From molecules to behavior: Towards systems level understanding of bacterial chemotaxis

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(Work done with Dr. Bernardo Mello)

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Physics  <->  Biology

Mathematics
Current Roadblocks for Systems Biology

- Many missing elements in the network
- Many missing links in the network
- Most kinetic constants are unknown
- Not enough quantitative experiments

Exceptions: Particular behaviors in simpler systems. Such as chemotaxis in bacteria (E. coli) “Hydrogen atom” in biology

- Important example of signal transduction and sensory system in biology
- Best chance in quantitatively understanding a complex biological system
- General principles in understanding complex biological systems
  Adaptation; Signal Amplification; Robustness; Effect of Noise ….
E. coli anatomy

Receptors

Flagellar Motor

Nucleoid region

Ribosomes

Flagella ~ 10μm

2μm

Reproduce every ~20 minutes
Under normal condition
An important biological behavior: cell motility and chemotaxis
The Bacterial Chemotaxis Motion

(The sensory system of bacteria)

• Two modes of motion

 (1) **Run**: flagella rotate counter clockwise
 smooth swimming \( \sim 20\mu m/s \)
 (2) **Tumble**: flagella rotate clockwise
 tumbling (randomly change direction)

• Switch frequency set by comparing instantaneous
 attractant concentration and its **memory**: temporal sensing

Biased Random walk
The E. Coli Chemotaxis Signaling Pathway

Signal transduction
(sensing, adaptation, signal transport,....)  Switch  Motor

(Bacterial chemotaxis)

(Serial 120x66 to 426x726)

(Bacterial chemotaxis)

(Serial 120x66 to 426x726)
We have a lot of molecular level knowledge about the chemotaxis pathway

CheW

CheA

Tsr
Number of Key Molecules for Chemotaxis in a single E. Coli cell

Stoichiometry

Total number of Receptors: 15,000-26,000

Ts\(\text{r}\):Ta\(\text{r}\):Trg(Tap,Aer)\(~2:1:0.1\)

(5 types of chemoreceptor)

(Li and Hazelbauer, Journal of Bacteriology, 186(12), 3687 (2004))
What type of computation does the cell do?

Temporal comparison: \[ \sim C(t) - C(t - \Delta t) \]

Because the cell moves \[ \Delta x = v \Delta t \]

The cell effectively calculates the spatial gradient of C

➢ How does cell keep a memory of its past history?

➢ How does it carry out the calculation?
Receptor complex: the memory and computation device

Ligand (MeAsp)

Current time
(fast time scale)

Memory of past
(slow time scale)

CheR
CheB

Current time

Memory of past

A
W
Quantitative Characteristics of Chemotaxis Response

- High sensitivity (~10’s nM, a few ligand molecules)
- Signal amplification (~40X)
- High sensitivity exists in a wide range of backgrounds
- Wide dynamic range (100nM → 1mM)
- Near perfect adaptation
Receptor Clustering as the Mechanism for High Gain

Chemoreceptors cluster in bacteria (~20,000 chemo-receptors in a E. Coli cell)
(Maddock & Shapiro, 1993)
(Lybarger & Maddock)
• Clustering of MCP+CheA+CheW
• Independent of CheR or CheB

One Problem: High gain against wide dynamic range

Conceptual model → quantitative model → direct compare with data

Needs quantitative data!
The recent *in vivo* response measurements using FRET

Direct *in vivo* measurement of CheY$^P$ level by FRET
(Fluorescence Resonance Energy Transfer)
(Sourjik&Berg, PNAS 99 123-127 (2002))

Molecular level measurement while the cell is alive and behaving
The response data for wt and different mutants

WT in different background

Different Tar/Tsr expression levels

Different methylation levels

......... (from H. Berg Lab)
Modeling the receptor complex quantitatively

- 4 states for each individual receptor \( i \)

- Kinase activity \( a_i = 0,1 \)

- Ligand binding \( l_i = 0,1 \)

Energy (Hamiltonian) of the states:

\[
H_i = a_i(E_m(m_i) + E_L(m_i)l_i) + \mu_l(m_i)l_i + \text{coupling term}
\]

Probability in each of the 4 states: \( P(a_i, l_i) \propto \exp(-H_i(a_i, l_i)) \)

- 3 independent parameters for each individual methylation level

\[
K_a; K_i; E_v; E_o (= E_v - \ln \frac{K_a}{K_i})
\]
A Simple Model of Receptor Interaction

