Biophysical Applications of the Physics of Diffusion

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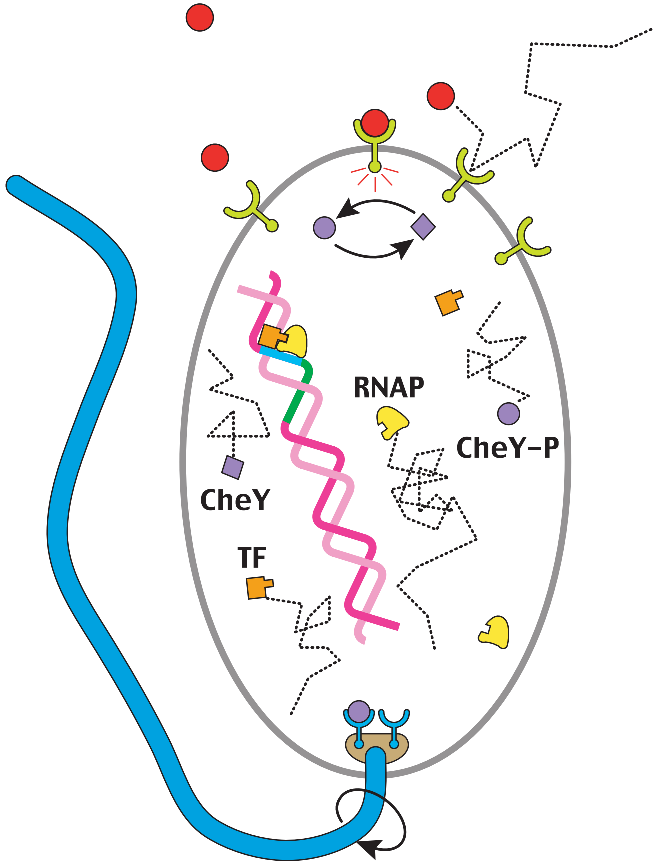
Biochemical Signaling and the Physics of Diffusion

Measurement of concentration of a diffusing signaling molecule by a receptor is a generic task.

In many cases, signaling molecules are present in small copy numbers, making fluctuations in their numbers significant.



How reliably can biochemical reactions be carried out given inherent fluctuations in numbers of crucial molecules?

What is the physical limit in measuring the concentration of signaling molecules by biological receptors?

First addressed by:

H. C. Berg & E. M. Purcell, ''Physics of Chemoreception'', Biophysical Journal (1977)

- Measurement of chemoattractant by single celled organism limited by statistical fluctuations
- Least fractional error attainable set by physics of diffusion
- E. coli chemotaxis machinery near optimal

Outline:

- Revisit BP result within general framework of statistical mechanics
- Generalize to cooperatively interacting receptor cluster
- Compare with recent experiments

E. coli as a Model Organism

Workhorse of molecular biology;
most studied cell in all of science:

--- small genome (~ 4300 genes),
--- normal lack of pathogenicity
--- ease of growth in the lab

- Basis of recent developments in biotechnology and genetic engineering, including living factory for producing human medicines

- Basis for understanding of fundamental cellular processes:
  --- cellular sensory systems,
  --- regulation of gene expression,
  --- cell division, etc.

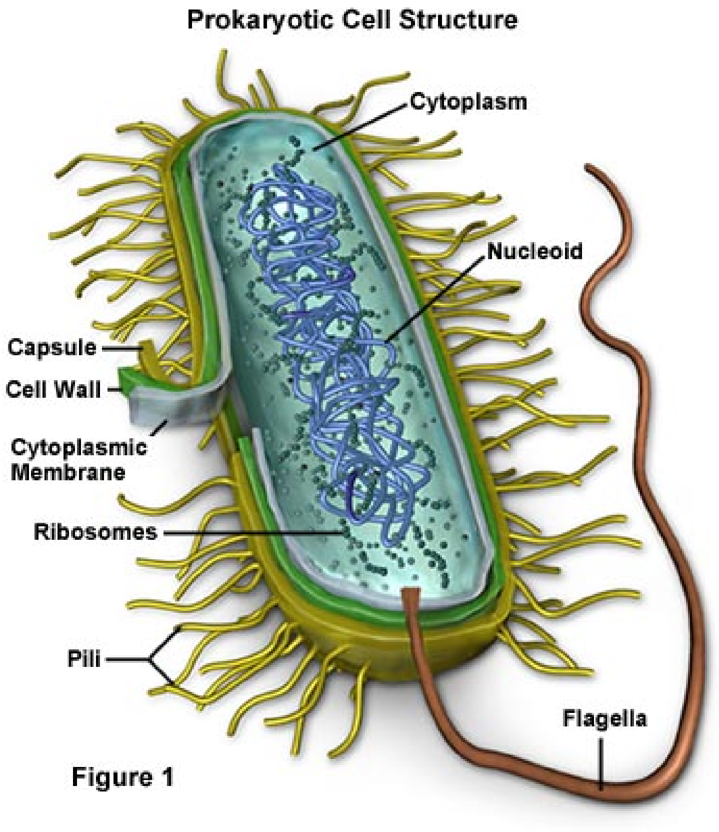


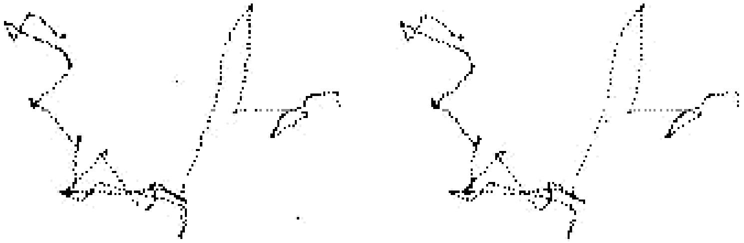
Figure 1

- Size:
  --- 0.5 μm in diameter,
  --- 1.5 μm in length

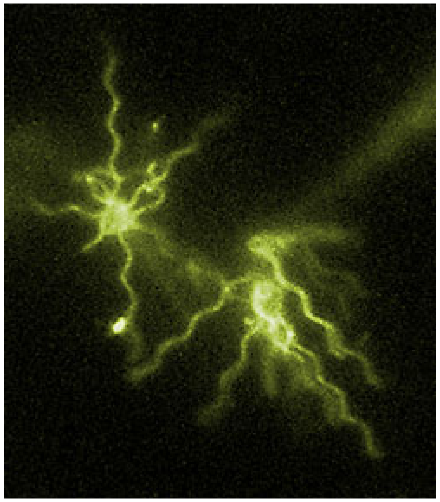
- Cell cycle:
  --- ~ 1 hour

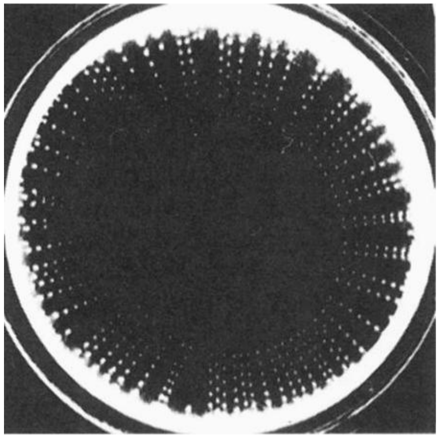
Sensory Mechanisms in Single Cells: Chemotaxis in *E. coli*

3D tracking microscope image
of a single cell's motion



From H. C. Berg, *Physics Today*, 2000.





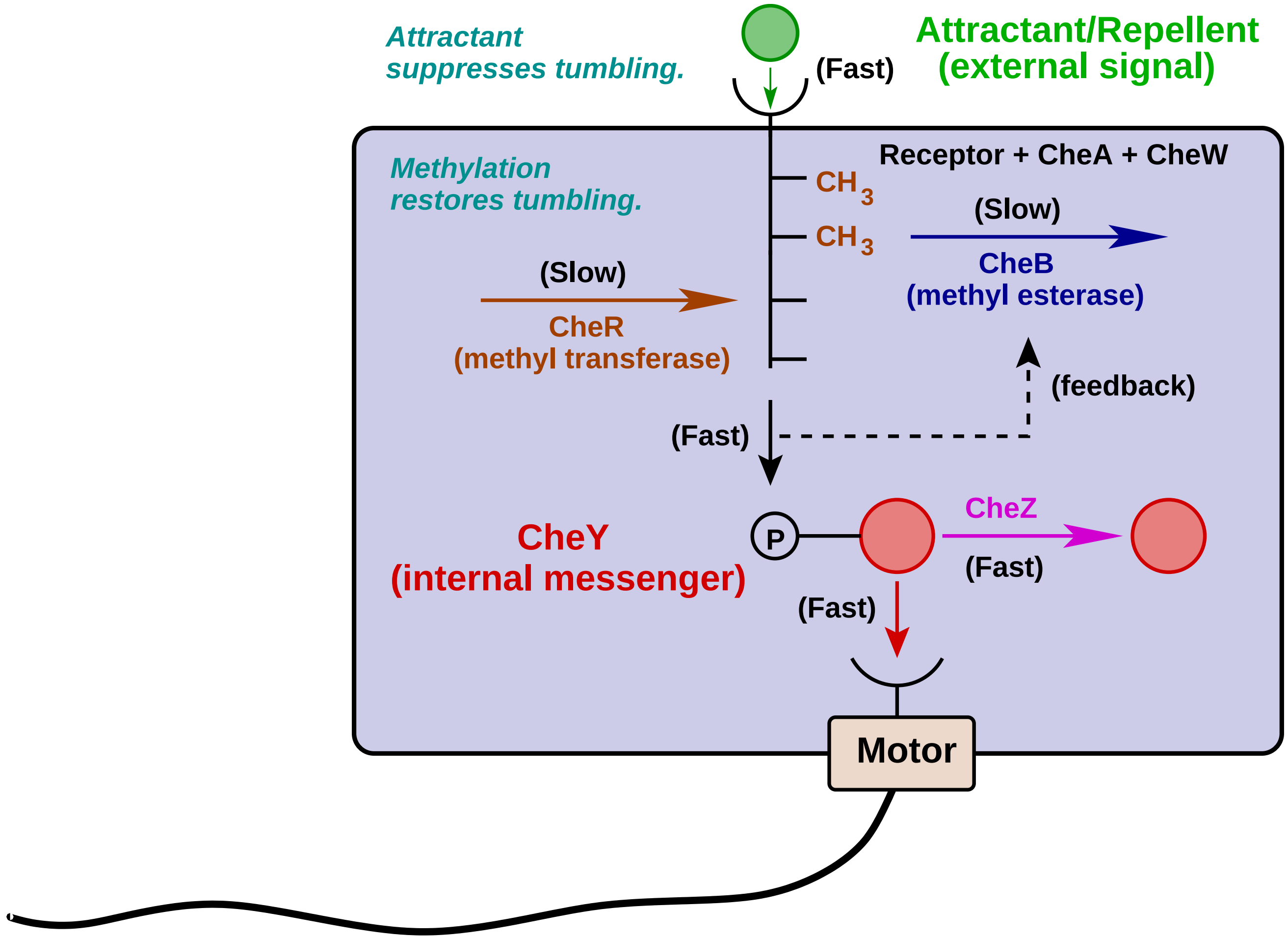
from E. O. Budrene and H. C. Berg,

Nature (1995)

Biased random walk of runs punctuated by tumbles:

- Temporal measurement of external concentration
- Response:
  Modulation of mean runtime
- Physical constants:
  $\tau_T \sim 0.1\, s$
  $\tau_R \sim 1\, s$ (in uniform environment)
  $v \sim 20\, \mu m/s$

E. coli Chemotaxis Signaling Network

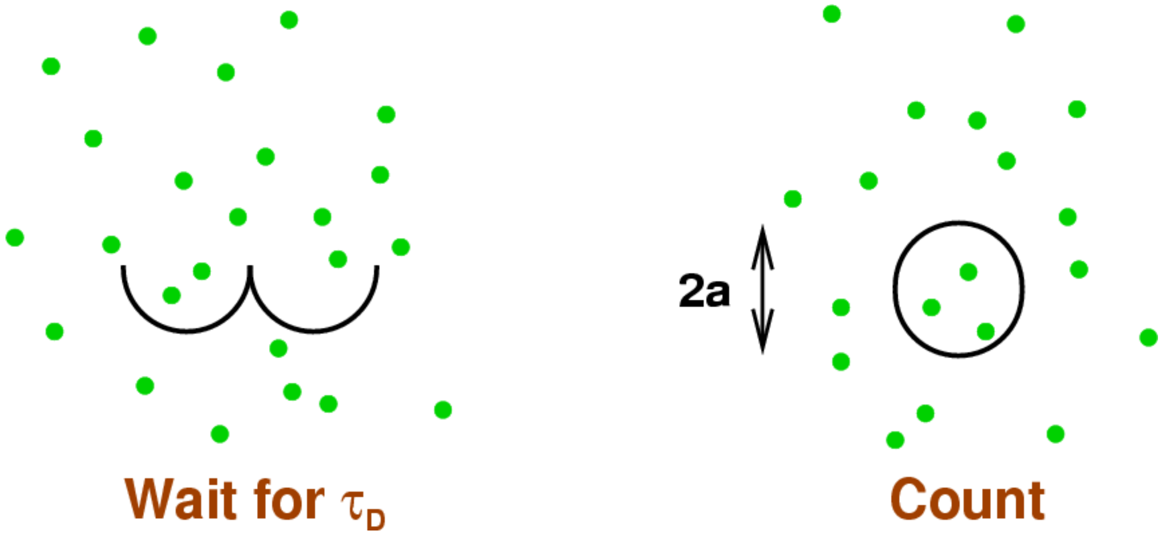


Example of

- Extracellular signaling: cell measures external attractant/repellent concentration
- Intracelluar signaling: motor measures [CheY-P]

Berg-Purcell Results Revisited

"Perfect" Device



- Single measurement of number of substrate molecules:
  $N = \overline{N} \pm \delta N_1, \qquad \overline{N} \sim \bar{c}a^3, \qquad \delta N_1 \sim \sqrt{\overline{N}}$

- Diffusion time: $\tau_D \sim a^2/D$

- Number of independent measurements in time τ:
  $N_M \sim \tau/\tau_D, \qquad \delta N_M \sim \sqrt{\overline{N}/N_M}$

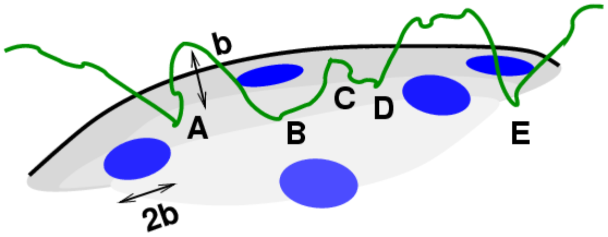
$$\boxed{\delta c/\bar{c} \sim 1/\sqrt{\bar{c}aD\tau}}$$

Questions

- How does this argument based on counting molecules in a volume apply to cells and receptors that count molecules on their surface?
- What about the details of the biochemical kinetics that govern the interaction of ligand with its receptor?
- How to generalize from a single receptor to a cluster of receptors? What about interactions between the receptors, or between the receptors and internal states of the cluster?

