Active depinning of bacterial droplets: the collective surfing of *Bacillus subtilis*

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Context: Surface translocation of microbes
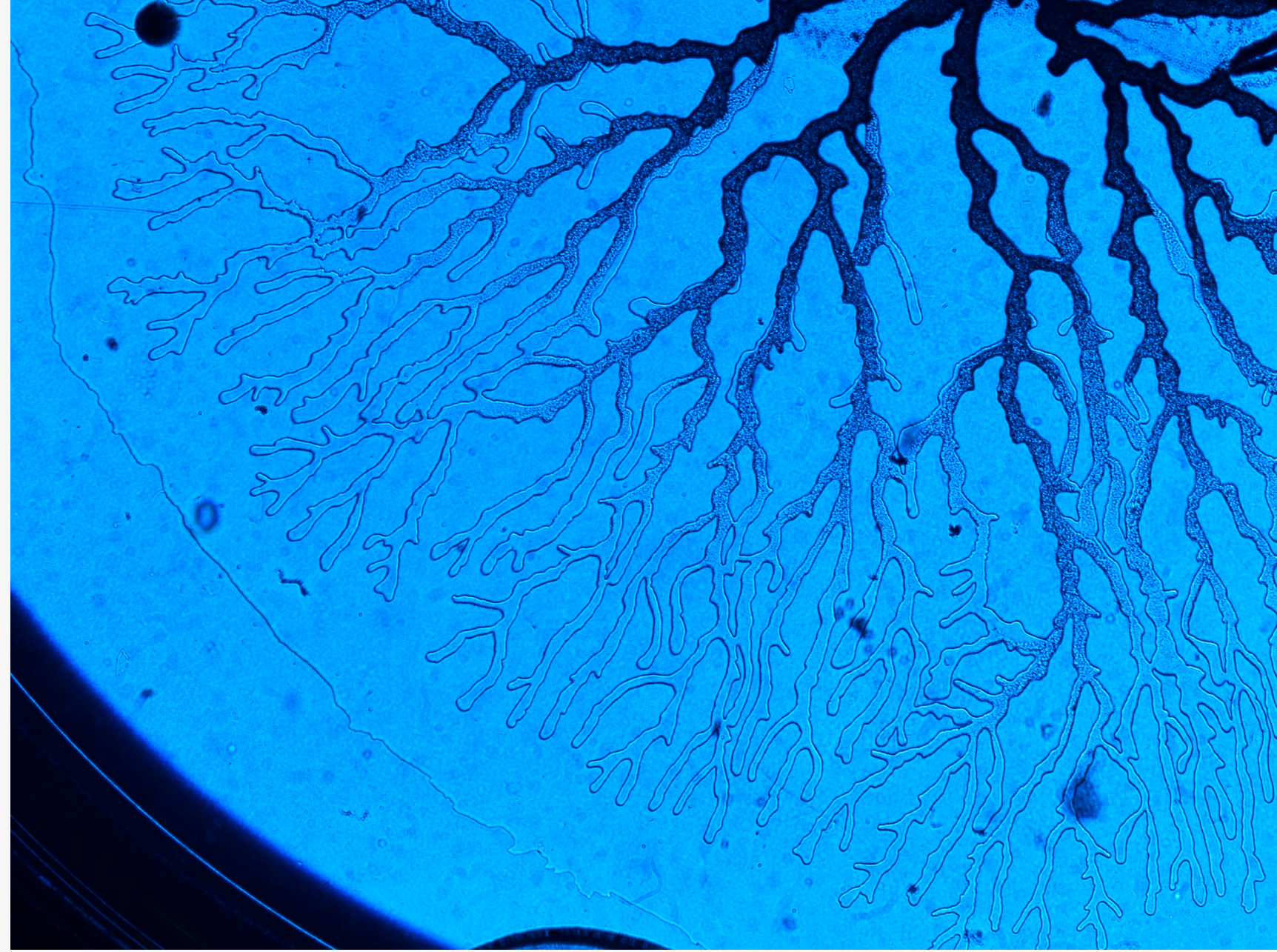
Swarming *B. subtilis*

