionless interaction strength \tilde{k} =

⁴ These equations do not generate the MinE-ring observed experimentally. By assuming that MinE preferentially binds to regions of a given MinD density, solutions representing MinE-rings are produced [12], see also [10], where a similar assumption was made. The MinE-ring is not essential for the Min oscillations, though [31]. ⁵ In the numerical integrations of the target

⁵ In the numerical integrations of the AC model, we assume that the cytoplasmic distributions are homogenous in a cross-section perpendicular to the system's long axis. This is appropriate for the diffusion constants of $D_D \approx 15 \,\mu\text{m}^2 \,\text{s}^{-1}$ and $D_E \approx 10 \,\mu\text{m}^2 \,\text{s}^{-1}$ that have been measured in *E. coli* [28].

⁶ There are also stationary solutions that break rotational symmetry. They correspond to protein blobs forming on the membrane.



Figure 2. Distribution of MinE on the membrane corresponding to the solution presented in figure 1. Here, the black line indicates an iso-concentration curve at $c_{de} = 120 \ \mu \text{m}^{-2}$.



Figure 3. Phase diagrams. (*a*) Numerically determined phase diagram for equations (1)–(4) in a bacterial geometry as a function of the dimensionless radius $\tilde{R} = R/r$, where $r = \sqrt{k_2/k_1}$, and the dimensionless interaction strength $\tilde{k} = c_{max}^2 k_1^2 / (\omega_{de} k_2)$. The distribution of Min-proteins is either stationary and homogenous (gray dots) or oscillates, while being rotationally symmetric (yellow stars) or chiral (blue triangles). The oscillations of data point (1.7, 650) are shown in figures 1 and 2. (*b*) As (*a*) but in a cylindrical geometry. Black lines indicate boundaries of linear stability of lateral and circumferential modes and are good approximations for the phase boundaries, see text for further details. In (*a*) parameters are $(\omega_D c_{max})/(\omega_d e^r) = 2.6$, $(\omega_E c_{max})/(\omega_d e^r) = 10.5$, $D_D/(r^2 \omega_{de}) = D_E/(r^2 \omega_{de}) = 2400$, $D_d/(r^2 \omega_{de}) = 30$, $\bar{k}_1 = -k_1$, $\bar{k}_2 = 10^{-4}k_2$, $(Dr)/c_{max} = 1.04$, $(\mathcal{E}r)/c_{max} = 0.4$. The protein concentrations correspond to a MinD concentration of 900 μ m⁻¹ and a MinE concentration of 350 μ m⁻¹ in a cell of 3 μ m length and 1 μ m diameter. In (*b*) we chose $\omega_D C_D/\omega_{de} = 0.79$, $\omega_E C_E/\omega_{de} = 0.79$, where C_D and C_E are the constant cytoplasmic concentrations. The other parameters are as in (*a*).

 $c_{\max}^2 k_1^2 / (\omega_{de} k_2)$ and the dimensionless cell radius $\tilde{R} = R/r$, where *r* is the characteristic length defined above. We see that the homogenous protein distribution is stable as long as the interaction strength \tilde{k} is sufficiently small. Above a critical value, the distributions are heterogeneous. For the parameter values chosen for figure 3, they oscillate. These solutions are rotationally invariant for small radii \tilde{R} , but lose this invariance beyond a critical radius. The value of the critical radius depends on \tilde{k} . In addition, these solutions rotate around the cylinder axis and spontaneously break chiral symmetry. Consequently, two solutions of different handedness and correspondingly of different senses of rotation coexist in this regime.

In order to gain more insight into the phase diagram, we analyze the dynamic equations in the simpler geometry of a cylinder without hemispherical caps. At the cylinder ends, we choose reflecting boundary conditions. Furthermore, we assume the distributions c_D and c_E to be homogenous, which is a good approximation in view of the large cytoplasmic diffusion constants measured for MinD and MinE [28]. In that