Multiple Surface Absorbers

Multiple Absorbers



For N_r discrete absorbers of size b,

- By analogy with electrostatics:

$$I / I^{max}_{sphere} = N_r b \,/\, (N_r b + \pi a)$$

- Also from:

$$1 - P_{esc} = N_r b \,/\, (N_r b + 4a)$$

P_{esc} : probability that substrate molecule at $r = a + b$ survives all subsequent contacts and escapes to ∞

Beat down diffusion noise by $1/\sqrt{N_{tot}}$:

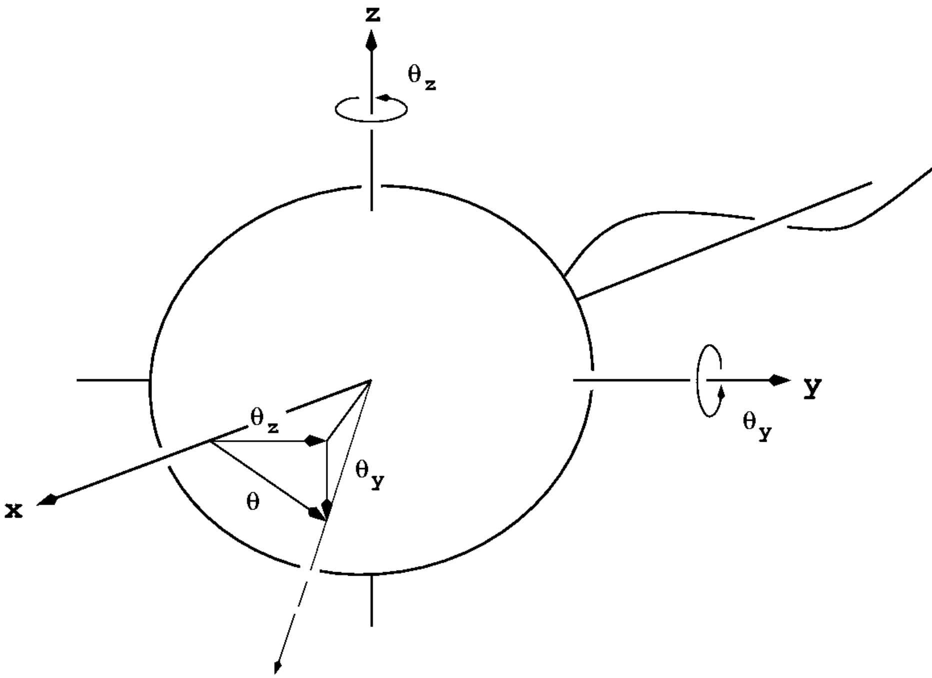
$$\frac{\delta c_{rms}}{\bar{c}} \sim \frac{1}{\sqrt{D\bar{c}\tau}} \left(\frac{\pi}{N_r b} + \frac{1}{a} \right)^{1/2}$$

Integration Time?

- Cell swimming along x axis wanders off course due to rotational diffusion about y and z axes:

$$D_r = k_B T/\gamma_r, \qquad \gamma_r = 8\pi\eta a^3$$

- Bacterium wanders off by $\langle \theta^2 \rangle = 4D_r\tau$ in time τ.
- Integration time must be shorter than the time for rotational diffusion to disorient the cell!



Question:

- How does the *E. coli*'s mean runtime compare with its characteristic timescale for rotational diffusion?

Statistical Mechanics Treatment

W. Bialek and S. Setayeshgar, Proc. Nat'l. Acad. Sci. (USA) 102, 10040 (2005).

Linear Response

Linear response of receptor occupancy to conjugate 'force':

$$\begin{aligned} \frac{d\,\delta n}{dt} &= -(k_+\bar{c} + k_-)\,\delta n + k_-(1-\bar{n})\,[\delta c + \bar{c}\,\beta\,\delta F] \\ \frac{d\,\delta c}{dt} &= D\nabla^2\,\delta c - \delta(\vec{x} - \vec{x}_0)\frac{d\,\delta n}{dt} \end{aligned}$$

Generalized susceptibility:

$$\begin{aligned} \tilde{\alpha}(\omega) = \frac{\delta\tilde{n}(\omega)}{\delta\tilde{F}(\omega)} &= \frac{k_+\bar{c}(1-\bar{n})}{k_B T}\,\frac{1}{-i\omega[1+\Sigma(\omega)] + (k_+\bar{c} + k_-)} \\ \Sigma(\omega) &= k_+(1-\bar{n})\int_0^\Lambda \frac{d^3k}{(2\pi)^3}\,\frac{1}{-i\omega + Dk^2}, \qquad \Lambda \sim \pi/a \end{aligned}$$

Fluctuation-dissipation theorem:

$$S_F(\omega) = -\frac{2k_B T}{\omega}\,\mathrm{Im}\left[\frac{\delta\tilde{F}(\omega)}{\delta\tilde{n}(\omega)}\right]$$

Effective spectral density of noise in measuring c, in terms of 'noise force' spectrum:

$$S_c^{\mathit{eff}}(\omega) = \left(\frac{\bar{c}}{k_B T}\right)^2 S_F(\omega)$$

Linear Response

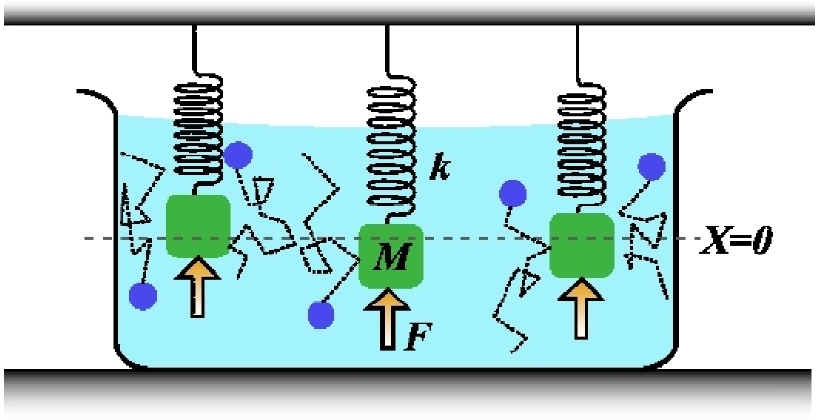
Linear response of receptor occupancy to conjugate ‘‘force’’:

$$\frac{d\,\delta n}{dt} = -\,(k_+\overline{c} + k_-)\,\delta n \;+\; k_+\,(1-\overline{n})\;\underbrace{[\delta c + \overline{c}\beta\,\delta F]}_{\delta\mathcal{F}\;\cdot\;\overline{c}\beta}$$

Analogous to Brownian motion in a harmonic potential:

$$M\,\delta\ddot{X} + \gamma\,\delta\dot{X} + k\,\delta X = \delta f(t)$$

(ignoring the inertial term, $M\,\delta\ddot{X}$)



Mechanical/Chemical Systems

Physical quantity	Mass-spring system	Chemical system
Coordinate	Displacement	Receptor occupancy: $\delta n = n - \overline{n}$
Conjugate force	$f(t)$	Free energy change: $\delta\mathcal{F} = k_B T(\delta k_+/k_+ - \delta k_-/k_-)$
'Spring' constant	k	$k_B T/[\overline{n}(1-\overline{n})]$
'Damping' constant	γ	$k_B T/(k_- \overline{n})$

- Fluctuation-dissipation theorem

$$\langle \delta f(t) \cdot \delta f(t') \rangle = 2 k_B T \, \gamma \, \delta(t - t')$$

- More generally,

$$\delta X(t) = \int dt' \, \alpha(t - t') \; \delta f(t')$$

$$S_X(\omega) = \frac{2k_B T}{\omega} \, \mathrm{Im} \, [\tilde{\alpha}(\omega)] \; , \qquad S_f(\omega) = -\frac{2k_B T}{\omega} \, \mathrm{Im} \left[\frac{1}{\tilde{\alpha}(\omega)} \right]$$

Connection with Berg-Purcell Result

Accuracy of a measurement which integrates for a time $\tau \gg \tau_c$:

$$\delta c_{rms} \approx \sqrt{S_c^{eff}(\omega = 0) \cdot \frac{1}{\tau}}$$

Two contributions to concentration noise power spectrum:

$$S_{c_{eff}}(\omega = 0) = \frac{2\overline{c}^2}{k_+\overline{c}(1 - \overline{n})} + \frac{\overline{c}}{\pi D a}$$

Lower bound set by diffusion only:

$$\boxed{\frac{\delta c_{rms}}{\overline{c}} > \frac{1}{\sqrt{\pi D a \overline{c} \tau}}}$$

Berg-Purcell results give:

- intuitive argument for lower bound set by counting noise due to diffusion (second term)
- limit set by binding/unbinding to surface receptors, using diffusion-limited binding rate (first term)

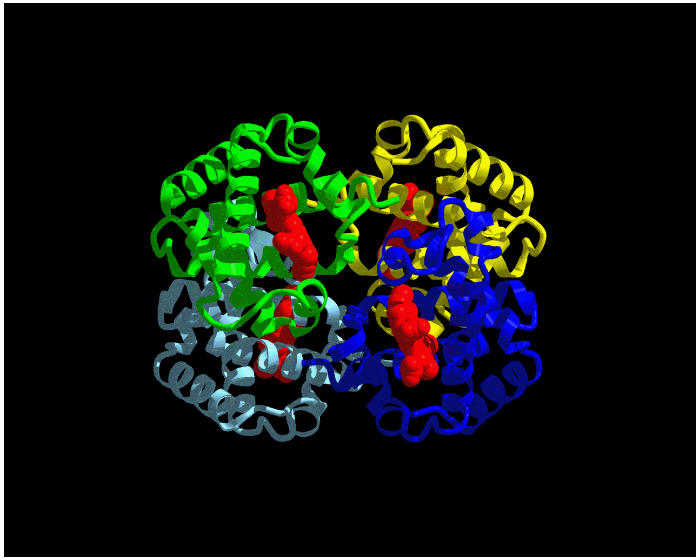
Simple derivation of Brownian motion leading to the Langevin Equation and the Fluctuation-Dissipation Theorem

B. G. de Grooth, Am. J. Phys. 67, 1248 (1999).

Cooperatively Interacting Receptors

W. Bialek and S. Setayeshgar, q-bio.MN/0601001.

Cooperativity in Biochemical Regulation



Displayed by enzymes and receptors with multiple binding sites:

when a substrate molecule binds to a site, the receptor's affinity for subsequent substrate molecules increases (decreases).

