# FEBS Letters

Review

# Surface topology assisted alignment of Min protein waves

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A B S T R A C T

Self-organization of proteins into large-scale structures is of pivotal importance for the organization of cells. The Min protein system of the bacterium *Escherichia coli* is a prime example of how pattern formation occurs via reaction–diffusion. We have previously demonstrated how Min protein patterns are influenced by compartment geometry. Here we probe the influence of membrane surface topology, as an additional regulatory element. Using microstructured membrane-clad soft polymer substrates, Min protein patterns can be aligned. We demonstrate that Min pattern alignment starts early during pattern formation and show that macroscopic millimeter-sized areas of protein patterns of well-defined orientation can be generated.

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## 1. Introduction

Pattern formation by self-organization of biomolecules is of key significance for cellular organization. During the process of self-organization, global order arises from an initially disordered system and results in organization of living systems on subcellular, as well as on multicellular level. Already in 1952, Alan Turing suggested a diffusion–reaction system as one mechanism to generate patterns of molecular concentrations [1].

An example for such a pattern forming reaction–diffusion driven protein network is the Min system of the bacterium *Escherichia coli*. This system consists of the three proteins MinC, the ATPase MinD, and MinE, which oscillate from pole to pole in the bacterium to spatially regulate cell division [2–5]. MinD and MinE dynamically attach and detach from the membrane by a reaction–diffusion driven process, which is powered by ATP. MinC is a negative regulator for division site placement, which binds to MinD [6,7]. On time-average, the Min protein oscillations result in a non-homogeneous concentration gradient of the division inhibitor MinC with the lowest concentration in the middle of the cell. Thus, misplaced cell division near the cell poles is inhibited [7].

Earlier computational models suggested that MinD and MinE constitute a minimal system for pattern formation and dynamic pole-to-pole oscillations [8–10]. In line with the theoretical simulations, more recent reconstitution experiments of MinD and MinE

in vitro clearly demonstrated that the two proteins MinD and MinE alone are indeed capable of forming dynamic surface patterns on supported membranes, and oscillate from pole-to-pole when they are enclosed in cell-shaped membrane compartments [11,12]. However while Min-oscillations have a predefined propagation axis on small elongated membrane patches and in cell-shaped compartments [12,13], the orientation of wave and pattern propagation in bigger systems, such as large areas of planar membranes, have no globally preferred orientation.

Here we report a novel in vitro assay to achieve orientation of Min protein patterns also in large-scale systems. In other words, we show that dynamic Min protein patterns can be aligned in predefined directions on large membrane areas using micro-fabricated devices of specific surface topology. In particular, we investigate reconstituted Min pattern on supports with two different arrays of membrane clad micro grooves. Interestingly, our data show that reaction–diffusion induced patterns can indeed be modulated by surface topology. Thus, although protein patterns are mainly regulated by biochemical properties of participating proteins, our data emphasize the potential of surface geometry as a superimposed regulatory cue to alter pattern formation.

## 2. Material and methods

### 2.1. Min proteins

eGFP-MinC, MinD and MinE were purified as his-tagged fusions. All proteins were expressed in BL21 cells grown in 2xYT medium

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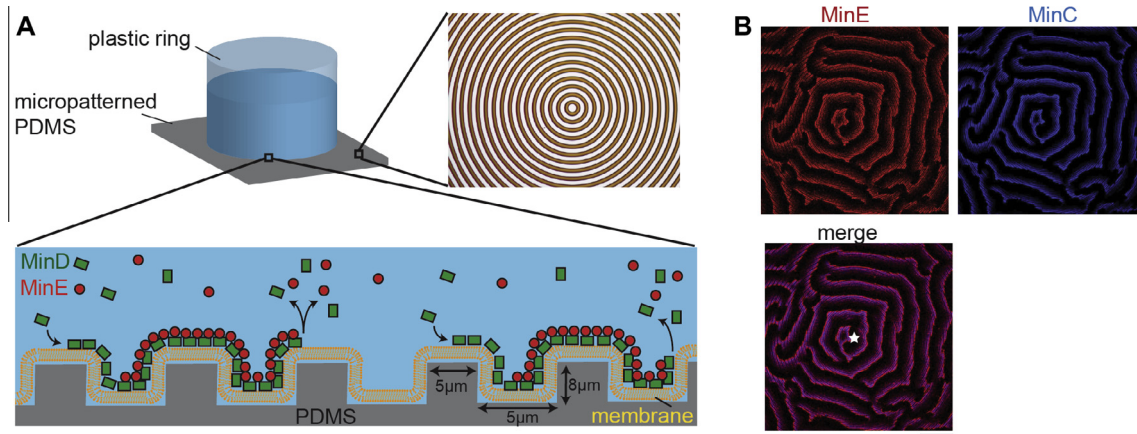*E-mail address:* schwille@biochem.mpg.de (P. Schwille).