case, the dynamic equations for the cytoplasmic distributions (1) and (2) decouple from the dynamic equations for the distributions of membrane-bound proteins (1) and (4), see [15]. The cytoplasmic concentrations evolve into a stationary state and we are left with the dynamics on the membrane. In spite of these simplifying assumptions, the corresponding phase diagram is qualitatively similar to the diagram obtained before, see figure 3(b). We now perform a linear stability analysis of the homogenous state. To this end, we decompose the distributions c_d and c_{de} in terms of the eigenfunctions $f_{n,m}(z, s, t) = \exp(\lambda_{n,m}t)\exp(ins/R)\cos(m\pi z/L)$, with $n, m = 0, 1, 2, \dots$, of the linearized time-evolution operator. Here, t is time, z with $0 \leq z \leq L$ the coordinate along the system's long axis and s the coordinate along the circumference. The parameters R and L denote the radius and the length of the cylinder coat, respectively. Finally, $\lambda_{n,m}$ is the growth exponent of the eigenfunction $f_{n,m}$. Note that each $f_{n,m}$ respects the boundary conditions.

The growth exponents $\lambda_{n,m}$ depend only on the absolute value q of the wave vector \mathbf{q} , which can take the discrete



Figure 4. Growth exponents and wave vectors of equations (1)–(4) in a cylindrical geometry and linearized with respect to the stationary homogenous distribution. (*a*) The real and imaginary part of growth exponent λ as a function of the wave number *q*. The red line indicates the interval of unstable modes. Parameters are as in figure 3(*b*), with $\tilde{k} = c_{\max}^2 k_1^2 / (\omega_{de} k_2) = 500$ and L = 7.5r ($r = (k_2/k_1)^{1/2}$). (*b*) and (*c*) Grid of wave vectors corresponding to eigenmodes in the cylindrical geometry. The red annulus indicates regions of unstable wave vectors as obtained from the linear stability analysis. q_z : wavenumber of the lateral eigenmode, q_s : wavenumber of circumferential eigenmode. In (*b*) all circumferential modes are stable, in (*c*) there are unstable circumferential modes. Cylinder radii are R = 1.25r (*b*), and R = 2r (*c*).

values $\mathbf{q}(n,m) = (n/R, m\pi/L)$. A typical dependence of the growth exponent's real and imaginary parts on q is illustrated in figure 4(a). If \tilde{k} is larger than a critical value, the real part of $\lambda(q)$ is positive in some interval. Examples of wave vector lattices for two different radii \tilde{R} together with the regions of $\lambda(q) > 0$ are presented in figures 4(b)and (c). As can be seen, different modes are unstable if the system's radius is changed. In particular, for large radii, modes with $m \neq 0$ can get unstable indicating the presence of circumferential waves. Remarkably, for \tilde{k} close to its critical value, the boundary between oscillating states with and without rotational symmetry is well approximated by the stability boundary of modes with $m \neq 0$, see figure 3(b). The chiral waves thus result from coupling between the longitudinal $(n = 0, m \neq 0)$ and circumferential modes $(n \neq 0, m = 0)$.

Waves breaking rotational invariance can also be found in cooperative attachment (CA) models. We performed numerical calculations in the cell-like geometry using the dynamic equations proposed by Huang and Wingreen [32]. Similar to the model discussed above, distributions breaking rotational symmetry are found if a certain critical radius of the system is exceeded. We found two kinds of such solutions, see figure 5. In both cases, the pole-to-pole oscillations of the MinD and MinE distributions are superimposed by standing waves along the circumference. The frequencies of the circumferential and the longitudinal oscillations are locked either in a ratio of 1:1, figure 5(a), or in a ratio 1:2, figure 5(b). In the former case, this gives the impression of a traveling wave along the bacterial long axis.

This characteristic difference between the patterns of the AC and the CA model in the bacterial geometry can be understood by again considering the simplified geometry of a cylinder without hemispherical caps. As indicated in figure 3 for the AC model, close to the instability, the dynamics along the long axis and along the circumference decouple from each other, since the dynamics is well approximated by the linearized equations. A corresponding analysis can be done for the CA model, where now the cytoplasmic concentrations are not considered to be homogenous. We investigated the dynamics along the circumferential direction by studying the solutions in one spatial dimension with periodic boundary conditions. We performed extensive numerical simulations and found that while the AC model generates traveling waves, the CA model produced standing waves in this geometry. This agrees nicely with the solutions found in the full bacterial geometry and highlights a fundamental difference between the two models on the level of the collective behavior generated. We will now discuss an experimental situation that exploits this difference and might therefore help to distinguish between the two mechanisms in *E. coli*.