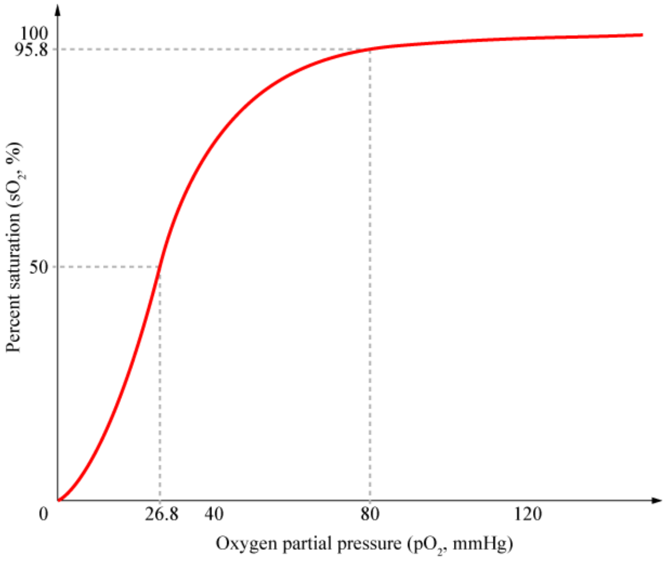
- Famous example of (positive) cooperativity: binding of oxygen to Hemoglobin

  (four O atoms can bind to Hb,
  with increasing affinity of binding
  as successive O atoms bind)

- Classical models:

  --- MWC (Monod-Wyman-Changeux), and
  --- KNF (Koshland-Nemethy-Filmer) models

  can be obtained as limiting cases of
  1d Ising model



Cooperativity in the Flagellar Motor

Monod-Wyman-Changeux model of bacterial flagellar motor:

- Free energy of R/T states of receptor cluster with n ligands bound:

$$F_{R,T}(n) = F_{R,T}(0) - nk_BT\,\ln\left(\frac{c}{K_{R,T}}\right),$$

$$K_{R,T} = \frac{k_-^{R,T}}{k_+^{R,T}}$$: *dissoc'n const. in* R/T *states*

- Partition function of the receptor cluster

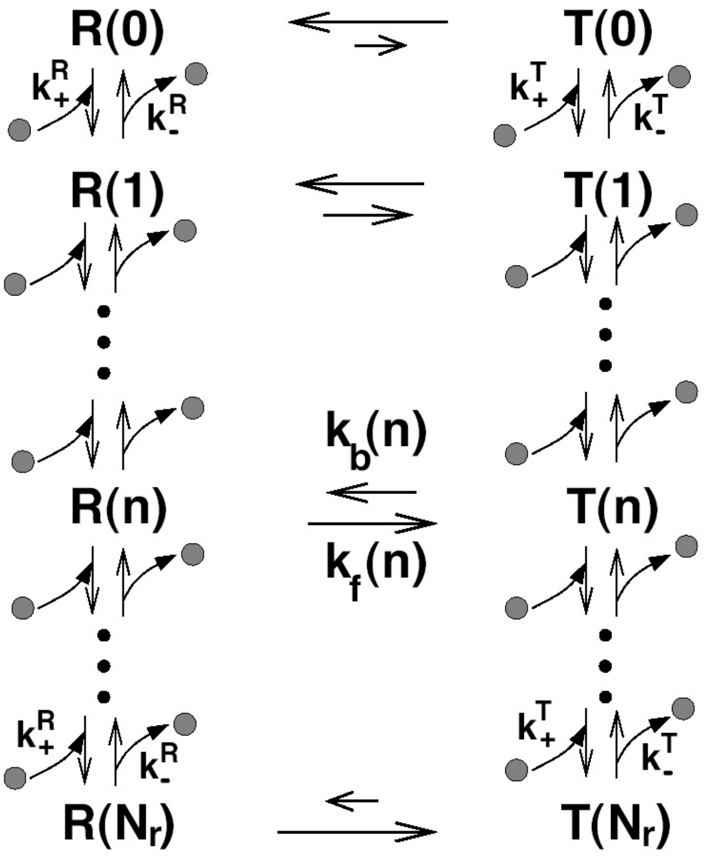
$$Z = Z_R + Z_T$$

$$Z_{R,T} = e^{-\beta F_{R,T}(0)}\left(1+\frac{c}{K_{R,T}}\right)^{N_r},$$

and

$$p_T(n) = \binom{N_r}{n}\left(\frac{c}{K_T}\right)^n \frac{e^{-\beta F_T(0)}}{Z},$$

$$p_T = \sum_{n=1}^{N_r} p_T(n) = \left[1+\frac{1}{L}\left(\frac{1+c/K_R}{1+c/K_T}\right)^{N_r}\right]^{-1}$$



$N_r = 34$ FliM sites
R: CCW state
T: CW state

Coupled Switching and Substrate Diffusion

- Ligand binding to i^{th} site:

$$\frac{dn_i}{dt} = k_+ \left(1 - n_i\right) c(\vec{x}_i) - k_- \, n_i, \qquad i = 1, \ldots, N_r$$

For fast binding kinetics, $n_i(t) \equiv n_i^{R,T}(t) \longrightarrow \overline{n}_i^{R,T} = \overline{c}(\vec{x}_i) / \left[\overline{c}(\vec{x}_i) + K_{R,T}\right].$

- Dynamics of receptor cluster as two-state system:

$$\frac{dp_T}{dt} = \overline{k}_f (1 - p_T) - \overline{k}_b \, p_T,$$

where $\overline{k}_{f,b}$ obtained as averages over equilibrium distributions, $p_{R,T}(n)$.

- Diffusion, coupled to dynamics of switching:

$$\frac{\partial c}{\partial t} = D\nabla^2 c - \dot{p}_T \sum_{j=1}^{N_r} \overline{n}_j^T \, \frac{\delta\left(|\vec{x} - \vec{x}_j| - b\right)}{4\pi b^2} - \dot{p}_R \sum_{j=1}^{N_r} \overline{n}_j^R \, \frac{\delta\left(|\vec{x} - \vec{x}_j| - b\right)}{4\pi b^2}.$$

Measurement Accuracy of Cooperative Receptor Cluster

Following same steps through FDT ...

- Averaging the state of the cluster (R/T) over a time $\tau \gg \tau_c$:

$$\frac{\overline{(\delta c)^2}}{\overline{c}^2} \approx \underbrace{\frac{2}{N_r^2\,(\overline{n}_T - \overline{n}_R)^2\,\overline{k}_f(1-\overline{p}_T)\tau}}_{\text{'Stuff'}\ >\ 0} + \frac{1}{2\pi D\overline{c}\tau}\left(\frac{1}{N_r b} + \frac{g_0}{a}\right)$$

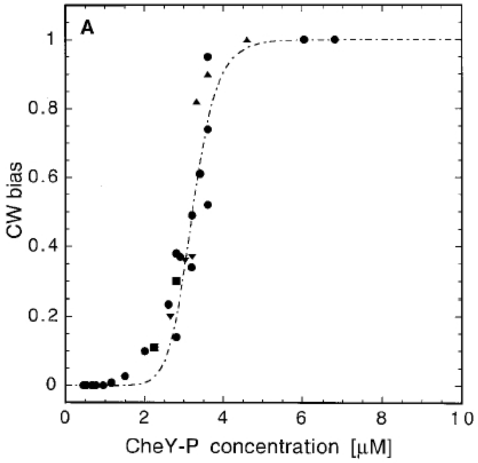
- Strength of cooperativity, as measured by dp_T/dc:

$$\frac{dp_T}{dc} = N_r\,(n_T - n_R)\,\frac{p_T}{c}\,\frac{n_R/n_T}{L + n_R/n_T}$$

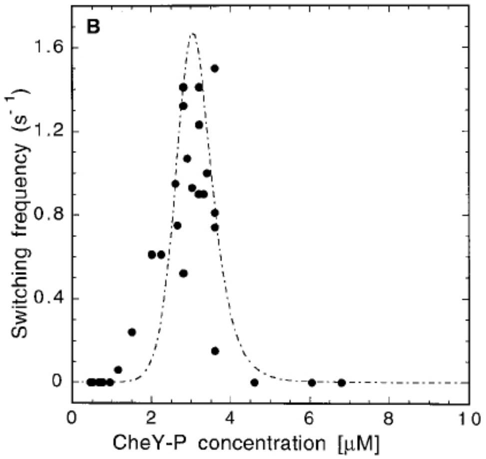
Although gain in response to input increases with N_r, $(n_T - n_R)$, accuracy in measuring $\overline{c}$ constrained by diffusive lower bound !!

E. coli Flagellar Motor

CW bias, $p_T(c)$

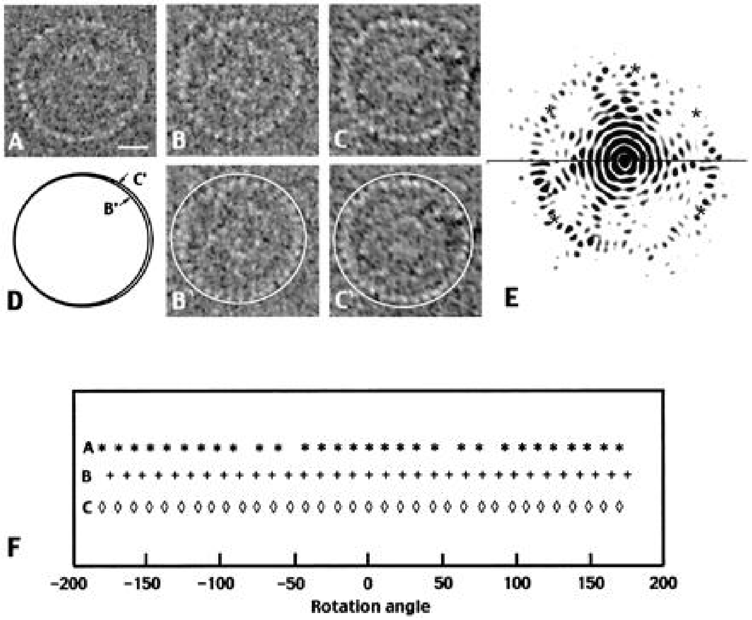


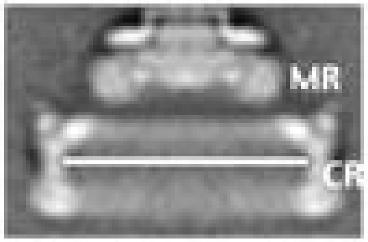
Switching frequency, $f(c)$



From P. Cluzel, *et al.*, *Science* (2000).

C-ring





From D. R. Thomas, *et al.*, *Proc. Natl. Acad. Sci.* (1999).

$N_r = 34$ FliM sites

$a \sim 22.5$ nm

Optimal Switching?

$$\frac{\overline{\delta c^2}}{c^2} \approx \frac{4}{N_r^2 \left(n_T - n_R\right)^2} \frac{1}{f(c)\tau} + \frac{1}{2\pi D c \tau}\left(\frac{1}{N_r b} + \frac{g_0}{a}\right)$$

With $\tau = 1$ s, $b = 1$ nm

- **First term** for:

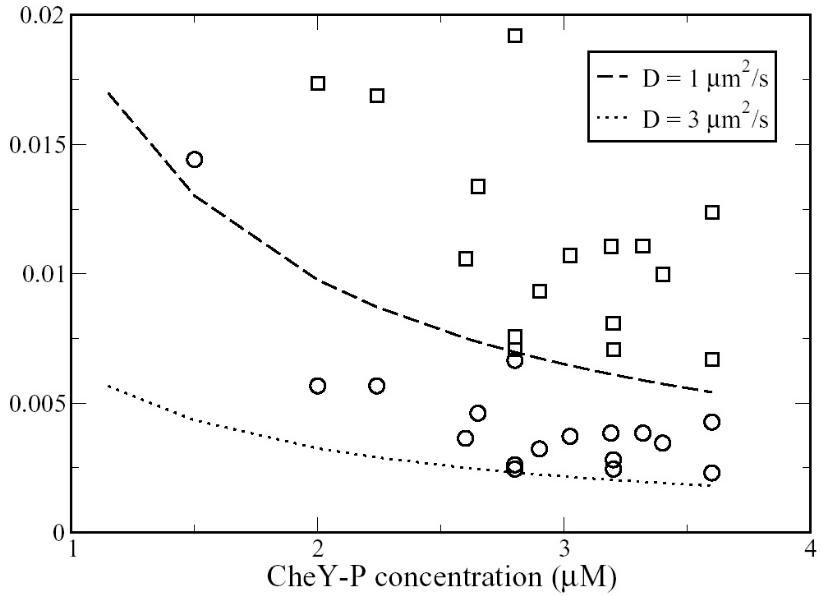
  Circle: $n_T - n_R = 1$

  Square: $n_{R,T} = c/(c + K_{R,T})$ with $(K_R, K_T) = (12, 0.8)\,\mu M$ obtained from fit to $p_T(c)$

- **Second term** for:

  Dashed: $D = 1\,\mu m^2/s$

  Dotted: $D = 3\,\mu m^2/s$

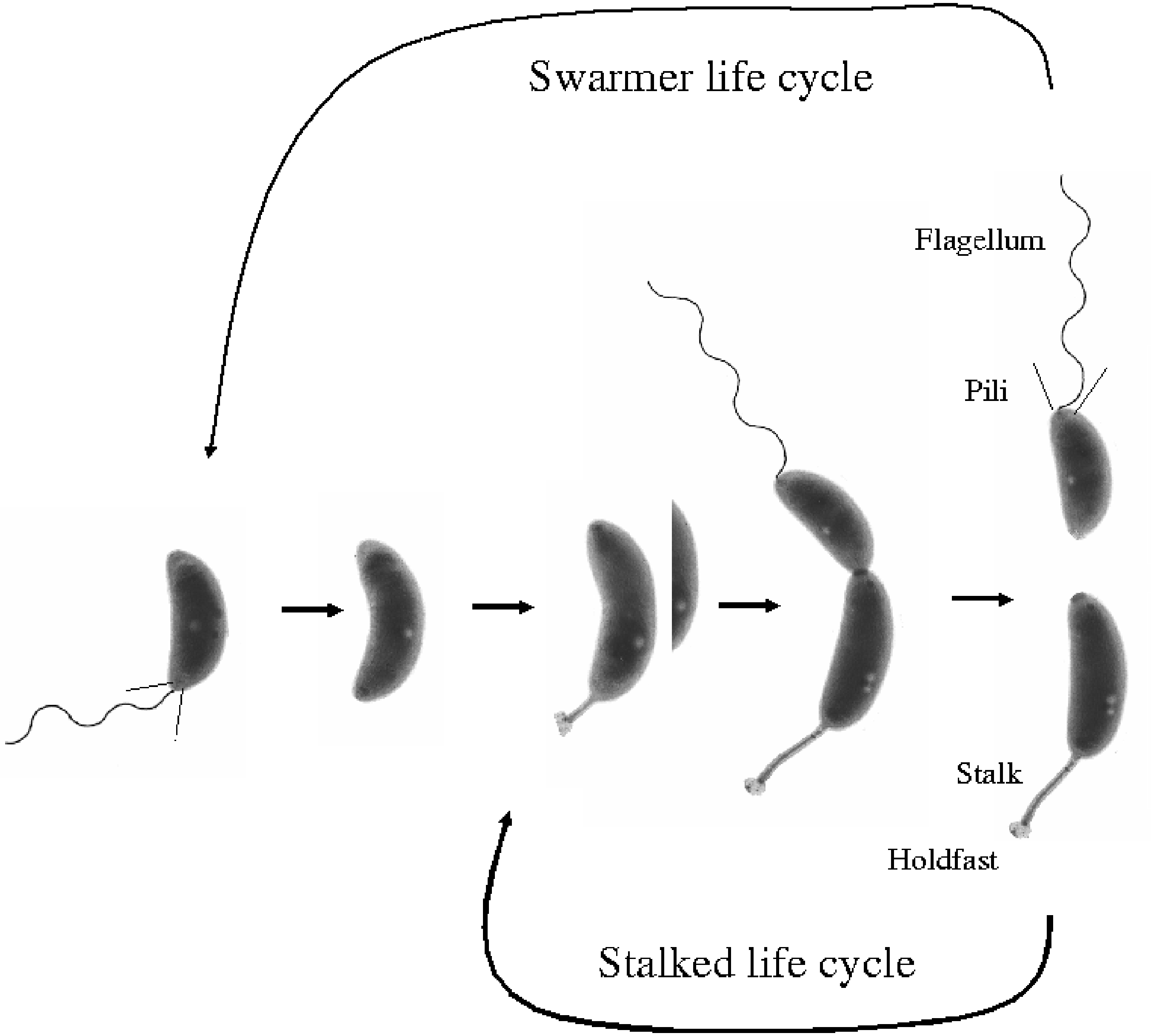


Limit from cooperative kinetics consistent with diffusive lower bounds (to within factor) ...

