
Mechanochemical feedback loop drives persistent motion of liposomes

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Supplementary Notes: Mechanochemical feedback loop drives persistent motion of liposomes

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Model for the Min reaction–diffusion dynamics

The dynamics of the Min protein concentration fields were modeled in a reaction–diffusion approach, as has been done previously to reproduce the Min patterns observed *in vitro*¹. To allow for stationary Min patterns, we extend the canonical Min skeleton model² by persistent binding of MinE proteins to the membrane. In the following, we provide the equations and parameters used to model the patterning dynamics.

We distinguish between three different cytosolic protein species (MinE (u_E), MinD-ADP (u_{DD}), and MinD-ATP (u_{DT})). Furthermore, we consider three membrane-bound protein species (MinE (u_e), MinD (u_d), and MinDE complexes (u_{de})). Bulk components are denoted by upper-case indices, whereas membrane components are characterized by lower-case indices. In the bulk, MinD-ADP is converted into MinD-ATP at a rate λ , corresponding to the nucleotide exchange. All other reactions involve protein binding to and on the membrane: MinD-ATP attaches from the bulk to the membrane at a rate k_D and can additionally be recruited to the membrane by membrane-bound MinD at a rate k_{dD} . For MinE, we assume that the dominant binding process is due to recruitment via MinD at a rate k_{dE} . When membrane-bound MinD captures MinE from the bulk, they form a MinDE complex on the membrane. MinE hydrolyses MinD, which breaks up the MinDE complex, leading to MinD-ADP detachment. This process occurs at a rate k_e . The experimentally observed stationary protein patterns are not captured by the widely-used Min skeleton model², and instead we extend this model by persistent MinE binding¹. Persistent MinE binding means that after hydrolysis, MinE remains on the membrane instead of unbinding together with MinD. The membrane-bound MinE proteins then dissociate from the membrane at a rate k_e , but can also bind to membrane-bound MinD proteins to form MinDE complexes at a rate k_{ed} . These reaction-diffusion dynamics are represented by a set of partial differential equations (PDEs):

$$\partial_t u_{DD} = D_c \nabla_c^2 u_{DD} - \lambda_{DD} \quad (\text{S1a})$$

$$\partial_t u_{DT} = D_c \nabla_c^2 u_{DT} + \lambda_{DD} \quad (\text{S1b})$$

$$\partial_t u_E = D_c \nabla_c^2 u_E \quad (\text{S1c})$$

$$\partial_t u_d = D_{m,d} \nabla_m^2 u_d + f_d(u_d, u_e, \tilde{u}_{DT}, \tilde{u}_E) \quad (\text{S1d})$$

$$\partial_t u_{de} = D_{m,de} \nabla_m^2 u_{de} + f_{de}(u_d, u_{de}, u_e, \tilde{u}_E) \quad (\text{S1e})$$

$$\partial_t u_e = D_{m,e} \nabla_m^2 u_e + f_e(u_d, u_{de}, u_e) \quad (\text{S1f})$$

Here, the reaction terms are

$$f_d(u_d, u_e, \tilde{u}_{DT}, \tilde{u}_E) = (k_D + k_{dD} u_d) \tilde{u}_{DT} - u_d k_{dE} \tilde{u}_E - k_{ed} u_d u_e \quad (\text{S2a})$$

$$f_{de}(u_d, u_{de}, u_e, \tilde{u}_E) = u_d k_{dE} \tilde{u}_E - k_{de} u_{de} + k_{ed} u_d u_e \quad (\text{S2b})$$

$$f_e(u_d, u_{de}, u_e) = k_{de} u_{de} - k_{ed} u_d u_e - k_e u_e. \quad (\text{S2c})$$

$\tilde{u}_{DT}$ and $\tilde{u}_E$ correspond to the respective MinD and MinE bulk concentrations right on the membrane, i.e. $\tilde{u}_i = u_i|_m$.

