From molecules to behavior: *E. coli*'s memory, computation and chemotaxis

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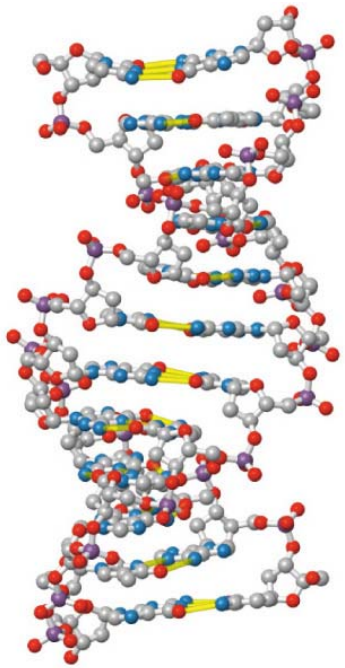
Howard Berg

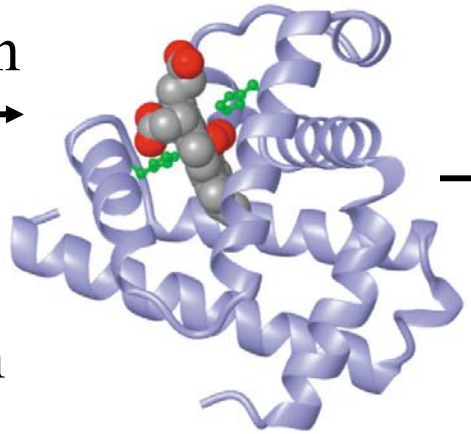
Physics ⟷ **Biology**

03/04/10 Toronto

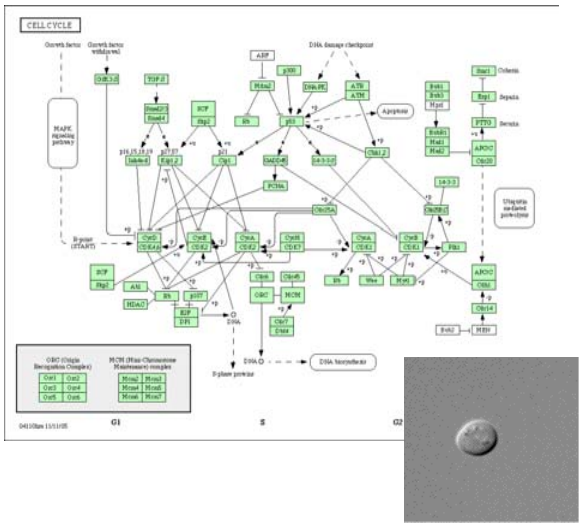
From building blocks of biological systems to understanding of biological behaviors



transcription
translation



regulation



DNA | **Proteins** | **Biological Network**

Genomic sequences — 3-D structures — Biological behaviors

Bioinformatics — **Protein Folding** — **Systems Biology**

How do bio-molecules interact to give rise to biological behaviors?

Biological systems are complex

- Many different types of molecules involved.
- Heterogeneous Interactions (temporally/spatially).
- Many missing elements (nodes)/links in the interaction network.
- Many kinetic constants are unknown.

We need "Hydrogen atom" in systems biology!

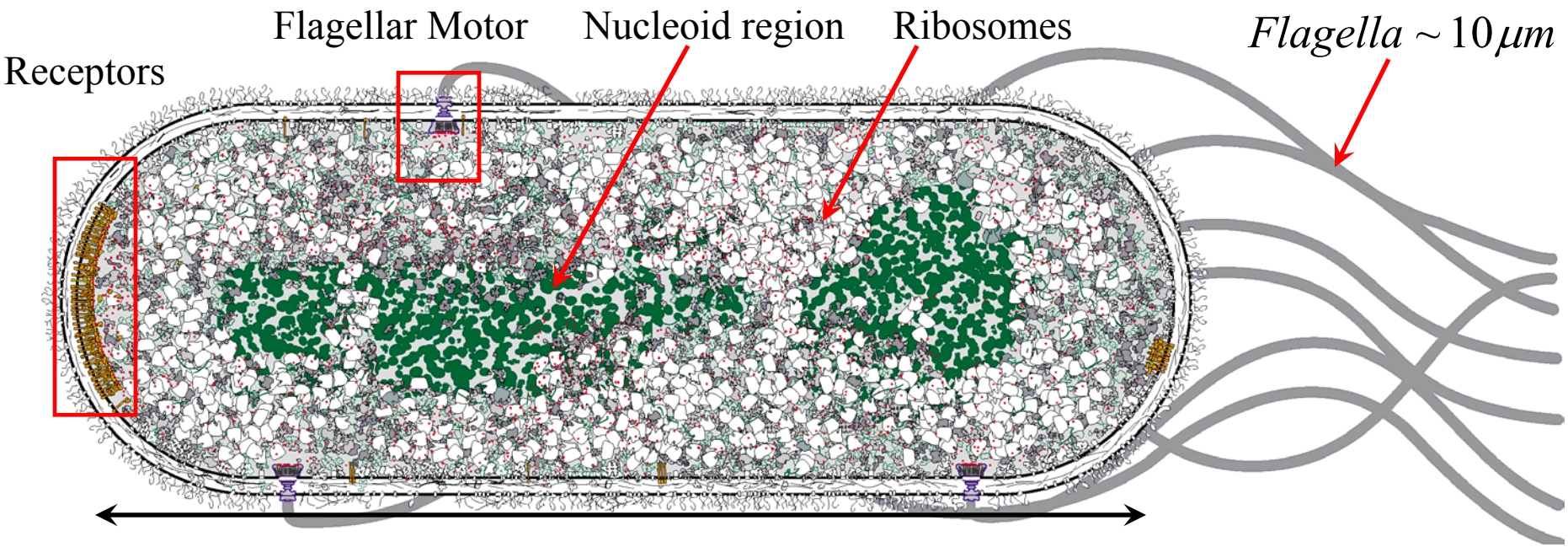
Chemotaxis in bacteria (E. coli)

General behavior in simple model systems

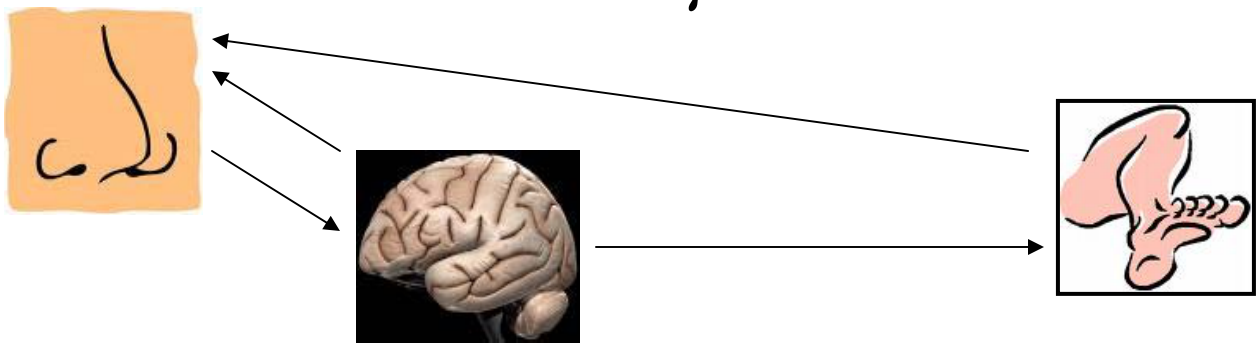
- **Important example of signal transduction and sensory system in biology**
- **Best chance in quantitatively understanding a complex biological system**
- **General principles in understanding complex biological systems**

Adaptation; Signal Processing; Robustness; Effect of Noise …..

E. coli anatomy and chemotaxis

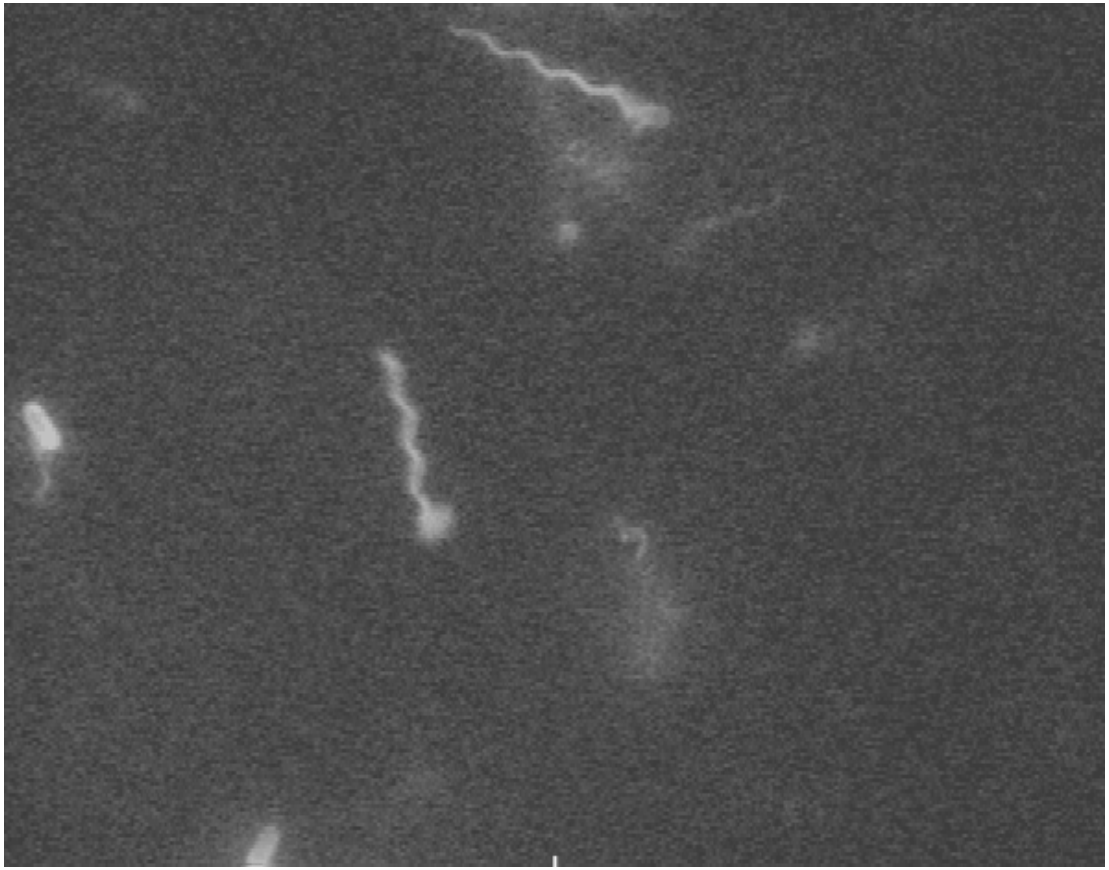


Reproduce every ~20 minutes
Under normal condition

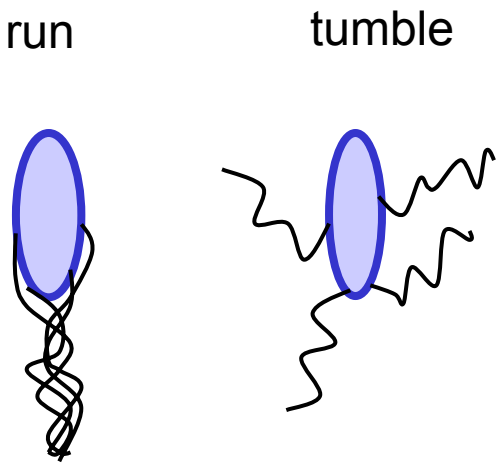


How cells 1) receive signal; 2) process signal and 3) react to signal

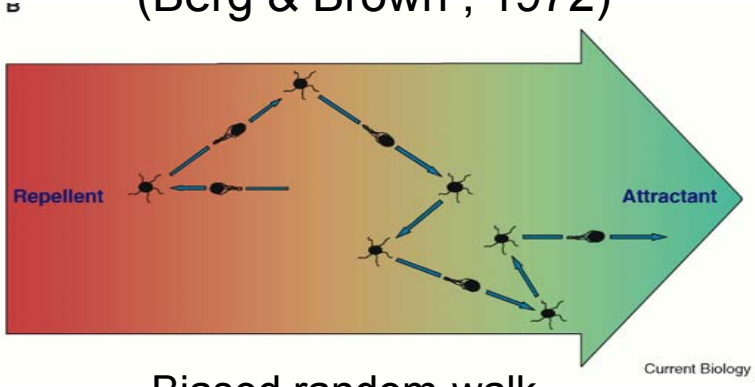
The biased random motion of *E. coli:* run & tumble



(From the Berg lab)



(Berg & Brown , 1972)



Biased random-walk

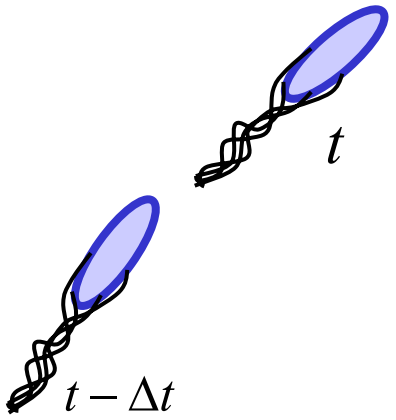
Switch between tumble and run by comparing current environment with some memory encoded internally

The cell as a molecular information processor

Temporal comparison $\sim C(t) - C(t - \Delta t)$ (Current: $C(t)$; Past: $C(t - \Delta t)$)

Because the cell moves $\Delta x = v\Delta t$

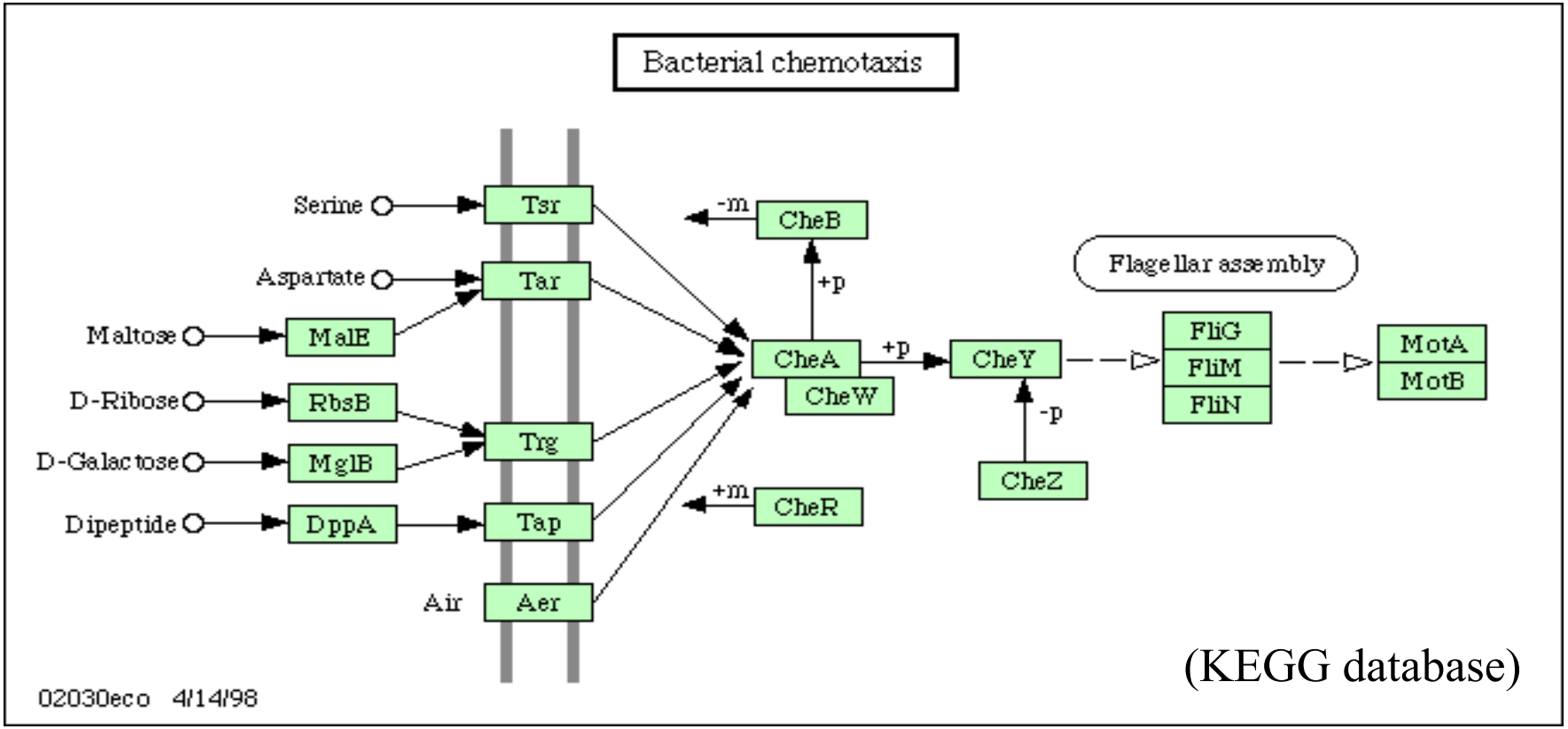
The cell effectively calculates the spatial gradient of C



- How does cell keep a memory of its past history?
- How does it carry out the calculation of gradient?

How is the memory/computation performed by the molecules?

The *E. Coli* chemotaxis signaling pathway (A molecular signal processing machine)



(KEGG database)

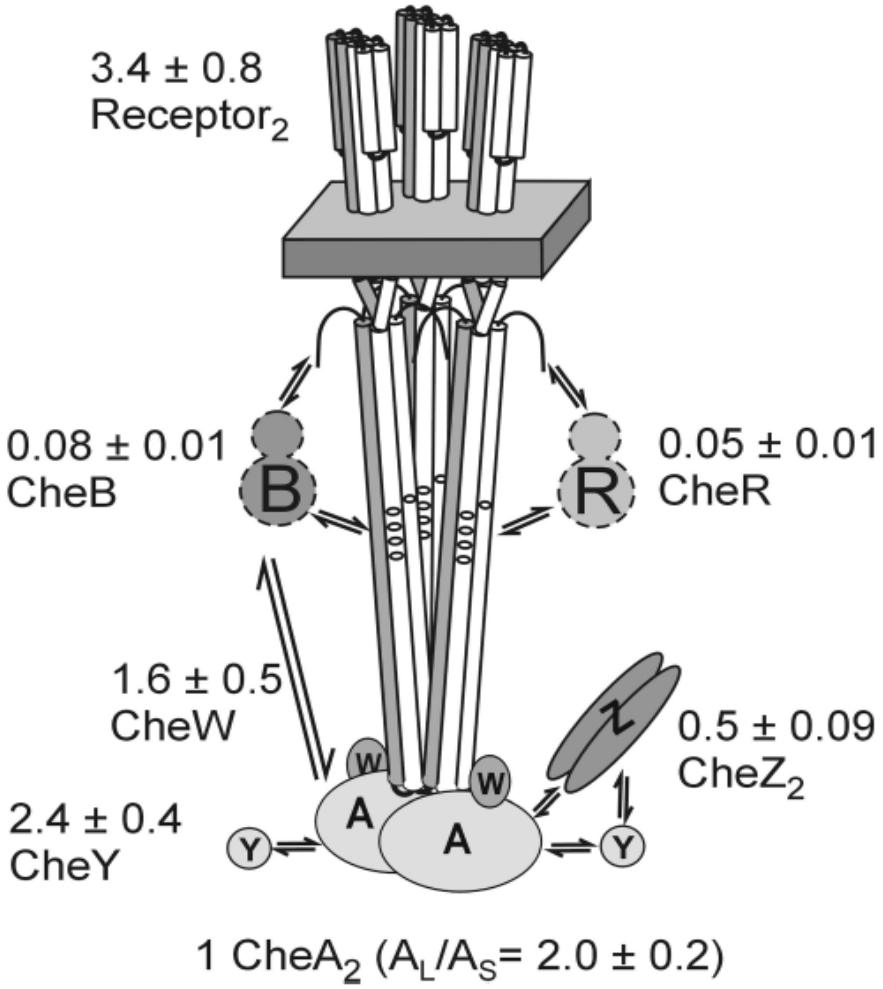
Signal transduction (sensing, amplification, adaptation…..)

