Spatial Regulators for Bacterial Cell Division Self-Organize into Surface Waves in Vitro

Loose, M., Fischer-Friedrich, E., Ries, J., Kruse, K., Schwille, P.

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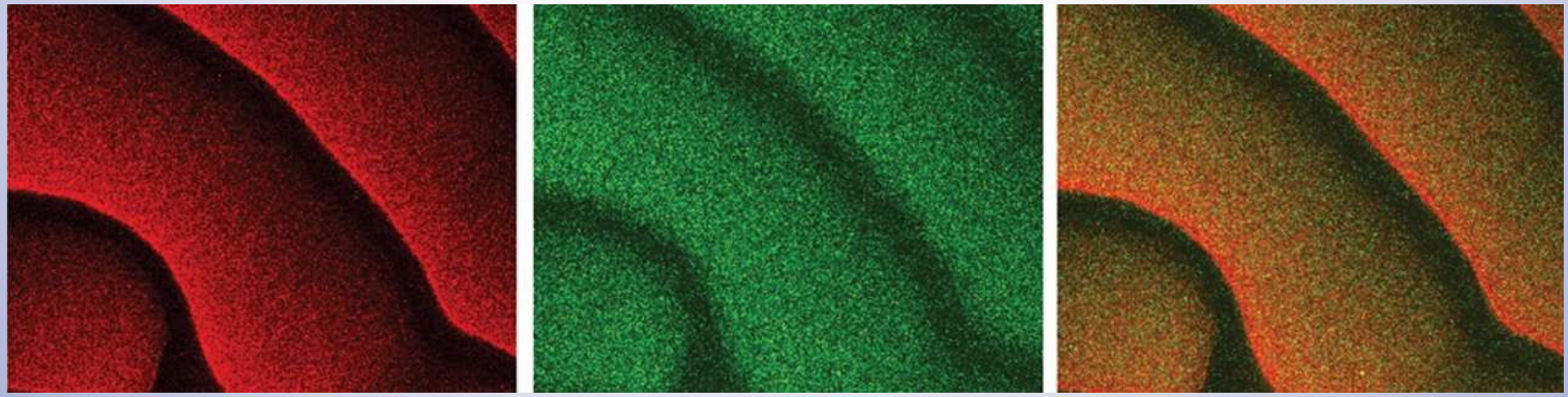

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Bacterial cell division

- Division septum
  - formation by invagination of the cytoplasmatic membrane and ingrowth of the cell wall
  - positioned fairly precisely at midcell

- How is this spatial regulation achieved?

  → positioning the division plane is a fundamental problem in biology

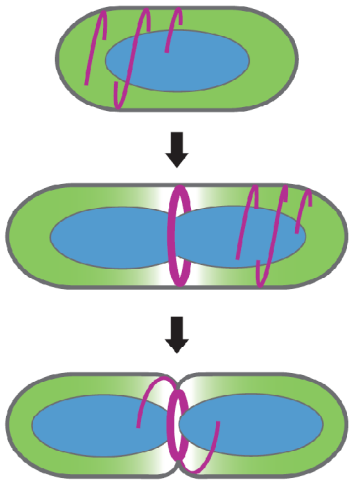
FtsZ

- FtsZ is the first protein localized at the future site of cell division

  - FtsZ: bacterial homologue of tubulin

  - assembles into a Z ring

  - recruitment of at least a dozen other proteins



Lutkenhaus, 2007

How is assembly of the Z ring limited to midcell?