We use reactive boundary conditions to couple the bulk protein dynamics to the membrane protein dynamics via a net diffusive flux $\nabla_n u_i|_m$, where ∇_n represents a spatial derivative normal to the reactive boundary:

$$D_c \nabla_n u_{DD}|_m = k_{de} u_{de} \quad (\text{S3a})$$

$$D_c \nabla_n u_{DT}|_m = -(k_D + k_{dD} u_d) \tilde{u}_{DT} \quad (\text{S3b})$$

$$D_c \nabla_n u_E|_m = -k_{dE} u_d \tilde{u}_E + k_e u_e \quad (\text{S3c})$$

To account for the geometry sensitivity of the Min patterns in the presence of liposomes, we model the liposomes as an excluded volume $V(x, y; t)$. The volume exclusion leads to additional fluxes in the bulk protein concentrations, where the strength of the exclusion is parametrized by ξ :

$$\partial_t u_{DD} = D_c \nabla_c^2 u_{DD} - \nabla_c [(\nabla_c V(x, y; t)) \cdot u_{DD} \cdot \xi] - \lambda_{DD} \quad (\text{S4a})$$

$$\partial_t u_{DT} = D_c \nabla_c^2 u_{DT} - \nabla_c [(\nabla_c V(x, y; t)) \cdot u_{DT} \cdot \xi] + \lambda_{DD} \quad (\text{S4b})$$

$$\partial_t u_E = D_c \nabla_c^2 u_E - \nabla_c [(\nabla_c V(x, y; t)) \cdot u_E \cdot \xi] \quad (\text{S4c})$$

We assume a generic coupling of the dynamic movement of the liposome (characterized by its center position $x_V(t)$) to the MinE pattern: motivated by the experimental observations, we assume that the velocity of the liposome is dictated by the MinE asymmetry, which we calculate as

$$\partial_t x_V(t) = - \int ds (s - x_V(t)) \cdot u_e(s) \cdot v \cdot \mathcal{S}(x, t). \quad (\text{S5a})$$

Here, $\mathcal{S}(x, t)$ is a kernel that limits the probing of the asymmetry to the vicinity of the liposome with radius R :

$$\mathcal{S}(x, t) = \begin{cases} 1 & \text{for } x_V(t) - R \leq x \leq x_V(t) + R \\ 0 & \text{else.} \end{cases} \quad (\text{S5b})$$

The factor v is a phenomenological proportionality constant representing the coupling strength between the MinE concentration gradient and the liposome speed.

The ensuing differential equations were solved in finite element simulations using COMSOL 5.6. As initial conditions, we explicitly broke the symmetry of the system by applying an exponentially decaying movement to the excluded volume at the start of the simulation (within the initial ~ 2000

seconds). This manual symmetry breaking was designed to move the excluded volume by exactly 1 μm , with any further movements being the outcome of coupling to the protein dynamics.

The system was screened for parameter regimes that show quasi-stationary Min patterns using a linear stability analysis¹, resulting in the following parameters that were used throughout all simulations.

Parameter	Symbol	Value
Box width	L	90 [μm] (1)
Box height	he	30 [μm]
Cytosolic diffusion	D_c	60 [$\mu\text{m}^2/\text{s}$] ^{1,3}
MinD membrane diffusion	D_d	0.013 [$\mu\text{m}^2/\text{s}$] ^{1,3}
MinDE membrane diffusion	D_{de}	0.013 [$\mu\text{m}^2/\text{s}$] ^{1,3}
MinE membrane diffusion	D_e	0.005 [$\mu\text{m}^2/\text{s}$] (2)
MinD attachment rate	k_D	0.065 [$\mu\text{m}/\text{s}$] ¹
MinD recruitment rate	k_{dD}	0.02 [$\mu\text{m}^3/\text{s}$] ¹
MinE recruitment rate	k_{dE}	0.126 [$\mu\text{m}^3/\text{s}$] ¹
MinDE desintegration rate	k_{de}	0.34 [1/s] ¹
MinDE complex formation rate	k_{ed}	0.01 [$\mu\text{m}^2/\text{s}$] (3)
MinE detachment rate	k_e	0.0001 [1/s] (4)
MinD nucleotide exchange rate	λ	6 [1/s] ^{2,4}
MinD total concentration	n_D	648 [1/ μm^3] ¹ (5)
MinE total concentration	n_E	100 [1/ μm^3] ¹ (5)
Vesicle speed proportionality factor	v	0.005 [1/s]
Excluded volume factor	ξ	300

Supplementary Table S 1: List of parameters used in finite element simulations.