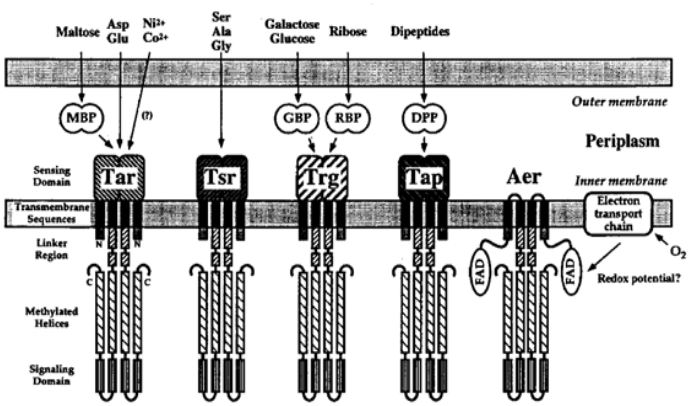
Switch

Motor

The key molecules for *E. Coli* chemotaxis signaling

The chemo-receptor (sensor)





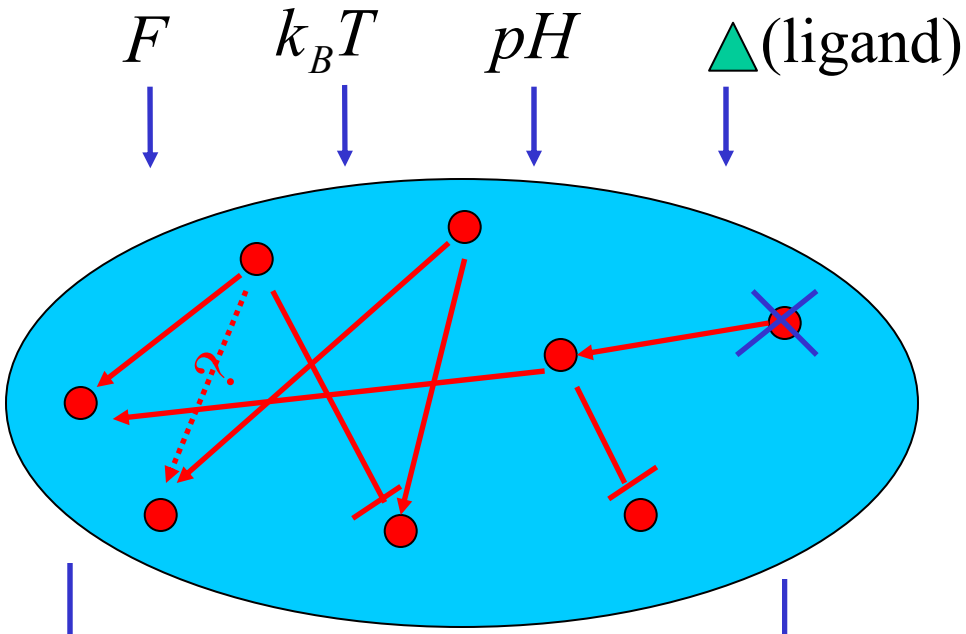
(5 types of chemoreceptor)

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

Tsr:**Tar**:Trg(Tap,Aer)~2:1:0.1

(Li and Hazelbauer, Journal of Bacteriology, 186(12), 3687 (2004))

How can physics (modeling) help biology?



•External perturbation (chemical,physical stimuli)

•Internal perturbation (mutations)

Quantitative Model

•Observed Response

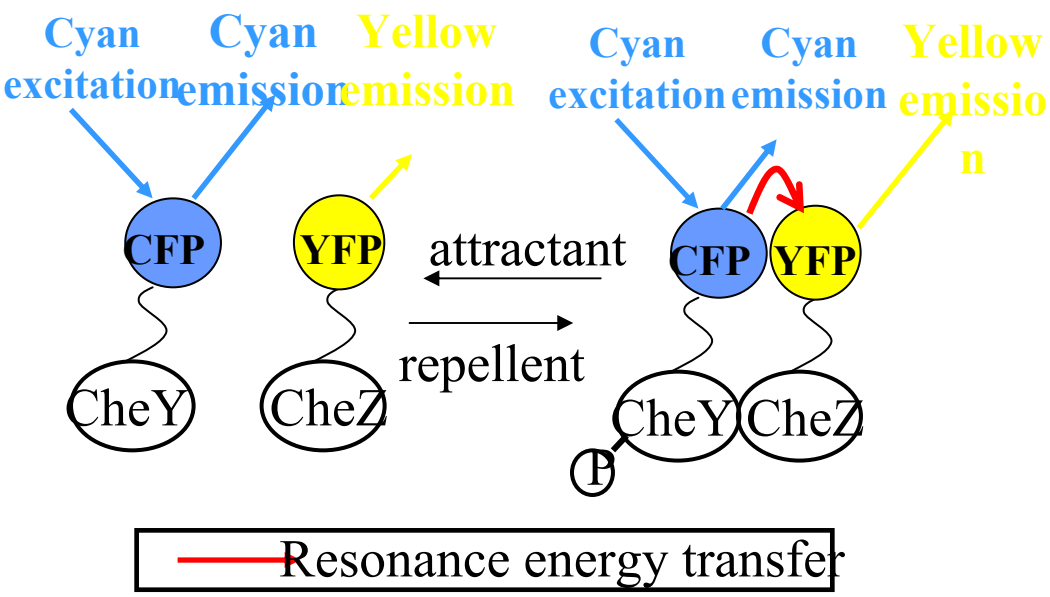
For complex systems, **quantitative modeling** is necessary to identify:

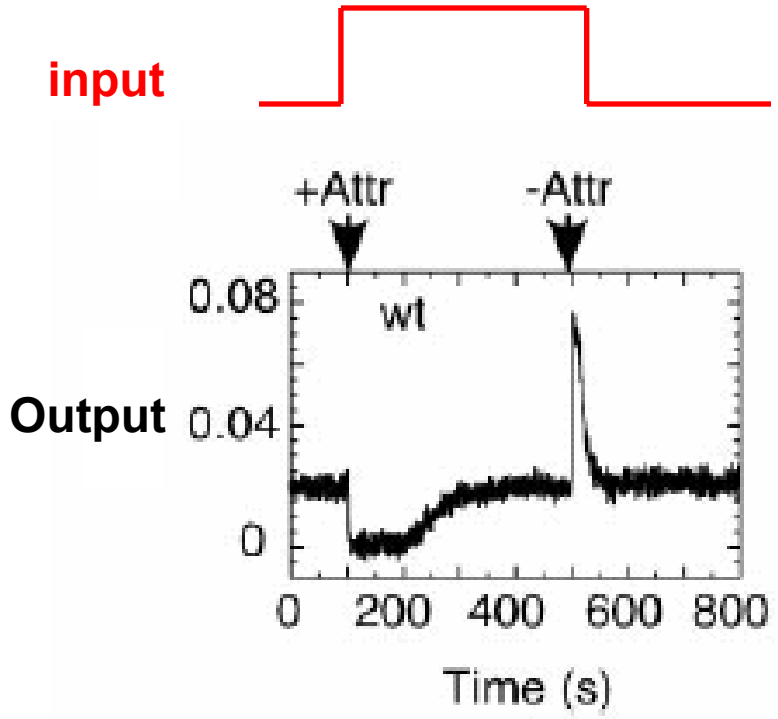
Network properties
•Missing links & nodes
•Relation between links
•The numbers on the links

Understand and predict complex **Biological behavior**

Probing the cell *in vivo* by perturbations

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

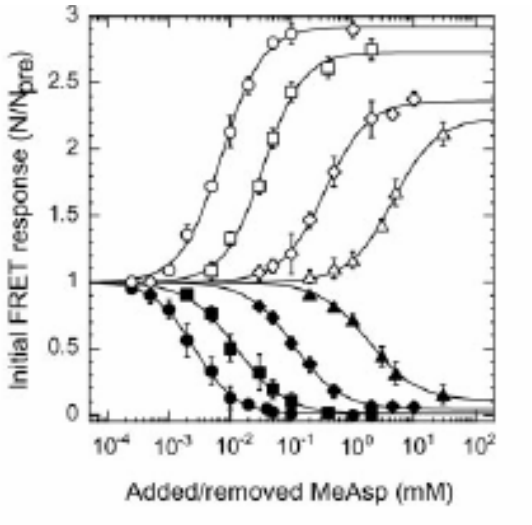




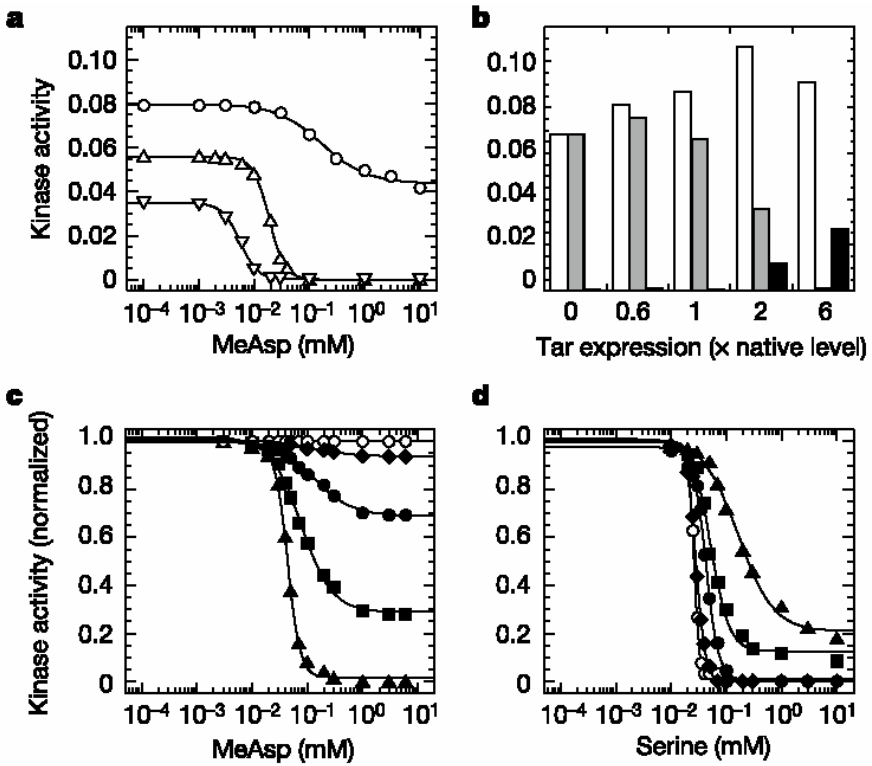
Molecular level measurement while the cell is alive and behaving

The response data for wt and different mutants

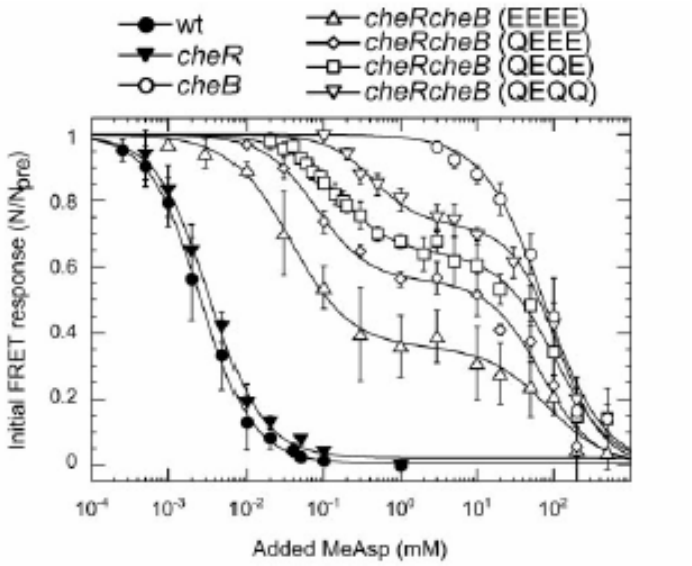
WT in different background



Different Tar/Tsr expression levels



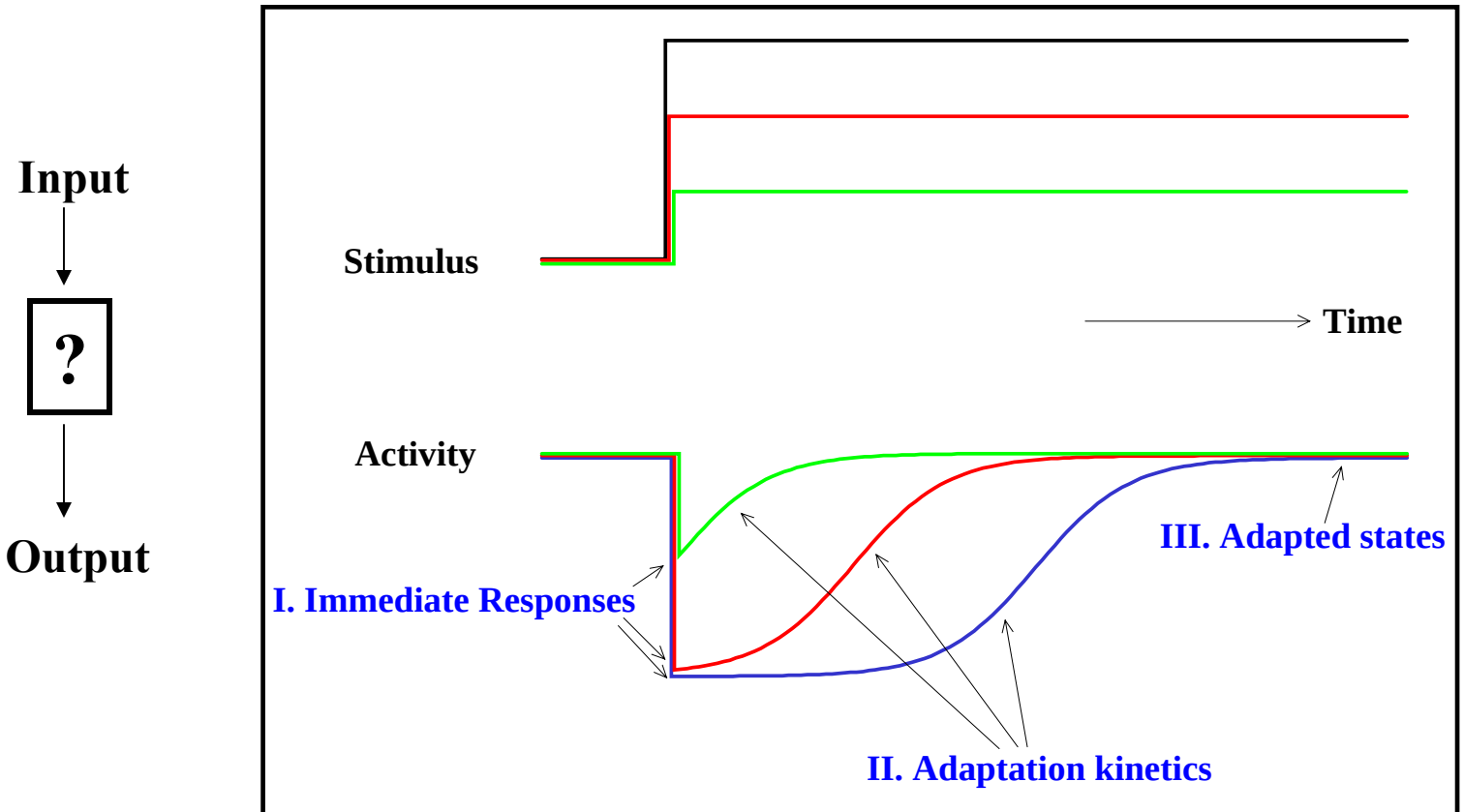
Different methylation levels



····· (from the Berg Lab)

High gain in a wide dynamic range for E. coli chemotaxis

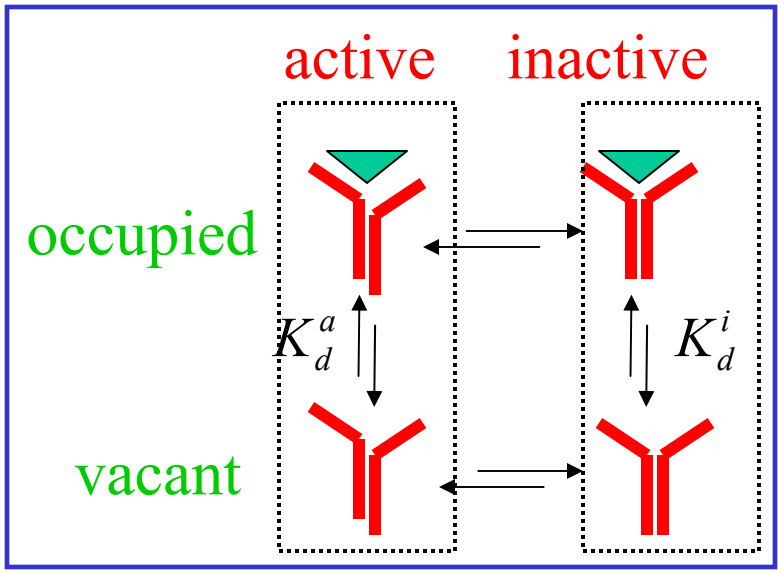
- High sensitivity (~10's nM, a few ligand molecules)
- Signal amplification (~40X)
- High sensitivity exists in a wide range of backgrounds
- Wide dynamic range (100nM→1mM)
- Near perfect adaptation



Energetics and the steady state behavior of the system

The energetics of a receptor dimer

- 4 states for each individual receptor i
  - Kinase activity $a_i = 0,1$
  - Ligand binding $l_i = 0,1$

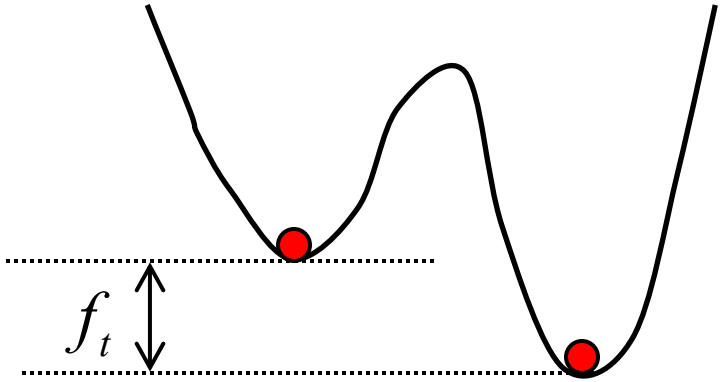


$$< a >= (1 + \exp(-f_t))^{-1}$$

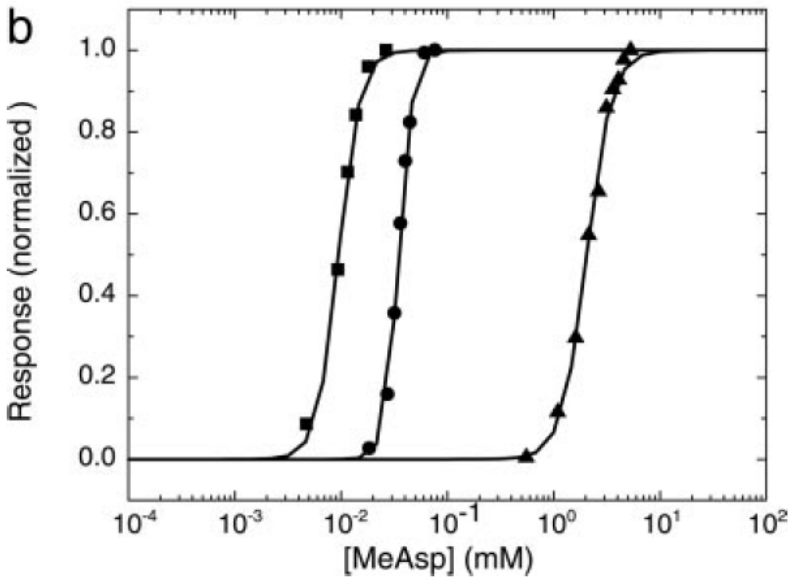
$$f_t = f_m(m) - \ln \frac{1 + [L]/K_d^i}{1 + [L]/K_d^a}$$

