# Motile bacteria in giant unilamellar vesicles

*A cautionary tale*

**Wilson Poon**

Lucas Le Nagard, Aidan Brown, Alexander Morozov, Vincent Martinez

*School of Physics & Astronomy, The University of Edinburgh*

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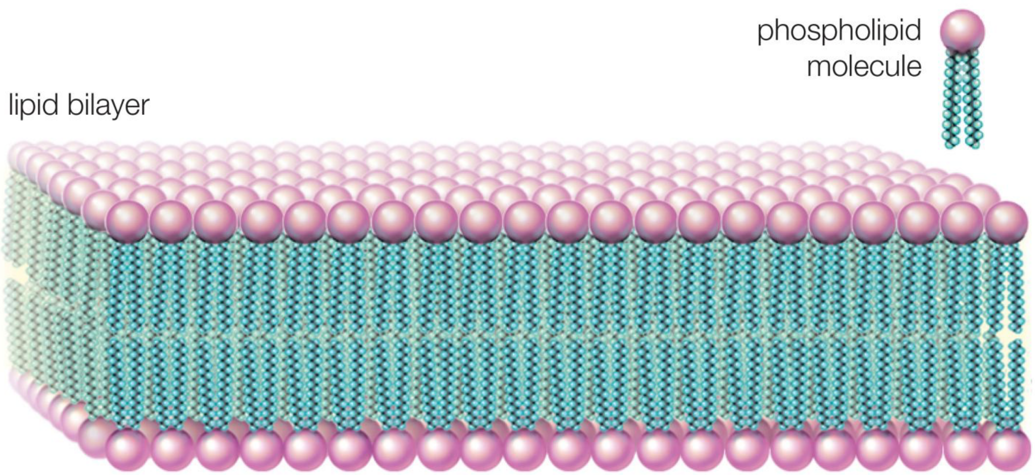
*Department of Physics, Durham University*

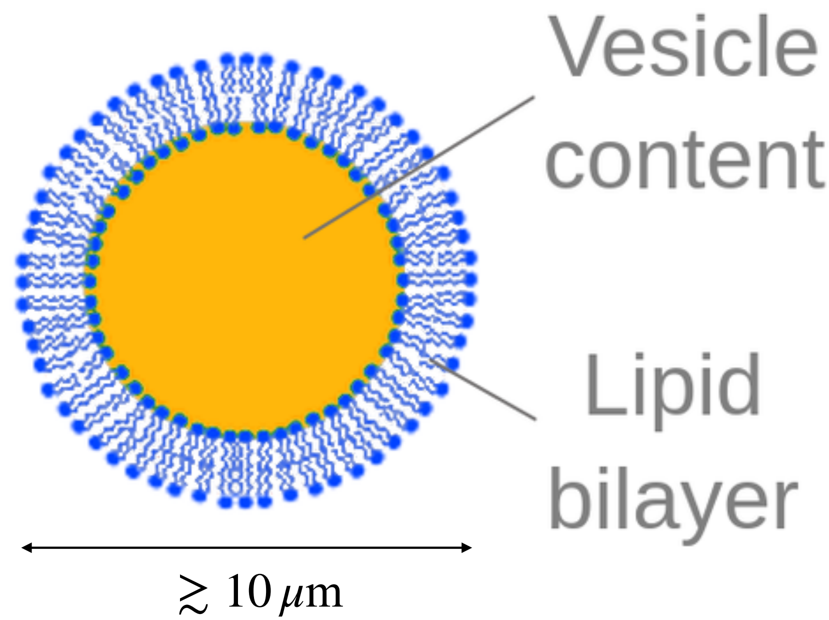

**The cautionary tale**

Passive systems are all alike; every active system is unique in its own way.*

*Happy families are all alike; every unhappy family is unhappy in its own way. (Tolstoy, *Anna Karenina*)

What is a giant unilamellar vesicle (GUV)?



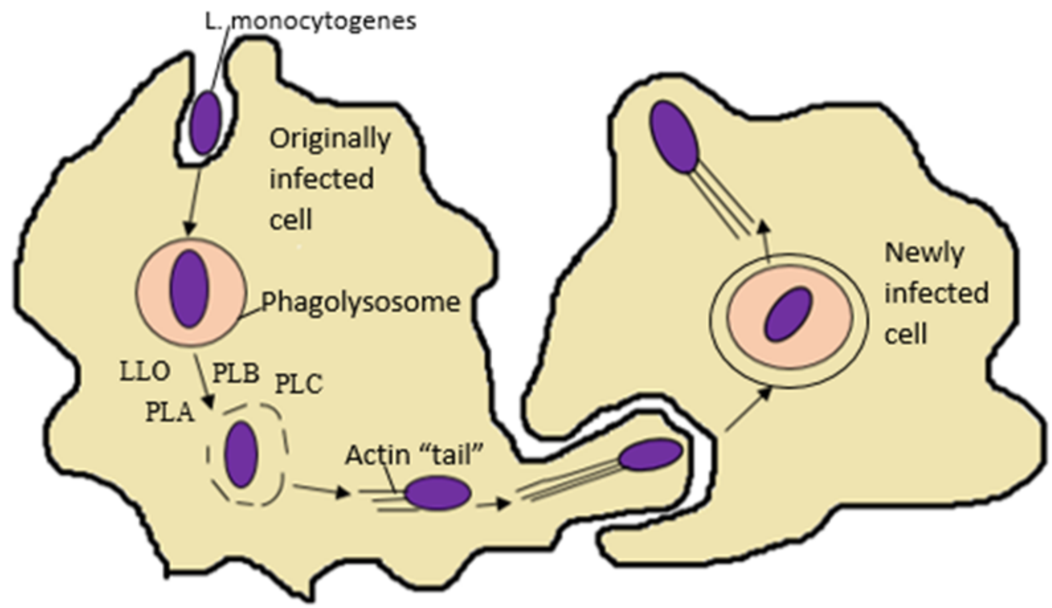

= empty (eukaryotic) cell!

Why may anyone want to put motile bacteria inside a GUV?

1. Potential medical relevance

*Listeria monocytogenes*

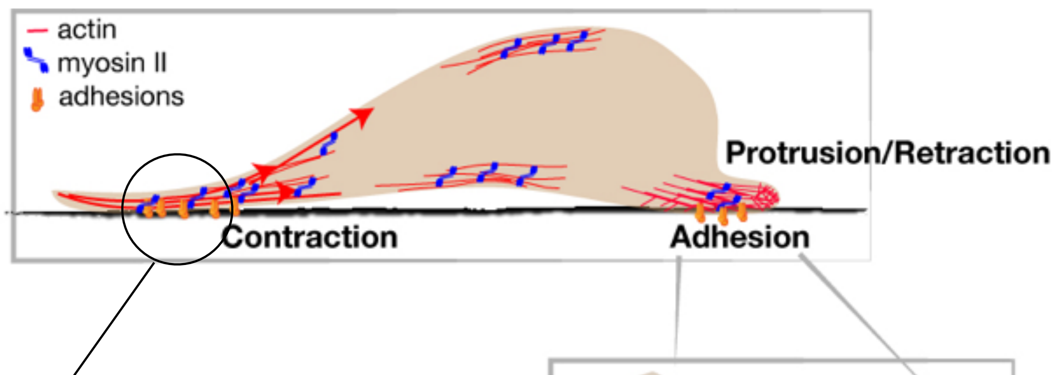
- Common contaminant of pâté
- Mortality rate 20-30%

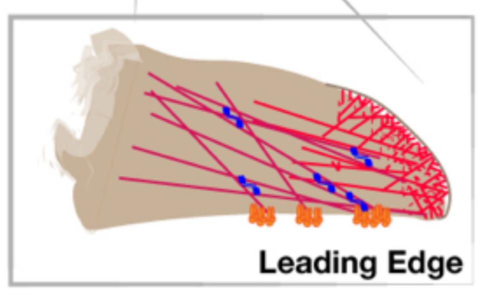


Why may anyone want to put motile bacteria inside a GUV?

2. Fundamental interest for active matter physics

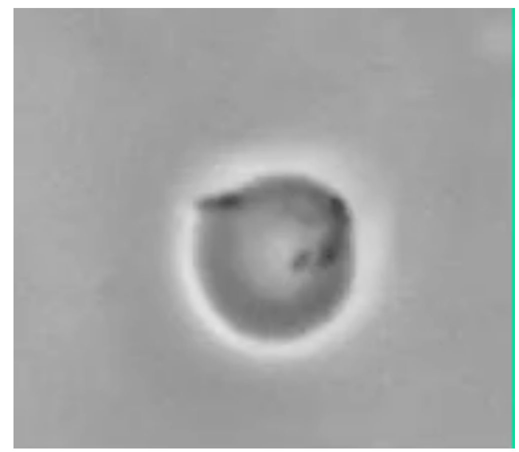
- Active-passive mixture




Cytoskeleton = active gel (myosin ‘walking’ on F-actin) + passive membrane

A different way of coupling activity to a passive membrane …



… is there anything new?

Why may anyone want to put motile bacteria inside a GUV?

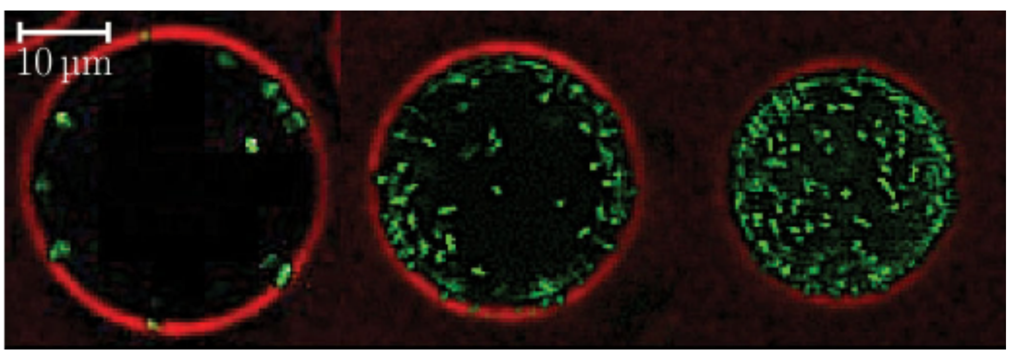
2. Fundamental interest for active matter physics

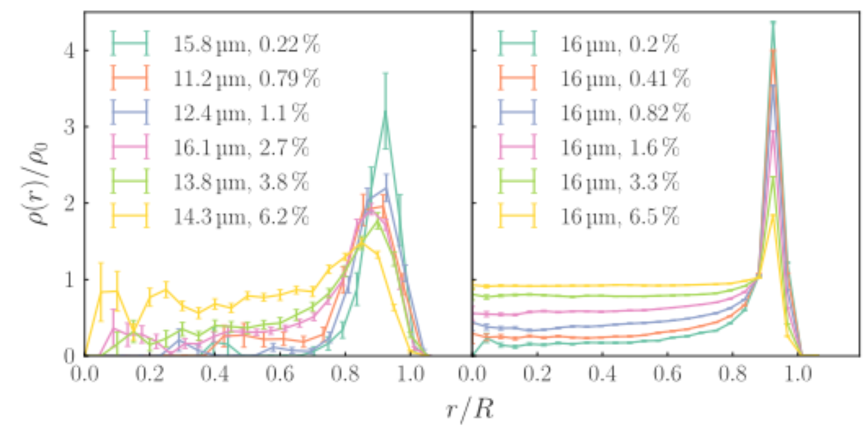
• Swimming in confinement

PRL **113,** 268101 (2014) PHYSICAL REVIEW LETTERS week ending 31 DECEMBER 2014

**Filling an Emulsion Drop with Motile Bacteria**

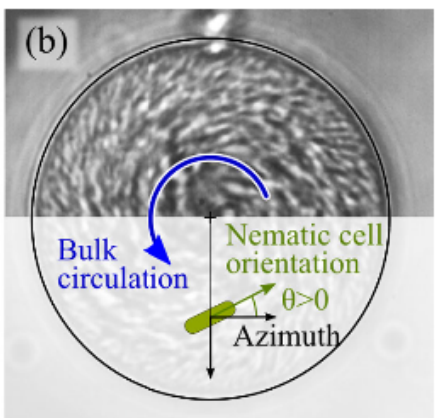
I. D. Vladescu,* E. J. Marsden,† J. Schwarz-Linek, V. A. Martinez, J. Arlt, A. N. Morozov, D. Marenduzzo, M. E. Cates, and W. C. K. Poon‡