2.2. Waves in open geometries

As chiral waves could be hard to detect *in vivo*, we looked for an experimental situation that would allow one to observe waves of Min-proteins. Such a situation might be given by a flat cytoplasmic membrane supported by a substrate immersed in a buffer solution that acts as a reservoir for MinD, MinE and ATP. We now discuss the dynamics of Min-proteins in such a geometry for two different initial conditions corresponding to different experimental settings. The first seems more convenient from an experimental point of view, while the second allows for a better theoretical analysis.

In the first scenario, we start with a membrane to which no proteins are attached. Experimentally, this situation can be realized by initially preparing the buffer solution without ATP. In that case, MinD and therefore MinE do not bind to the membrane [33]. At time t = 0, ATP is added and the Min-proteins start to bind to the membrane, which is assumed to lie in the (x, y)-plane. We analyze this situation by solving the dynamic equations (3) and (4) in the (x, y)-plane. As initial distribution, we use $c_d(x, y) = \epsilon c_{\max} r(x, y)$ and $c_{de}(x, y) = 0$, where $\epsilon \ll 1$ and r(x, y) is a field of random numbers between 0 and 1. This distribution is used to mimic an initially sparse irregular cover of the membrane by MinD. A homogenous initial distribution results in homogenous distributions for all time when evolved by equations (3) and (4). For simplicity, we assume in the following that the solutions are invariant with respect to translations in the y-direction, leaving us with an essentially one-dimensional problem. The cytosolic concentrations are assumed to be fixed by the buffer, $c_D(x, t) = C_D$ and $c_E(x, t) = C_E$ for all x and t.



Figure 5. Solutions to the cooperative attachment model of [32] breaking rotational invariance. Shown are concentrations of membrane-bound MinD, $c_d + c_{de}$. Black lines indicate iso-concentration contours with $c_d + c_{de} = 740 \ \mu m^{-2}$ (*a*) and $c_d + c_{de} = 360 \ \mu m^{-2}$ (*b*). Superimposed onto the longitudinal pole-to-pole oscillations are standing waves along the circumference. In (*a*), where $c_{max} = 2000 \ \mu m^{-2}$, the period of the longitudinal oscillation is twice that of the circumferential oscillation, in (*b*), where $c_{max} = 1000 \ \mu m^{-2}$, they are equal. Other parameters are (same notation as in [32]) $\sigma_{de} = 0.4 \ \mathrm{s}^{-1}$, $\sigma_D^{\text{ADP} \to \text{ATP}} = 1 \ \mathrm{s}^{-1}$, $\sigma_D = 0.025 \ \mu \ \mathrm{s}^{-1}$, $\sigma_{dD} = 0.0015 \ \mu \mathrm{m}^3 \ \mathrm{s}^{-1}$, $\sigma_E = 0.3 \ \mu \mathrm{m}^3 \ \mathrm{s}^{-1}$, $D_D = D_E = 2.5 \ \mu \mathrm{m}^2 \ \mathrm{s}^{-1}$, $D = 1300 \ \mu \mathrm{m}^{-3}$ and $\mathcal{E} = 500 \ \mu \mathrm{m}^{-3}$. The cell has a length of 2.5 \ \mu m and a radius of 1 \ \mu m.



Figure 6. Time evolution of the MinD concentration on a flat membrane in an open geometry using equations (3) and (4). The initial condition was an essentially empty membrane with small fluctuations in the MinD concentration. The asymptotic state is either stationary (*a*) or oscillatory (*b*). In (*b*), the transient phase in the beginning is left out and only the asymptotic state is shown. Parameters are $\omega_{de} = 0.04 \text{ s}^{-1}$, $D_d = 0.2 \,\mu\text{m}^2 \text{ s}^{-1}$, $\bar{k}_1 = \bar{k}_2 = 0$, $c_{\text{max}} = 477 \,\mu\text{m}^{-2}$ with $\omega_D C_D = 0.014 \text{ s}^{-1}$, $\omega_E C_E = 0.024 \text{ s}^{-1}$, $k_1 = 11.2 \times 10^{-6} \,\mu\text{m}^6 \text{ s}^{-1}$, $k_2 = 18 \times 10^{-7} \,\mu\text{m}^8 \text{ s}^{-1}$ in (*a*) and $\omega_D C_D = 0.08 \text{ s}^{-1}$, $\omega_E C_E = 0.32 \text{ s}^{-1}$, $k_1 = 13.4 \times 10^{-5} \,\mu\text{m}^6 \text{ s}^{-1}$, $k_2 = 21.5 \times 10^{-6} \,\mu\text{m}^8 \text{ s}^{-1}$ in (*b*).