Methylation energy

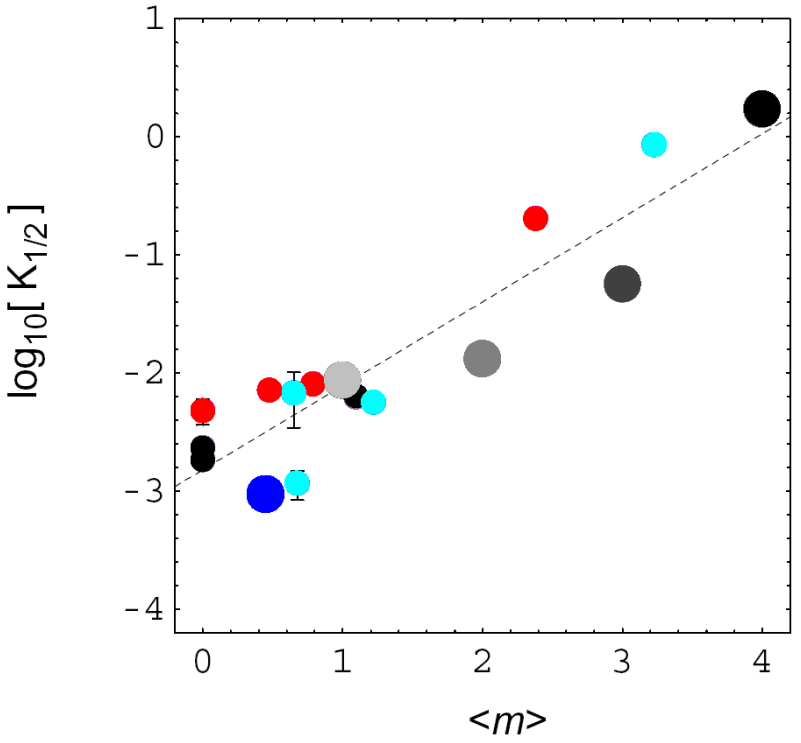
Ligand binding energy



The methylation energy function



(Shimizu et al. PNAS 2006)



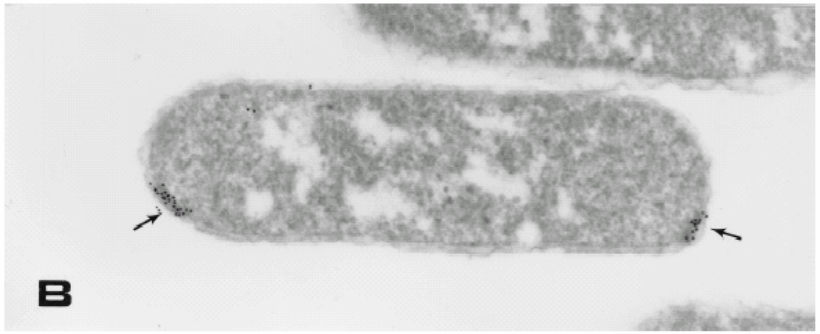
(Shimizu et al. unpublished)

$$f_m(m) \approx \alpha(m - m_0)$$

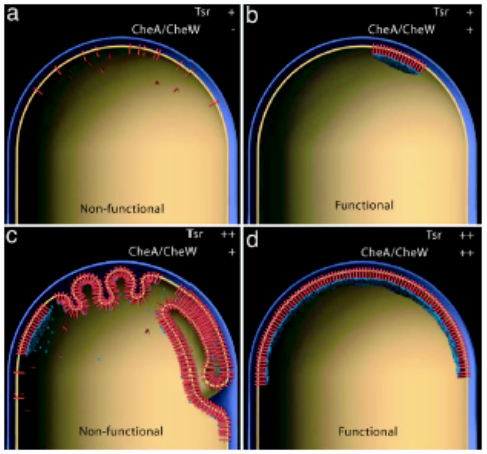
$$\alpha \approx 1.5 - 2.0 \;\; ; \;\; m_0 \approx 1 - 2$$

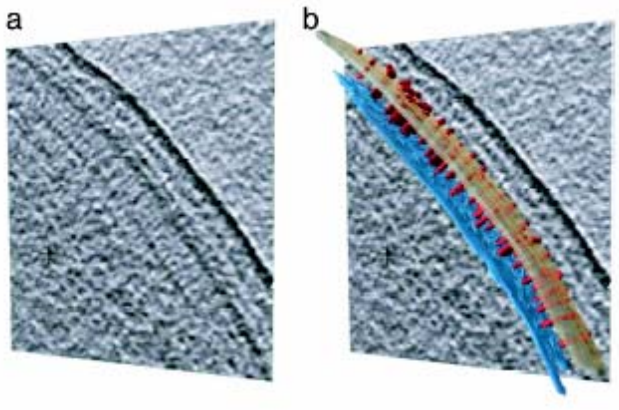
Chemotaxis receptors form clusters at the cell poles

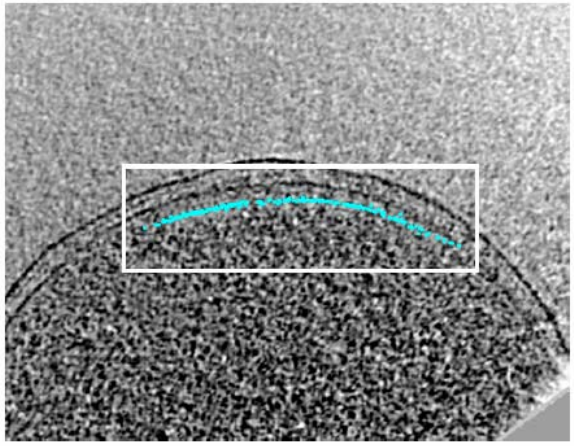
Chemoreceptors cluster in bacteria
(~20,000 chemo-receptors in a E. Coli cell)

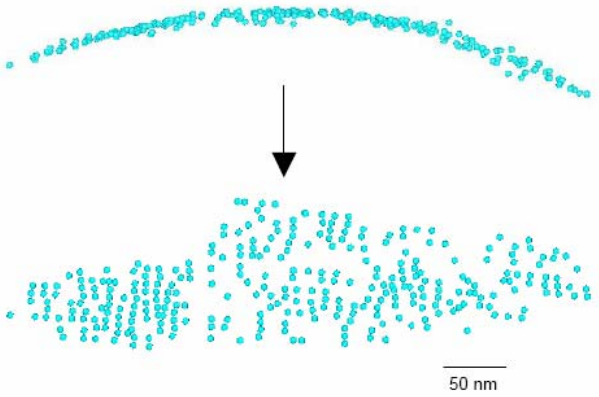


(Maddock & Shapiro, 1993)
(Lybarger & Maddock)





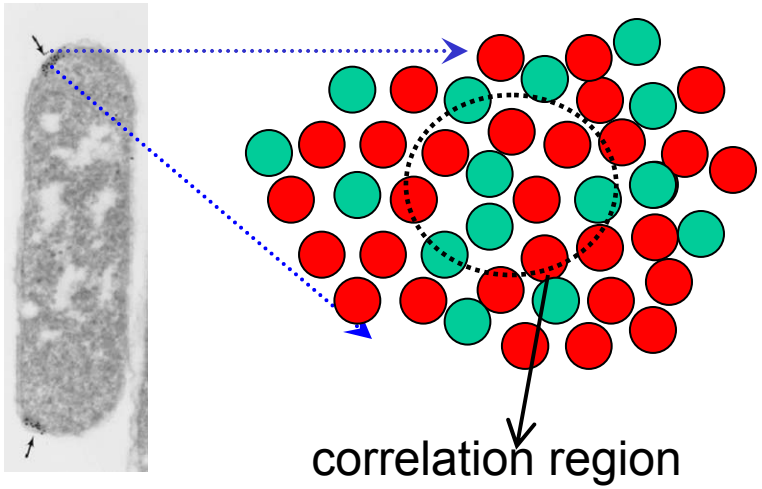




(Subramanian Lab, NCI)

The receptor-receptor interaction within the polar cluster

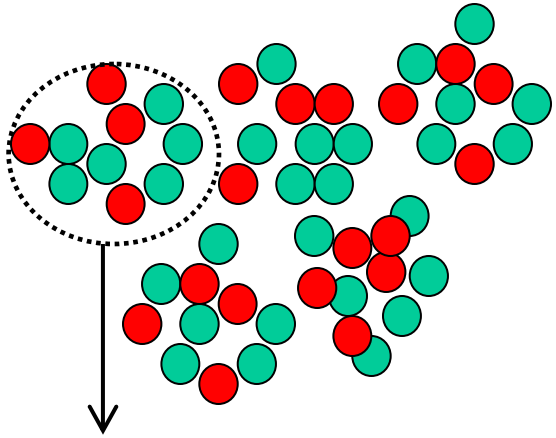
Ising type model (nearest neighbor)



$$+ a_i \sum_j C^{(nn)}_{q_i q_j} (a_j - \tfrac{1}{2})$$

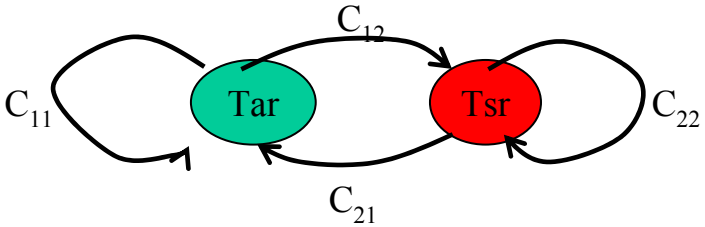
More realistic

MWC type model (all-or-none)



$$C \to \infty \quad \text{within cluster size } N$$

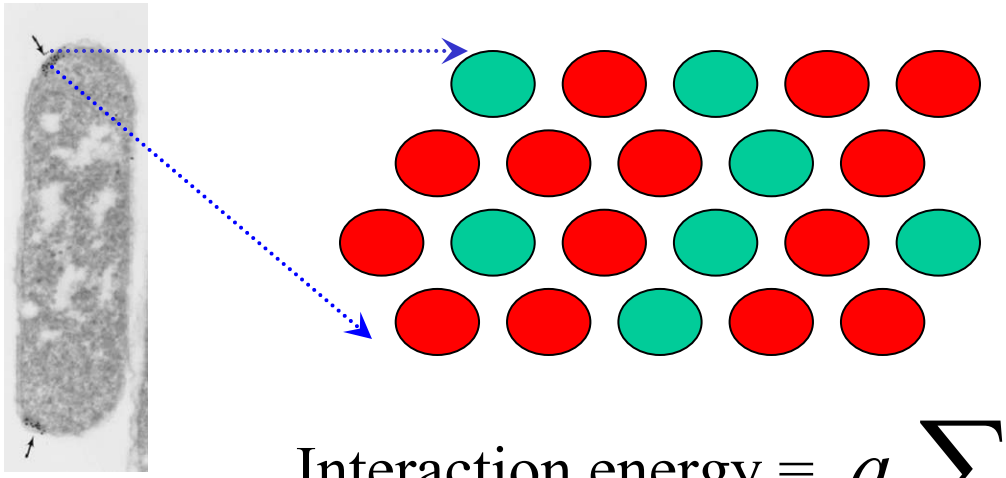
Simplicity (analytical solution)

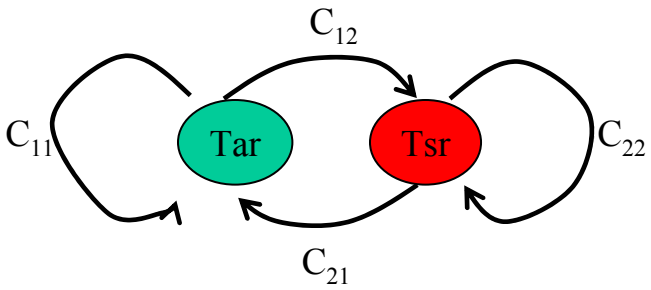


Non-discriminative Interaction between heterogeneous receptors
They act together

The Ising-like model for receptor Interaction

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





$$\text{Interaction energy} = a_i \sum_j C_{q_i q_j} (a_j - \tfrac{1}{2})$$

•j labels all the "neighboring" receptors of i'th receptor

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

"Spin" "local magnetic field" "coupling to neighbors"

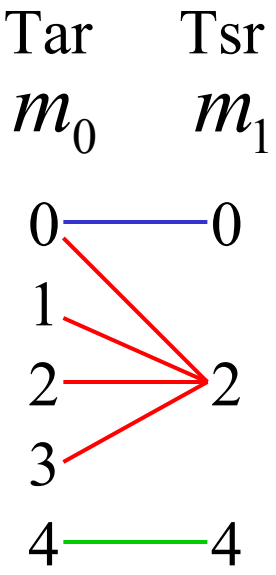
Analogous to the Ising model for magnetism in physics

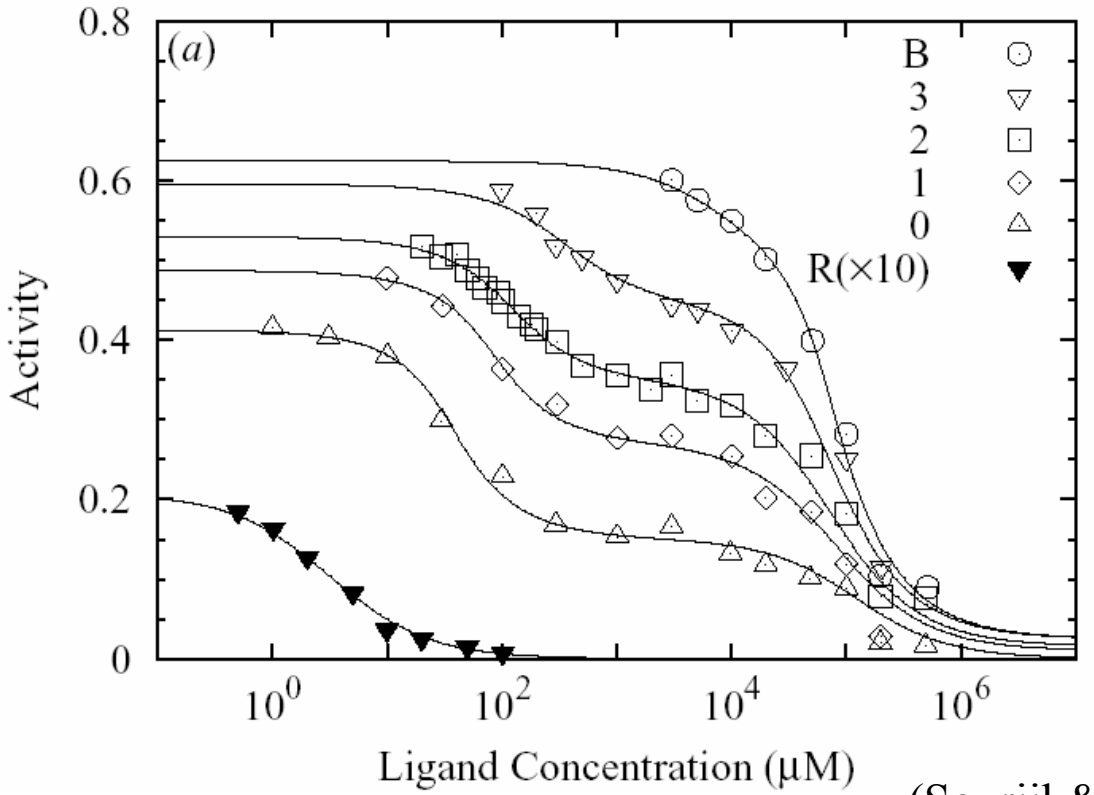
The model results for the cheRB- mutant strains

Adaptation disabled: Receptor methylation level fixed

Data can only be explained with interaction between Tar and Tsr receptors

Different types of chemo-receptors act together





Exp.---symbols
Theory ---lines

(Sourjik& Berg, PNAS 2002)
(Mello & Tu, PNAS, 2003)

# of parameters in the model: 3x8+4=28
of **independent** data points: ~6x7=42

Responses in wild-type (wt) *E. coli* cells that can adapt accurately

Monod-Wyman-Changeaux (MWC) model for N highly correlated receptors:

$$a_{wt}([L],[L]_0) = \frac{L(1+[L]/K_a)^N}{(1+[L]/K_i)^N + L(1+[L]/K_a)^N}$$

$[L]_0$-- background ligand signal concentration; $K_{i,a}$—dissociation constants