- ▶ Rich phenomenology, mechanisms unclear
- ▶ Often sharp colony boundary: no leaving colony alone
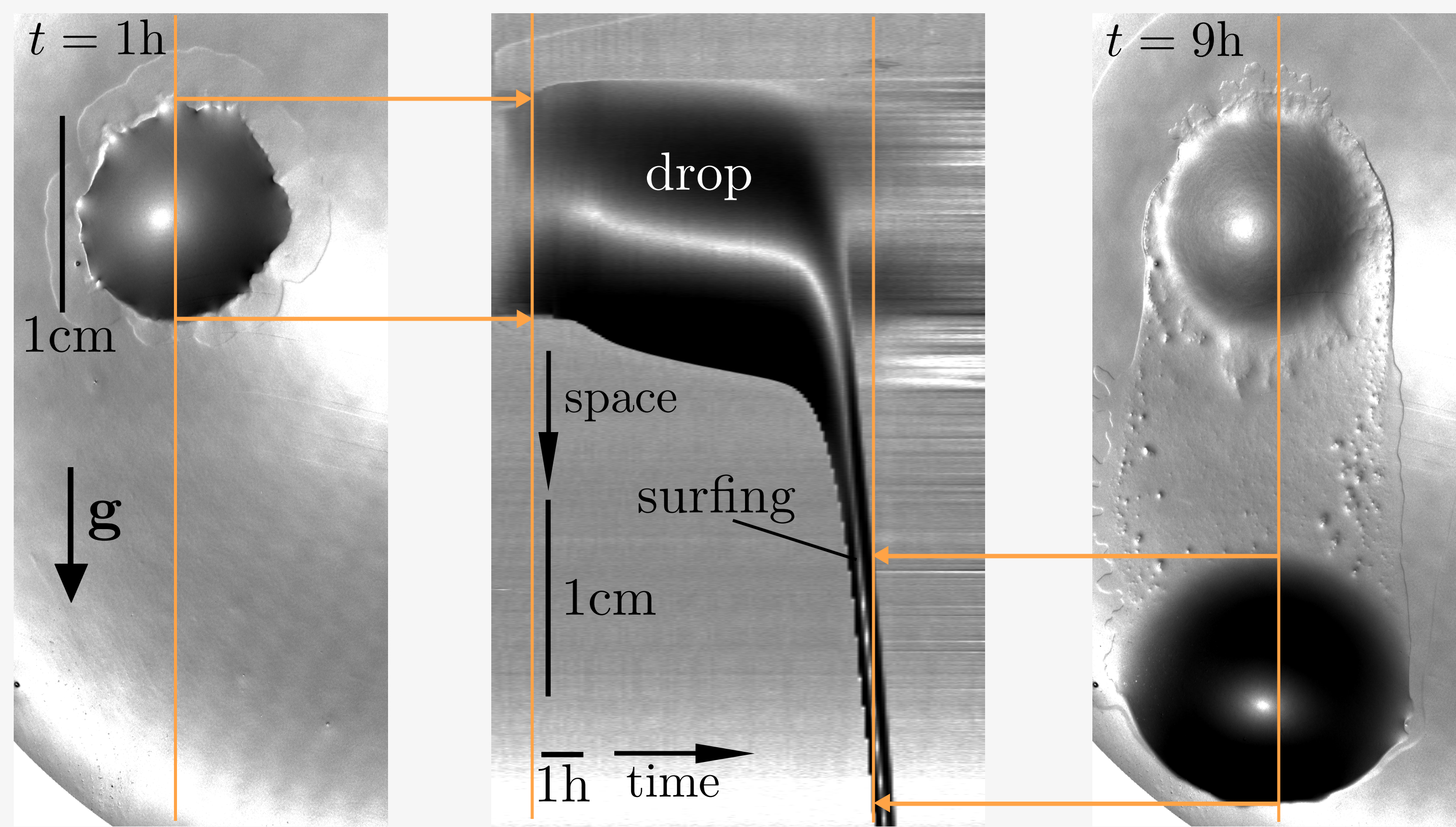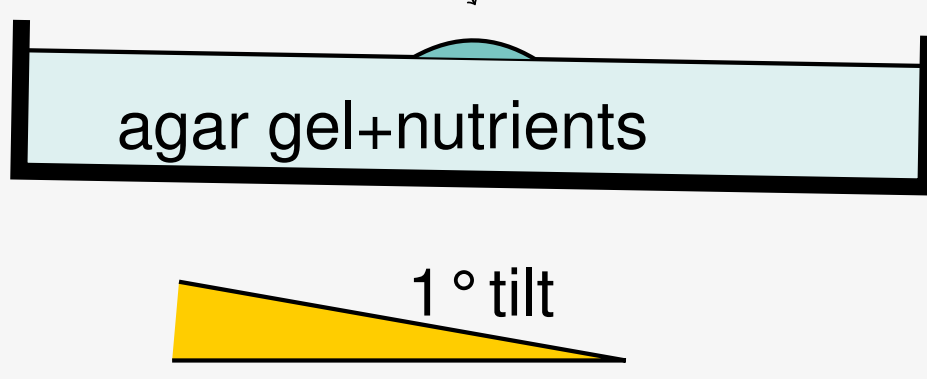- ▶ Mouvement strongly constrained by interfacial forces

$\gamma L \sim$

$10^{-6}\,$N	$2 \cdot 10^{-7}\,$N
$10^{-9}\,$N	Flagellar propulsion
$10^{-12}\,$N	$10^{-12}\,$N
$10^{-15}\,$N	Weight $5 \cdot 10^{-14}\,$N

Capillary forces enormous at bacterial scale

Sketch of capillary forces (vertical cut)
Bacteria confined between substrate and free surface.

