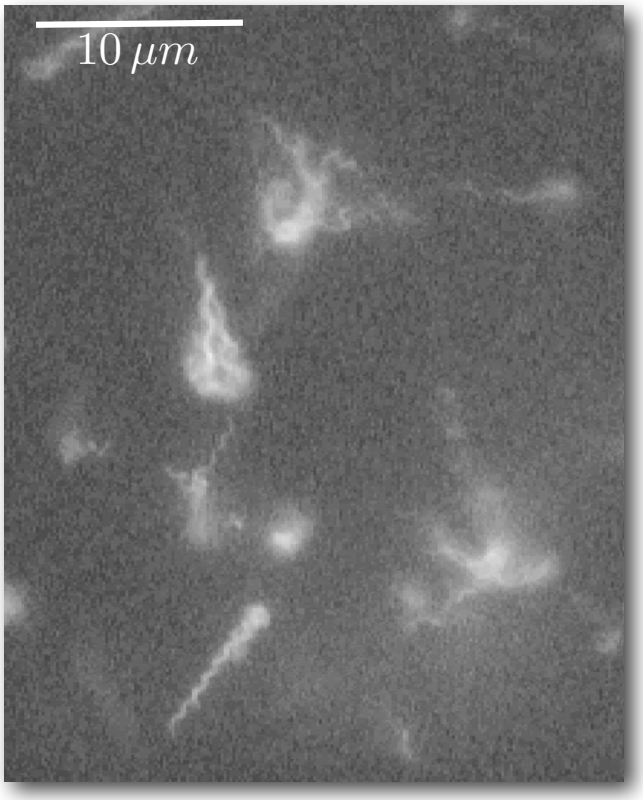
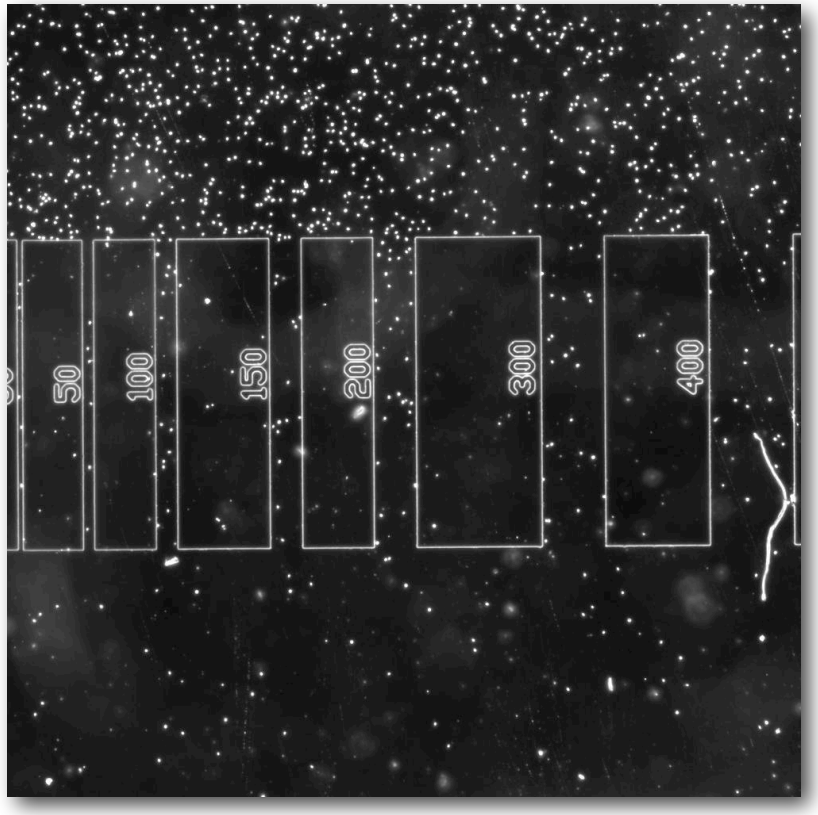
Biophysics of swimming cells
(selected topics)

E. coli



Turner, Ryu and Berg *J. Bacteriol.* **182**, 2793 (2000)

C. reinhardtii
(eukaryotic microalga)



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The Plan:

- Low-Re very quick recap
- Bacterial flagella
- Run-and-tumble

- Eukaryotic flagella
- Flagellar synchrony
- Flagellar growth

Microorganisms swim for a variety of different reasons….

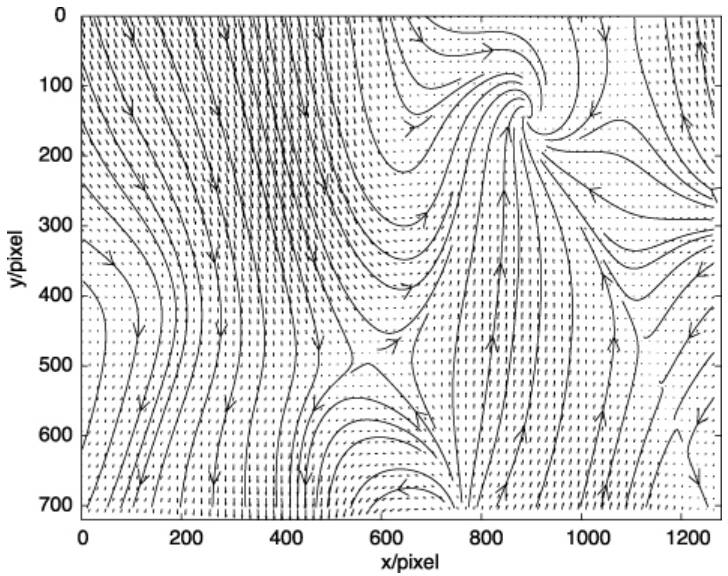
For example?

..however..

…in order to understand swimming…..

…we need to describe fluid flows

flow field



fluid velocity

$\mathbf{u}(\mathbf{x}, t)$

fluid pressure

$p(\mathbf{x}, t)$

Linked through Navier-Stokes equations

Density conservation

$$\nabla \cdot \mathbf{u} = 0$$

Momentum conservation

$$-\nabla p + \mu \nabla^2 \mathbf{u} = \rho \left(\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right)$$

viscosity

density

The Reynolds number

(Navier-Stokes equations) $$-\nabla p + \mu \nabla^2 \mathbf{u} = \rho \left(\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right)$$

Q: How important is viscosity vs inertia?

Need typical values: U typical velocity

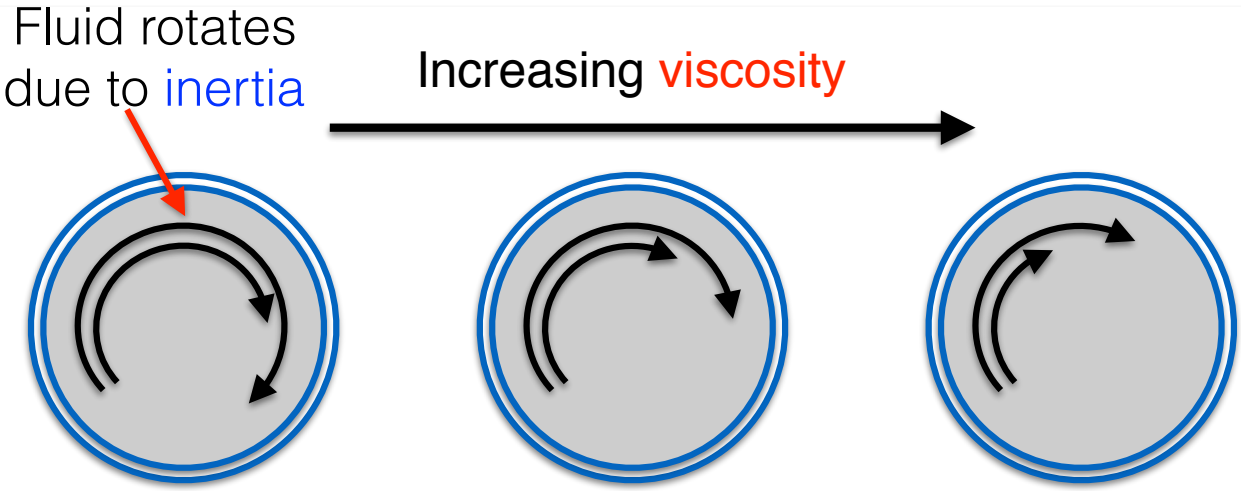
L typical length

viscous term $\mu \frac{U}{L^2}$ inertial term $\rho \frac{U^2}{L}$

Reynolds number

$$Re = \frac{\text{inertia}}{\text{viscosity}} = \left(\rho \frac{U^2}{L} \right) \left(\frac{L^2}{\mu U} \right) = \rho \frac{UL}{\mu}$$

Reynolds number: relative importance of viscous and inertial forces



Fluid will stop earlier ↔ Change in Re

$$Re = \frac{\text{inertia}}{\text{viscosity}} = \rho \frac{UL}{\mu}$$

U typical velocity

L typical length

$\rho = 10^3\,\mathrm{Kg/m^3}$ water density

$\mu = 10^{-3}\,\mathrm{Pa\,s}$ water viscosity

$\mathrm{Re} \gg 1$	Inertia wins
Swimming person	$\mathrm{Re} \sim 10^6\ (1.000.000)$
stirred coffee cup	$\mathrm{Re} \sim 10^3\ (1.000)$
ciliate	$\mathrm{Re} \sim 10^{-2}\ (0.01)$
bacterium	$\mathrm{Re} \sim 10^{-5}\ (0.00001)$
$\mathrm{Re} \ll 1$	Viscosity wins

Cells live in a low Reynolds number world:
dominated by viscosity!

$$-\nabla p + \mu \nabla^2 \mathbf{u} \simeq 0$$

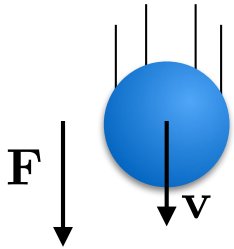
Stokes Equation

$$(-\nabla p + \mu \nabla^2 \mathbf{u} + \mathbf{f} \simeq 0)$$

(Stokes Equation with external force)

two consequences

Velocity is (effectively) proportional to Force



$$F = \gamma v$$

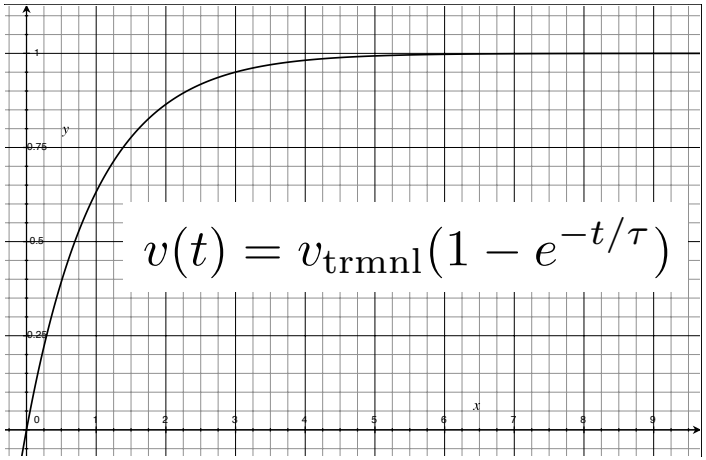
force velocity

γ drag coefficient

$\gamma_{\text{sphere}} = 6\pi\mu a$ a = radius of sphere

Actually…. finite time to reach terminal velocity

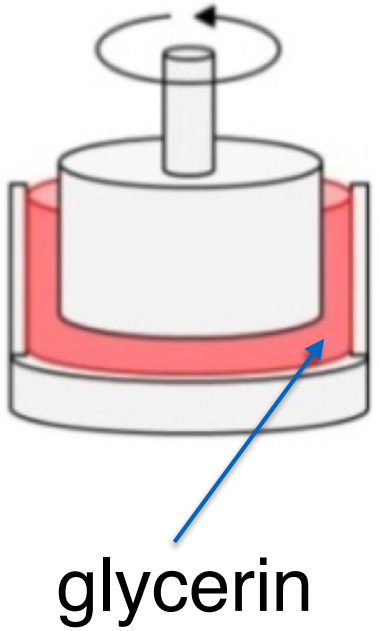
$$\tau = \frac{\text{mass}}{\text{drag coefficient}} = \frac{m}{\gamma}$$



Your turn: estimate the characteristic time τ for a bacterium and compare it to typical motility timescales (>1ms)

$$\tau \simeq 10^{-7}\,\text{s} \ll 10^{-3}\,\text{s}$$

Time reversibility at low Reynolds number



Completely reversible!

