Physical Limits to Biochemical Signaling

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Biochemical Signaling

Single molecular events can have macroscopic consequences.

- mutations in single DNA molecule
- photon absorption by single rhodopsin molecule
- olfaction triggered by single odorant molecule

More generally, cell’s functions are catalyzed by enzymes often present in small copy numbers.

How reliably can biochemical reactions be carried out in the presence of inherent fluctuations in numbers of crucial molecules?
This Work

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

Motivated by:


- 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

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Chemotaxis in *E. coli*

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


**Biased random walk of runs punctuated by tumbles:**

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

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

- Extracellular signaling: cell measures external attractant/repellent concentration
- Intracellular signaling: motor measures [CheY-P]
Berg-Purcell Results Revisited
Hypothetical “Perfect” Device

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

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

- Number of independent measurements in time \( \tau \):
  \[ N_M \sim \tau / \tau_D, \quad \delta N_M \sim \sqrt{\frac{\bar{N}}{N_M}} \]

\[ \delta \bar{c} / \bar{c} \sim 1 / \sqrt{\bar{c}aD\tau} \]
Questions

• How does this argument based on counting molecules in a volume work for cells and receptors that count molecules on their surface?

• How general is this argument? Does it apply equally well to a single receptor molecule and to an entire cell?

• 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?

• What about the details of the biochemical kinetics that govern the interaction of ligand with its receptor?
Measurement of Concentration by Surface Receptor(s)

Cell Surface = Single Receptor

- total inward current: \( I_{sphere} = 4\pi Da\bar{c} \)
- diffusion limited binding rate: \( I_{sphere} \)
- unbinding rate: \( \tau_b^{-1} \)
- average occupancy: \( \bar{n} \)

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

\[
\sigma_n^2 = \frac{\bar{n}(1 - \bar{n})}{N_M}, \quad \sigma_c^2 = \left( \frac{\partial c}{\partial n} \right)^2 \sigma_n^2
\]

\[
\frac{\delta c_{rms}}{\bar{c}} \sim \frac{1}{\sqrt{\tau(1 - \bar{n})I_{sphere}}} = \frac{1}{2} \frac{1}{\sqrt{\pi Da\bar{c}\tau (1 - \bar{n})}}
\]

Accuracy limit set by number of "new" particles that occupy receptor in time \( \tau \)
Multiple Receptors

For $N_r$, discrete receptors of size $b$,

- By analogy with electrostatics:

$$I/I_{sphere} = \frac{N_r b}{(N_r b + \pi a)}$$

- Also from:

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

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

Beat down diffusion noise by

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

March 16, 2006 Meeting of the American Physical Society 10
Statistical Mechanics Treatment

Single Receptor

Receptor binding and substrate diffusion:

\[
\frac{dn(t)}{dt} = k_+ c(\vec{x}_0, t) [1 - n(t)] - k_- n(t)
\]

\[
\frac{\partial c(\vec{x}, t)}{\partial t} = D \nabla^2 c(\vec{x}, t) - \delta(\vec{x} - \vec{x}_0) \frac{dn(t)}{dt}
\]

Detailed balance:

\[
\frac{k_+ c}{k_-} = \exp \left( \frac{\Delta F}{k_B T} \right)
\]

where \( \Delta F \) is the change in free energy associated with binding.

Fluctuations:

- Thermal fluctuations leading to rate fluctuations:

\[
\frac{\delta k_+}{k_+} - \frac{\delta k_-}{k_-} = \frac{\delta F}{k_B T}
\]

- Fluctuations in concentration, \( \delta c \)
Linear Response

Linear response of receptor occupancy to conjugate ‘force’:

\[
\frac{d \delta n}{dt} = -(k_+ c + k_-) \delta n + k_-(1 - \bar{n}) [\delta c + c \beta \delta F]
\]

\[
\frac{d \delta c}{dt} = D \nabla^2 \delta c - \delta(x - \bar{x}_0) \frac{d \delta n}{dt}
\]

Generalized susceptibility:

\[
\tilde{\alpha}(\omega) = \frac{\delta \tilde{n}(\omega)}{\delta \tilde{F}(\omega)} = \frac{k_+ c(1 - \bar{n})}{k_B T} \frac{1}{-i\omega[1 + \Sigma(\omega)] + (k_+ c + k_-)}
\]

\[
\Sigma(\omega) = k_+(1 - \bar{n}) \int_0^\Lambda \frac{d^3 k}{(2\pi)^3} \frac{1}{-i\omega + Dk^2}, \quad \Lambda \sim \pi/a
\]

