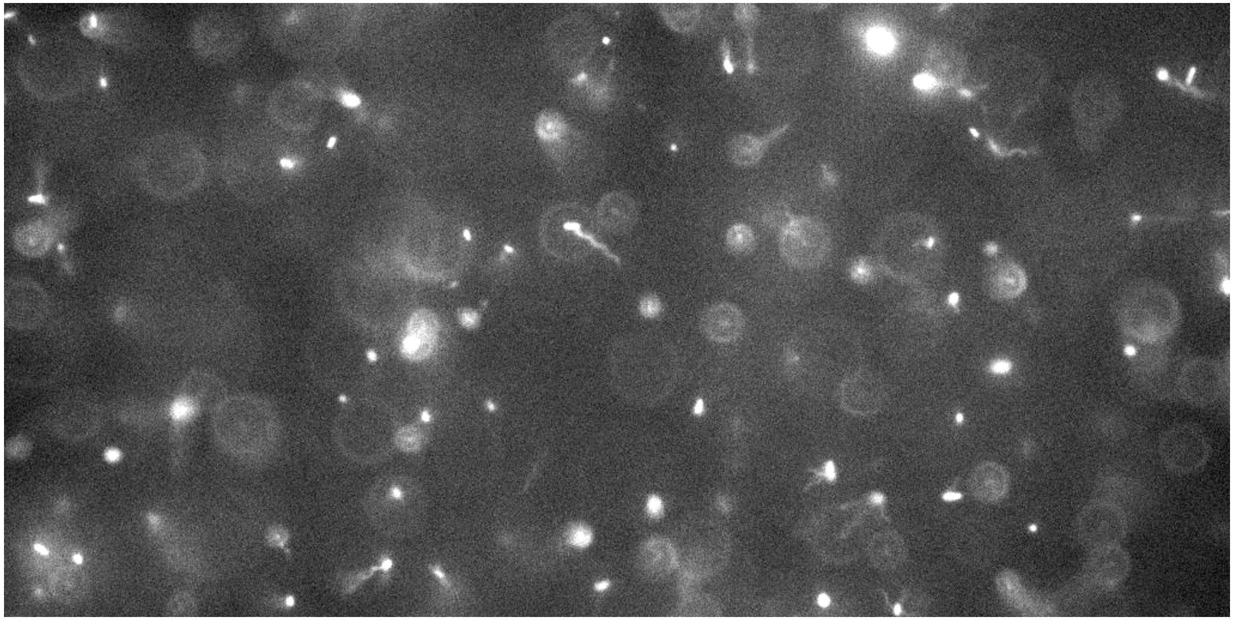
Eric Clément eric.clement@upmc.fr

Active Fluids Group at the PMMH-ESPCI, Paris

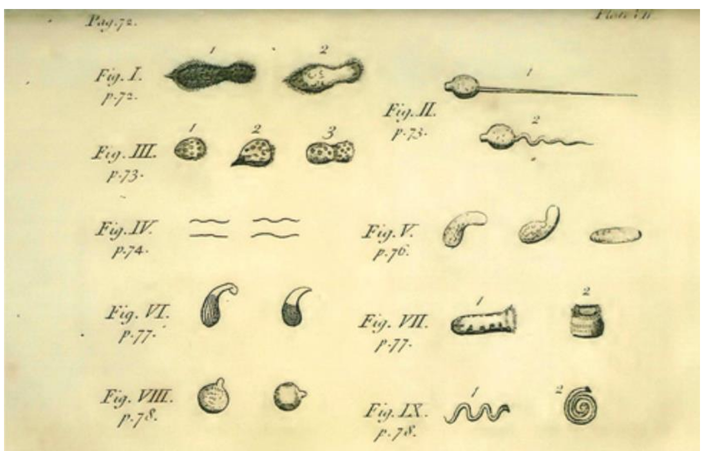
Some elements on bacteria motility



The first microbiologist !

Antoni van Leeuwenhoek (1632-1723)
father of protozoology and bacteriology

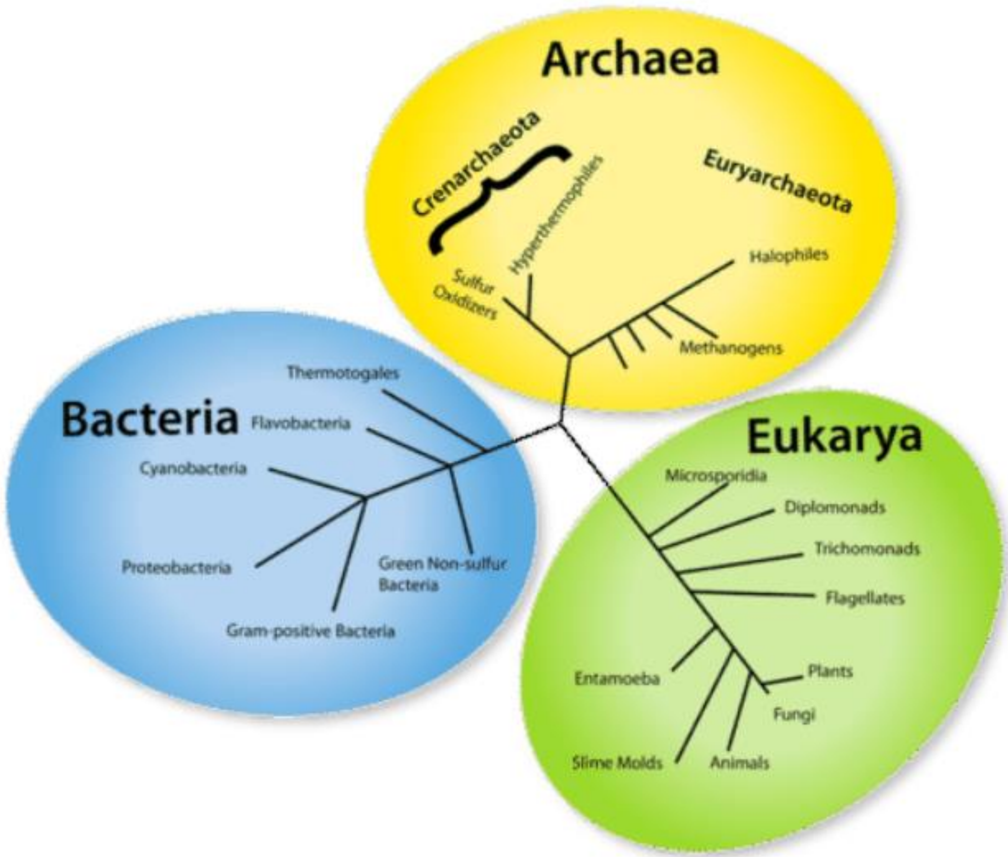
Animalcula



- ❑ Discovery of « little animals »
- ❑ First description of autonomous motion by the « *rotation of small wheels issuing out of their bodies*»

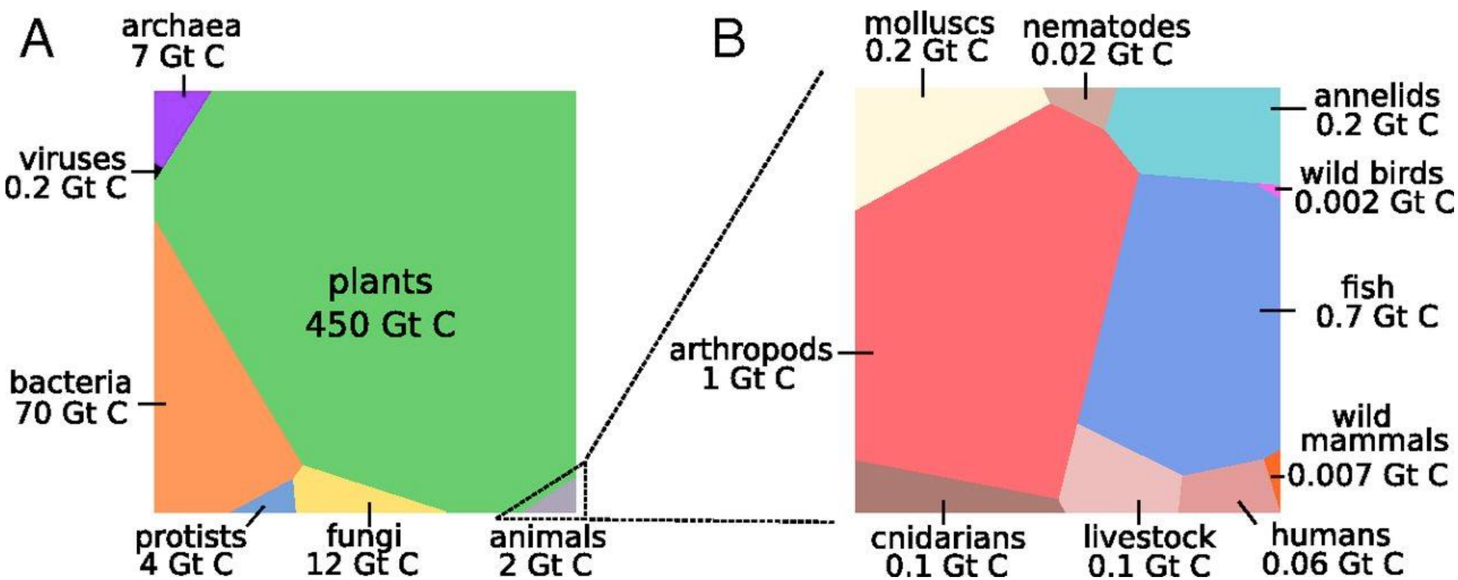
van Leeuwenhoek Lett. Roy. Soc., **24** (1705)
(2 citations)

The three kingdoms of life (on earth)



Bacteria

- Bacteria are micro-organisms (typically 1-2 µm) ubiquitous in nature
- Appeared 3.8 billion years ago! => strong capacity of **adaptation** to diverse environments
- ~30,000 formally named species (*i.e.* for which the physiology has been studied) Dykhuizen, *Proc. Calif.Acad.Sci.*

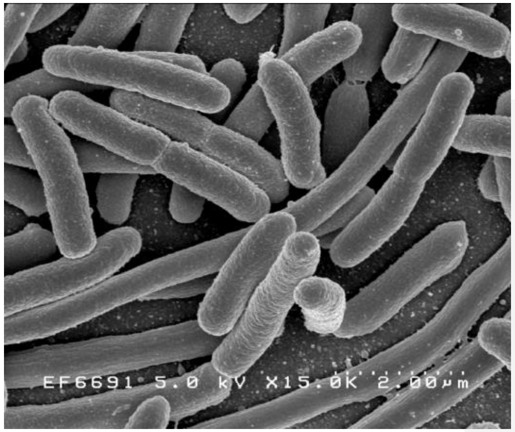


The Biomass distribution on Earth Bar-On *et al.*, *PNAS* (2019)

Bacteria and Human Beings

- Bacteria are essential to human life : 90% of our body is composed by non-human cells among which bacterial cells. Turnbaugh *et al., Nature* (2007)
- *E. coli* is a common example of bacteria living in the human gut
- We often use bacteria in industrial processes like cheese fabrication or wine malolactic fermentation

Cheese



Escherichia coli

Vine

Bacteria populations dynamics

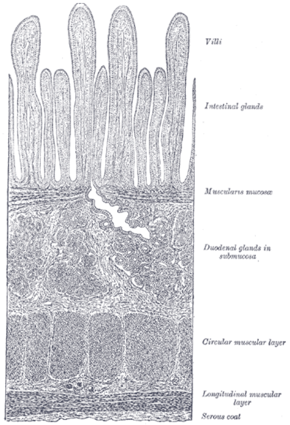
Soil ecology

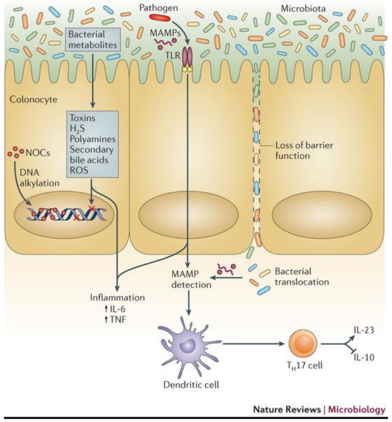
CO2 production/storage

Biodiversity (symbiosis with plant roots, fungi)

Microbiota

Intestin wall



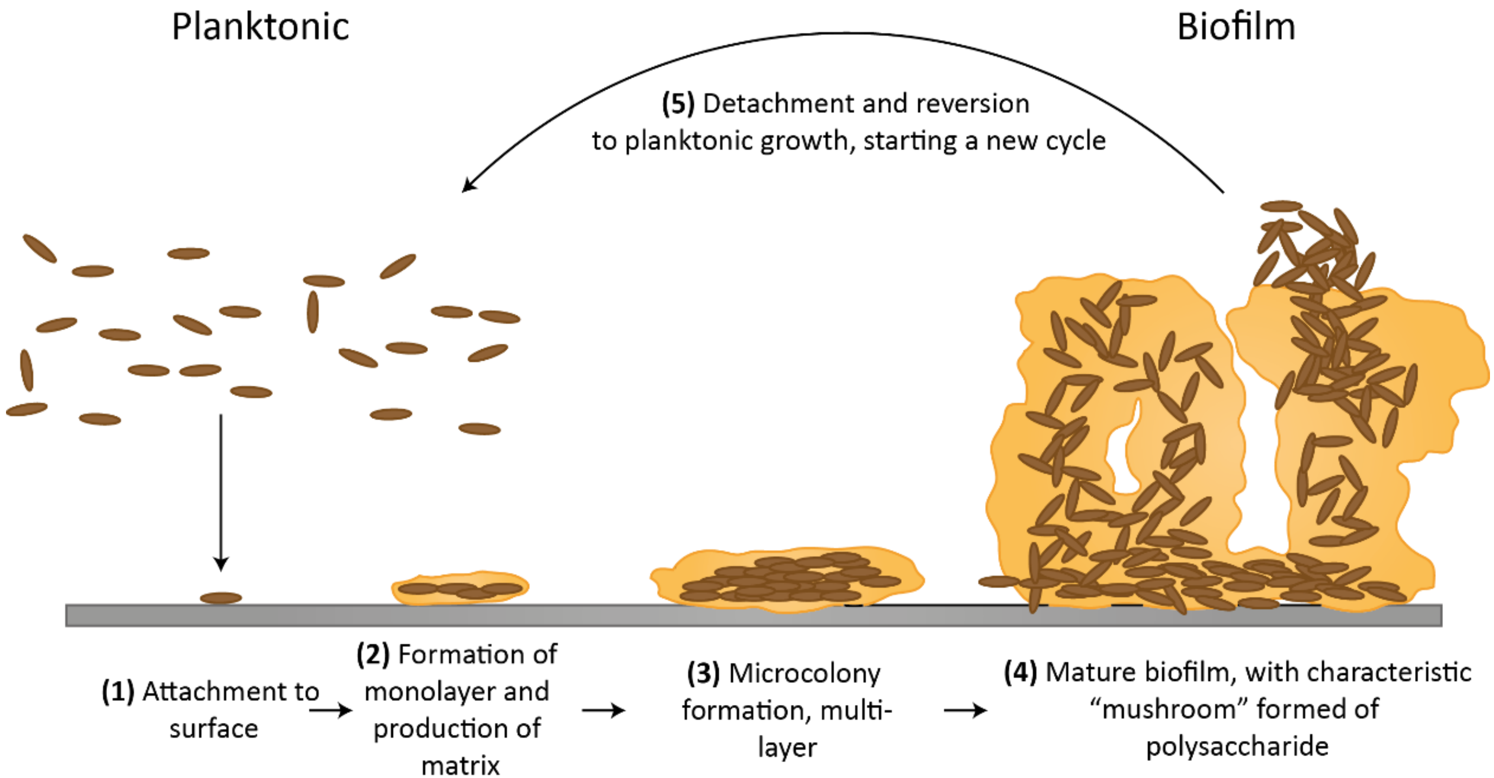


Key role in many pathologies

Chronical deseases
Obesity
Cancer

New therapeutic pathways

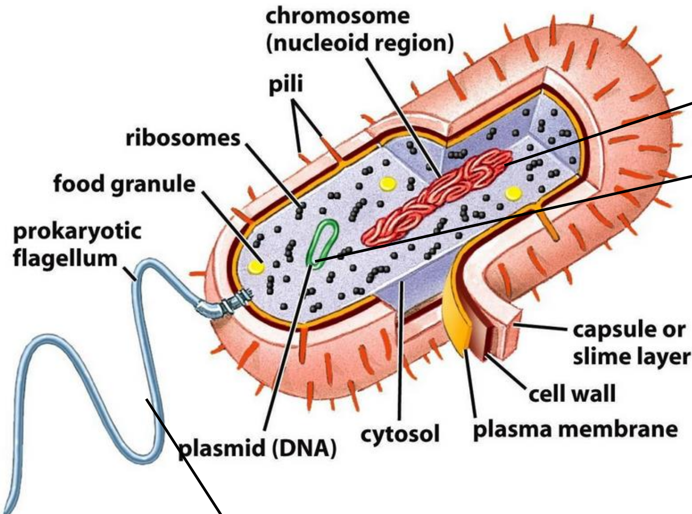
Planktonic state vs biofilm



Hollmann et al. _Brit.Soc.Immun

- Planktonic state is essential to bacteria in order to explore and colonize environments

Motile bacteria

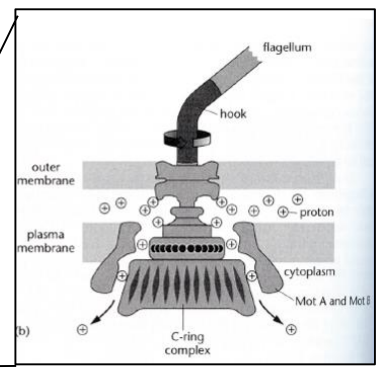


Genetics, epigenetics

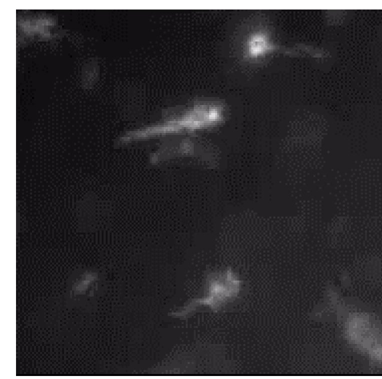
Adaptative evolution, mutations

Synthetic biology
Programable target function

Proton gradient motor



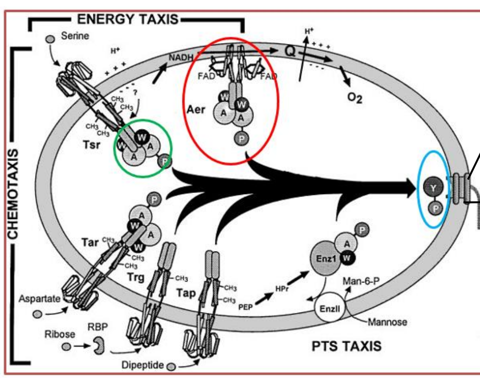
Motility



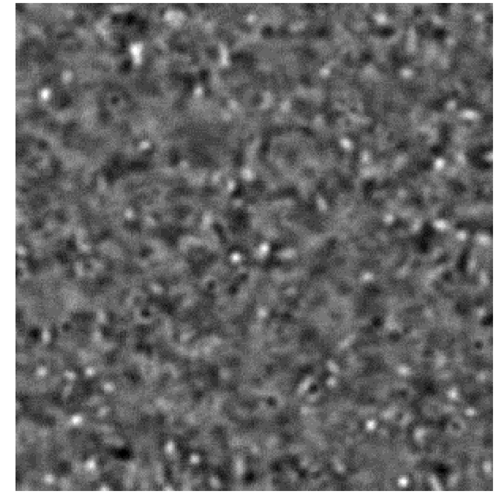
Biochemical machinary

Chemical
environment

Attractant/repellent
Redox reactions



A model for « active colloids »



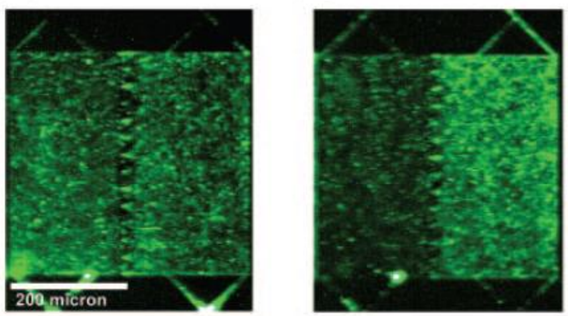
- ❑ Revisit the statistical physics of colloids
- ❑ Emergence of new universality classes
  - Symmetry of microscopic interactions
  - Range of interactions
- ❑ Hydrodynamic description
  - Macroscopic constitutive relations
  - Transport relations
- ❑ See for a practical introduction : Schwarz-Linek et al., Colloids and Surfaces B: Biointerfaces, **137**,2 (2016).