Model substrate: Agar hydrogel
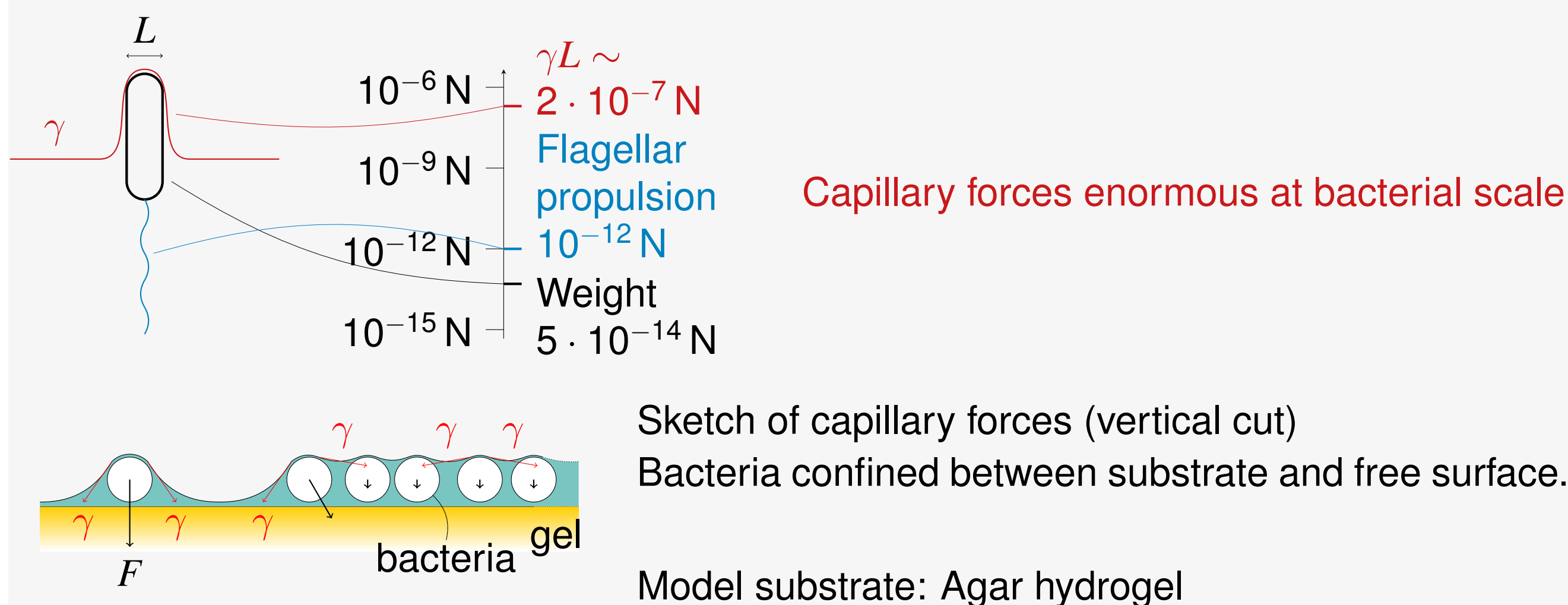
- ▶ Role of wetting and contact line depinning ?

Wetting of bacterial droplets

- ▶ Wetting hysteresis pins even millimetric water droplets to vertical

Petri dish with gel

water 2 μl

- ▶ Bacteria depin their drop

bacterial suspension 2 μl, 1 % vol.

agar gel+nutrients

1° tilt

$t = 1$h $t = 9$h

1cm

g

drop

space

surfing

1cm

1h   time

- ▶ Bacteria achieve depinning on very shallow slopes . . .

↑ drop speed

$Ca = \dfrac{\eta U}{\gamma}$

T, RH

V_i, OD t_d, t_g

α

water drop sliding threshold

Bond number

$$Bo = \frac{\text{drop weight}}{\text{capillary forces}} = \frac{\rho V g \sin \alpha}{V^{1/3}\,\gamma}$$