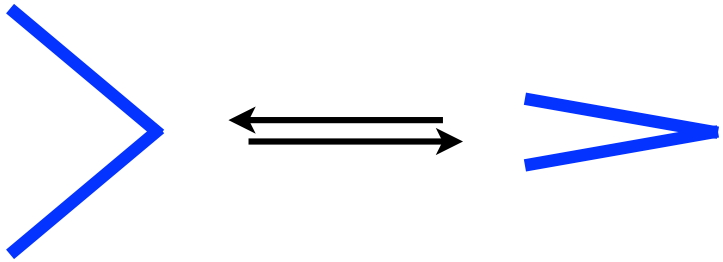
…mathematically:

Navier-Stokes equations $\xrightarrow{Re \simeq 0}$ $-\nabla p + \mu \nabla^2 \mathbf{u} = 0$ Stokes equations

No explicit temporal dependence

The "scallop theorem"

(Edward Purcell, 1977)



symmetric under time reversal

If $\mathcal{R} \ll 1$:

Time doesn't matter. The pattern of motion is the same, whether slow or fast, whether forward or backward in time.

The Scallop Theorem

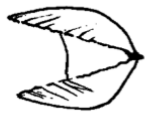


Figure 6.

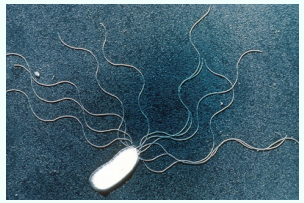
Actually real scallops don’t care….

Prokaryotes and Eukaryotes: two solutions to the swimming problem

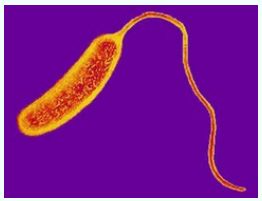
average width human hair

$\sim \mu$m $\sim 10\mu$m $\sim 100\mu$m

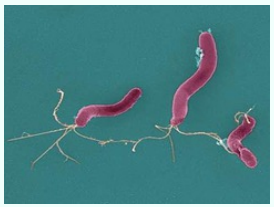
Prokaryotes



E. coli



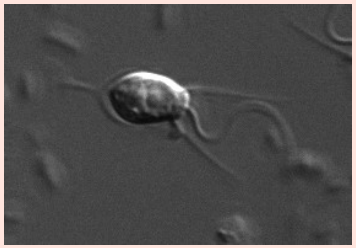
V. cholerae



H. pilori

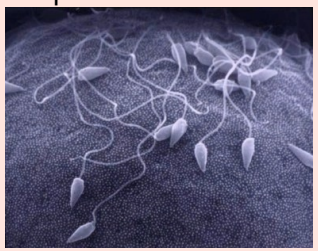
Eukaryotes

choanoflagellates



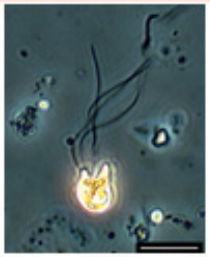
kinglab.berkeley.edu

spermatozoa



stanford.edu/group/Urchin

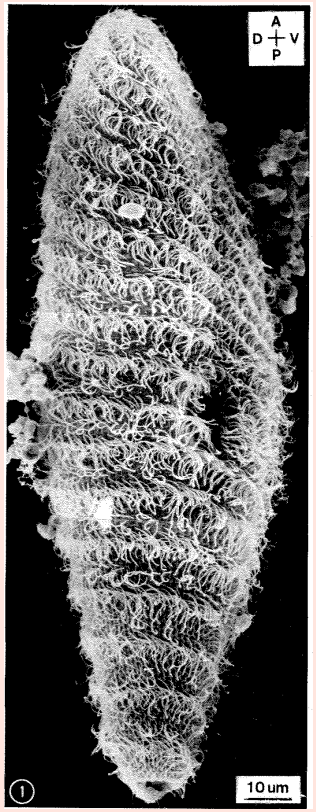
eukaryotic microalgae



The prasinophyte *Cymbomonas tetramitiformis*. Scale bar 15 µm. Photo Seija Hällfors.

The prasinophyte *Pyramimonas* sp. Scale bar 30 µm. Photo Seija Hällfors.

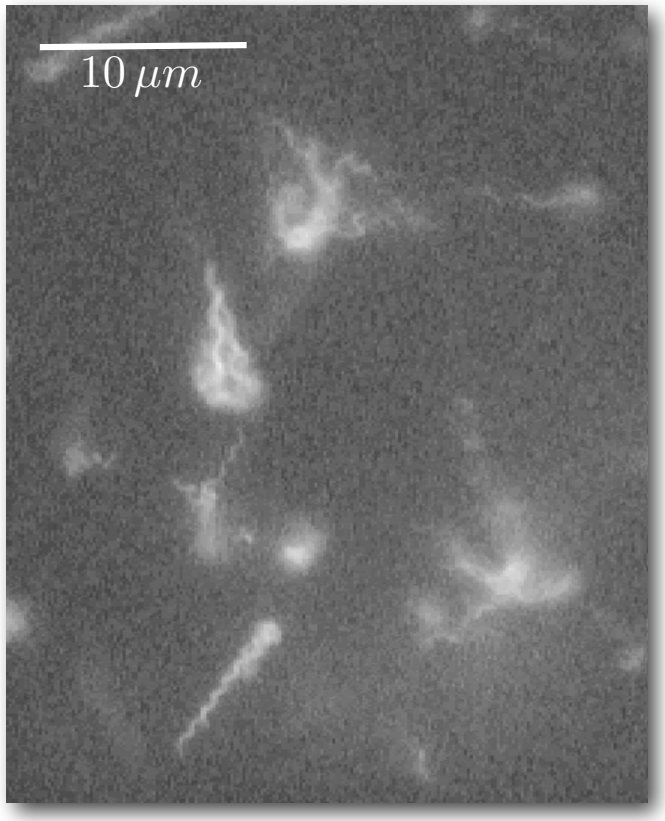
protists



Brennen and Winet (1977)

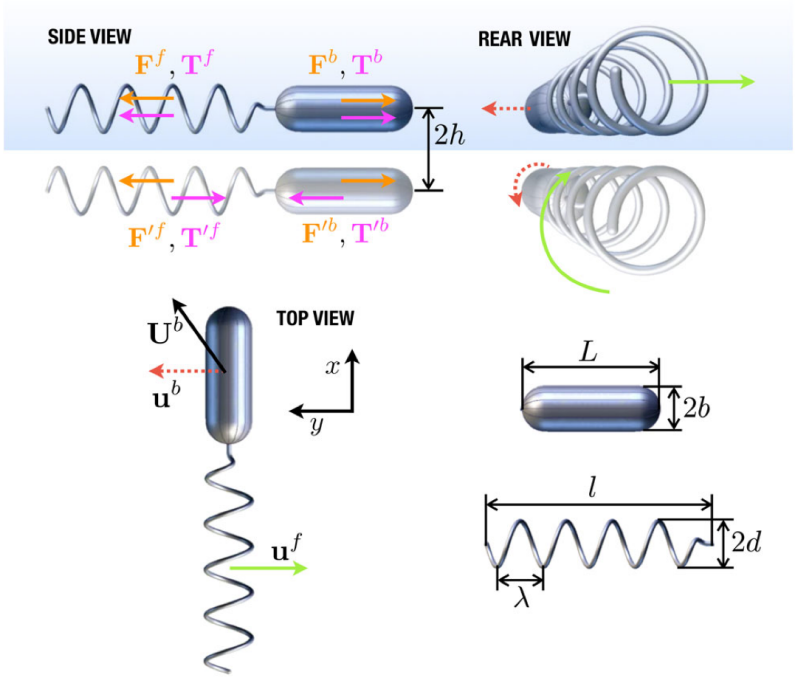
Bacterial motility

E. coli



Turner, Ryu and Berg *J. Bacteriol.* **182**, 2793 (2000)

Helical filament(s)…



Di Leonardo *et al.* PRL (2011)

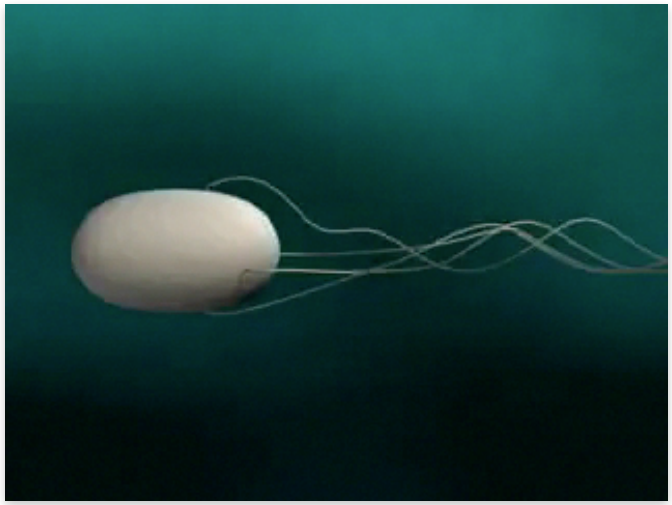
$$\text{thickness} \sim 15\,\text{nm}$$

$$d \sim 0.2\,\mu\text{m}$$

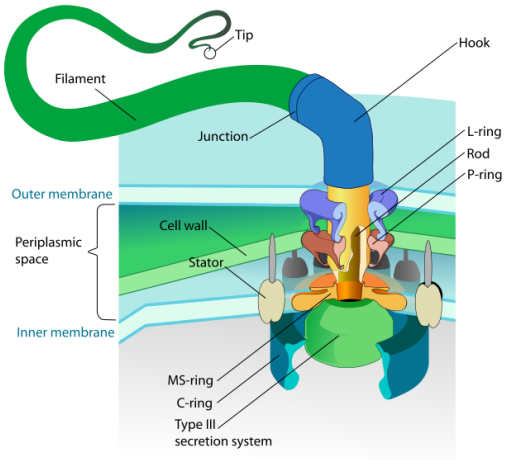
$$\ell \sim 10\,\mu\text{m/s}$$

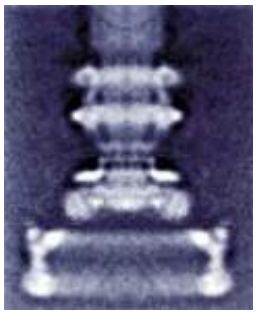
$$\lambda \sim 1 - 2\,\mu\text{m}$$

…driven at the base by a rotary motor



Protonic NanoMachine Group, Osaka Univ.