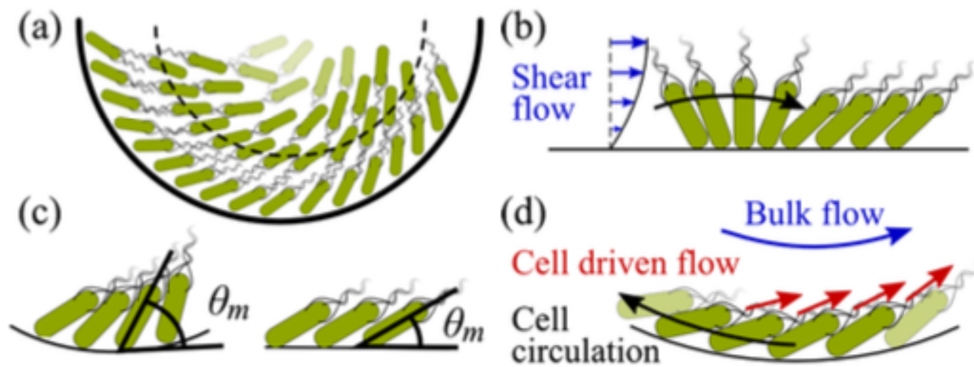


# Confinement Stabilizes a Bacterial Suspension into a Spiral Vortex

Hugo Wioland,[1] Francis G. Woodhouse,[1] Jörn Dunkel,[1] John O. Kessler,[2] and Raymond E. Goldstein[1]

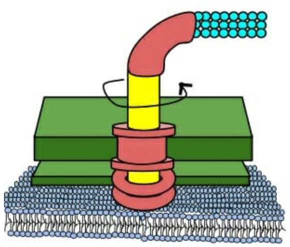


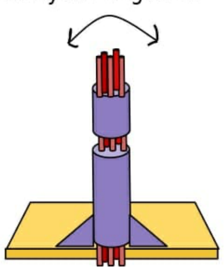
# Why may anyone want to put motile bacteria inside a GUV?

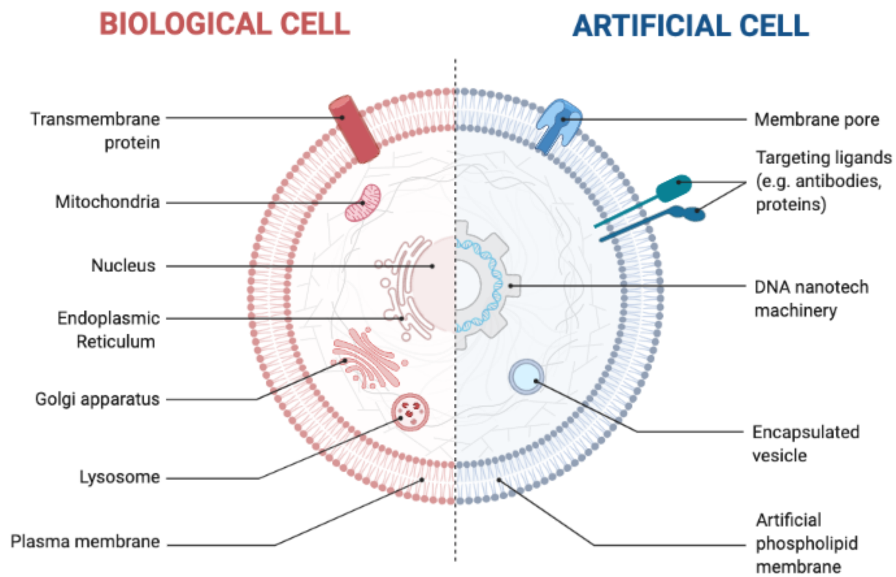
## 3. Conferring motility on artificial cells?

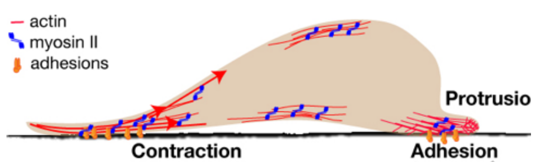
Prokaryoric flagellum



Eukaryoric flagellum



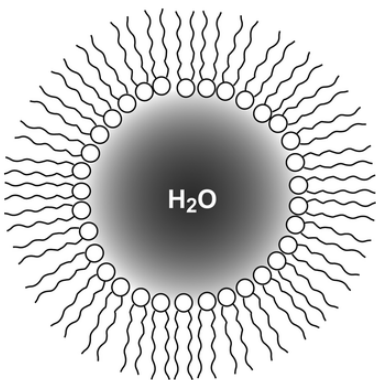


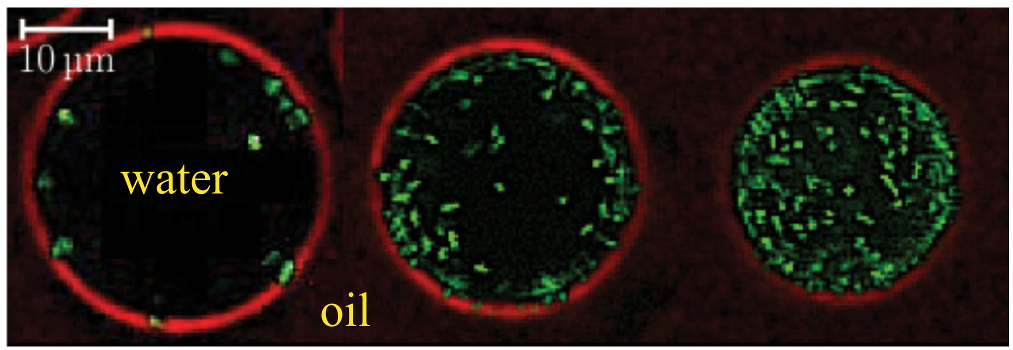




Siton-Mendelson & Bernheim-Groswasser, *Cell Adhesion & Migration* **10** (2016) 461-474

# Previous work

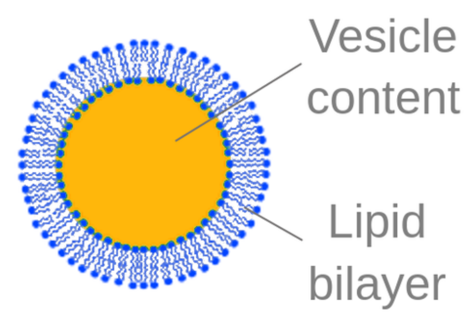
Swimming *Escherichia coli* in **emulsion** drops




Advantage: easy to prepare …
… shake up a bacterial culture with sunflower oil …
… great for studying confinement effects!

Disadvantage: not a bilayer …
… can't use to study coupling activity to *membrane*



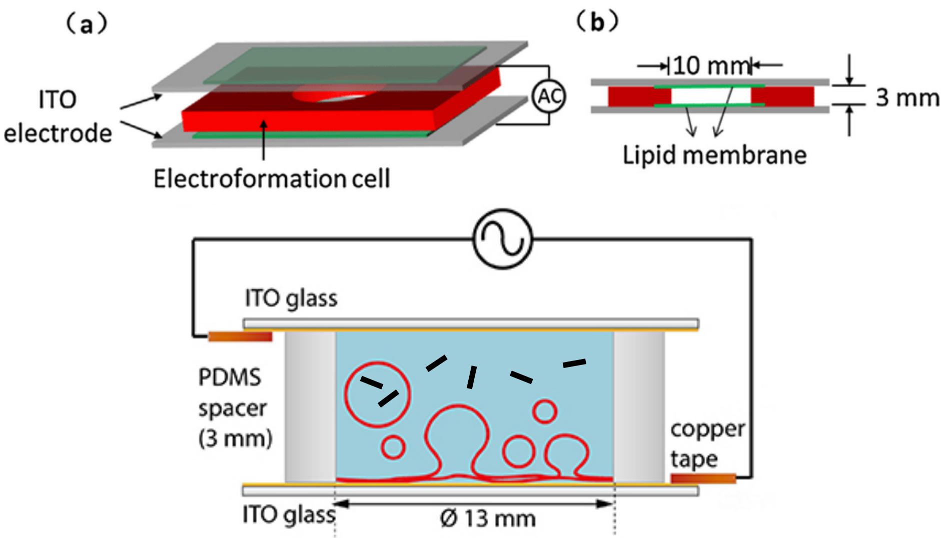
PHYSICAL REVIEW LETTERS **124,** 158102 (2020)

Induced by *Bacillus subtilis*

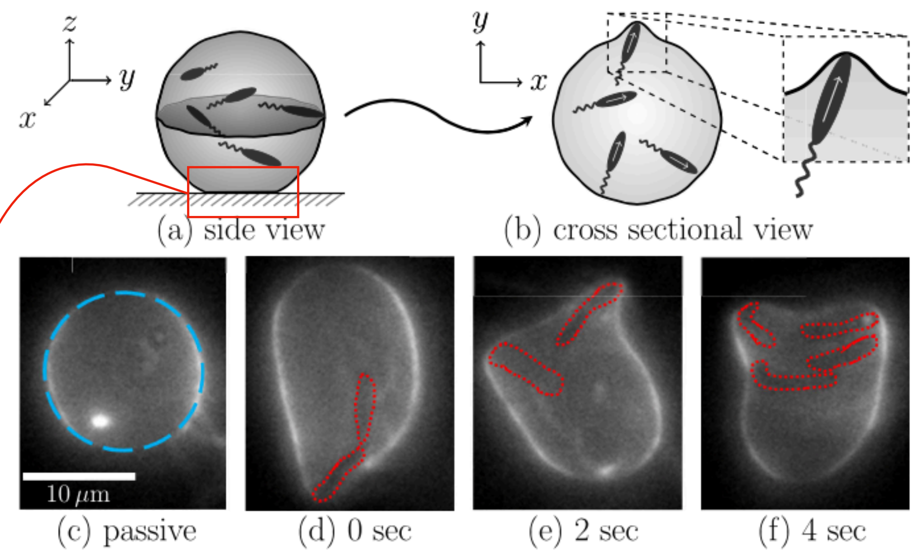
**Active Contact Forces Drive Nonequilibrium Fluctuations in Membrane Vesicles**

Sho C. Takatori[1,*] and Amaresh Sahu[2,†]
[1]*Department of Chemical Engineering, University of California, Santa Barbara, California 93106, USA*
[2]*Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California 94720, USA*

"Electroformation"
= AC applied to lipid-coated electrodes

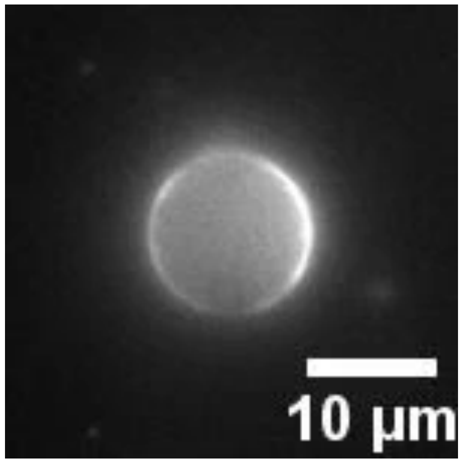


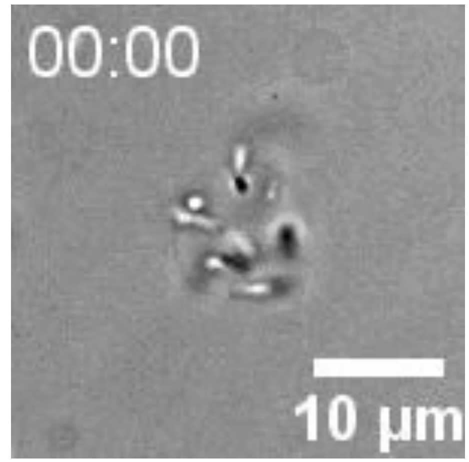

Mechanism (without or with bacteria) remains mysterious!