Cell Morphology and the Physics of Diffusion

J.K. Wagner, S. Setayeshgar, L. A. Sharon, J.P. Reilly, and Y.V. Brun, PNAS 11772 (2006).

Caulobacter Life Cycles



Swarmer cell: dispersal

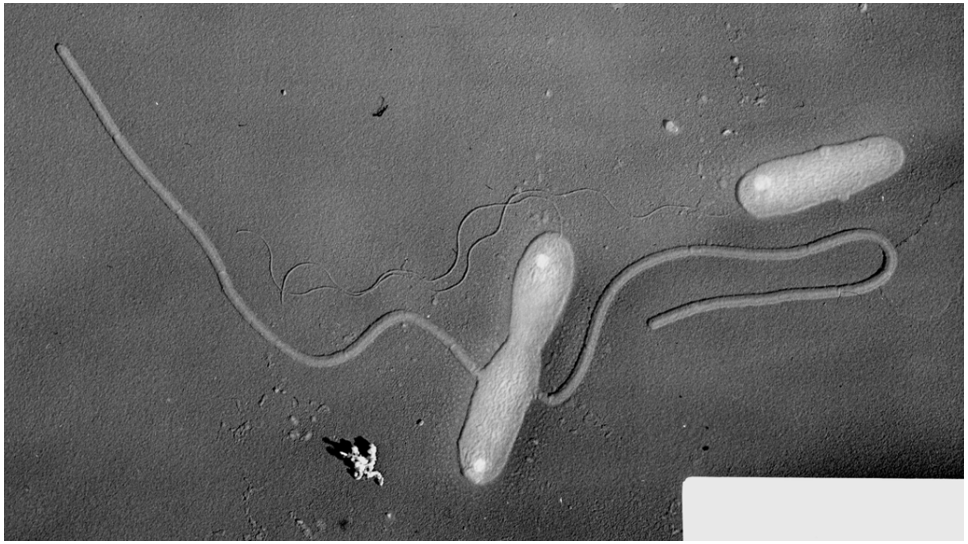
- No DNA replication
- No cell division
- Motility

Stalked cell: reproduction

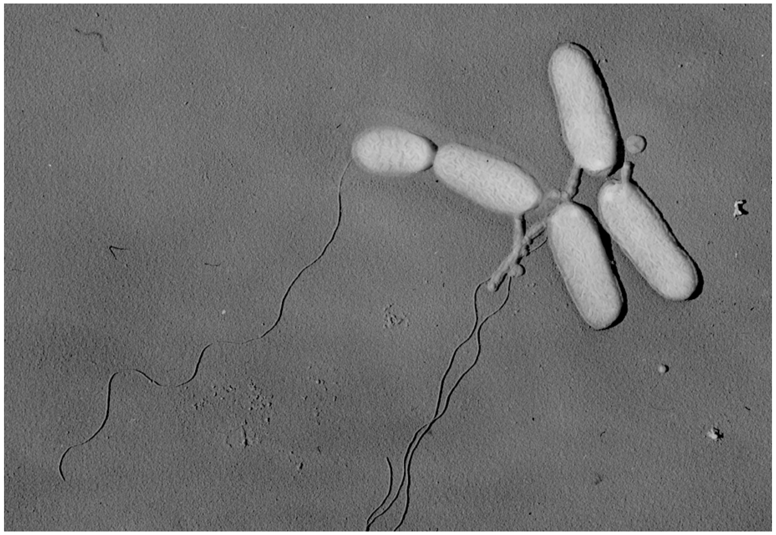
- DNA replication
- Cell division
- Surface adhesion

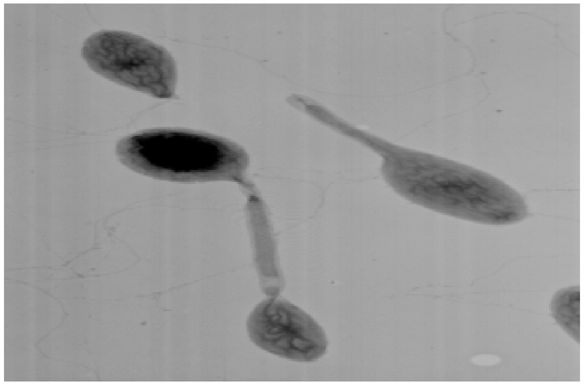
Various prosthecate bacteria

Asticaccaulis biprosthecum

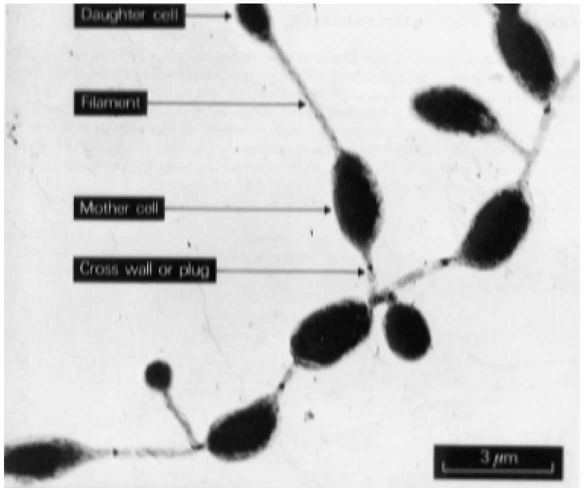


Asticaccaulis excentricus

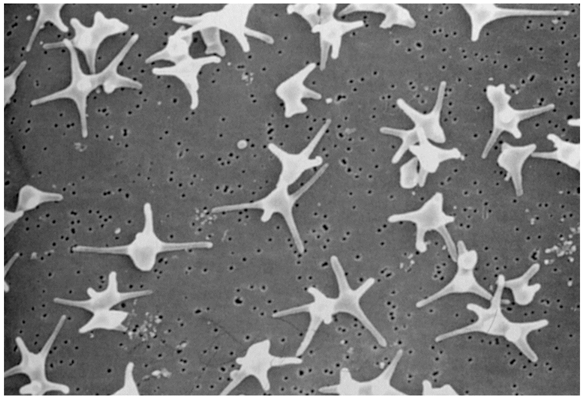




Hyphomonas neptunium

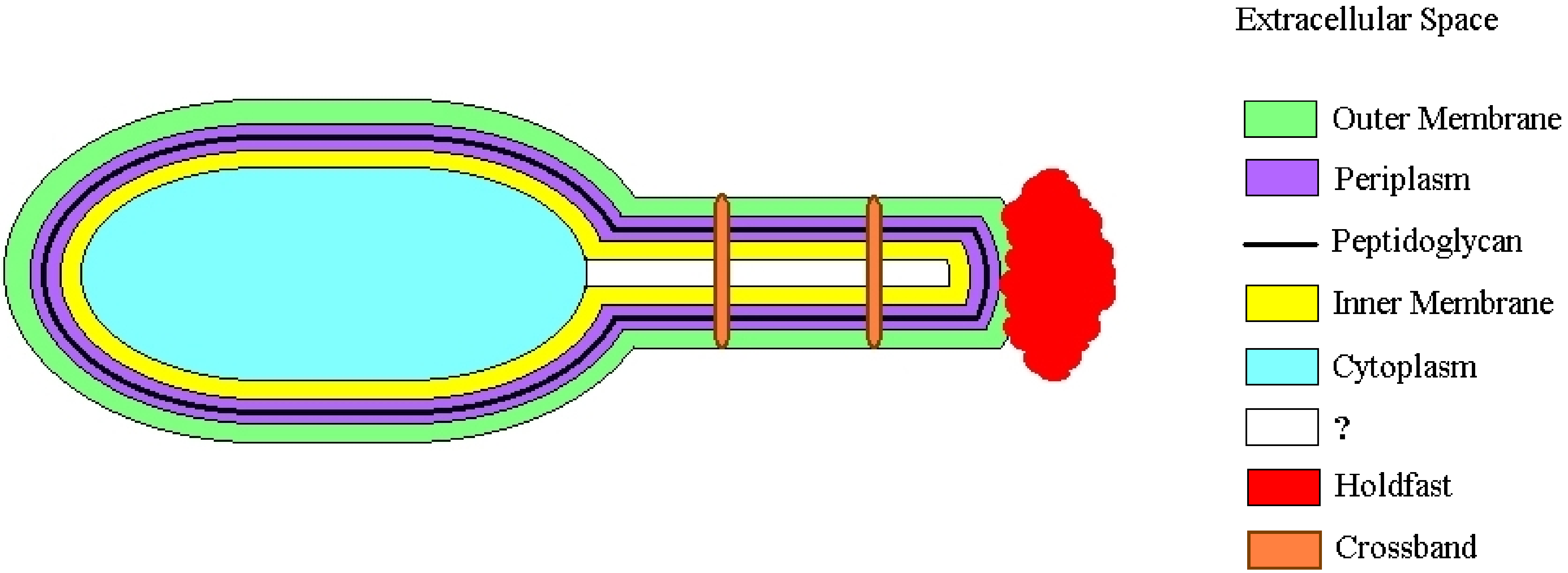


Rhodomicrobium vanniellii

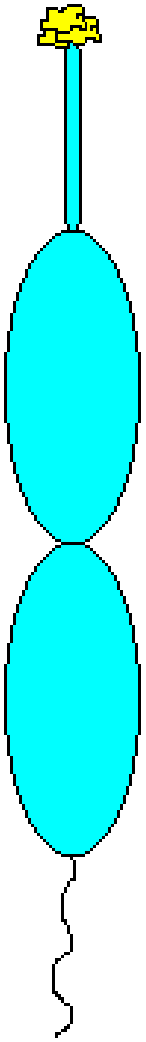


Ancalomicrobium adetum

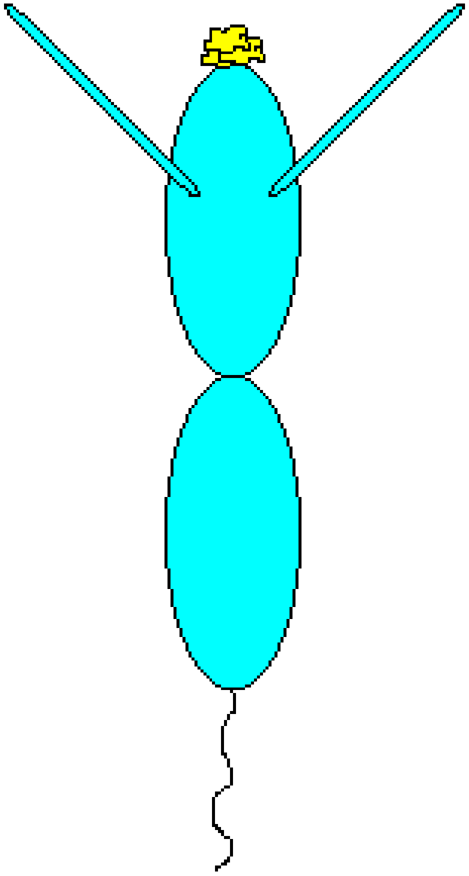
The stalk is an extension of the cell surface



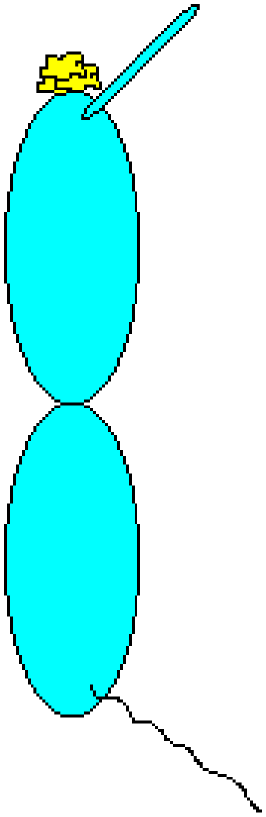
The role of the stalk is NOT surface adhesion