Many novel effects

(some examples)

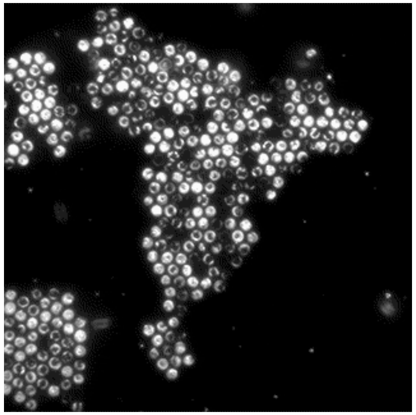
Diffusion rectification

t = 0 t > 0



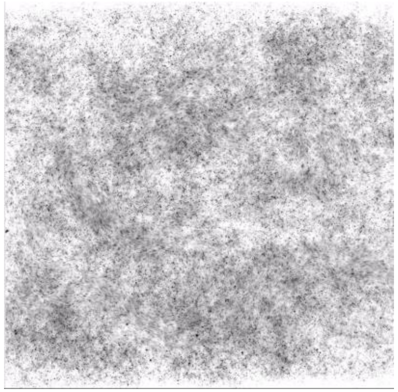
Galajda et al. J. o f Bact ., **189** 8704 (2007)

Living crystals



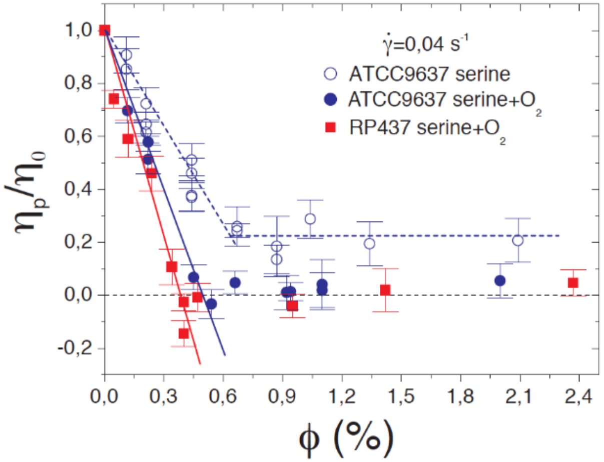
Petroff et al. PRL **114**, 158102 (2015)

Collective motion



Martinez et al. PNAS **117**, 2326 (2020)

« Superfluid »



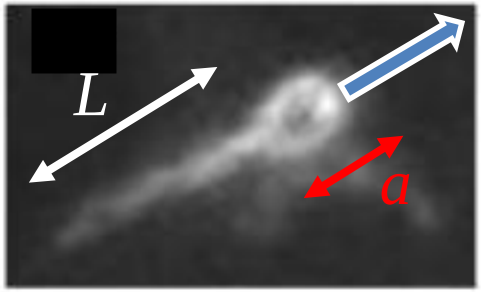
Lopez et al. PRL. **115**, 028301 (2015)

Bacteria swarms activate gear

Sokolov et al. PNAS **107**, 969 (2010)

Hydrodynamics of swimming

$$\vec{V}_B \cong 25 - 30\,\mu m / s$$



$$a \cong 2\,\mu m$$

$$L \cong 6 - 7\,\mu m$$

Reynolds number $\mathrm{Re} = \dfrac{\text{Inertial forces}}{\text{Viscous forces}} = \dfrac{\rho\, a V_B}{\eta} \cong 10^{-4}$

- ❑ Low Reynolds microswimmer

The small forces kingdom

$$F = \frac{\eta^2}{\rho}$$

Intrinsic force scale for a viscous fluid
$F = 10^{-9}\ N$ for water

$Re >> 1$ $\quad f \propto F R_e^2$

$Re << 1$ $\quad f \propto F R_e$ « Small forces » kingdom !

- ❑ You need to develop forces of order $f = Re\, F$ to propel or move an object in a viscous fluid

$f \cong 10^{-13}\ N$

Ex

$a = 1\ \mu m$

$V = 30\ \mu m/s \quad \Rightarrow \quad \mathrm{Re} \approx 10^{-4}$

« Coasting time » $\tau_\delta = \frac{\rho a^2}{\eta} = 10^{-6} s$

« Coasting length » $\delta = V\tau \cong 10^{-11} m$

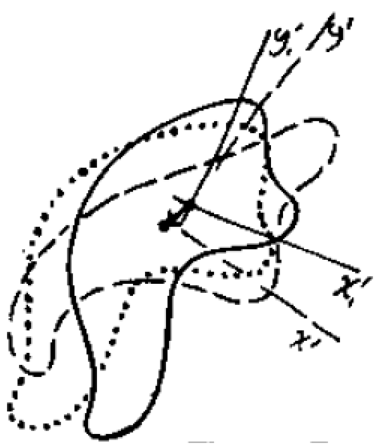
Stokes equations $Re = 0$

$$-\vec{\nabla}p + \eta\nabla^2\vec{u} = \vec{0}$$

$$\vec{\nabla}.\vec{u} = 0$$

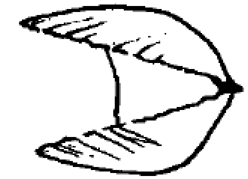
No time explicitely !

+ Instantaneous boundary conditions



- ❑ Swimming means « move your body in a cyclic manner and hope for a net motion »
- ❑ The distance travelled by the swimmer between two different surface configurations does not depend on the rate at which the surface deformation occurs, but only on the geometry of the intermediate shapes.

The « scallop theorem »

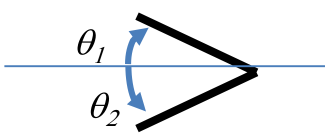


E.M.Purcell, Am. J. Phys. 45, 11 (1977)

Purcell, PNAS, 94 11307 (1997)

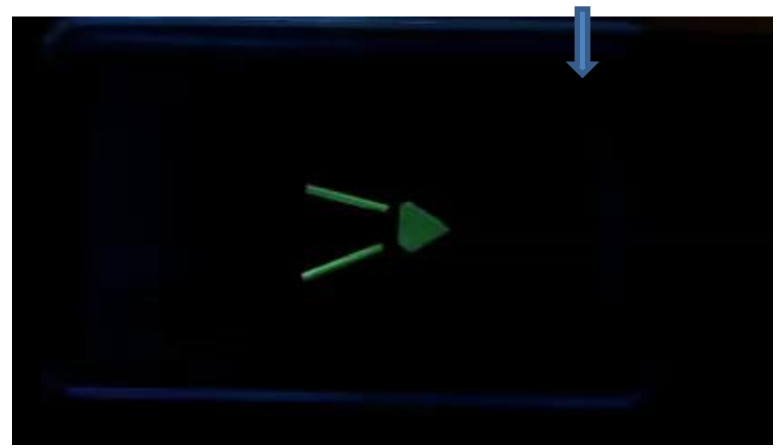
$Re=10^3$

L=10 cm

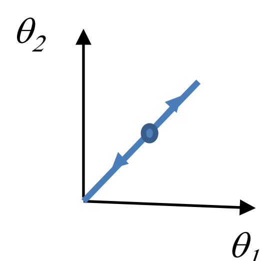


$Re << 1$

L=10 cm



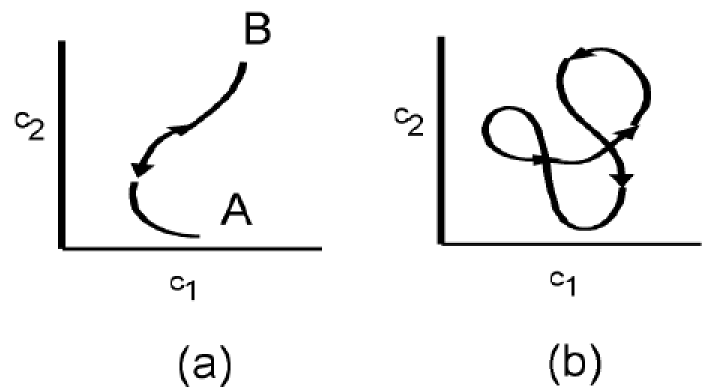
Qiu et al Nature Com (2014)



Reversible motion

The « scallop theorem » or reciprocal motion theorem

Ex. (C1, C2) 2D configuration space

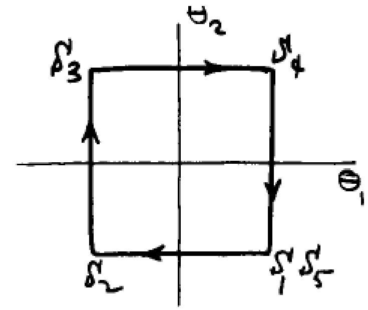


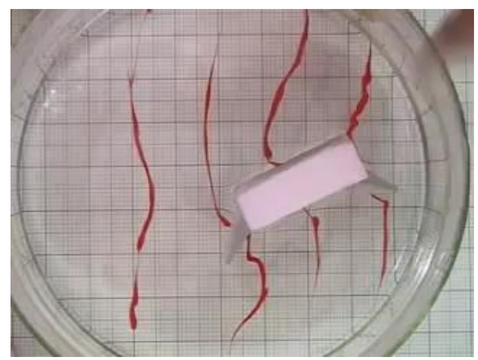
(a) Reciproqual motion
(b) Non-reciprocal motion

« *If the standard movement of a periodic Stokesian locomoter is reciprocal, steady locomotion with non-zero velocity is not possible.* » (Purcell 1977)

A minimal swimmer model

the 3 – link swimmer (Purcell 1977)





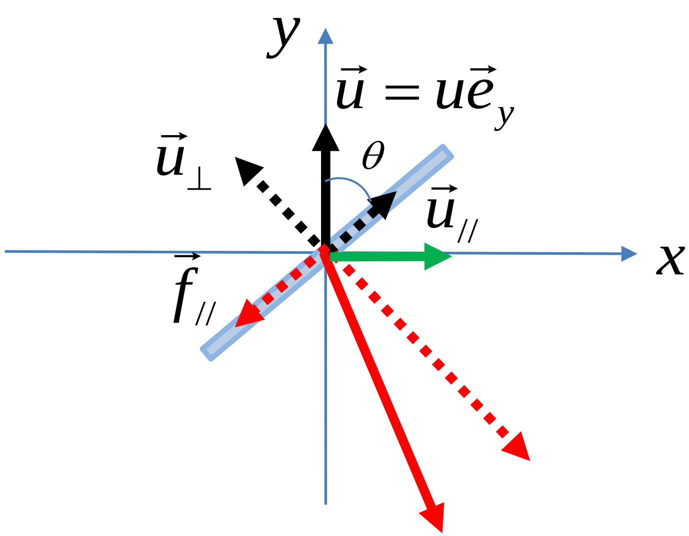
Peko Hosoi MIT

Helical swimming

G.I. Taylor movie series on hydrodynamics

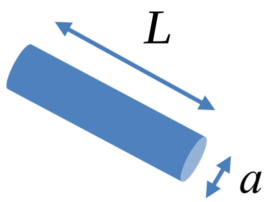
- The corkscrew strategy is efficent at zero Re

Key concept



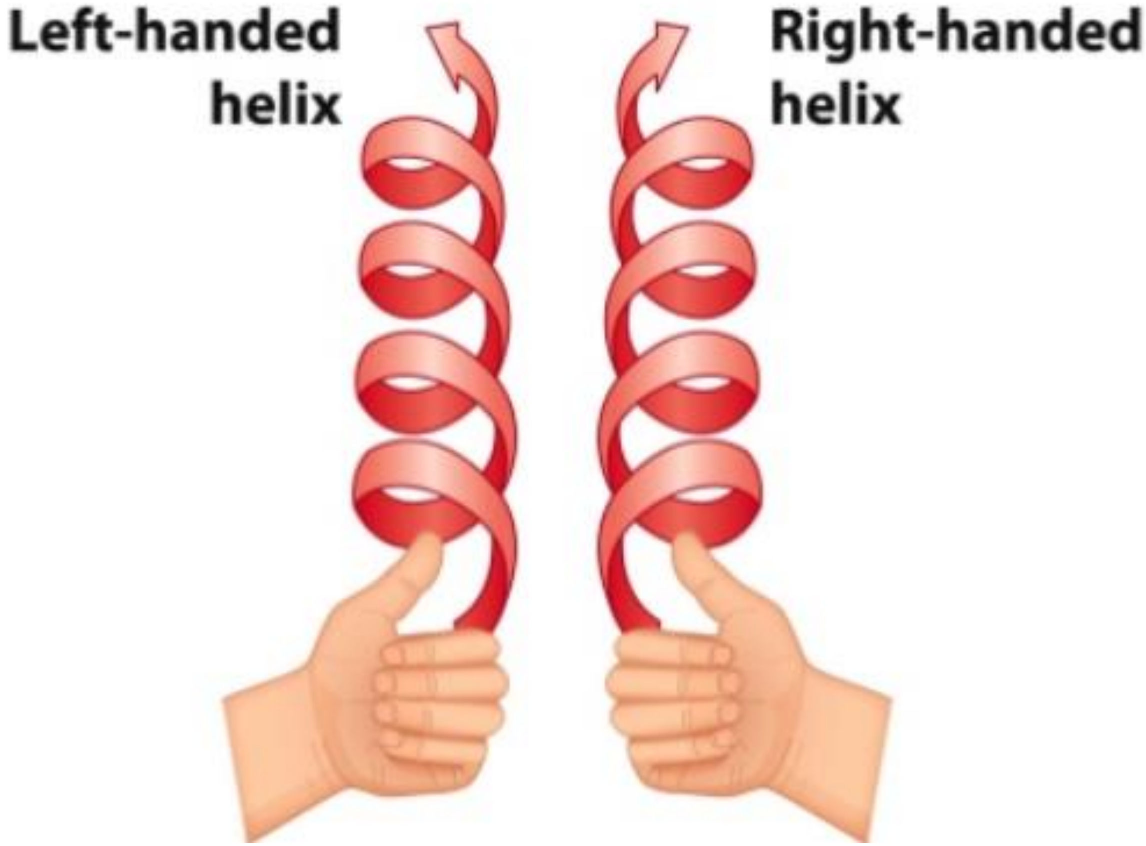
$$\vec{f}_{prop} = \eta(\xi_\perp - \xi_{//}) u \sin\theta \cos\theta \, \vec{e}_x$$ **Force/length** along x direction

Ex.



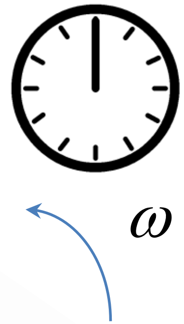
$$\xi_\perp = 2\xi_{//} = \frac{4\pi}{ln 2L/a}$$

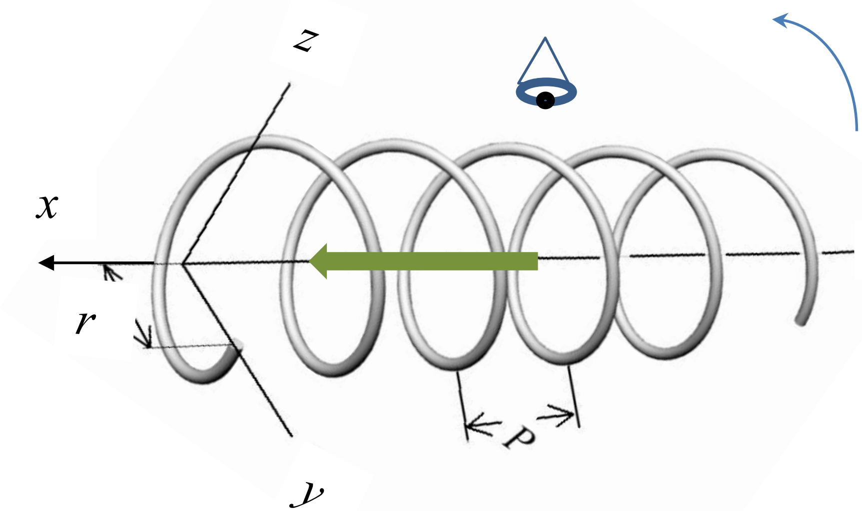
❑ Drag anisotropy


Left-handed
helix
Right-handed
helix

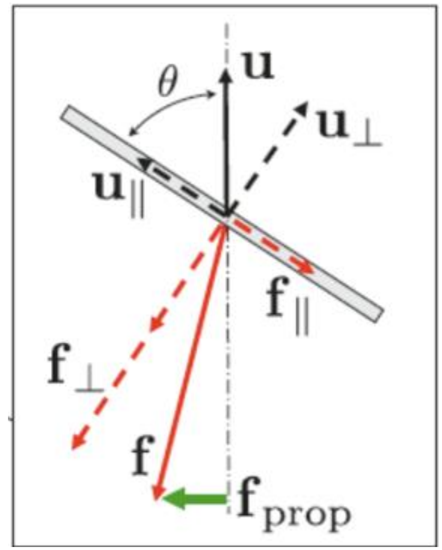
Helical propulsion

Right handed helix CW rotation





Top view



Angular velocity

$$u = r\omega$$

- ❑ Propulsive force / length

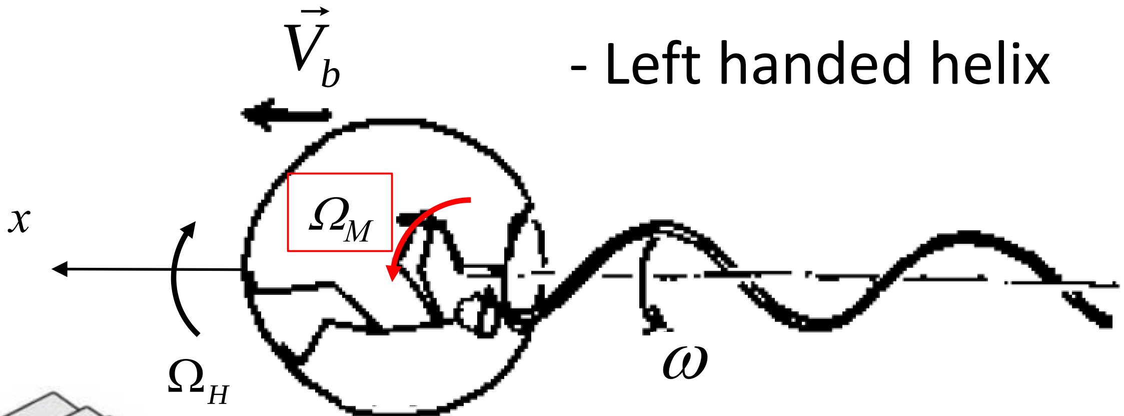
$$\vec{f}_{prop} = \eta(\xi_\perp - \xi_{//})u\sin\theta\cos\theta\,\vec{e}_x$$