- (1). The box width was varied between 30 μm and 90 μm , and small boxes were preferred for the sake of computational speed where feasible. Larger boxes were used when boundary effects significantly affected the protein patterns, in particular for the transient mesh patterns.
- (2). The MinE diffusion constant was varied in a range of $0.0005 \mu\text{m}^2/\text{s} < D_e < 0.013 \mu\text{m}^2/\text{s}$. Stationary labyrinth patterns formed ad hoc in the range of $0.001 \mu\text{m}^2/\text{s} < D_e < 0.013 \mu\text{m}^2/\text{s}$. At smaller diffusion constants, a transient mesh pattern preceded the stationary labyrinth pattern. Since a large spread in the diffusion constants makes systematic simulations inefficient, we chose D_e to be significantly larger than the experimentally estimated value while still obtaining stationary patterns in simulations.
- (3). This MinDE complex formation rate was chosen to ensure the two processes of MinE binding, i.e. from the cytosol (k_{dE}) and from the membrane (k_{ed}), can both contribute to the MinDE complex formation.
- (4). The MinE detachment rate was chosen to be much smaller than the time scale of pattern formation (ca. 1000s), which ensures that the MinE recycling does not interfere with the pattern formation. Otherwise the traditional Min skeleton model is recovered.
- (5). Based on previous work¹, we swept over the MinD and MinE total protein concentrations to identify a parameter region for the quasi-stationary pattern formation.

Three types of patterns (mesh, labyrinth, and travelling waves) have been observed in experiments⁵. In simulations, when the system approaches its steady state, labyrinth patterns are always obtained for the tested parameter regime. Mesh patterns, in turn, can be observed transiently during pattern

establishment (**Supplementary Fig. 2**). It is likely that the persistent binding model does not yet capture all relevant Min interactions. A candidate for additional extensions is the MinE switch, which allows a wider range of patterns in simulation¹.

Dynamic coupling of the protein patterns to the liposome shape

To demonstrate the mechanochemical feedback between the Min protein patterns and the liposomes, we modeled the liposome as an excluded volume with fixed shape, where the position of the liposome was coupled to MinE gradients (**Fig. 4d**). This is the most generic implementation of a mechanochemical feedback and makes minimal assumptions about the molecular protein-protein and protein-membrane interactions. Here, we demonstrate that making an explicit choice for the protein-membrane interaction yields results that are consistent with the generic implementation.

We implement a protein-dependent liposome adhesion in 2D as a test case for the generic mechanochemical feedback. To implement adhesion, we model the liposome as a phase field, where we adopt a formalism that has been widely used to model dynamics of adhering cells⁵⁻⁸. The dynamics of the liposome phase field u are determined by relaxational dynamics of the following free energy⁶:

$$\mathcal{F} = \int d\mathbf{x} \gamma \left(\frac{1}{2} |\nabla u|^2 + G(u) \right) + \frac{\kappa}{2} (\nabla^2 u - G'(u))^2 + \frac{\eta}{2} u \cdot u_0 - \sigma(x) |\nabla u|^2 |\nabla u_0|^2.$$

Here, $G(u) = 18u^2(1-u)^2$ constrains the phase field to 0 or 1, penalizing any deviations from these two values. The first two terms represent liposome surface tension and bending rigidity. The supported lipid bilayer (SLB) is represented by an auxiliary (but non-dynamic) phase field

$$u_0(\mathbf{x}) = \frac{\left(1 - \tanh\left(\frac{z}{z_0}\right)\right)}{2}.$$

In the free energy, the third term ensures non-overlap between the liposome and the SLB, whereas the last term represents adhesion between the SLB and the liposome membrane with strength $\sigma(x)$.