Caulobacter crescentus



Asticcacaulis biprosthecum



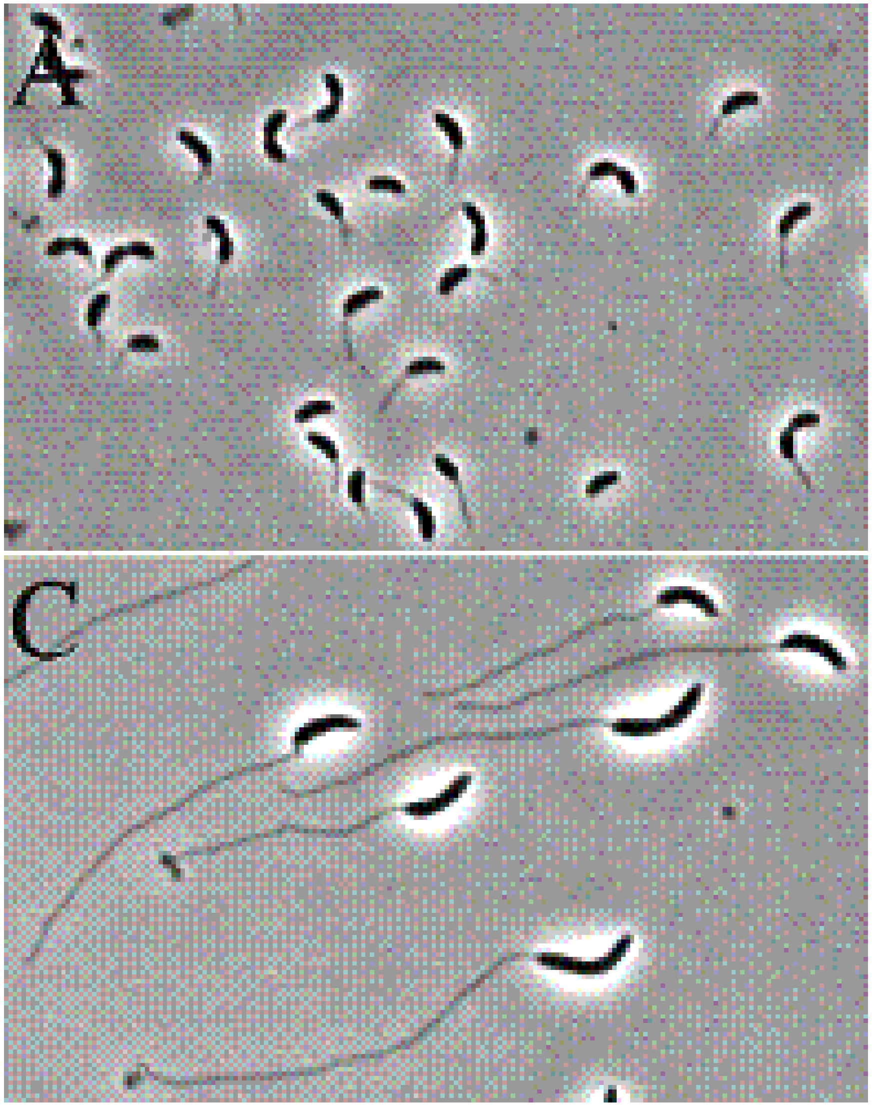
Asticcacaulis excentricus

So what is the function of the stalk?

Not required for attachment

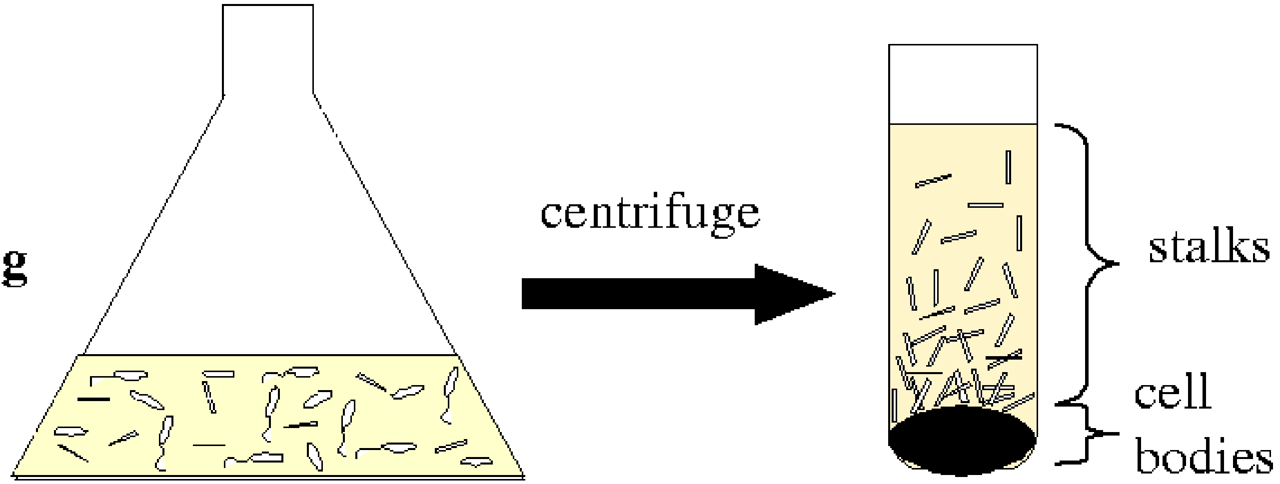
Induced by phosphate starvation

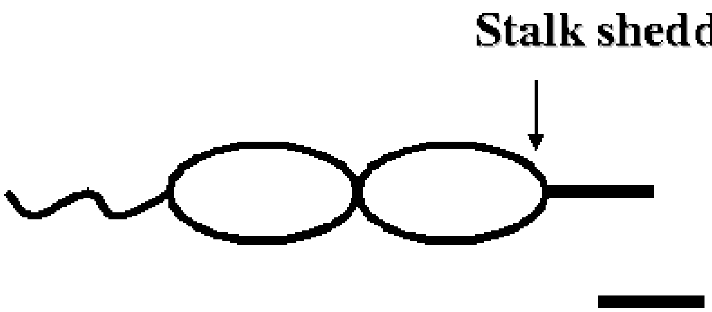
- Top: High PO_4, $1 - 2\ \mu m$
- Bottom: Low PO_4, $\sim 30\ \mu m$

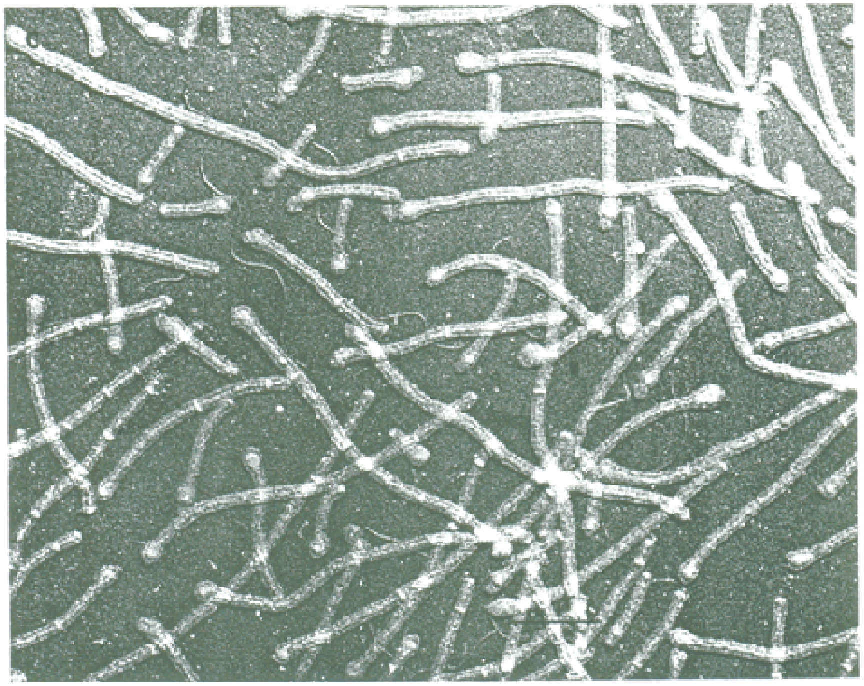


Purifying Stalks

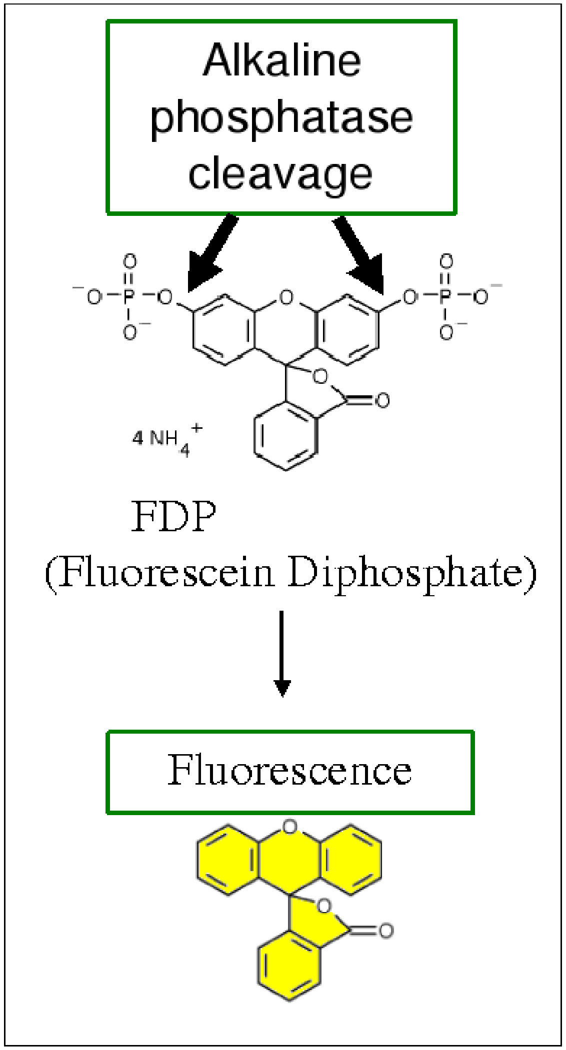
Grow stalk shedding mutant







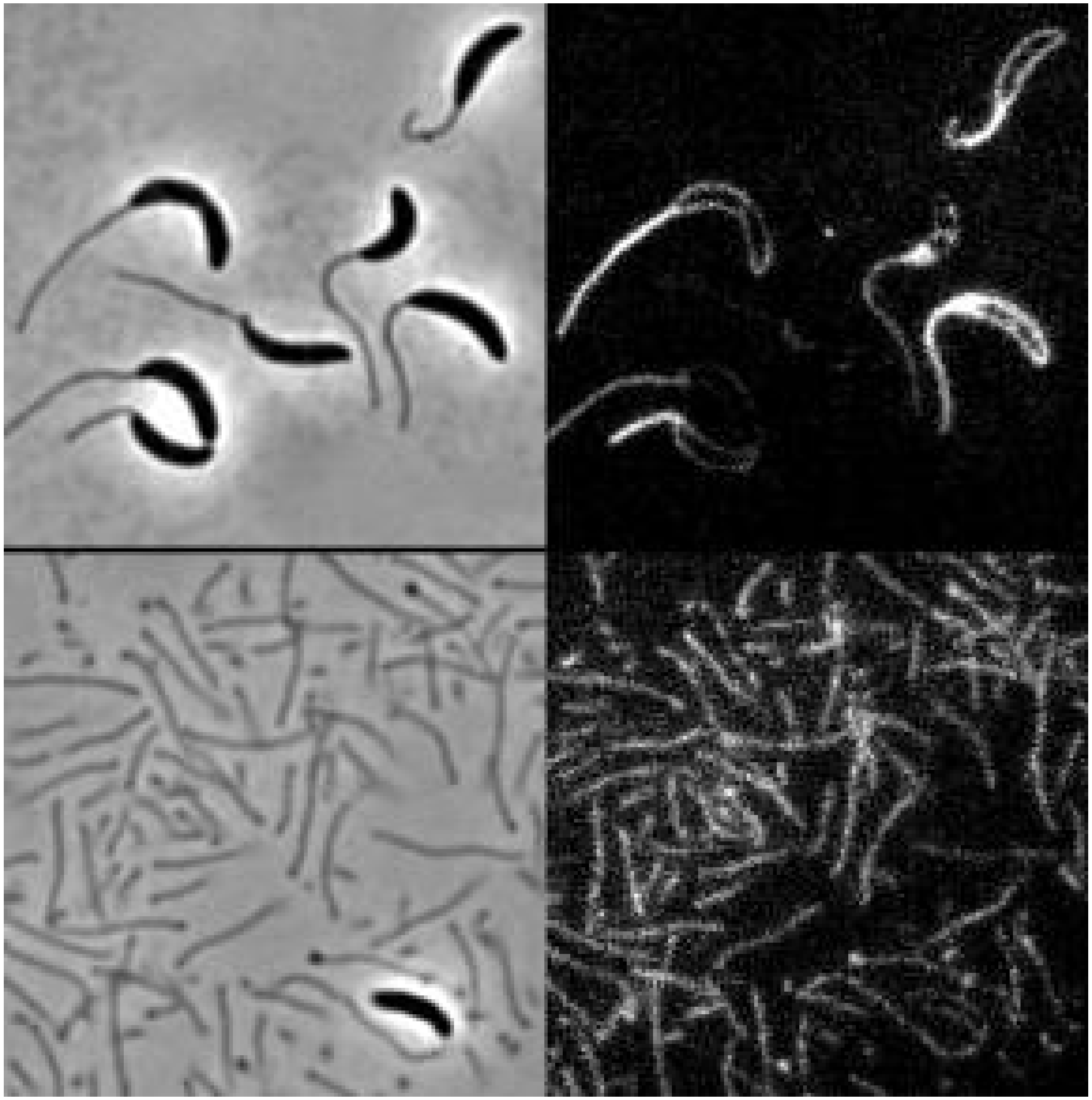
Are stalks able to take up and hydrolyze FDP, a synthetic phosphate-ester?