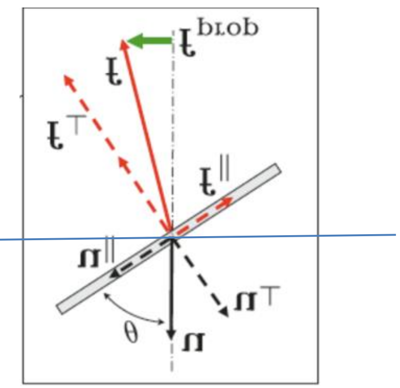
E.Lauga and T.R.Powers, Rep. Prog. Phys. 72, 096601 (2009)

Model for a self-propelled bacterium

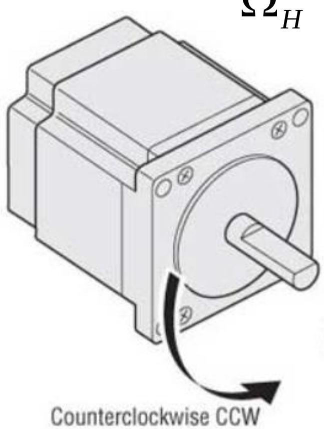
Purcell PNAS **94** 11307 (1997)

- Left handed helix



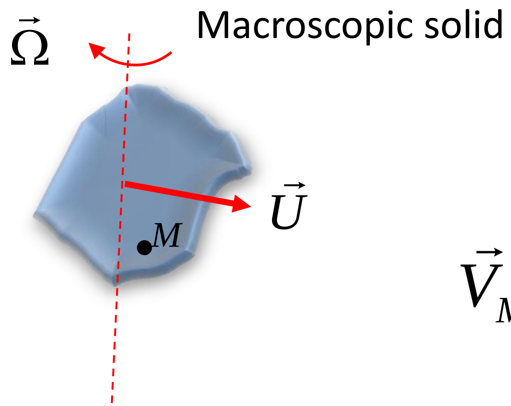


CCW motor rotation



- ❑ Flagella rotate counter clockwise (CCW)
- ❑ The body rotates clockwise (CW)

The resistance matrix $Re = 0$



$\vec{F}$	Force on the solid	$\vec{U}$	Translation velocity
$\vec{T}$	Torque on the solid	$\vec{\Omega}$	Angular roration rate

$$\vec{V}_M = \vec{U} + \vec{\Omega} x \vec{r}$$

$$\begin{pmatrix} \vec{F} \\ \vec{T} \end{pmatrix} = -\eta \begin{pmatrix} \overline{\overline{A}}\,\vec{U} + \overline{\overline{B}}\,\vec{\Omega} \\ {}^{t}\overline{\overline{B}}\,\vec{U} + \overline{\overline{C}}\,\vec{\Omega} \end{pmatrix}$$

$$\overline{\overline{A}}, \overline{\overline{B}}, \overline{\overline{C}}$$

Rank 2 tensor (3x3matrices)

$$\left[\overline{\overline{A}}\right] = L, \ \left[\overline{\overline{B}}\right] = L^2, \ \left[\overline{\overline{C}}\right] = L^3$$

Physical dimensions

General properties

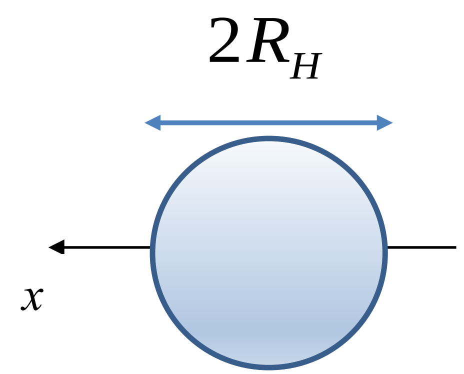
$$A_{ij} = A_{ji}, \quad C_{ij} = C_{ji}$$

Physical symetries

❑ Resistance matrix for the sphere

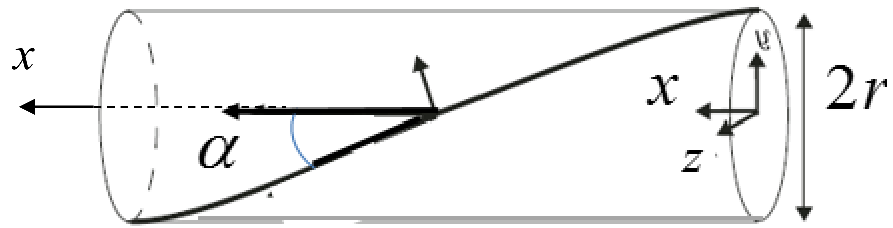
$$\begin{pmatrix} F \\ T \end{pmatrix} = -\eta \begin{pmatrix} A & 0 \\ 0 & C \end{pmatrix} \begin{pmatrix} U \\ \Omega \end{pmatrix}$$

$$A = 6\pi R_H$$
$$C = 8\pi R_H^3$$



❑ Resistance matrix for the helix (left handed)

$$\begin{pmatrix} F \\ T \end{pmatrix} = -\eta \begin{pmatrix} a & b \\ b & c \end{pmatrix} \begin{pmatrix} U \\ \omega \end{pmatrix}$$

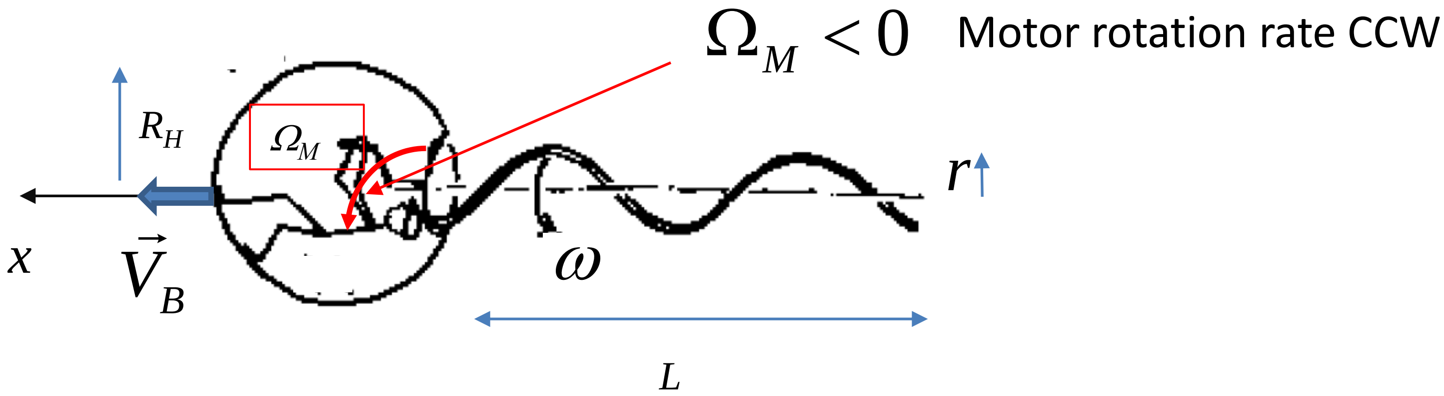


$$a \approx \xi_{//} L$$
$$b \approx \alpha(\xi_{\perp} - \xi_{//}) r L$$
$$c \approx \xi_{\perp} r^2 L$$

Helix angle $\alpha << 1$

Rodenborn et al. PNAS, **110**, 338 (2013)

Swimming velocity

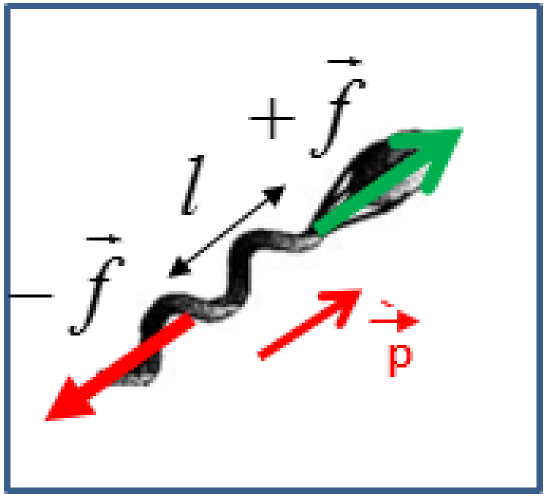


$$V_B = -\frac{bC}{(A+a)(C+c)-b^2}\,\Omega_M \propto (\xi_\perp - \xi_{//})\,\Omega_M$$

Motor rotation rate

Drag anisotropy

Swimmers a as force dipoles



An elliptical cow !

Stokeslet solution $Re=0$

Stokes equations

$$-\vec{\nabla}P + \eta\nabla^2\vec{u} + \vec{F} = \vec{0} \qquad \vec{F}(\vec{r}) = \vec{f}\delta_{\vec{r}-\vec{r}\,'}$$

One force singularity in $\vec{r}\,'$

$$\vec{\nabla}.\vec{u} = 0$$

$$\vec{u} = \overline{\overline{G}}(\vec{r}-\vec{r}\,')\vec{f}$$

$$P = \frac{\vec{f}.(\vec{r}-\vec{r}\,')}{4\pi\, r^3}$$

Oseen tensor

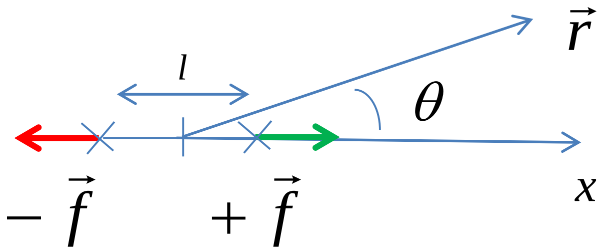
$$\overline{\overline{G}}(\vec{r}) = \frac{1}{8\pi\eta_0}\left(\frac{\overline{\overline{1}}}{r} + \frac{\vec{r}\vec{r}}{r^3}\right)$$

$$u \propto \frac{f}{\eta_0 r}$$

- Linear superposition of stokeslets -> solution of Stokes equation

$$\vec{u} = \sum_i \overline{\overline{G}}(\vec{r}-\vec{r}_i)\,\vec{f}_i$$

Moving stresslets $Re=0$



$l \to 0$

No net force on the fluid

$$\vec{u}(\vec{r}) = \frac{fl}{8\pi\eta_0 r^3}(-1+3\cos\theta^2)\vec{r}$$

$$P = 2\eta\frac{fl}{r^3}\left[-1+3\cos^2\theta\right]$$

$$u \propto \frac{\sigma_0}{\eta_0 r^2}$$

$$\sigma_0 = f\,l$$

Dipole strength

$$\sigma_0 \propto \eta_0 V_B\, l^2$$

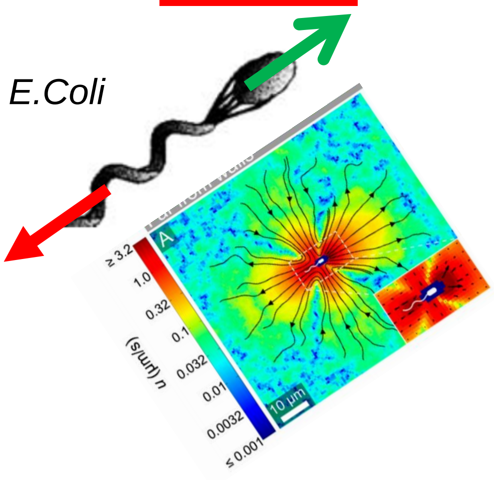
Swimming activity

Force dipole sign

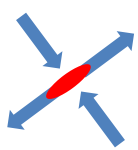
(Far field)

Pusher

E.Coli



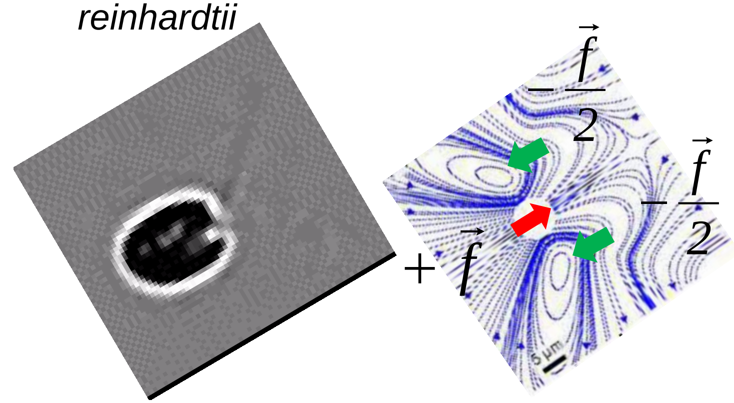
K.Drescher et al. PNAS **108**, 10945 (2011)



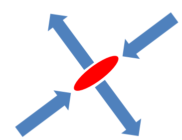
Extensile

Puller

Clamydomonas reinhardtii

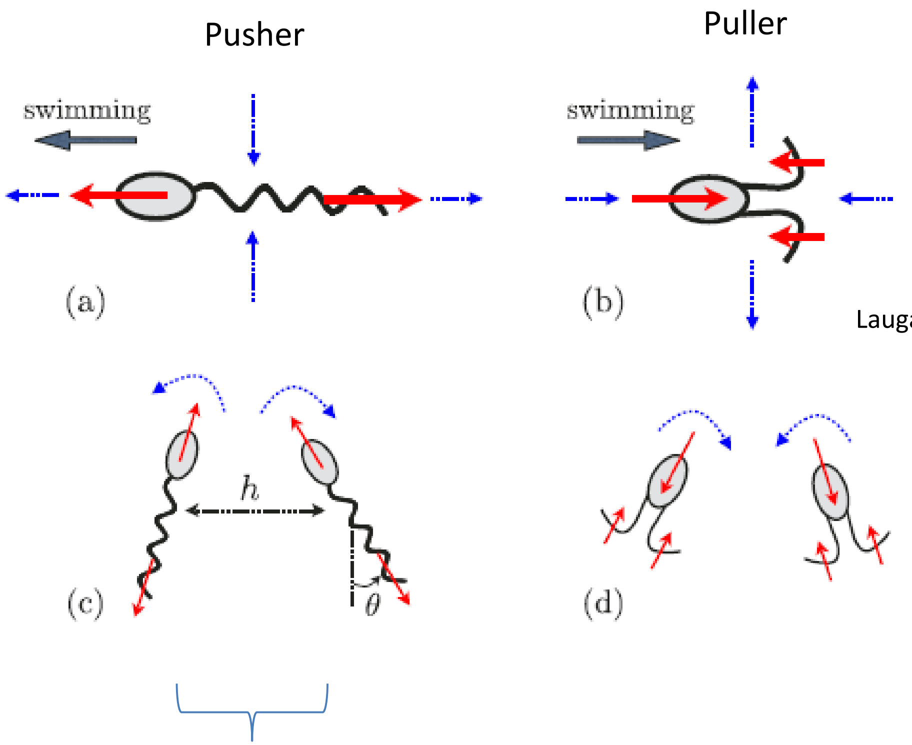


K.Drescher et al.PRL **105**, 168101 (2010)



Contractile

Swimmers in interaction

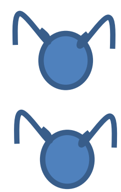


Lauga Rep. Prog. Phys. 096601(2009)

Hydrodynamic attraction

- Pushers favor nematic alignement

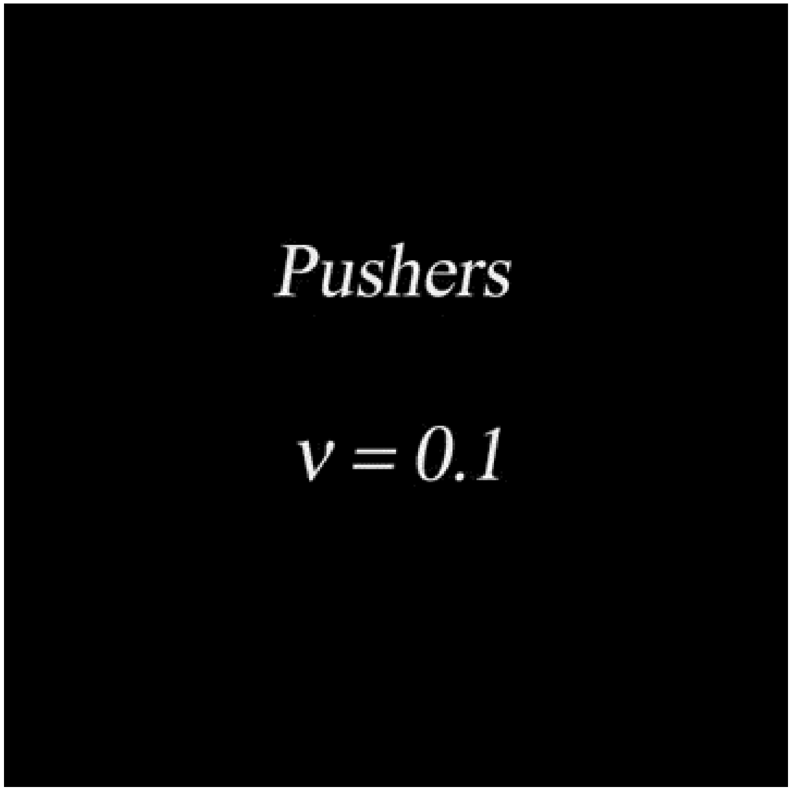
- long range hydrodynamic attraction
- short range hard-core (elongated particle)

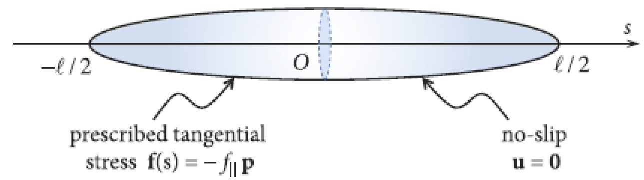


- pullers favor line forming

- long range hydrodynamic attraction
- short range hard-core (spherical particle)

3D slender-body numerical simulation



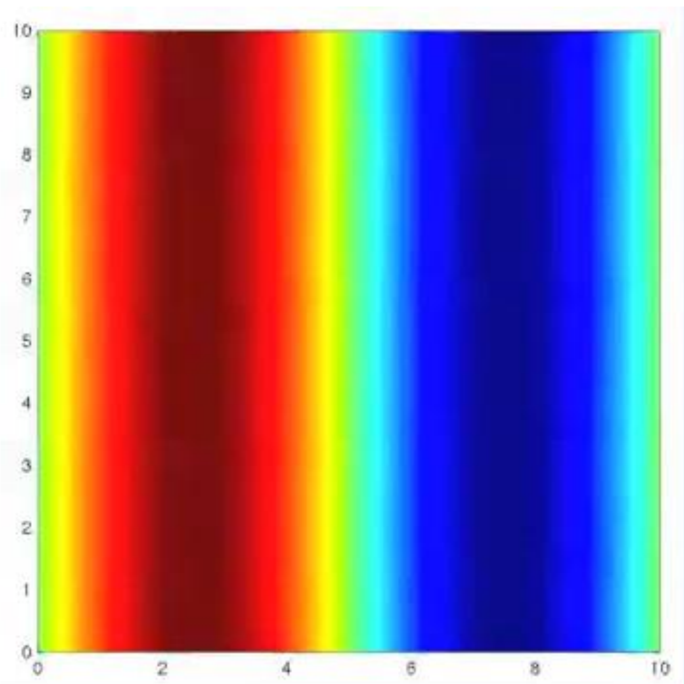


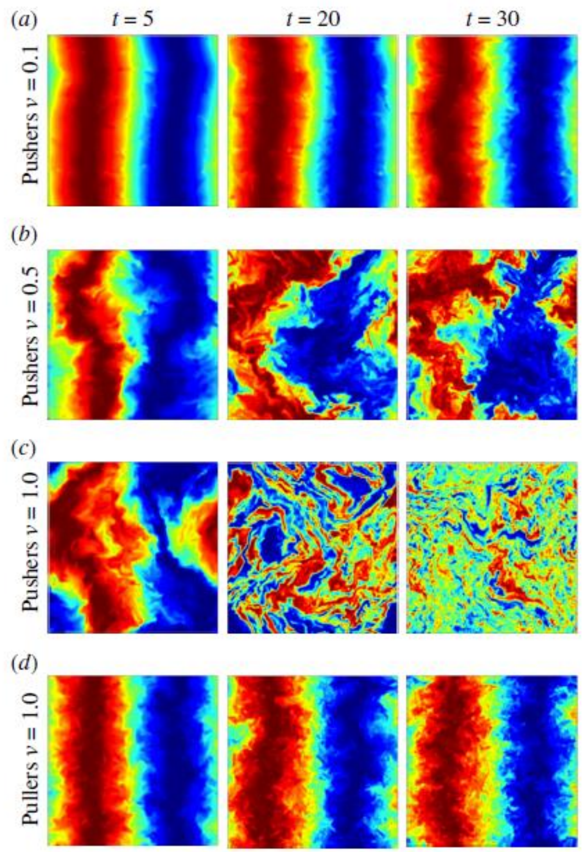
Excluded volume fraction

$$\nu = n\frac{\pi}{6}l^3$$

Saintillan et al.. J.Roy.Soc. Interface (2013)