WT cell adapt perfectly:

$$a_{wt}([L]_0,[L]_0) = a_0$$

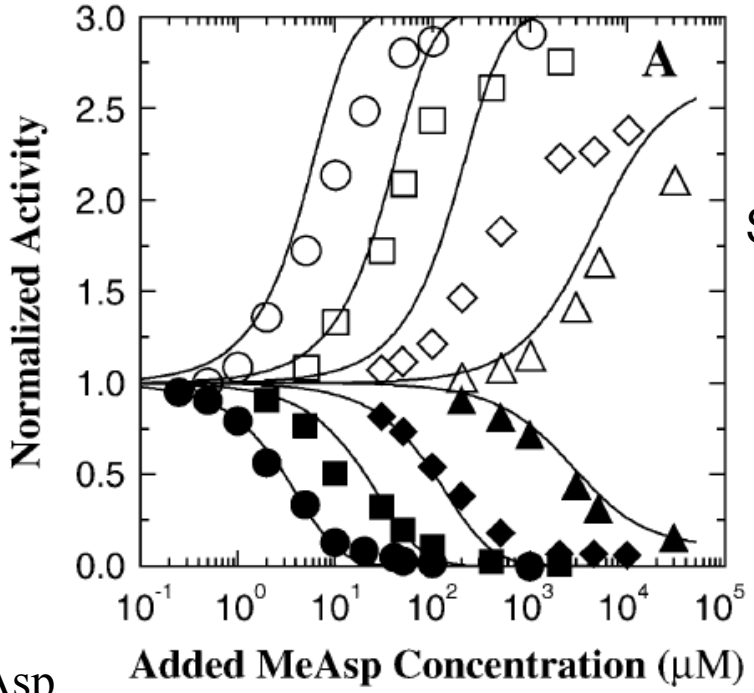
$$\Downarrow$$

$$L = \frac{a_0}{1-a_0}\left(\frac{1+[L]_0/K_i}{1+[L]_0/K_a}\right)^N$$

Microscopic parameters determined

$N_t = 3N \approx 20;\ K_i \approx 18\mu M;\ K_a \approx 3mM$ for MeAsp

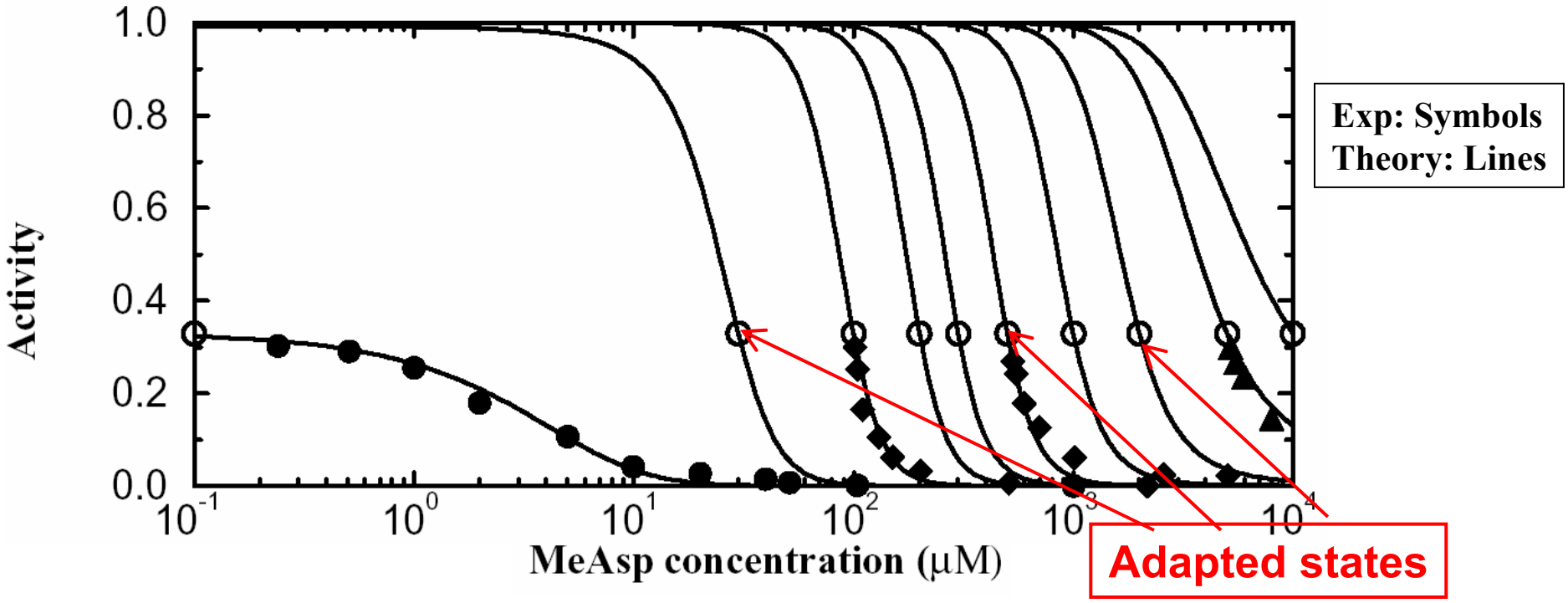
(Mello&Tu, BioPhys. J. 2007)



($[L]_0$=0, 0.1, 0.5, 5mM)

Adaptation enables high sensitivity over a wide range of backgrounds

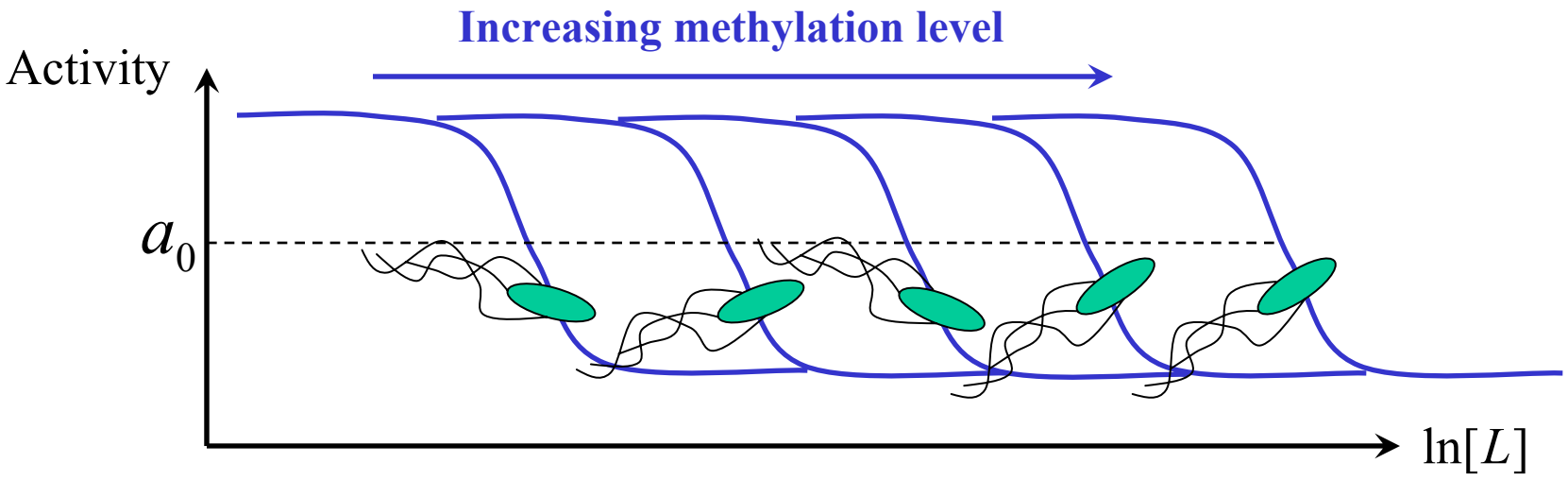
Increasing receptor methylation level →
(increasing background concentrations $[L]_0$=0, 0.1, 0.5, 5mM)



Mechanism for sustained high gain:

Self-tuned near-critical behavior: the "smart" Ising model

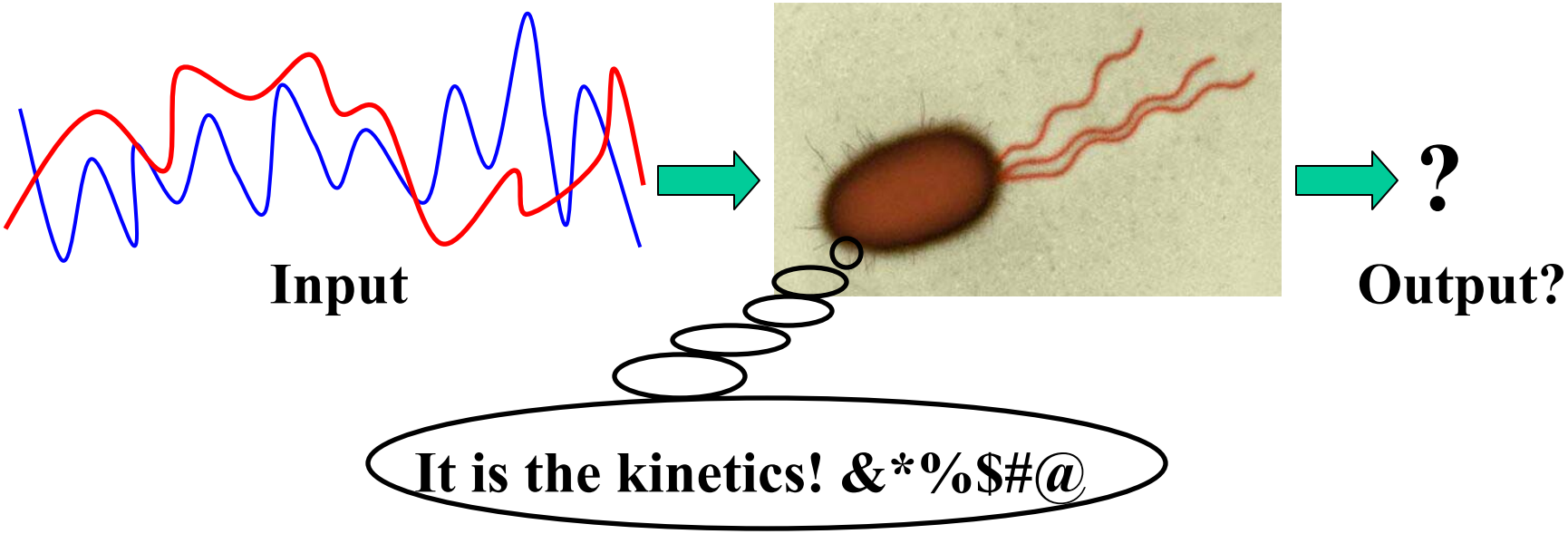
E. Coli surfing the adaptation wave



Kinetics and responses to time varying signals

Responses to time varying signals

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



What type of signal processor is bacterial chemotaxis pathway?

Amplifier; filter; nonlinear effects; signal integration/differentiation

The dynamics of the receptor complex

Ligand binding (Input)

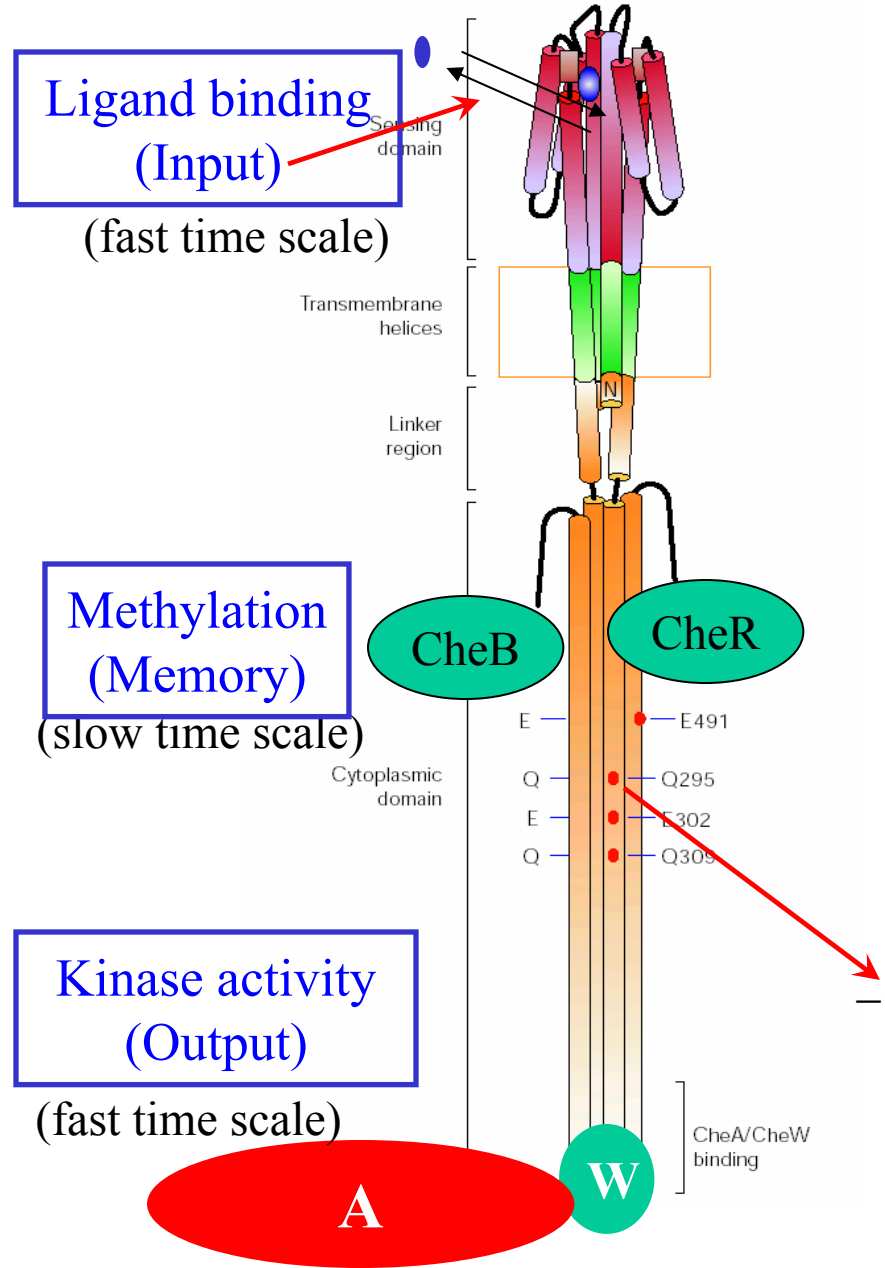
(fast time scale)

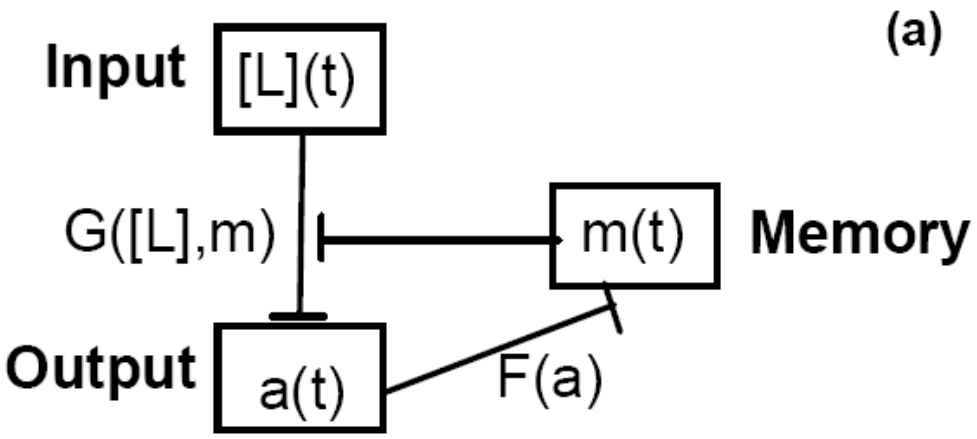
Methylation (Memory)

(slow time scale)

Kinase activity (Output)

(fast time scale)





$-CH_3$

A coarse-grained dynamical model for chemotaxis

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

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

Perfect adaptation

$$\frac{dm}{dt} = 0 \Rightarrow F(a) = 0 \Rightarrow a = a_0$$

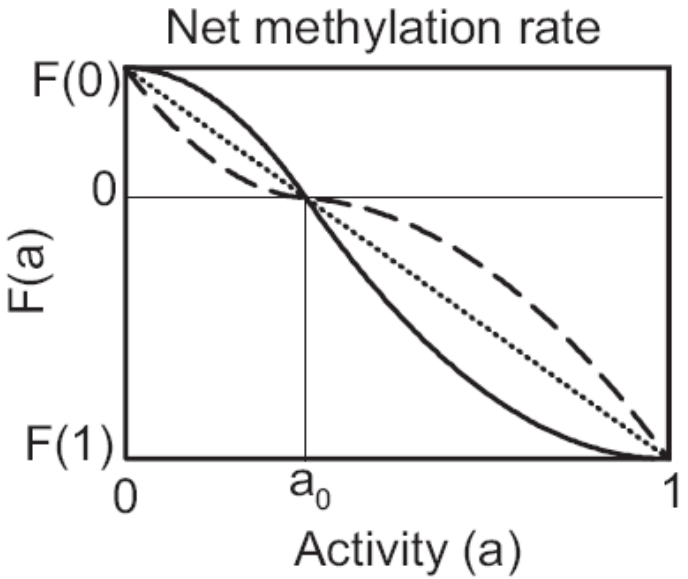
Independent of [L]: perfect adaptation

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

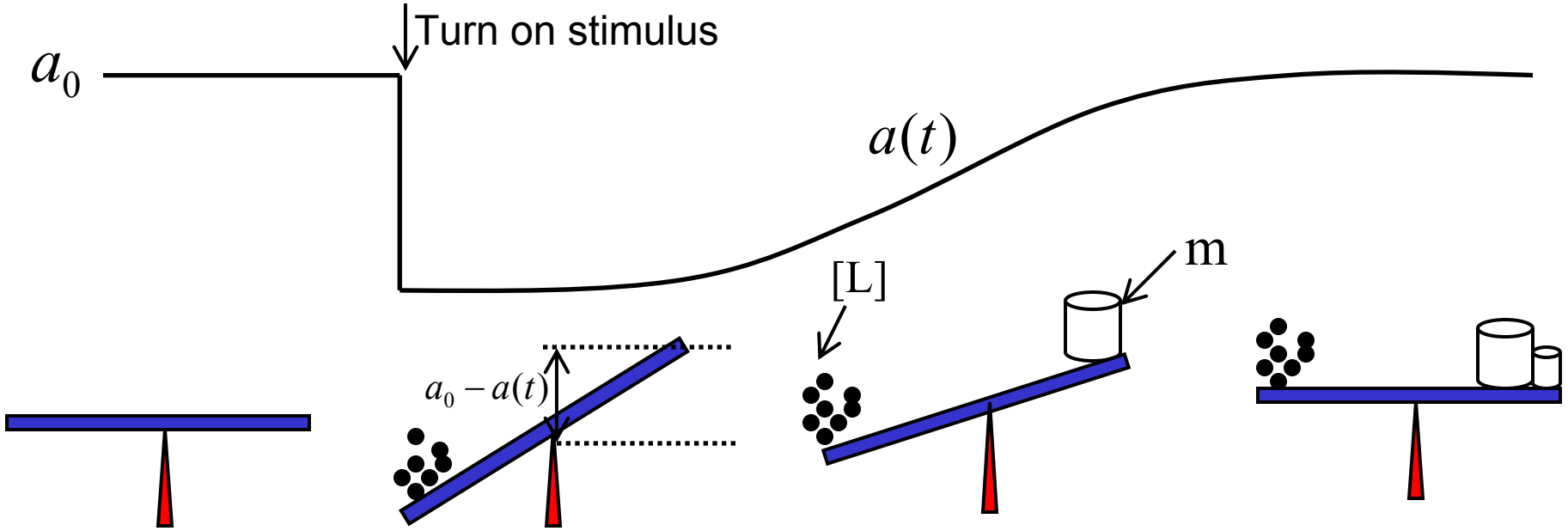
Number of receptor in the all-or-none cluster

$$F(a_0) = 0, F(0) > 0, F(1) < 0$$

$$-F(1) > F(0)$$



The operation of a measurement device



- Measure external object by balancing it with internal weights.
- Operate by feedback: add or subtract weights based on the imbalance.