Fluctuation-dissipation theorem:

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

Effective spectral density of noise in measuring \(c\), in terms of ‘noise force’ spectrum:

\[
S_c^{\text{eff}}(\omega) = \left( \frac{\bar{c}}{k_B T} \right)^2 S_F(\omega)
\]
Connection with Berg-Purcell Result

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

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

Two contributions to concentration noise power spectrum:

$$S_c^{\text{eff}}(0) = \frac{2\bar{c}^2}{k_+\bar{c}(1 - \bar{n})} + \frac{\bar{c}}{\pi Da}$$

Lower bound set by diffusion only:

$$\frac{\delta c_{\text{rms}}}{\bar{c}} > \frac{1}{\sqrt{\pi Da\bar{c}\tau}}$$

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)
Extension to Multiple Non-Interacting Receptors

Receptor Cluster

- Receptors at $\vec{x}_i$, $i = 1, \ldots, N_r$
- Take $n_i = \bar{n}$; $\delta n = \sum_i \delta n_i / N_r$
- 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,

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

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

Correlations in receptors occupancies taken into account automatically.
Cooperatively Interacting Receptors

W. Bialek and S. Setayeshgar, physics/0601001.
Cooperativity

Monod-Wyman-Changeux model of bacterial flagellar motor:

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

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

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

- 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

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

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

$\begin{align*}
N_r &= 34 \text{ Fli}M \text{ sites} \\
R &= \text{CCW state} \\
T &= \text{CW state}
\end{align*}$
Ligand binding to $i^{th}$ site:

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

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

Dynamics of receptor cluster as two-state system:

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

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

Diffusion, coupled to dynamics of switching:

$$\frac{\partial c}{\partial t} = D \nabla^2 c - \dot{p}_T \sum_{j=1}^{N_r} \frac{n_j^{T}}{\bar{n}_j} \delta (|\vec{x} - \vec{x}_j| - b)/4\pi b^2 - \dot{p}_R \sum_{j=1}^{N_r} \frac{n_j^{R}}{\bar{n}_j} \delta (|\vec{x} - \vec{x}_j| - b)/4\pi b^2.$$
Measurement Accuracy for Cooperative Receptor Cluster

Following same steps through FDT …

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

\[
\frac{(\delta c)^2}{\bar{c}^2} \approx \frac{2}{N_r^2 (\bar{n}_T - \bar{n}_R)^2 \bar{k}_f (1 - \bar{p}_T) \tau} + \frac{1}{2\pi D \bar{c} \tau} \left( \frac{1}{N_r b} + \frac{g_0}{a} \right)
\]

- 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 \(\bar{c}\) constrained by diffusive lower bound !!
Some Physical Examples

Internal and external signaling in *E. coli*: Comparison with recent experiments on

- Flagellar motor
- Transcription
E. coli Flagellar Motor

CW bias, $p_T(c)$

Switching frequency, $f(c)$


C-ring


$N_r = 34$ FliM sites

$\alpha \sim 22.5$ nm
Optimal Switching?

\[ \frac{\delta c^2}{c^2} \approx \frac{4}{N_r^2 (n_T - n_R)^2 f(c) \tau} + \frac{1}{2\pi Dc\tau} \left( \frac{1}{N_r b} + \frac{g_0}{\alpha} \right) \]

With \( \tau = 1 \text{s}, \ b = 1 \text{nm} \)

- **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) ...
Proteins are transcribed as needed depending on cell cycle, external cues.

RNA polymerase transcribes $\sim 3000$ genes in \textit{E. coli}.

Transcription factors activate/inhibit and confer specificity to RNAP.

transcription $\leftrightarrow$ read-out of promoter site occupancy
Noise in Gene Expression
from Elowitz et al., Science (2002)

LacI repressible:
Cyan allele of GFP
Yellow allele of GFP

\[ \eta_{\text{int}}^2 = \langle (c - y)^2 \rangle / 2 \langle c \rangle \langle y \rangle \]

A, B: low/high intrinsic noise

Red: Intrinsic noise, \( \eta_{\text{int}} \)
Fluorescence intensity varied with addition of LacI deactivating agent to cell culture.