- Activity of a receptor affected by the activities of its neighbor in the receptor cluster. Cooperativity in a continuum lattice.

\[ \text{Interaction energy} = a_i \sum_j C_{q_i q_j} (a_j - \frac{1}{2}) \]

- \( j \) labels all the “neighboring” receptors of \( i \)’th receptor

\[ H = \sum_i a_i [E_m(m_i) + E_L(m_i)l_i + \sum_{j(i)} C_{q_i q_j} (a_j - \frac{1}{2})] + \mu_l(m_i)l_i \]

“Spin” \quad “local magnetic field” \quad “coupling to neighbors”

Analogous to the Ising model for magnetism in physics
The Results for the 6 CheRB- Mutant Strains

Adaptation disabled: Receptor methylation level fixed

- Solve our model by mean field theory, MC simulation
- Find parameters to fit to all 6 mutant strains together

\[
\begin{array}{cc}
\text{Tar} & \text{Tsr} \\
0 & 0 \\
1 & 1 \\
2 & 2 \\
3 & \color{red}3 \\
4 & \color{green}4 \\
\end{array}
\]

![Graph showing experimental (symbols) and theoretical (lines) data for the adaptation of the CheRB- mutant strains.

# of parameters in the model: \(3 \times 8 + 4 = 28\)

# of independent data points: \(\sim 6 \times 7 = 42\)

(Sourjik & Berg, PNAS 2002)
(Mello & Tu, PNAS, 2003)
The Model for the Wild-type Cell (with CheR & CheB)

• Receptor methylation level follows a distribution $f_{qml}$, determined by the methylation/demethylation kinetics.

$$\sum_{l}^{m+1} (1-a_{0ml}) f_{0ml} = k_{B} \sum_{l} a_{0(m+1)l} f_{0(m+1)l}$$

Assuming only active receptor can be demethylated; only inactive receptor can be methylated

(Barkai and Leibler, Nature, 1997)
(Mello & Tu, *Biophysical Journal*, 84(5), 2843-2856 (2003))

• Steady state distribution can be determined

Perfect adaptation

Increasing background $[L]_0$
Wild-type responses: Theory versus Experiments

- Consistent with experimental data over full range of ambient concentration
- Reveal mechanism for the wide dynamical range over which high sensitivity is sustained.
Sensitivity: Experiments versus Theory

\[ S \equiv \frac{\Delta A / A_0}{\Delta [L] / [L]_0} \]

agreement over full range of ambient MeAsp concentrations
The effect of near perfect adaptation: High gain over a wide range of backgrounds

Increasing receptor methylation level (higher background concentrations)

The “smart” Ising model: Self-tuned near-critical behavior

\[ H = \sum_i a_i [E_m(m_i) + E_L(m_i)l_i] + \sum_{j(i)} C_{q,q_j} (a_j - \frac{1}{2})] + \mu_i (m_i)l_i \]

- Receptor interaction results to high gain: PHYSICS
- Adaptation maintain the high gain: BIOLOGY
E. Coli surfing the adaptation wave

Increasing methylation level

Activity

$\alpha_0$

$\ln[L]$
Towards understanding behavior: the fun has just started

We learned something about:

I. Signal amplification  III. Effects of adaptation

---

I. Fast Response
II. The kinetics $a(t)$
III. Steady State
Responses to complex temporal signals

Simple step function stimulus is useful to understand the pathway. But, such simple stimuli is un-physiological.

- What kind of signal processor is bacterial chemotaxis pathway?
  Amplifier; filter; nonlinear effects; signal integration/differentiation
- Why is it designed the way it is?
  What is it good for?
Theoretical model is necessary

Controlled experiments

Simple Step stimuli

More complex stimuli

(Exponential ramps)

Model

Spatial Gradient

Realistic temporal stimulus profile for a chemotaxing cell

Molecular level knowledge about the signaling pathway
A simple integrated (systems level) model

Ligand binding (fast time scale)

methylation (slow time scale)

Kinase activity (fast time scale)

Input: $[L](t)$

Output: $a(t)$

Memory: $m(t)$

$G([L],m)$

$F(a)$
The kinetic model for adaptation

Receptor cooperativity

\[ a = G(m, [L]) = [1 + \exp(-\Delta E(m, [L]))]^{-1} \]

\[ \frac{dm}{dt} = F(a, m, [L]) \]