Advantage: again, easy to do …
Disadvantages: GUV often remains attached …
… and properties hard to control

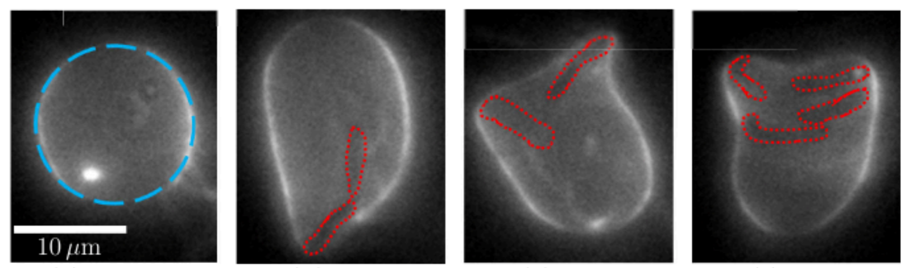
GUVs show thermal fluctuations




Passive: $\frac{1}{2}\kappa\langle x^2\rangle = \frac{1}{2}k_BT$

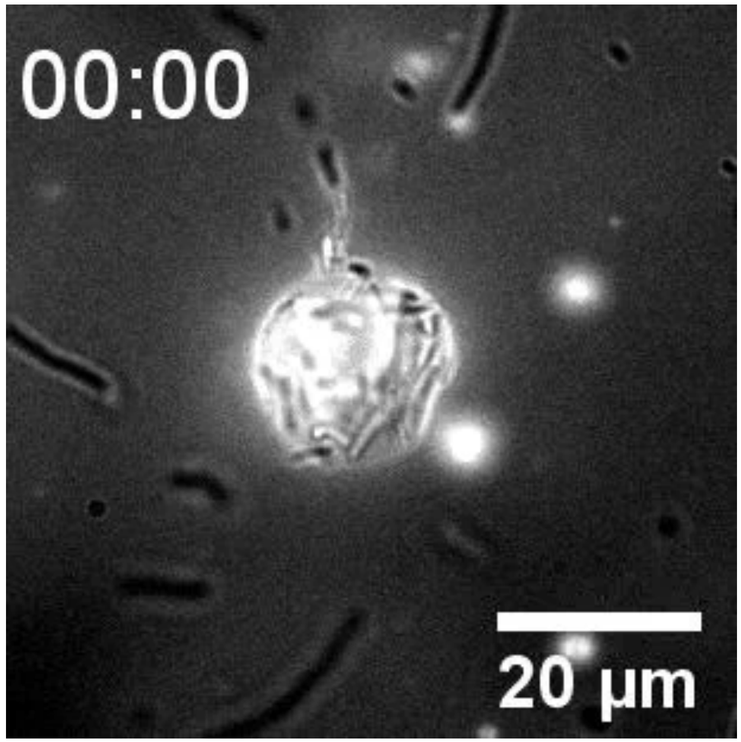
Measure $\langle x^2\rangle \rightarrow \kappa$




(c) passive (d) 0 sec (e) 2 sec (f) 4 sec

Measure $\langle x^2\rangle \rightarrow$ 'active fluctuations'

‘Extreme fluctuations’ …




… bacteria create and swim into *transient* membrane tubes!

52 | Nature | Vol 586 | 1 October 2020

Article

# Active particles induce large shape deformations in giant lipid vesicles

https://doi.org/10.1038/s41586-020-2730-x
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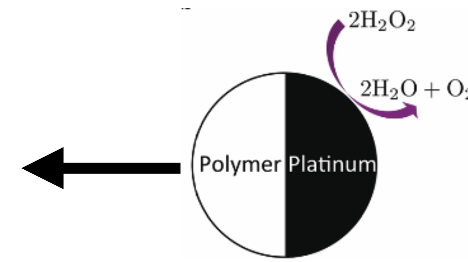
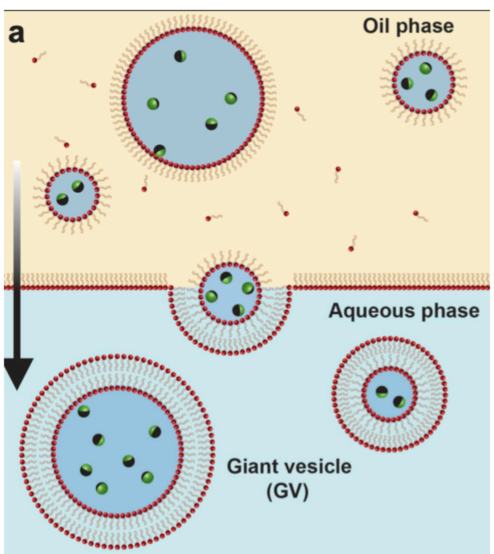
Accepted: 24 July 2020
Published online: 30 September 2020

Hanumantha Rao Vutukuri[1,3✉], Masoud Hoore[2,3], Clara Abaurrea-Velasco[2], Lennard van Buren[1], Alessandro Dutto[1], Thorsten Auth[2], Dmitry A. Fedosov[2], Gerhard Gompper[2✉] & Jan Vermant[1]

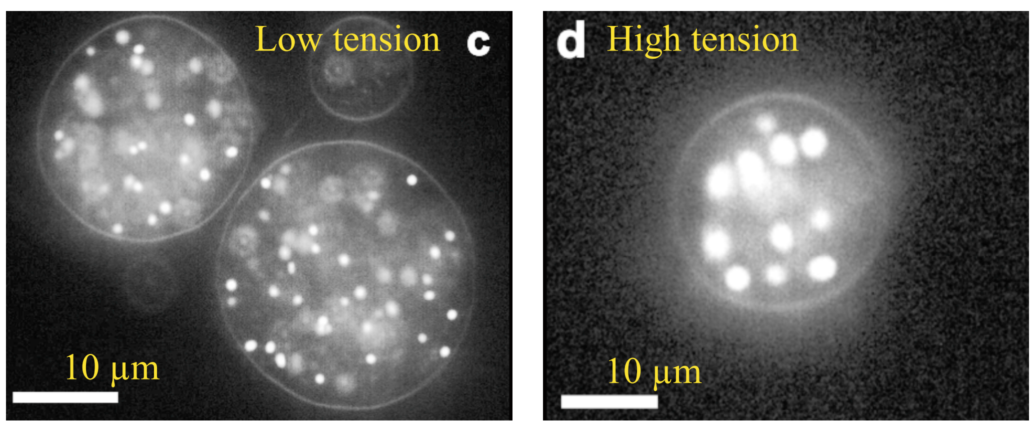


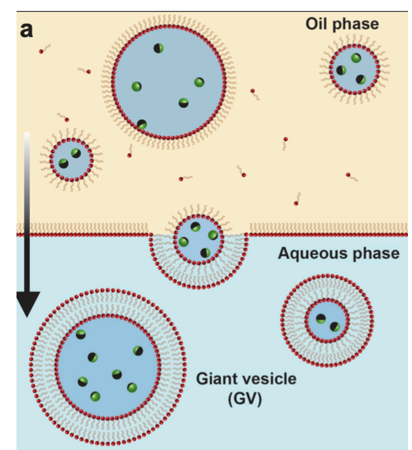

Certainly *not* diffusiophoresis …
… probably combination of electrophoresis and 'rocket propulsion' [See Brown & Poon, *Soft Matter* (2014) and Eloul et al., *PRL* (2020)]

'Inverted emulsion method'



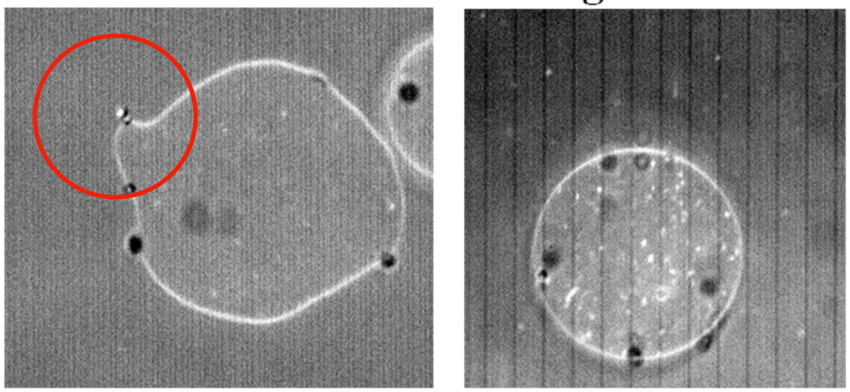

Chief advantage: can control the membrane *tension*



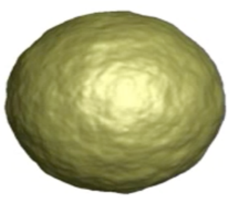




Evaporate → osmolarity ↑
→ deflation (= tension ↓)