Bo

drop weight →

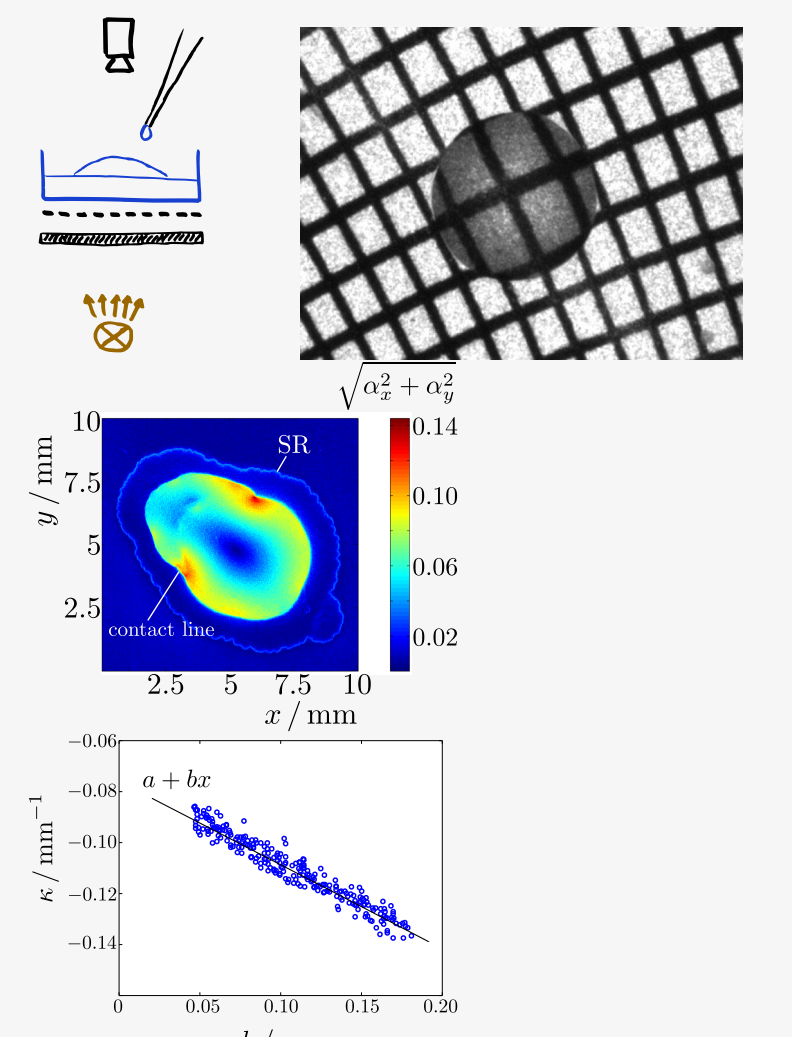- ▲ . . . initial droplet of bacterial suspension
- ★ . . . water
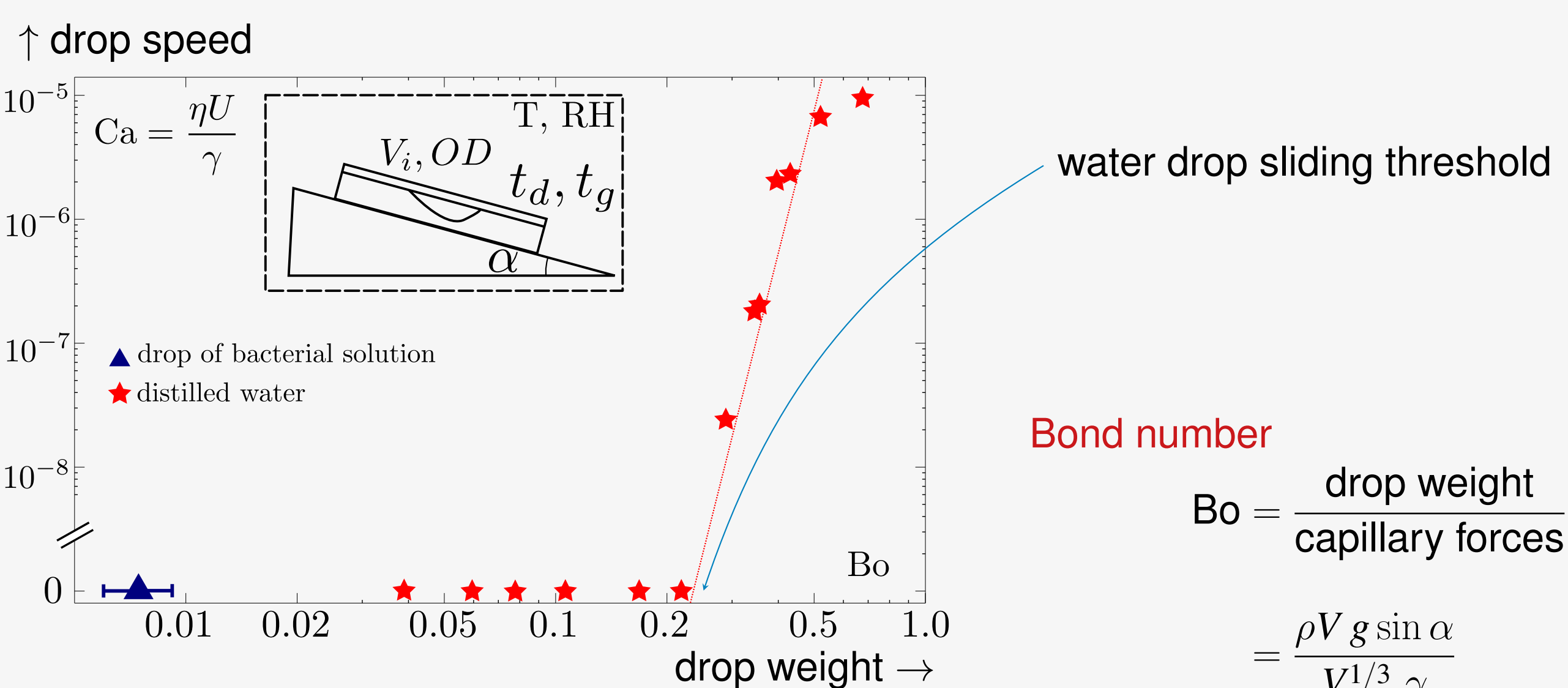
- ▶ . . . but this *colony surfing* occurs only after several hours.

