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 accuracy limit for the measurement of concentration by biological sensors, which in turn control downstream biochemical mechanisms?

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 intra- as well as extracellular signaling
- Compare with recent experiments
Chemotaxis in \textit{E. coli}

from Berg, Physics Today (2000)

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)
  \( v \sim 20 \mu \text{m/s} \)

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:
  \[ N = \bar{N} \pm \delta N, \quad \bar{N} \sim \bar{c}a^3, \quad \delta N \sim \sqrt{\bar{N}} \]

• Diffusion time: \( \tau_D \sim a^2/D \)

• Number of independent measurements:
  \[ N_M \sim \tau/\tau_D, \quad \delta N \sim \sqrt{\bar{N}/N_M} \]

\[ \frac{\delta c}{\bar{c}} \sim \frac{1}{\sqrt{\bar{c}aD\tau}} \]
"Absorption" by Surface Receptor(s)

Cell Surface = Single Receptor

- total inward current: \( J_{sphere} = 4\pi Da\bar{c} \)
- diffusion limited binding rate: \( J_{sphere} \)
- unbinding rate: \( \tau_b^{-1} \)
- average occupancy: \( \bar{n} = \bar{c}/(\bar{c} + c_{1/2}) \)

- Detailed balance: \( \bar{n}/\tau_b = (1 - \bar{n}) J_{sphere} \)
- Correlation time: \( \tau_c^{-1} = \tau_b^{-1} + J_{sphere} \)
- Binomial statistics for indep. binding events in time, \( \tau \):
  \[
  \frac{\delta c_{rms}}{\bar{c}} \sim \frac{1}{\sqrt{\tau (1 - \bar{n}) J_{sphere}}} = \frac{1}{2} \frac{1}{\sqrt{\pi Da\bar{c}\tau (1 - \bar{n})}}
  \]

Accuracy limit set by number of "new" particles that occupy receptor in time \( \tau \)
"Absorption" by Surface Receptors, (cont’d)

Multiple Receptors

- For \( m \) discrete receptors, by analogy with electrostatics:

\[
\frac{J}{J_{\text{sphere}}} = \frac{mb}{(mb + \pi a)}
\]

- Also from:

\[
1 - P_{\text{esc}} = \frac{mb}{(mb + 4a)}
\]

\( P_{\text{esc}} \): probability that substrate molecule at \( r = a + b \) survives all subsequent contacts.

Beat down diffusion noise by

\[
\frac{\delta c_{\text{rms}}}{c} \sim \frac{1}{2} \frac{1}{\sqrt{\pi Dc\tau (1 - n)}} \left( \frac{\pi}{mb} + \frac{1}{a} \right)^{1/2}
\]
Statistical Mechanics
Treatment
Single Receptor

Receptor binding and substrate diffusion:

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

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

Free energy \( F \) associated with binding, from detailed balance:

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

Fluctuations:

- Thermal fluctuations leading to rate fluctuations:

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

- Fluctuations in concentration, \( \delta c \)
Fluctuation-Dissipation Theorem

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

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

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:
  \[\langle \delta X(t) \rangle = \int dt' \alpha(t - t') \delta \mathcal{F}(t')\]
  \[S_X(\omega) = \frac{2k_B T}{\omega} \text{Im} [\tilde{\alpha}(\omega)]\]
  \[S_\mathcal{F}(\omega) = - \frac{2k_B T}{\omega} \text{Im} \left[ \frac{1}{\tilde{\alpha}(\omega)} \right]\]
Linear Response

Generalized susceptibility:

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

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

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

\[ S_{\tilde{F}}(\omega) = -\frac{2k_B T}{\omega} \text{Im} \left[ \frac{\delta \tilde{F}(\omega)}{\delta \tilde{n}(\omega)} \right], \quad S_{c}^{\text{eff}}(\omega) = \left( \frac{\bar{c}}{k_B T} \right)^2 S_{\tilde{F}}(\omega) \]

Accuracy of a measurement which integrates for a time \( \tau \gg 1 \):

\[ \delta c_{\text{rms}} \approx \sqrt{S_{c}^{\text{eff}}(0) \cdot \frac{1}{\tau}} \]

''Self-energy'' \( \Sigma(\omega) \):

\[ \Sigma(\omega \ll D/a^2) \approx \Sigma(0) = \frac{k_+(1 - \bar{n})}{2\pi D a} \]
Connection with Berg-Purcell Result

Two contributions to concentration noise power spectrum:

\[ S_{c_{\text{eff}}} = \frac{2\bar{c}^2}{k_+\bar{c}(1 - \bar{n})} + \frac{\bar{c}}{\pi Da} \]

Lower bound set by diffusion only:

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

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 Receptors

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

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

- Eg., uniformly distributed receptors,

$$\phi(m) = m g_0 / a, \quad g_0 \sim \mathcal{O}(1)$$

(Numerically verified for ring, hemispherical geometries, with regularly and uniformly randomly distributed receptors)

Correlations in receptors occupancies taken into account automatically.
Some Physical Examples

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

- Transcription
- Flagellar motor
• Proteins are transcribed as needed depending on cell cycle, external cues.

• RNA polymerase transcribes $\sim 3000$ genes in *E. coli*.

• Gene regulation: transcription factors activate/inhibit and confer specificity to RNAP.

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

LacI repressible:
Cyan allele of GFP
Yellow allele of GFP

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

A, B: low/high intrinsic noise

Red: Intrinsic noise, \( \eta_{\text{int}} \)
Fluorescence intensity varied with addition of LacI deactivating agent to cell culture.
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>$\alpha$</td>
<td>3 nm</td>
</tr>
<tr>
<td>$D$</td>
<td>3 $\mu$m$^2$/s</td>
</tr>
</tbody>
</table>

- For $\delta c_{rms}/\bar{c} > 1/\sqrt{\pi \bar{c} \alpha D \tau} \sim 10\%$ require: $\tau \sim 1$ min
- Integration time provided by $\tau_{1/2}$ of mRNA ($\sim$minutes)?
**E. coli Flagellar Motor**

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

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


Putting in Numbers ...

Motor switching between CW / CCW as random telegraph process:

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

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

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

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

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.
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:\(^{(a)}\)

- Statistical mechanics derivation of accuracy in measurement of concentration through binding of substrate to a receptor(s).
- Comparison with available experimental data.

Extensions:

- Cooperatively interacting receptors\(^{(b)}\).

(a) W. Bialek, SS, ”Physical Limits to Biochemical Signaling,” PNAS (to appear).
(b) W. Bialek, SS, ”Accuracy and Amplification in Biological Switches,” in preparation.