Mixing of a passive scalar field





Strong mixing properties of pushers

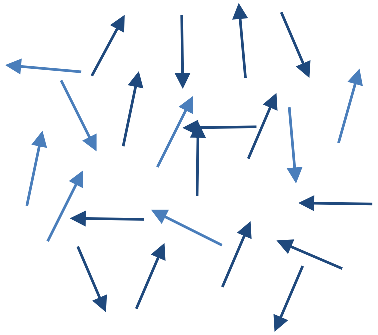
Moderate mixing of pullers

Active dipoles hydrodynamics

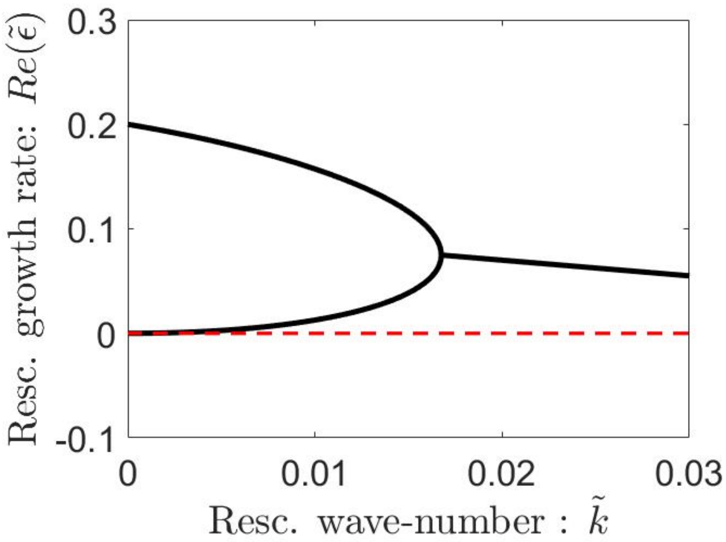
σ_0 Dipolar strength

$n = \phi l^{-3}$ Dipoles density

No noise !



Velocity fluctuations growth rate: $\delta \widetilde{U_k} \propto e^{i\vec{k}\vec{r}} \exp(\varepsilon_k \tilde{t})$



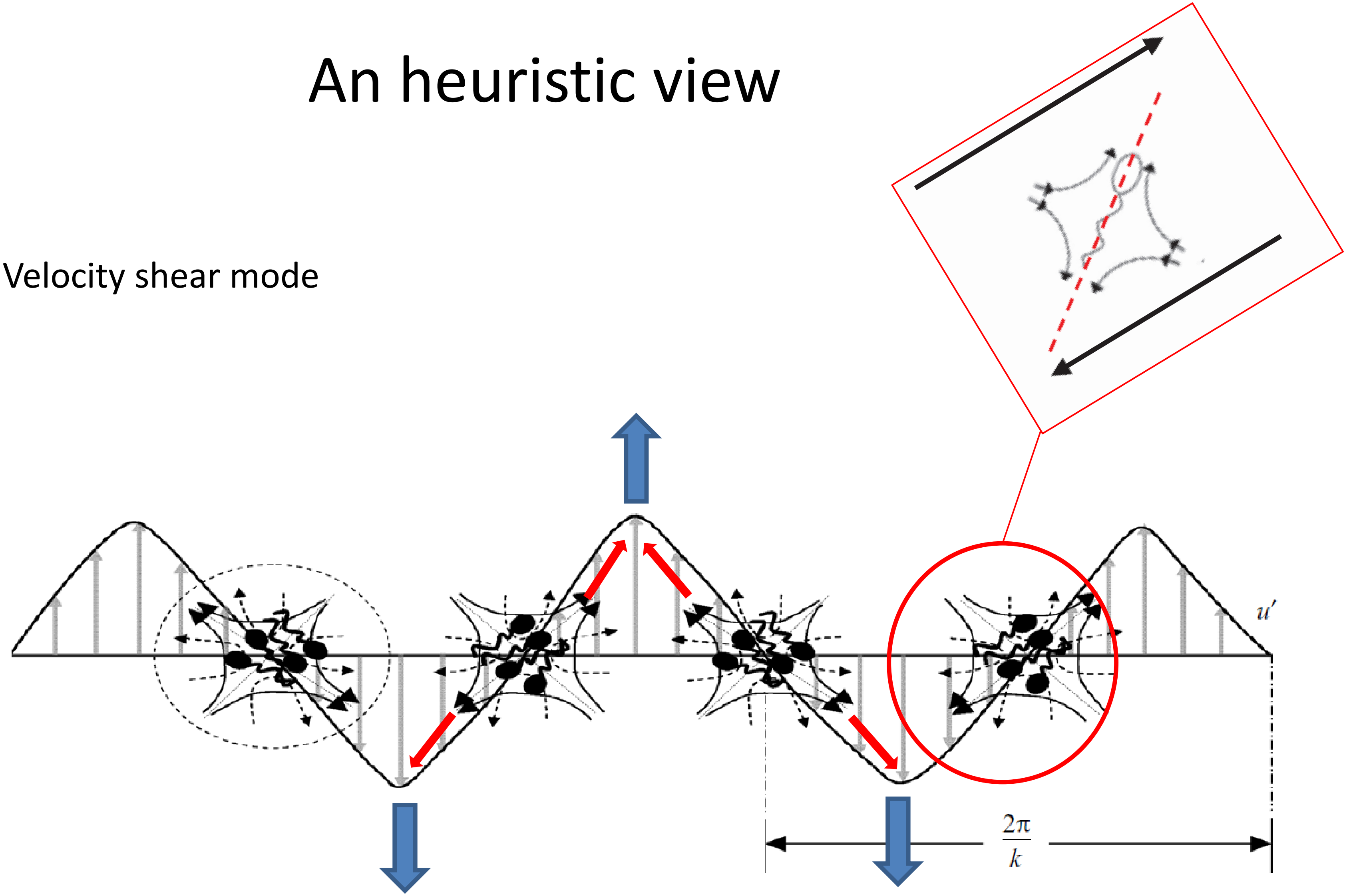
Fastest growing linear mode
$Re(\varepsilon(k)) > 0$ (unstable)

Saintillan and Shelley, Phys. Fluids **20**, 123304 (2008).
Subramanian and Koch JFM, **632**,359 (2009)

- An active suspension of elongated swimming pushers is **linearly instable** at all concentrations

An heuristic view

Velocity shear mode



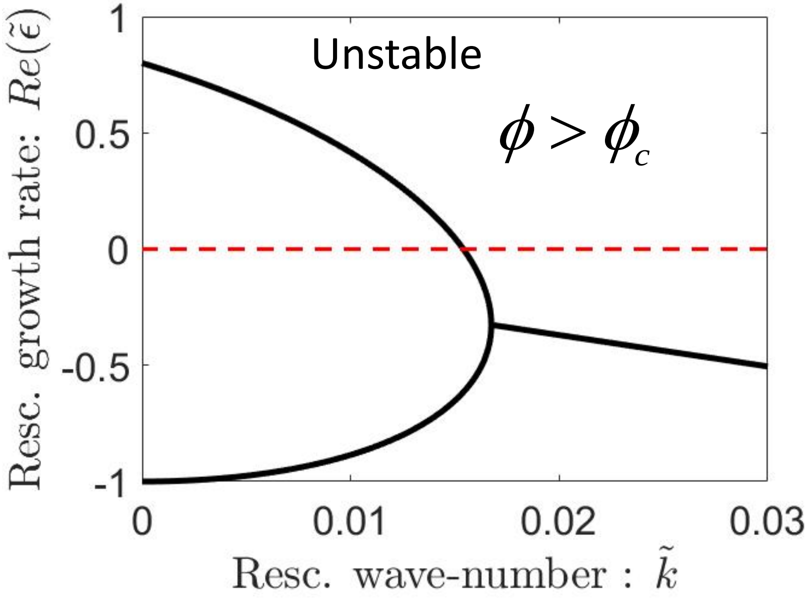
Subramanian and Koch JFM, **632**,359 (2009)

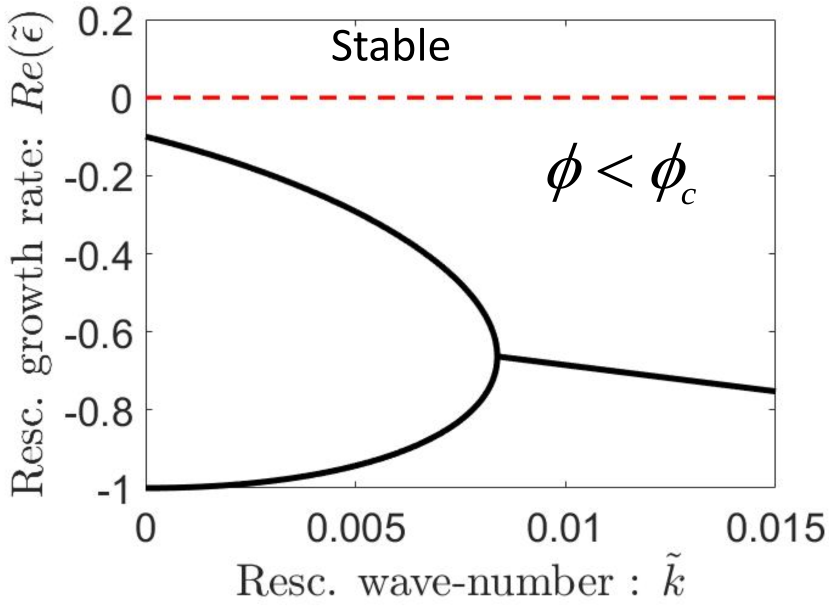
Rotational noise $D_r \propto 1/\tau_p$

Activity number $A = \frac{\sigma_0}{\eta_0 D_r l^3} \propto \frac{V_B}{D_r l} \propto \frac{V_B \tau_p}{l} = l_p / l$

τ_p :directional persistence time
$l_p = V_B \tau_p$: persistence length

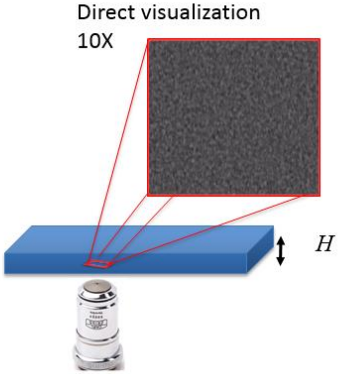
Linear instability $\phi_c \propto 1/A = l/l_p$



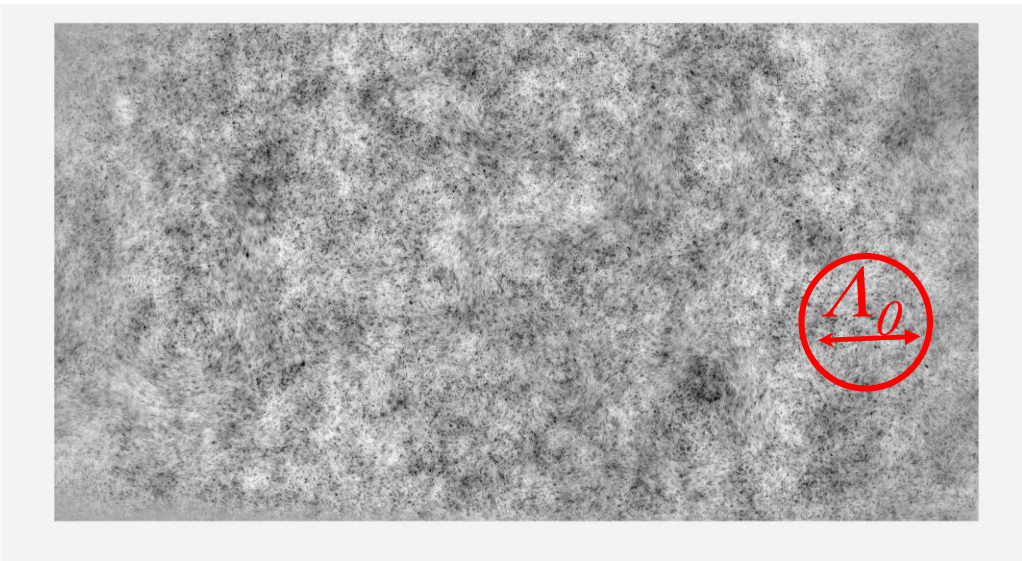


- Rotational noise stabilizes the suspension below a critical volume fraction ϕ_c

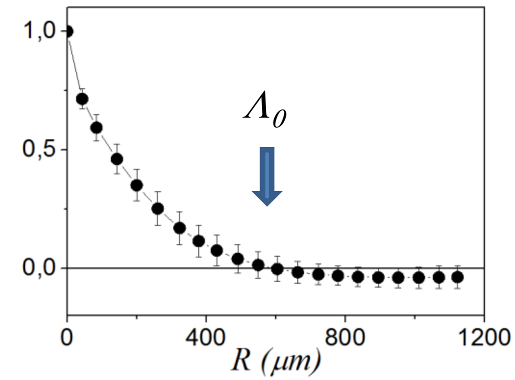
Collective motion of E.coli



H=600 μm



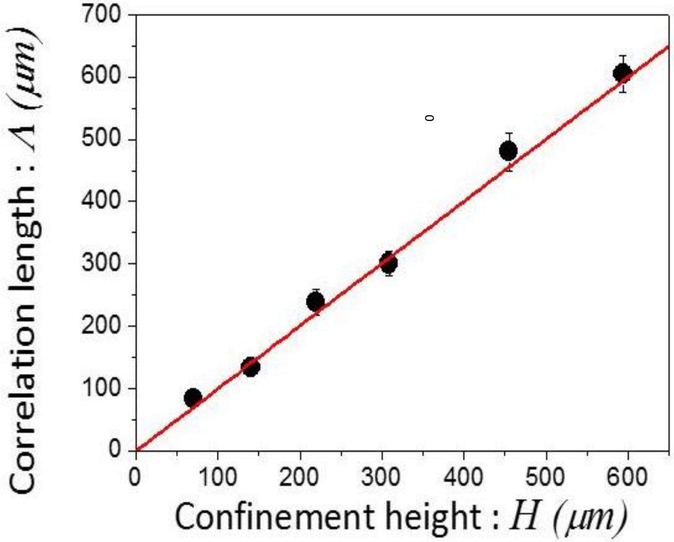
Velocity correlation



Vortex scale $\Lambda_0(H)$?

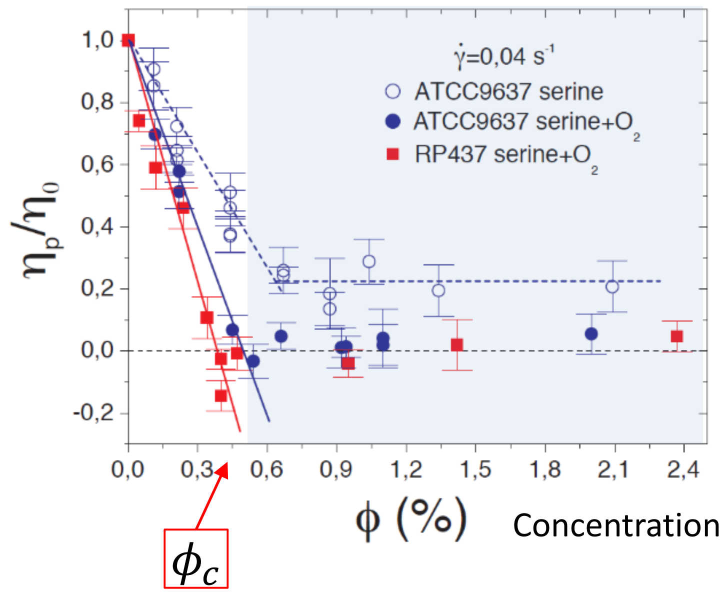
- Emergence of large scale vortices in dense suspensions of motile E.coli

Emergenge of a critical « superfluid »



Martinez et al. (2021)

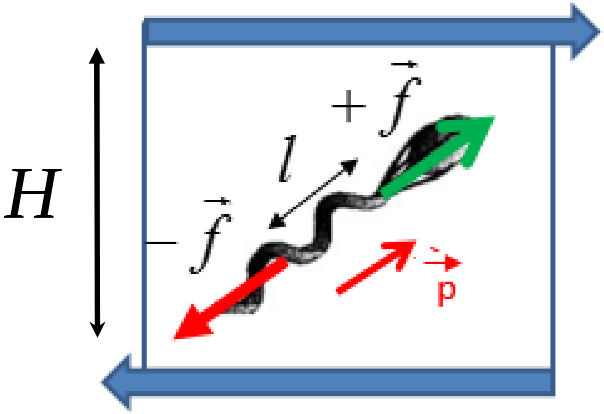
Viscosity Lopez et al. PRL. **115**, 028301 (2015)



Concentration:

- ❑ Correlation length Λ_0 increases linearly with confinement height H
- ❑ The fluid becomes « critical »
- ❑ Macrocopic viscosity can reach zero !