Asymptotically, the initial distribution evolved either into a homogenous or into a heterogeneous distribution. In the latter case, the distribution was either stationary or oscillatory. The case of a stationary solution is presented in figure 6(a), where a spacetime plot of the MinD-concentration profile can be seen. As time increases, a spatially periodic pattern of high



Figure 7. Analysis of front propagation into the unstable homogenous stationary state on a flat membrane. (*a*) and (*b*) Time evolution of the concentration of bound MinD. Initial condition: homogenous distribution with localized perturbation in the interval (0 μ m, 2 μ m) where the concentration was increased by 1%. The asymptotic state is either stationary (*a*) or oscillatory (*b*). Blue lines indicate the rightmost location with $c_d = (1 + 5 \times 10^{-4})C_d$, where $c_d = C_d$ is the concentration of membrane-bound MinD in the stationary state ($C_d = 143 \ \mu$ m⁻² (*a*) and $C_d = 37 \ \mu$ m⁻² (*b*)). (*c*) Propagation speed and pattern wavelength as a function of k_1 . Numerically obtained data (black dots for speed, gray rhombi for wavelength, error bars indicate fitting errors) are compared to results from the linear analysis (red and green lines), see section 2.2 and appendix. Deviations are due to approximations in our calculations and nonlinear effects. The parameters are chosen for (*a*) as in figure 6(*a*), and for (*b*) as in figure 6(*b*) but with $k_1 = 10.7 \times 10^{-6} \ \mu$ m⁶ s⁻¹, $k_2 = 17 \times 10^{-7} \ \mu$ m⁸ s⁻¹ and $k_1 = 12.1 \times 10^{-5} \ \mu$ m⁶ s⁻¹, $k_2 = 19.3 \times 10^{-6} \ \mu$ m⁸ s⁻¹ for (*a*) and (*b*), respectively. The boundary conditions are such that the first and third spatial derivative of the concentrations vanish at x = 0 and $x = 80 \ \mu$ m.

and low concentrations develops throughout the system. An oscillatory solution is shown in figure 6(b).

These solutions can be contrasted to solutions of the CA model of [13] in the same geometry. Using the same assumptions as above the corresponding dynamic equations simplify to

$$\partial_t c_d = (\overline{\sigma}_D + \overline{\sigma}_{dD}(c_d + c_{de}))(c_{\max} - c_d - c_{de})/c_{\max} - \overline{\sigma}_E c_d + D_d \partial_x^2 c_d$$
(6)

$$\partial_t c_{de} = -\sigma_{de} c_{de} + \overline{\sigma}_E c_d + D_{de} \partial_x^2 c_{de}, \tag{7}$$

where, as in [32], we have introduced a maximal surface density of membrane-attached molecules. Furthermore, in comparison to the original equations, we have added diffusion terms for c_d and c_{de} . Solving the dynamic equations of [13] plus surface diffusion terms in a bacterial geometry, oscillations were found for $D_d = D_{de} = 0.01 \ \mu \text{m}^2 \text{s}^{-1}$ [19]. The symbols $\overline{\sigma}_D, \overline{\sigma}_{dD}$ and $\overline{\sigma}_E$ denote the effective rates $\sigma_D C_D, \sigma_{dD} C_D$ and $\sigma_E C_E$, where C_D and C_E are the constant cytoplasmic protein concentrations. In our numerical calculations, the initial distributions asymptotically always evolved toward homogenous distributions. In these calculations, we scanned a part of the parameter space such that the parameter values did not vary from the ones given in [32] by more than 10%. Compatible with the numerical results, a linear stability analysis shows that, for the dynamic equations (6) and (7), the stationary homogenous state is stable. Similar differences between the AC and CA models can also be found in the second scenario. There, we start with a stationary homogenous distribution of MinD and MinDE complexes to which we add a localized perturbation. For the CA model of [32], we again always find that the perturbation relaxes, approaching after long times a homogenous distribution. In contrast, for the AC model studied above, the perturbation need not relax. Figure 7 shows cases where the perturbation grows and spreads. For the chosen parameter values, the propagating front leaves a striped (stationary or oscillatory) pattern in its wake. For an emerging stationary pattern, the propagation velocity v and the wavelength ℓ of the pattern as a function of the interaction strength k_1 is presented in the same figure.