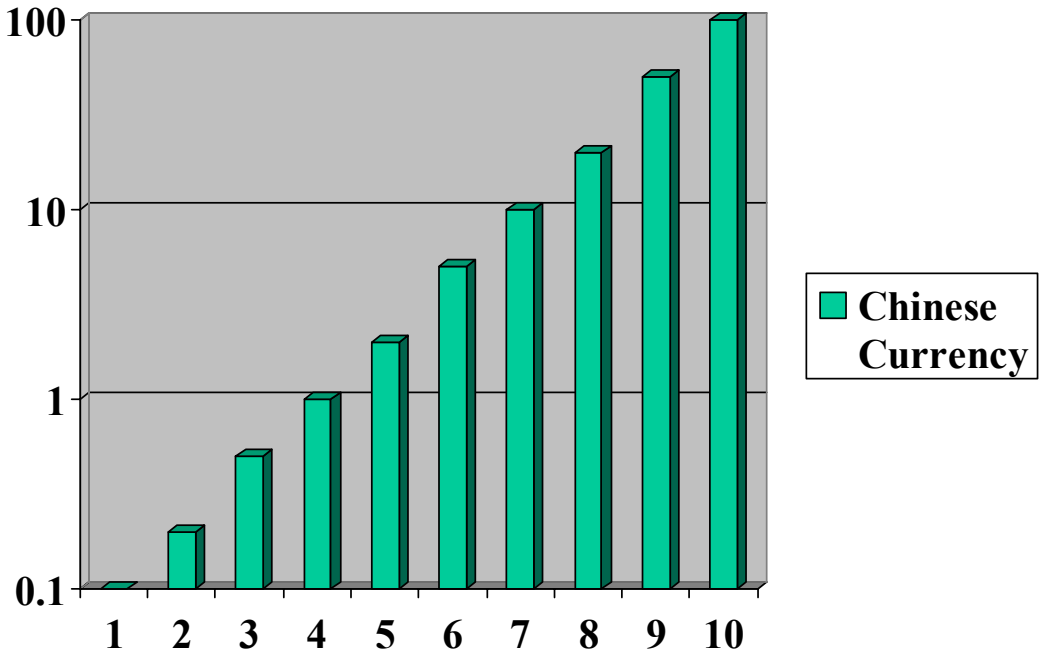
Methylation level measure external ligand concentration in log-scale

$$a = a_0 \Rightarrow m \approx \alpha^{-1} \ln[L] + const.$$

("Logarithmic-sensing in E. coli chemotaxis", Kalinin et al, Bio. J. 2009)

The measuring the outside world in log-scale

Log-scale weight is an efficient way of representing a wide range of values by a small number of units: e.g., the Chinese currency units



Some "forgotten" experiments and its recent incarnation: response to time varying signals

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

JOURNAL OF BACTERIOLOGY, Apr. 1983, p. 312–323
0021-9193/83/040312-12$02.00/0
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Vol. 154, No. 1

Adaptation Kinetics in Bacterial Chemotaxis

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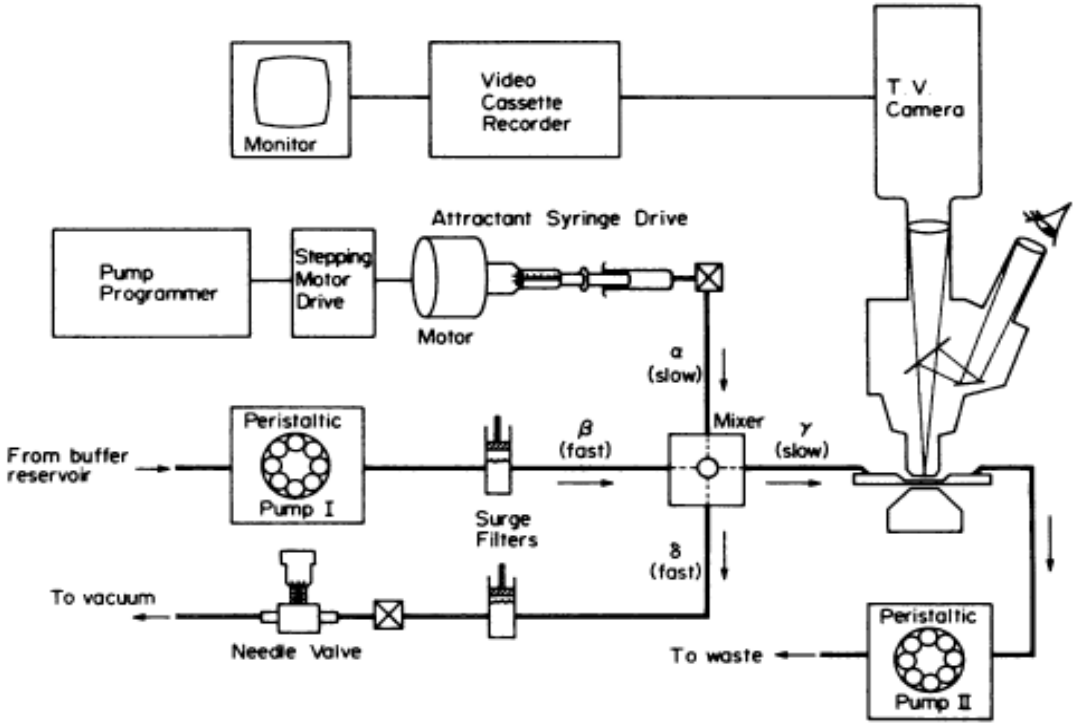
Received 18 October 1982/Accepted 21 January 1983



- **Exponential ramp**
- **Exponentiated sine wave**
- **Steps and impulses**

The response are now measured by using FRET now. (by T. Shimizu, H. Berg)

Theory prediction: constant activity shift in response to exponential ramp

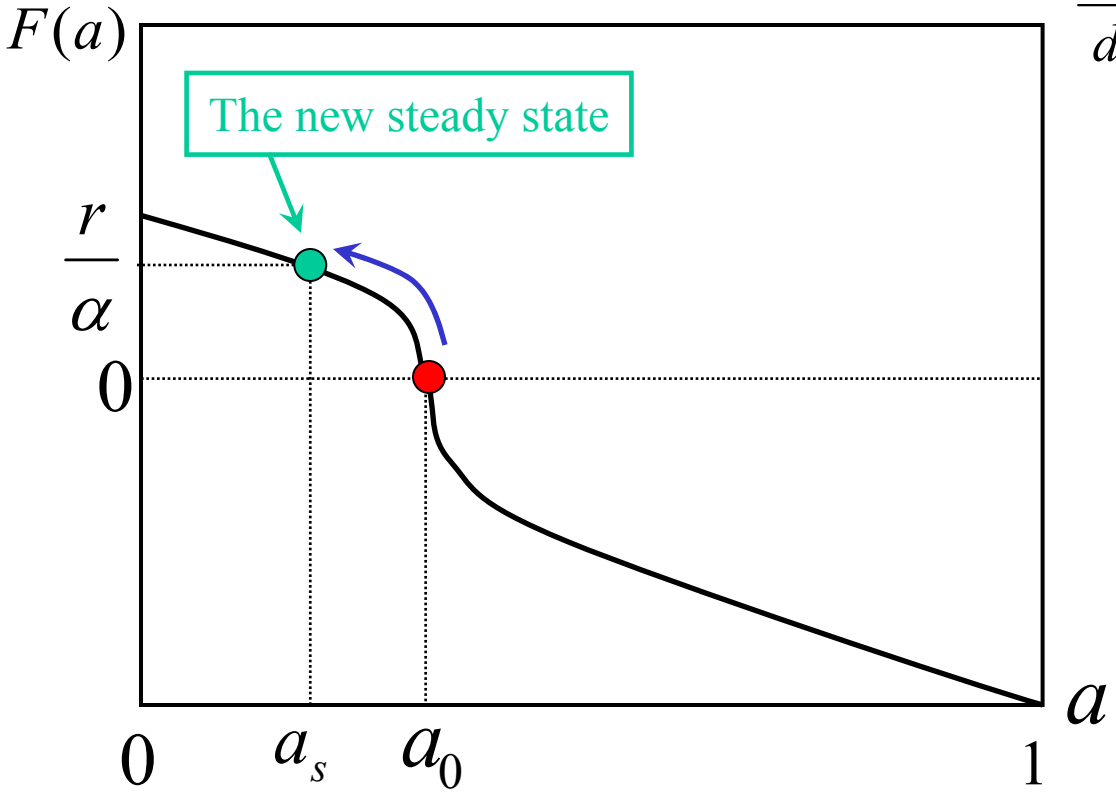
$$\Delta E \sim N[\alpha m - \ln([L]/K_d)] + const.$$

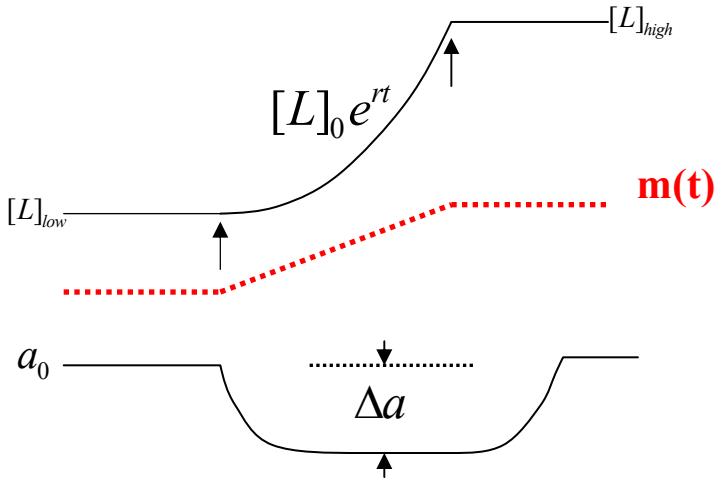
$$\ln[L] = rt$$

$$\frac{dm}{dt} = r/\alpha$$

$$\frac{dm}{dt} = F(a)$$

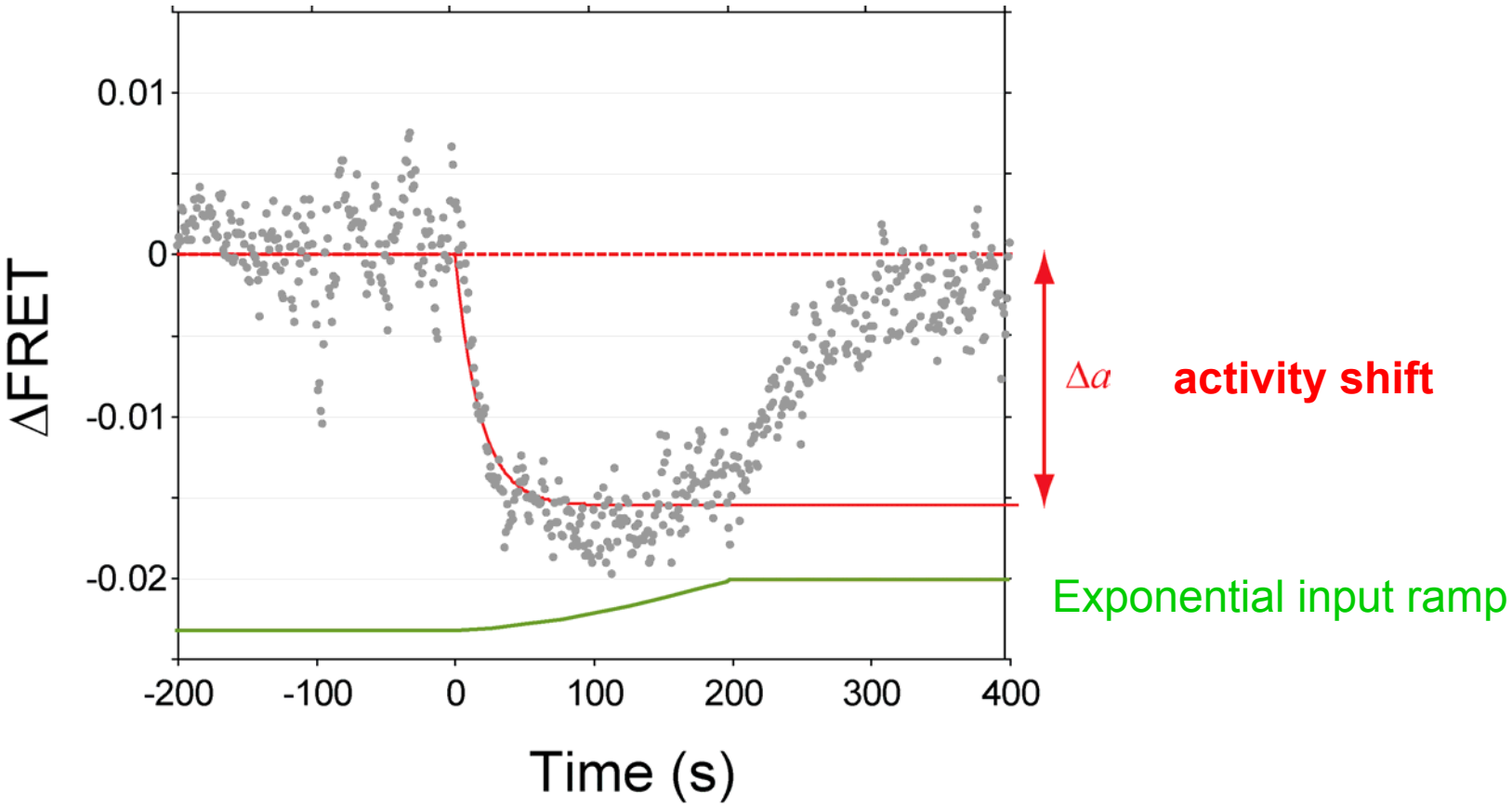
$$F(a_s) = \frac{r}{\alpha}$$





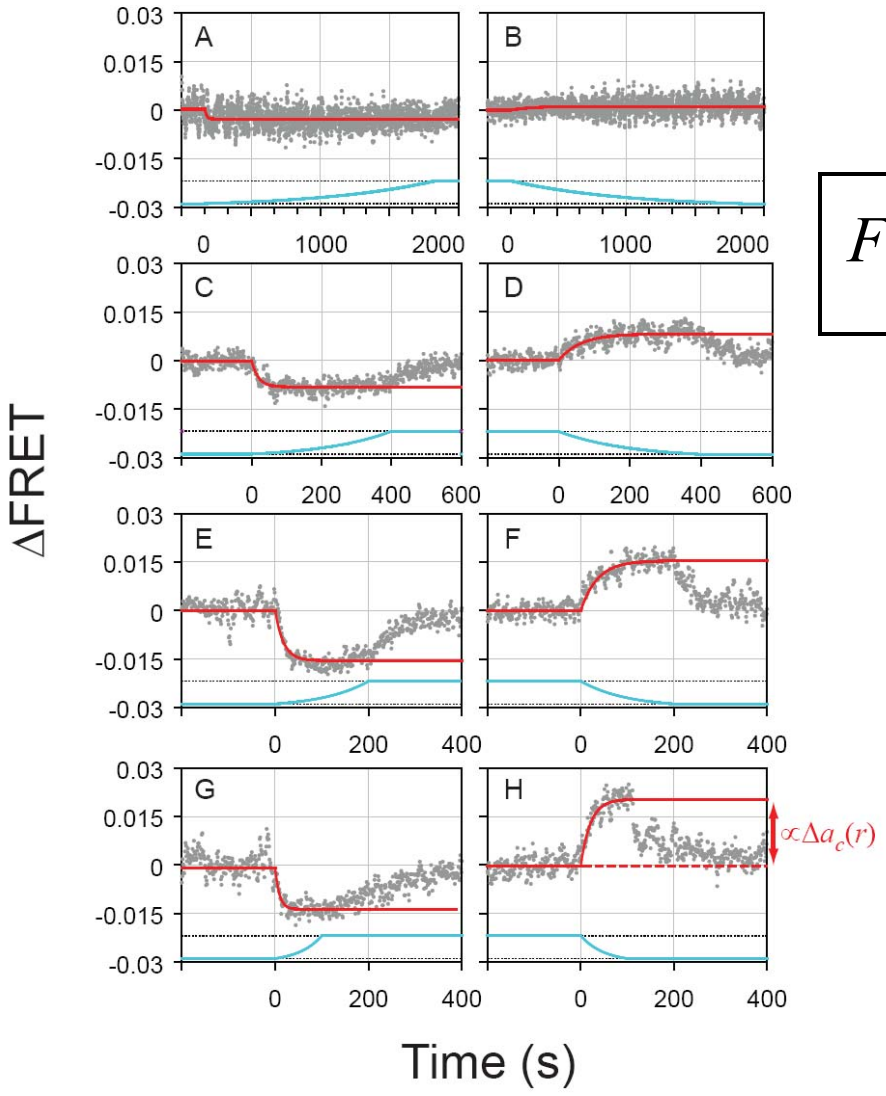
Methylation tries to catch up with the exponentially changing external stimulus
But it lag behind it, which leads to the activity shift

Response to exponential ramps: FRET experiments

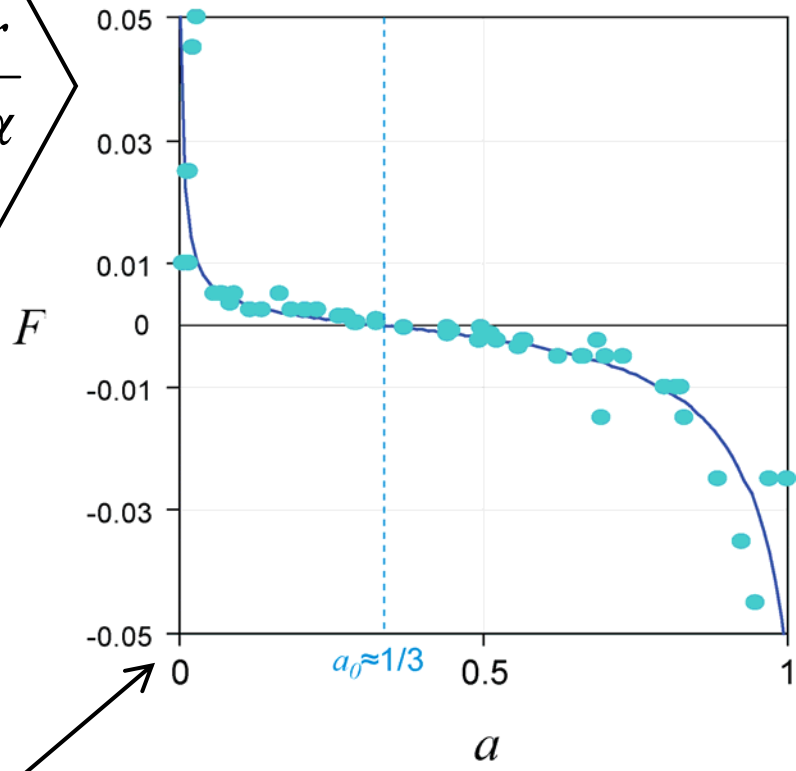


Constant activity shift in response to exponentially increasing signal

The dependence of the activity shift on ramp rate



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



The methylation rate function F(a) revealed: soft control at a0

Response to sine waves: the spectral analysis

Input: $[L](t) = [L]_0 e^{\beta \sin^2(\pi f t)}$

Output: kinase activity a(t)

Memory (Control): methylation m(t)

$$\Delta a = G'(f_t^0)\alpha[\Delta a - \frac{A_L}{\alpha}\cos(2\pi f t)]$$

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

$$A_a = \frac{ifc_a}{if + f_m} A_L, \quad A_m = \frac{f_m \alpha^{-1}}{if + f_m} A_L$$