The protein dynamics are coupled to the liposome in the same way as discussed in the Methods. However, the liposome dynamics are now coupled to the protein pattern via a concentration-dependent adhesion term

$$\sigma(x) = \tilde{\sigma}(u_e(x) + u_{de}(x)) = \max\left(\sigma_0 - \Delta\sigma \frac{u_e + u_{de}}{u_{e,0}}, 0\right).$$

This choice is motivated by the observation that MinD binding is required to enable liposome adhesion, and that MinE interferes with the MinD binding at the liposome-SLB contact edges.

From the free energy expression above, we derive a set of partial differential equations for the phase field assuming Model A-like relaxational dynamics,

$$\frac{\partial u}{\partial t} \sim -\frac{\delta \mathcal{F}}{\delta u}.$$

With this model, we obtain similar liposome and pattern dynamics as for the generic coupling mechanism discussed in the main text. In **Supplementary Fig. 7** we show a side-by-side comparison of

the kymographs for generic coupling and the kymographs for the phase field implementation with adhesion-mediated coupling, confirming that the generic coupling is an appropriate representation of the true coupling mechanism. For the phase field simulations, the parameters listed in **Supplementary Table S2** were used along with the parameters from **Supplementary Table S1**.

Parameter	Symbol	Value
Surface tension	γ	2 [pN]
Bending rigidity	κ	0.1 [pN μm^2]
Overlap penalty	η	0.4 [pN/ μm]
SLB interface width	z_0	0.25 [μm]
Basal adhesion factor	σ_0	2 [nN μm^2]
Protein-induced adhesion change	$\Delta\sigma$	[40 nN μm^2]
MinE normalization factor	$U_{e,0}$	1250 [$1/\mu\text{m}^{-2}$]

Supplementary Table S 2: Parameters for the phase-field simulation of the dynamic mechanochemical coupling.

References

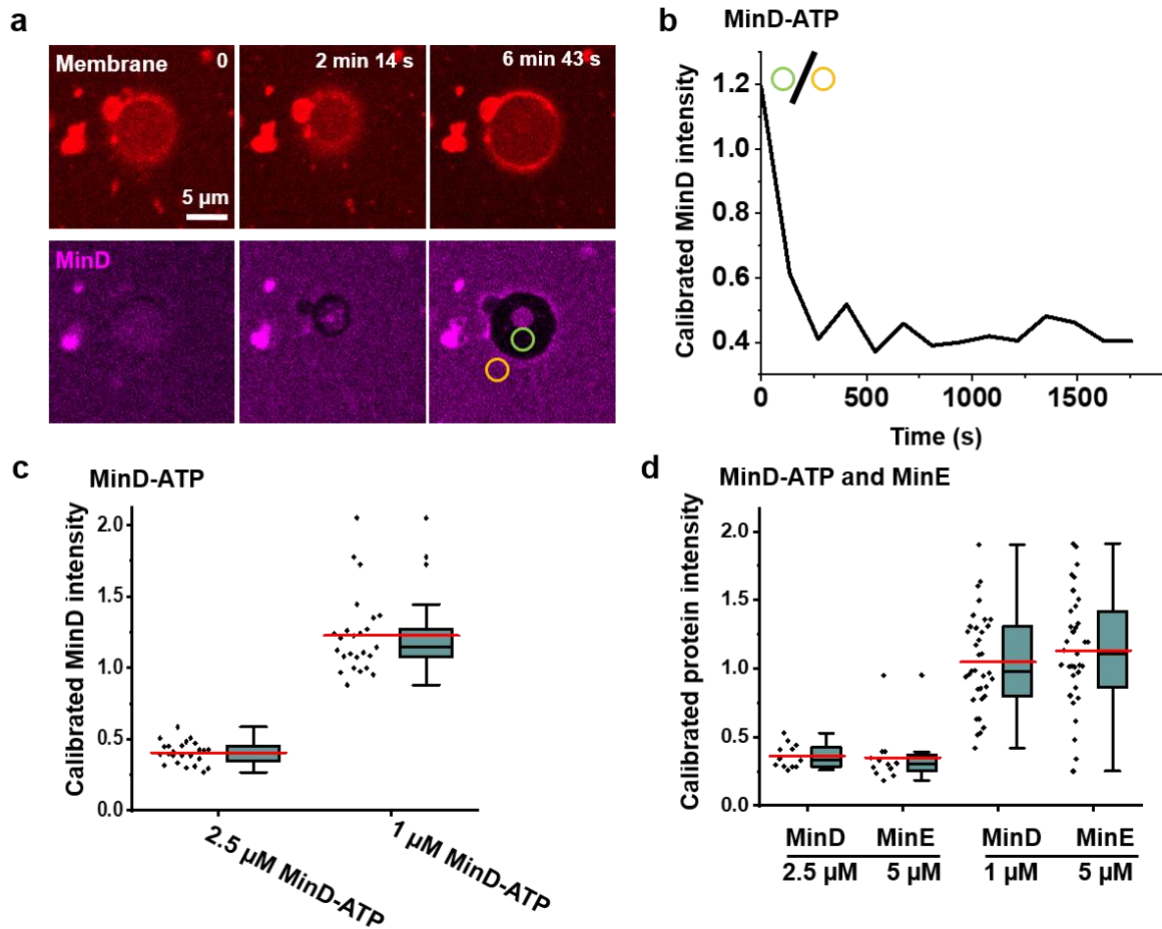
1. Denk J, *et al.* MinE conformational switching confers robustness on self-organized Min protein patterns. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 4553-4558 (2018).
2. Halatek J, Frey E. Highly canalized MinD transfer and MinE sequestration explain the origin of robust MinCDE-protein dynamics. *Cell Rep.* **1**, 741-752 (2012).
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5. Nonomura, M, Study on multicellular systems using a phase field model. *PloS one* **7**, e33501 (2012).
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8. Moure, A and Gomez, H, Phase-field modeling of individual and collective cell migration. *Archives of Computational Methods in Engineering* **28**, 311-344 (2021).