Viscosity decrease with concentration



Heuristic view

Y.Hatwalne *et al*, Phys. Rev. Lett. **92**, 118101 (2004)

Relative viscosity at low shear rate

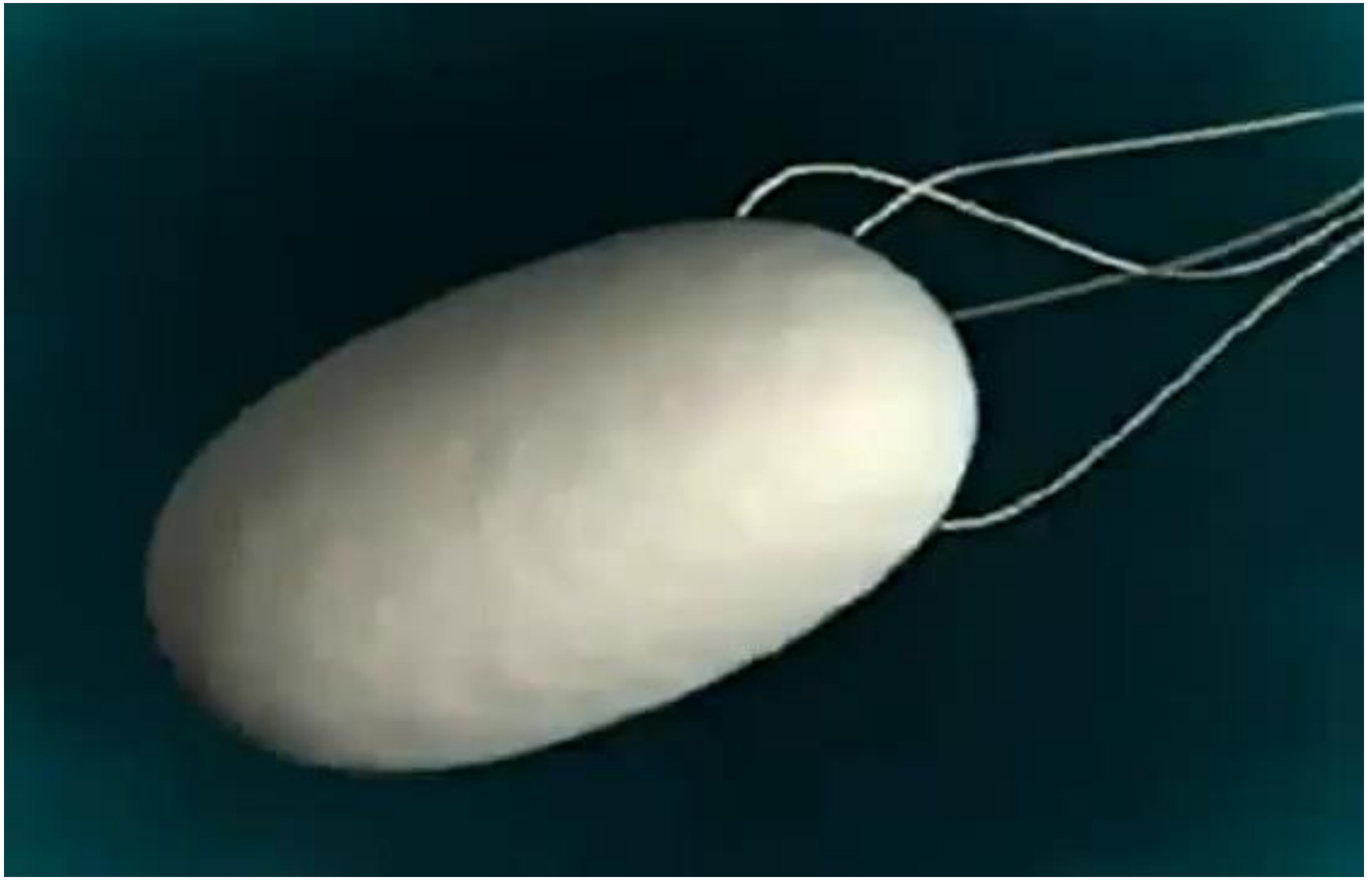
$$\eta_r = 1 + \left(a - b\frac{V_B \tau_p}{l}\right)\phi$$

B. M. Haines et al., PR E **80**, 041922 (2009).
D.Saintillan, Model Exp. Mech. **50**, 1275 (2010).

Activity number $A = l_p / l$

- Zero viscosity at $\phi = \phi_c$ predicted by active pushers dipoles hydrodynamic model (but for $\phi > \phi_c$???)

What is under the hood ?

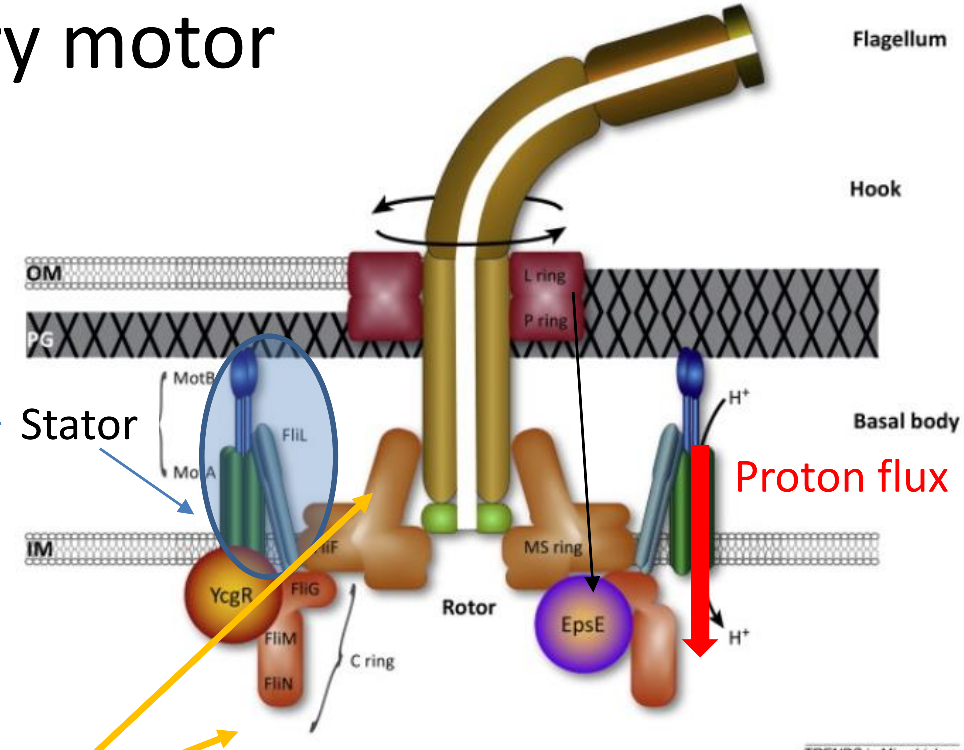


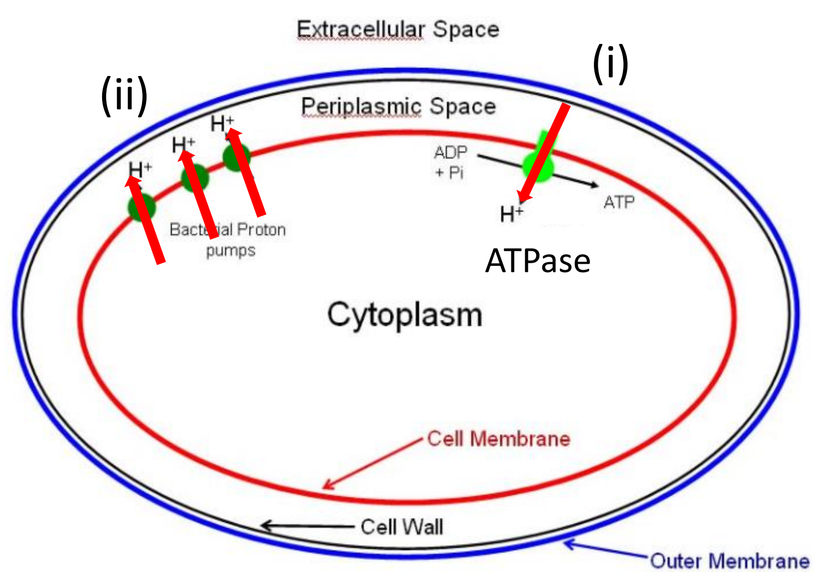
The rotary motor

Rotation rate : *100-200 Hz*

Stator

Rotor





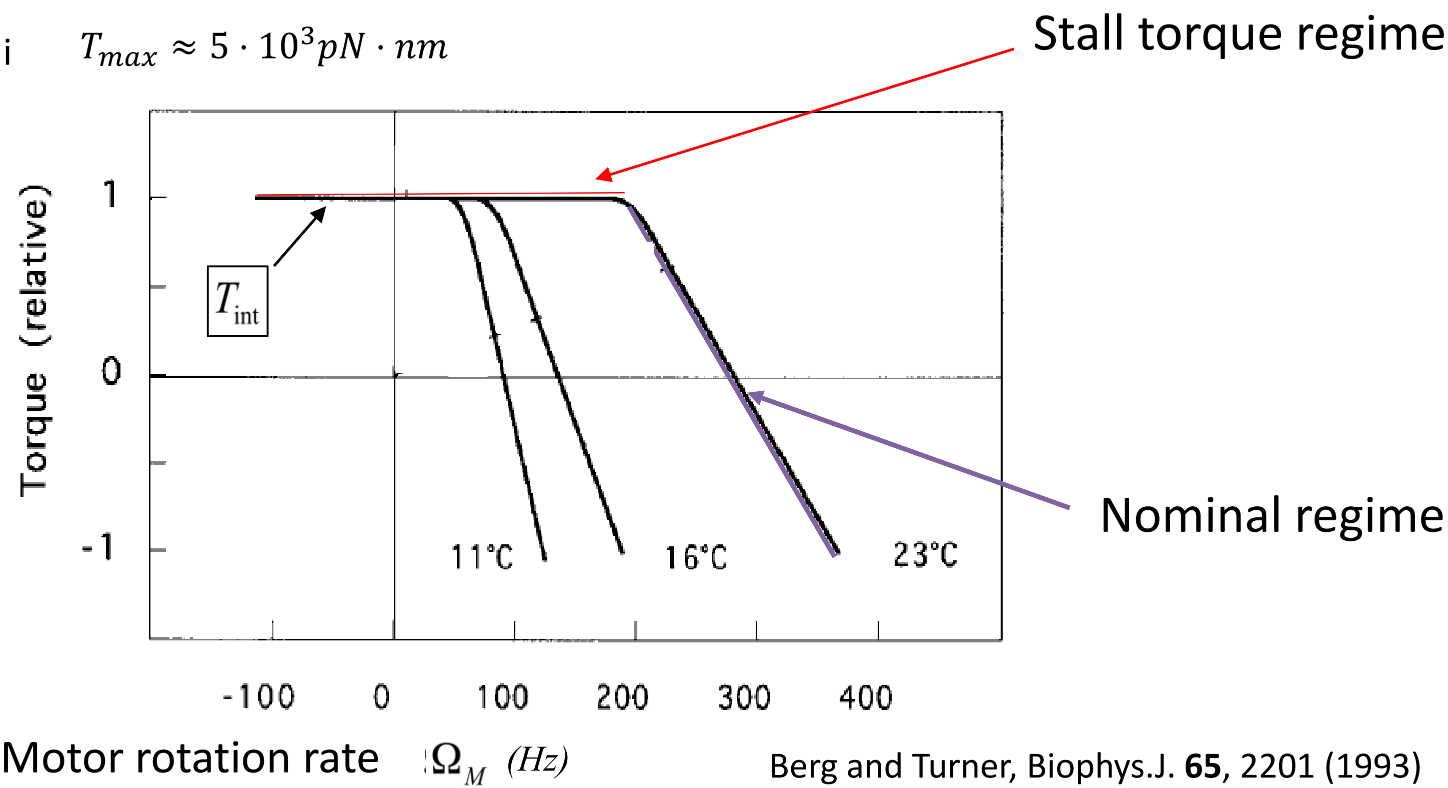
(i) Proton gradient motor

(ii) Proton pumps

Berg, Annu. Rev. Biochem. **72**, 19 (2003)

Rotary motor performances

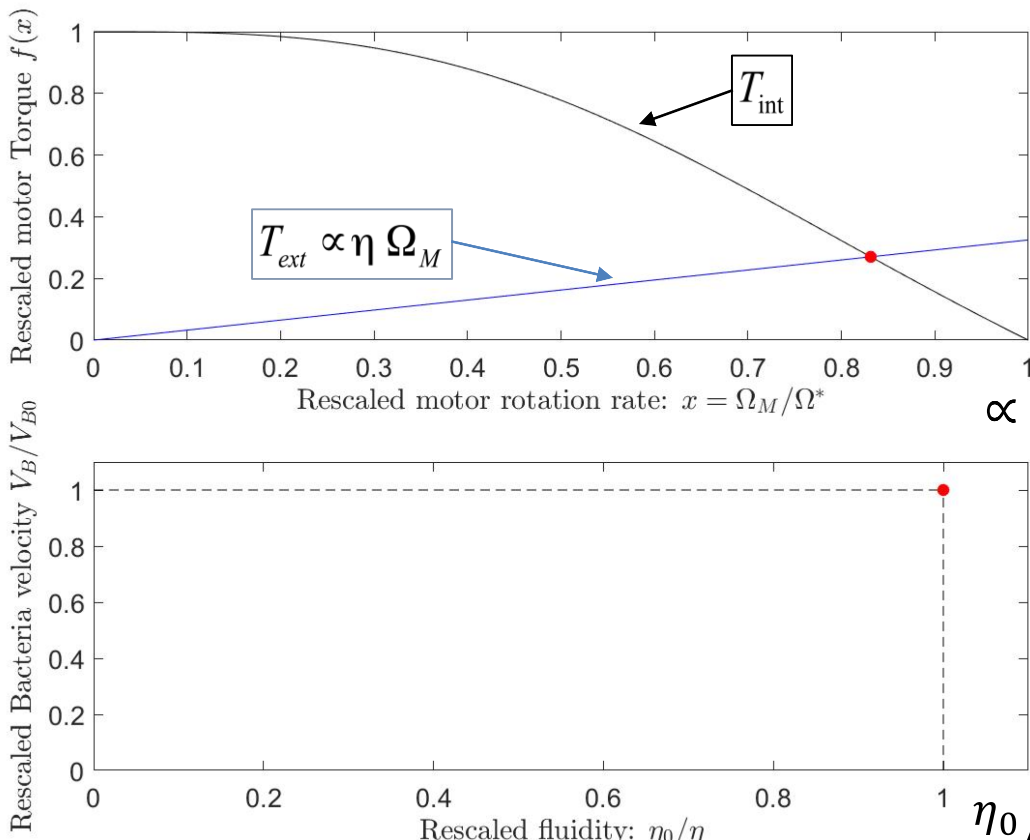
E.coli $T_{max} \approx 5 \cdot 10^3 pN \cdot nm$



Berg and Turner, Biophys.J. **65**, 2201 (1993)

Swimming velocity dependence on viscosity η

$$f(x) = \frac{T_{int}}{T_{max}}$$ Relative torque

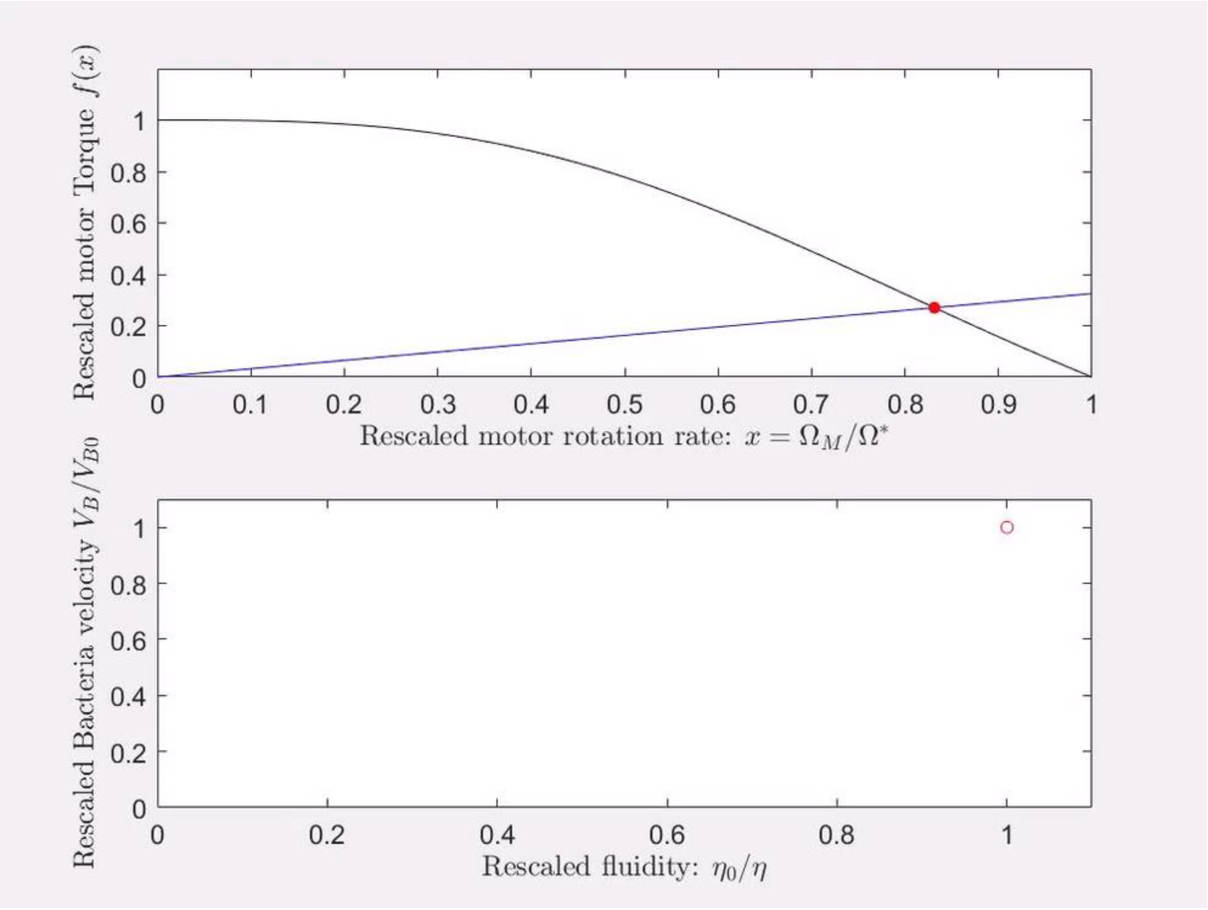


$\propto \Omega_M$ Relative motor rotation rate

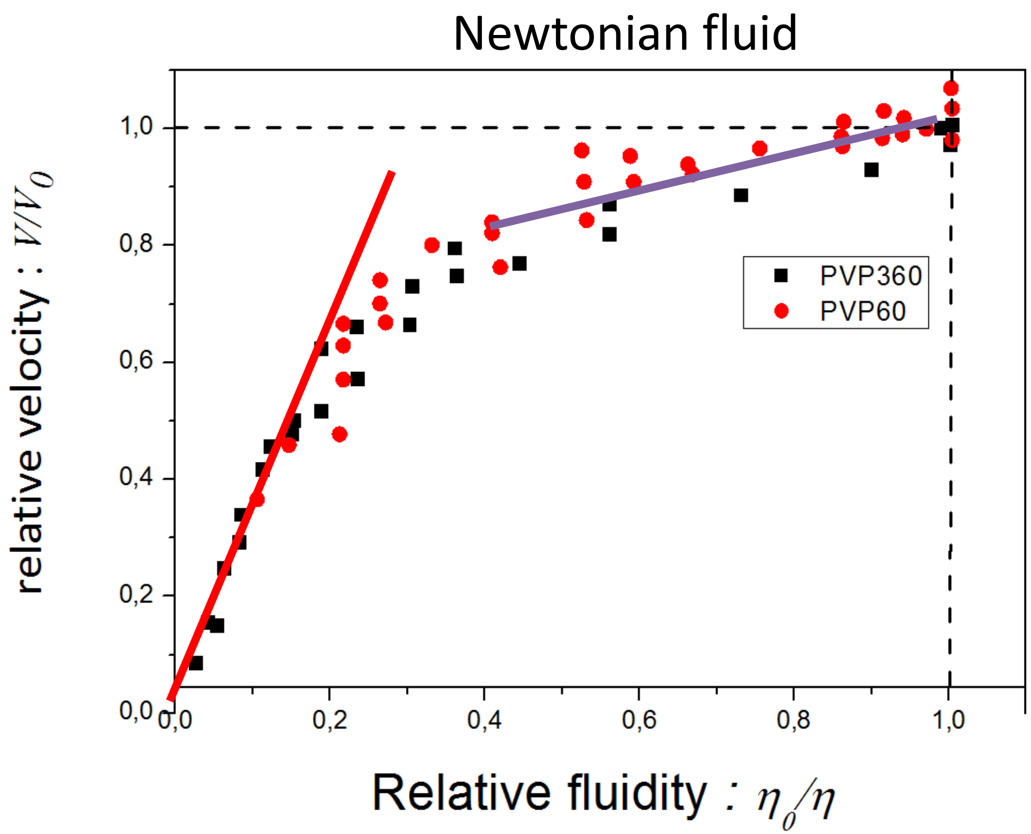
$$\frac{V_B}{V_B^0}$$

η_0/η Relative fluidity

Swimming velocity dependence on viscosity η



Swimming velocity dependence on viscosity η

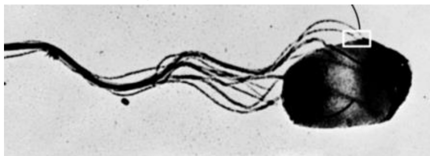


After Martinez et al.
PNAS , **111**, 17775 (2014)