→ determined by a gradient of negative regulators of Z ring assembly

Min system

- Min proteins oscillate between cell poles

  → select the cell center as division site

- MinD

  - ATPase, binds to the membrane in an ATP-dependent manner

  → recruits MinC and MinE

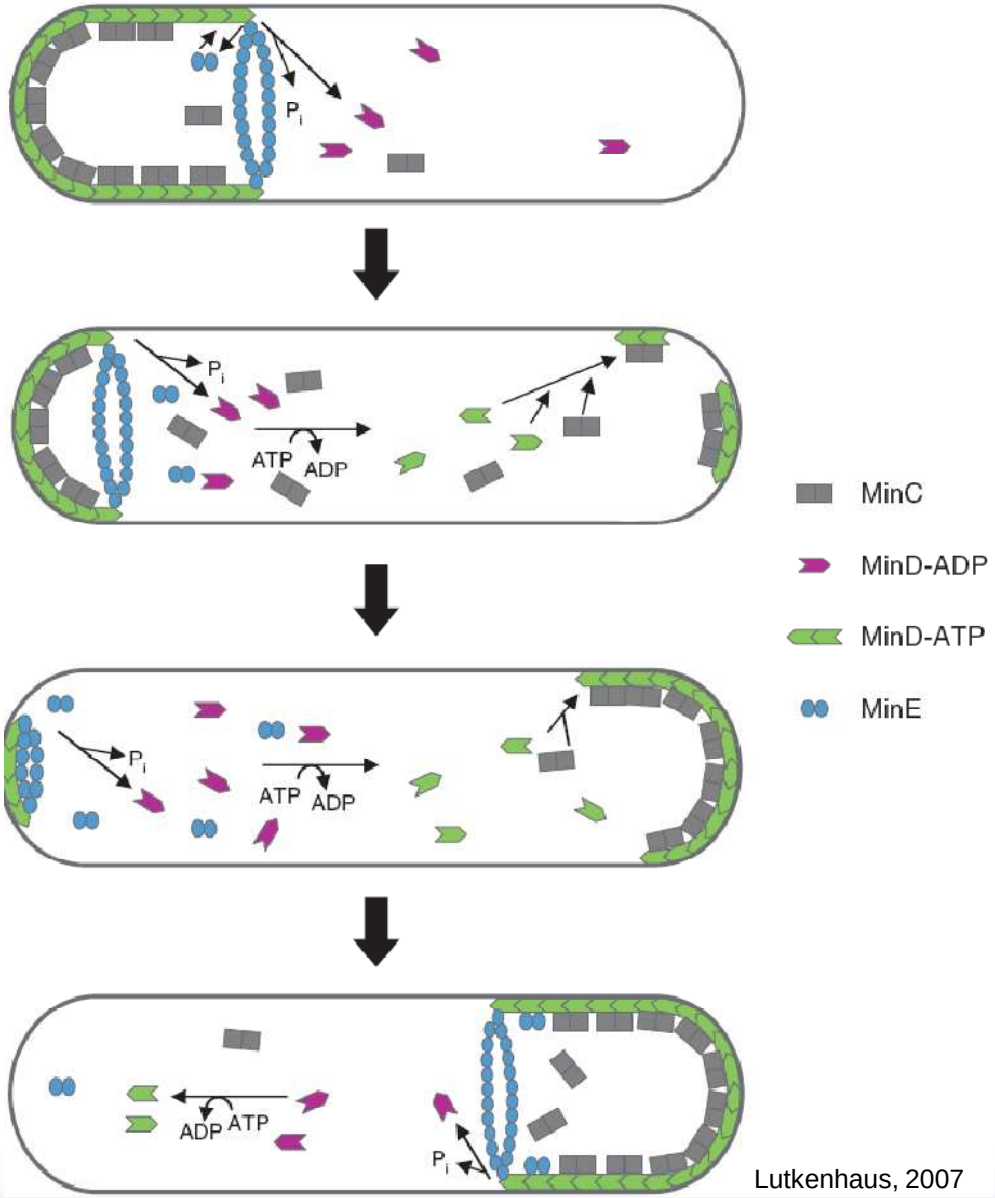
- MinC

  - inhibitor of FtsZ assembly

- MinE

  - induces ATP hydrolysis by MinD → dissociation

Min system



Lutkenhaus, 2007

- MinD-ATP binds to membrane at the left polar zone

  → recruits MinC and MinE

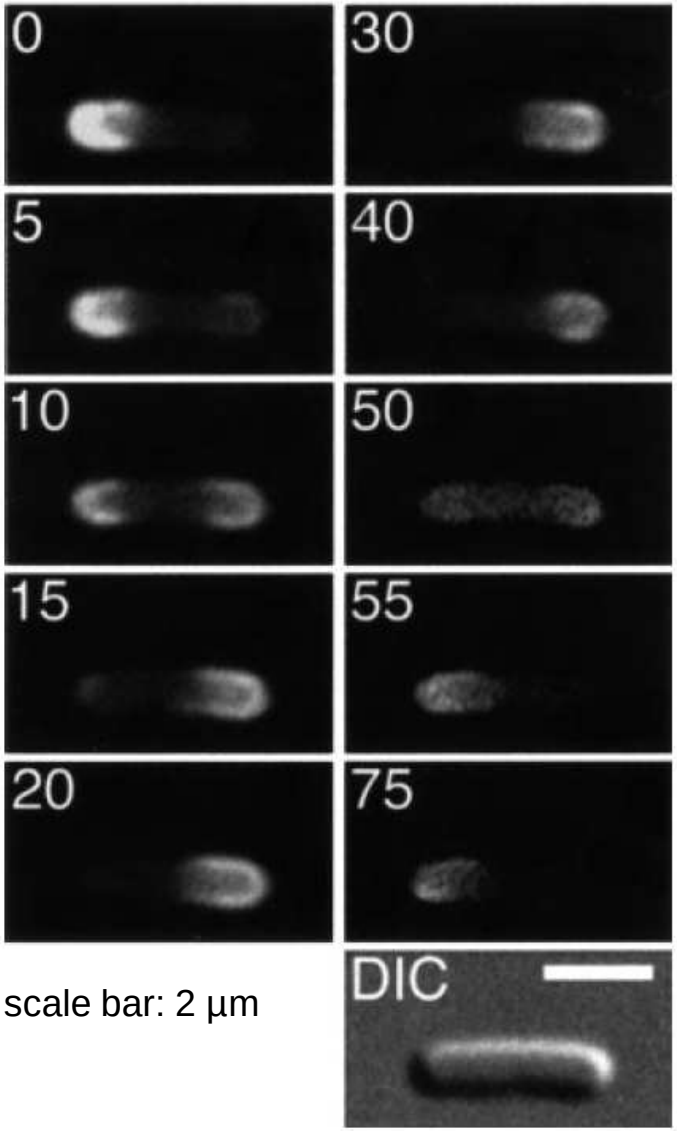
- MinE displaces MinC and stimulates MinD ATPase

  → proteins release membrane

- MinD-ADP undergoes nucleotide exchange

- new polar zone of MinD is established, extending towards midcell

Oscillation of GFP-MinD



scale bar: 2 µm

Hale, 2001

Min system

- inhibition of FtsZ assembly at the cell poles due to MinC

  → formation of division septum is restricted to cell center

- periodicity of the oscillation: ~40 s

- MinC plays no role in the oscillation

  → passenger in the oscillation

Min system

- various theories to explain Min oscillation have been suggested

- some models propose that no spatial markers are required for oscillation

→ Min-protein self-organization should also work in vitro

In vitro Min system

- Loose *et al.*: experimental approach with a minimal number of components

  → systematical exploration of the Min system

- components: - supported lipid bilayer
  - Min E (Alexa647)
  - Min D (Bodipy-FL)