We have calculated the asymptotic linear spreading velocity of the perturbation propagating into the unstable state, see [34] for a review of this method. Essentially, the time evolution of the perturbation is analyzed by solving the dynamic equations that are linearized with respect to the unstable state. The asymptotic linear spreading velocity v^* is the average velocity of the level curve $x_{\epsilon}(t) = \max\{x | c_d(x, t) = C_d + \epsilon\}$ in the limit of large times⁷, where C_d is the concentration of the stationary state. The value of ϵ has to be chosen sufficiently small such that the propagating front is described well by the linearized

⁷ Note that the velocity is the same if x_{ϵ} is defined with c_{de} instead of c_d .

dynamic equations. Details of our calculation are presented in the appendix. We find that the actual spreading velocity is well approximated by the linear spreading velocity v^* , see figure 7(*d*). Furthermore, our linear analysis gives a good approximation for the wavelength ℓ of the periodic pattern that is formed in the wake of the perturbation front, see figure 7(*d*). Generally, we find that the spreading velocity increases with the values of $\tilde{k} = c_{\max}^2 k_1^2 / (\omega_{de} k_2)$ and ω_{de} . In contrast, the velocity decreases with an increasing diffusion constant D_d . Furthermore, the wavelength ℓ increases with \tilde{k} and decreases with the diffusion constant D_d .

3. Conclusion and outlook

In this work we have analyzed an aggregation current (AC) model for the dynamics of the Min-proteins in E. coli. We found that waves on the cytoplasmic membrane are a genuine feature of the AC mechanism. Similar patterns have been found in surface chemical reactions in the presence of attractive interactions between the adsorbed particles [35, 36]. In our analysis of the AC model in a bacterial geometry, we found chiral surface waves that propagate around the bacterial circumference and that are superimposed on the pole-topole oscillations characteristic for the Min-proteins in E. coli. These solutions have a definite handedness and coexist with a solution of reversed sense of rotation. Solutions breaking rotational symmetry can also be found in the cooperative attachment (CA) model suggested by Huang et al [13, 32]. In contrast to the chiral waves, all such solutions we observed result from a coupling between a standing longitudinal and a standing circumferential wave and are consequently nonchiral. Correspondingly, an analysis of the two models in one dimension with periodic boundary conditions revealed traveling waves for the AC model and standing waves for the CA model. The different wave patterns reflect thus a characteristic difference between the two model classes. As in both models analyzed here only generic terms appear, we expect this result to be valid for a large number of CA and AC models and to be independent of many of the biochemical details making up the dynamics of MinD and MinE.

Are distributions of Min-proteins breaking rotational symmetry observed experimentally? The helical arrangements of membrane-bound MinD and MinE reported in [22] do break this symmetry. Neither of the solutions presented here, however, resembles this pattern even though the chiral waves share the same symmetry with the helices. We therefore speculate that the effects presented here should rather be visible in bacteria with an enlarged radius compared to wild-type *E. coli*. Observation of the Min dynamics in such cells might therefore provide valuable hints on the oscillation mechanism. Our calculations show that circumferential modulations of the surface densities should become visible for bacterial radii which are of the same order as the characteristic length of the longitudinal pattern.