**Fig. 1.** Reconstitution of Min protein pattern on topologically structured supports (A) Experimental setup: A reaction chamber was placed on top of microstructured PDMS supports with concentric rings. The microstructured support was clad with membrane and the buffer volume on top of the membrane was supplemented with MinC, MinD, MinE and ATP. Inset: Light microscopy image of PDMS structures. (B) Confocal microscopy image of MinC/D/E protein pattern on PDMS structures. 0,1 μM eGFP-MinC, 1 μM MinD, 1 μM MinE. The star marks the midpoint of the concentrical rings. Height of structures: 8 μm. Scale bar: 100 μm.
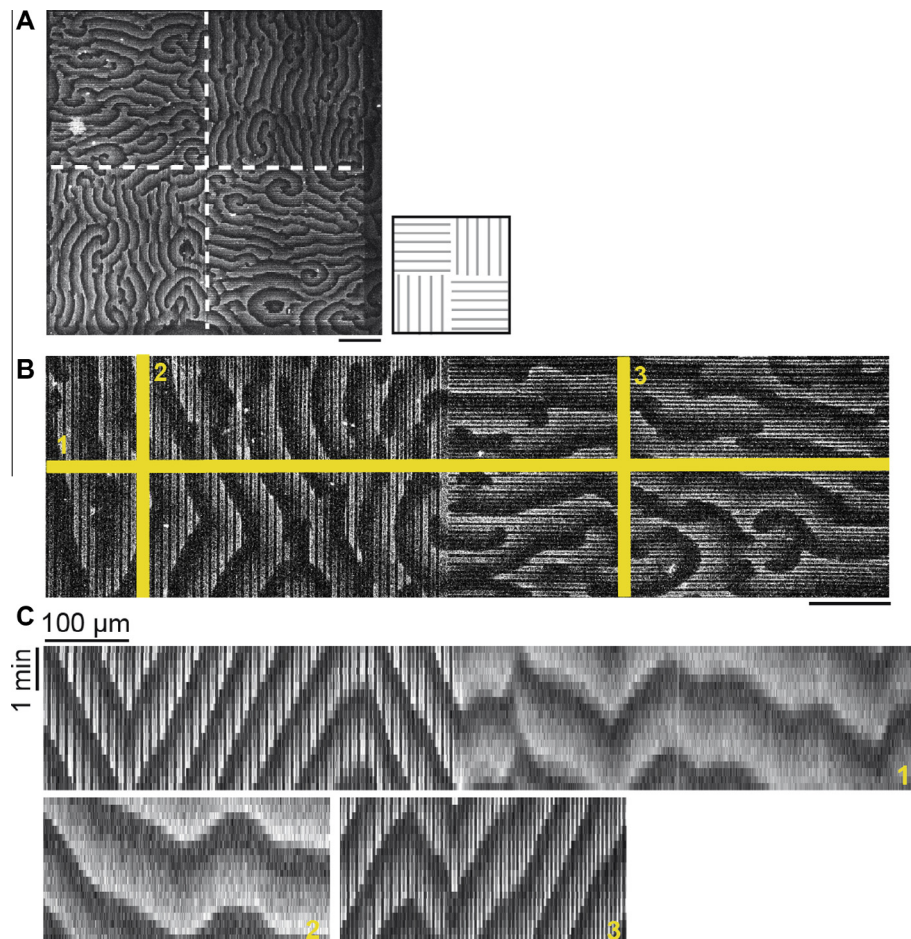


**Fig. 2.** Min protein waves propagate perpendicular to arrays of Microfabricated parallel grooves. (A) MinD/E patterns on fields of parallel, membrane clad micro walls. The orientation of the walls is depicted in a schematic graph. Scale bar: 200 μm. (B) Image of MinD/E patterns on vertically (left) and horizontally (right) patterned surface. Scale bar: 100 μm (C) Kymographs (time vs. space plots) along the lines depicted in (B).

and purified as described previously [11,14]. MinE was labeled with AlexaFluor 488 C5 maleimide (Molecular Probes) according to the manufacturer's instructions.