  } fluorescently labeled
  - ATP

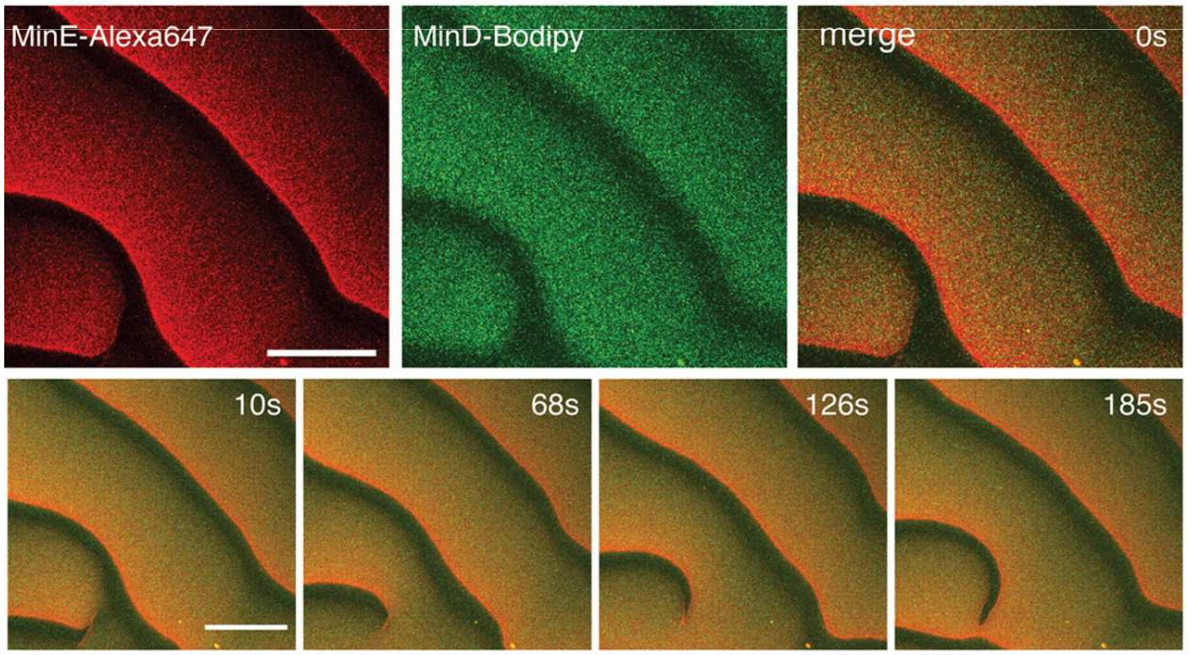
Experimental approach

- MinD and ATP in the buffer

  → homogeneous protein layer on supported bilayer

- adding MinE to the buffer

  → observation of planar surface waves within 1 h



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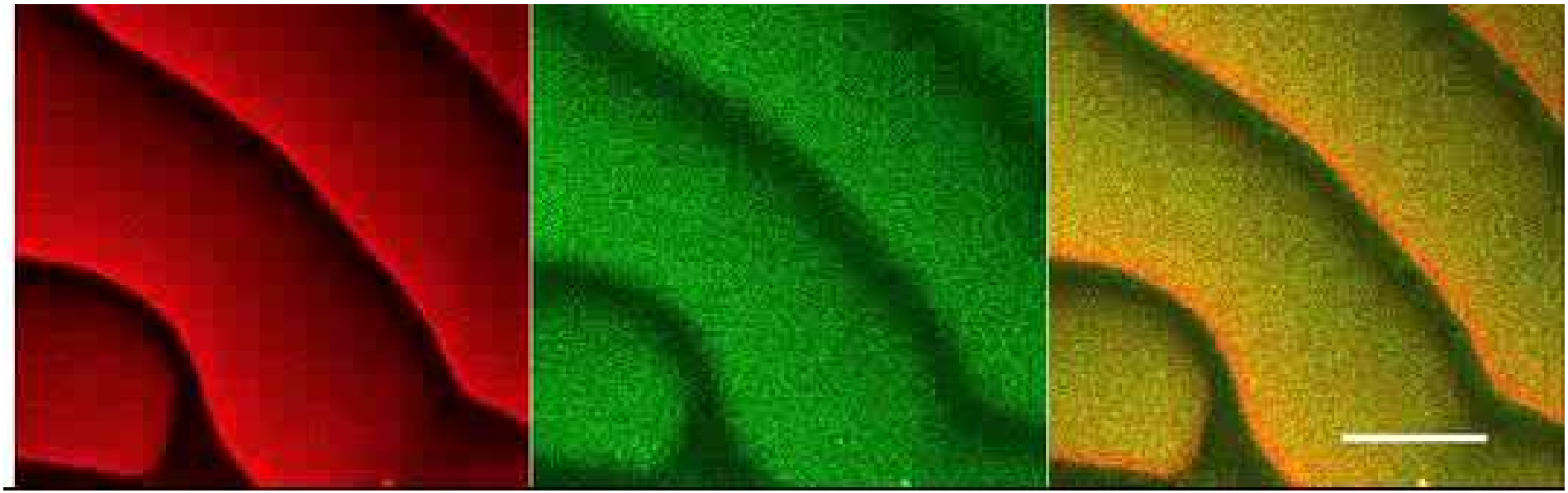
Loose, 2008