A possibly easier way to study Min-protein surface waves is offered by *in vitro* experiments where a flat membrane supported by a solid substrate is exposed to a buffer containing MinD, MinE and ATP. Analyzing such a situation, we found the formation of interesting patterns and surface waves of bound proteins in the AC model. In contrast, the CA model evolves the system into a stationary homogenous state. By calculating the linear spreading velocity of a perturbation front moving into an unstable state, we were able to characterize the dependence of the wave velocity on the system parameters. This should help to design experiments aiming for detecting surface waves.

The analysis performed here was based on a deterministic description of the Min dynamics. For the μ M concentrations of MinD and MinE present in E. coli, fluctuations are likely to be non-negligible. It will therefore be important to extend our analysis to a stochastic description of the Min-proteins in the bacterial as well as in the open geometry. A stochastic analysis for surface reactions showed that the phenomenon of surface waves persists in the presence of fluctuations [37]. In a stochastic model based on similar assumptions as that of [13], which assumed rotational symmetry of the solution, traveling waves are observed for some interspersed time intervals [17]. Their origin is, however, unclear and might lie in stochastic transitions between different standing wave patterns. Studies of a stochastic variant of the CA model in a bacterial geometry have not reported on solutions breaking rotational symmetry [16, 19]. In these studies the dimensions of the system were chosen similar to those of a real bacterium, further suggesting that solutions breaking rotational symmetry can only be observed in cells with a larger than normal radius.

The surface wave patterns of Min-proteins discussed in this work seem to offer a good handle to experimentally study the validity of the models for Min-protein dynamics. If observed they would in particular strongly support the mechanism of self-organization of the Min oscillations. Furthermore, an analysis of waves in the presence of fluctuations together with carefully performed experiments in the *in vitro* setting suggested above should provide strong hints on the relative importance of cooperative effects during binding and of aggregation currents of membrane-bound proteins. In our opinion, these experiments are of particular interest as they do not involve a biochemical analysis of the molecular Min-protein interactions, but are based on their collective behavior.

Appendix. Calculation of the linear spreading velocity

In this appendix, we calculate the linear spreading velocity of a localized perturbation into an unstable stationary homogenous state of the dynamic equations (3) and (4) in the situation discussed in section 2.2. The distributions c_D and c_E are assumed to be homogenous, their values are free parameters. In order to simplify our calculations, we set $\bar{k}_1 = \bar{k}_2 = 0$ and restrict ourselves to parameter values such that $\bar{\omega}_D \bar{\omega}_E < \omega_{de}^2$, where $\bar{\omega}_D = \omega_D C_D$ and $\bar{\omega}_E = \omega_E C_E$. Consequently, for each wave vector q at most one of the eigenmodes grows in time and the corresponding growth exponent is real. In an open geometry, the asymptotic solutions are stationary if the rates satisfy the condition above.

The linear spreading velocity is obtained from the solution of the dynamic equations that are obtained by linearizing (3) and (4) with respect to a stationary homogenous state. In general, the solution $\mathbf{c} = (c_d, c_{de})$ to these equations can be written as

$$\mathbf{c}(x,t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \mathrm{d}q \, \mathrm{e}^{\Lambda(q)t + \mathrm{i}qx} \bar{\mathbf{c}}(q), \tag{A.1}$$

where $\mathbf{\tilde{c}}(q) = \int_{-\infty}^{\infty} dx \, \mathbf{c}(\mathbf{x}) \exp(-iqx)$ denotes the Fourier transform of $\mathbf{c}(x, t = 0)$. The matrix $\Lambda(q)$ is the time-evolution operator of the linearized dynamic equations in Fourier representation

$$\Lambda(q) = \begin{pmatrix} -\overline{\omega}_D - \overline{\omega}_E + (\tilde{k} - D_d)q^2 + \tilde{k}q^4 & -\overline{\omega}_D \\ \overline{\omega}_E & -\omega_{de} \end{pmatrix}, \quad (A.2)$$

where \tilde{k} is the dimensionless control parameter $\tilde{k} = c_{\max}^2 k_1^2 / (\omega_{de} k_2)$. Let $\mathbf{e}_1(q)$ denote the normalized eigenmode of $\Lambda(q)$ associated with the eigenvalue λ_1 , that has the larger real part of the two eigenvalues. Then, we can write

$$\mathbf{c}(x,t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \mathrm{d}q \, \mathrm{e}^{\lambda_1(q)t + \mathrm{i}qx} (\bar{\mathbf{c}}(q) \cdot \mathbf{e}_1(q)) \mathbf{e}_1(q). \quad (A.3)$$