**Low-tension** **High-tension**



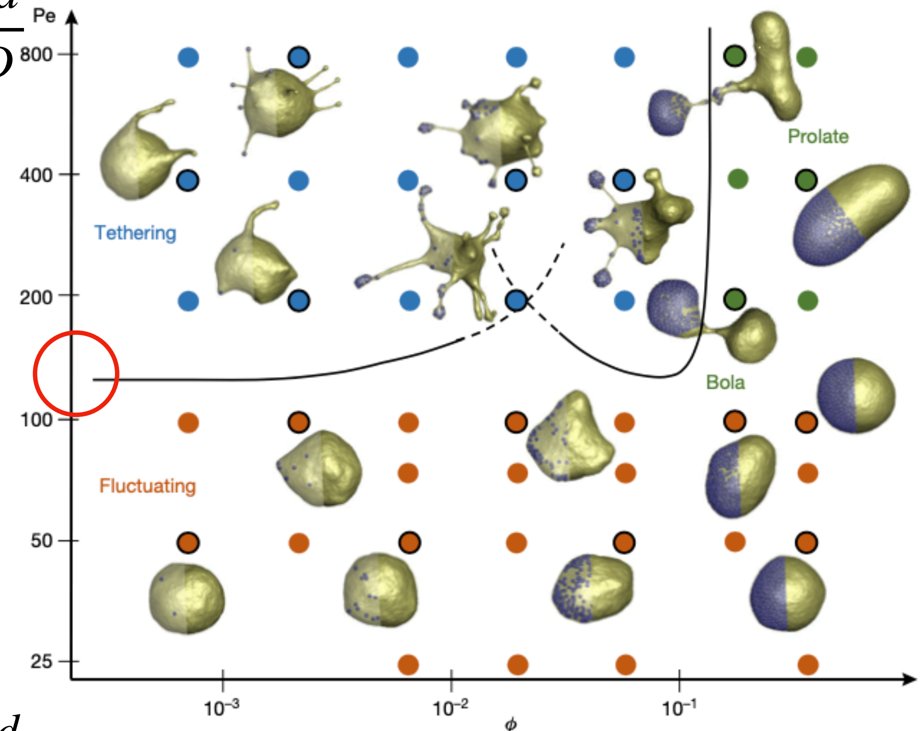
Simulations



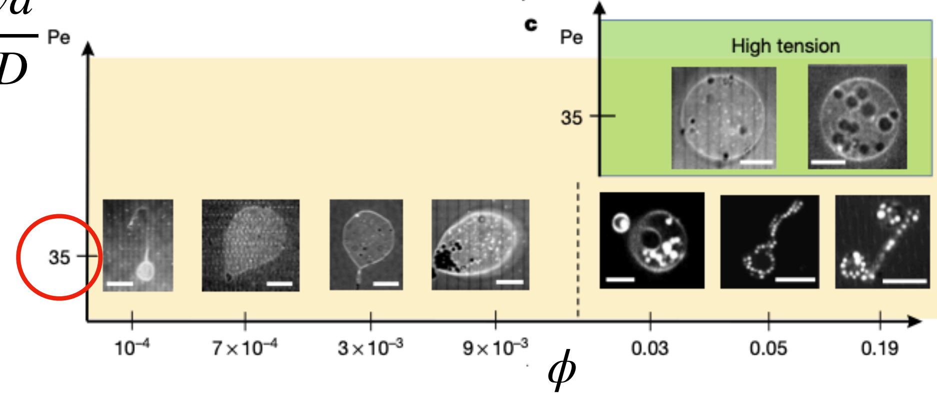
<u>Janus swimmers</u>
Diameter $d$
Speed $v$
Diffusivity $D$

Mystery: membrane tubes form at far too low Pe

$$\mathrm{Pe} = \frac{vd}{D}$$




$$\mathrm{Pe} = \frac{vd}{D}$$



VOLUME 88, NUMBER 23 PHYSICAL REVIEW LETTERS 10 JUNE 2002

# Formation and Interaction of Membrane Tubes

Imre Derényi,[1,2] Frank Jülicher,[1,3] and Jacques Prost[1]

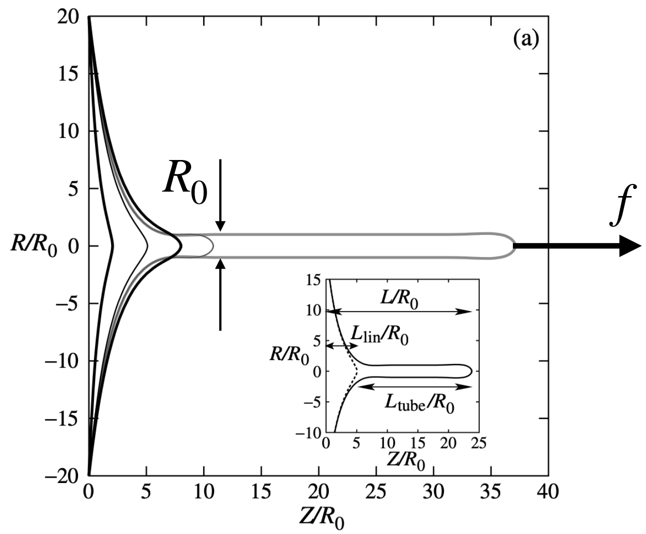
Membrane free energy

$$\mathscr{F} = \int \frac{1}{2}\kappa K^2 \, dA + \sigma A - fL$$

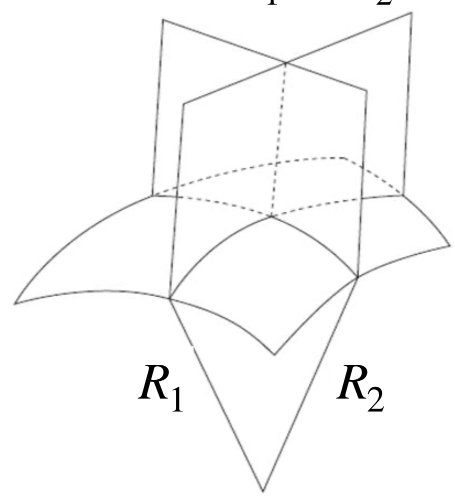
$$= \underbrace{\frac{1}{2}\frac{\kappa}{R^2}(2\pi RL)}_{\mathscr{F}_{\min} \Rightarrow R\uparrow} + \underbrace{\sigma(2\pi RL)}_{\mathscr{F}_{\min} \Rightarrow R\downarrow} - fL$$

$K =$ 'extrinsic' curvature
$\kappa =$ bending rigidity
$\sigma =$ (internal) membrane tension
$f =$ (external) force

Solve $\frac{\partial \mathscr{F}}{\partial R} = \frac{\partial \mathscr{F}}{\partial R} = 0$




$$K = \frac{1}{R_1} + \frac{1}{R_2}$$




$$f_0 = 2\pi\sqrt{2\sigma\kappa} \qquad R_0 = \sqrt{\frac{\kappa}{2\sigma}}$$

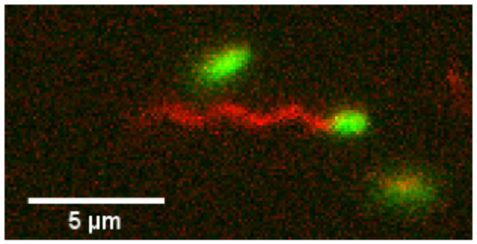
✓

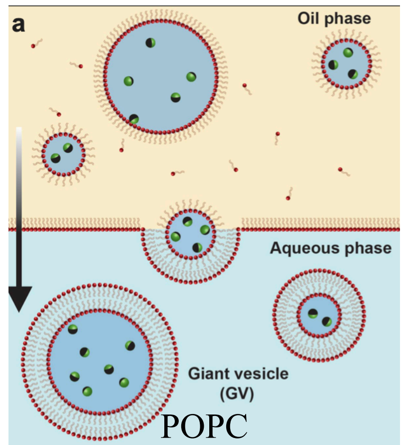
Fixed Janus propulsion $f \Rightarrow$ need soft membrane …

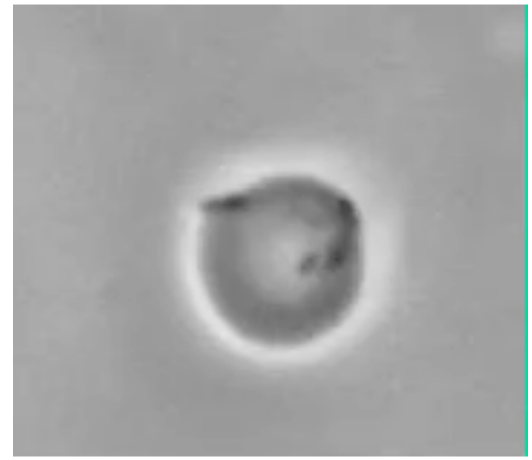
… quantitatively wrong for Janus

# Our experiments

*Escherichia coli*



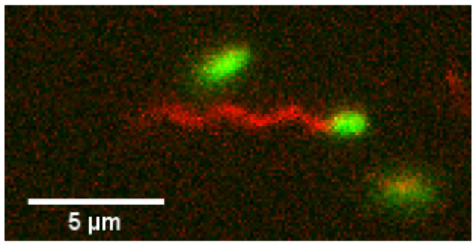



"Other people have done it with Janus swimmers and *B. subtilis* …"

Theorem: Any experiment that is worth doing is worth doing again!

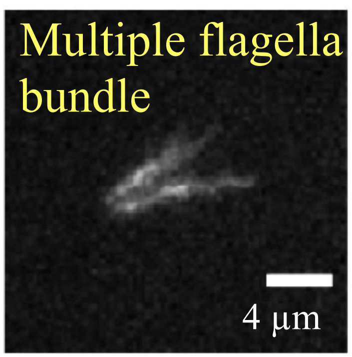
## Why it is still worth doing …

*Escherichia coli*

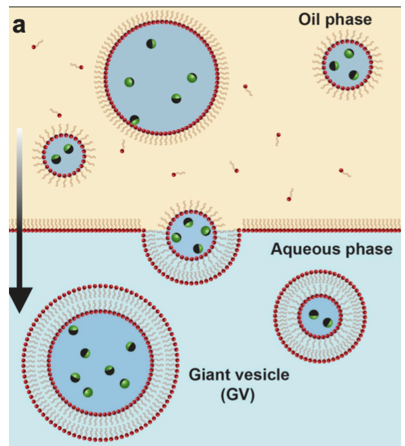



Single flagella bundle …

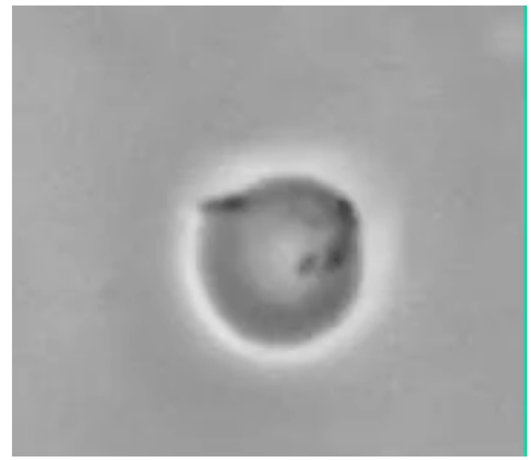
*B. subtilis*




Li, *Soft Matter* **7** (2011) 5228




Same as Janus paper

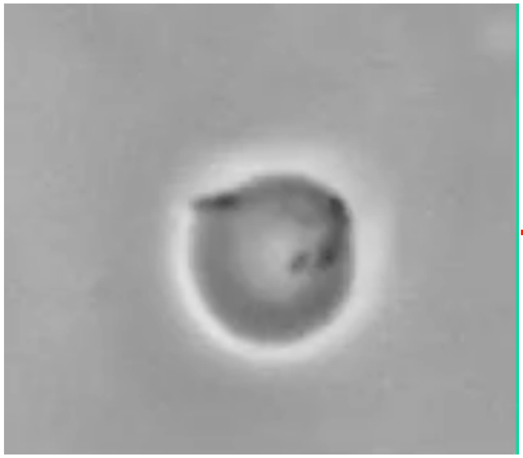


Density ≈ Janus paper …
… but < *Bacillus* paper

\+ leftover business:

(a) From Janus: too low $f$ for tubes!