Planar surface waves

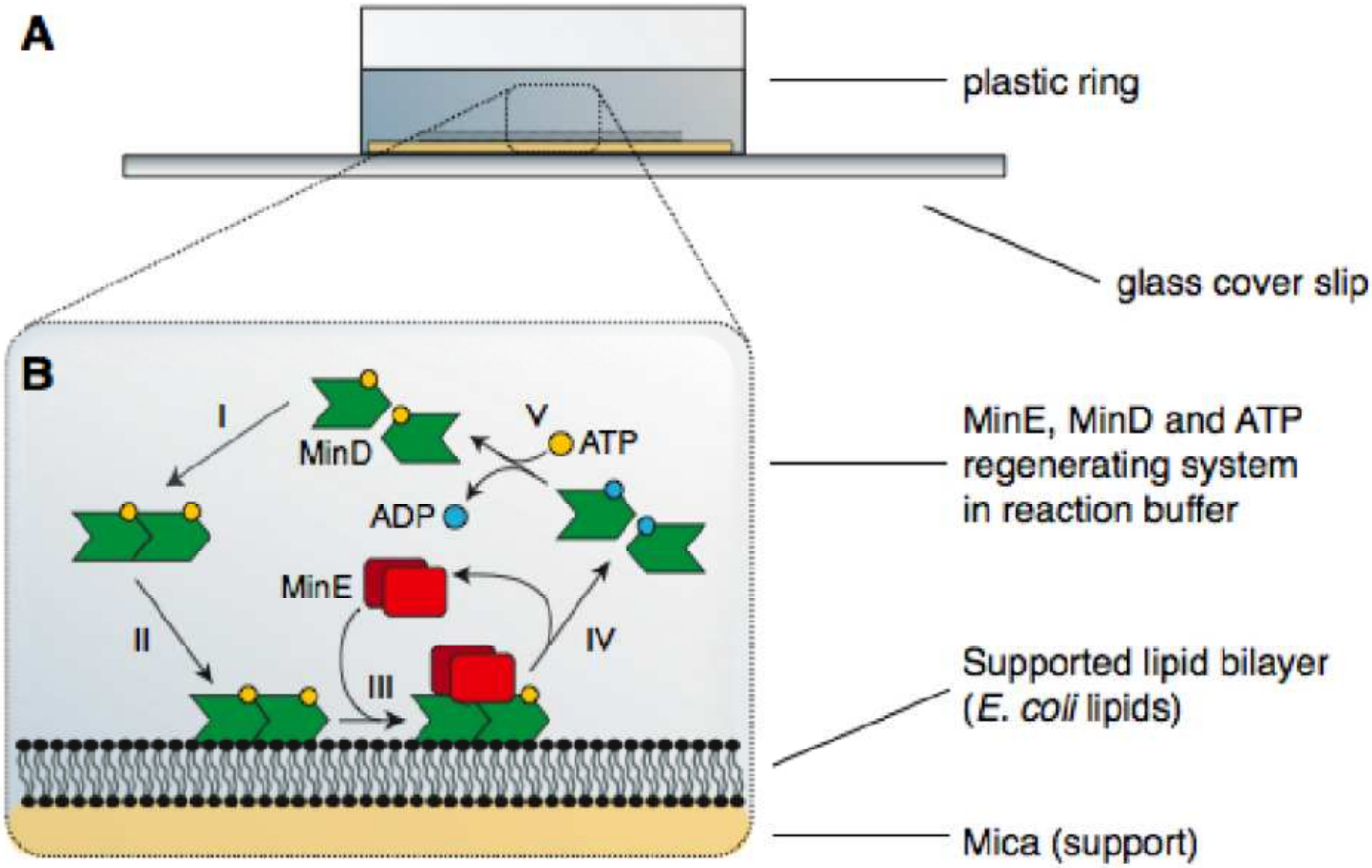
MinE-Alexa647 MinD-Bodipy merge



scale bar: 50 µm

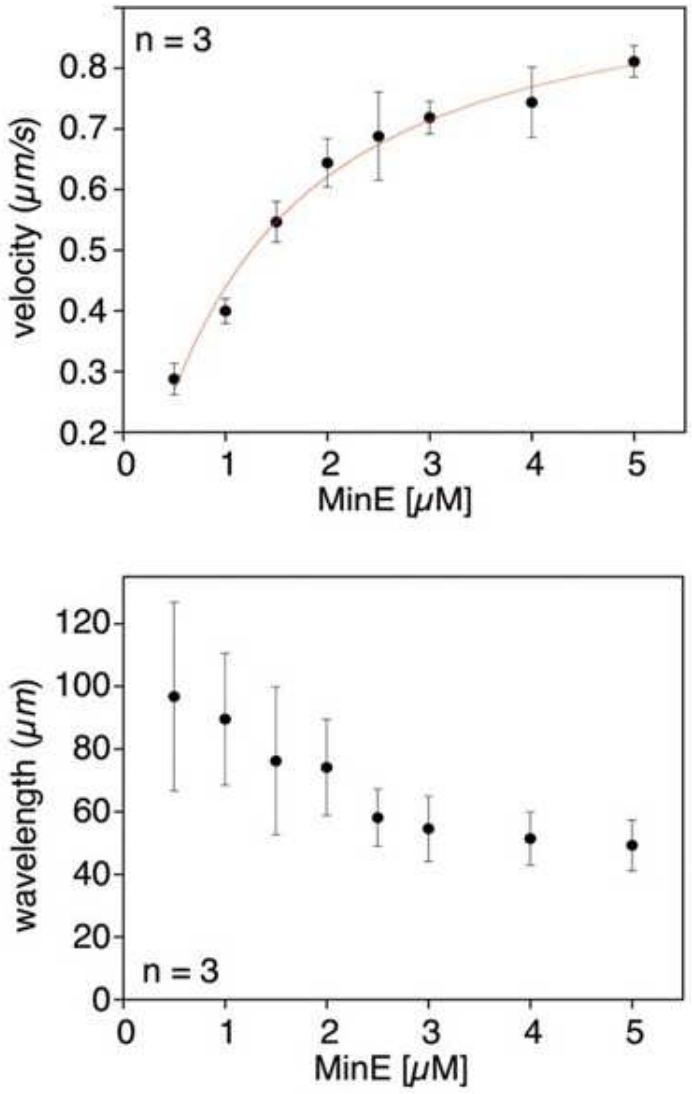
- movement in a distinct direction
- present for several hours