B. subtilis produces biosurfactant *surfactin* ▶ reduces surface tension

$\gamma/(\mathrm{mN/m})$

surfactin at 0.1 g/L

$OD \approx 0.27 (SN)$

$OD \approx 0.27$

$OD \approx 1.5$

t/min
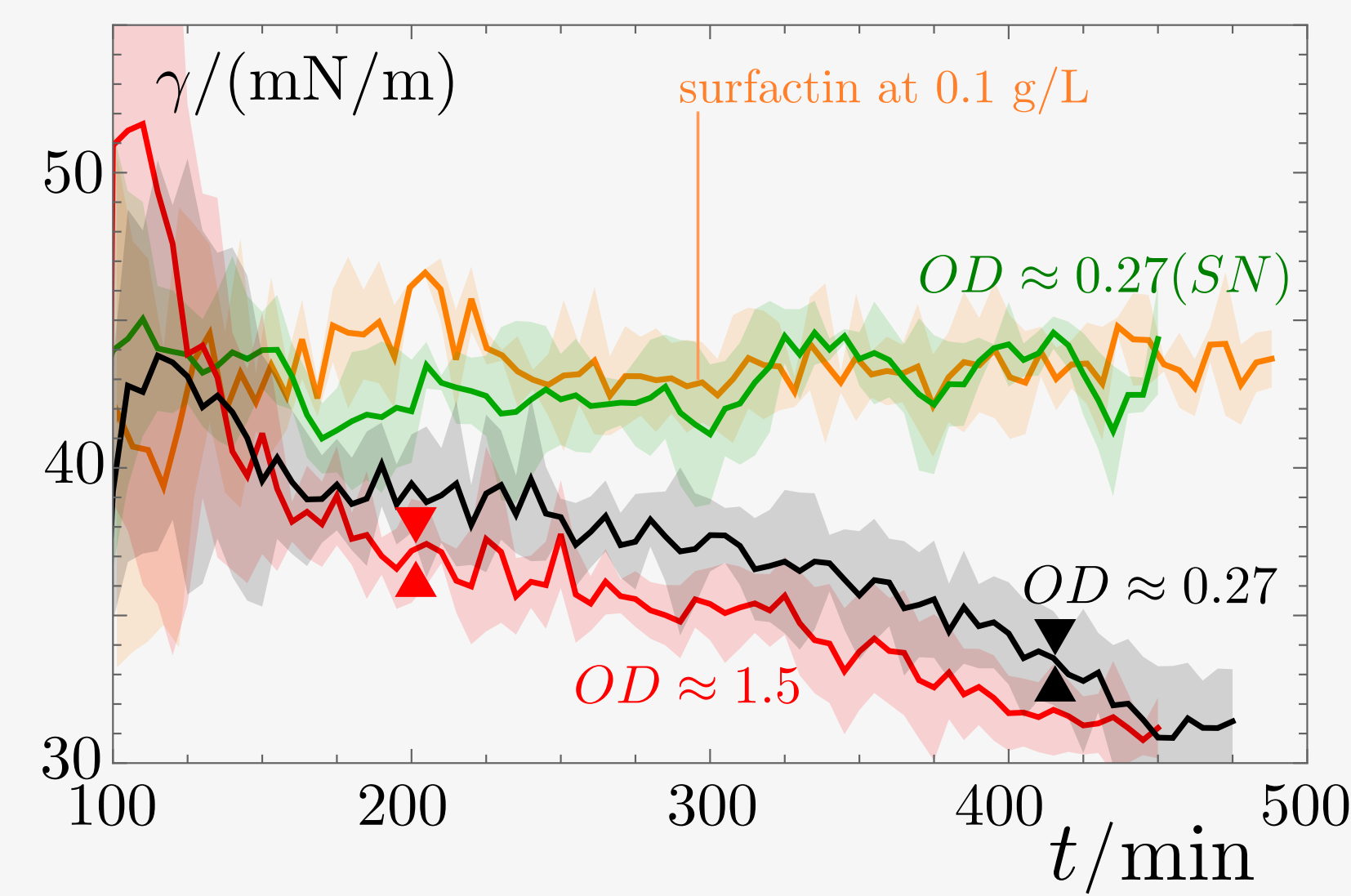
Moving grid profilometry

Drop volume increases prior to depinning

$\dfrac{V}{V_i}$

surfactin solution 0.1 g/L

strain 168 (sfp⁻)