Additional Supplementary Tables

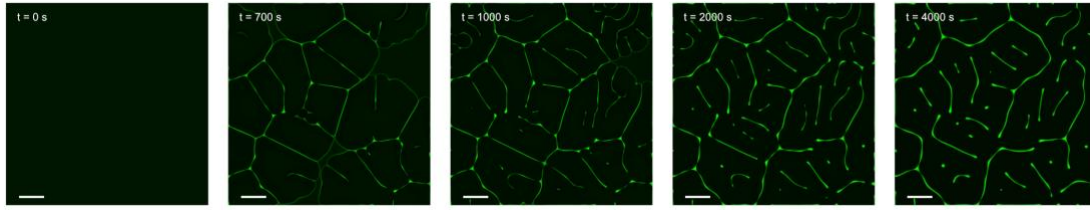
Parameter class	Simulation results upon parameter variation	
	Below default value	Above default value
Protein reaction rates: <ul style="list-style-type: none"> • Change pattern time scale • Default: $k_e = 10^{-4}$ [1/s] • Extended Data Fig. 9 	Patterns adapts faster than the liposome can move, and the liposome comes to a halt.	The liposome moves faster than the pattern can adapt, resulting in liposome “hopping”.
Protein diffusion rates: <ul style="list-style-type: none"> • Change pattern intensity • Default: $D_e/D_d \approx 0.38$ • Extended Data Fig. 3, Supplementary Fig. 2 	Patterns become more pronounced, transient mesh patterns emerge.	Pattern intensity decreases, no pattern for $D_e/D_d \geq 1$.
Mechanical coupling: <ul style="list-style-type: none"> • Change movement time scale • Default: $\nu = 0.005$ [1/s] • Extended Data Fig. 10 	Patterns adapts faster than the liposome can move, and the liposome comes to a halt.	The liposome moves faster than the pattern can adapt, resulting in liposome “hopping”.

Supplementary Table S 3: Parameter dependence of the liposome movement types.