Theoretical background



Loose, 2008

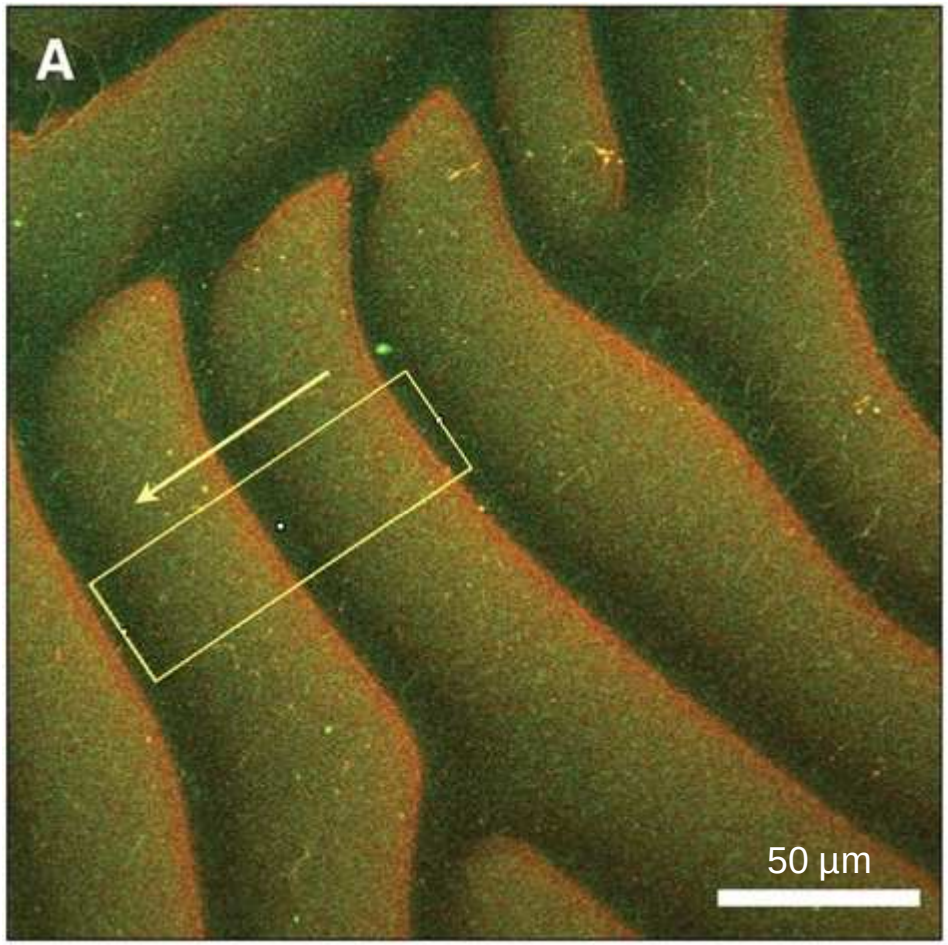
Concentration dependency on MinE

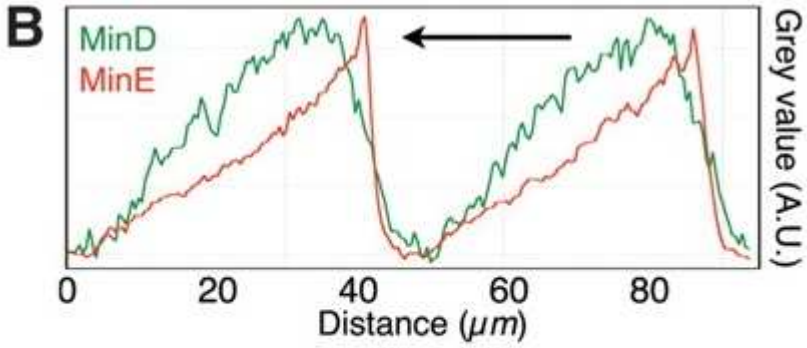


Loose, 2008

- for given concentration of MinD and MinE, waves moved at constant velocity and wavelength
- with increasing concentration of MinE, velocity increased while wavelength decreased

Quantitative characterization





- characteristic protein-density distribution parallel to propagation direction
- density maximum of MinE followed maximum of MinD
- MinE formed a sharp line along trailing edge of the wave (red)

→ pattern resembles situation in cell, where MinE ring moves toward the pole, detaching MinD from the cell membrane

Loose, 2008

ATP dependency

- ATP is necessary for the nucleotide exchange of MinD-ADP to MinD-ATP

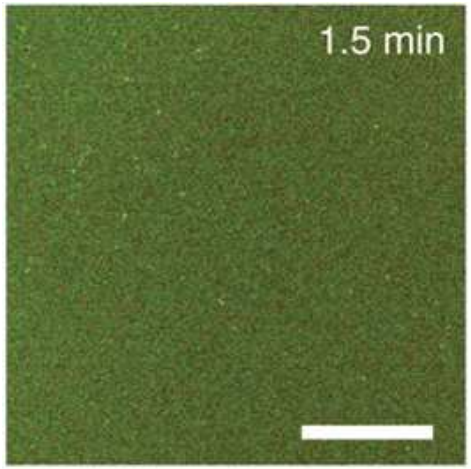
- only MinD-ATP can attach to cell membranes

→ in the absence of ATP as well as under the use of a nonhydrolyzable ATP analog no wave formation could be observed

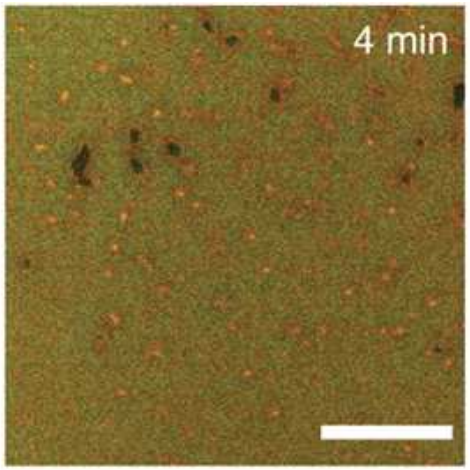
How did MinE lead to surface waves?