Perfect adaptation

\[ \Delta E = N[f_m(m) + f_L([L])] \]

\[ = N[\alpha(m - m_0) - \ln \frac{1+[L]/K_d}{1+C[L]/K_d}] \]

(Monod-Wyman-Changeaux allosteric model)

Increasing methylation levels

\[ F(a_0) = 0, F(0) > 0, F(1) < 0 \]

\[ -F(1) > F(0) \]

\[ F(a) \]

\[ F(0) \]

\[ F(1) \]

\[ 0 \]

\[ a_0 \]

\[ 1 \]

Increasing methylation levels
Some “forgotten” experiments

Experiments done in the 80’s by Howard Berg’s group

Adaptation Kinetics in Bacterial Chemotaxis

STEVEN M. BLOCK, JEFFREY E. SEGALL, AND HOWARD C. BERG*
Division of Biology, California Institute of Technology, Pasadena, California 91125
Received 18 October 1982/Accepted 21 January 1983

- Exponential ramp
- Exponentiated sine wave
- Steps and impulses
I. Response to exponential ramp: the constant activity shift

\[ \Delta E \sim N[\alpha m - \ln([L]/K_d)] + \text{const.} \]

\[ \ln[L] = rt \]

\[ \frac{dm}{dt} = \frac{r}{\alpha} \]

\[ \frac{dm}{dt} = F(a) \]

\[ F(a_s) = \frac{r}{\alpha} \]

• Determine the new steady state analytically
• Measuring activity in different ramp rate will give us F(a)!

Methylation tries to catch up with the exponentially changing external stimulus
But it lag behind it, which leads to the activity shift
The graphs illustrate exponential ramp up and exponential ramp down functions.

- **Exponential Ramp Up**
  - Formula: $[L] \sim \exp(rt)$
  - Graph showing $m(t)/4$ and $a(t)$ over time.

- **Exponential Ramp Down**
  - Formula: $[L] \sim \exp(-rt)$
  - Graph showing $m(t)/4$ and $a(t)$ over time.

The experiments were conducted by S. Block et al., 1983.
The dependence of the activity shift on ramp rate

Experiment

Theory

Inactivity (1-a)

Ramp rate (r)

(ε₁=0.05;ε₂=0.1;f₁=0.15)
II. Response to sine waves: the spectral analysis

--- **Input:** \([L](t) = [L]_0 e^{\beta \sin^2(\pi ft)}\)  
--- **Memory (Control):** methylation \(m(t)\)

--- **Output:** kinase activity \(a(t)\)

\[
\Delta a = G'(f_t^0)\alpha[\Delta a - \frac{A_L}{\alpha} \cos(2\pi ft)]
\]

\[
\frac{d\Delta m}{dt} = F''(a_0)\Delta a
\]

\[
A_a = \frac{ifc_a}{if + f_m} A_L, \quad A_m = \frac{f_m c_m}{if + f_m} A_L
\]

\[
f_m = -\frac{\alpha F''(a_0) N a_0 (1-a_0)}{2\pi}
\]

Low frequency: \(f << f_m\)  
\(A_a \sim ifA_L, \quad A_m \sim A_L\)

High frequency: \(f >> f_m\)  
\(A_a \sim A_L, \quad A_m \sim A_L / if\)
The phase and amplitude of the responses

Response to low frequency signal

Response to high frequency signal

Time delay
Phase shift

Amplitude
Frequency dependence of responses

Response to low frequency signal

Response to high frequency signal

Phase Shift

Amplitude Response

Theory

Exp.

“calculate” derivative

“slave” of input

(S. Block et al, 1983)
III. Response to noisy signal

![Graph showing responses to noisy signal](image-url)
The chemotaxis filter function

- Methylation level: Low pass filter
- Kinase activity: Calculate derivative in low frequency regime.
IV. Response to impulse signal: the Green’s function for chemotaxis response

The CheY-P dynamics:

\[
\frac{d[Y]}{dt} = k_a a - \frac{[Y]}{\tau_z}
\]

*source* \hspace{1cm} *sink*

\[A_Y = \frac{k_a}{f_Z + if} \quad A_a = k_a c_a \quad \frac{1}{f_Z + if} \times \frac{if}{f_m + if} \quad A_L\]

Provide high frequency cutoff
The chemotaxis Green’s function

\[ \Delta[Y](t) = \int_0^t R(t-t') \Delta f_L(t') dt' \]

\[ R(t \geq 0) = R_0 [\tau_m^{-1} \exp(-t/\tau_m) - \tau_Z^{-1} \exp(-t/\tau_Z)]; \quad R(t < 0) = 0 \]

\[ R(f) \propto \frac{f_Z}{f_Z + if} \times \frac{if}{f_m + if} \]