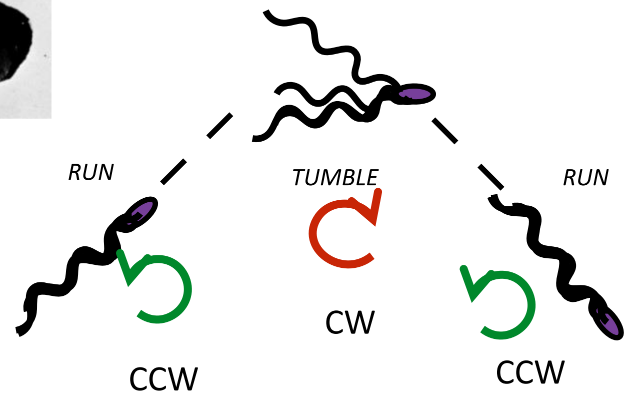
- ❑ Nominal regime : weak dependence
- ❑ Stall torque regime : $V_B \propto \Omega_M \propto \dfrac{T_{max}}{\eta}$

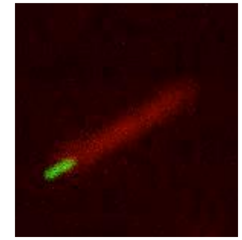
Run and tumble process

Flagella bundle



Wild-type E.coli



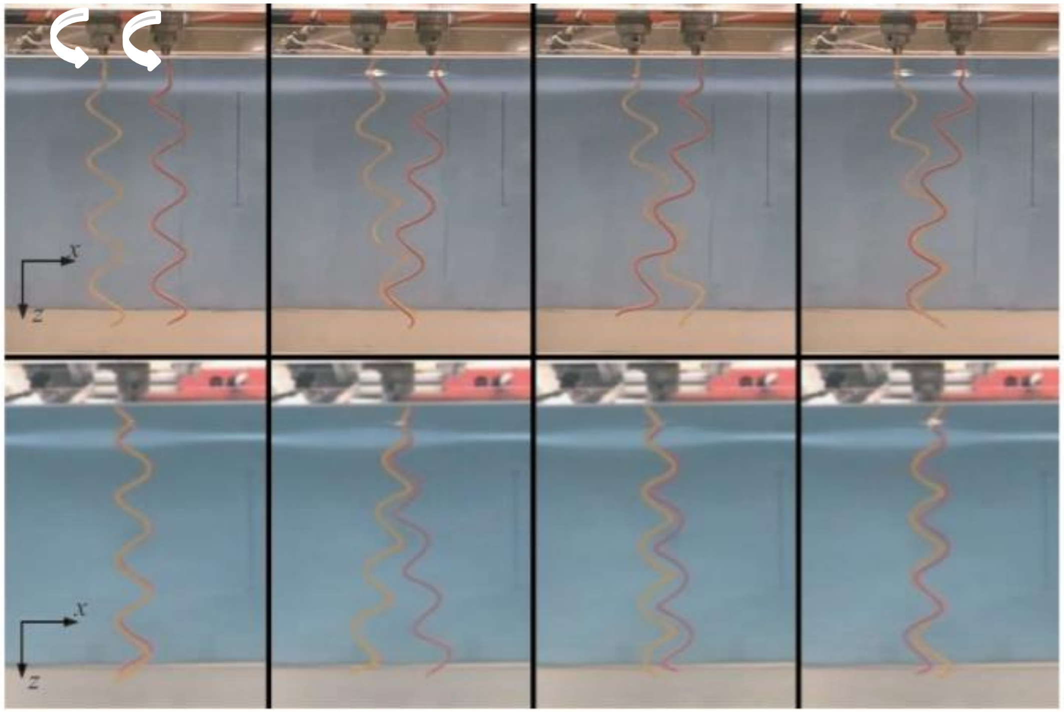


Wild type E.coli RP437

- Alternated switches in motor rotation create stochastic bundling / unbundling processes
- Explore the environment as a random walk
- Bias of the random walk in presence of chemical gradients (**chemotaxis**)

Hydrodynamic bundling process

E.Coli –”petricious” - 4 – 5 helical flagella



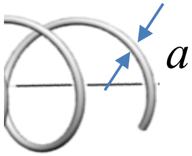
B. Liu et al. PNAS , **108**, 19516 (2011)

$$R_e \cong 10^{-3}$$

$$M = \tau\omega = \frac{\eta L^4}{K}\omega \cong 150$$

Bending modulus

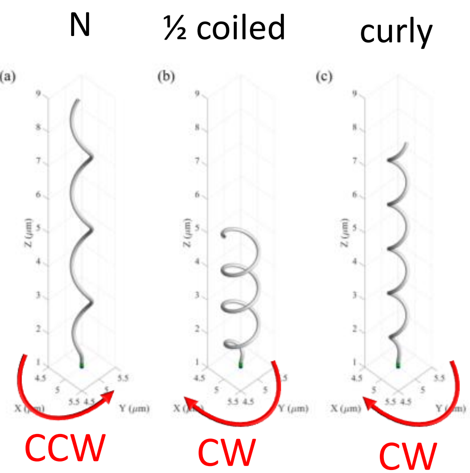
$$K = EI \propto a^4$$

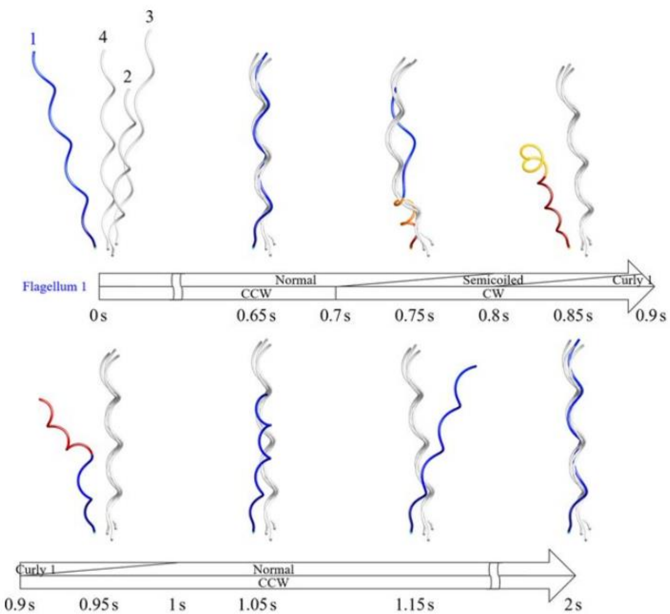


- ❑ Hydrodynamic attraction between rotating flagella => bundling

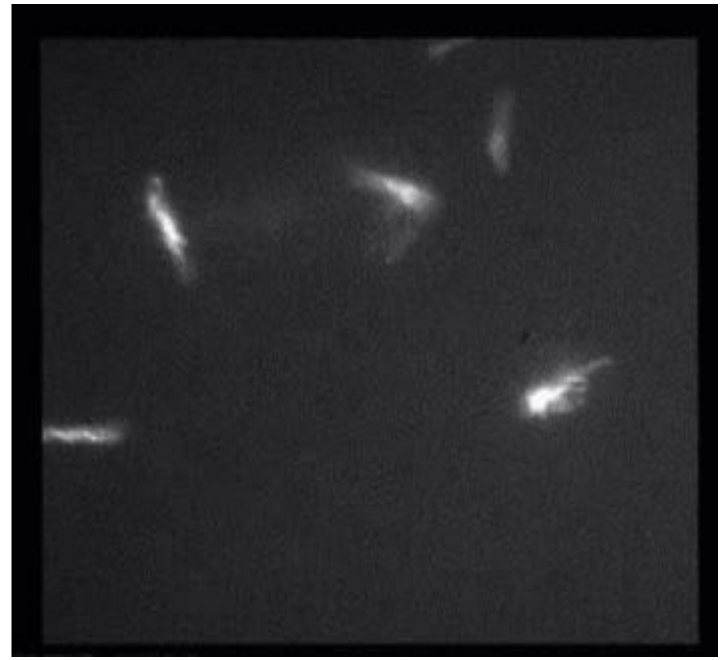
Polymorphic transformation

Turner et al. J. Bacteriology, **182**, 2793 (2000)





Lee et al. PRE **98**, 052405 (2018)

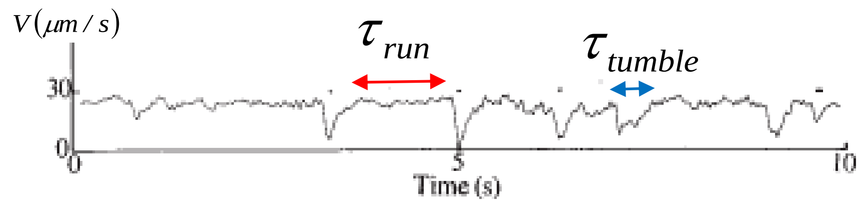


G.Miño PMMH-ESPCI

- ❑ Change shape =>
  change of swimming direction

Standard vison for the run and tumble process

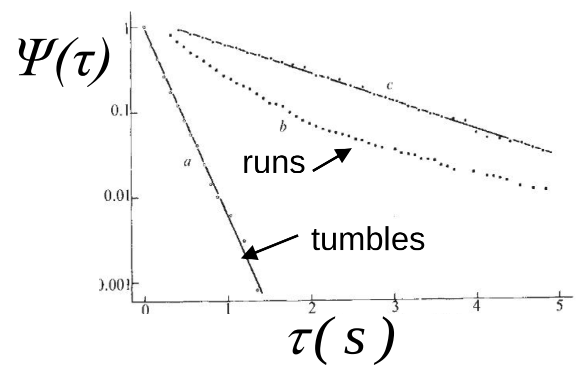
Lagrangian 3D tracking



Berg & Brown, Nature **239**, 500 (1972)

Fraction of time run > τ

$$\Psi(\tau) = 1 - \int_{\tau}^{+\infty} \Pi(\tau')d\tau'$$

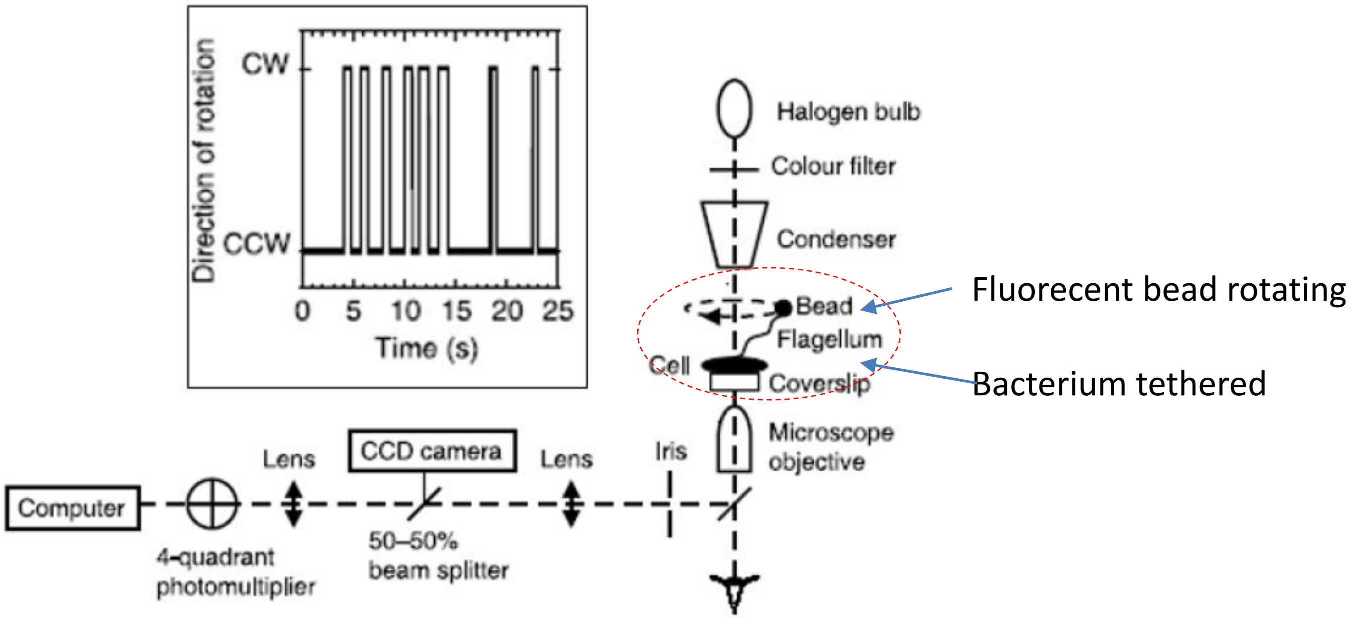


- Poisson processes (currently assumed)

$$\Pi(\tau) \propto exp(-\tau/\tau_\alpha) \rightarrow \tau_{run} \cong 1s, \quad \tau_{tumb} \cong 0.1s$$

Berg, *Ecoli-in-motion* (2004)

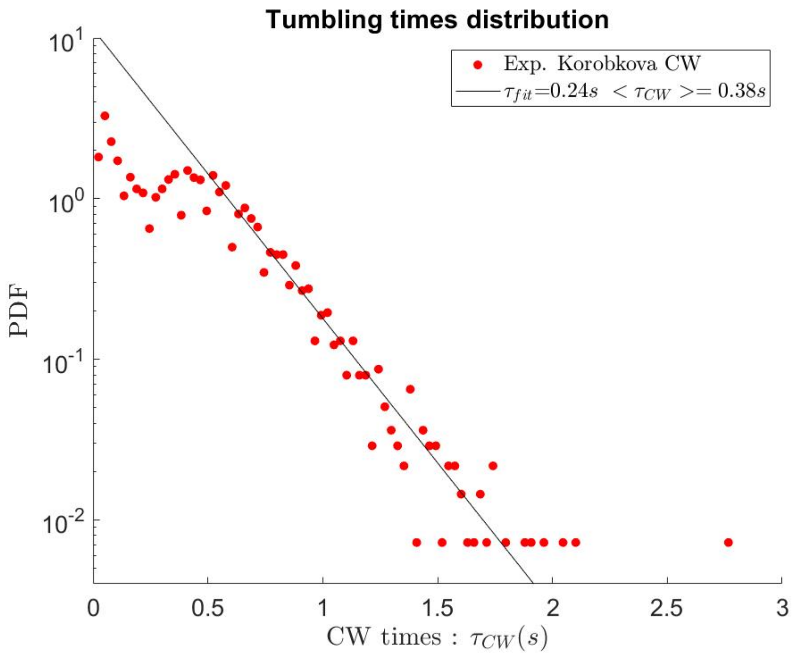
Switch times distributions

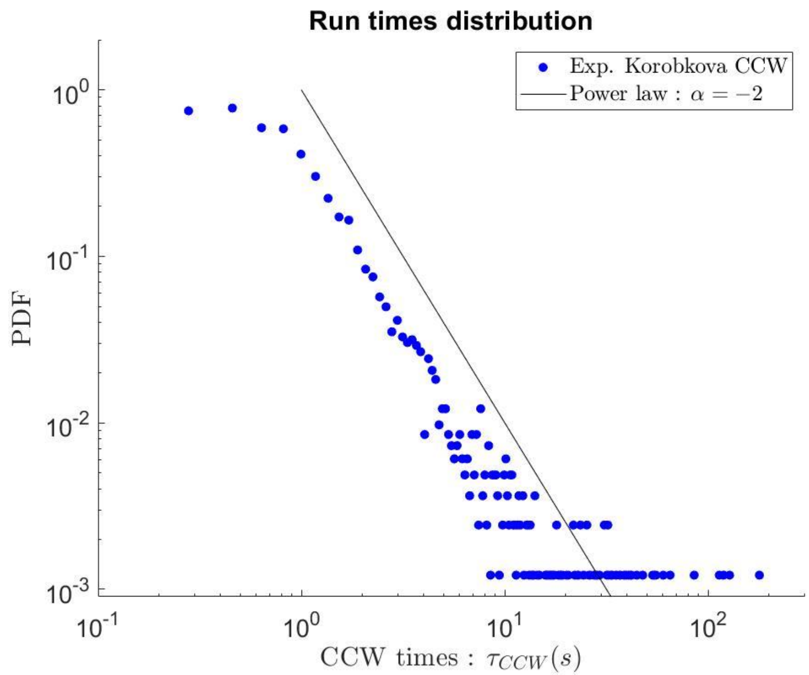


Korobkova *et al.*, Nature, **428**, 574, 2004

❑ Obtain the distribution of run (CCW) times and tumbling (CW) times

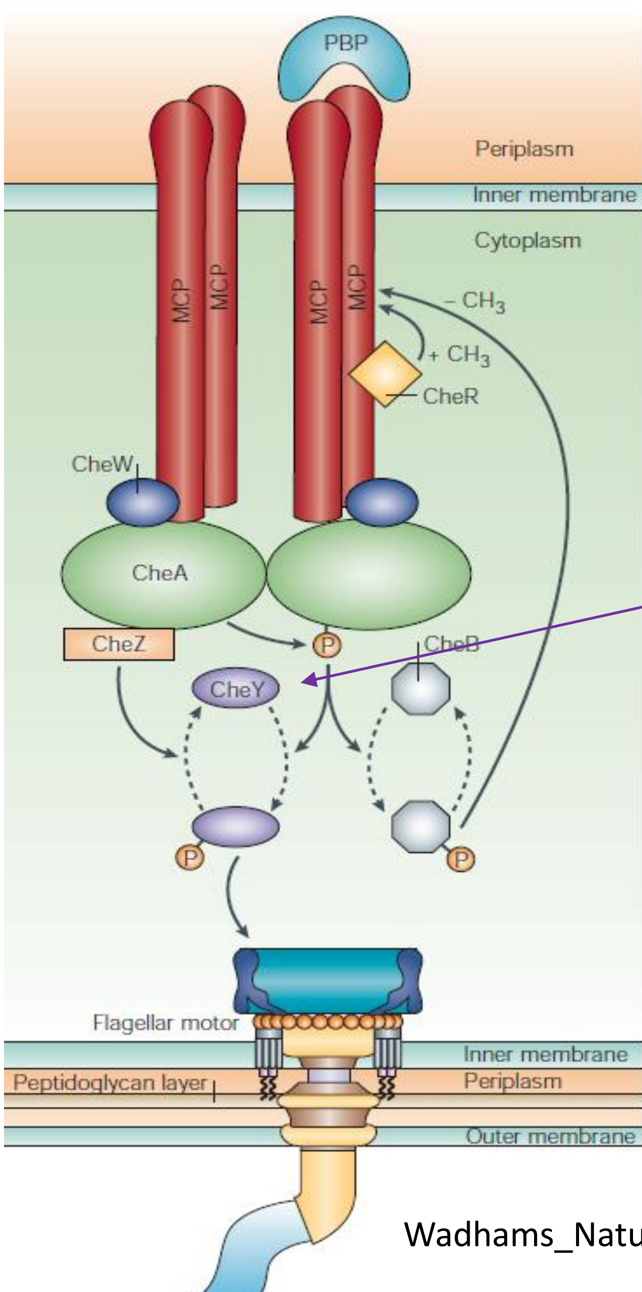
Switch times distributions





- ❑ Tumbling times displays an exponential tail
- ❑ Large tail «run – time » distributions
- ❑ At odd with simple Poisson R/T statistics