$OD \approx 1.5$

$OD \approx 0.27$

$OD \approx 0.27 (SN)$

$OD \approx 0(H_2O)$
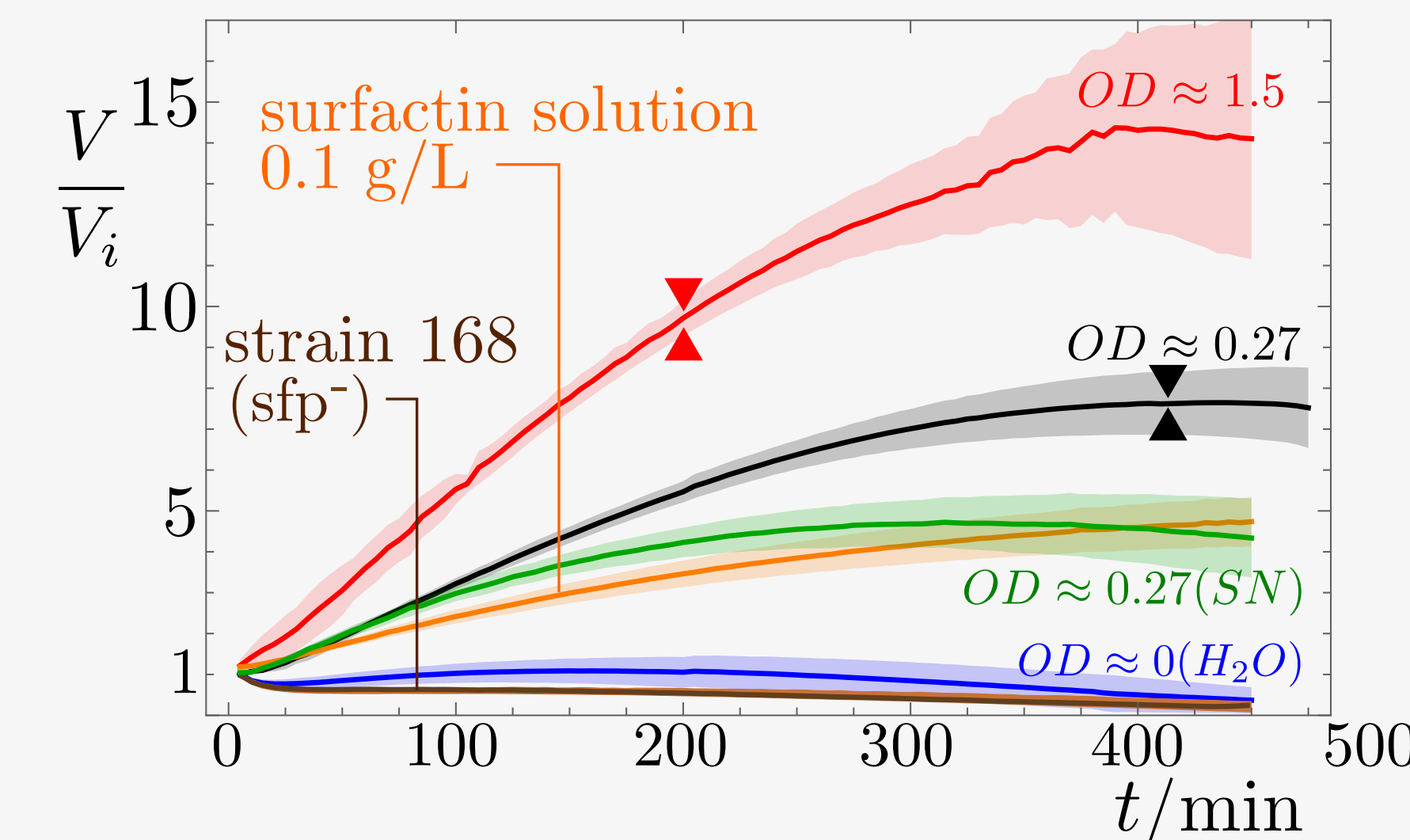
Initial volume $V_i = 2\,\mu l$

t/min

- ▶ slow volume decrease for water
- ▶ the more bacteria (OD 1.5 > OD 0.27), the faster volume increases
- ▶ supernatant (SN = bacteria removed) behaves the same initially
- ▶ surfactant deficient strain 168 ⇒ no volume increase, as for water
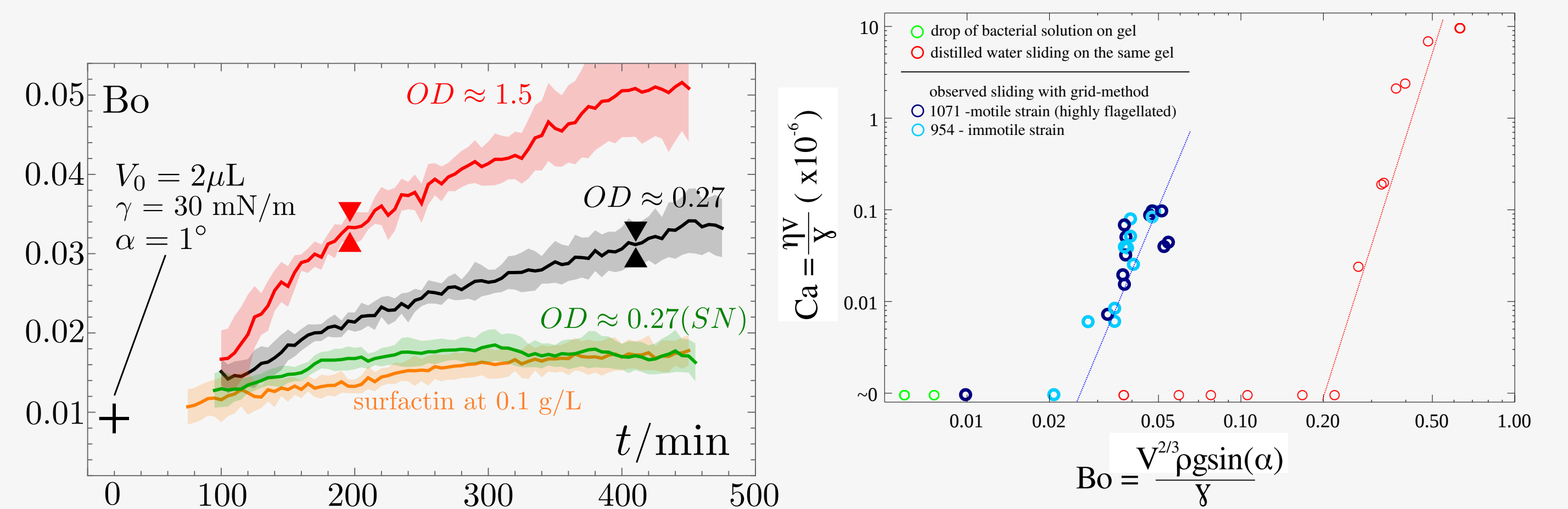- ▶ pure surfactant solution ⇒ volume increase

Bacterial droplets harness 3 distinct effects to unpin

drop depinning condition:

Weight > Contact line pinning force

$$\rho V g \sin \alpha > \mathrm{cst.} \cdot V^{(1/3)}\,\gamma\,(\cos\theta_r - \cos\theta_a)$$

Volume ↗, Surface tension ↘ ⇒ $Bo = \rho V^{2/3} g \sin \alpha / \gamma$ increases,

Wetting hysteresis ↘ ⇒ critical Bond from 0.2 down to ∼0.03

Bo

$V_0 = 2\mu L$
$\gamma = 30$ mN/m
$\alpha = 1°$

$OD \approx 1.5$

$OD \approx 0.27$

$OD \approx 0.27 (SN)$

surfactin at 0.1 g/L

t/min

$Ca = \dfrac{\eta V}{\gamma}$ (×10⁻⁶)

drop of bacterial solution on gel
distilled water sliding on the same gel

observed sliding with grid-method
1071 -motile strain (highly flagellated)
954 - immotile strain

$Bo = \dfrac{V^{2/3} \rho g \sin(\alpha)}{\gamma}$

Control experiments

- ▶ Are bacteria really needed ?

1. Centrifuge & filter, keep supernatant (SN)
2. Put droplets of bacterial suspension and of SN side-by-side

$t = 1$h30 $t = 9$h40

CL

SN

g
$\alpha = 1°$

$t = 10$h00
contamination

$t = 10$h50   1h time   B

SN

contamination

surfing

⇒ Contamination triggers drop sliding

- ▶ How many bacteria are needed ?

Fraction of colony surfing events ($N = 5$) for different bacterial concentrations (OD) in the deposited drop.

$p = \dfrac{N_{surf}}{N}$

$p = \dfrac{5}{5}$

$p = 0$

OD
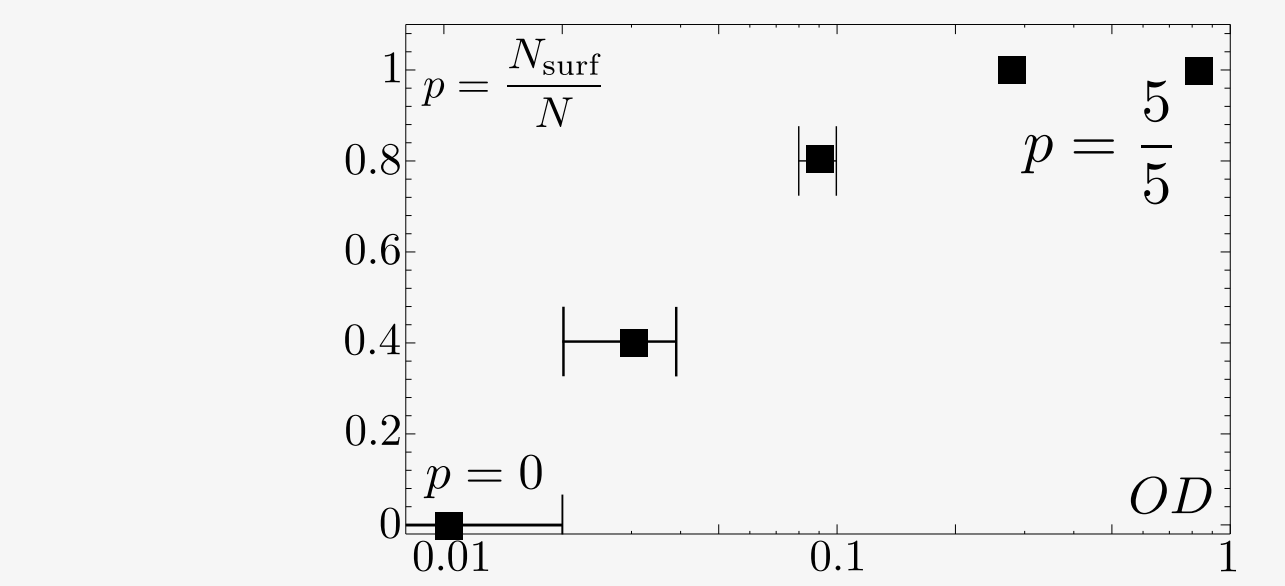
- ▶ OD ≤ 0.02   no surfing (out of five exp.)
- ▶ OD ≥ 0.27   surfing always occurs

- ▶ Do propelling forces contribute to depinning ?