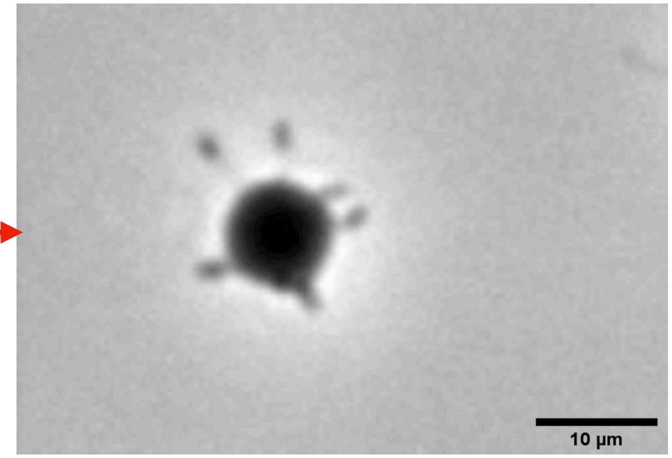
(b) From *Bacillus*: transient tubes only

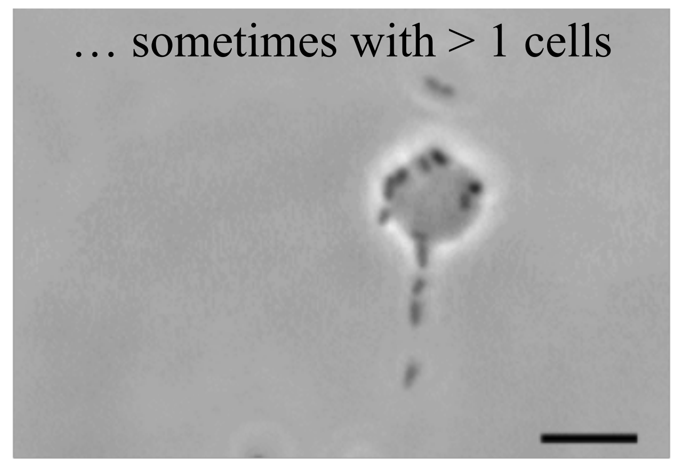


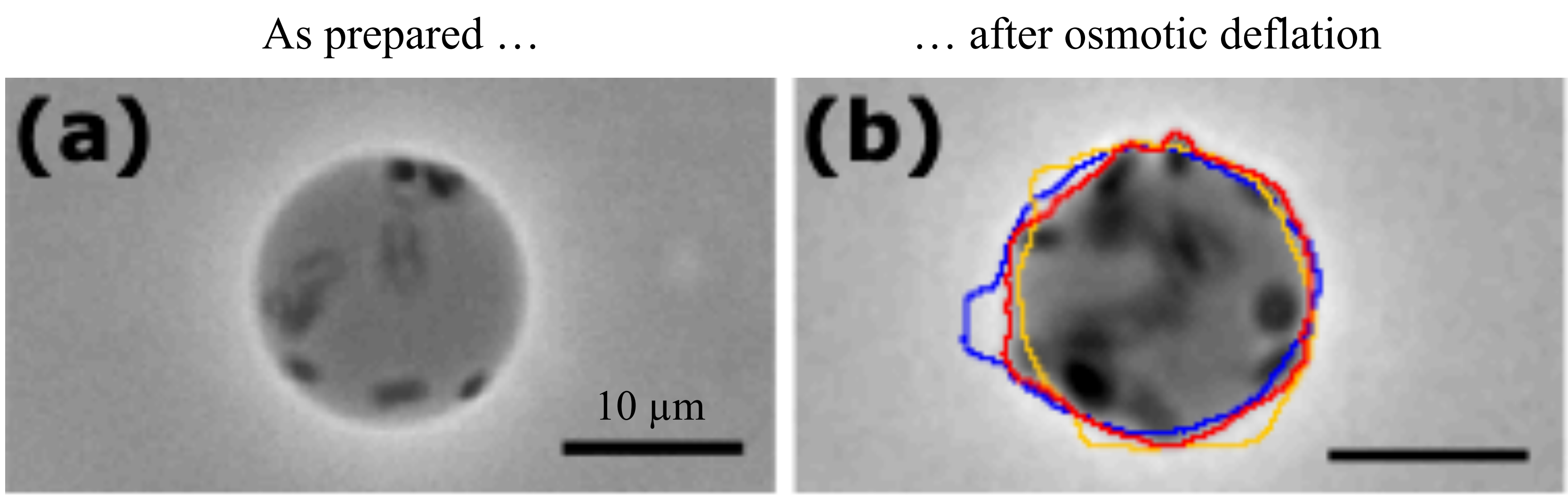
Osmotic deflation to
lower membrane tension

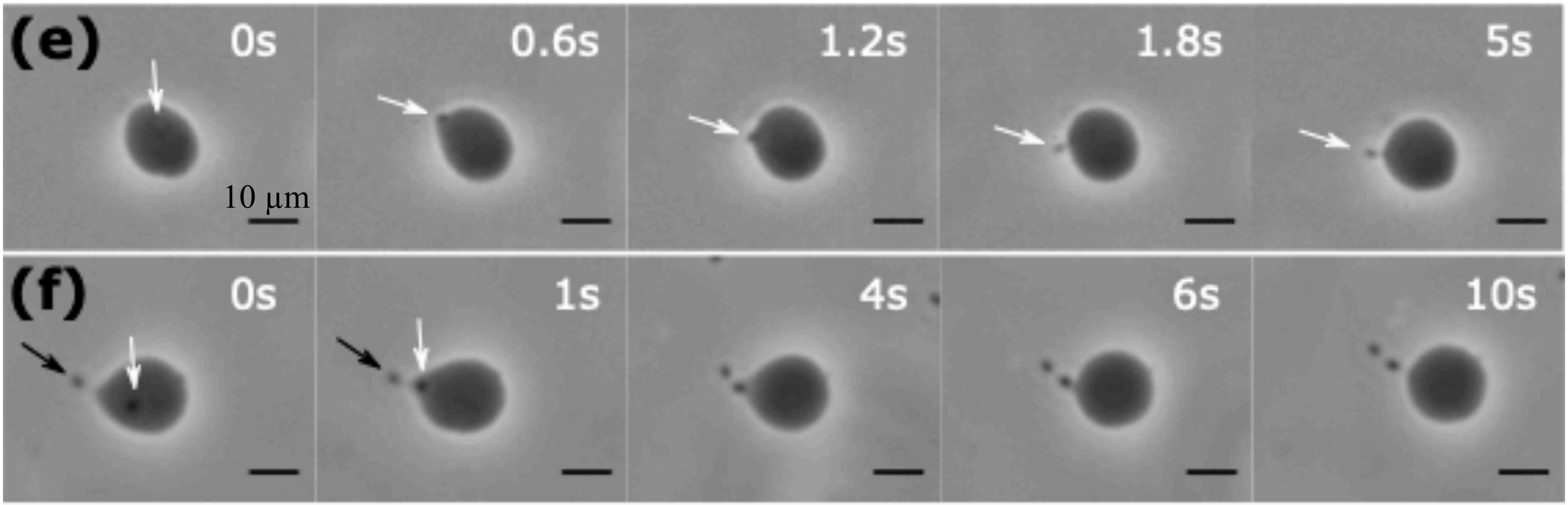
$t = 0$: 17% with tubes
Evaporate for
20 mins: 49%
40 mins: 67%

Stable membrane tubes …




As prepared …
… after osmotic deflation
(a)
(b)
10 μm

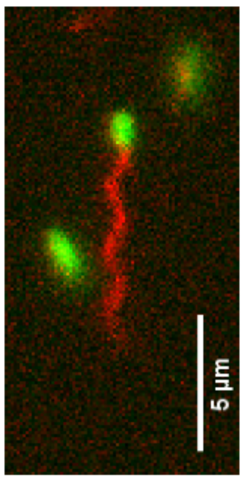


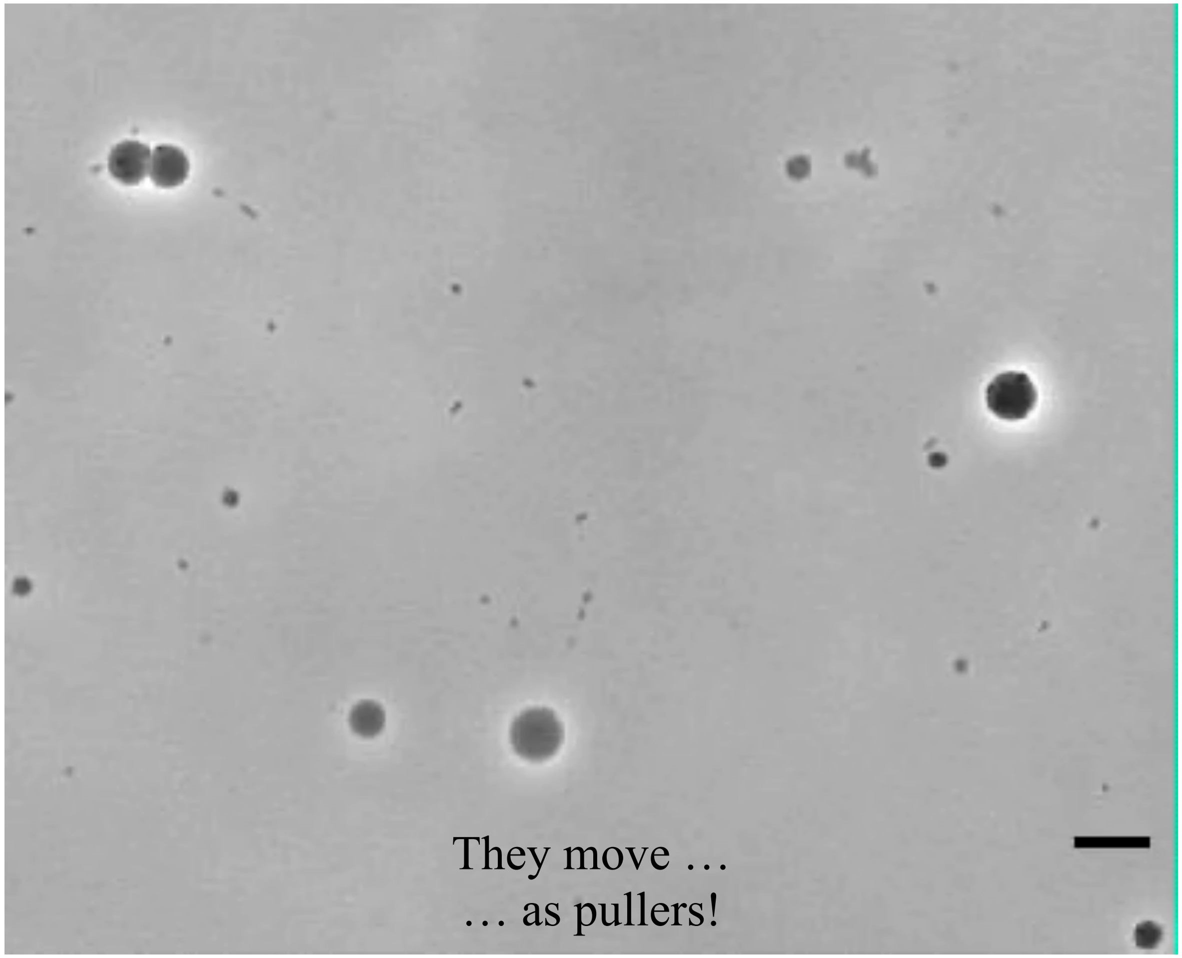
(e)
0s
0.6s
1.2s
1.8s
5s
10 μm
(f)
0s
1s
4s
6s
10s

Note how *thin* the tube is …

The really exciting bit …

Pusher

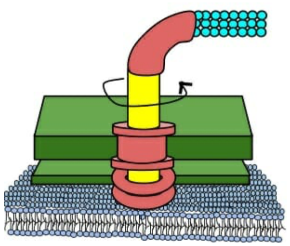


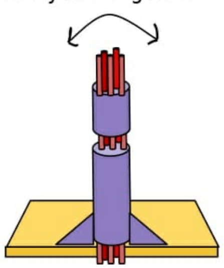
Why may anyone want to put motile bacteria inside a GUV?