Chemotactic biological machinery

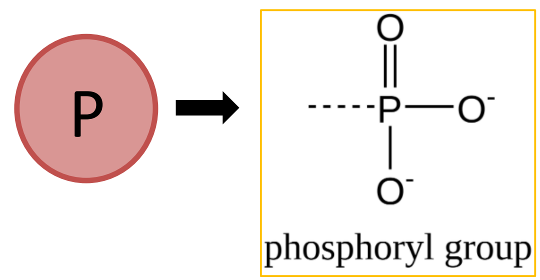


MCP : Methyl-accepting Chemotaxis Proteins

CheA, CheW

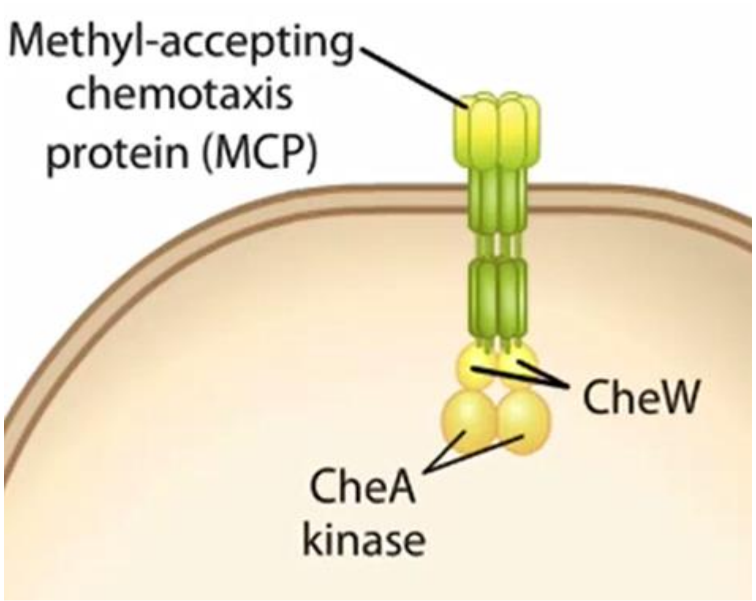
CheY, CheB, CheR

Functional proteins



Rotary motor

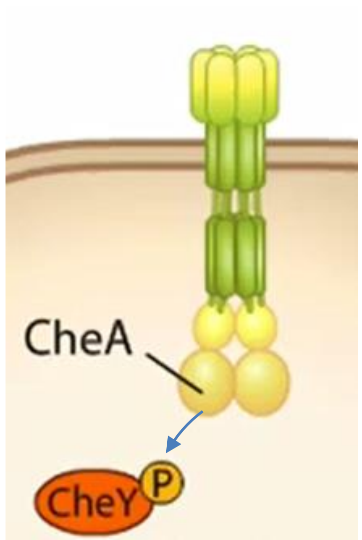
Wadhams_NatureReviews_2004

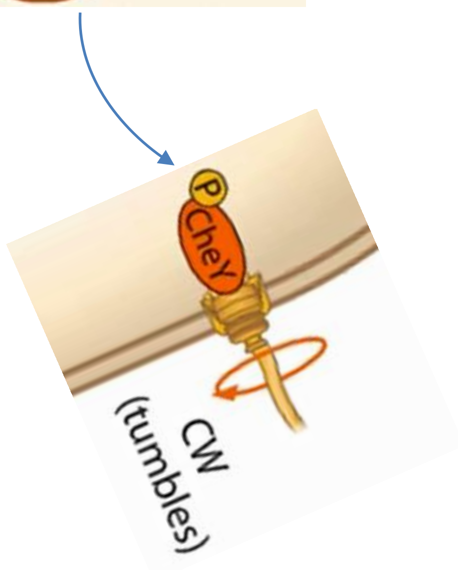


$$ATP + (CheA) \rightarrow ADP + P + (CheA)$$

CheA = Kinase protein = cut ATP and releases P

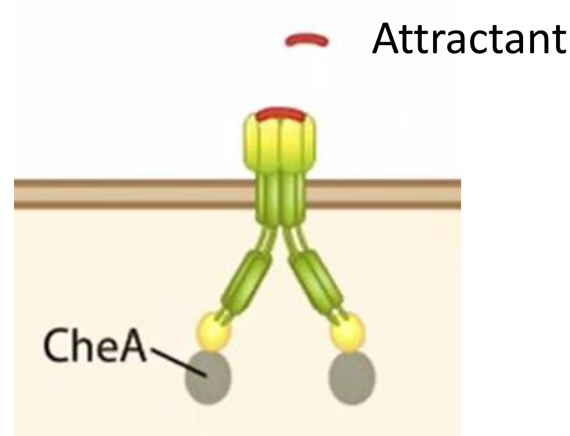
MCP **active state**





Promotes **tumbling** phase

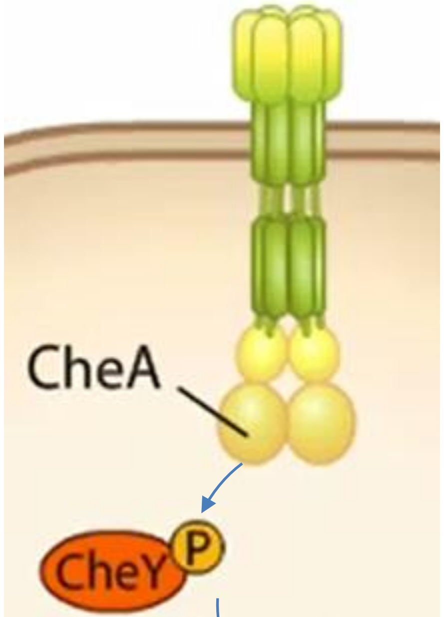
MCP **passive state**



no P release

Promotes **run** phase

Adapted bacterium

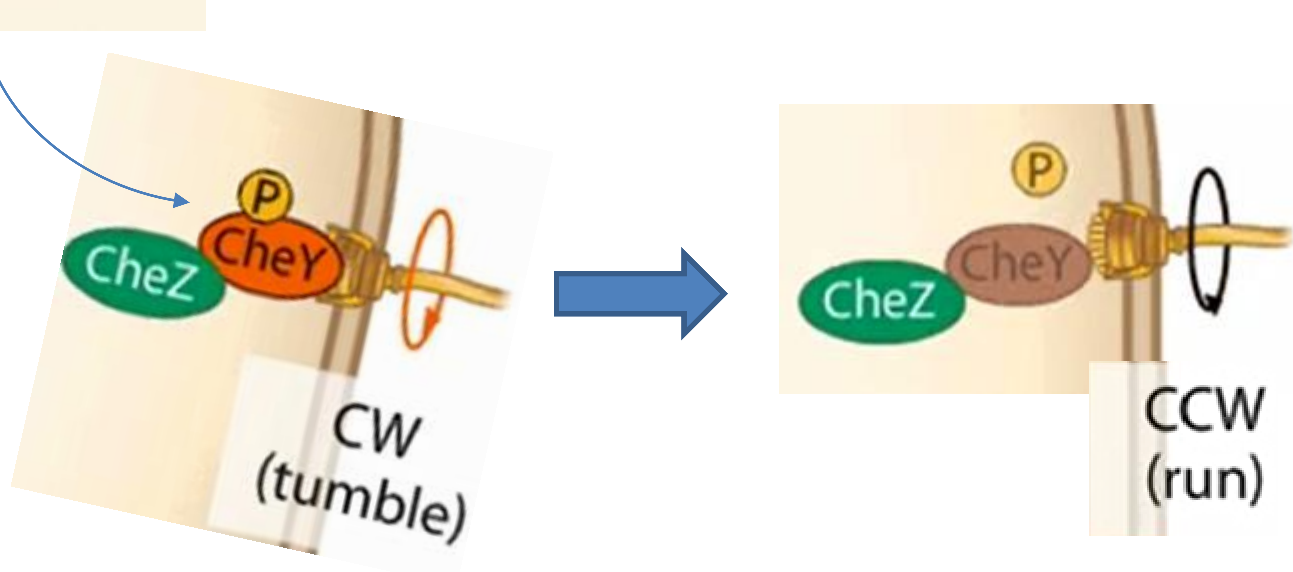


CheY phosphorylation from the CheA production of P

$$CheY + P \rightarrow CheY - P$$

CheY de-phosphorylation by CheZ

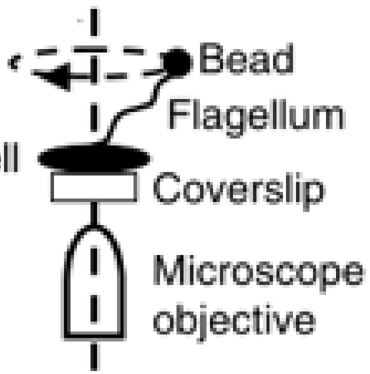
$$CheY - P + CheZ \rightarrow CheY + CheZ + P$$



- An adapted bacterium undergoes phosphorylation / dephosphorylation of CheY, hence fixing the run/tumble rate

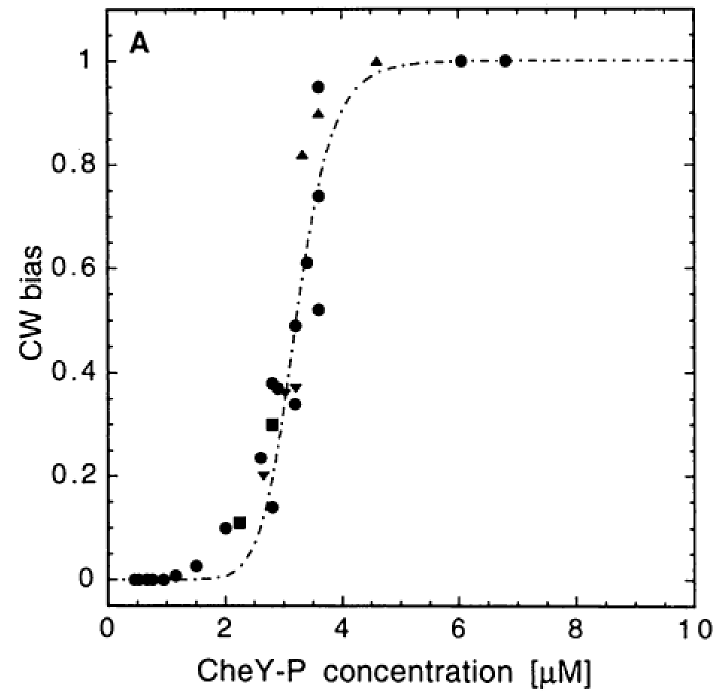
Switching sensitivity to CheY-P

RP437



Tumble
mode

Run
mode



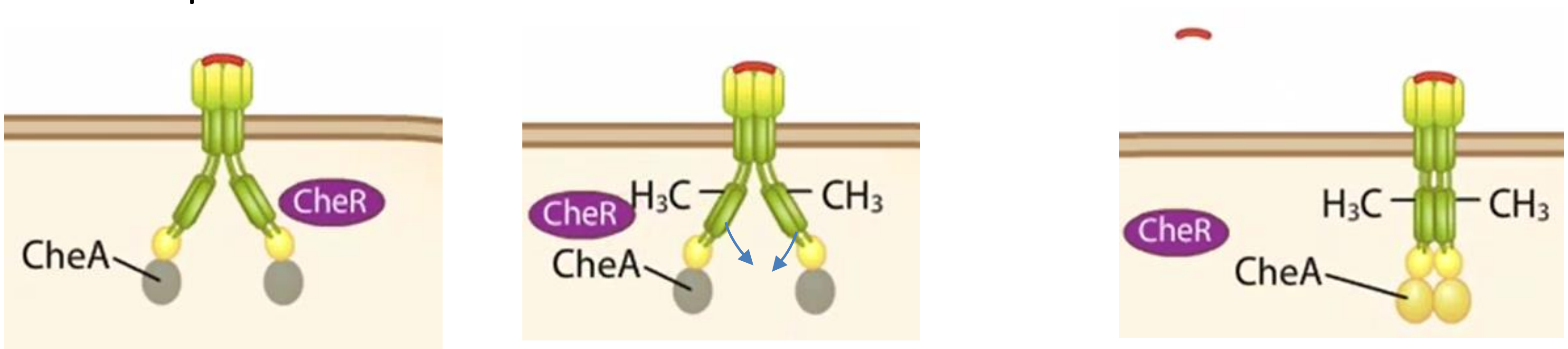
Cluzel et al. Science **287** 1655 (2000)

- **Highly sensitive** to the presence of CheY-P near the motor

Sensory adaptation process

MCP passive state

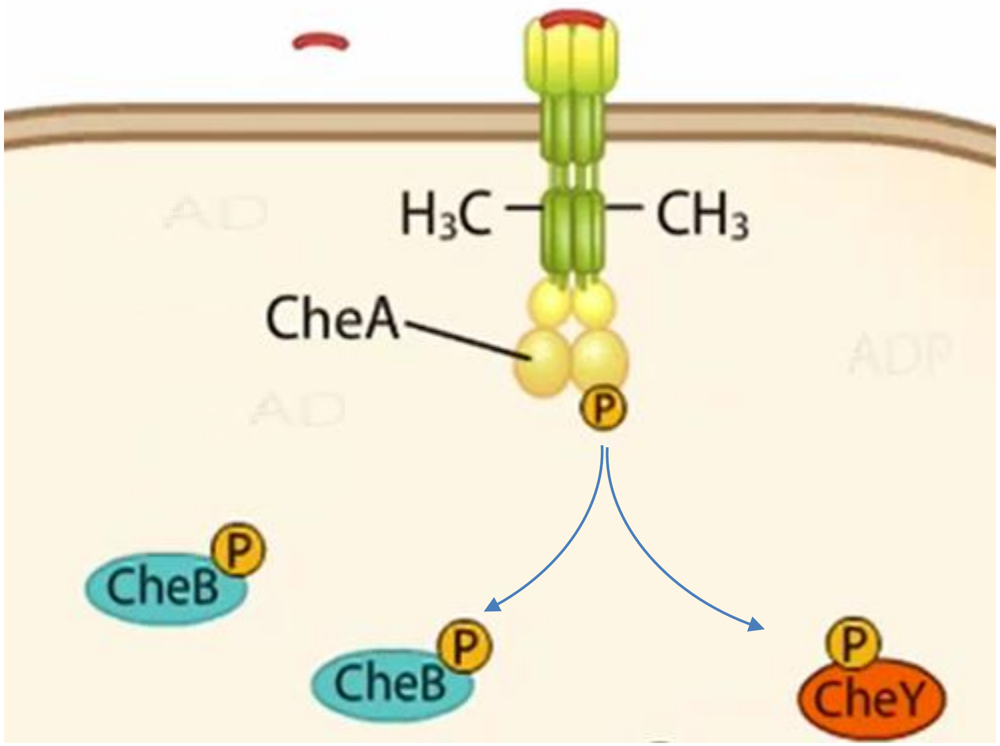
MCP active state



- ❑ CheR slowly mehtylates MCP such as to reach an active state

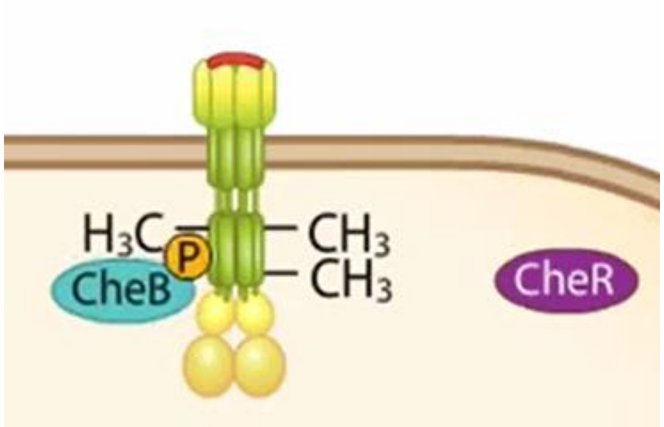
- ❑ At high level of methylation the bacterium is re-adapted and run/tumble exploration resumes

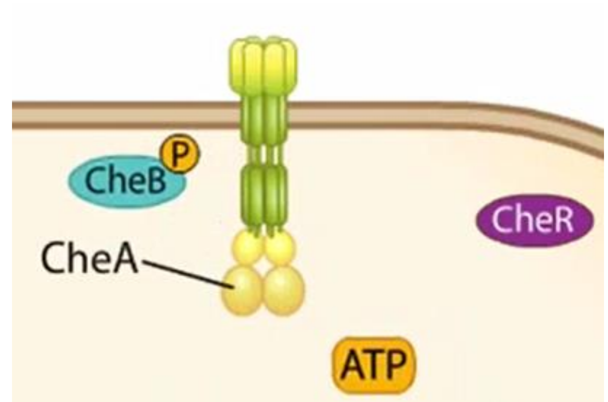
Regulation process



- CheY competes with CheB for the P from kinase CheA

Regulation process





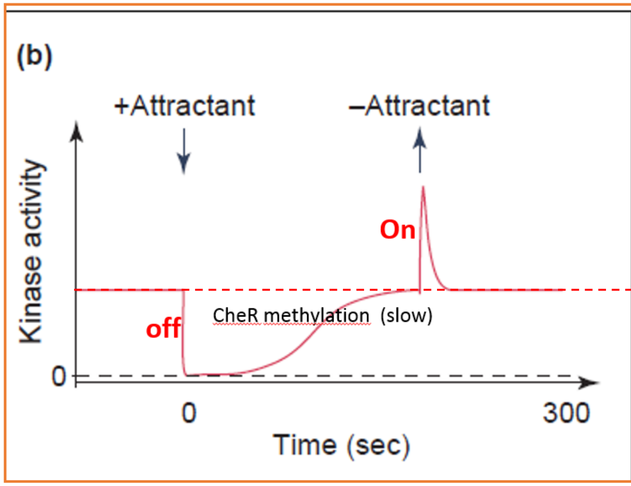
- CheB-P opposes the activity of CheR and **de-methylates** MCP

- In **lower attractant regions CheR is less likely to methylate** MCP then CheB-P demethylation dominates and the bacterium is re-adapted at low methyl level

Regulated chemotactic response

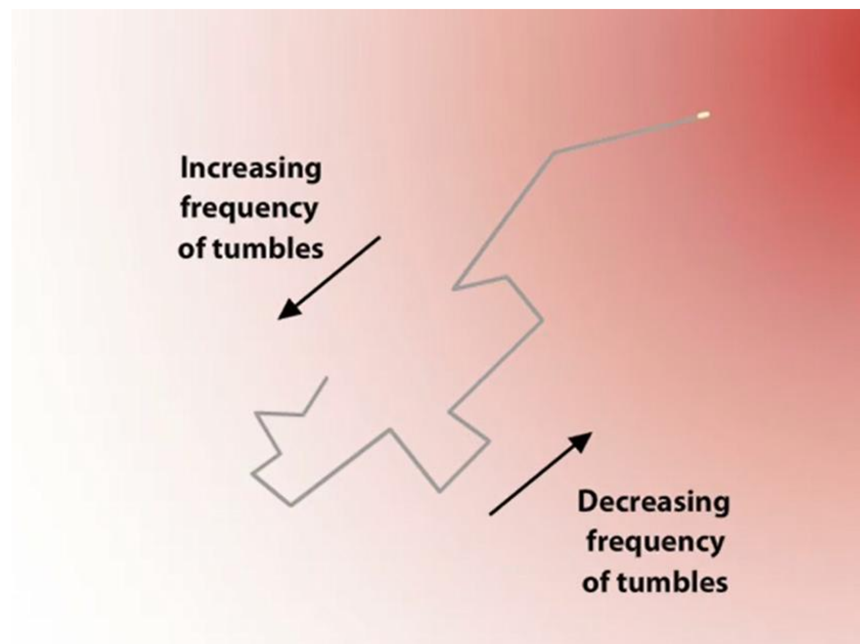
Tumble

Run

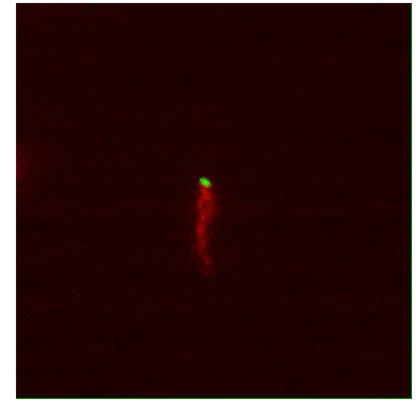


« adapted level »

