Design Principles of a Bacterial Signalling Network

Why is chemotaxis more complicated than needed ?

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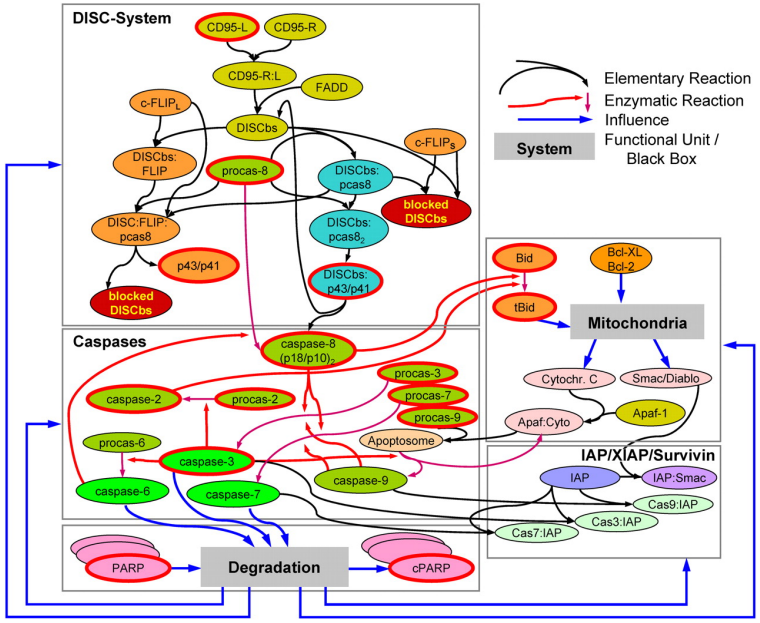
Outline

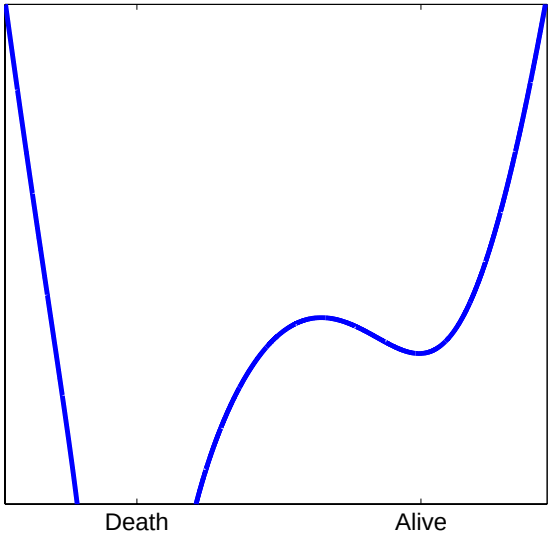
- **The Eighth Question**
- **Bacterial Chemotaxis**
- **Barkai/Leibler Model**
- **Cell-to-Cell Variability**
- **Design Principles of Robustness**