The chemotaxis transfer functions

$$c_a = Na_0(a_0 - 1); \; f_m = -(\alpha F'(a_0) N a_0 (1 - a_0))/2\pi$$

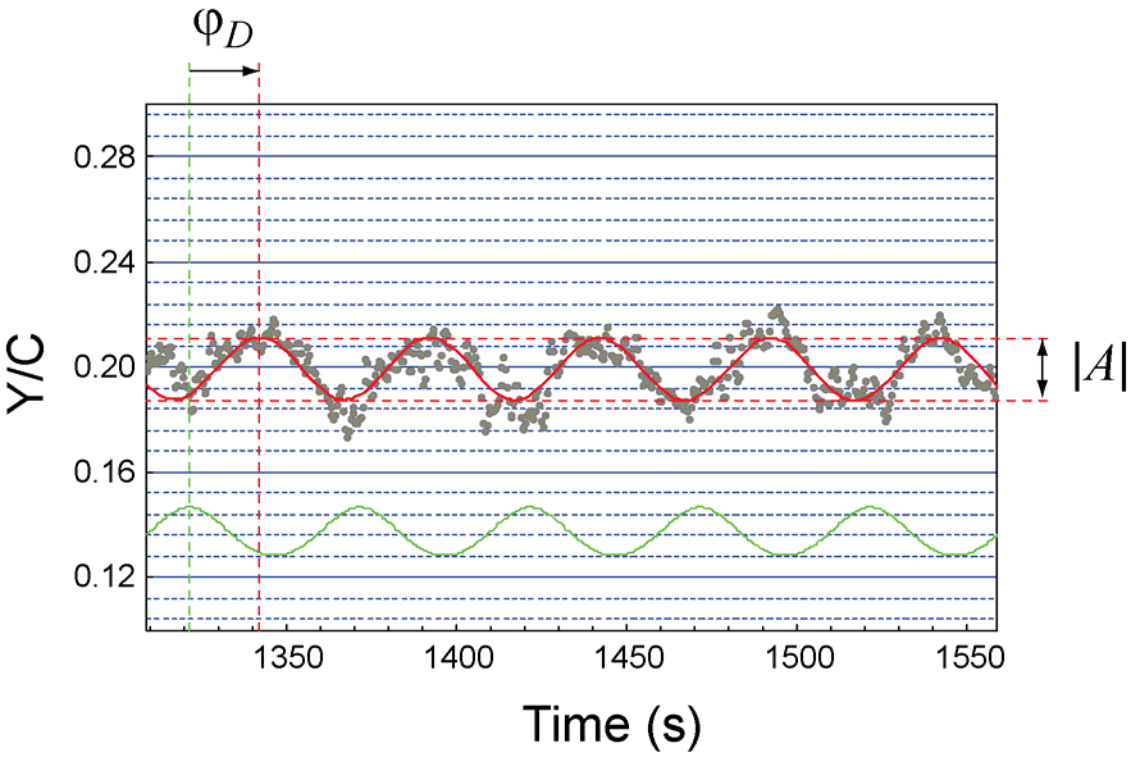
Low frequency : $f << f_m$

$A_a \sim ifcA_L, \quad A_m \sim A_L$

High frequency : $f >> f_m$

$A_a \sim A_L, \quad A_m \sim A_L / if$

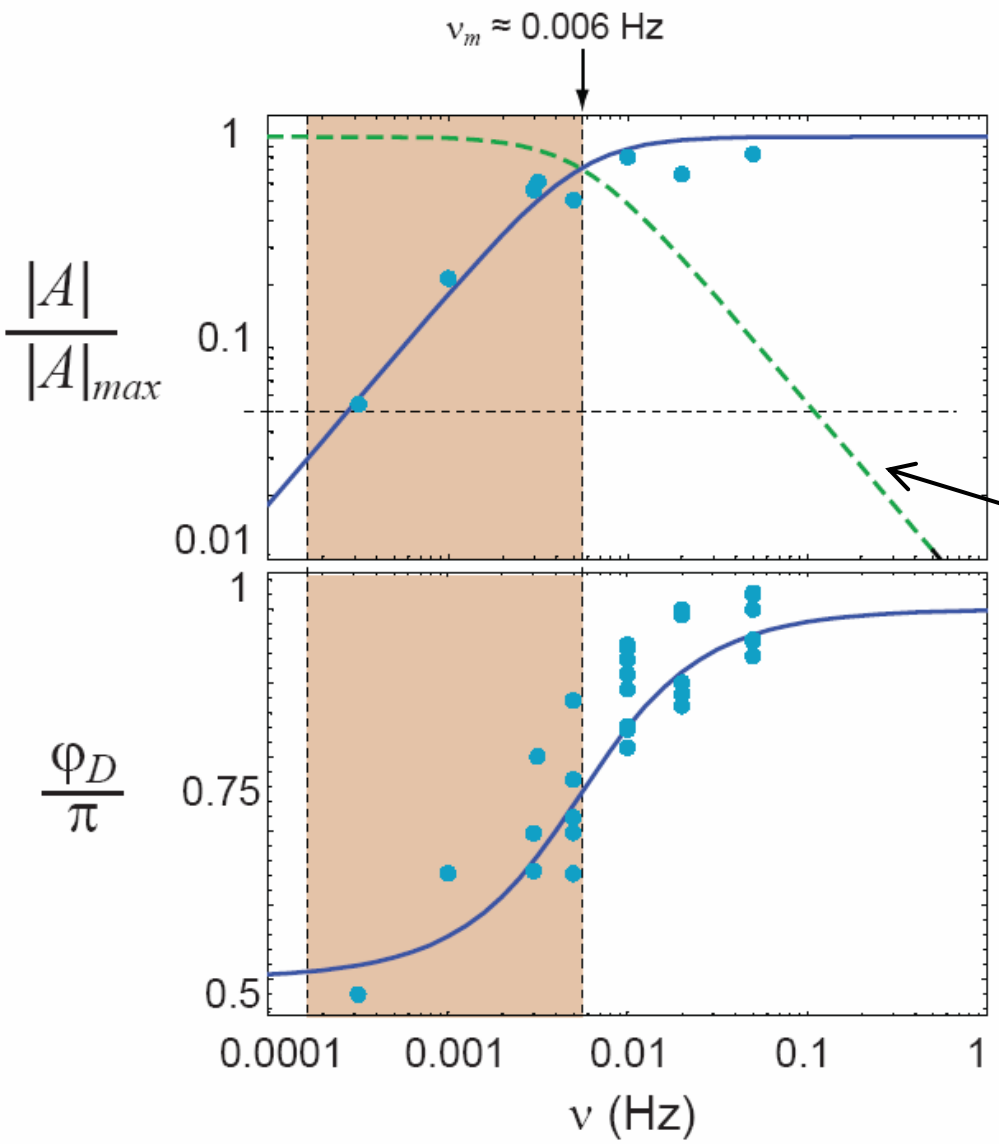
The phases and amplitudes of the responses and their dependences on frequency



$$a(t) = |A| \cos(2\pi\nu t - \varphi_D)$$

$$[L](t) = [L]_0 \exp[A_L \sin 2\pi\nu t]$$

Theoretical predictions and experiments



Data

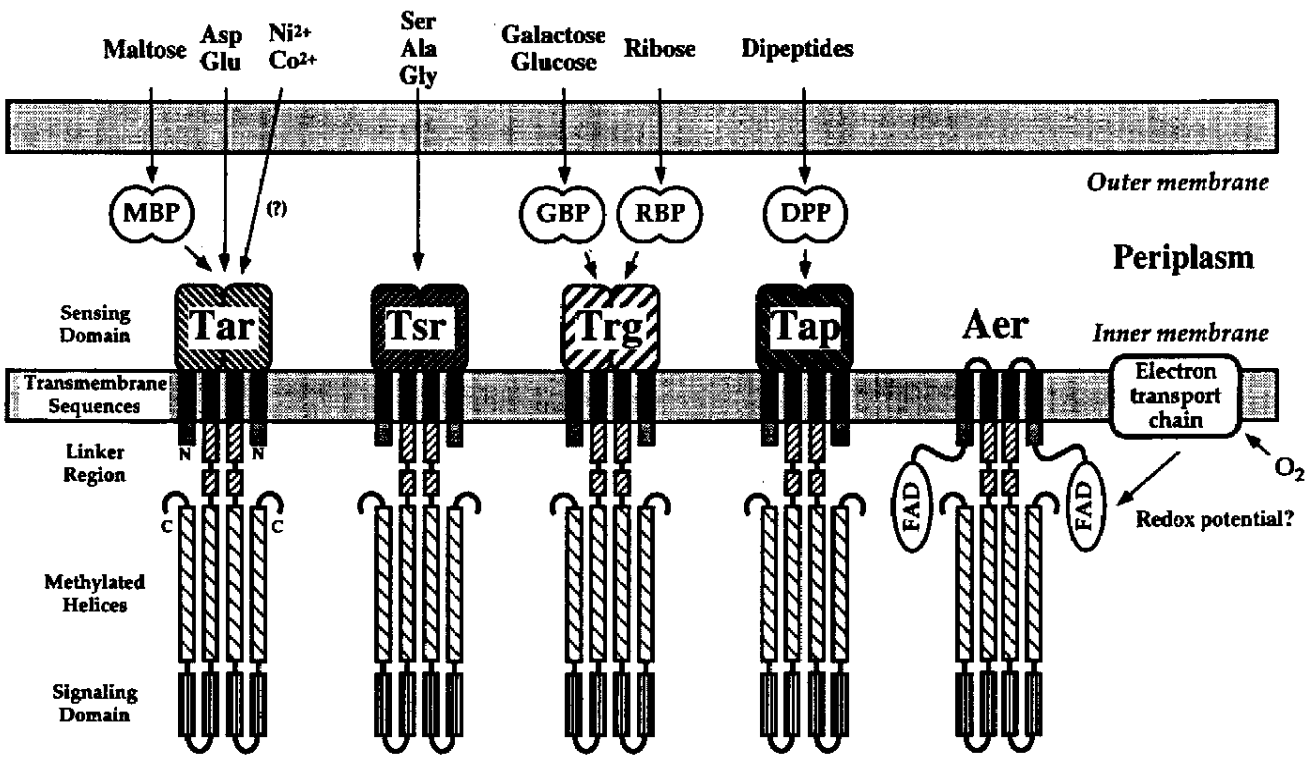
Theory

A low pass filter for the derivative of the signal

(Y. Tu, T. Shimizu, H. Berg, PNAS 08)
(T. Shimizu, Y. Tu, H. Berg, submitted 09)

Signal differentiation: Adaptation and response to mixed signals

There five different types of chemo-receptors forming mixed receptor clusters



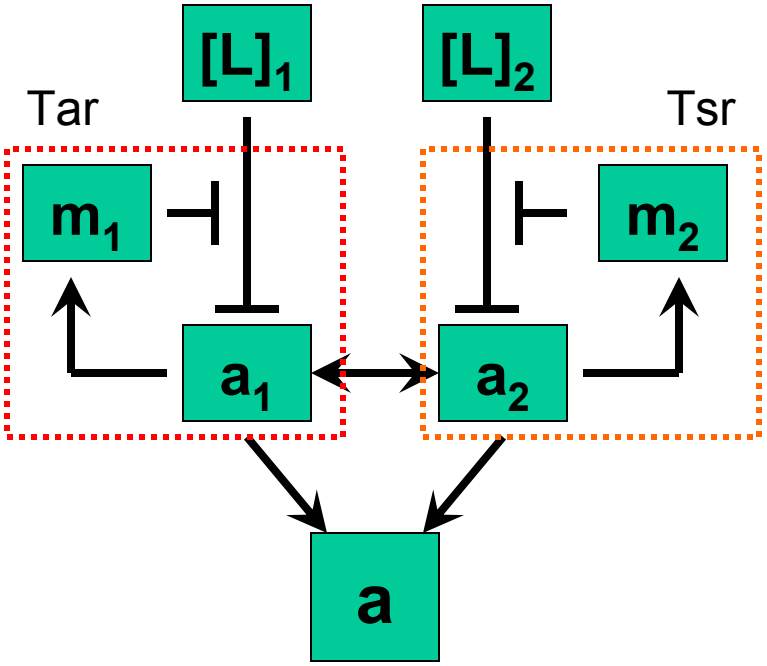
(5 types of chemoreceptor, each sensing different signals)

Total number of Receptors: 15,000-26,000. **Tsr**:**Tar**:Trg(Tap,Aer)~2:1:0.1

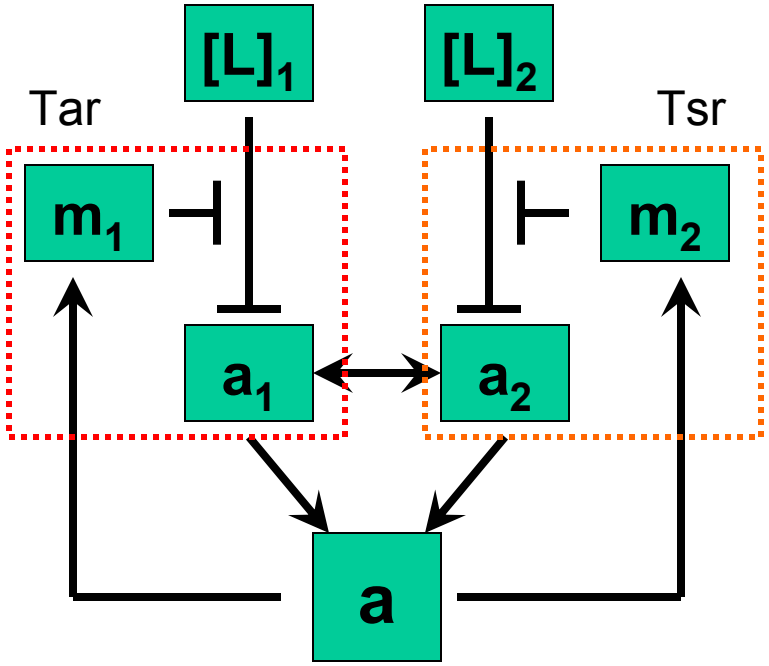
Can the cell tell different signals apart? How? and Why?

The local versus global methylation dynamics

The local adaptation model



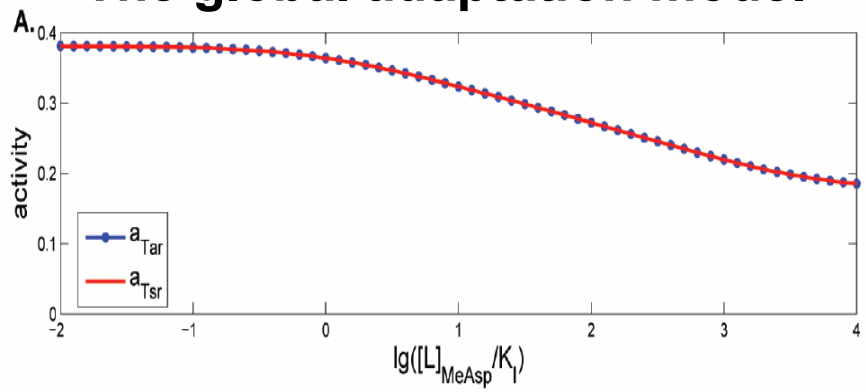
The global adaptation model



1—Tar/Asp; 2—Tsr/Serine

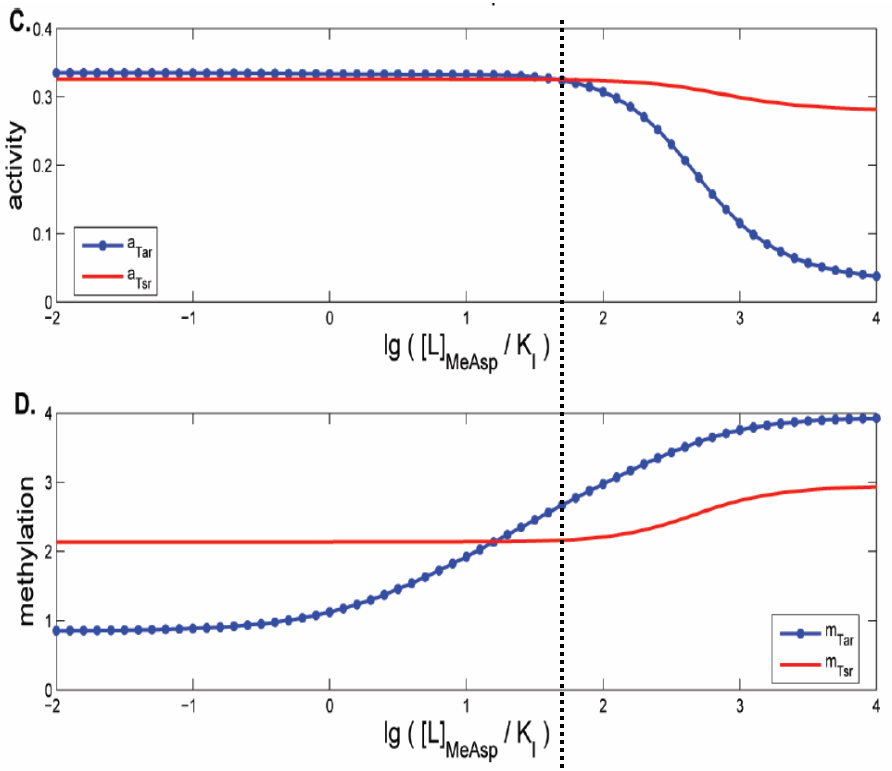
The properties of the adapted (steady) state

The global adaptation model



Methylation level of both Tar and Tsr respond (equally) to either MeAsp or Serine—always crosstalk.

The local adaptation model

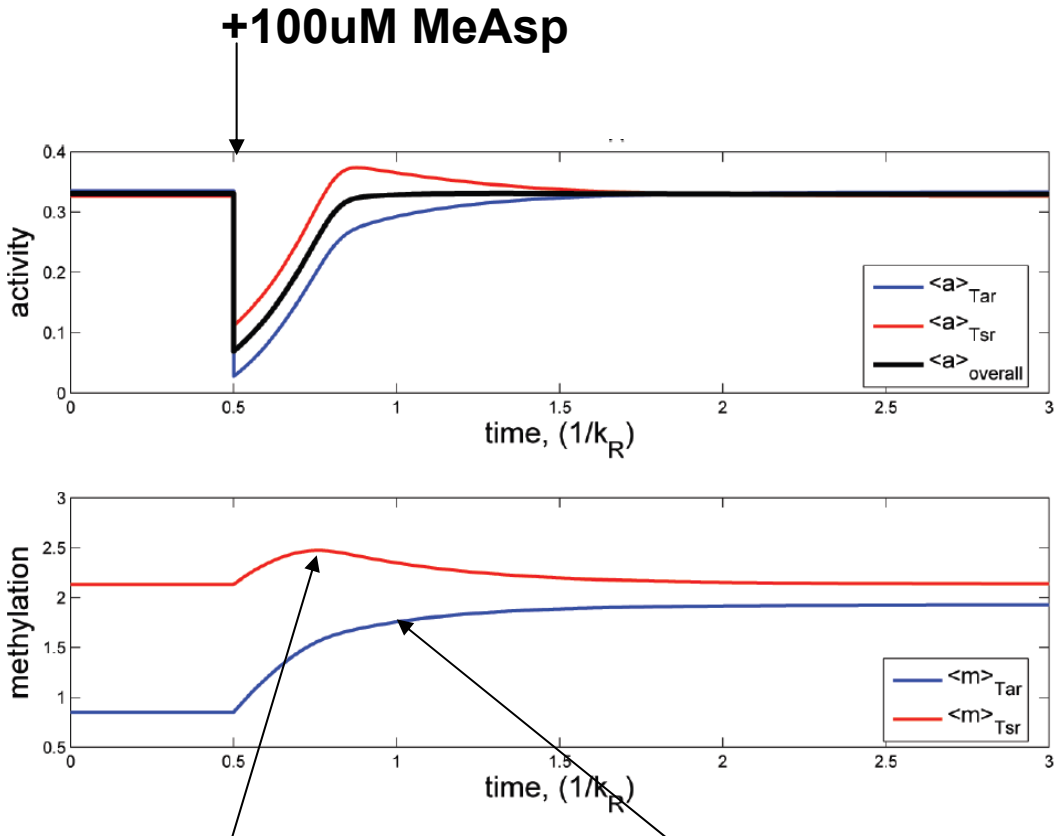


Perfect adaptation
No methyl-crosstalk

Inaccurate adap.
Methyl-crosstalk

- Only methylation level of Tar changes in response to MeAsp when adaptation is accurate, no crosstalk.
- Methylation crosstalk occurs only when accurate adaptation fails.