An overexpression vector encoding His6-eGFP-MinD was generated by amplifying MinD from a pET28a-MinD [11] and ligating it into a pET28a-eGFP vector using the restriction sites for HindIII and

EcoRI. The resulting ORF encoded a MinD fusion with an N-terminal hexahistidine-tag and eGFP. The construct was transformed in B21 cells, which were grown in LB medium and His6-eGFP-MinD was purified according to the protocol for MinD.

To reconstitute Min protein waves 1 μM MinD, 1 μM MinE and 2.5 mM ATP were incubated in reaction buffer (25 mM Tris–HCl pH7.5, 150 mM KCl, 5 mM MgCl) on top of supported bilayers. eGFP-MinC was used at a concentration of 0.1 μM.

## 2.2. Micro structured supports

Photoresist patterns of 8 μm height on silicon wafers were produced using the resist ma-P 1275 (micro resist technology GmbH). Afterwards the wafer was coated with chlorotrimethylsilane (Sigma–Aldrich).

PDMS monomer and crosslinker solution (Sylgard184, Dow Corning) was mixed at a ratio of 9:1, degased in a vacuum, and poured on top of the wafer. A glass cover slip was pressed on top of the si-wafer to sandwich a thin layer of PDMS between the glass cover slip and the wafer. Then the PDMS was cured for 3 h at 80 °C. The cured PDMS layer together with the glass cover slip was carefully peeled off. Before the microstructured PDMS was used as a membrane support, it was sonicated for 5 min in ethanol, washed with water, air dried, and hydrophylized using an air plasma.



**Fig. 3.** Min waves on supports with vertical (left) and horizontal (right) grooves. As the PIV-analysis reveals, waves propagate perpendicularly to the orientation of the trenches. (A) Source image (B) Angle map. (C and D) Histogram of angle frequency. For vertical trenches, lower angles (C) dominate whereas for horizontal, angles close to 90° dominate (D).

## 2.3. Supported lipid membranes

Supported lipid membranes were produced by vesicle fusion. We prepared small unilamellar vesicles from *E. coli* lipid extract (Avanti polar lipids) by sonication in reaction buffer (25 mM Tris–HCl pH7.5, 150 mM KCl, 5 mM MgCl2). 0.5 mg/ml vesicles were filled into a reaction champer on top of plasma cleaned, micro structured surfaces. CaCl2 was added and the sample incubated for 15 min at 37 °C. Finally the formed lipid membrane was washed with reaction buffer to remove residual vesicles.

To test the mobility of membranes *E. coli* polar lipids were supplemented with 0.1 mol% fast-DiI (Invitrogen) and motility of the membrane was verified with FRAP experiments.

## 2.4. PIV analysis

Direction of wave propagation was analyzed by particle image velocimetry (PIV) using a custom made MATLAB code based on the MatPIV-function developed by J Kristian Sveen [15,16]. The routine detects spatial shifts of signals in *x* and *y* on a local level between two subsequent frames of a *xyt*-stack. For this, each *xy*-plane is subdivided into two-dimensional elements (here of 32 × 32 pixels) and for each element, a vector is calculated representing the direction of local shifts. The ensemble of vectors can be plotted in a vector map. For an image stack of *n* frames, $(n - 1)$ of such vector maps are calculated. Since the direction of wave propagation remains unchanged throughout the acquisition of the respective time-lapses, all vector maps corresponding to one image stack could be averaged to one single vector map (Fig. 3B and Supplementary Fig. 4B and C). From this mean angle map, angles were calculated by the arctan of the respective vectors and color-coded for an angular spectrum between 0° and 90°. An angle of 0° (blue) represents horizontal direction of the travelling wave, whereas an angle of 90° (pink) corresponds to a vertical propagation. The PIV-elements at the border of a frame produces artifacts, therefore they were omitted for the analysis (white frame in the color-coded images of Fig. 3).