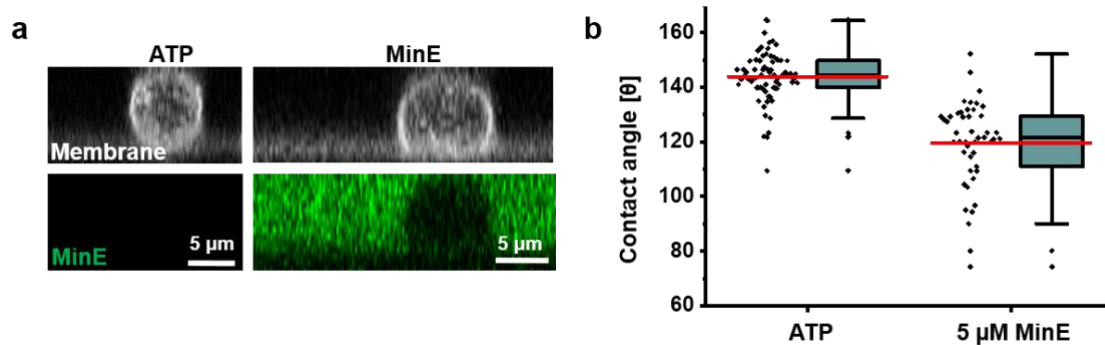
Supplementary Figures



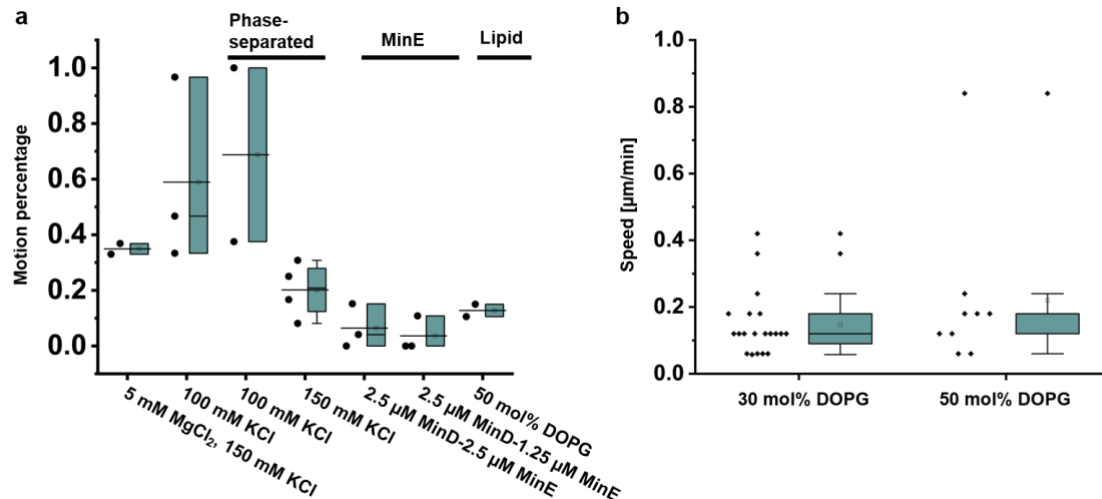
Supplementary Figure 1. **a)** MinD proteins are excluded from the liposome-SLB contact areas as shown with the fluorescence time sequence images; **b)** We calibrated the MinD fluorescence intensity of the liposome-SLB contact areas (green circle in **a**) with that of the SLBs (yellow circle in **a**), that is, the MinD intensity on the SLBs was 1 and the calibrated MinD intensities of the contact areas indicated the protein exclusion degree. Upon MinD membrane binding (2.5 μ M), the calibrated MinD intensity dropped to 0.4 within 500 s, and did not change with further incubation (0.4 ± 0.1 ; $n=24$; 2 experiments); **c)** At lower concentration of MinD (1 μ M), the calibrated MinD intensity was around 1.2 (1.2 ± 0.3 ; $n=25$; 3 experiments), indicating reduced MinD exclusion; **d)** We further tested whether MinE will also be excluded from the contact areas. MinD-ATP and MinE were added together and their fluorescence intensities on the contact area were calibrated with that of the SLBs. At 2.5 μ M MinD and 5 μ M MinE, the calibrated intensities for MinD and for MinE were 0.4 ± 0.1 and 0.4 ± 0.2 respectively ($n=12$; 2 experiments). At lower concentration of MinD (1 μ M MinD and 5 μ M MinE), however, the calibrated intensity increased to 1.0 ± 0.3 for MinD and 1.1 ± 0.4 for MinE ($n=35$; 2 experiments). Therefore, MinD and MinE were both excluded from the liposome-SLB contact areas at high MinD concentration. For all the box figures, whiskers are 1.5 x IQR (interquartile range), median is shown as a black line, mean is shown as a red line.