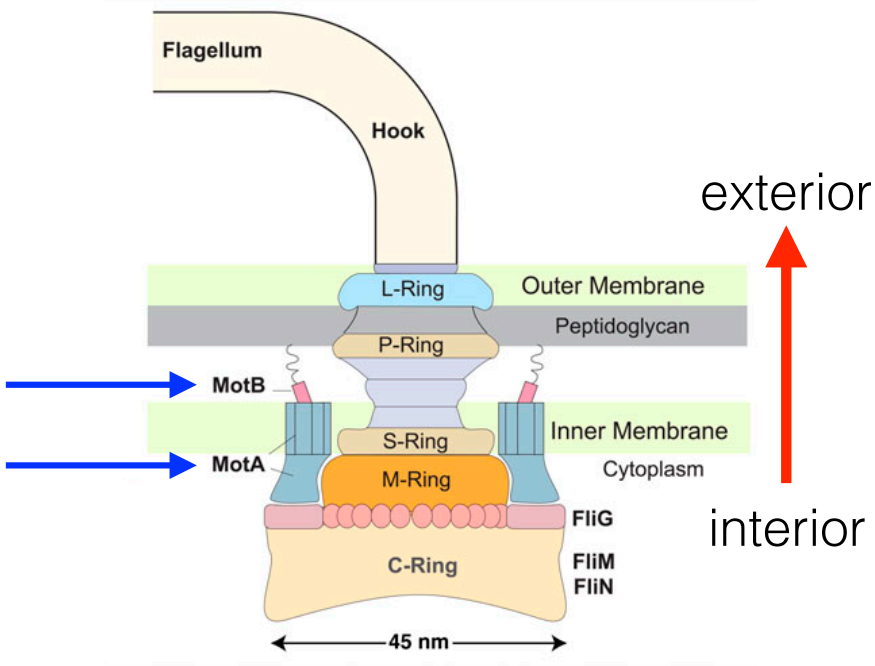




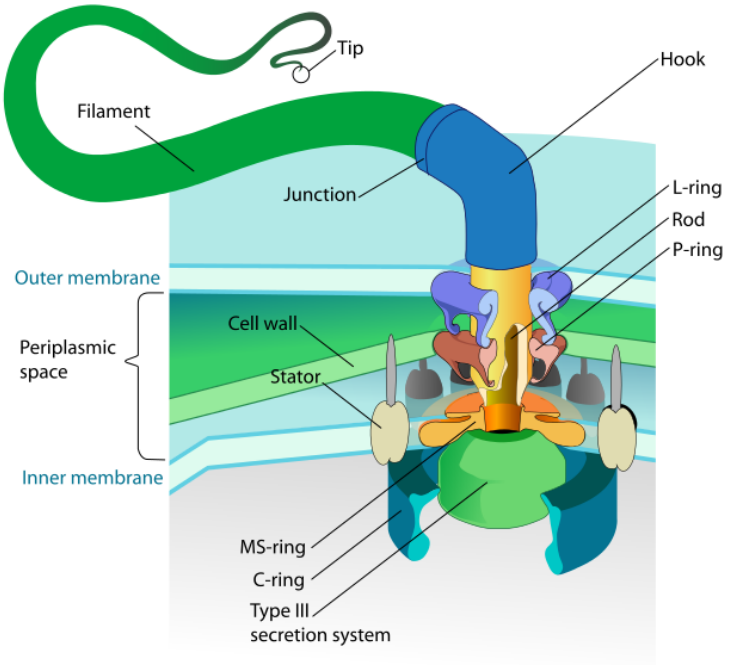
$$\nu_{\text{rot}} \sim 100\,\text{Hz}$$

$$v \sim 30\,\mu\text{m/s}$$

Flagellar structure I: hook and basal body



Mandadapu *et al.* PNAS (2015)

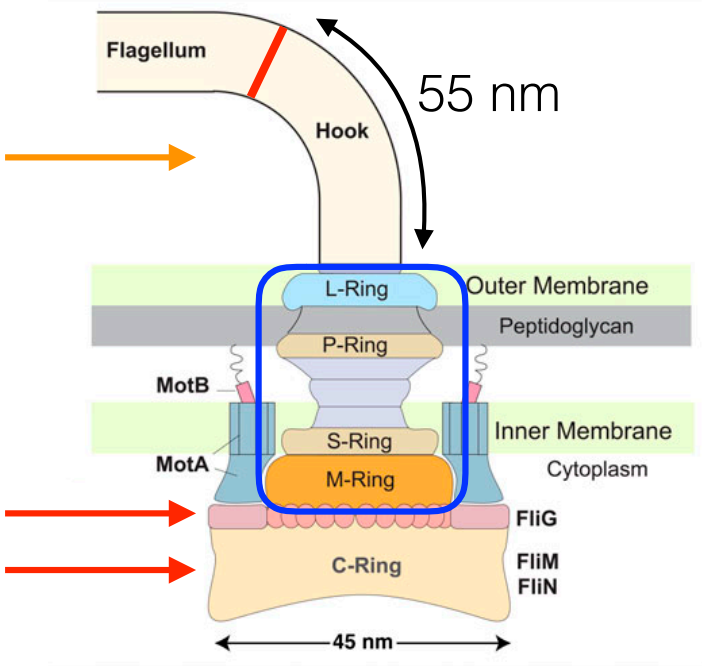


Wikipedia

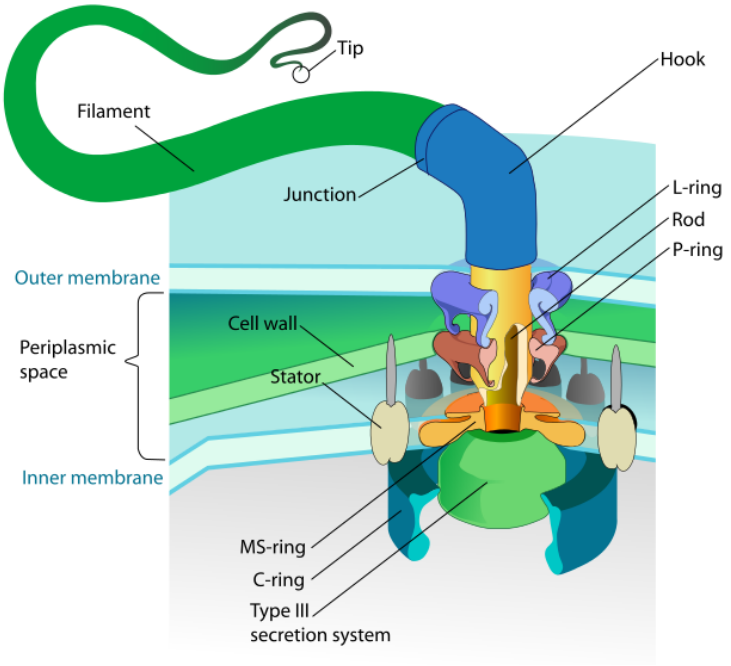
1) Cuts across the membranes surrounding the cell: cytoplasmic membrane and the cell wall (plus outer membrane for gram negative bacteria)

2) Cytoplasmic membrane contains stator units (4 MotA and 2 MotB per unit)
3) Stator: ring of up to 11 units (depending on load; more later).
4) Stator units: connected to the cell wall

Flagellar structure I: hook and basal body



Mandadapu *et al.* PNAS (2015)



Wikipedia

5) Basal body unit. Templated by the MS-ring; contains: outer membrane L-ring; cell-wall P-ring; a rod spanning the periplasmic space. (variant of type III injection system)

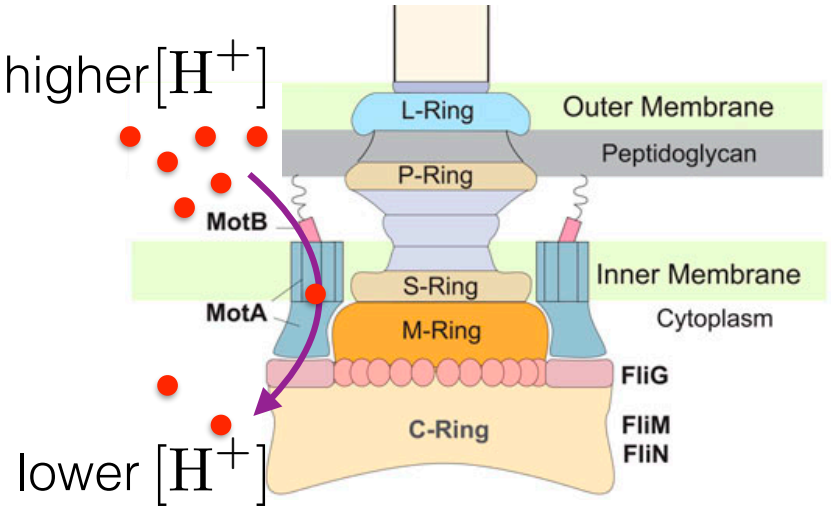
6) Rotor and switch unit (C-ring and FliG proteins; 26-fold symmetry)

7) Flexible hook outside: allows formation of bundles of flagella; connects to the flagellum proper through a junction (HAP1; HAP3)

Powering flagellar rotation

Across the membrane:

- difference in proton concentration
- difference in electrostatic potential



Difference in electrochemical potential of protons

(also known as: Proton Motive Force -PMF-)

$$\Delta\mu = V_m - \ln(10)\frac{k_B T}{e}\Delta\mathrm{pH}$$

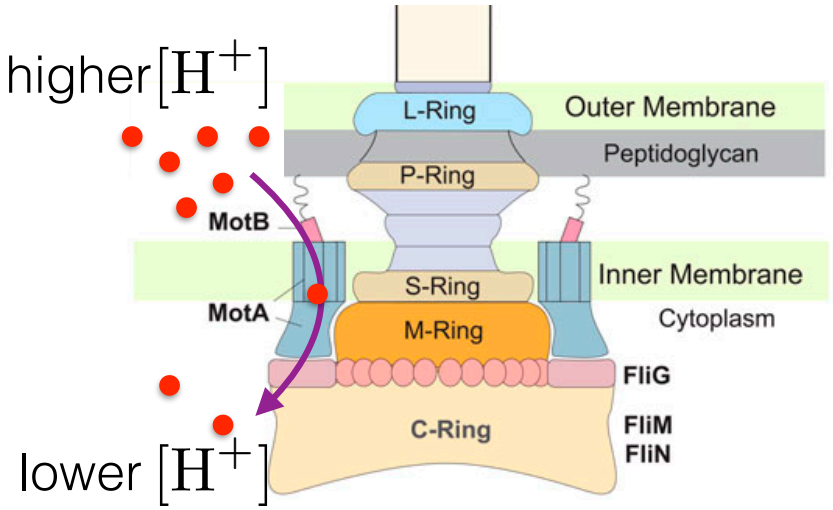
trans-membrane potential

Q: what is this ?

Powering flagellar rotation

Across the membrane:

- difference in proton concentration
- difference in electrostatic potential



Difference in electrochemical potential of protons
(also known as: Proton Motive Force -PMF-)

$$\Delta\mu = V_m - \ln(10)\frac{k_B T}{e}\Delta\mathrm{pH}$$

trans-membrane potential

entropic contribution
(see entropy of the ideal gas)

Typical values of:

$$\Delta\mu \simeq -170\,\mathrm{mV}$$

$$\tau \simeq 170\,\mathrm{pN\,nm}$$

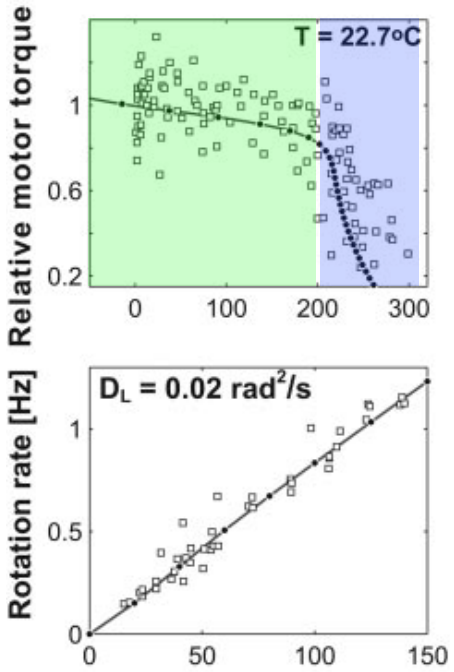
(motor torque, more next slide)

Q: How many protons are used per revolution?