## 3. Results

### 3.1. Topologically structured membranes guide Min patterns in predefined directions

To examine the effect of membrane topology on the spatiotemporal organization of Min protein patterns, we used grooved membrane supports with groove sizes of 5 micrometers and a distance of 5 micrometers, corresponding to about the tenth fraction of Min protein wavelength. PDMS layers with arrays of microfabricated PDMS grooves were clad with *E. coli* polar membranes using a vesicle fusion technique. The fluidity, i.e., lipid mobility within the membranes, which adopt the topology of the underlying PDMS grooves, was confirmed by labeling membranes with 0.1% DiI and subsequent FRAP experiments (data not shown).

At first we engineered PDMS grooves that were arranged in concentric rings (Fig. 1a). The ring-like grooves were 8 μm high, 5 μm wide and had a distance to the next groove of 5 μm. To reconstitute Min protein pattern on top of the membrane clad structures, we added 1 μM MinD, 0.9 μM MinE, 0.1 μM MinE-Atto655 and 2.5 mM ATP. After the incubation time of 30 min the Min proteins were imaged and shown to form dynamic protein patterns on the topological structured membranes.

Previous experiments demonstrated that the Min system self-organizes into similar dynamic surface waves on flat supported membranes. However, on top of planar membranes, Min proteins form patterns without globally preferred orientation, such as
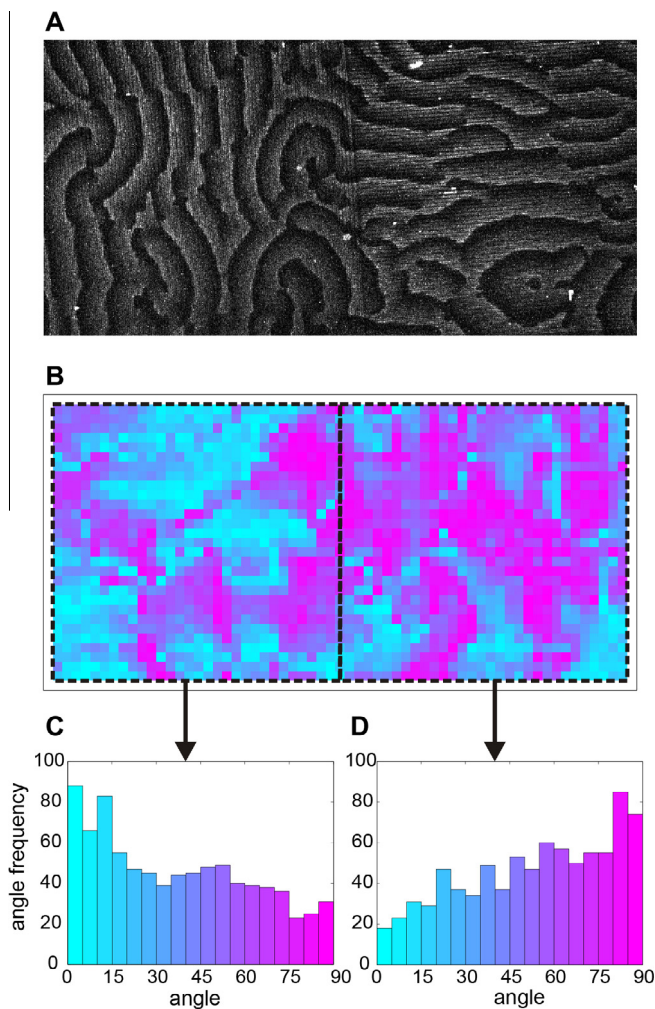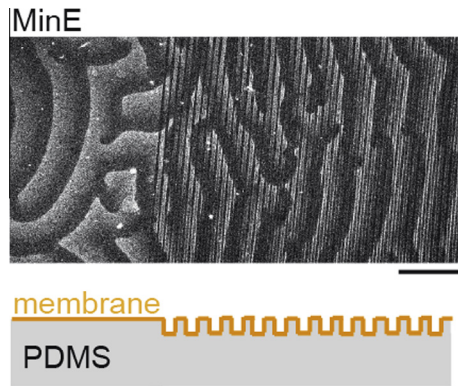
**Fig. 4.** Min patterns on microstructured membranes and flat membranes have similar wavelength. Confocal image of reconstituted MinD/E waved doped with MinE.Atto655 on parallel walls (right) next to a flat membrane region and schematic image of the corresponding membrane topology. Scale bar: 100 μm.