Alkaline phosphatase cleavage

FDP
(Fluorescein Diphosphate)

Fluorescence



Role of stalk in nutrient uptake?

Calculate diffusive current to stalk: In prolate spheroidal coordinates

$$\begin{aligned}\xi &= \frac{1}{2}\left[-(\ell^2+b^2)+\rho^2+z^2+\sqrt{-4\ell^2(b^2-\rho^2)+4b^2z^2+(\ell^2+b^2-\rho^2-z^2)^2}\right] ,\\ \zeta &= \frac{1}{2}\left[-(\ell^2+b^2)+\rho^2+z^2-\sqrt{-4\ell^2(b^2-\rho^2)+4b^2z^2+(\ell^2+b^2-\rho^2-z^2)^2}\right] ,\\ \phi &= \tan^{-1}\frac{y}{x} ,\end{aligned}$$

where $\rho^2 = x^2 + y^2$, and $x^2/b^2 + y^2/b^2 + z^2/\ell^2 = 1$ $(\xi = 0)$.

We find

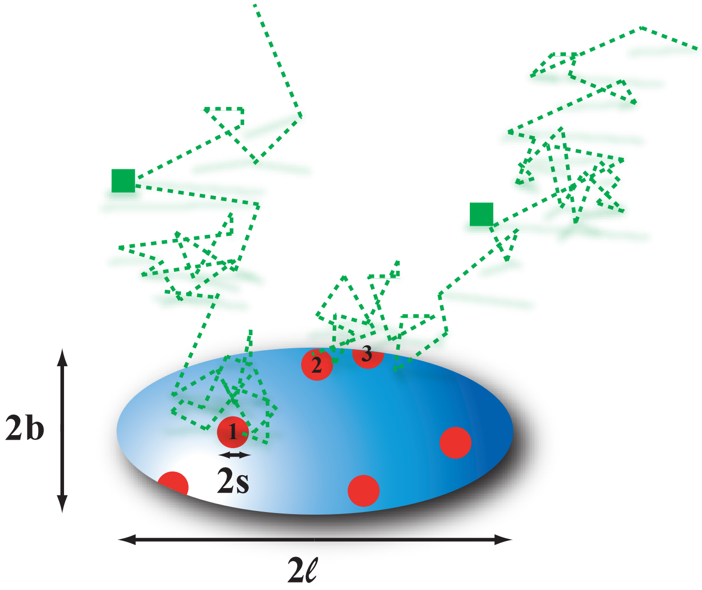
$$I^{max}_{spheroid} = \frac{4\pi D c_0 \ell \sqrt{1-b^2/\ell^2}}{\tanh^{-1}\left(\sqrt{1-b^2/\ell^2}\right)} .$$

What about with N discrete absorbers?

Probability of capture

Obtained by solving recursion relation (1D):

$$P(x) = \frac{1}{2}\left(P(x+\delta) + P(x-\delta)\right)$$



giving

$$\nabla^2 P = 0 \qquad \begin{cases} P(\infty) & = 0 \\ P(\text{surface}) & = 1 \end{cases}$$

We find the probability of a diffusing particle arriving at the spheroidal surface ($\xi = 0$) from $\xi(\rho, z)$:

$$P(\xi) = \frac{1}{\tanh^{-1}\sqrt{1 - b^2/\ell^2}} \tanh^{-1}\sqrt{\frac{1 - b^2/\ell^2}{1 + \xi/\ell^2}},$$

Probability of not contacting an aborber upon arriving at surface

After 1 hit:

$$\begin{aligned} A &: \textit{area of spheroid} \\ \frac{N\pi s^2}{A} &: \textit{probability encounter is w/ receptor} \\ \underbrace{\left(1 - \frac{N\pi s^2}{A}\right)}_{\beta} P(\xi) &: \begin{array}{l}\textit{probability diffusing particle at } \xi(z,\rho) \\ \textit{does } \underline{\textit{not}} \textit{ hit an absorber}\end{array} \end{aligned}$$

Many independent hits?

For random walker to encounter different absorbers (*i.e.*, consecutive hits on surface separated by distance approximately equal to s):
require excursion above surface also by s.

P_s: probability of contacting surface from distance s above

$$s \;=\; h_\xi(\xi = 0)\,\delta\xi \qquad h_\xi\ :\ \text{element of length in } \xi \text{ direction}$$

Find average over surface of spheroid:

$$\langle \delta\xi \rangle = \begin{cases} (8bs)/\pi\,, & (b \ll \ell) \\ (4b\ell s)/(b+\ell)\,, & (b \sim \ell) \end{cases}$$

$$\begin{aligned} P_s = P(\xi = \langle \delta\xi \rangle) \;&\underset{b\ll\ell}{\approx}\; 1 - \left(\frac{4s}{\pi b}\right)\frac{1}{\ln\left(2\ell/b\right)}\,, \\ &\underset{b\sim\ell}{\approx}\; 1 - \frac{2bs}{\ell\,(b+\ell)}\,. \end{aligned}$$

Probability of escape to ∞

$$P_{esc} = \sum_{n=0}^{\infty} \left[\underbrace{(\beta P_s)^n}_{make\ \ n\ \ hits} \cdot \underbrace{(1 - P_s)}_{escape\ \ to\ \ \infty} \right] = \frac{1 - P_s}{1 - \beta P_s},$$

$$\begin{aligned} 1 - P_{esc} \ &\underset{b \ll \ell}{\approx} \ \left[1 + \frac{4\ell}{Ns} \frac{1}{\ln(2\ell/b) - \frac{4s}{\pi b}} \right]^{-1}, \\ &\underset{b \sim \ell}{\approx} \ \left[1 + \frac{4b^2}{N\ell s} \frac{1}{1 - \frac{2bs}{\ell(b+\ell)}} \right]^{-1}. \end{aligned}$$

Therefore,

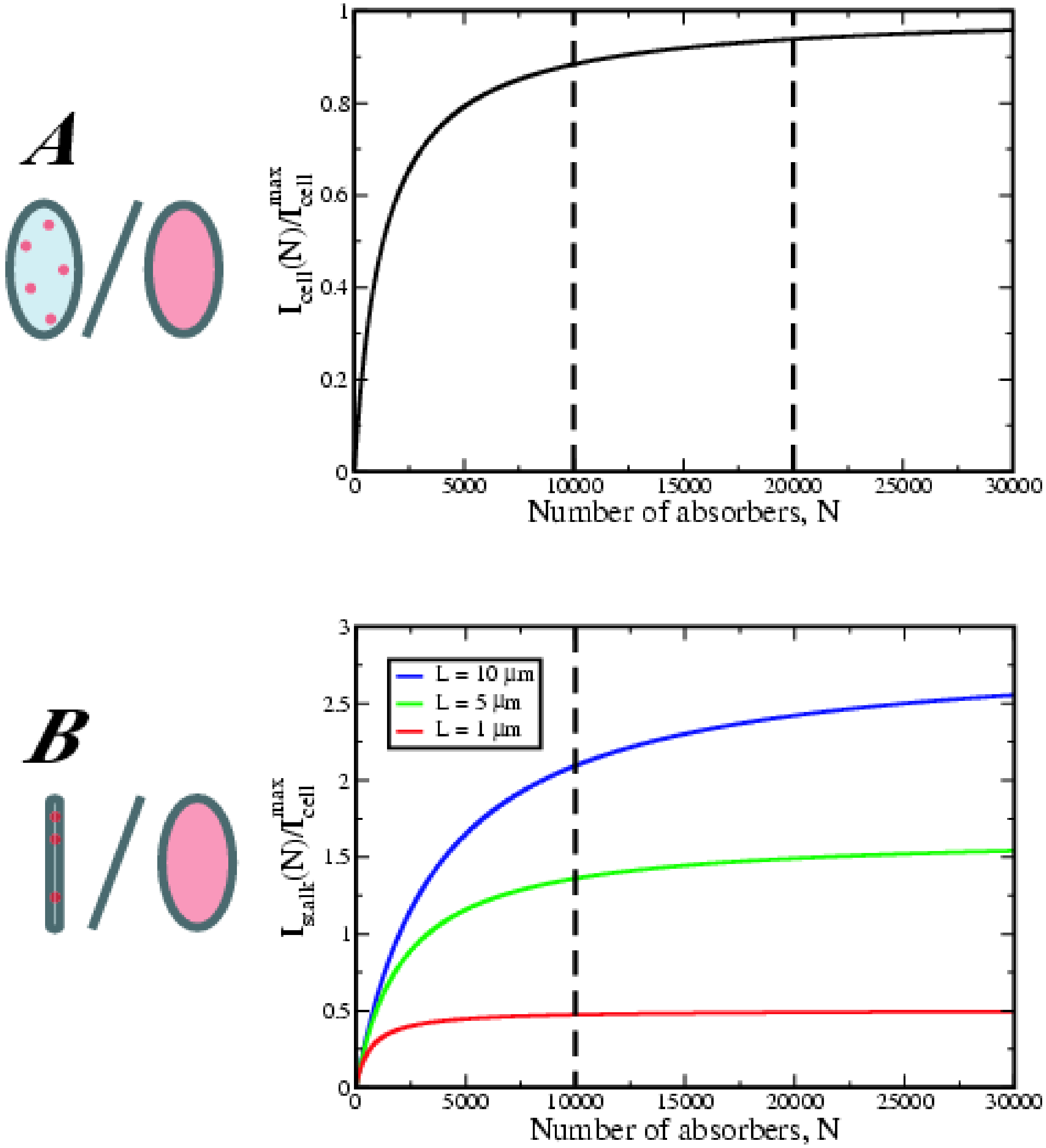
$$I_{spheroid}(N; \ell, b, s) = (1 - P_{esc})\, I_{spheroid}^{max}(\ell, b):$$

(Note that b, s, ℓ determine rate of saturation of the current to N discrete absorbers to the maximum current.)

Comparison of diffusive current to stalk and cell body

Observations:

- Current saturates to maximum current with increasing N
- Advantageous to grow stalk than add additional transporters to cell body.

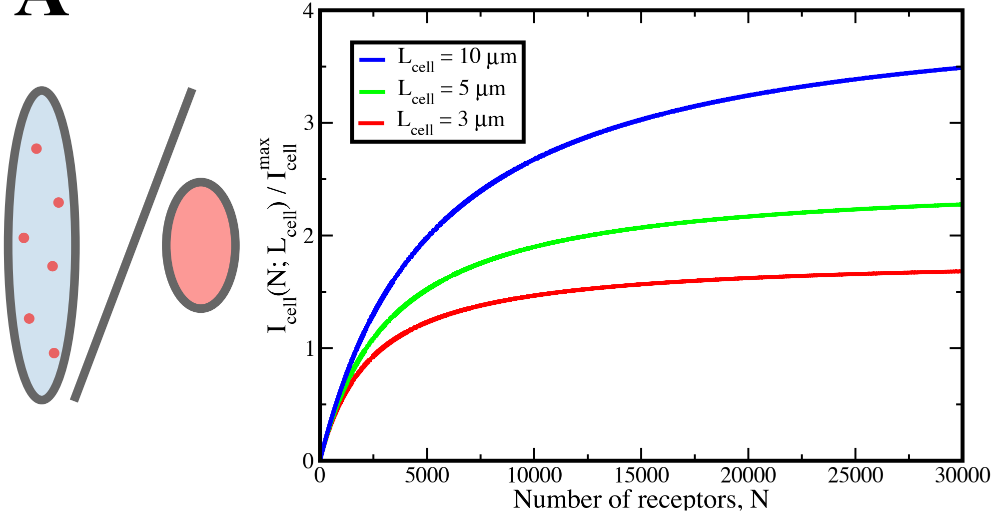


Comparison of diffusive current to stalked cell and elongated cell body

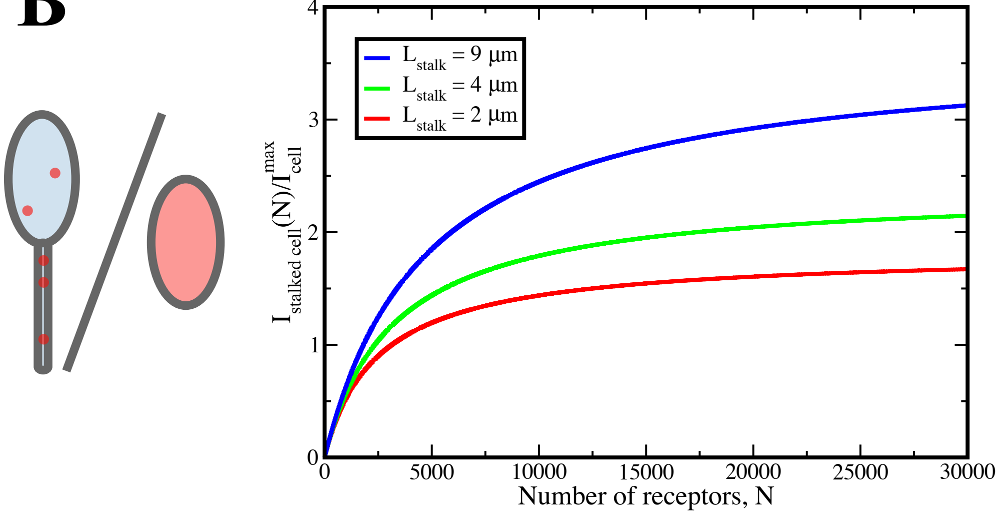
Observations:

- At given total length, stalked and elongated cell do comparably.
- Smaller cost in surface area and volume to generate stalk.