~40

Powering flagellar rotation

- Stepwise rotation in units of 1/26th of a full turn (reflecting symmetry of the rotor)
- Power stroke driving rotation is generated in the MotA/MotB stator units



Xing *et al.* PNAS 103:1260 (2006)

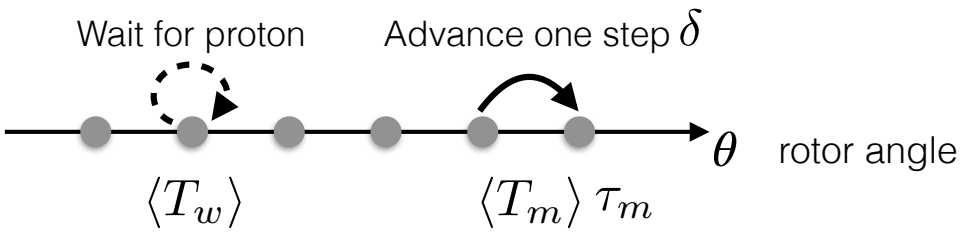
Torque (τ) vs. speed (ω):

typical "knee" dependence

τ almost independent of ω

τ decreases with ω

Idea behind the knee: two regimes



$$\tau \simeq \frac{\tau_m \langle T_m \rangle}{\langle T_w \rangle + \langle T_m \rangle}$$

$$\omega \simeq \frac{\delta}{\langle T_w \rangle + \langle T_m \rangle}$$

high load $\langle T_m \rangle \gg \langle T_w \rangle$

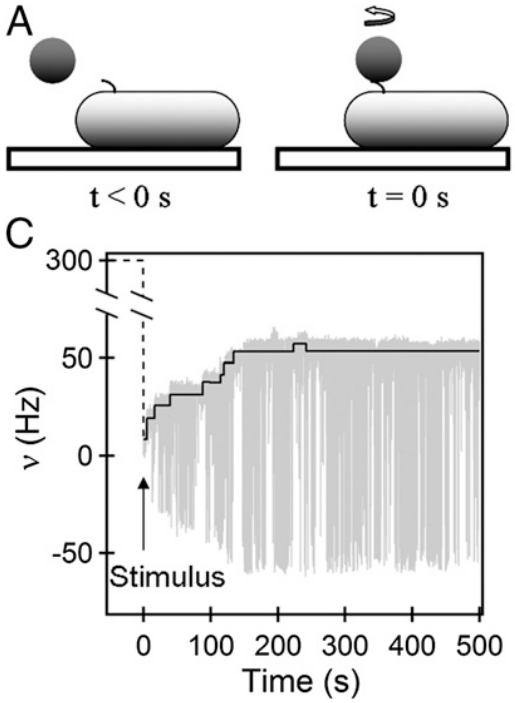
$$\tau \simeq \tau_m$$

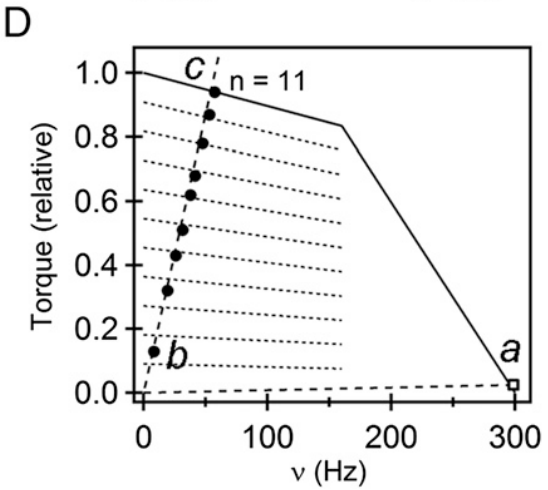
low load $\langle T_m \rangle \ll \langle T_w \rangle$

$$\tau \simeq \tau_m \left(1 - \frac{\omega \langle T_w \rangle}{\delta}\right)$$

Meacci, Tu PNAS (2009)

Not so simple!!
Flagella are mechanosensitive





Lele, Hosu, Berg PNAS 110:11839 (2013)

!! Recruitment of stators is load-dependent !!

- Recent studies: mutants with only ONE stator
- For a detailed mechanistic model of power stroke for 1 stator: Mandadapu et al. PNAS 2015

Back to the structure: A long list of flagellar proteins

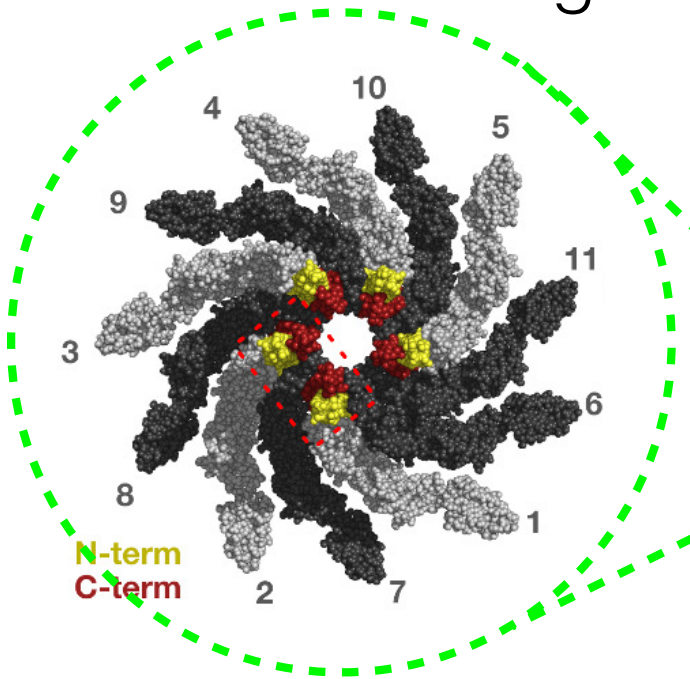
hook-basal body apparatus

flagellum proper

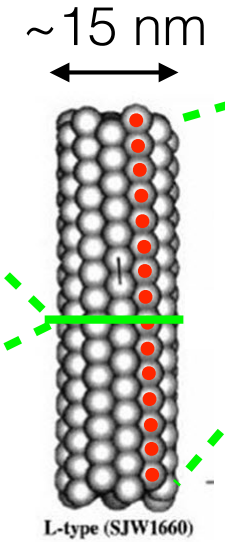
TABLE 1 Proteins of the flagellum and its export apparatus, arranged by cellular location

Protein symbol[a]	Common name and function	Cellular location	Stoichiometry (approx.)[b]	Assembly/ export method
FliI	ATPase; drives type III flagellar export	Cytoplasm		
FliH	Negative regulator of FliI	Cytoplasm		
FliJ	General chaperone	Cytoplasm		
FlgN	FlgK-, FlgL-specific chaperone	Cytoplasm		
FliS	FliC-specific chaperone	Cytoplasm		
FliT	FliD-specific chaperone	Cytoplasm		
FliG	Rotor/switch protein; torque generation; strong interaction with MS ring	Peripheral	26	Self-assembly
FliM	C ring; rotor/switch protein; target for CheY-P binding	Peripheral	37	Self-assembly
FliN	C ring; rotor/switch protein	Peripheral	110	Self-assembly
FliF	MS-ring protein; mounting flange for rotor/ switch and rod; housing for export apparatus	Cytoplasmic membrane	26	Sec
MotA	Stator protein; exerts torque against rotor/switch	Cytoplasmic membrane	64	Sec
MotB	Stator protein; converts proton energy into torque	Cytoplasmic membrane	32	Sec
FlhA	Export component; target for soluble export complex	Center of MS ring[c]	≥2	Sec?
FlhB	Export component; substrate specificity switch; target for soluble export complex	Center of MS ring	≥2	Sec?
FliO	Export component	Center of MS ring	≥1	Sec?
FliP	Export component	Center of MS ring	~4	Sec?
FliQ	Export component	Center of MS ring	≥1	Sec?
FliR	Export component	Center of MS ring	≥1	Sec?
FliE	MS-ring rod junction protein; export gate	Periplasmic space	~9	Type III
FlgB	Rod protein; transmission shaft	Periplasmic space	7	Type III
FlgC	Rod protein; transmission shaft	Periplasmic space	6	Type III
FlgF	Rod protein; transmission shaft	Periplasmic space	6	Type III
FlgG	Distal rod protein; transmission shaft	Periplasmic space	26	Type III
FlgJ	Rod capping protein; muramidase	Periplasmic space	5?	Type III
FlgI	P-ring protein; part of bushing; internal disulfide bridge	Periplasmic space	24	Sec
FlgA	Chaperone for P-ring protein	Periplasmic space	?	Sec
FlgH	L-ring protein; part of bushing; lipoprotein	Outer membrane	28	Sec
FlgD	Hook-capping protein	Cell exterior	5?	Type III
FlgE	Hook protein	Cell exterior	132	Type III
FliK	Hook-length-control protein	Cell exterior	?	Type III
FlgK	HAP1; first hook-filament junction protein	Cell exterior	13	Type III
FlgL	HAP3; second hook-filament junction protein	Cell exterior	~10	Type III
FliD	HAP2; filament-capping protein;	Cell exterior	5	Type III
FliC		Cell exterior	20,000	Type III

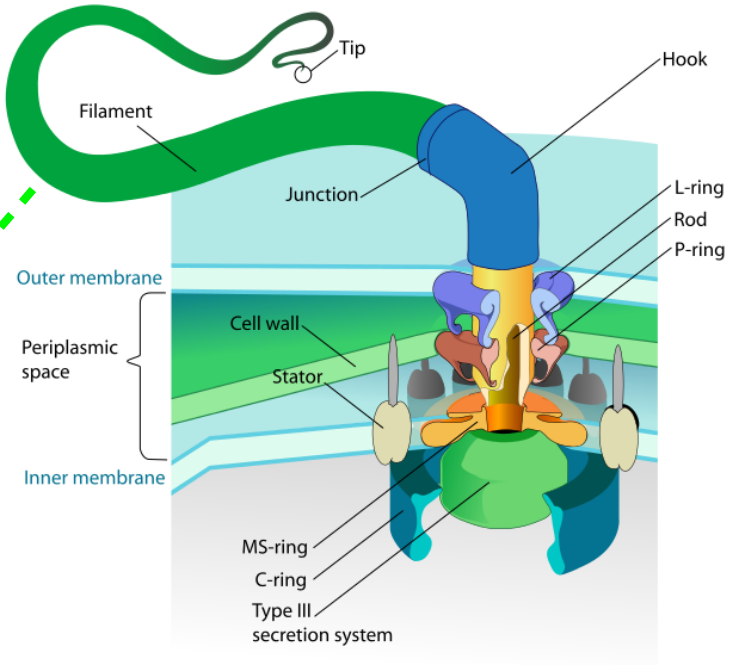
Flagellar structure II: flagellar filament



Renault *et al.* eLife (2017)



Macnab (1999)



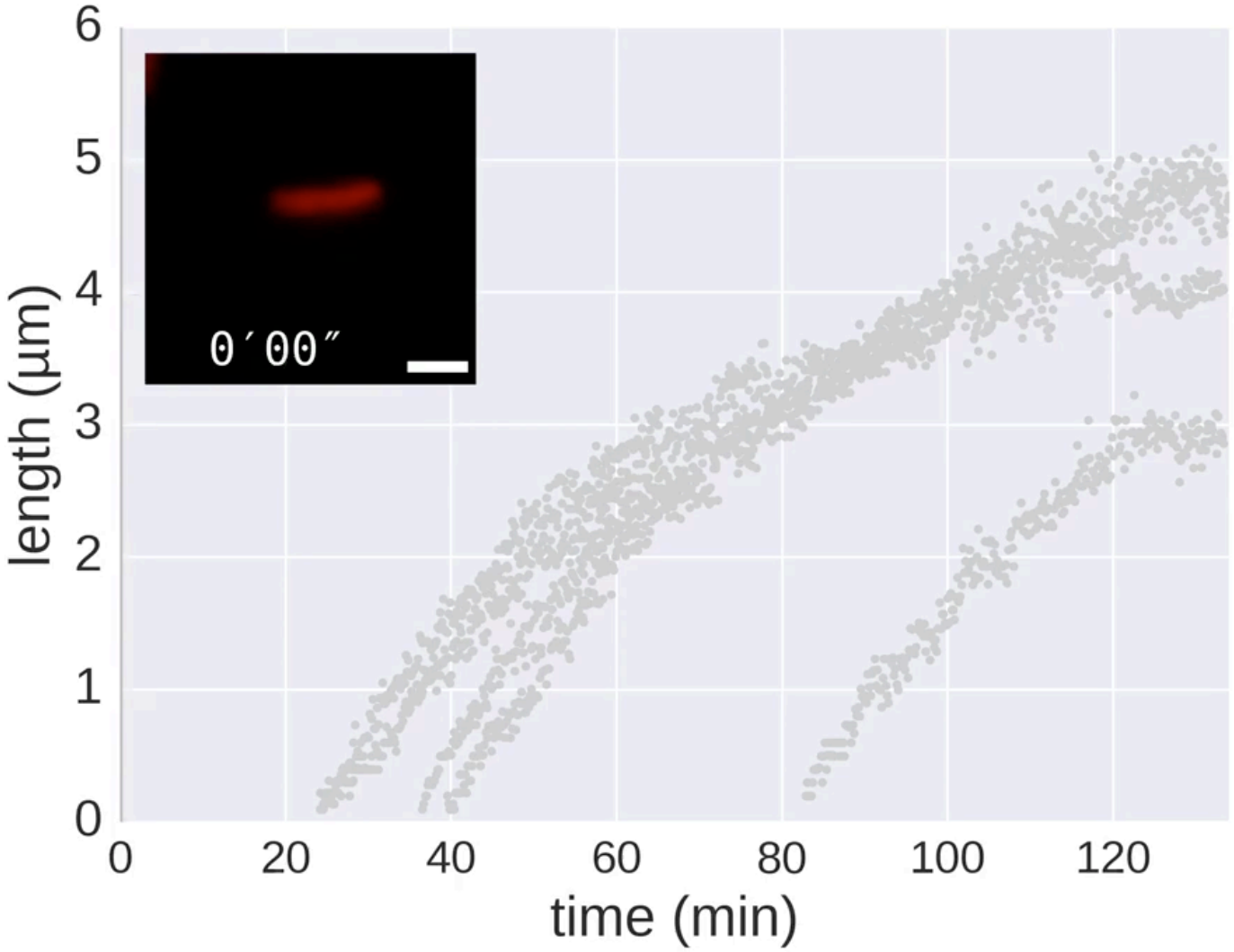
1) Flagellin subunits (FliC) which assemble into a left-handed helical filament (~1-2um pitch) composed of 11 fibrils.