- ❑ High attractant level: decreases tumble frequency
- ❑ Low attractant level : increases tumble frequency



Mutant swimmers

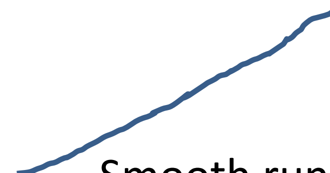


CheY

CheW

CheA

No CheY-P formation



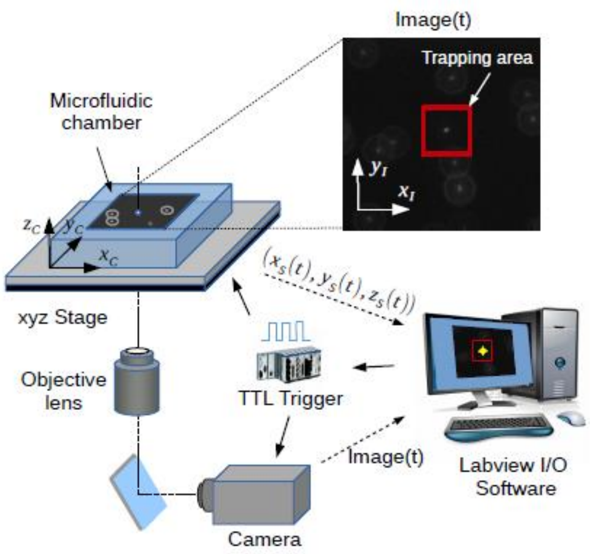
Smooth runner

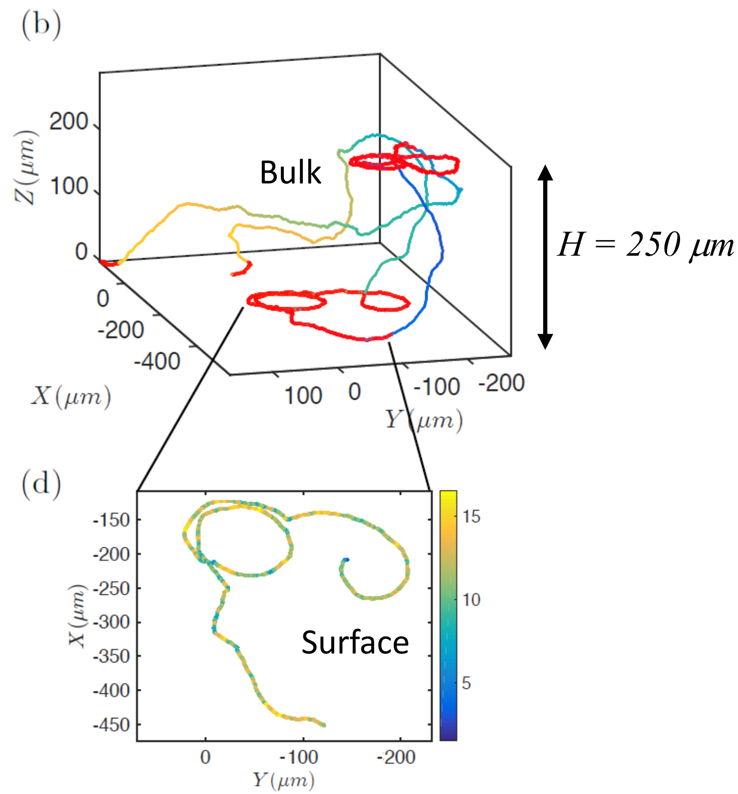
CheZ

No de-phosphorilation of Chey-P

Tumbler swimmer

Automated 3D lagrangian tracking





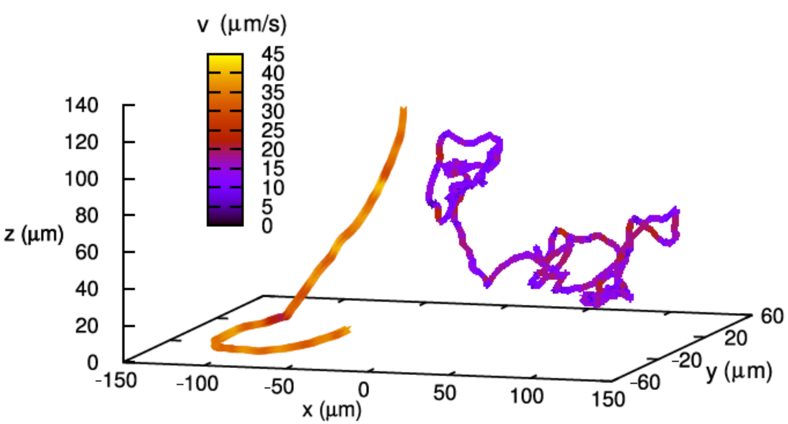
Darnige, Figueroa-Morales et al. Rev.Scient.Instr. **88**, 055106 (2017)
Junot et al. preprint (2021)

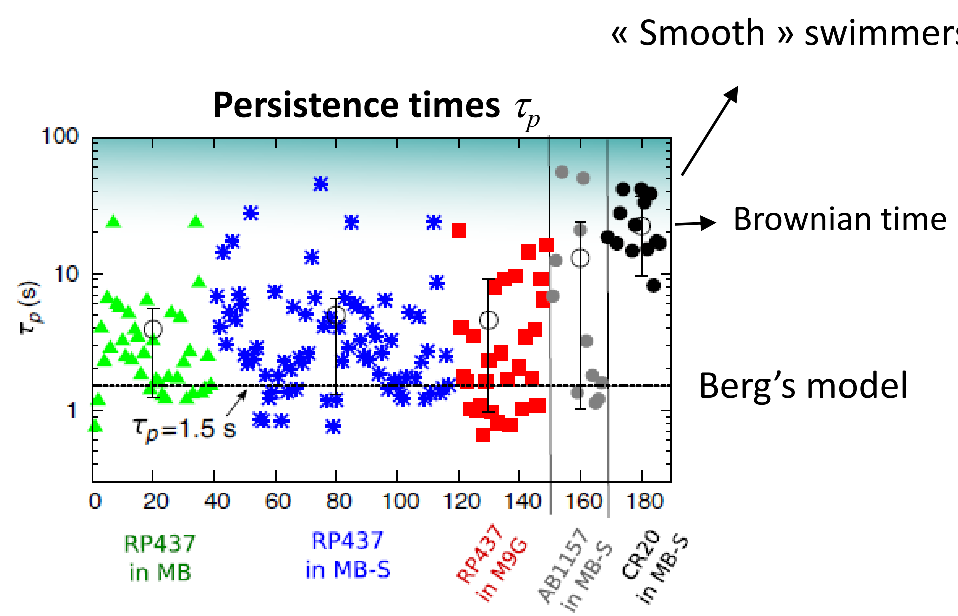
- ❑ Long time tracks (> 20 min) of fluorescent E.coli bacteria swimming between two glass plates
- ❑ Extract trajectories at surfaces and in the bulk

Motility features in a population of monoclonal WT E.coli

N. Figueroa-Morales et al Phys.Rev.X, **10**, 021004 (2020)

Visualization over **short-times** (<100 s)

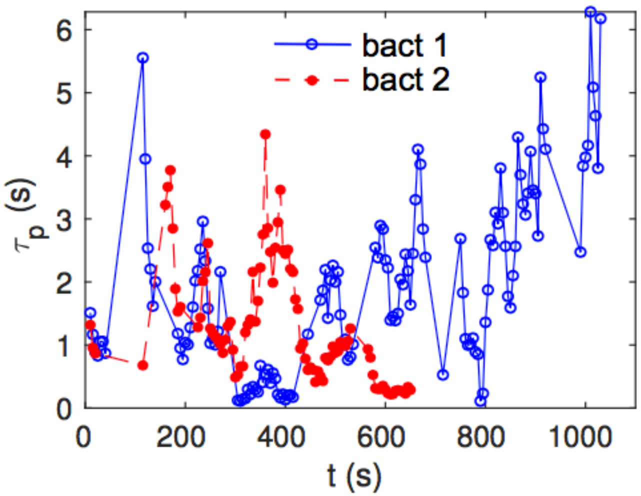




- Very large distribution of persistence times τ_p
- Robust to environmental and E.coli strain changes

Behavioral variability

Visualization over very long times (up to 25 min)

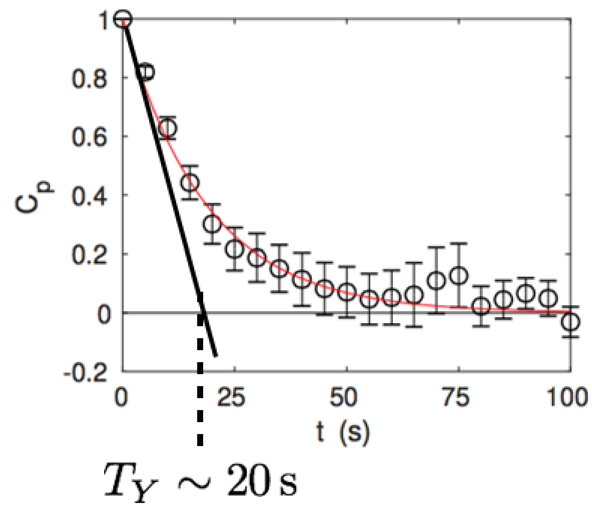


Never tumbles: high τ_p

Often tumbles: low τ_p

- ❑ Persistence time τ_p varies widely in time for the **same** bacterium

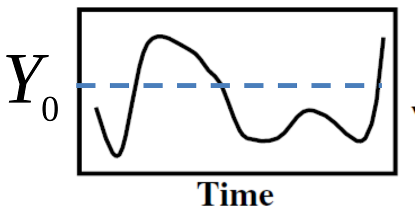
Persistence time auto-correlation

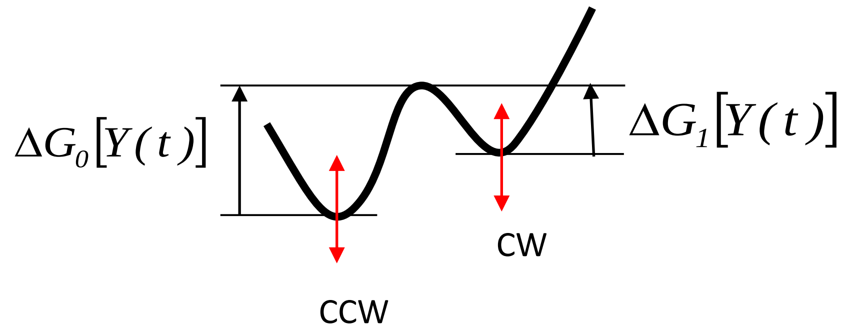


- The persistence time τ_p for a bacterium is correlated over a memory time: $T_Y \sim 20\,\mathrm{s}$

Modeling fluctations in R/T regulation

CheY-P=*Y(t)*





Orstein-Uhlenbeck equation for CheY-P concentration :

White noise

$$\dot{Y}(t) = -\frac{(Y - \overline{Y})}{T_Y} + \eta(t)$$

T_Y Memory time

Tu and Grinstein, PRL **94**, 208101 (2005)

- ❑ Reaction barriers for the phosphorylation reaction process vary with fluctuating CheY_P concentration

Behavioral Variability Model

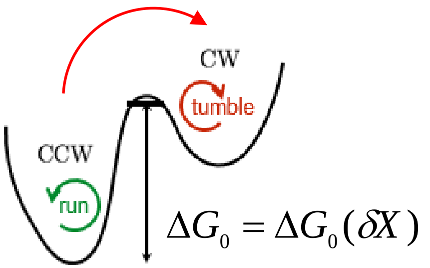
N. Figueroa-Morales et al Phys.Rev.X, **10**, 021004 (2020)

Collab. with R. Soto Univ. Chile

$\delta X \propto [CheY-P]$

Relative fluctuations in CheYP: **the « mood »**

$$\frac{\Delta G_0[Y(t)]}{k_B T} \approx \frac{\Delta G_0[Y_0]}{k_B T} + \Delta_0 \delta X(t) + \dots$$



Switching rate run (CCW) to tumble (CW) : $\frac{1}{\tau_s} \approx \frac{1}{\tau_0}\exp(\Delta_0 \delta X(t))$

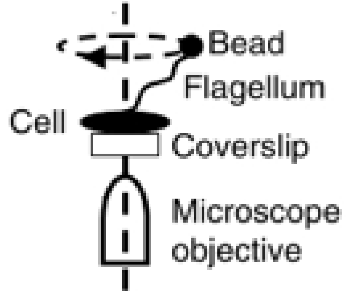
$$ln(\tau_S/\tau_*) \propto \delta X(t)$$

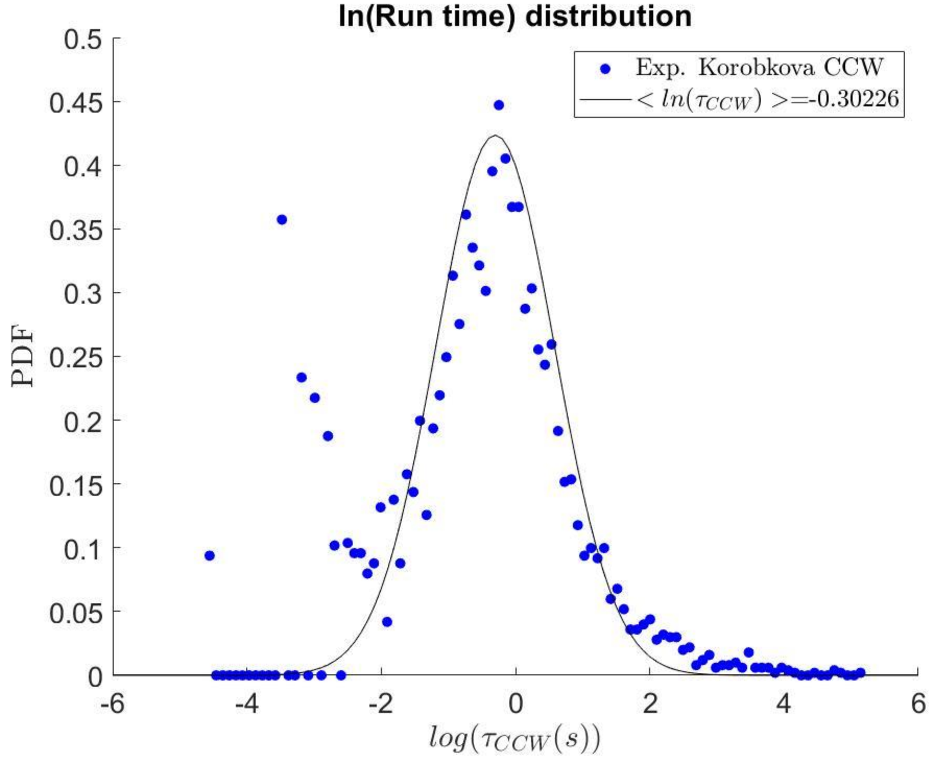
Normal Gaussian distribution
(Orstein Uhlenbeck process)

- ❑ CCW->CW switching times follow a **log-normal distribution**

Back on Korobkova's data

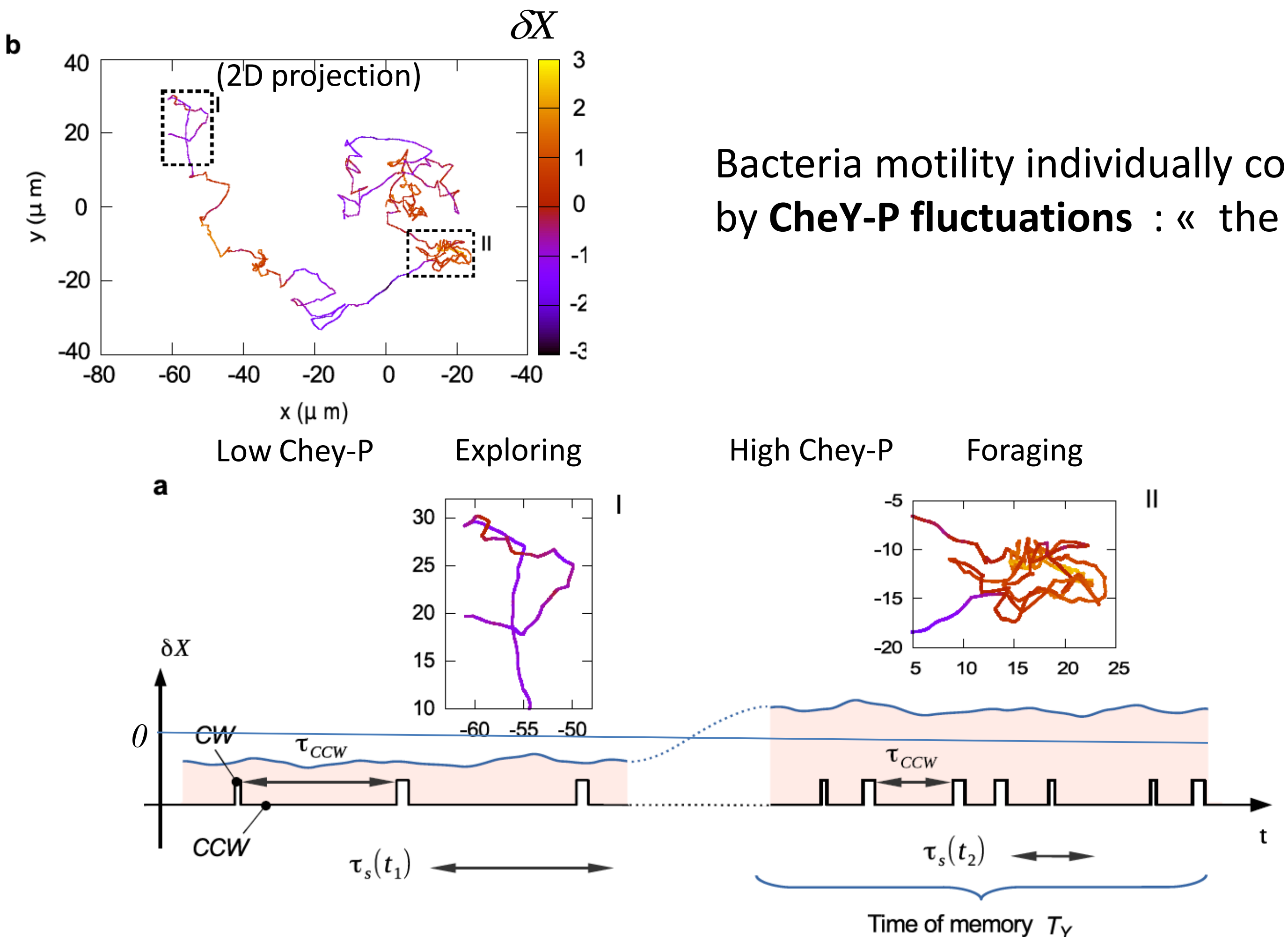
RP437





- ❑ CCW->CW single cell time series consistent with a **log-normal distribution**

In the « mood » for walk



Bacteria motility individually controlled by **CheY-P fluctuations** : « the mood »

❑ Emergence of a random walk with memory