A



B



To make a stalk or not...

- Maximum rate of diffusive uptake by an object (cell, stalk) is proportional to the linear dimension, and NOT the surface area.
- The rate of uptake saturates with increasing number of discrete absorbers.

''From a microscopic viewpoint, both sets of results reflect the fact that a diffusing particle that finds itself in a given region of space is destined, by that very circumstance, to wander around that region for a time, probing it thoroughly before wandering away for good.''

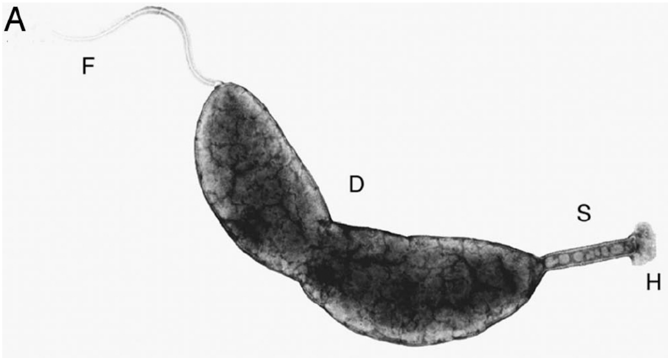
- From H. C. Berg, Random Walks in Biology

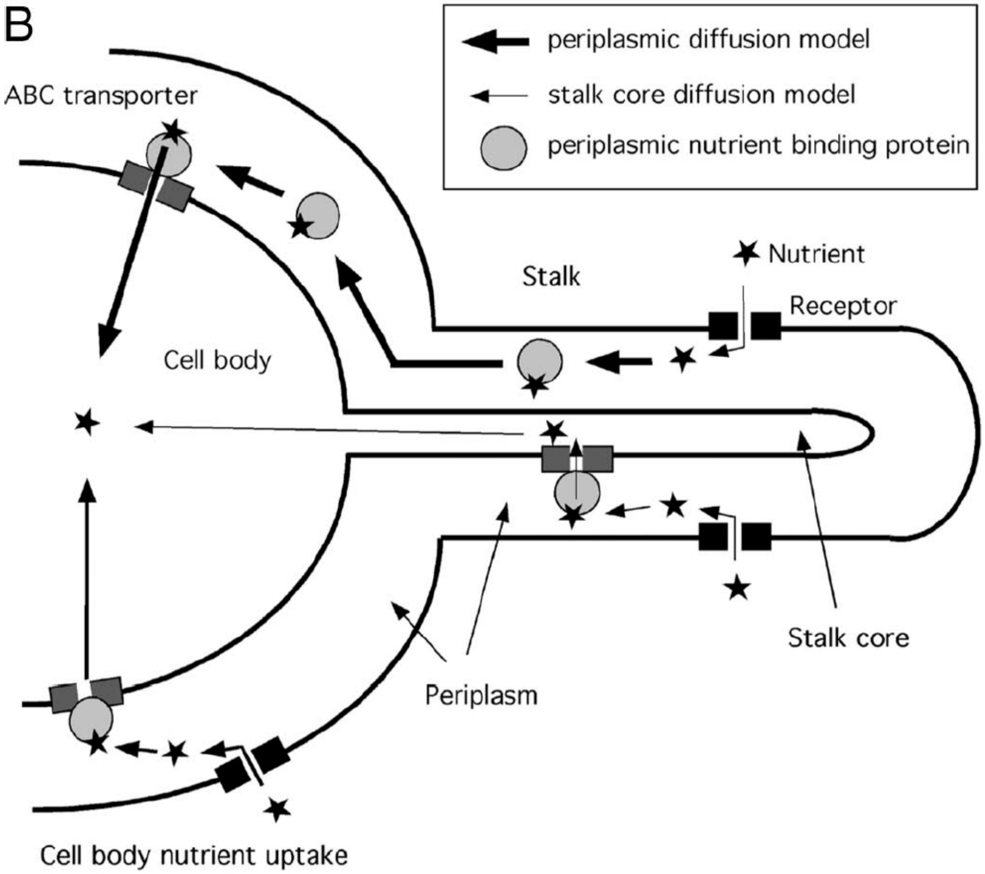
Proteomic and biochemical analysis

Stalk has

- Very few cytoplasmic proteins
- Outer membrane porins
- No high affinity inner membrane transporters in stalk
- Periplasmic metabolic enzymes
- Proteins involved in membrane structure
- Periplasmic binding proteins

Diffusion in periplasm?





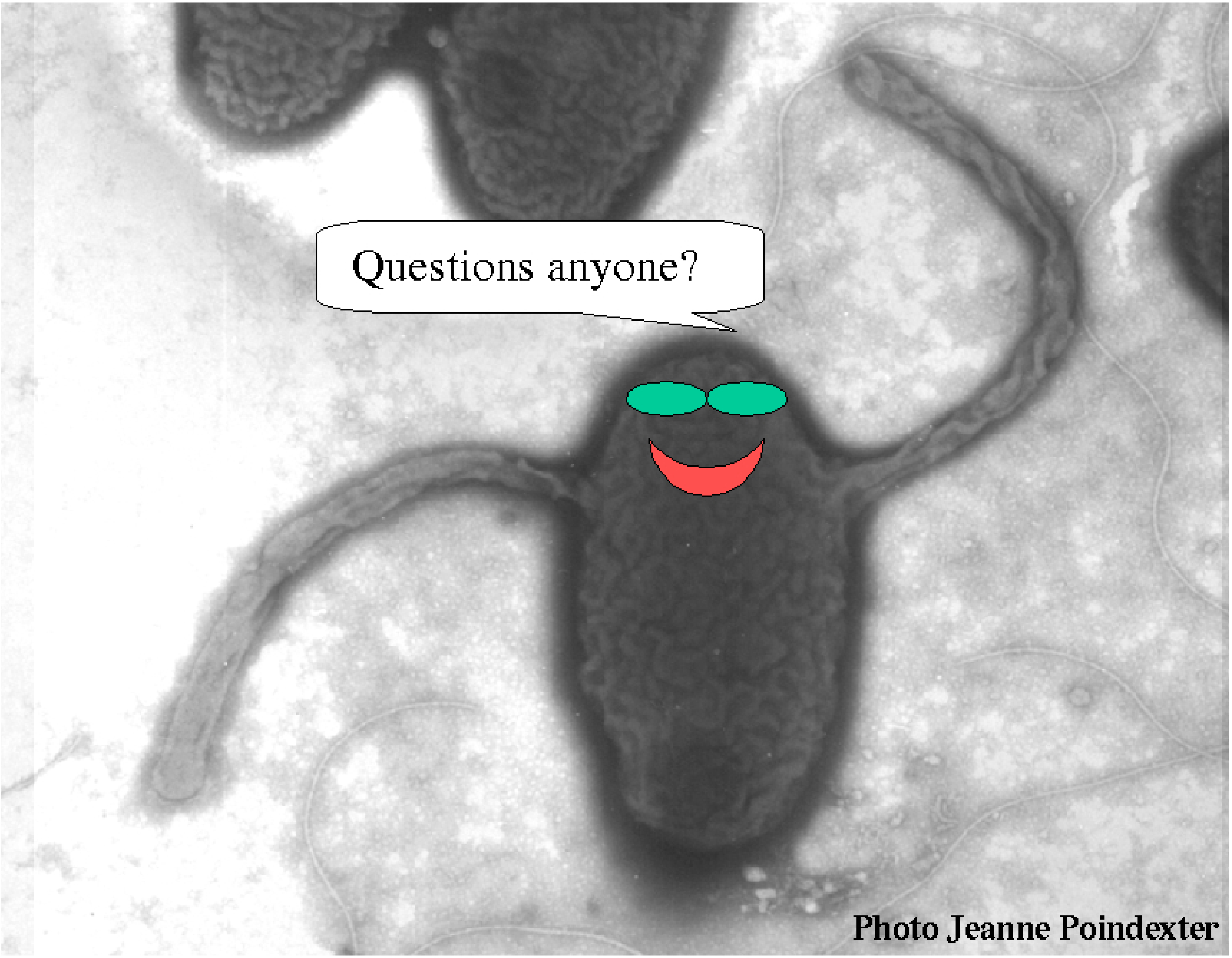
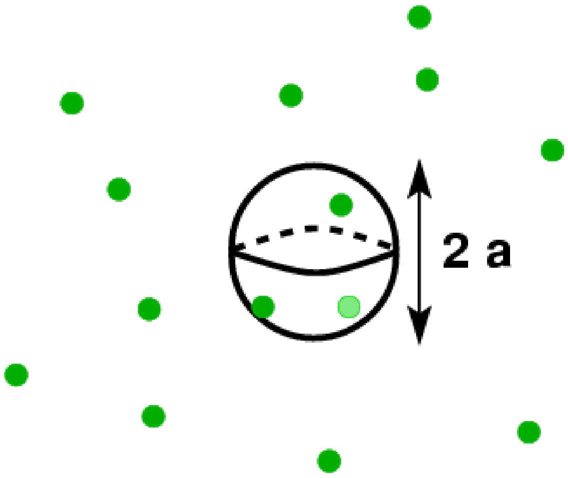


Photo Jeanne Poindexter

Measurement of Concentration by Surface Receptor(s)

Cell Surface = Single Receptor



- total inward current: $I_{sphere} = 4\pi D a \bar{c}$
- diffusion limited binding rate: I_{sphere}
- unbinding rate: τ_b^{-1}
- average occupancy: $\overline{n}$

- Detailed balance: $\overline{n}/\tau_b = (1 - \overline{n})\, I_{sphere}$
- Correlation time: $\tau_c^{-1} = \tau_b{}^{-1} + I_{sphere} = I_{sphere}/\,\overline{n}$
- Number of independent binding events in time τ: $N_M = \tau/\tau_c$,

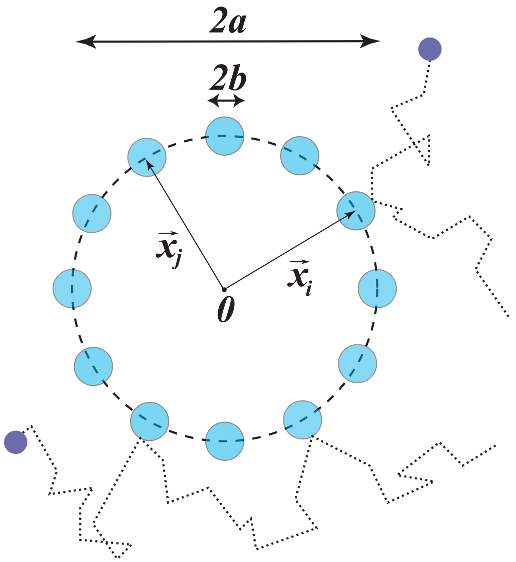
$$\sigma_{\overline{n}}^2 = \frac{\overline{n}\,(1-\overline{n})}{N_M}, \qquad \sigma_{\overline{c}}^2 = \left(\frac{\partial c}{\partial n}\right)^2 \sigma_{\overline{n}}^2$$

$$\boxed{\frac{\delta c_{rms}}{\overline{c}} \sim \frac{1}{\sqrt{\tau(1-\overline{n})I_{sphere}}} = \frac{1}{2}\frac{1}{\sqrt{\pi D a \bar{c} \tau\,(1-\overline{n})}}}$$

Accuracy limit set by number of ''new'' particles that occupy receptor in time τ

Extension to Multiple Non-Interacting Receptors

Receptor Cluster



- Receptors at $\vec{x}_i$, $i = 1, \ldots, N_r$
- Take $\overline{n}_i = \overline{n}$; $\delta n = \sum_i \delta n_i / N_r$
- Assume distribution such that:

$$\sum_{i=1}^{N_r} \sum_{j \neq i} \delta n_j \frac{1}{|\vec{x}_j - \vec{x}_i|} = \phi(N_r) \cdot \sum_{i=1}^{N_r} \delta n_i$$

e.g., regularly distributed receptors,

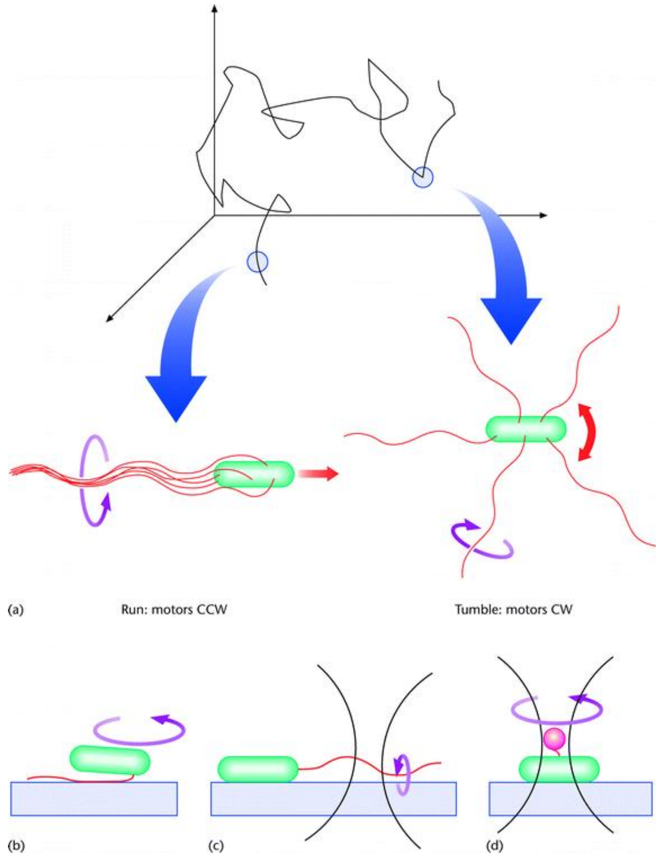
$$\phi(N_r) \approx N_r \, g_0 / a, \qquad g_0 \sim \mathcal{O}(1)$$

(can be numerically verified for ring, hemispherical geometries, with regularly and uniformly randomly distributed receptors)

$$\frac{\delta c_{rms}}{\overline{c}} > \frac{1}{\sqrt{\pi D \overline{c} \tau}} \left(\frac{1}{N_r b} + \frac{g_0}{2a} \right)^{1/2}$$

Correlations in receptors occupancies taken into account automatically.