2) Helical shape comes from the quaternary structure of the fibrils (a bit more later) (quaternary structure = structure of protein assemblies)

3) Extension ~10um; terminates with a capping structure at the tip

4) The flagellum breaks easily! …but then…. how is it (re)made ?!?

Flagellar assembly by *Salmonella enterica*

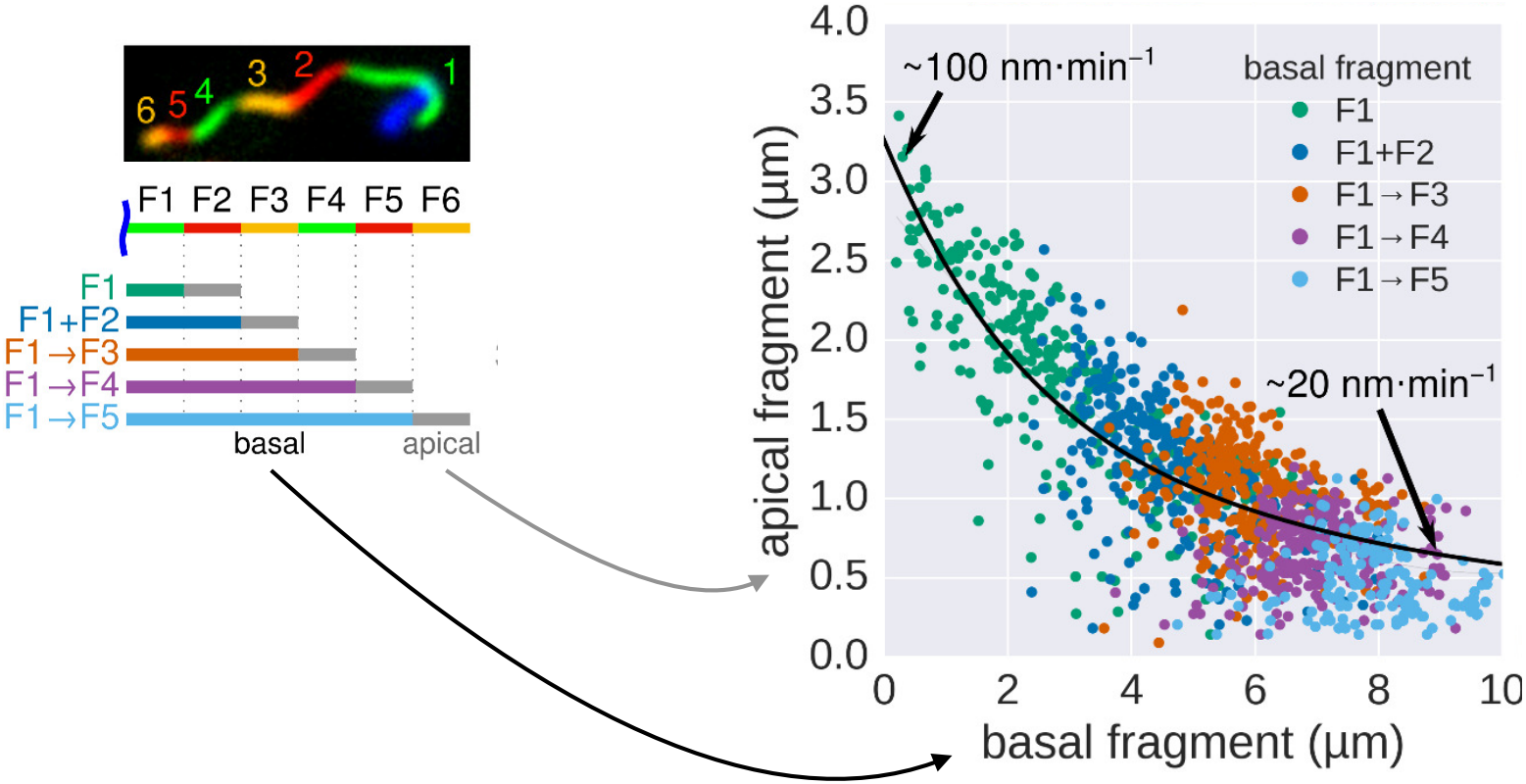


Renault *et al.* eLife 6:e23136 (2017)

Flagellar assembly

Renault *et al.* eLife 6:e23136 (2017)

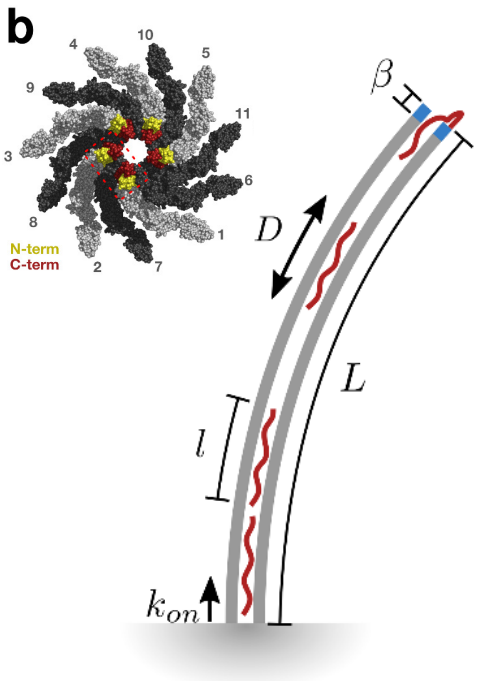
Apical growth vs. length of preceding portion of the flagellum



Assembly rate
decreases with flagellar length

Flagellar assembly

Renault *et al.* eLife 6:e23136 (2017)



Injection diffusion model

some definitions
- ℓ = length of a flagellin monomer
- u(x,t) = number of flagellin monomers at (x,t)
- L(t) = length of the (assembled) flagellum at time t

injection
- Flagellin injected at the base: type III injection system, pmf-powered
- Crowding at the injection point ⇒ injection rate k_{on} → **$k_{on}(1-u(0,t))$**

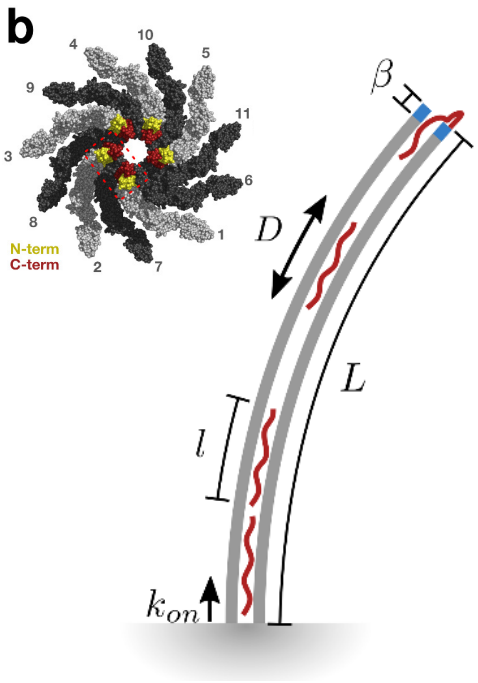
diffusion
- Monomers diffuse along the central hole (~2nm ∅) with diffusivity **D**

assembly
- Monomers at the tip fold in place (helped by capping structure), adding a length **β**

Your Turn: Write down the equations modelling the "injection-diffusion-flagellar growth" problem.

Flagellar assembly

Renault *et al.* eLife 6:e23136 (2017)



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diffusion
- Monomers diffuse along the central hole (~2nm ∅) with diffusivity **D**

assembly
- Monomers at the tip fold in place (helped by capping structure), adding a length **β**

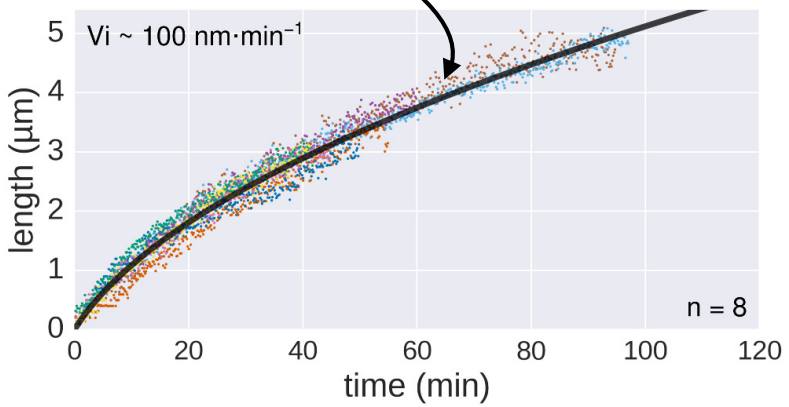
monomer diffusion $\frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial x^2}$

monomer injection at the base $-D\frac{\partial u/\ell}{\partial x}_{|x=0} = k_{on}(1-u(0,t))$

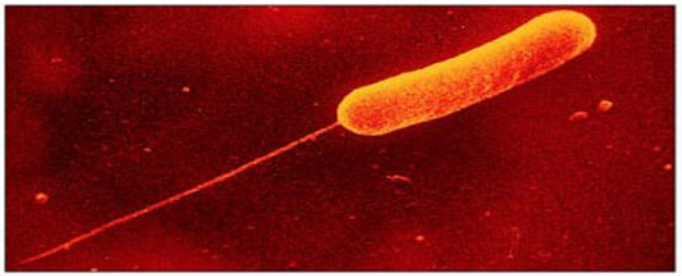
tip growth $\frac{dL}{dt} = \beta\left(-D\frac{\partial u/\ell}{\partial x}\right)_{|x=L}$

(quasi-steady state for u(x,t))