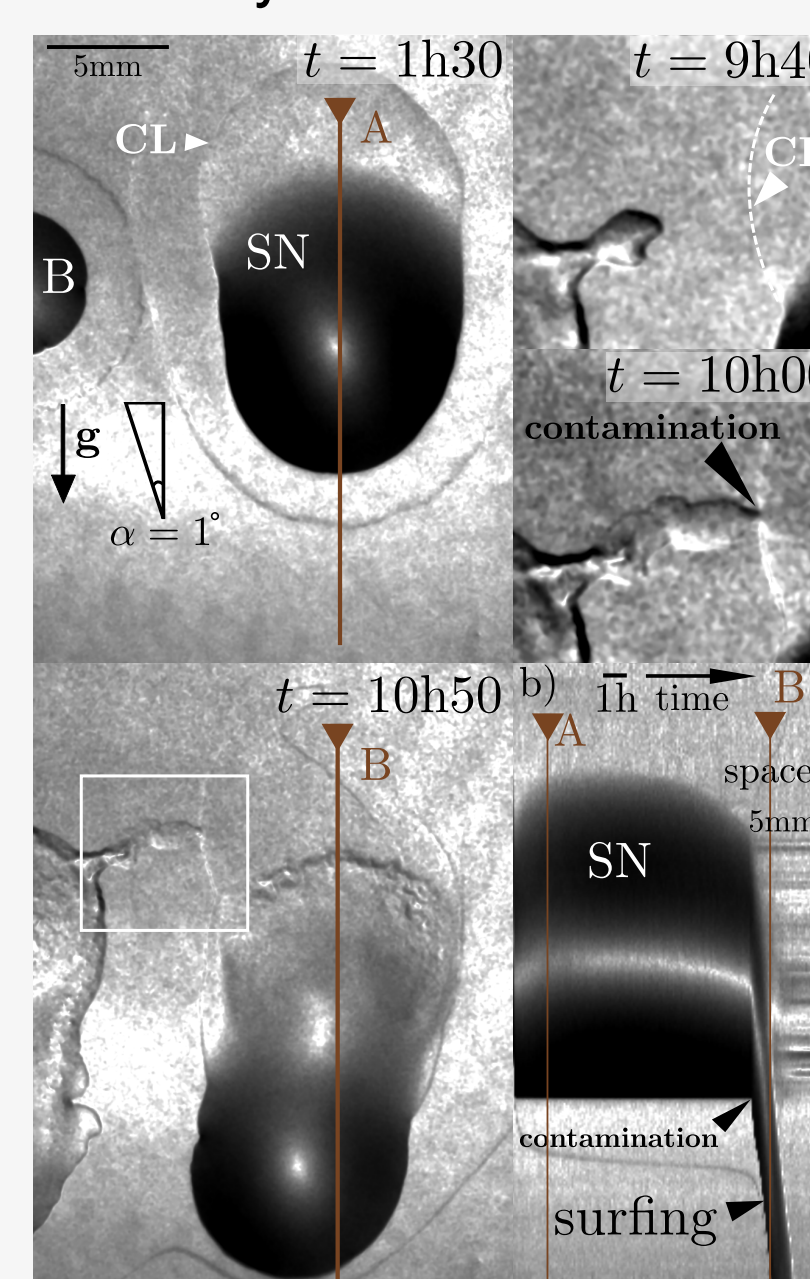Pressure from active swimmers could help depin contact line

⤳ Compare with surfing probability for non-flagellated mutant

$p = \dfrac{N_{surf}}{N}$

motile
non-motile

→ gel slope α / deg
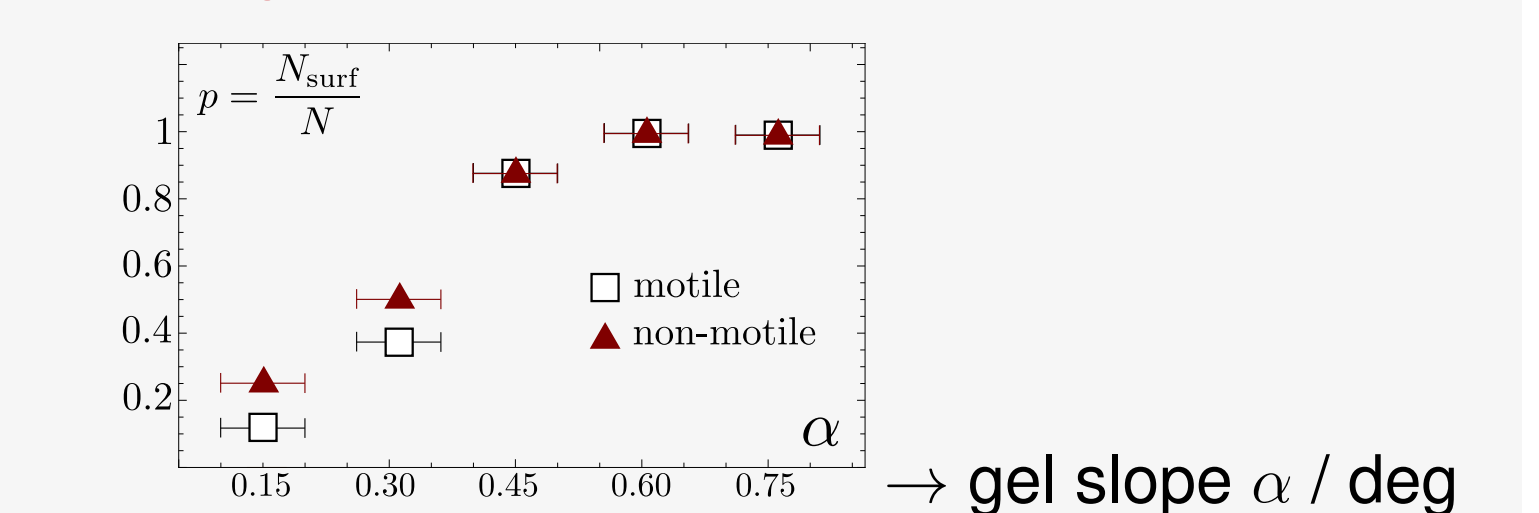
⇒ Colony surfing independent of individual motility