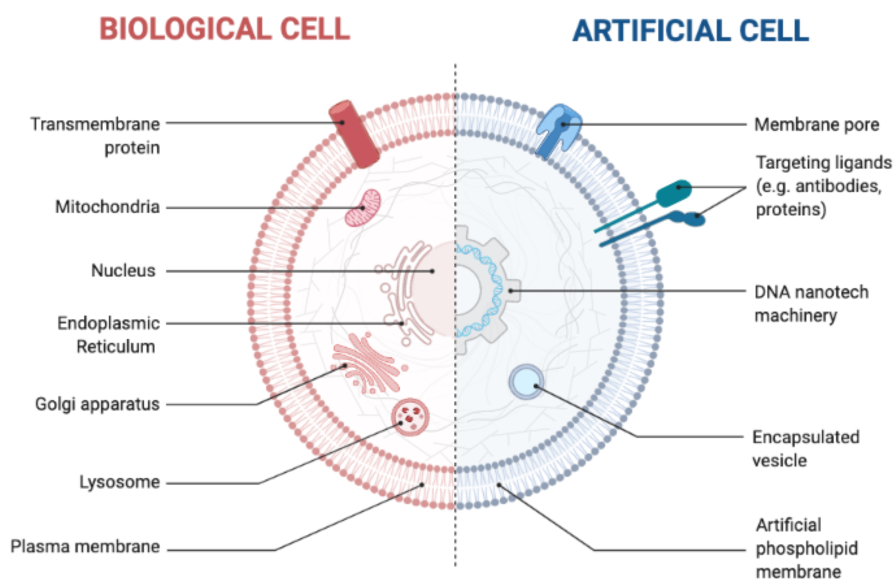
3. Conferring motility on artificial cells?

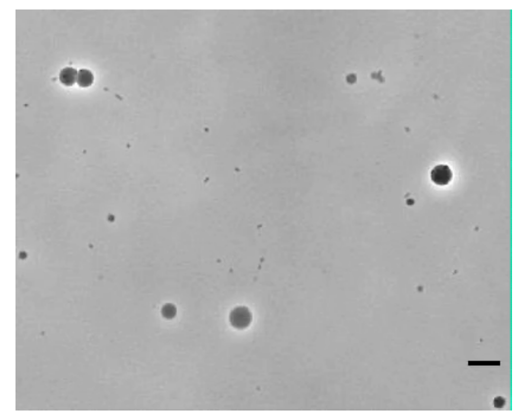
Prokaryoric flagellum

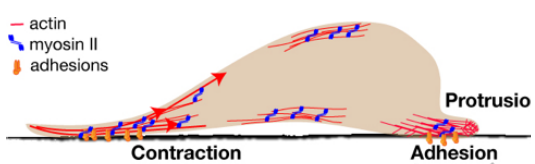


Eukaryoric flagellum

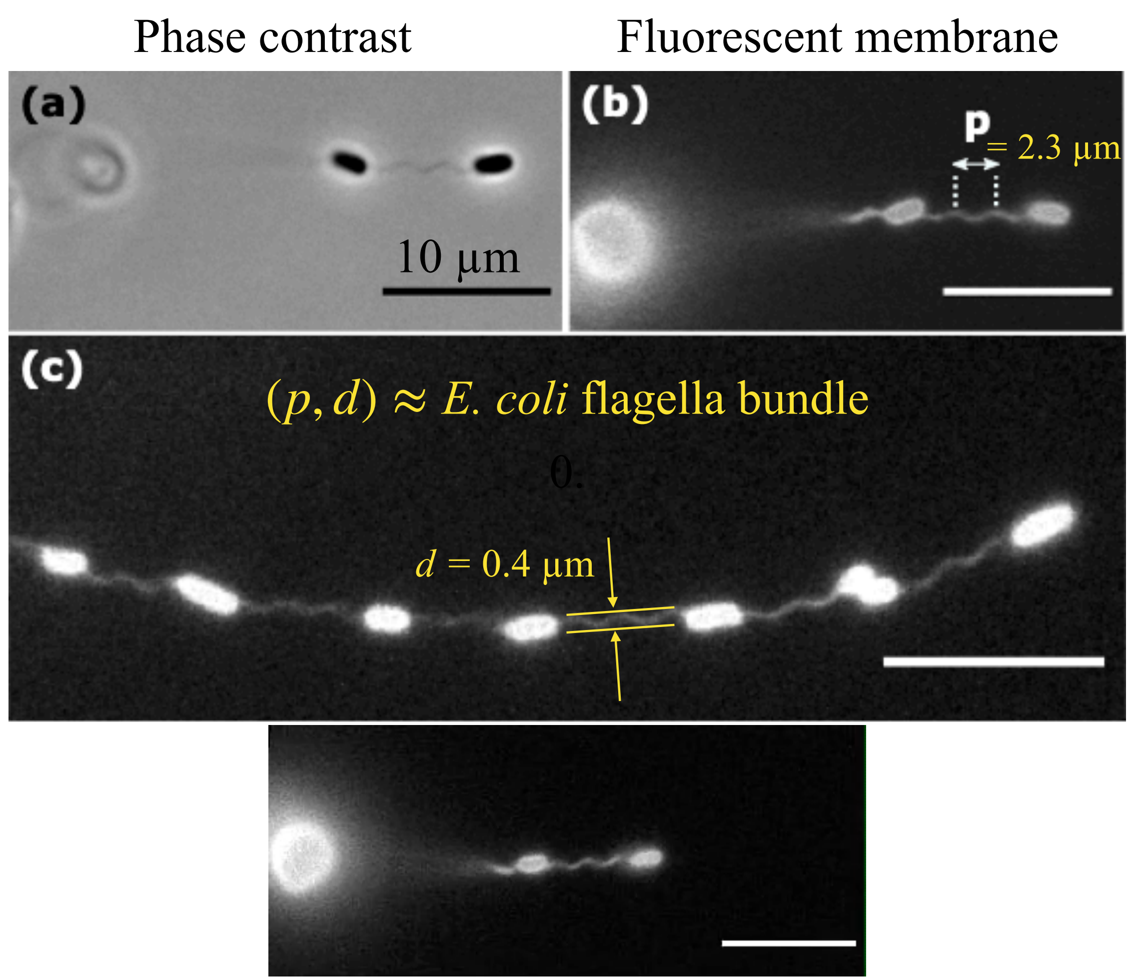


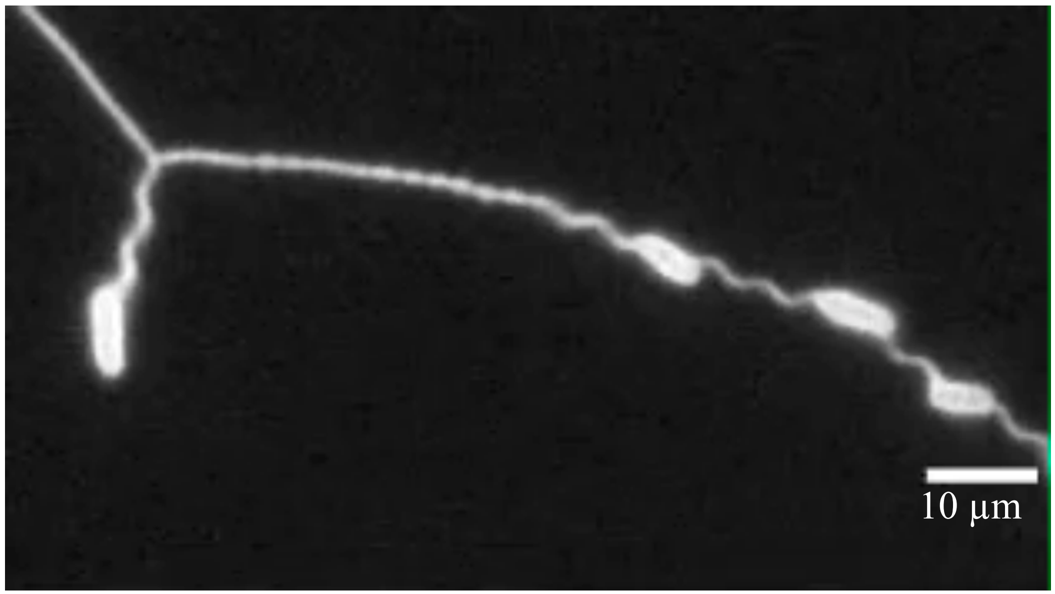


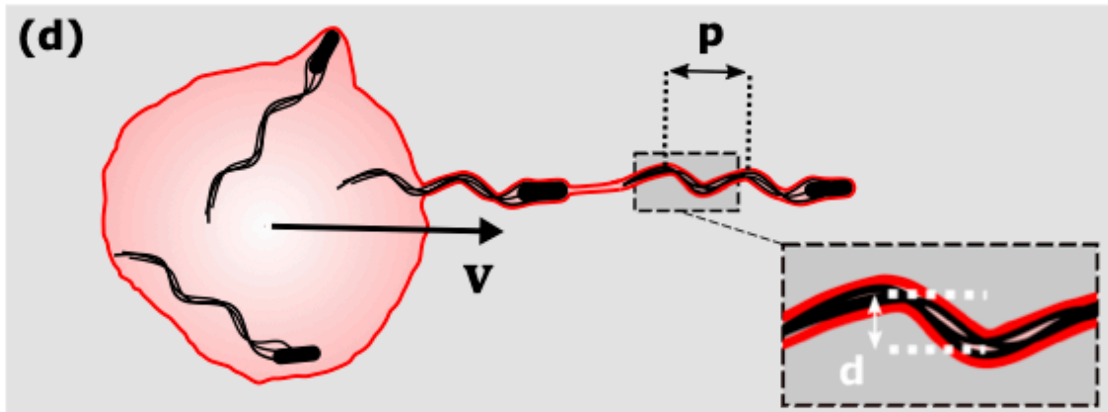



Siton-Mendelson & Bernheim-Groswasser, *Cell Adhesion & Migration* **10** (2016) 461-474

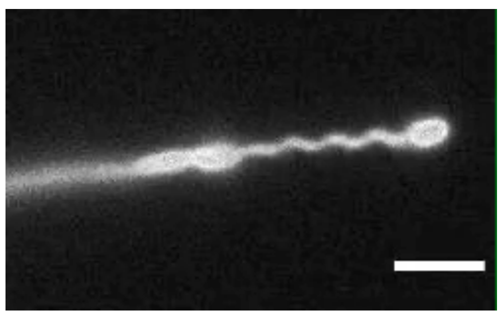

Phase contrast
Fluorescent membrane
(a)
10 μm
(b)
p
= 2.3 μm
(c)
(p, d) ≈ E. coli flagella bundle
0.
d = 0.4 μm