$L = -b + \sqrt{b^2 + 2at}$ $\quad a = \beta D/\ell$ $\quad b = D/k_{on}\ell$



Different arrangements of bacterial flagella

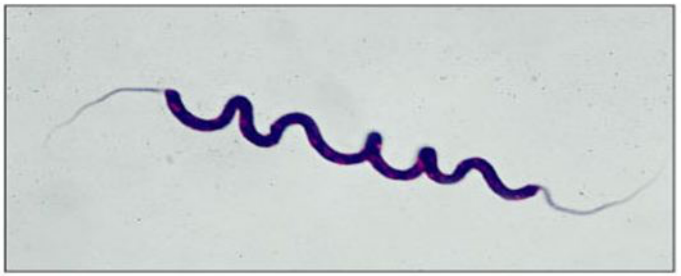


(a) Monotrichous

V. cholerae

C. crescentus

P. aeruginosa



(b) Amphitrichous

Alkaligenes faecalis

(c) Lophotrichous

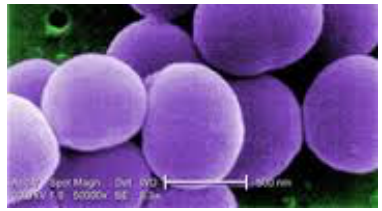
Spirillum

(d) Peritrichous

E. coli

S. typhi

atrichous

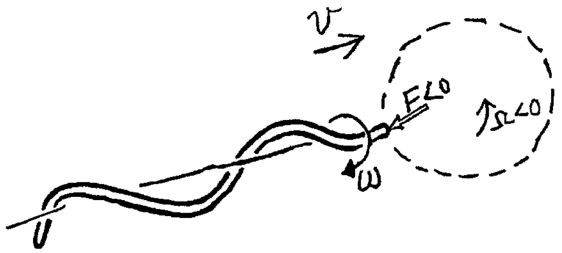


S. aureus

From flagellum to motion

All (swimming) bacteria swim by coupling translation and rotation

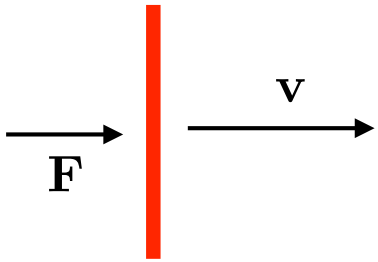
Rotation of a helical filament generates thrust…



E. M. Purcell PNAS (1997)

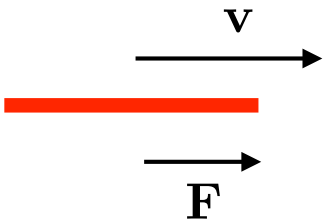
…due to difference in drag of a filament for:

"transverse" motion



$F = \gamma_{\perp} v$

"parallel" motion



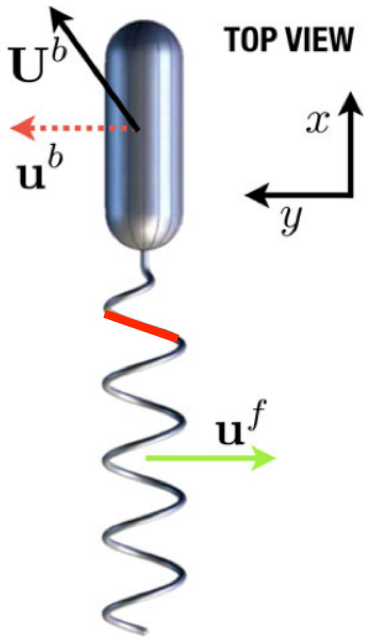
$F = \gamma_{||} v$

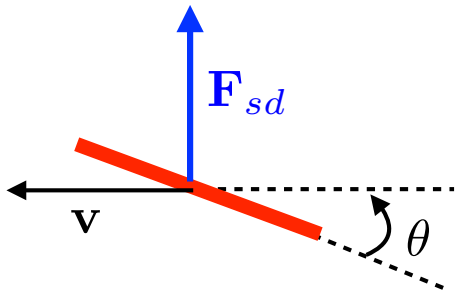
$$\gamma_{\perp} \simeq 2\gamma_{||}$$

"slender" filament

How??

Drag force on a section of rotating flagellum



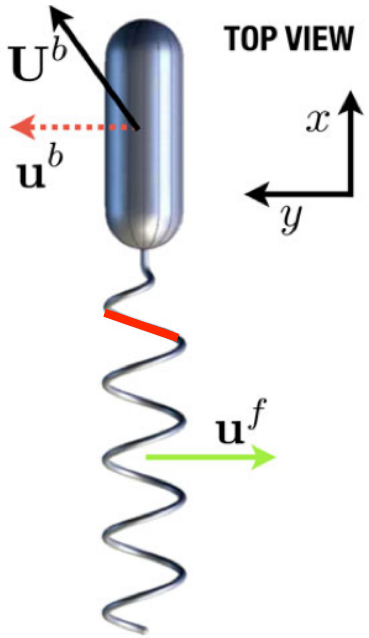


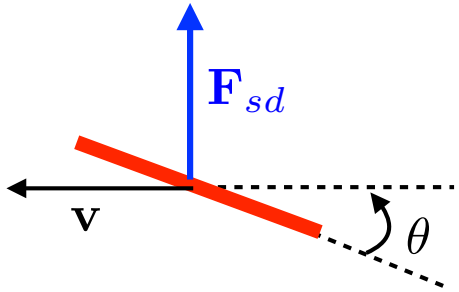
$\gamma_\perp$ perpendicular drag

$\gamma_{||}$ parallel drag

Your turn: find the force along the swimming direction $\mathbf{F}_{sd}$ due to motion of the filament shown above.

Drag force on a section of rotating flagellum





$\gamma_\perp$ perpendicular drag

$\gamma_{||}$ parallel drag

Along the swimming direction the total force is

Contribution to flagellar thrust ⟶ $F_{sd} = (\gamma_\perp - \gamma_{||})\frac{v}{2}\sin(2\theta)$

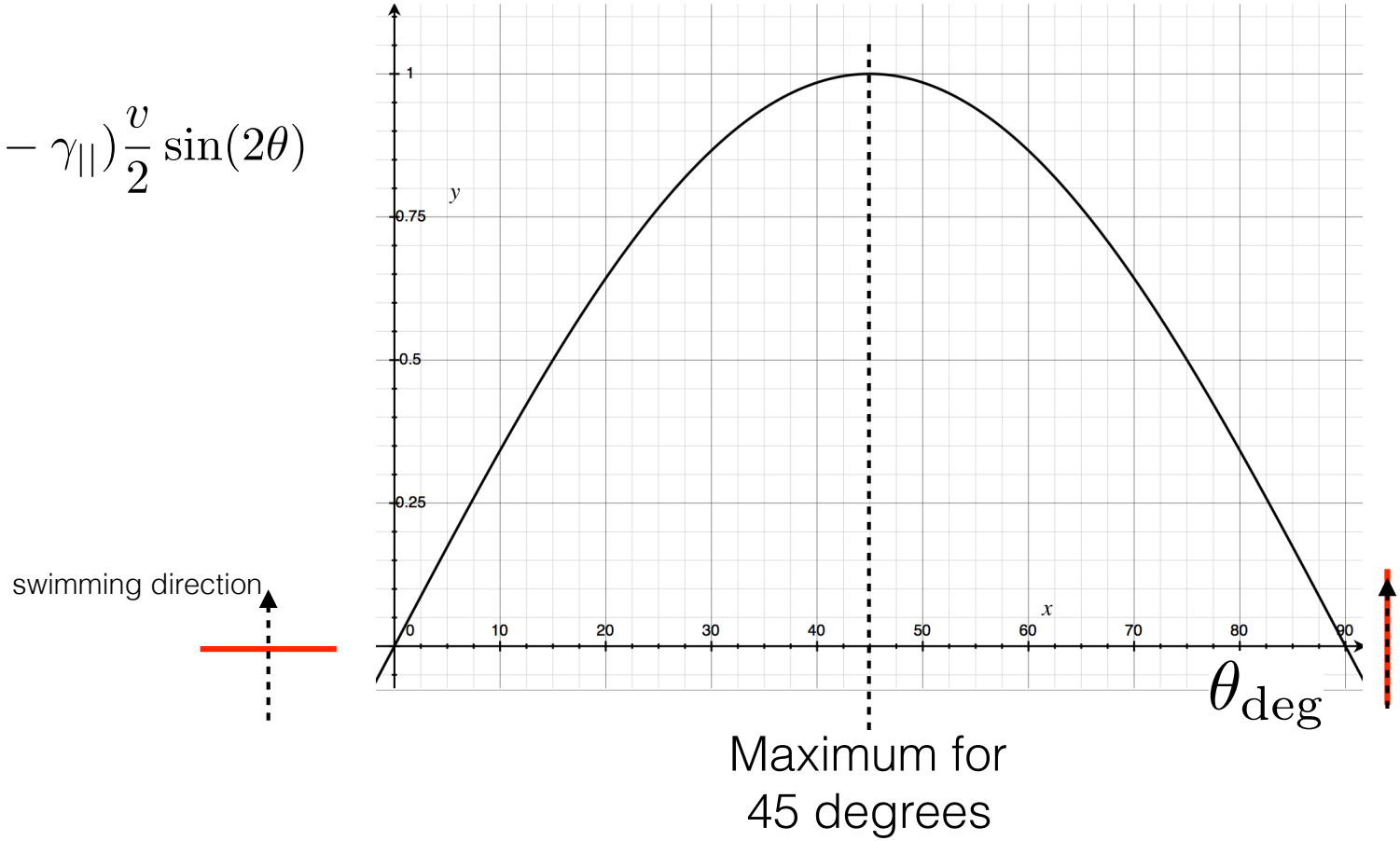
No thrust for

$\gamma_\perp = \gamma_{||}$

Difference in drag is essential for propulsion

Drag force on a section of rotating flagellum

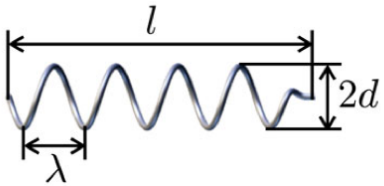
$$F_{sd} = (\gamma_{\perp} - \gamma_{||})\frac{v}{2}\sin(2\theta)$$



$$\tan(\theta) = \frac{\lambda}{2\pi\, d}$$

$$d_{\max} = \frac{\lambda}{2\pi} \simeq 160 - 320\,\mathrm{nm}$$

$$d_{\exp} \simeq 200\,\mathrm{nm}$$

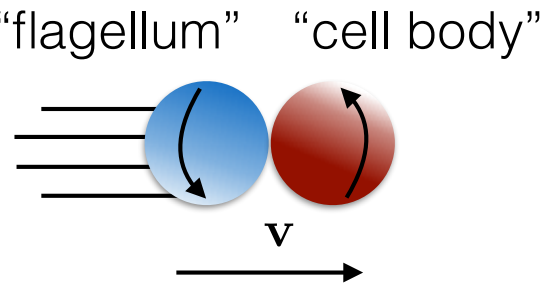


$$\lambda \sim 1 - 2\,\mu\mathrm{m}$$

operates close to maximum thrust!!

Is it an efficient propulsion method?
Rough estimate

bacterium = two spheres counter-rotating at frequency ν
translating at speed v



$\omega = 2\pi\,\nu$ angular speed (rad/s)

Swimming efficiency:

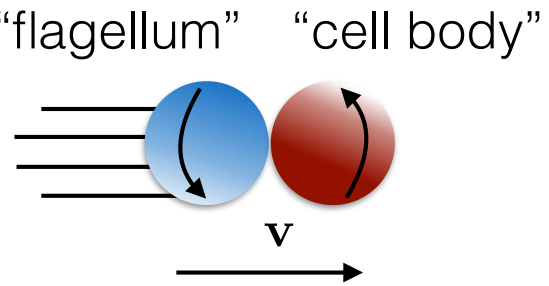
$$\epsilon = \frac{\text{power dissipated for translation}}{\text{total power dissipated}} \simeq \frac{F_{\text{drag}}\,v}{T_{\text{rot}}\omega}$$

$F_{\text{drag}} = 6\pi\gamma a\,v$ drag force on a single sphere translating at speed v

$T_{\text{rot}} = 8\pi\gamma a^3\omega$ drag torque on a single sphere rotating at angular speed ω

Is it an efficient propulsion method?
Rough estimate

$$\epsilon = \frac{\text{power dissipated for translation}}{\text{total power dissipated}} \simeq \frac{F_{\rm drag}\, v}{T_{\rm rot}\omega}$$



$F_{\rm drag} = 6\pi\gamma a\, v$ drag force on a single sphere translating at speed v

$T_{\rm rot} = 8\pi\gamma a^3\omega$ drag torque on a single sphere rotating at angular speed ω

$\omega = 2\pi\,\nu$ angular speed (rad/s)