spirals and parallel, randomly oriented waves. In contrast, a significant preferential orientation of the Min wave fronts was observed on membrane supports with concentrically aligned grooves: The wave fronts predominately oriented themselves parallel to the grooves. In other words, the waves mainly propagated towards or away from the center of the surface structures (Fig. 1b, Supplementary Figs. 1 and 2). While on long term the pattern on structured supports resembled those on flat bilayers, a distinct alignment of protein patterns persisted for an observation time of about 2 h.

### 3.2. Min protein patterns follow discontinuous groove alignments

To address the question whether the alignment of Min waves by micro structured membrane supports is prominent enough to follow discontinuous orientation, we designed rectangular fields of parallel straight-lined PDMS grooves, oriented with 90° to each other like on a chessboard (Fig. 2).

Interestingly, the discontinuity in the orientation of pattern did not disturb the effect of Min wave alignment. In agreement with experiments on concentrically structured membrane supports, the Min wave fronts again aligned preferentially parallel to the PDMS grooves over almost the full range of the rectangular patches. Thus, although these highly organized protein patterns ultimately originate from specific properties of nanometer-sized proteins, macroscopic alignment pattern on square-millimeter big areas were observed.

To investigate the alignment of propagating Min protein patterns on microstructured supports more quantitatively, we performed a particle image velocimetry (PIV) analysis and examined the exact angle of wave propagation (Fig. 3). The distribution of propagation angles was plotted in histograms using a bin width of 5° (Fig. 3C,D and Supplementary Fig. 4). In line with our qualitative observation and kymograph analysis, the PIV data demonstrate that Min waves preferentially propagated perpendicular to the orientation of the groves. Although velocity vectors of all orientations were detected on microscopic scale, the occurrence of vectors perpendicular to the walls was about three to four times larger than the frequency for vectors parallel to the walls.

Notably, the wavelength of Min protein patterns on flat membranes was similar to the wavelength on topologically structured structures when measured by beeline (Fig. 4), although the patterns traveled though the grooves and were also observed on the bottom of grooves (Supplementary Fig. 3). This observation seems to be counterintuitive, because the actual distance along the surface of the membrane is, compared to beeline, more than two times larger. However, we have previously shown that Min waves can couple across flat membranes patches when the patches were separated by smaller than 10 μm wide gold barriers [13]. Note that the distance between two adjacent microfabricated grooves is 5 μm and that this distance is small compared to the Min wavelength of about 50–100 μm. The observation of a contained beeline wavelength is therefore consistent with the previous experiments and originated from the three-dimensional reaction–diffusion principles of the Min system.
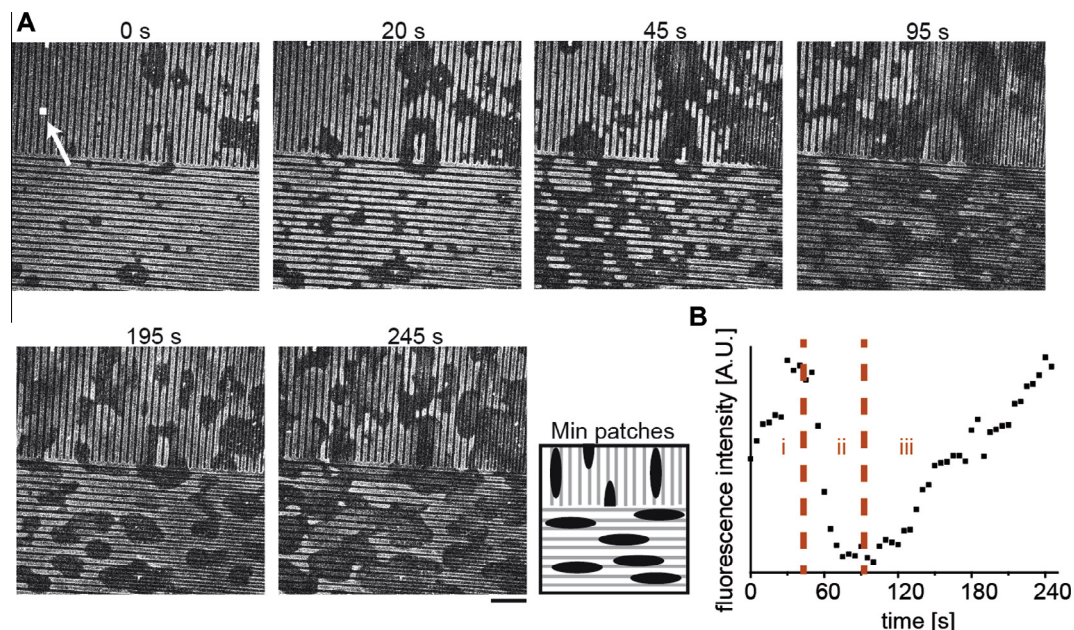