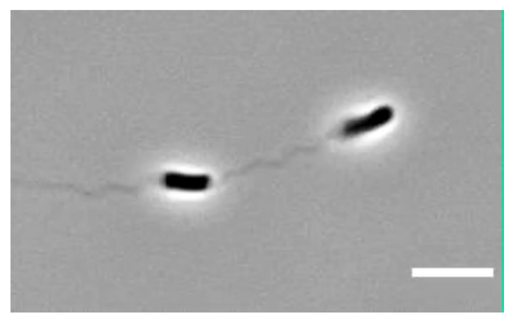

10 μm

Lipid bilayer thickness ≈ 4 nm, $(p, d) \approx$ *E. coli* flagella bundle

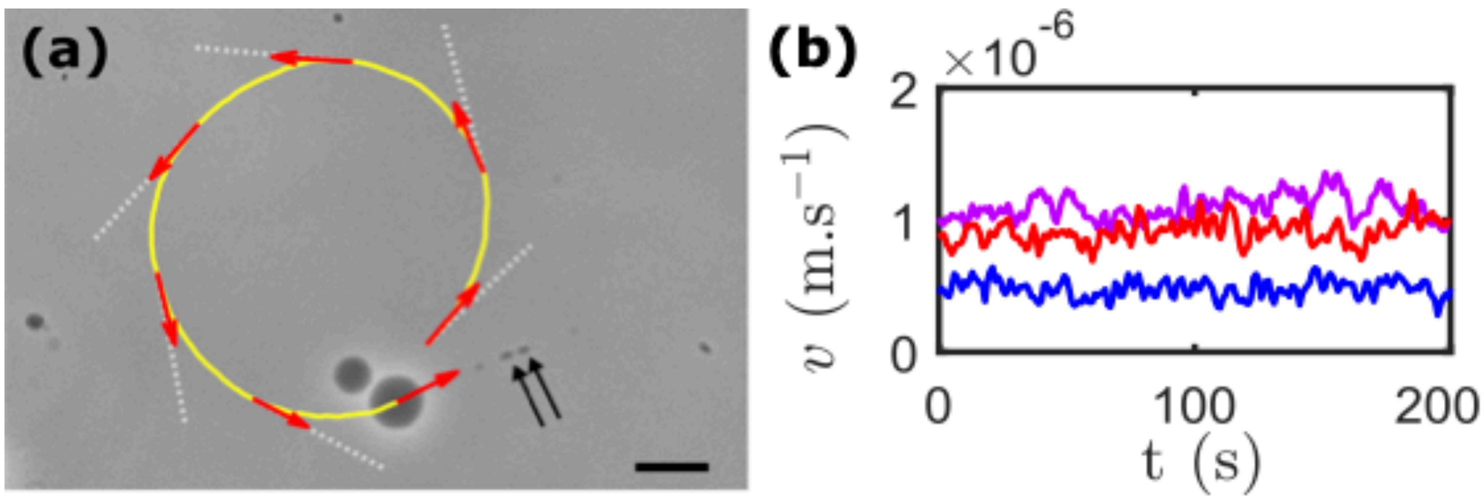



Lipid membrane wraps round flagella bundle → helical ‘effective flagellum’





(How do the ‘joints’ work?!)

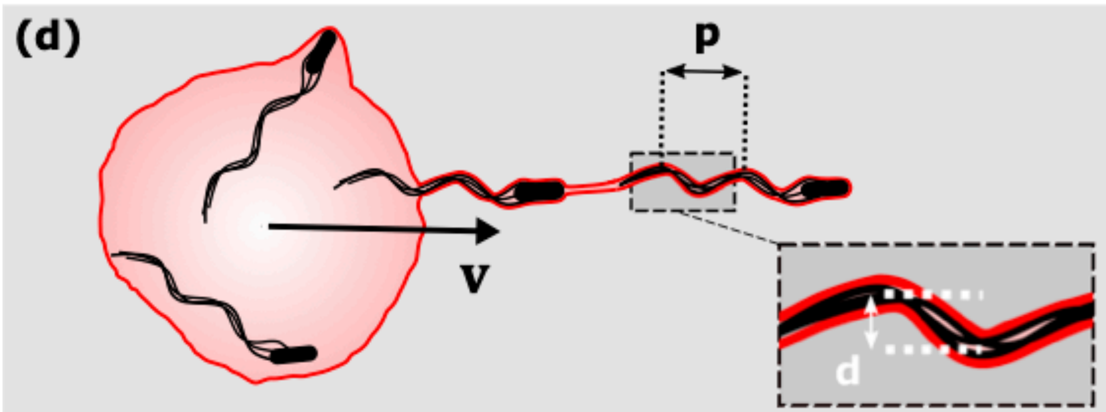


Equal number cw and ccw

$N$ bacteria in a tube pulling a vesicle of radius $R_v$

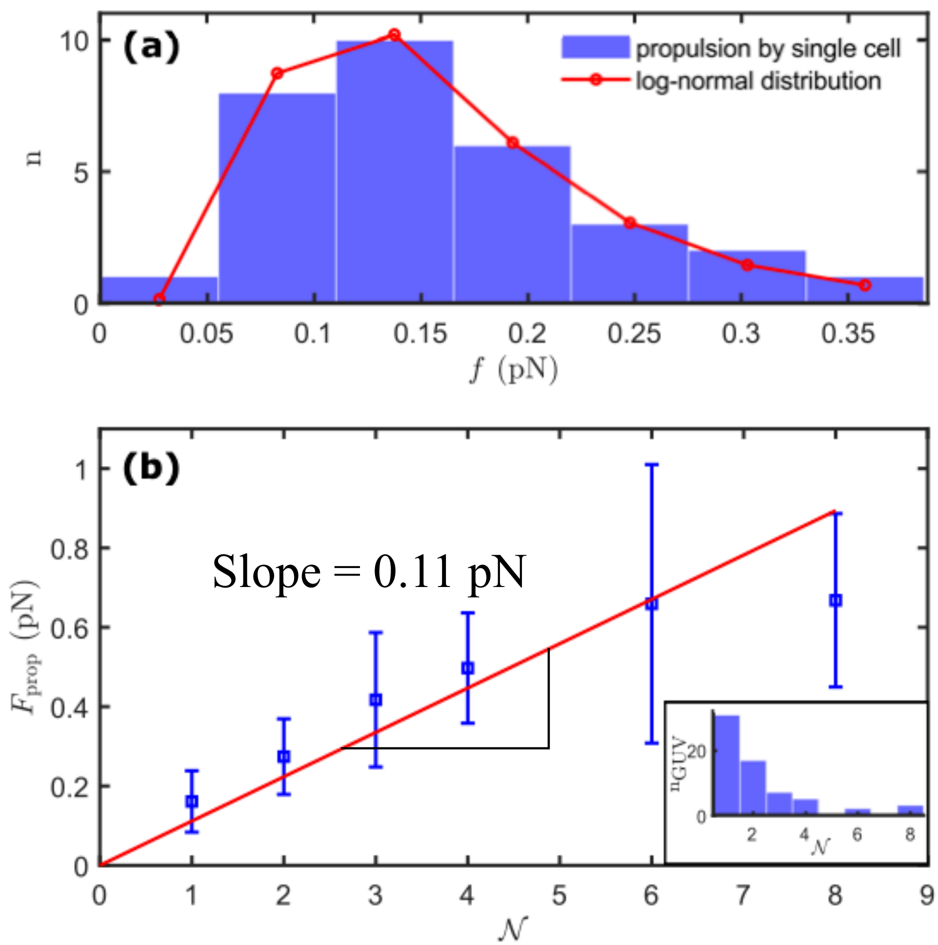
$$F_{\text{drag}} = (\xi_{\text{ves}} + N\xi_{\text{bac}})v = F_{\text{prop}}$$

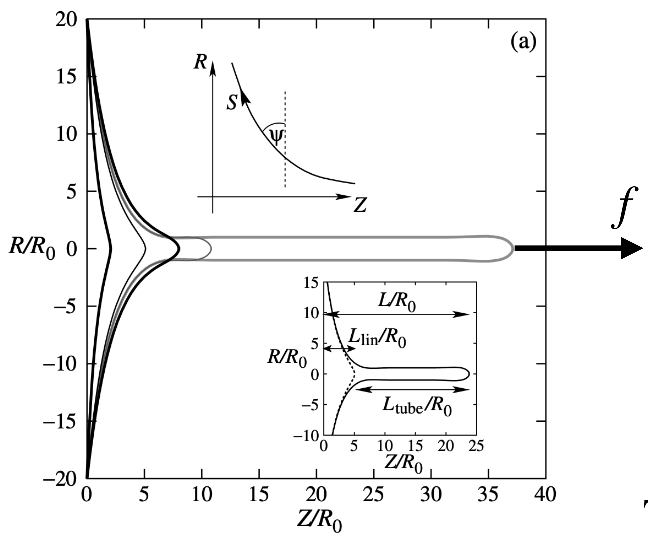
$$\xi_{\text{ves}} \approx 6\pi\eta R_{\text{ves}} \qquad \xi_{\text{bac}} \approx \xi_{\text{body}} \approx \frac{2\pi\eta b}{\ln(2b/a) - 1/2} \quad \text{for } (a, b) \text{ prolate ellipsoid}$$




Measure $v$ and geometric parameters $(a, b; R_{\text{ves}})$, calculate $F_{\text{prop}}$

Free *E. coli* $F_{\text{prop}} \approx 0.5$ pN



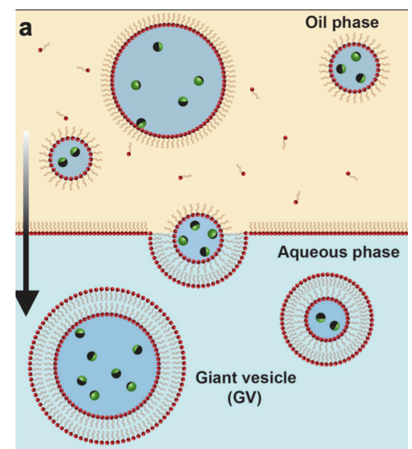

$$f = 2\pi\sqrt{2\sigma\kappa} \qquad R_0 = \sqrt{\frac{\kappa}{2\sigma}}$$

$$\Rightarrow R_0 = \frac{2\pi\kappa}{f}$$

$$f \lesssim 0.5\,\text{pN} \qquad \kappa \sim 10^{-19}\,\text{J} \qquad \Rightarrow R_0 \gtrsim 1.3\,\mu\text{m}$$

Tubes are too thin; tubulation @ too low $f$
... just like in Janus expts.