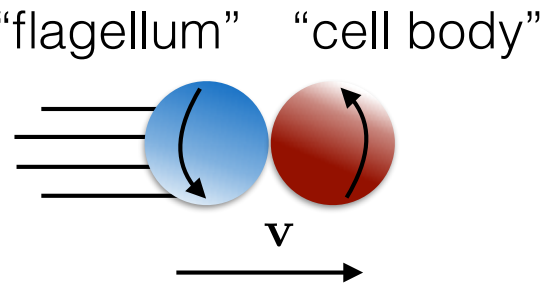
$\nu \simeq 100\,\mathrm{Hz}$ rotation frequency

$v \simeq 30\,\mu\mathrm{m/s}$ swimming speed

Your turn:
estimate the efficiency of
bacterial propulsion

Is it an efficient propulsion method?
Rough estimate

$$\epsilon = \frac{\text{power dissipated for translation}}{\text{total power dissipated}} \simeq \frac{F_{\rm drag}\, v}{T_{\rm rot}\omega}$$



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$\omega = 2\pi\,\nu$ angular speed (rad/s)

$\nu \simeq 100\,\text{Hz}$ rotation frequency

$v \simeq 30\,\mu\text{m/s}$ swimming speed

$$\epsilon \simeq 0.8\%$$

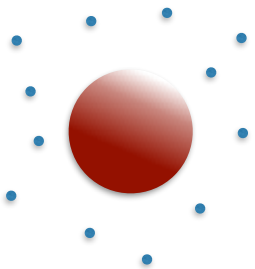
$$(a = 0.5\,\mu\text{m})$$

experimentally $\epsilon_{\rm exp} \simeq 2\%$

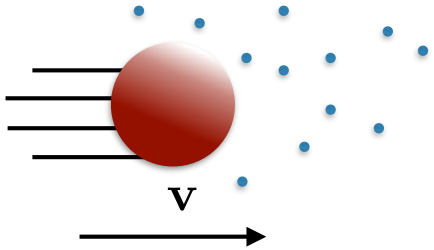
Not very efficient

Why do they move?

Could it be to increase absorption of nutrients?



c_0 background concentration



Diffusive uptake rate

$$r_{dif} = 4\pi a D c_0$$

Advective uptake rate

$$r_{dr} = S v c_0$$

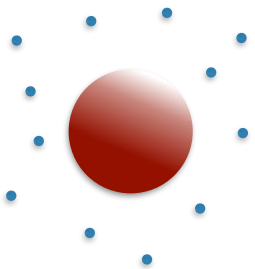
$D \simeq 4 \times 10^3 \, \mu\mathrm{m}^2/\mathrm{s}$ diffusion constant for molecules

S cross-section of the sphere

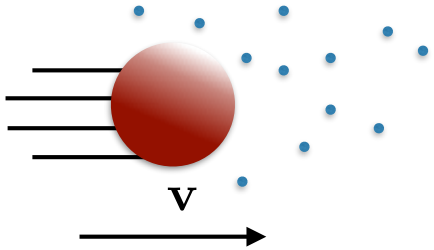
Your turn: estimate the importance of nutrient uptake by advective vs. diffusive uptake

Why do they move?

Could it be to increase absorption of nutrients?



c_0 background concentration



Diffusive uptake rate

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Advective uptake rate

$$r_{dr} = S v c_0$$

$D \simeq 4 \times 10^3 \, \mu\mathrm{m}^2/\mathrm{s}$ diffusion constant for molecules

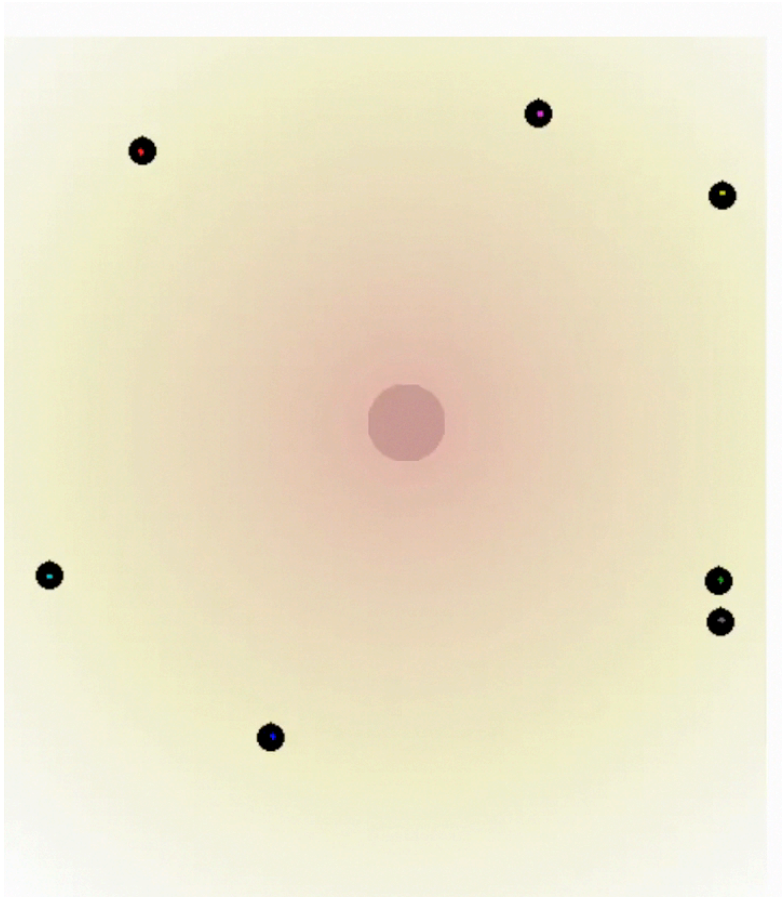
S cross-section of the sphere

$$\frac{r_{dr}}{r_{dif}} \simeq 0.3\%$$

Not significant!

Ideas??

Motility allows to move to better locations



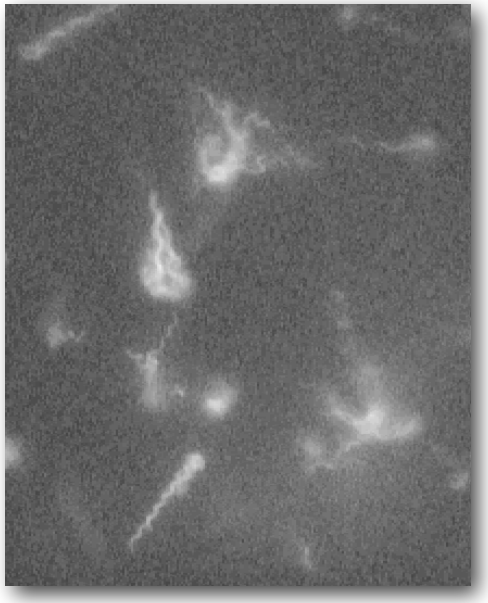
Swimming
+
directional changes

bacterial cells

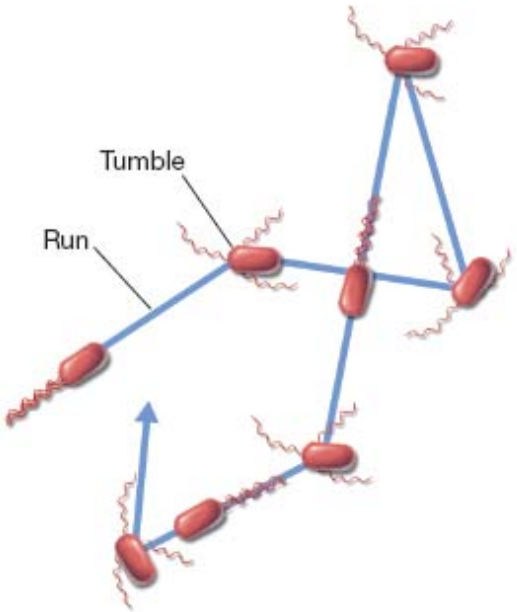
source of nutrients (chemoattractant)

How do you change the direction of motion?

"tumbles"…

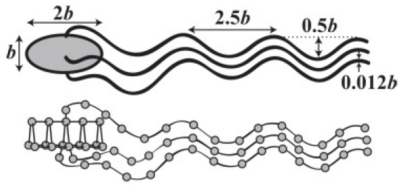


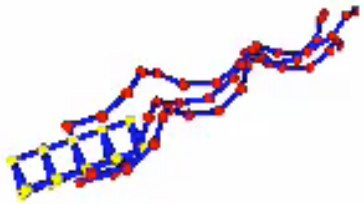
Turner *et al.* (2000)



…due to individual flagella randomly rotated in a direction opposite to normal

Simulated tumble



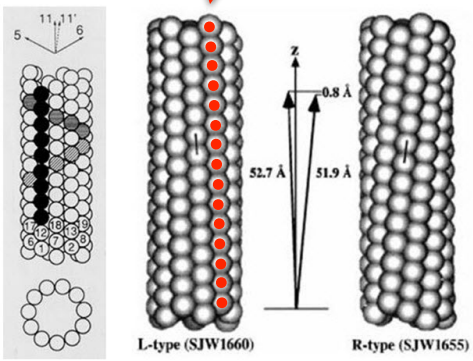


Watari, Larson *Biophys. J.* **98**, 12 (2010)

Notice: **shape** of counter-rotating flagellum changes!

Flagellar polymorphism facilitates “run and tumble”

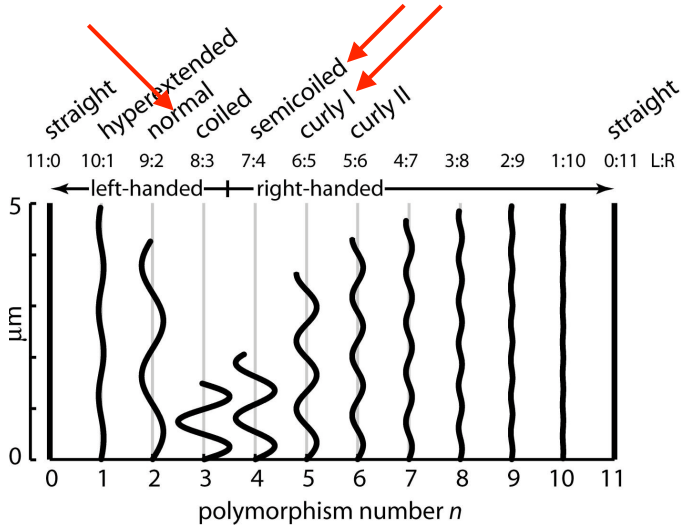
11 flagellin filaments



Macnab R.M. *Flagella and Motility* (1999)

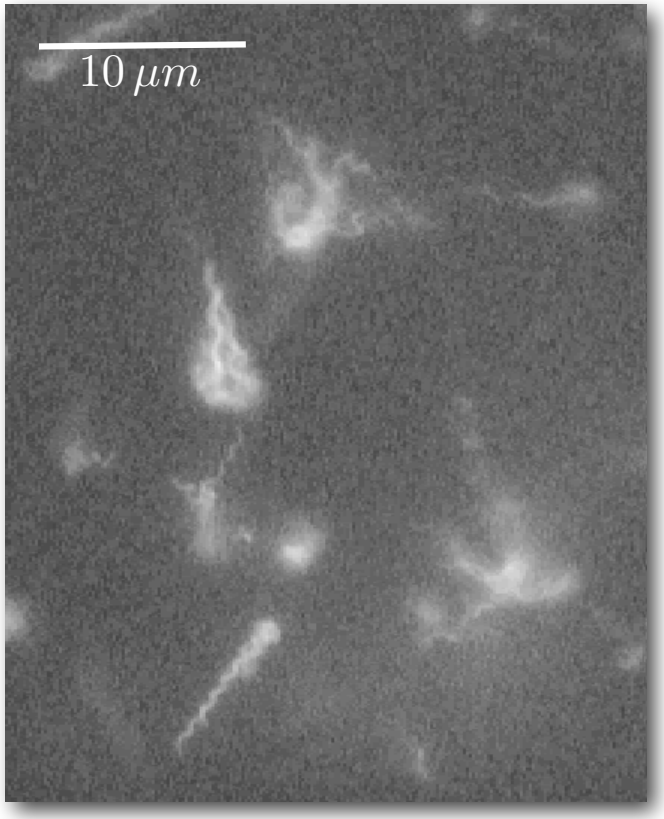
Calladine C.R. in *Prokaryotic and eukaryotic flagella* (1982)