\( \tau_m \approx 3 \text{ sec} \)

\( \tau_Z \approx 0.5 \text{ sec} \)

(Segall et al. PNAS ’86)
V. Response to large steps: stimulus dependent memory time scales in nonlinear regime

I. Immediate Responses

II. Adaptation kinetics

III. Adapted states

\[ \tau \]
Additivity of adaptation time

- Spudich & Koshland, PNAS, 1975
- Berg & Tedesco, PNAS, 1975

Two successive steps \[ \tau = \tau_1 + \tau_2 \]

One big step

Stimulus

[\( [L]_1 \)]

[\( [L]_2 \)]

[\( [L]_1 + [L]_2 \)]

Activity

\( \tau_1 \)

\( \tau_2 \)

\( \tau \)
The mechanism for additivity in adaptation time

Activity is suppressed to a=0 by large jump in attractant,
There, the adaptation time is determined by the constant rate F(0)

\[ \frac{dm}{dt} = F(a) = F(0) \]

\[ \tau \approx \frac{m_0([L]_2) - m_0([L]_1)}{F(0)} \]
Recovery time: Model versus Exp.

\[ \tau_1 = 1.25, \tau_2 = 0.68 \]

\[ \tau = 1.87 \]

\[ \tau_1 + \tau_2 \approx \tau \]

(Spudich & Koshland, PNAS, 1975)
Transition (recovery) time independent of intermediate ligand concentration variations

(data from Berg&Tedesco, PNAS 1975)
(Model parameter from Mello&Tu, Bio. Journal 2007)

\[ \tau_{\text{max}} \approx 350 \text{ sec} \Rightarrow F(0) \approx 0.0075 \text{ sec}^{-1} \]
The chemotaxis pathway as a information processor

1) It amplifies the signal in a wide range of background receptor-receptor interaction in receptor cluster near perfect adaptation

2) It senses the concentration in log-scale: the Weber’s law
   - Responses depend on $\frac{\Delta[L]}{[L]} = \Delta(\ln[L])$ for a wide range of $[L]$. The Weber-Fechner Law in sensory system
   - Information compression: wide range of concentration, limited bandwidth: 5 methylation levels.
   - caused by the effect of receptor methylation level on its kinase activity: it shifts the response curve in $\ln[L]$ space.
3) It is a low pass filter for the derivative.
   Calculate derivative of the input in low frequency regime

- It is a high pass filter for the input itself
- More importantly, It is a low pass filter for the derivative of the input
- The phase information tells it all.
4) The recovery time depends on the stimulus strength
- A range of time scale (seconds to minutes)

- Integral memory

\[ m(t) = \int_{0}^{t} F(a(t)) \, dt \]

- It does not have a fixed memory time (3 sec.)
- It memorize all its history in an integrated way
Some Current Challenges

• Molecular level


2) What is the molecular basis for the detailed methylation/demethylation dynamics? How is perfect adaptation achieved?

(S. Subramaniam Lab)
3) Methylatio/demethylation kinetics and how they depend on (correlate with) kinase activity (J. Hazelbauer lab)

4) What’s the molecular origin of steepness near $a_0$? (H. Berg lab)

$F(a_s) = \frac{r}{\alpha}$

CheB phosphorylation in Competition with CheY?
• **Behavior Level:**

• **How does the system differentiate different signal?**
  
  How the cell distinguish between different signal?  
  How smart is the bacteria?

• **Can the system be “rewired” (changing “coupling”) due to learning (exposure to some stimulus)?**

• **Can the same pathway be used to perform other task?**
  
  e.g. Thermotaxis---going to a particular temperature

• **Why such a large gain? What about noise?**
  
  What about signal gain in response to real stimulus encountered in the wild,  
  e.g., as the bacterium (biased) random walking towards a nutrient source.
Develop more quantitative experiments and compare with theory.

What happen to a cell when it is moving in a spatial profile (gradient, traps, etc.)

Direct comparison with quantitative microfluidics experiments

(Done with M. Wu lab in Cornell)
Quantitative, Systems Level Modeling of Bacterial Chemotaxis

Sensing

Adaptation

Signal transport

Cell motion

Signal amplification

Motor switching kinetics

Systems Approach (Integration)

Predictive in-silico chemotaxing bacteria