The adaptation kinetics in the local model



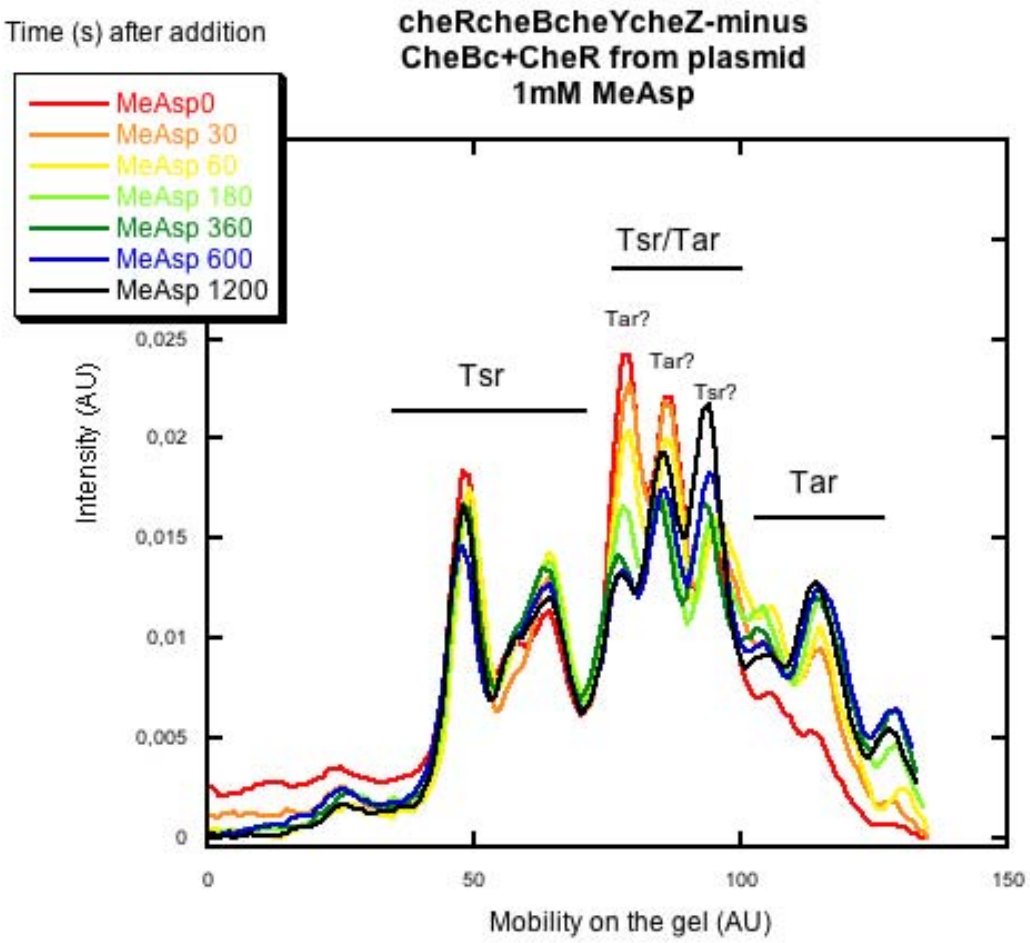
Methylation of the un-hit receptor Tsr increases initially before going back to its original value.

Methylation of the hit receptor Tar increases monotonically to its final value.

Some experimental evidence

Time series of receptor methylation states after addition of stimuli
(From Sourjik lab)

+1mM MeAsp

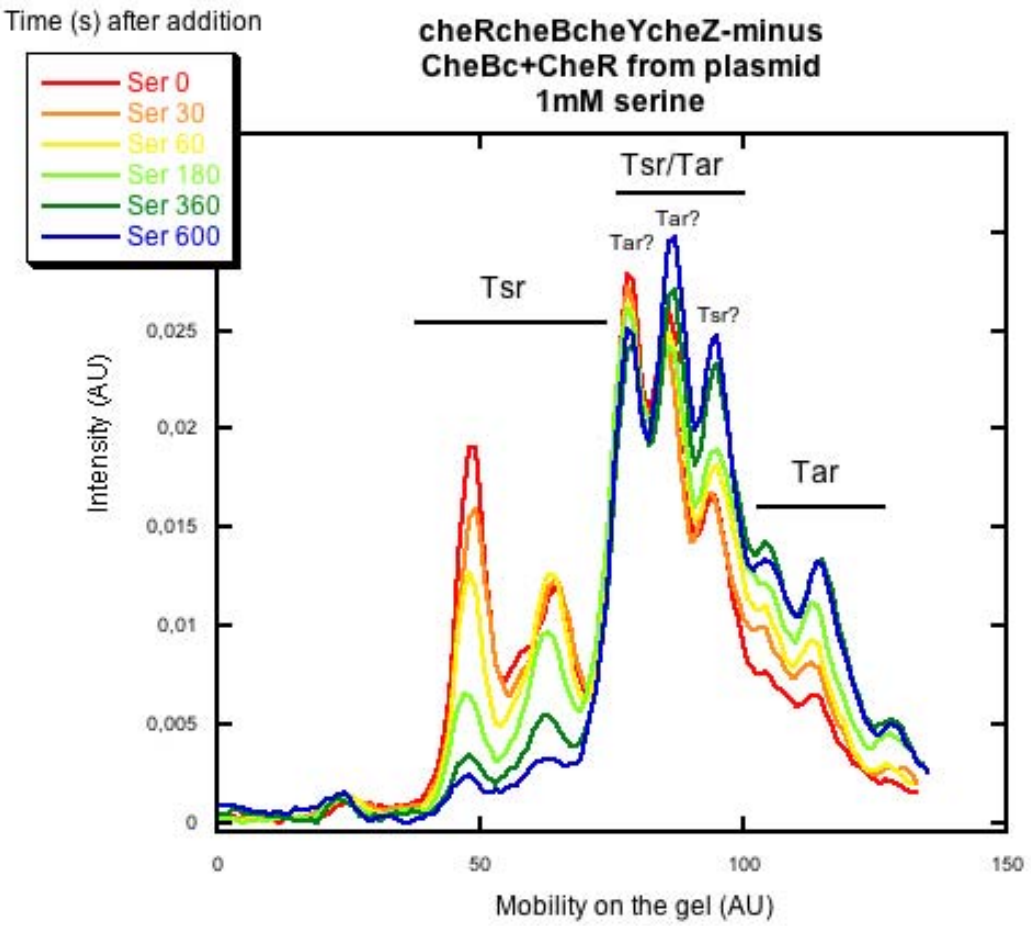


No methylation cross talk by adding MeAsp

More experimental evidence

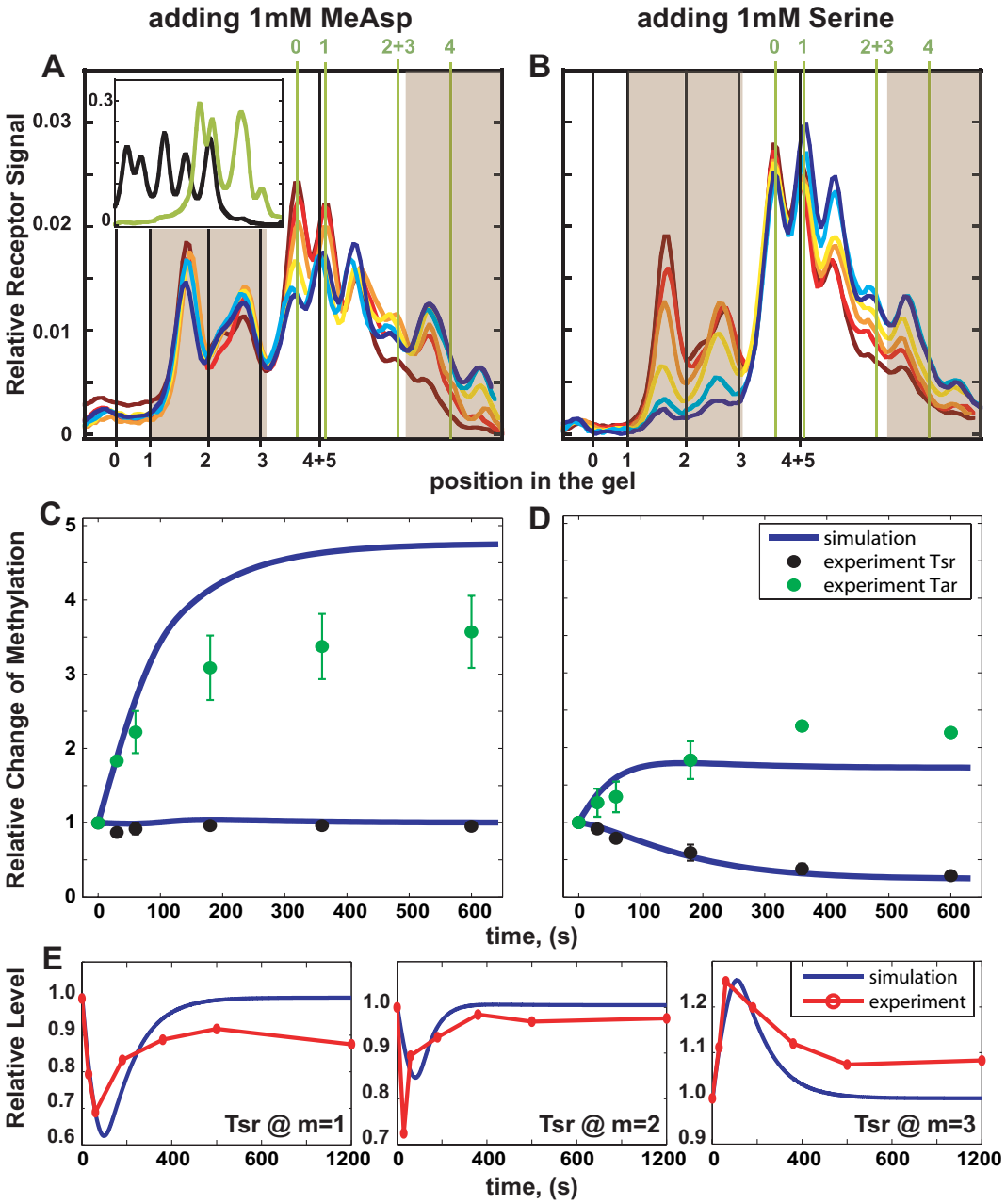
Time series of receptor methylation states after addition of stimuli

+1mM serine

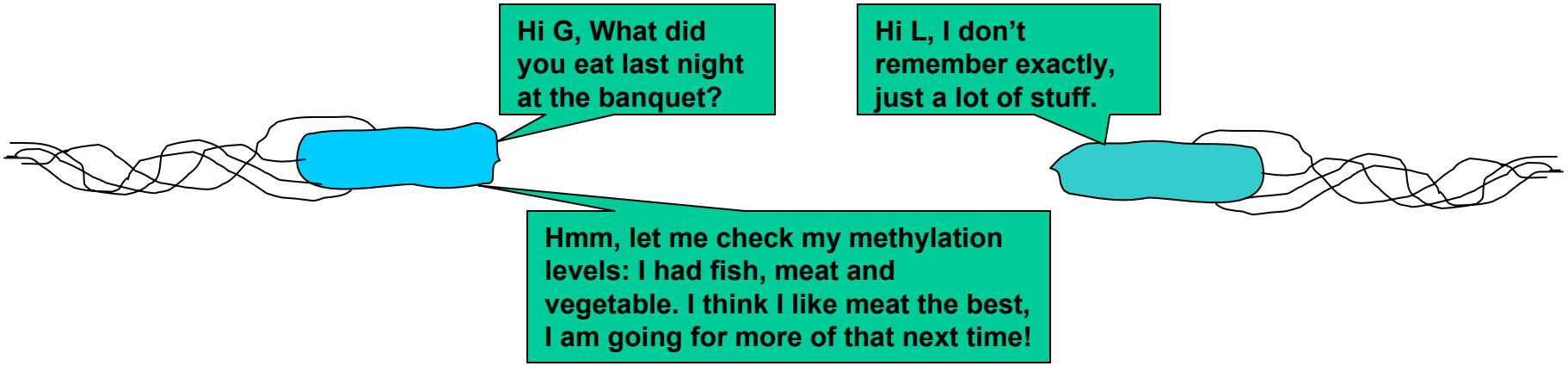


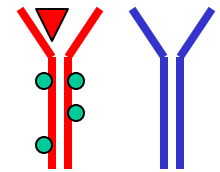
Tsr responds primarily (strongly).
Tar also responds, because the cell does not adapt perfectly to Serine.

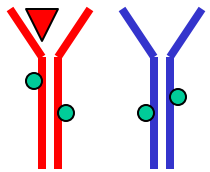
Comparison with the local adaptation theory (model)



The advantage of having a clear memory







Local field in Ising model

0 0

h -h

Average Activity <a>

$$\frac{1}{2} \quad (\because h = 0)$$

$$\frac{1}{2}\left[\frac{e^{-h}}{e^{h}+e^{-h}}+\frac{e^{h}}{e^{h}+e^{-h}}\right]=\frac{1}{2}$$

Sensitivity (susceptibility) d<a>/dh

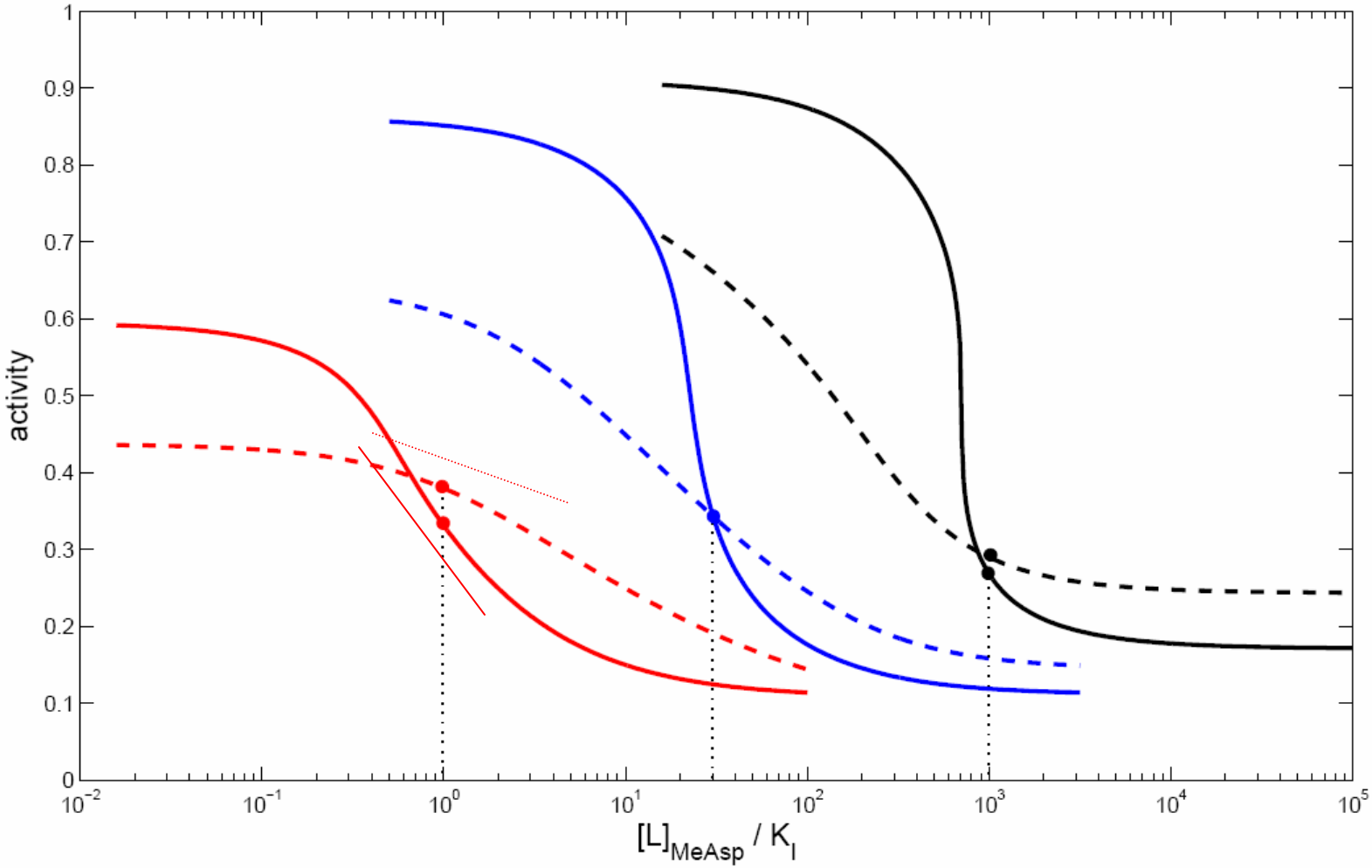
$$\frac{1}{4}$$

$$\left(e^{h}+e^{-h}\right)^{-2} \le \frac{1}{4}$$

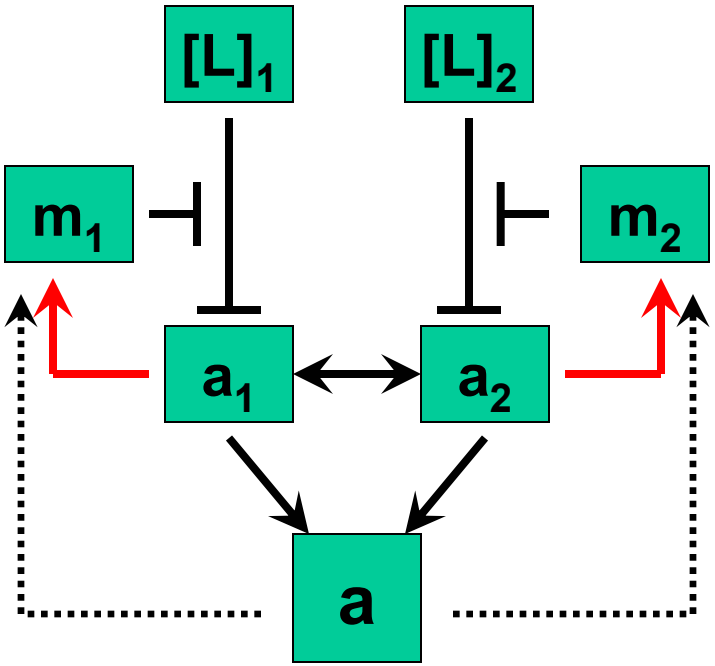
Heightened sensitivity with local memory

Solid lines --- Local model; Dashed lines – Global model
(3 different background MeAsp concentrations)

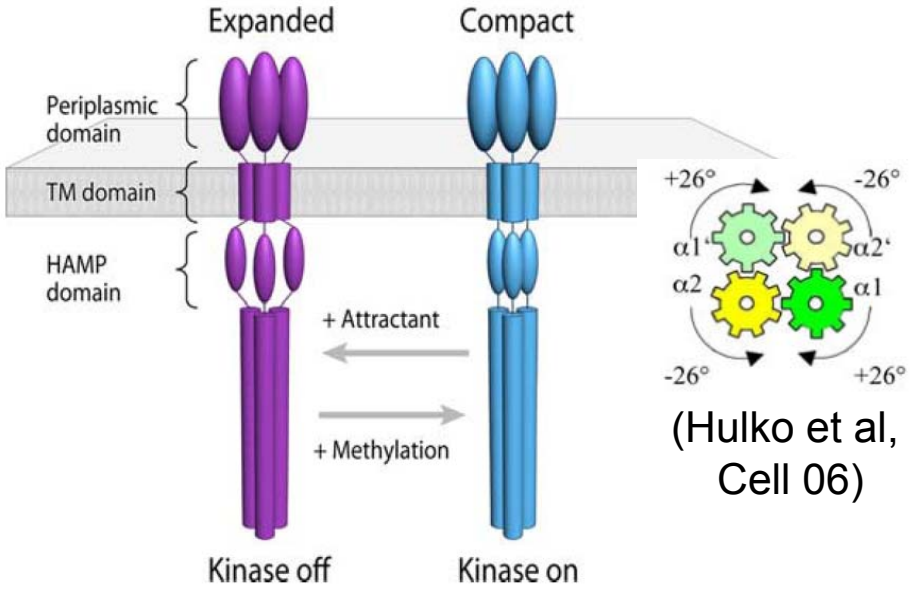
A.



Local conformational change of individual receptor controls its methylation dynamics



Local conformational change
HAMP domain?