Polymorphic states of bacterial flagella



Darnton N., Berg H. *Biophys. J.* (2007)

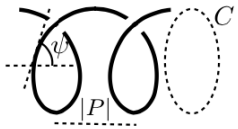
E. coli



Turner, Ryu and Berg *J. Bacteriol.* **182**, 2793 (2000)

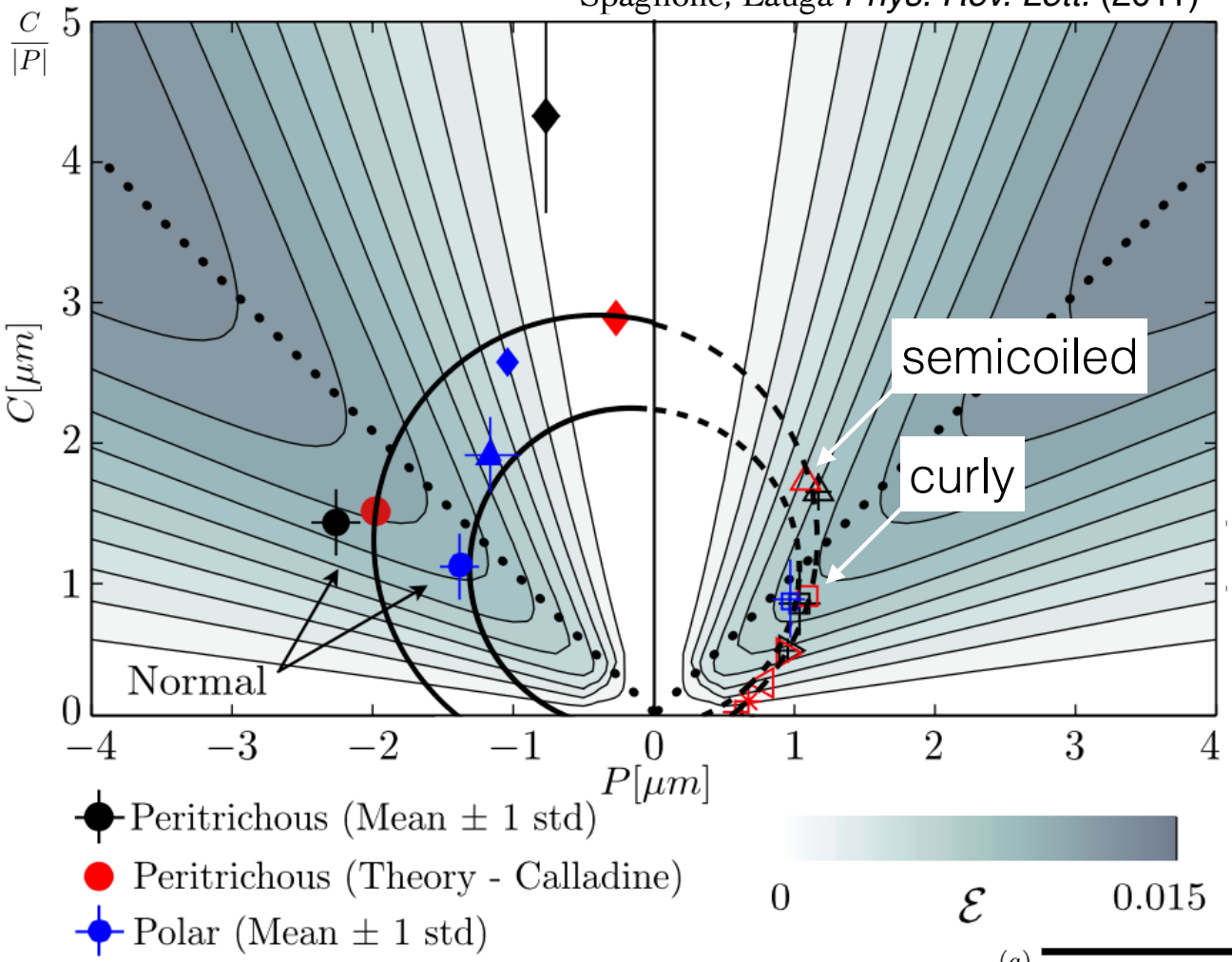
(run and tumble)

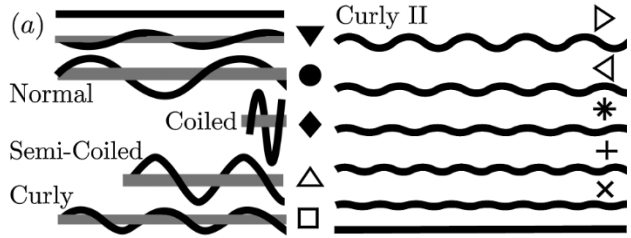
“Normal” is actually
the most hydrodynamically efficient shape



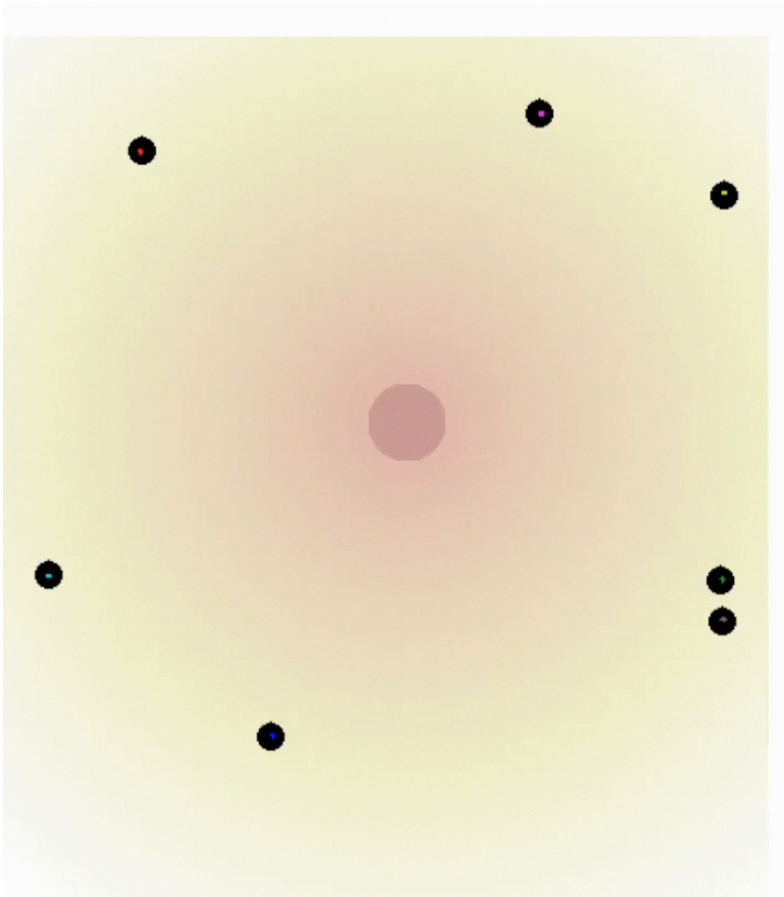
$$\tan(\psi) = \frac{C}{|P|}$$

Spagnolie, Lauga *Phys. Rev. Lett.* (2011)





Chemotaxis:
control of the "average" motion by regulation of tumbling frequency



Cell "memory" ~1s

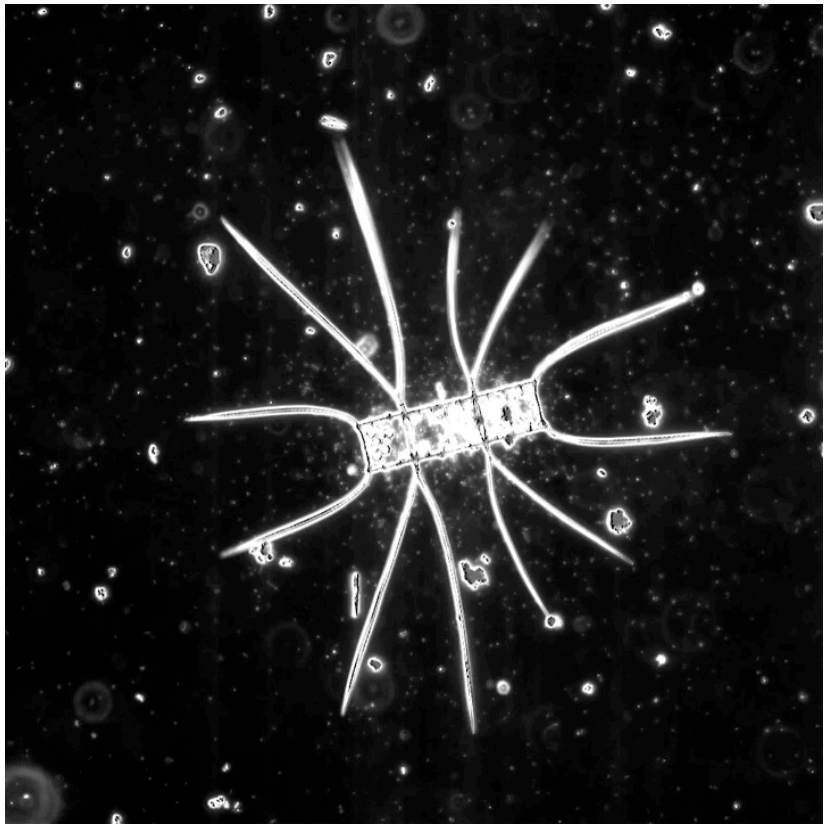
- if concentration increases: tumble less
- if concentration decreases: tumble more

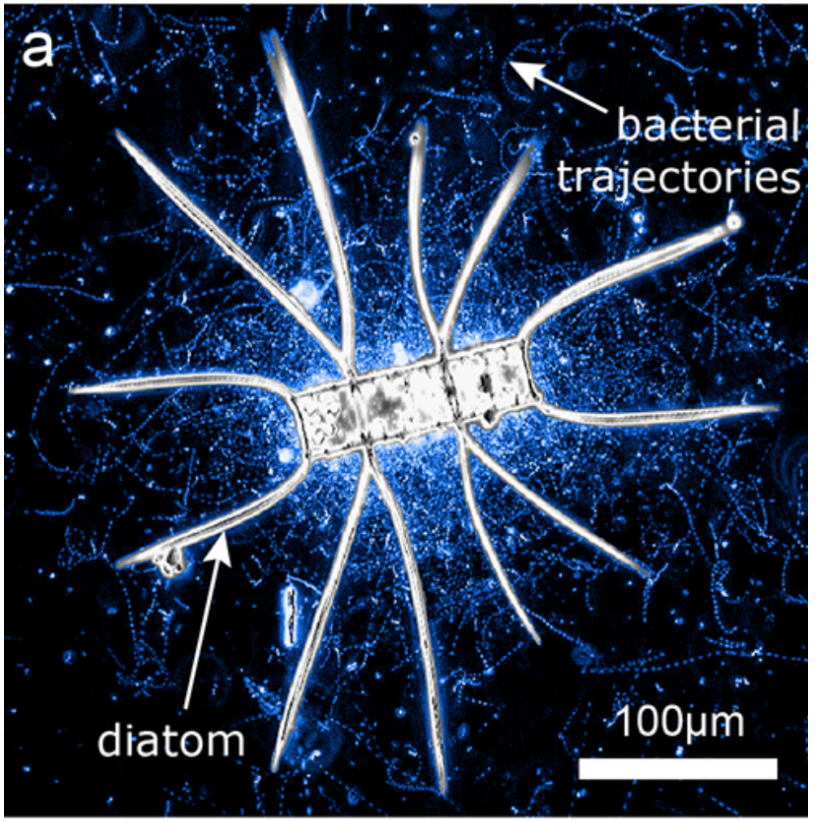
Directionality is achieved through a modulation of randomness

bacterial cells

nutrient source (chemoattractant)

Example: Marine bacteria chemotaxing to a dead diatom





Son, **Brumley**, Stocker *Nat. Rev. Microbiol.* **13**, 761 (2015)

It works!