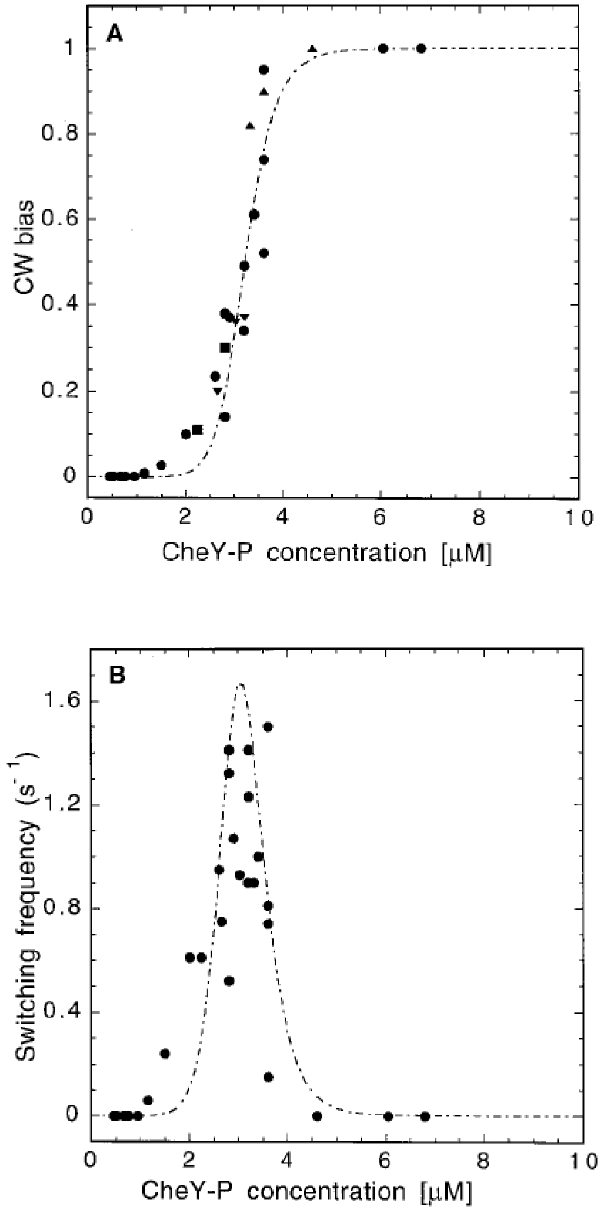
E. coli **Flagellar Motor**



From R. M. Berry, *Encyclopedia of Life Science* (2001).

$$b = \frac{[CheY - P]^h}{K^h + [CheY - P]^h}$$

$h \sim 10, \qquad K \sim 3\,\mu$M, $\qquad \overline{f} \sim 1.5$ Hz



From P. Cluzel, *et al.*, *Science* (2000).

Putting in Numbers ...

- **Motor switching between CW / CCW as random telegraph process:**

$$\delta b_{rms} \;=\; \sqrt{b\,(1-b)}\,\left(\frac{\tau_0}{\tau}\right)^{1/2}, \qquad \delta c_{rms} = \left(\frac{\partial b}{\partial c}\right)^{-1} \delta b_{rms}$$

$$\tau_0 \;:\; \text{correlation time} \;=\; 2b\,(1-b)/f$$

  - Motor states as read-out of [CheY-P], with $c = c_{1/2} = 3\ \mu$M, $b = 1/2$:

$$\delta c_{rms}/\bar{c} = \frac{2}{h}\left(\frac{1}{f\tau}\right)^{1/2}$$

  - For, $f \sim 1.5$ sec^{-1} and $h \sim 10$, motor provides readout of [CheY-P] accurate to $\sim 10\,\%$ within 2 sec.

- **Motor as receptor cluster**

$$a \sim 45\,\text{nm}, \qquad b \sim 1\,\text{nm}, \qquad m \sim 34$$

yields same accuracy, to within factor of 3.

Kinetics of R/T Switching

- Switching rates:

$$k_f(n) = k_f(0)\,\mu^n, \qquad \textit{and} \qquad k_b(n) = k_b(0)\,\nu^n,$$

where $p_T(n)\,k_f(n) = p_R(n)\,k_b(n)$, giving

$$\mu/K_R = \nu/K_T.$$

- Substrate binding/unbinding faster than rates of switching

$$\overline{k}_{f,b} = \frac{\sum_{n=0}^{N_r} k_{f,b}(n)\,p_{R,T}(n)}{\sum_{n=0}^{N_r} p_{R,T}(n)} = k_{f,b}(0)\left(\frac{1+\mu c/K_R}{1+c/K_{R,T}}\right)^{N_r}.$$

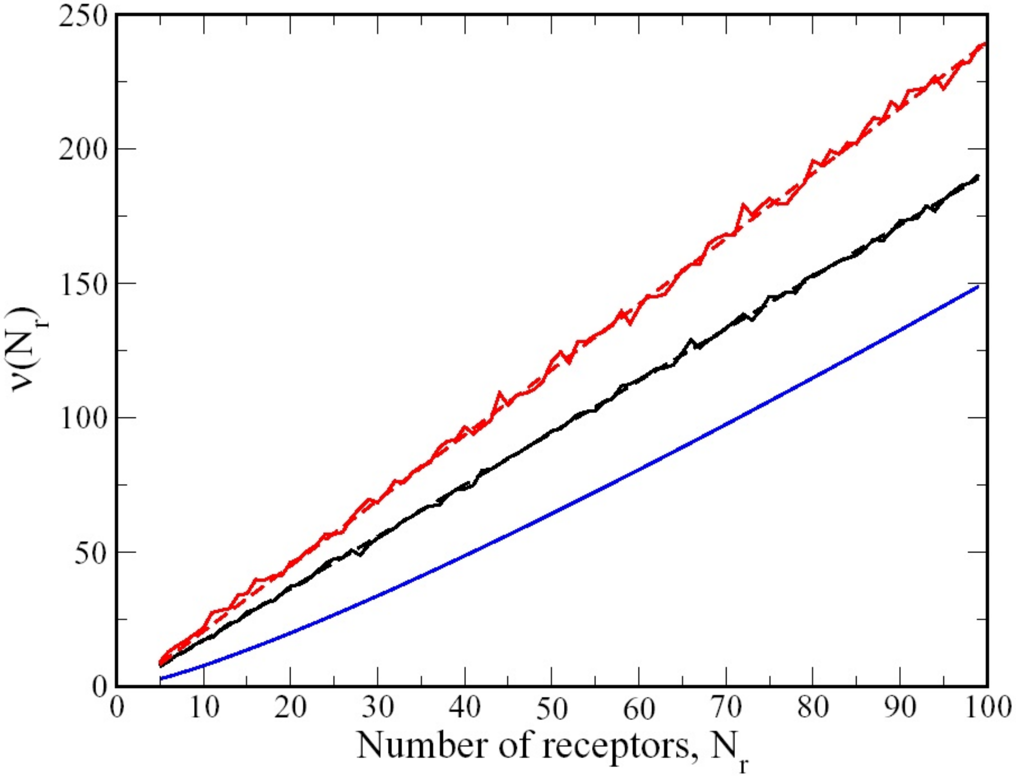
- Equilibrium constant for switching

$$K_{eq} = \frac{p_T}{p_R} = \frac{k_f}{k_b} = e^{-\beta(F_T - F_R)},$$

$F_{R,T}$: free energy of switch in R/T state

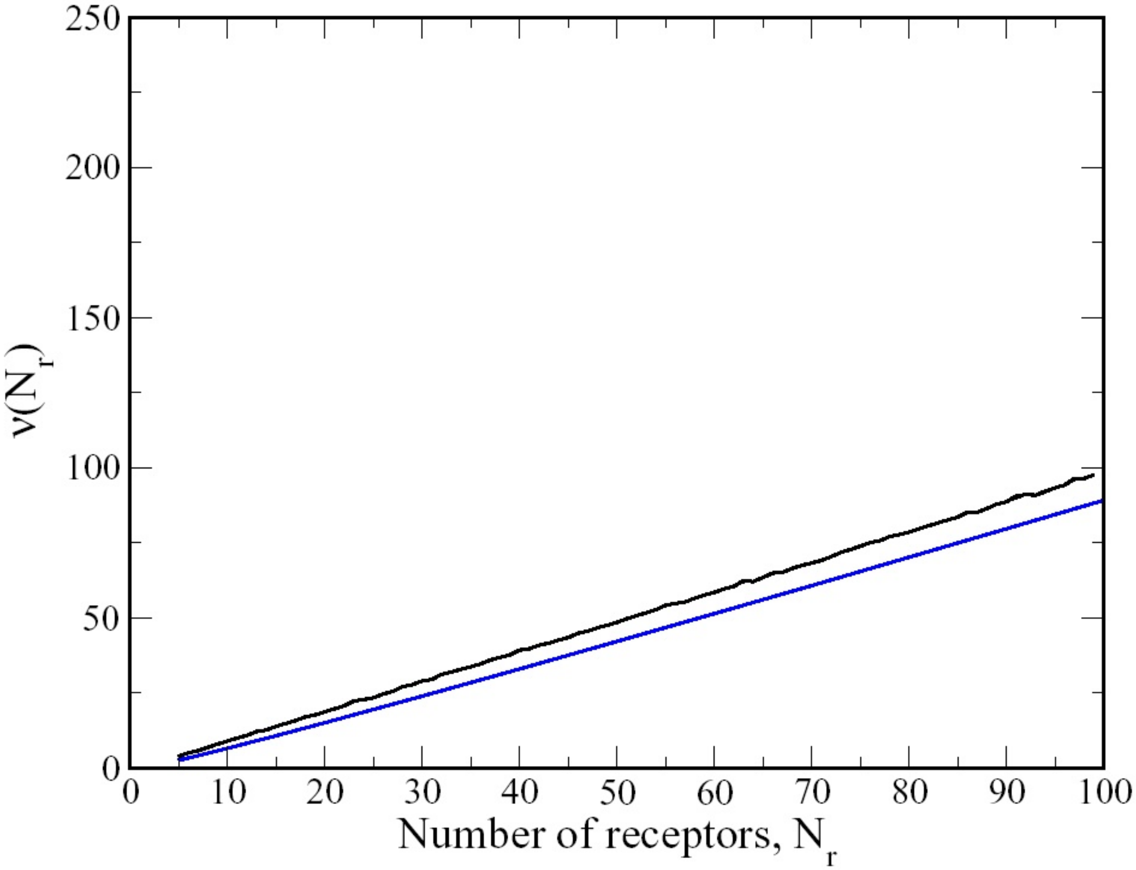
$$F_{R,T} = -k_B T \ln Z_{R,T} = F_{R,T}(0) - k_B T N_r \ln\left(1 + c/K_{R,T}\right),$$

Circular Cluster



Circular cluster of receptors with ring radius $a = 1$: The solid lines give the numerically evaluated sum, for uniformly randomly distributed receptors of radius $b = 0.005$ (black) and $b = 0.001$ (red), and regularly distributed receptors (blue). For randomly distributed receptors, the average of $\nu(N_r)$ is shown over 1000 realizations. The dashed lines show linear fits to the numerically evaluated sums, with slope given by $g_0 = 1.931 \pm 0.004$ (for $b = 0.005$) and $g_0 = 2.433 \pm 0.002$ (for $b = 0.001$). The theoretically predicted values of these slopes are 1.907 and 2.419, respectively. The linear fit to the sum for regularly distributed receptors yields $g_0 = 1.244$.

Spherical Cluster



Spherical cluster of receptors with radius $a = 1$: The solid lines give the numerically evaluated sum, for uniformly randomly distributed receptors of radius $b = 0.005$ (black) and regularly distributed receptors (blue). For randomly distributed receptors, the average of $\nu(N_r)$ is shown over 1000 realizations. The algorithm for generating uniformly distributed receptors is based on minimization of the electrical potential due to N equal charges. The dashed line shows the linear fit to the numerically evaluated sum, with slope given by $g_0 = 0.996 \pm 0.001$. The theoretically predicted value is 0.995.

Toward an Organizing Principle

We have seen several examples of optimal performance in the accuracy of measuring concentrations of signaling molecules, consistent with diffusive counting noise. . .

. . . Is optimality a general principle?

Other examples of performance close to limits:

- **photon counting in vision**
- **echo timing in bats**
- **identifying pitch of complex sounds**

Framework:

- **Theory: optimality → predictions/constraints for how unknown/partially known mechanisms might work**
- **→ New experiments**