MinD

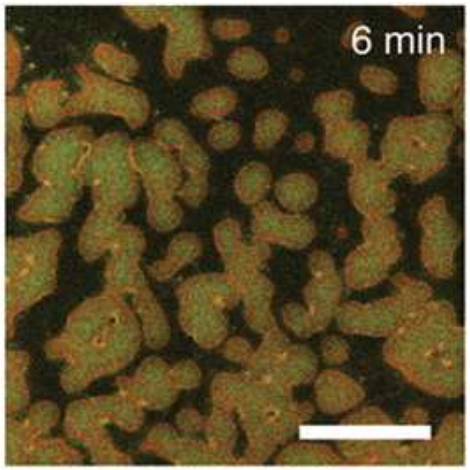
MinE



1.5 min after MinE addition

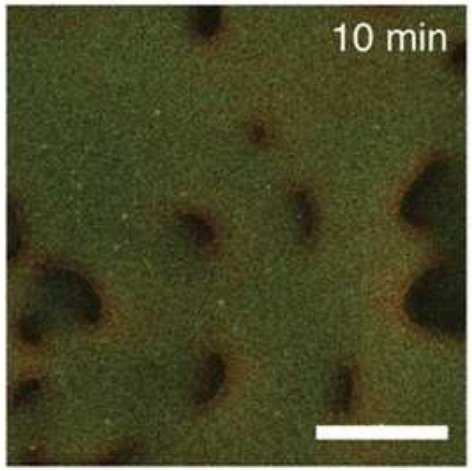


MinD layer became unstable

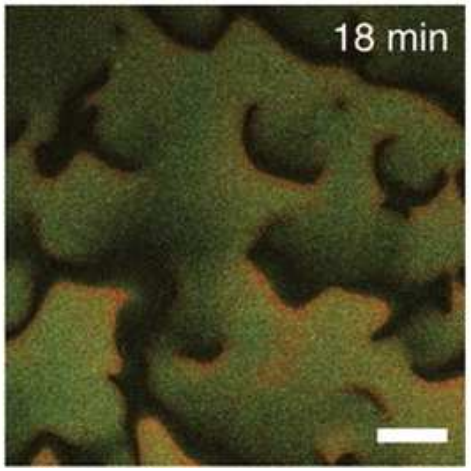


areas without MinD increased in size

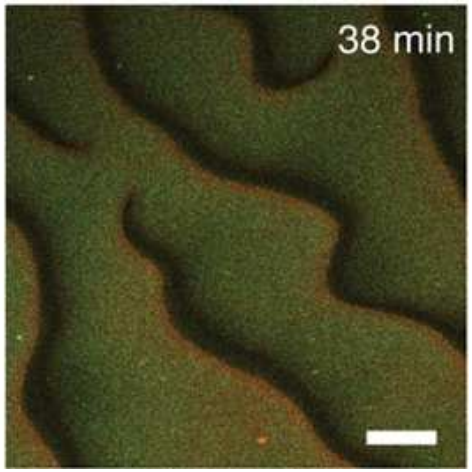
scale bar: 50 µm



MinD reattached to protein free parts



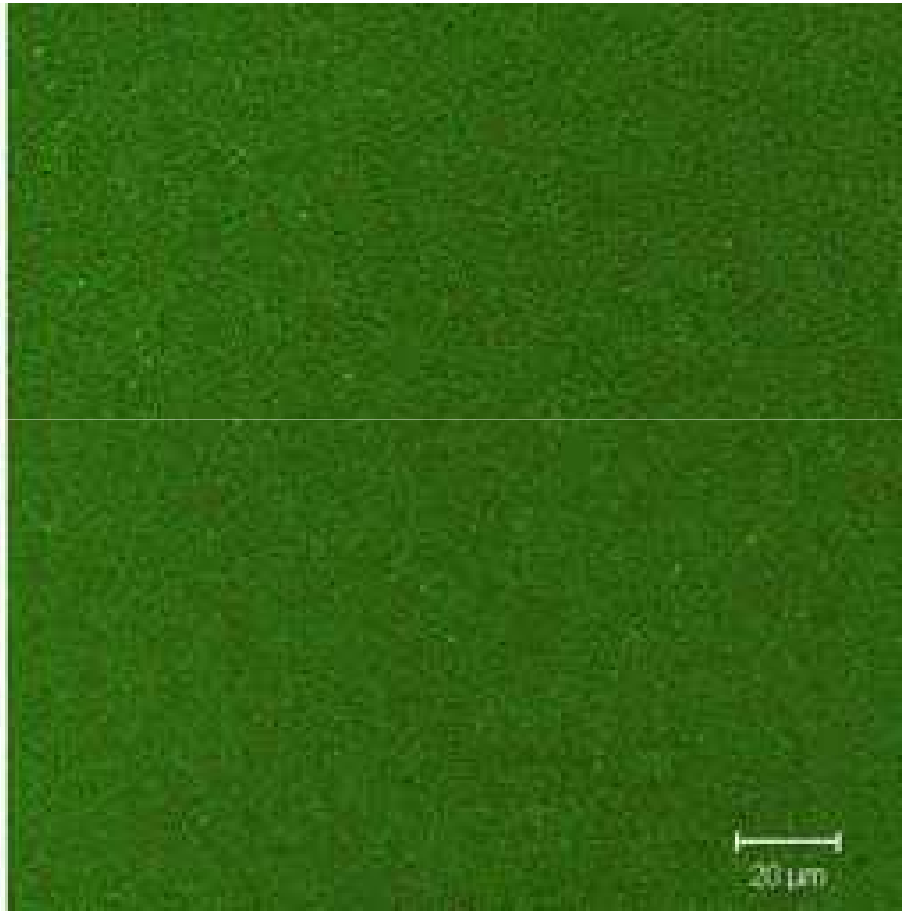
waves moved independently