Now consider a uniformly translated reference frame with coordinates $\xi = x - v^* t$. Here, v^* is the average spreading velocity of the perturbation in the limit of large times. Adapting coordinates to this frame, equation (A.3) becomes

$$\mathbf{c}(x,t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \mathrm{d}q \, \mathrm{e}^{\mathrm{i}q\xi} \, \mathrm{e}^{(\lambda_1(q) + \mathrm{i}v^*q)t} (\bar{\mathbf{c}}(q) \cdot \mathbf{e}_1(q)) \mathbf{e}_1(q).$$
(A.4)

As we have chosen the frame such that we ride on the front, we must have for consistency $v^* = \frac{\lambda_{1,r}}{q_i}$, where subscripts *r* and *i* denote the real and imaginary parts, respectively, i.e. $q = q_r + iq_i$ and $\lambda_1 = \lambda_{1,r} + i\lambda_{1,i}$. Otherwise the absolute value of $e^{(\lambda_1(q)+iv^*q)t}$ would decay or grow exponentially. v^* is the velocity with which the front spreads in the linearized case. As for the chosen parameter regime the emerging pattern in the wake of the front is stationary with wavelength ℓ , the concentration $\mathbf{c}(\xi, t)$ in the moving frame oscillates with frequency $\omega = v^*/\ell$. From (A.4), we can read off ω as $(\lambda_{1,i}(q^*) + v^*q_r^*)$. This determines ℓ provided we know v^* .

For large times, the integral (A.4) can be approximated by focusing on the factor $E(t) = e^{(\lambda_1(q)+iv^*q)t}$. Assuming a holomorphic integrand, the integration contour is first moved into the complex plane such that integration is along a contour of constant phase of E(t). Then a saddle-point approximation is performed [38]. At a saddle point on a contour of constant phase, the gradients of the real and the imaginary part of E(t)have to vanish. Therefore, the exponent $(\lambda_1(q) + iv^*q)t$ has to obey $\partial(\lambda_{1,r} - v^*q_i)/\partial q_r|_{q^*} = \partial(\lambda_{1,r} - v^*q_i)/\partial q_i|_{q^*} = 0$. Consequently,

$$\partial \lambda_{1,r} / \partial q_i |_{q^*} = \lambda_{1,r} / q_i |_{q^*}$$
(A.5)

$$\partial \lambda_{1,r} / \partial q_r |_{q^*} = 0, \tag{A.6}$$

where

$$\lambda_1(q) = p(q)/2 + \sqrt{p(q)^2/4} - \overline{\omega}_D \overline{\omega}_E \quad \text{with} \\ p(q) = \omega_{de} - \overline{\omega}_D - \overline{\omega}_E + (\tilde{k} - D_d)q^2 - \tilde{k}q^4.$$

We have solved equations (A.5) and (A.6) numerically. To this end we neglected $\overline{\omega}_D \overline{\omega}_E$ in the expression for $\lambda_1(q)$. The good agreement between the values we obtain for the asymptotic linear spreading velocity and the results from our numerical solutions of the dynamic equations (3) and (4) justifies this approximation, see figure 7(*d*).

Glossary

Aggregation current (AC). Aggregating transport of Min-proteins in the membrane-bound state due to particle interactions.

Cooperative attachment (CA). Attachment of cytosolic Min-proteins under the influence of membrane-bound Min-proteins due to particle interactions.

Cytosol. Internal fluid of a cell. It is surrounded by the cytoplasmic membrane, a lipid bilayer.

Chiral pattern. A pattern is called chiral if it differs from its mirror image.

Linear stability analysis. In a linear stability analysis, the stability of a state against small perturbations is assessed by linearizing the dynamic equations with respect to the state.

Linear spreading velocity. The velocity at which a localized perturbation spreads into a stationary, unstable state of a system according to the linearized dynamics.

Standing and traveling wave. A traveling wave is a concentration profile that moves with a certain velocity along a direction. A standing wave is a concentration profile whose amplitude is periodically modulated in time.

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