Putting in Numbers ...

Transcription as readout mechanism for promotor site occupancy:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_{TF}$</td>
<td>100</td>
</tr>
<tr>
<td>$a$</td>
<td>3 nm</td>
</tr>
<tr>
<td>$D$</td>
<td>3 $\mu$m$^2$/s</td>
</tr>
</tbody>
</table>

- For $\delta c_{rms}/\bar{c} > 1/\sqrt{\pi \bar{c} a D \tau}$ $\sim$ 10 % require: $\tau \sim$ 1 min

- Integration time provided by $\tau_{1/2}$ of mRNA ($\sim$ minutes)?
Concluding Remarks

Emerging theme, which can be made increasingly quantitative:
For its crucial tasks performance of the cell really does approach limits set by physical laws.

This work:

- Physical limit to biochemical signaling arising from diffusive counting noise, first suggested by Berg and Purcell, is surprisingly general.

- Chemical processing of the measurement of concentration of a signaling molecule adds to this noise floor; cooperativity can serve to suppress this contribution, allowing signaling systems to approach this limit.

- Details of interactions of multiple site with one another or with some further internal states of the receptor cluster do not change this overall picture.

(a) W. Bialek, SS, ’’Physical Limits to Biochemical Signaling,’’ PNAS 102, 10040-10045 (2005).

(b) W. Bialek, SS, ’’Cooperativity, Sensitivity and Noise in Biochemical Signaling,’’ physics/0601001.
Fluctuation-Dissipation Theorem

Linear response of receptor occupancy to conjugate "force":

\[
\frac{d \delta n}{dt} = -(k_+ c + k_-) \delta n + k_+ (1 - n) \frac{\delta c + \bar{c} \beta \delta F}{\delta \mathcal{F} \cdot \bar{c} \beta}
\]

Brownian Motion:

\[
M \ddot{\delta X} + \gamma \dot{\delta X} + k \delta X = \delta \mathcal{F}(t)
\]

- **Fluctuation-dissipation theorem**
  \[
  \langle \delta \mathcal{F}(t) \delta \mathcal{F}(t') \rangle = 2k_B T \gamma \delta(t - t')
  \]

- **More generally**
  \[
  \langle \delta X(t) \rangle = \int dt' \alpha(t - t') \delta \mathcal{F}(t')
  \]
  \[
  S_X(\omega) = \frac{2k_B T}{\omega} \text{Im} \left[ \tilde{\alpha}(\omega) \right]
  \]
  \[
  S_F(\omega) = -\frac{2k_B T}{\omega} \text{Im} \left[ \frac{1}{\tilde{\alpha}(\omega)} \right]
  \]
**E. coli Flagellar Motor**


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

\( h \sim 10, \quad K \sim 3 \mu\text{M}, \quad \bar{f} \sim 1.5 \text{ Hz} \)

Motor switching between CW / CCW as random telegraph process:

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

\[\tau_0 : \text{correlation time} = 2b(1-b)/f\]

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

Motor as receptor cluster

\[
a \sim 45 \ \text{nm}, \quad b \sim 1 \ \text{nm}, \quad m \sim 34
\]

yields same accuracy, to within factor of 3.
Kinetics of $R/T$ Switching

- Switching rates:
  
  \[ k_f(n) = k_f(0) \mu^n, \quad \text{and} \quad k_b(n) = k_b(0) \nu^n, \]

  where \( p_T(n) k_f(n) = p_R(n) k_b(n) \), giving
  
  \[ \frac{\mu}{K_R} = \frac{\nu}{K_T}. \]

- Substrate binding/unbinding faster than rates of switching
  
  \[ \bar{k}_{f,b} = \frac{\sum_{n=0}^{N_R} k_{f,b}(n) p_{R,T}(n)}{\sum_{n=0}^{N_R} p_{R,T}(n)} = k_{f,b}(0) \left( \frac{1 + \mu c/K_R}{1 + c/K_{R,T}} \right)^{N_R}. \]

- 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
  
  \[ F_{R,T} = -k_B T \ln Z_{R,T} = F_{R,T}(0) - k_B T N_r \ln (1 + c/K_{R,T}) , \]
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 $g_0 = 1.244$. 

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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.