collision of two waves → synchronizing

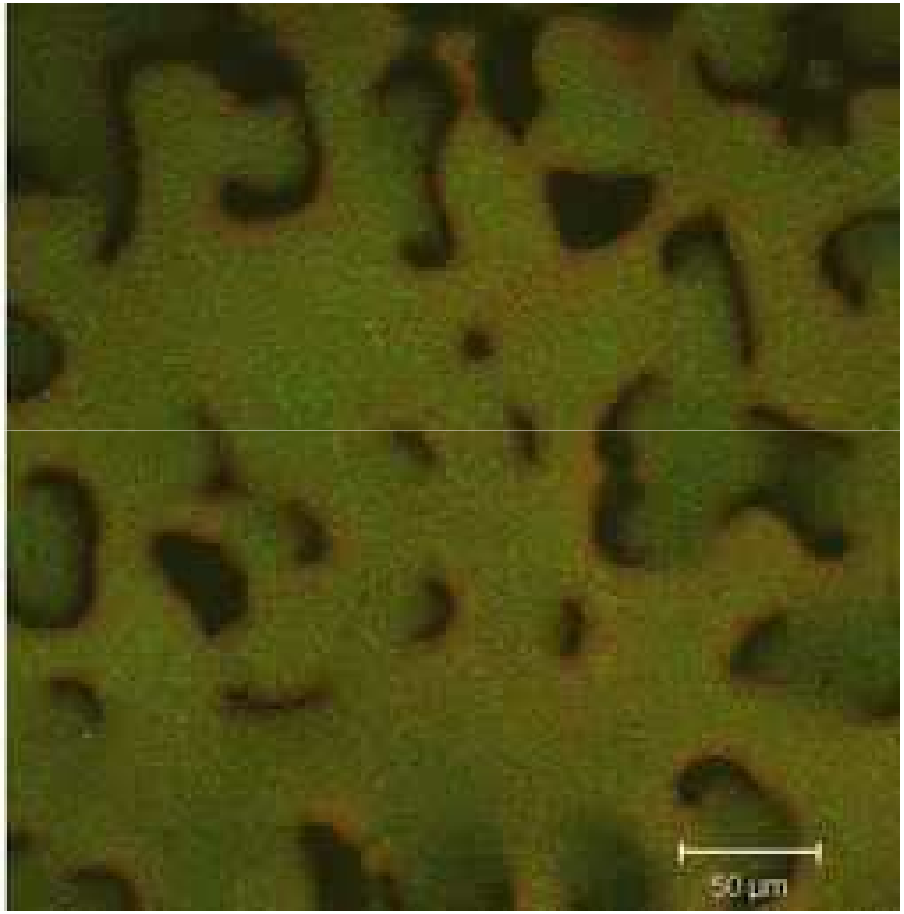
Loose, 2008

Initiation of Min protein waves



Loose, 2008

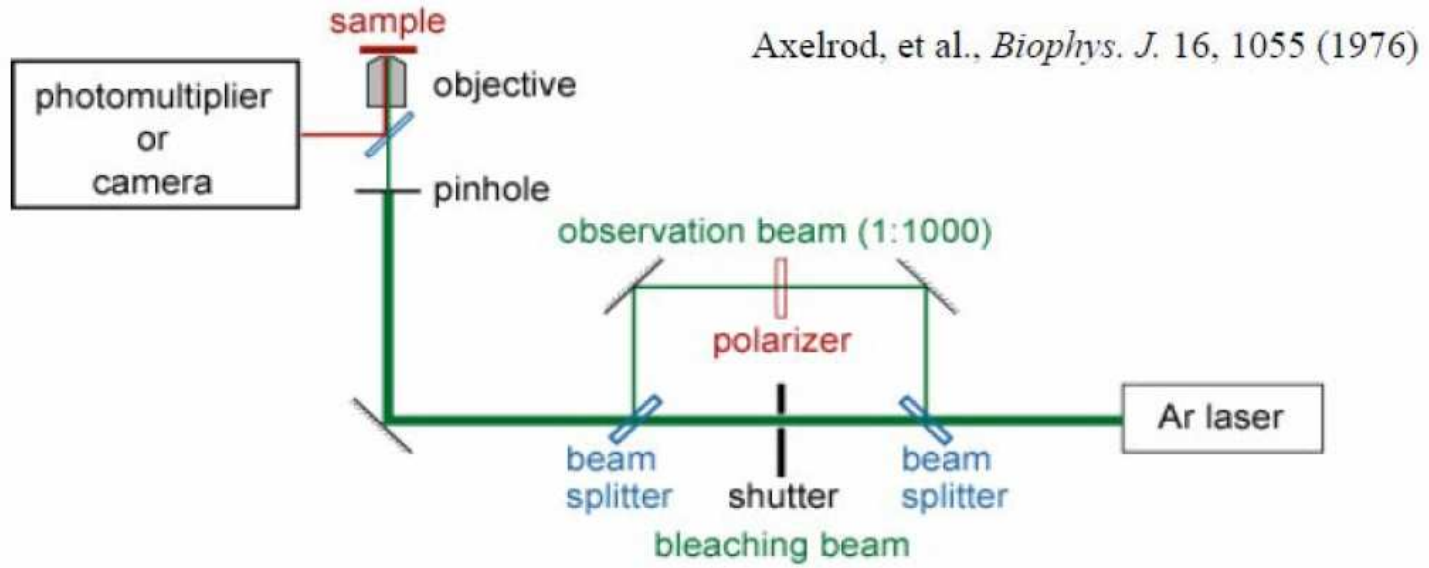
Synchronization of Min protein waves



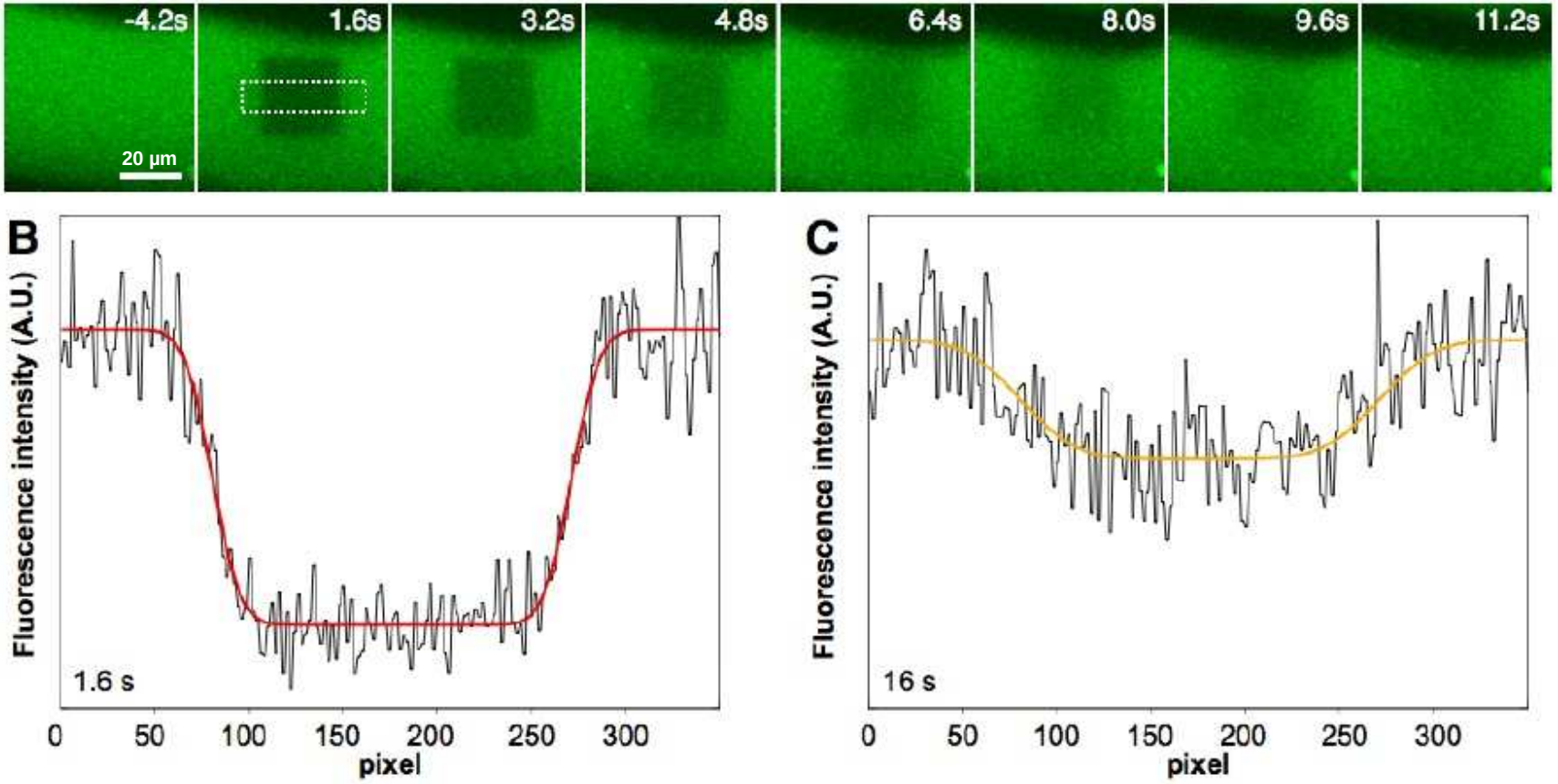
Loose, 2008

Investigation of protein mobility

- fluorescence photobleaching experiments to study protein mobility during wave propagation