**Fig. 5.** Initiation of Min pattern alignment. (A) Confocal time-lapse images of first oscillation cycle. Pattern of 1 μM MinD and 1 μM MinE (supplemented with 10% MinE.Atto655) were oriented within the attachment-detachment cycle. Scale bar: 50 μm (B) Development of fluorescence intensity of a square area with 10 μm width (depicted in A, first frame). (i) Increase of intensity due to increasing protein attachment, (ii) detachment of the Min protein from the membrane, (iii) reattachment of Min proteins to free membrane.

### 3.3. Min pattern alignment originates early during pattern formation

To investigate how the alignment of Min protein patterns parallel to the underlying grooves is initiated, we analyzed the onset of Min pattern formation. On top of flat supported bilayers it has been shown that dynamic protein patterns originate radially from several "hot spots" at arbitrary locations, due to stochastic variations in the protein concentrations on the membrane, and only later converge towards parallel wave fronts [11]. Thus, the intrinsic mechanism of the proteins to cooperatively attach and detach from the membrane then results in the fusion of patch-like pattern and finally in the formation of protein surface waves with no globally preferred orientation. Typically, a flat membrane is covered with areas of spirally and parallel, randomly oriented wave-like patters, when the patterns are equilibrated.

In comparison, the beginning of pattern formation on micro structured PDMS supports initially resembles Min pattern formation on flat supported membranes. First, when the proteins are incubated on top of the membrane, MinD proteins start to attach to the membrane and the protein concentration on the membrane increases. Then the proteins detach at individual localizations due to the inhibitory action of the antagonist MinE, which results in patch-like patterns. The patterns propagate and in repeating cycles, new MinD protein reattaches at free membrane areas, and thus generate dynamic surface waves after several oscillation cycles. However, in contrast to flat supported membranes the formation of Min protein patterns on micro structured supports preferentially started at the edges of microstructures. Thus, when the Min patches increase in size and merge into patterns with intrinsic wavelengths, the wave fronts tend to be oriented parallel to the micro walls. Already after the first cycle of membrane attachment and detachment, an orientation of the Min patches was detectable (Fig. 5).

### 4. Discussion

Using topologically micro-structured devices as membrane supports, we have established a novel lab-on-chip assay for aligning self-organizing Min protein patterns in predefined orientations. In particular, we employed two different arrays of membrane clad micro grooves and demonstrated that the patterns preferentially propagated perpendicularly to the walls. Thus, in addition to planar bilayers with specific shapes, large-scale orientation of Min patterns can also be achieved on topologically structured membranes. Notably, while the biological function of the Min system is to organize the space of a few micrometer-sized cells, we exploited the combination of intrinsic properties of the Min system with external cues to generate millimeter-sized areas with patterns of predefined orientation. Furthermore we demonstrated that pattern alignment occurs early during pattern formation. Taken together, our results show how nanometer sized protein machineries can be reconstituted to generate macroscopic patterns of well-defined orientation.

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.06.026.

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