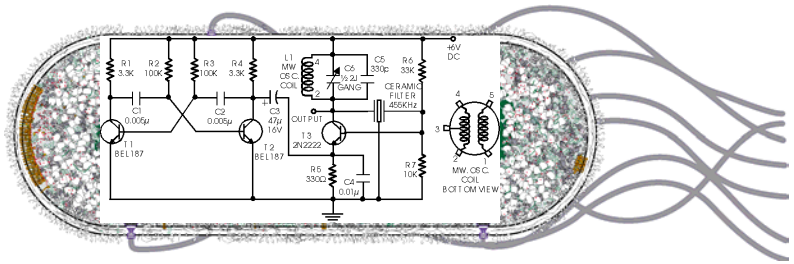
(Hulko et al, Cell 06)

(Khursigara et al, PNAS '08)

Ising model, where each receptor is assigned a local order parameter, is better suited to describe the methylation dynamics for mixed signals, than the all-or-none MWC model.

Summary

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

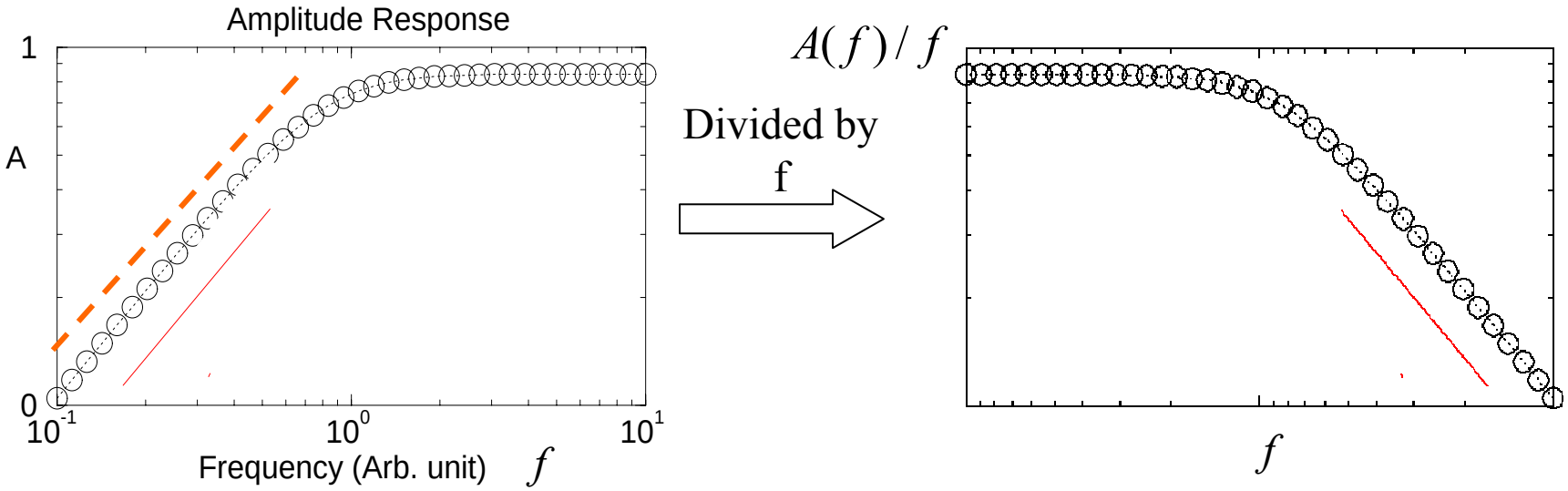
- Responses depend on $\Delta[L]/[L] = \Delta(\ln[L])$

  The Weber-Fechner Law in sensory system

- Information compression: wide range of concentration, limited scale of methylation levels.

3) It is a low pass filter for the derivative of the signal

Compute derivative of the input in low frequency regime



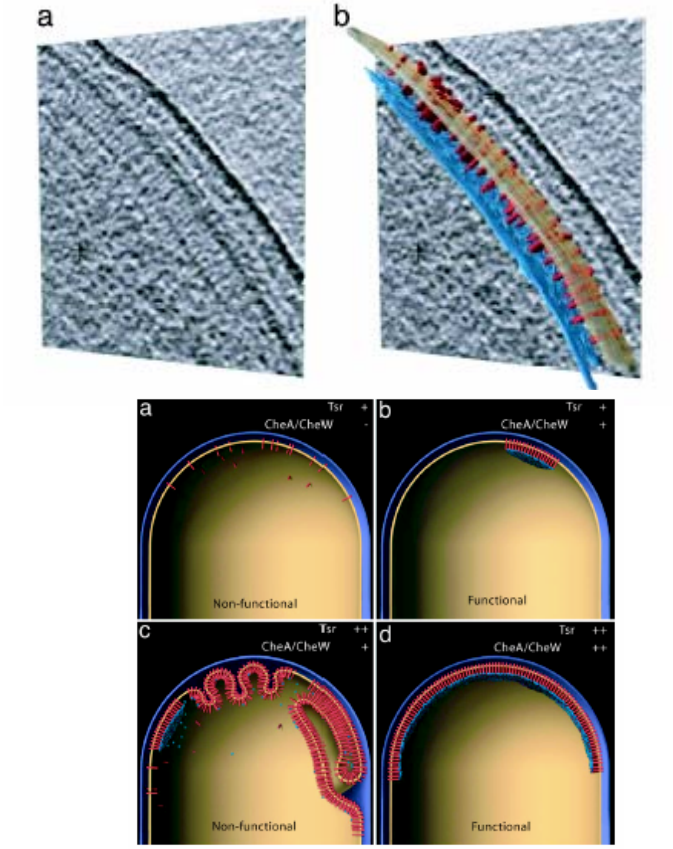
4) It records information on different ligand by the methylation levels of the corresponding receptors

Local memory; Global action

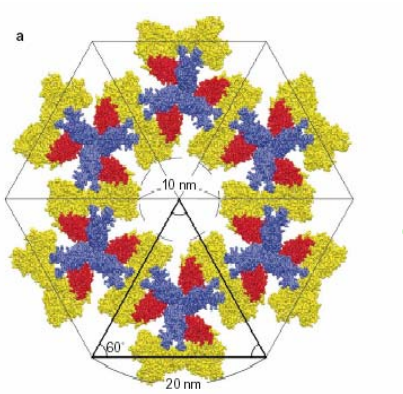
Some remaining challenges

At the molecular level

1)What's the structure of the cluster? How do they form? What affects the formation of the functional complex? What's the role of Cell membrane? Role of CheW and CheA?



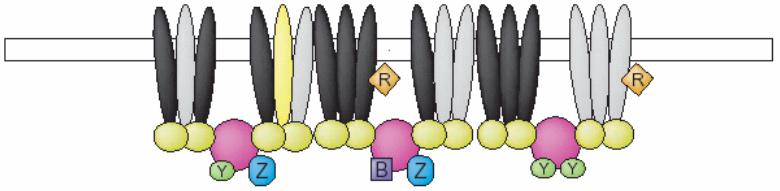
(S. Subramaniam Lab)



Ordered?

(shimitzu et al, 2000)

Disordered?



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

At the systems 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

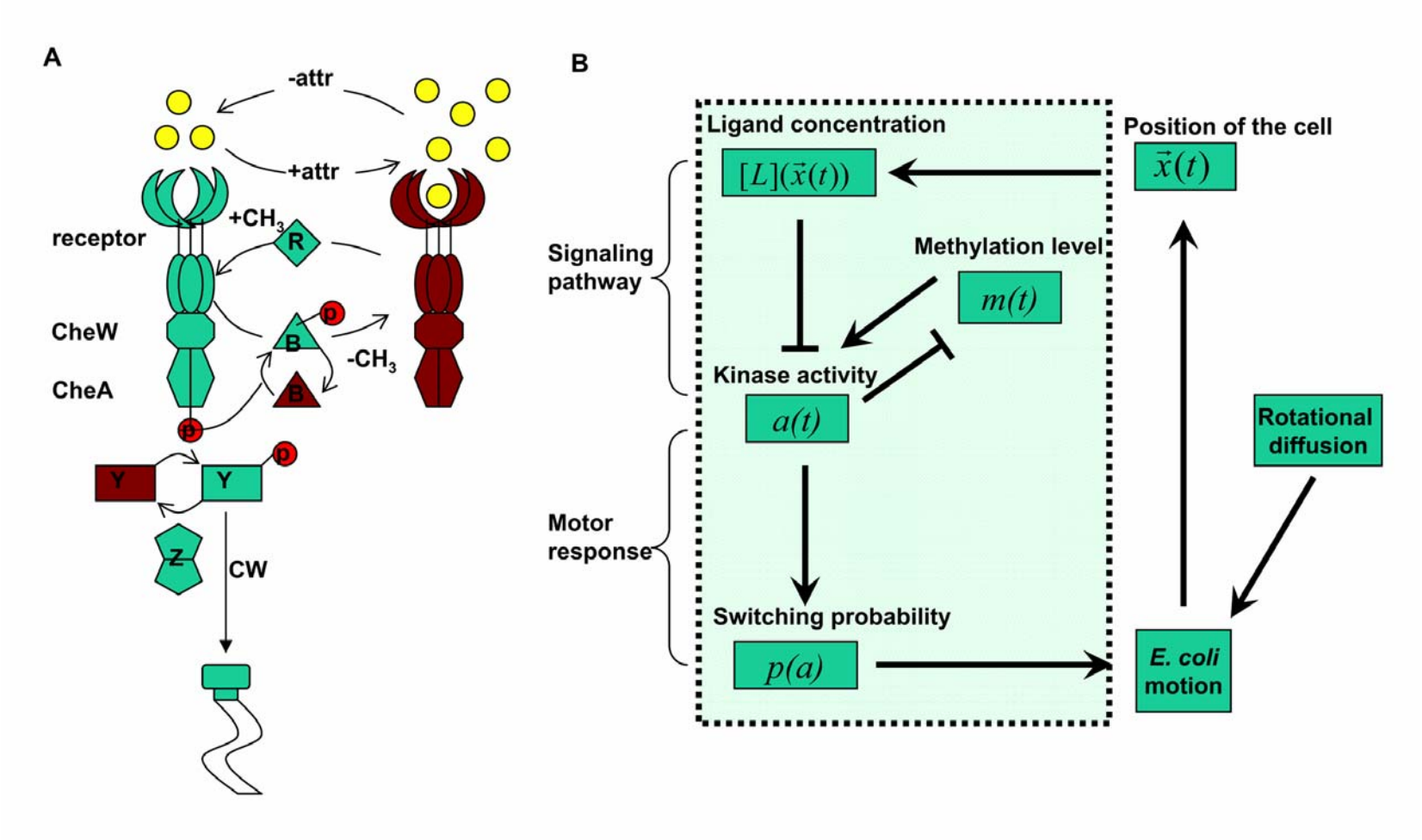
- **Why such a large gain? What about noise?**

  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.

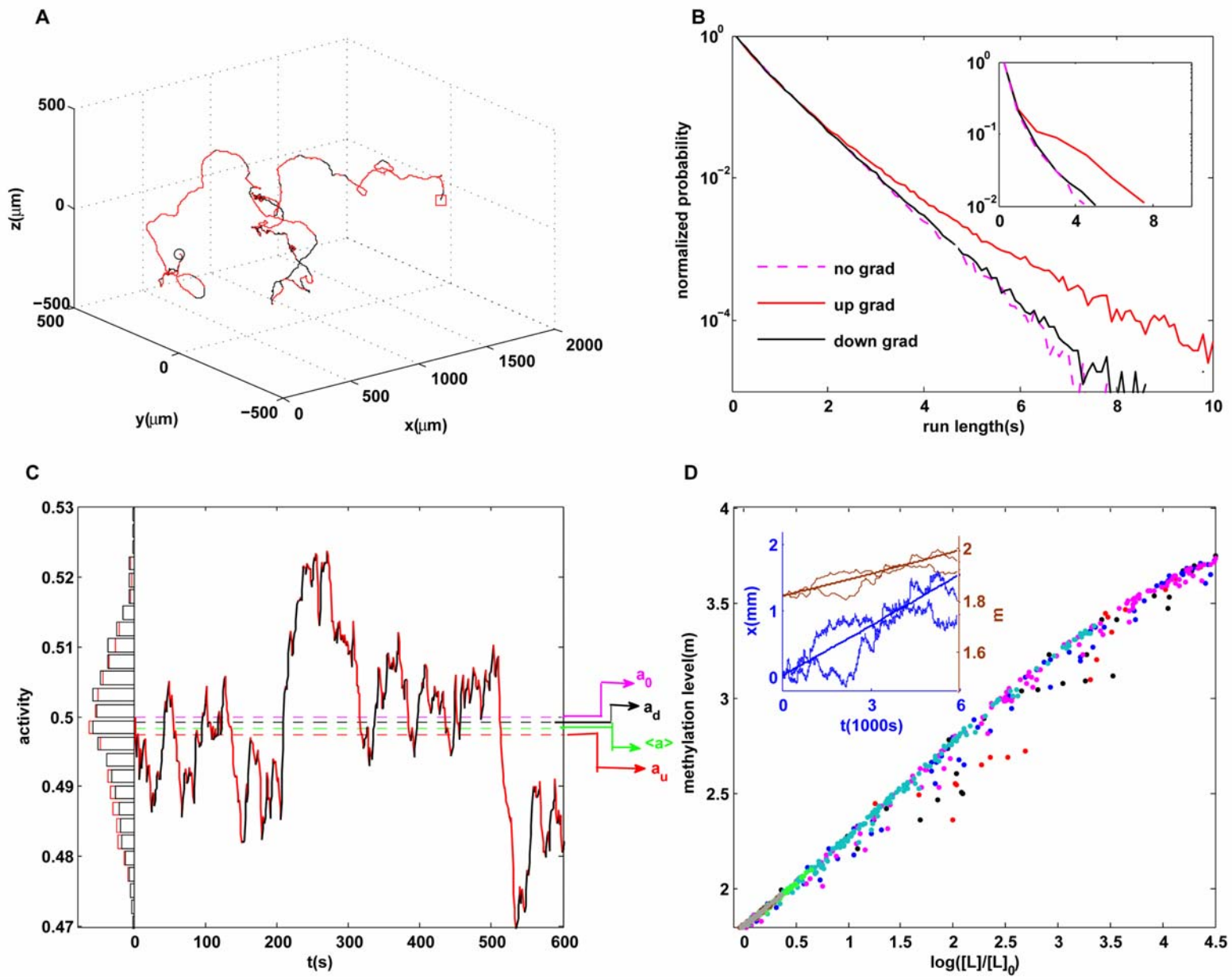
Thank You

From molecular pathway to understand behavior (cell motion)

From molecules to behaviors: A *E. coli* chemotaxis model based on intracellular signaling pathway dynamics



The single cell behavior

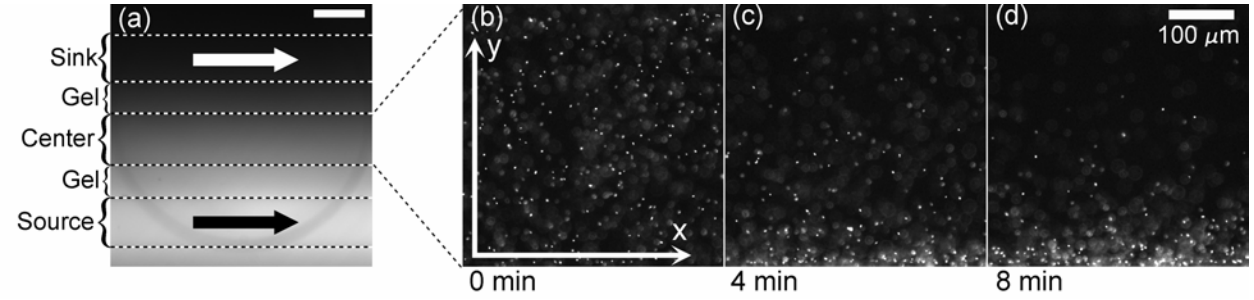


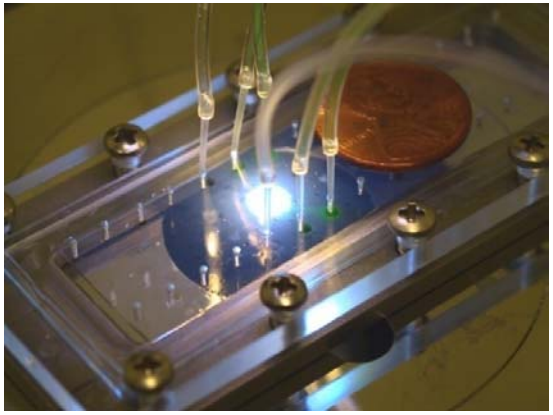
Comparison with microfluidics experiments

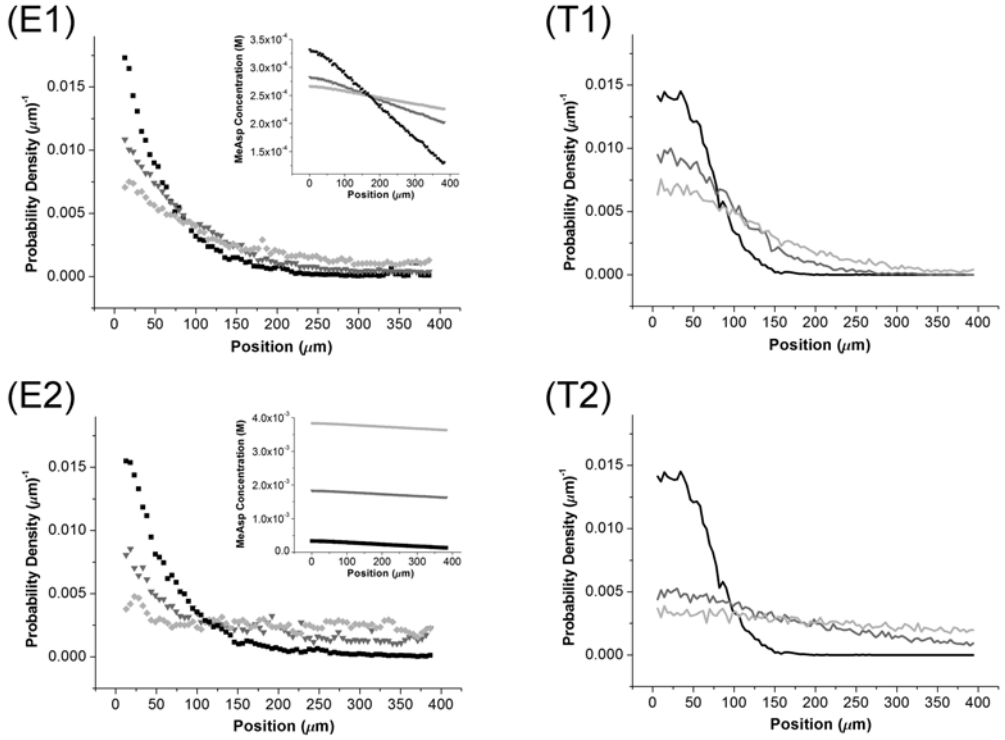
What happen to a cell when it is moving in a spatial profile (gradient, traps, etc.)

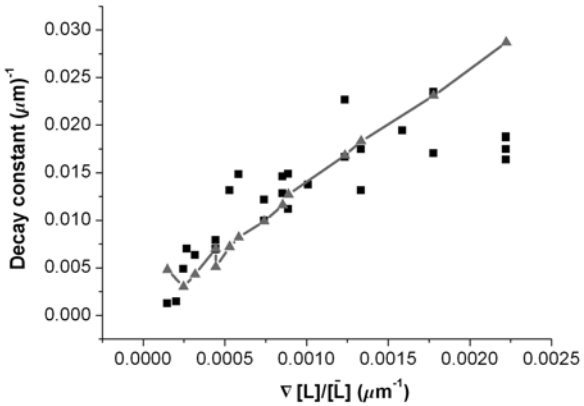
Direct comparison with quantitative microfluidics experiments

(Done with M. Wu lab in Cornell)









Comparison with the classical capillary experiments