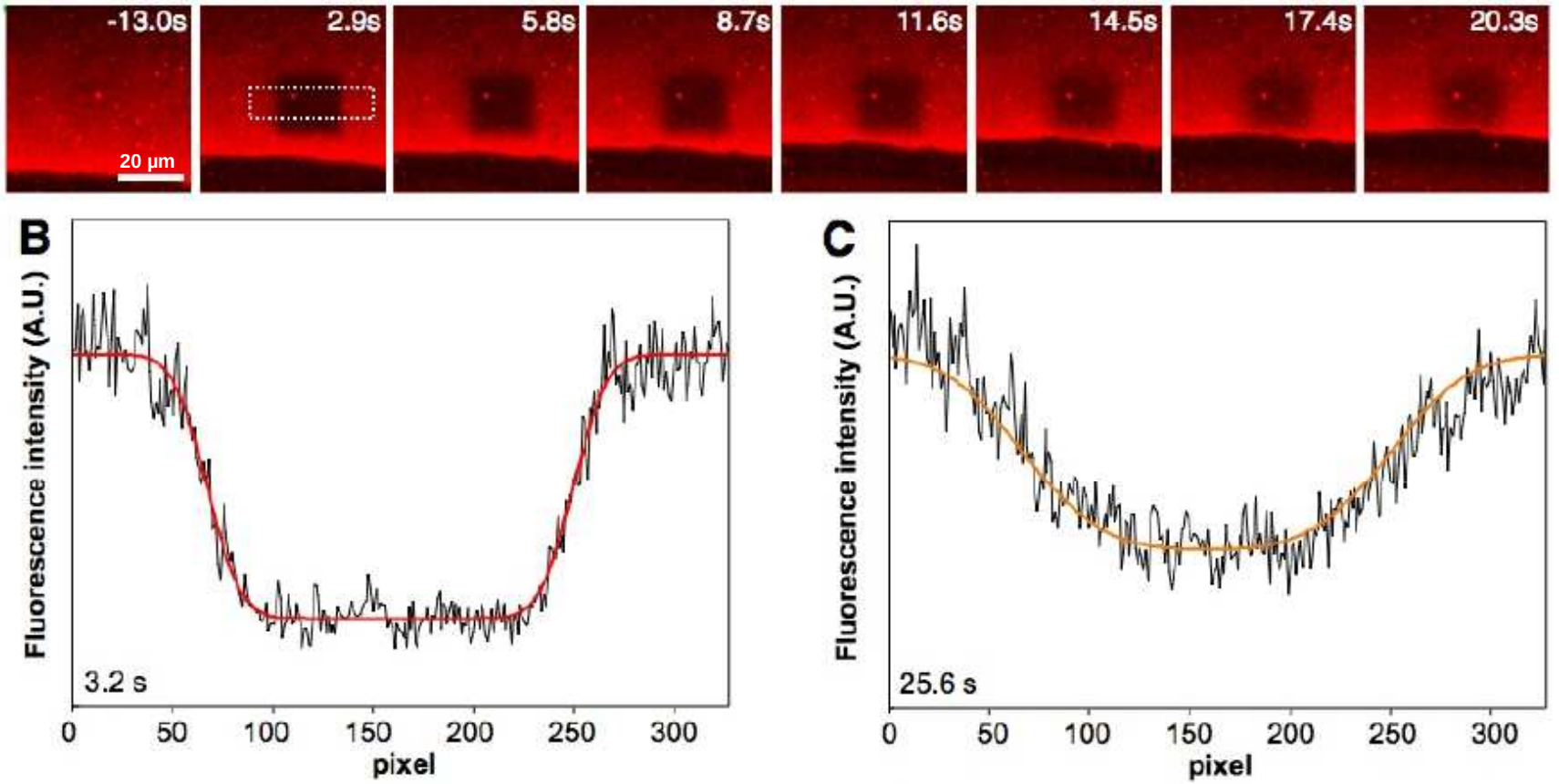
Investigation of MinD mobility



- bleached area remained at original position on the membrane during wave propagation

Loose, 2008

Investigation of MinE mobility



- same results for MinE photobleaching experiments

Loose, 2008

Investigation of protein mobility

- waves were not result of protein translocation

→ surface waves were generated by sequential rounds of detachment and reattachment of proteins from the soluble pool

Computational model

- observed surface waves were qualitatively different from the behavior predicted by existing theories

- Loose *et al*. developed a new computational model

  → can describe the situation in vitro and in vivo

  → mechanism in vitro may also drive Min oscillation in vivo

Summary

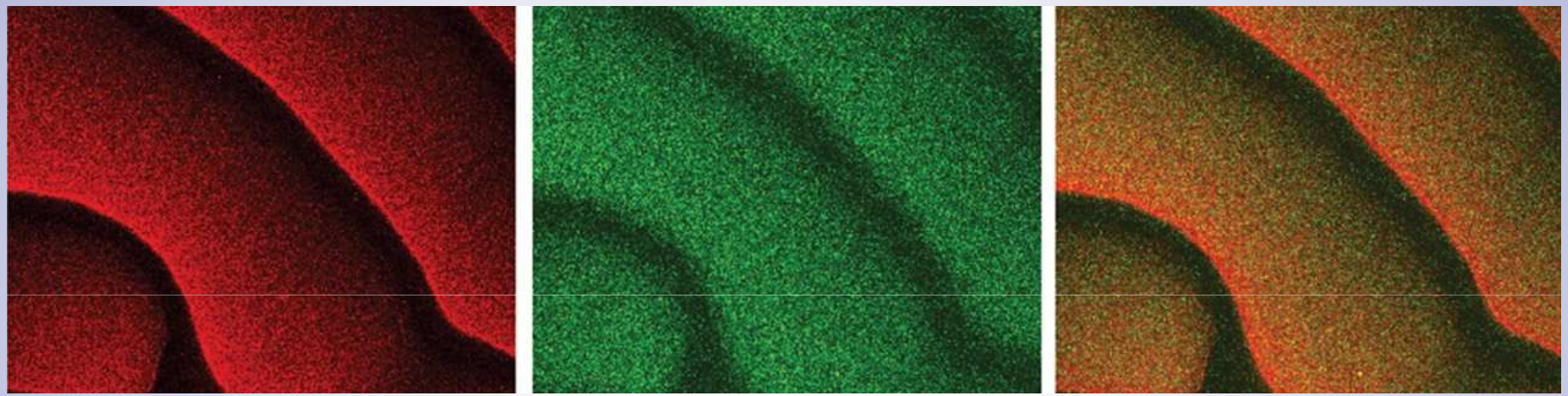
- Min oscillation is a significant example of self-organization in bacteria

- complex biological behavior can emerge from a limited number of components:

  - two proteins

  - a membrane

  - ATP



Thank you for your attention!