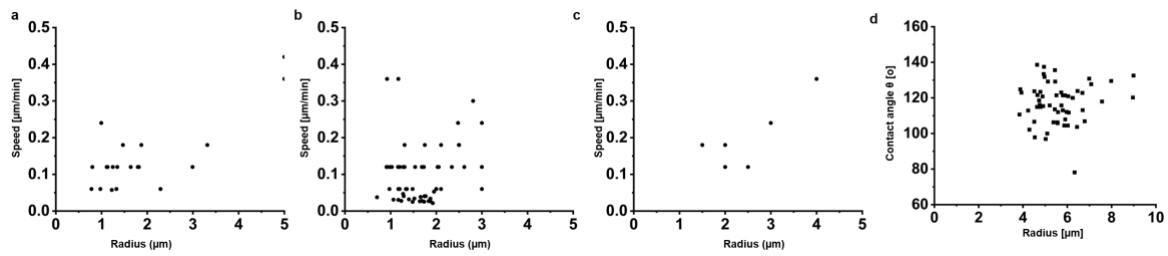
Supplementary Figure 2. Transient mesh patterns. At low MinE membrane diffusion constants ($D_e = 0.0009 \mu\text{m}^2 \text{s}^{-1}$), Min proteins firstly self-organized into a transient mesh pattern ($t = 700 \text{ s}$), and then turned into a labyrinth pattern. Scale bars: $10 \mu\text{m}$.



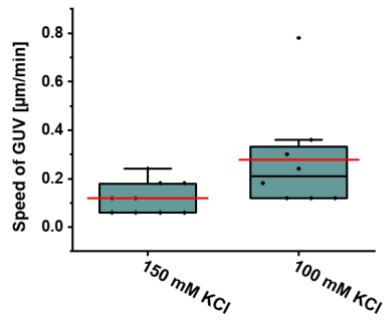
Supplementary Figure 3. a) Liposome flattening upon MinE addition in the absence of MinD-ATP; **b)** The liposome contact angles were decreased upon MinE binding (for ATP: $n = 73$, 4 experiments; for MinE binding: $n = 46$, 2 experiments). Note that in contrast with MinD-ATP, there is no distinguishable MinE membrane binding. MinE proteins have low affinity with lipid membranes in the absence of MinD, but MinE may transiently expose its MTS and induce membrane deformation, especially with high MinE concentration. For the box figure, whiskers are $1.5 \times$ IQR (interquartile range), median is shown as a black line, mean is shown as a red line.