Both used inverted emulsion



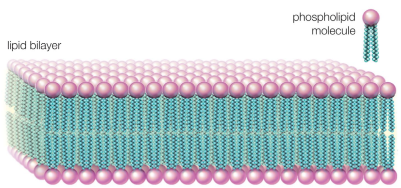
*Faraday Discuss.*, 2013, **161**, 305–331

# Spontaneous tubulation of membranes and vesicles reveals membrane tension generated by spontaneous curvature†

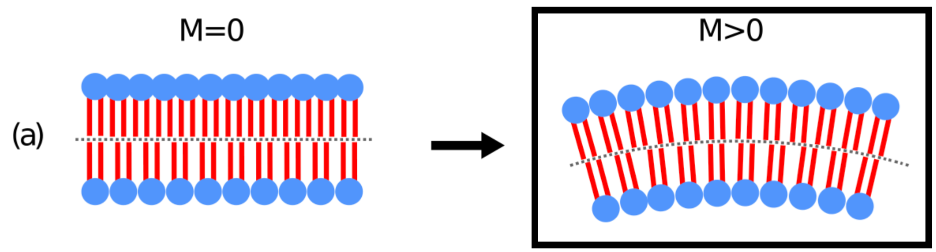
**Reinhard Lipowsky**

$$f = 2\pi\sqrt{2\sigma\kappa} \qquad R_0 = \sqrt{\frac{\kappa}{2\sigma}}$$

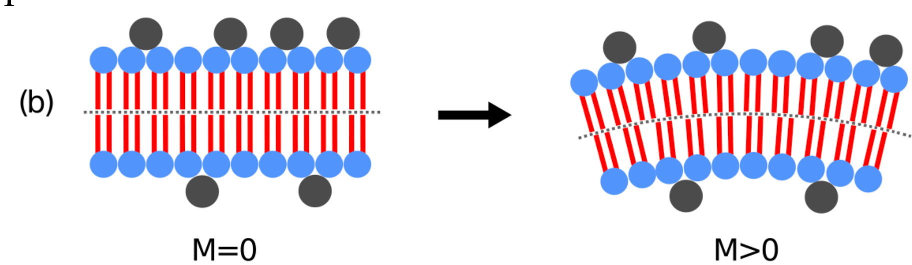
$$f = 2\pi\sqrt{2\kappa\sigma} - 4\pi\kappa M \qquad R_0 = \sqrt{\frac{\kappa}{2(\sigma + 2\kappa M^2)}}$$



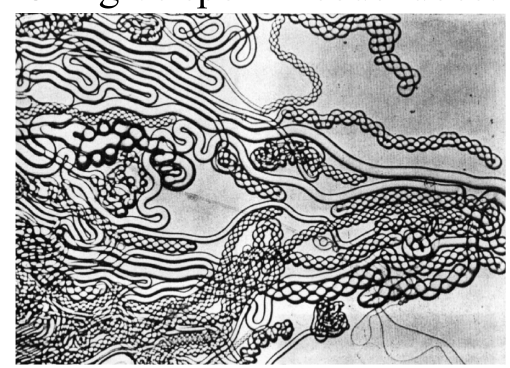

Spontaneous curvature $M = 0$



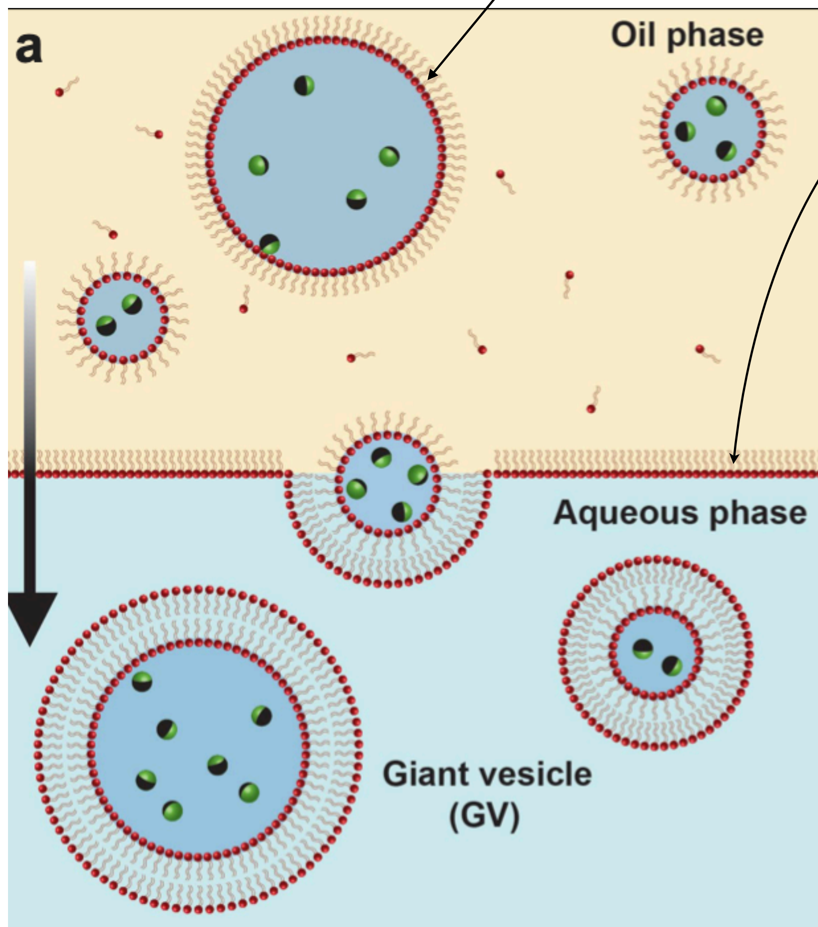

Changing composition of interior/exterior solutions ruled this out …




Can give spontaneous tubes!



Minimal equilibration




Extensive equilibration

→ different lipid densities

↓

Spontaneous curvature

↓

Thinner tubes?

PHYSICAL REVIEW E VOLUME 58, NUMBER 6 DECEMBER 1998

# Spontaneous curvature-induced pearling instability

Sahraoui Chaïeb* and Sergio Rica
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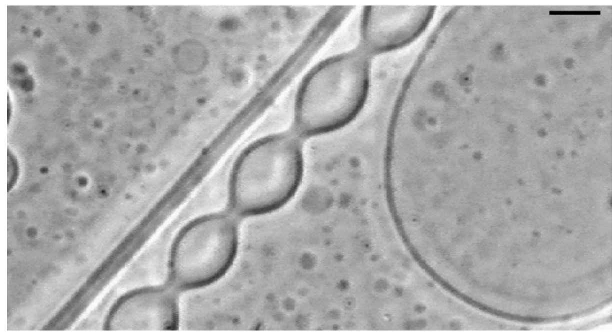
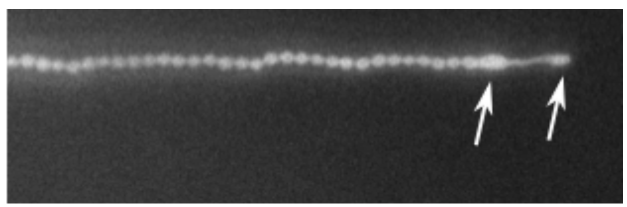
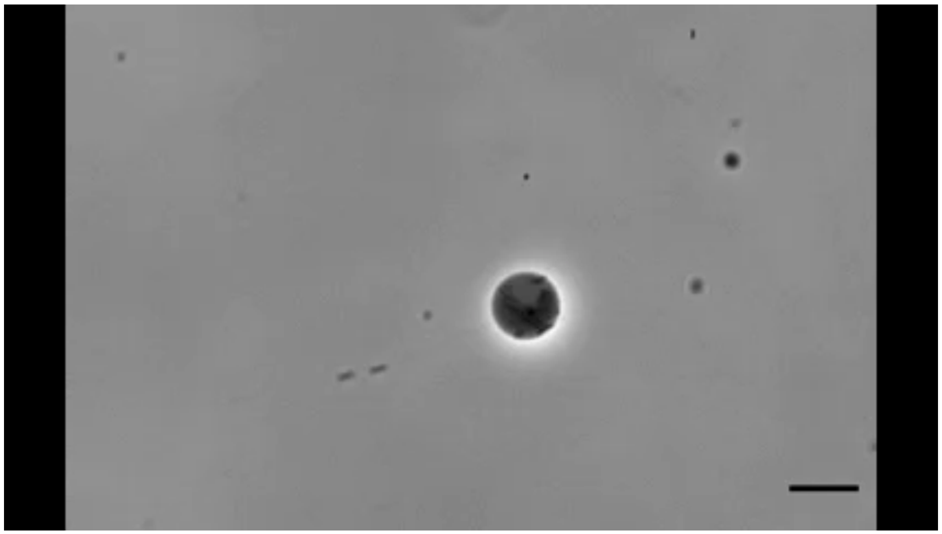
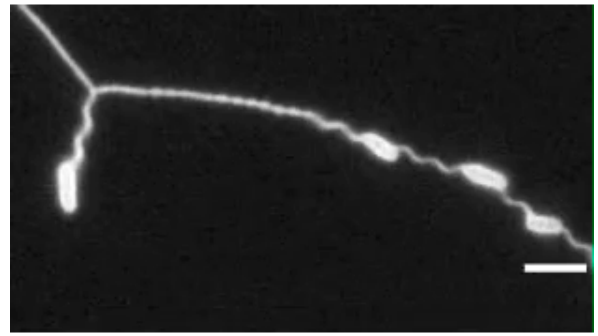
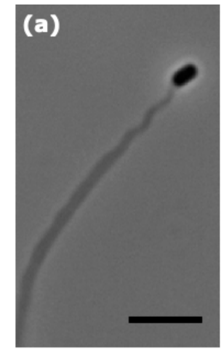




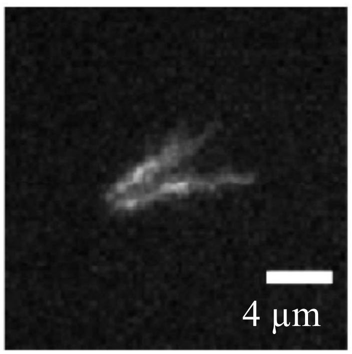
Other weird & wonderful things …





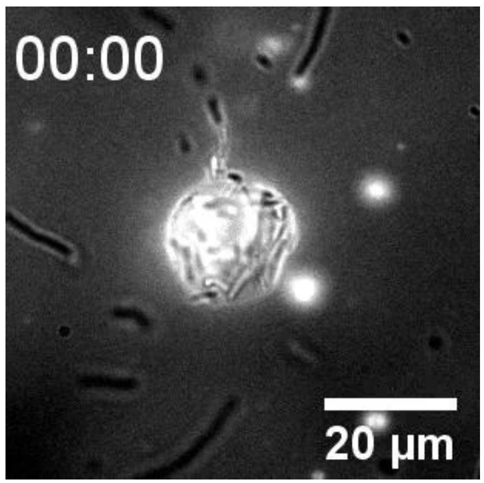


Why the difference with *B. subtilis*?



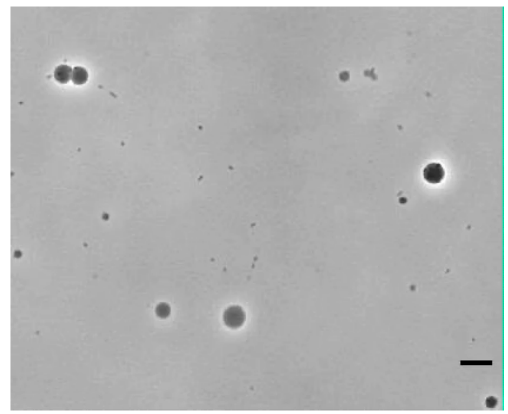

Multiple bundles

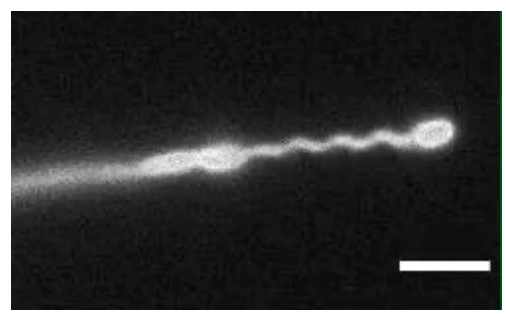
*B. subtilis*




Higher cell density
Higher GUV tension

*E. coli*





**The cautionary tale**

Passive systems are all alike; every active system is unique in its own way.*

Janus swimmer *vs. B. Subtilis vs. E. Coli*
Electroformation *vs.* Inverted emulsion

There is no such thing as ‘the physics of a pusher swimmer at a bilayer membrane’

| Details are washed out | | “Everything matters!” |
| --- | --- | --- |
| Equilibrium | Passive non-equilibrium | Active non-equilibrium |