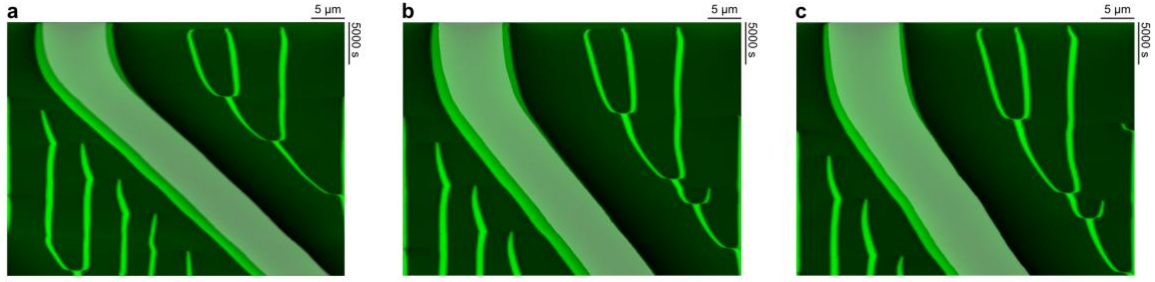
Supplementary Figure 4. a) Liposome motion percentage at different experimental conditions. From left to right: **Normal liposome at Min buffer, 2.5 μM MinD-5 μM MinE** (2 independent experiments; total liposome number: 12, 57); **Normal liposome at Min buffer without MgCl₂, 100 mM KCl, 2.5 μM MinD-5 μM MinE** (3 independent experiments; total liposome number: 15, 15, 30); **Phase-separated liposome at Min buffer without MgCl₂, 100 mM KCl, 2.5 μM MinD-5 μM MinE** (2 independent experiments; total liposome number: 8, 31); **Phase-separated liposome at Min buffer without MgCl₂, 150 mM KCl, 2.5 μM MinD-5 μM MinE** (4 independent experiments; total liposome number: 37, 39, 16, 14); **Normal liposome at Min buffer, 2.5 μM MinD-2.5 μM MinE** (3 independent experiments; total liposome number: 38, 28, 49); **Normal liposome at Min buffer, 2.5 μM MinD-1.25 μM MinE** (3 independent experiments; total liposome number: 59, 29, 37); For **1 μM MinD-5 μM MinE**, no motile liposome were observed (not listed in the figure; 3 independent experiments; total liposome number: 15, 12, 8); **b) Liposome motion speed at different DOPG concentrations.** The experimental conditions were all: Normal liposome at Min buffer without MgCl₂, 100 mM KCl, 2.5 μM MinD-5 μM MinE. For **30 mol% DOPG**, 4 independent experiments and total liposome number was 20. For **50 mol% DOPG**, 2 independent experiments and total liposome number was 9. For all the box figures, whiskers are 1.5 x IQR (interquartile range), median is shown as a black line.



Supplementary Figure 5. Liposome motion speed in related with radius at different experimental conditions, and the larger liposomes tend to move faster. The experimental conditions were: **a)** Normal liposome at Min buffer without MgCl_2 , 100 mM KCl (4 independent experiments; total liposome number: 20); **b)** Phase-separated liposome at Min buffer without MgCl_2 , 150 mM KCl (2 independent experiments; total liposome number: 62); **c)** Phase-separated liposome at Min buffer without MgCl_2 , 100 mM KCl (2 independent experiments; total liposome number: 8); **d)** **Contact angle in related with liposome radius** with MinD-ATP binding (3 independent experiments; total liposome number is 60), which did not yield a clear trend. It should be noticed that we did not measure the contact angles of small liposomes (around 1-3 μm) due to the resolution limitation of the confocal imaging as well as the contact angle measurement software, and any existing correlation between contact angle might be veiled by limited z-Stack resolution, in particular for small vesicles.



Supplementary Figure 6. Motion speed of phase-separated liposomes slightly increased with decreased KCl concentration (For 150 mM KCl, $n = 9$, 1 experiment; For 100 mM KCl, $n = 8$, 1 experiment; the Min buffer used here was in the absence of MgCl_2). It should be noticed that 2.5 mM MgCl_2 was always supplied from the ATP solution to form the Min protein patterns. For all the box figures, whiskers are 1.5 x IQR (interquartile range), median is shown as a black line, mean is shown as a red line.



Supplementary Figure 7. Kymographs of the MinE pattern and the liposome motion in the phase-field model. The MinE concentration is depicted in green, with the SLB-liposome contact area indicated in transparent white. The kymographs show liposome motion for identical initial conditions with parameters **(a)** $\gamma = 0.01$, $\sigma_0 = 10$, **(b)** $\gamma = 0.01$, $\sigma_0 = 40$, and **(c)** $\gamma = 0.004$, $\sigma_0 = 10$. Note that in **(b)** and **(c)**, the liposome is slower than for the default case shown in **(a)**, but the qualitative dynamics and the Min pattern remain unchanged. This indicates that the parameters of the phase-field effectively reduce to a characteristic time scale. In addition, this shows that the effect of changing physical parameters (σ_0 in this case) may not be distinguished from a change in the unphysical parameters (γ).