Examples of Networks I: Apoptosis

Pathway cartoon



System's behavior

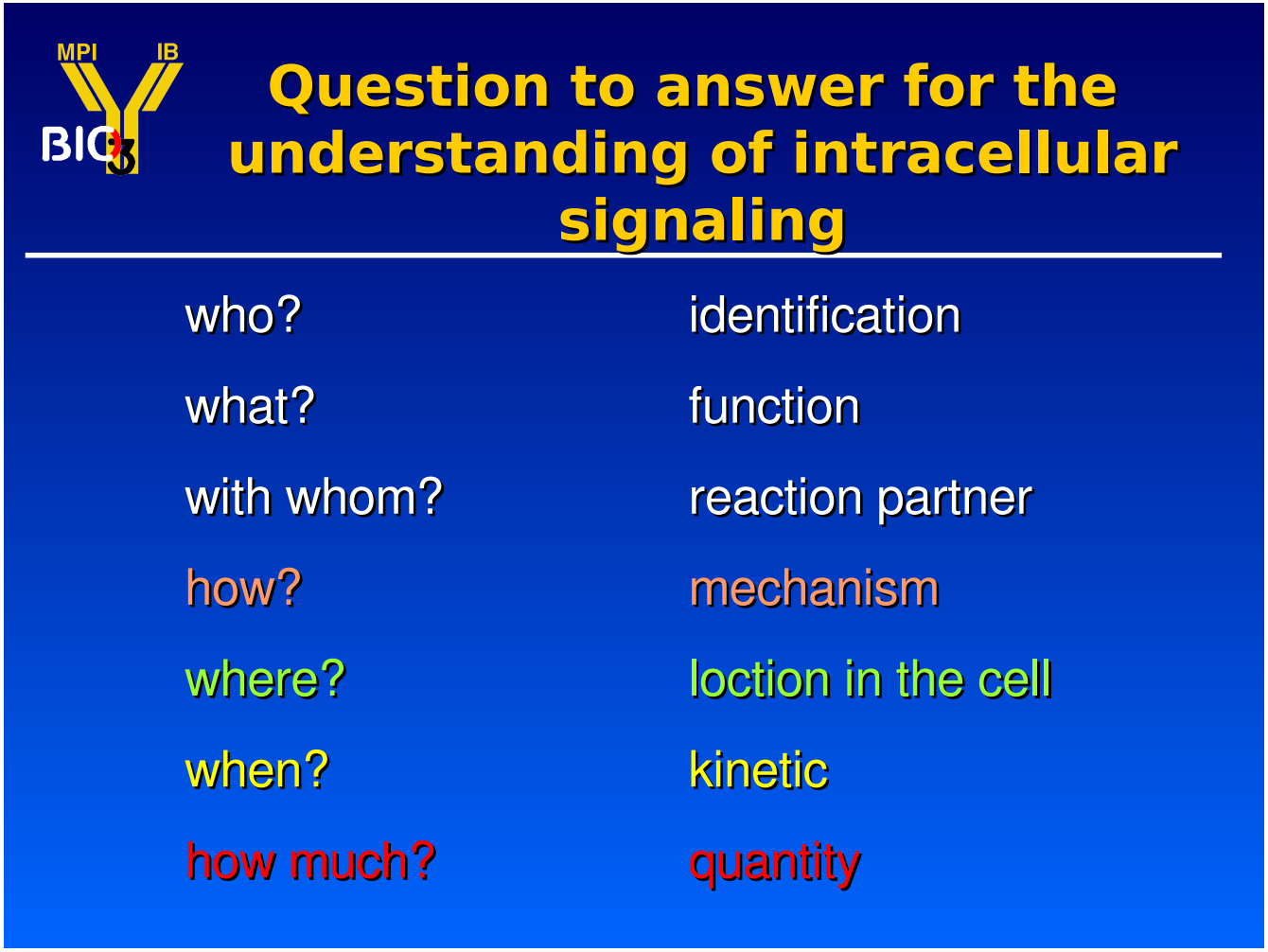


Threshold behavior, one-way bistable

Why Mathematical Modelling in Biology ?

- Make assumptions explicit
- Understand essential properties, failing models
- Condense information, handle complexity
- Understand role of dynamical processes, e.g. feed-back
- Impossible experiments become possible
- Prediction and control
- Understand what is known
- Discover general principles
- "You don't understand it until you can model it"

Michael Reth's Seven Questions



Courtesy of Michael Reth

Two Differences between Physics and Biology

Physics: Understand the empirical world by mathematics

- **Fundamental laws of nature vs. principles**
- **In biology there is "function" due to evolution**

Physics in biology = Systems Biology:

Understand function by mathematics

The Eighth Question

WHY ?

Why is chemotaxis more complicated than needed ?

Bacterial Chemotaxis – The Phenomenon

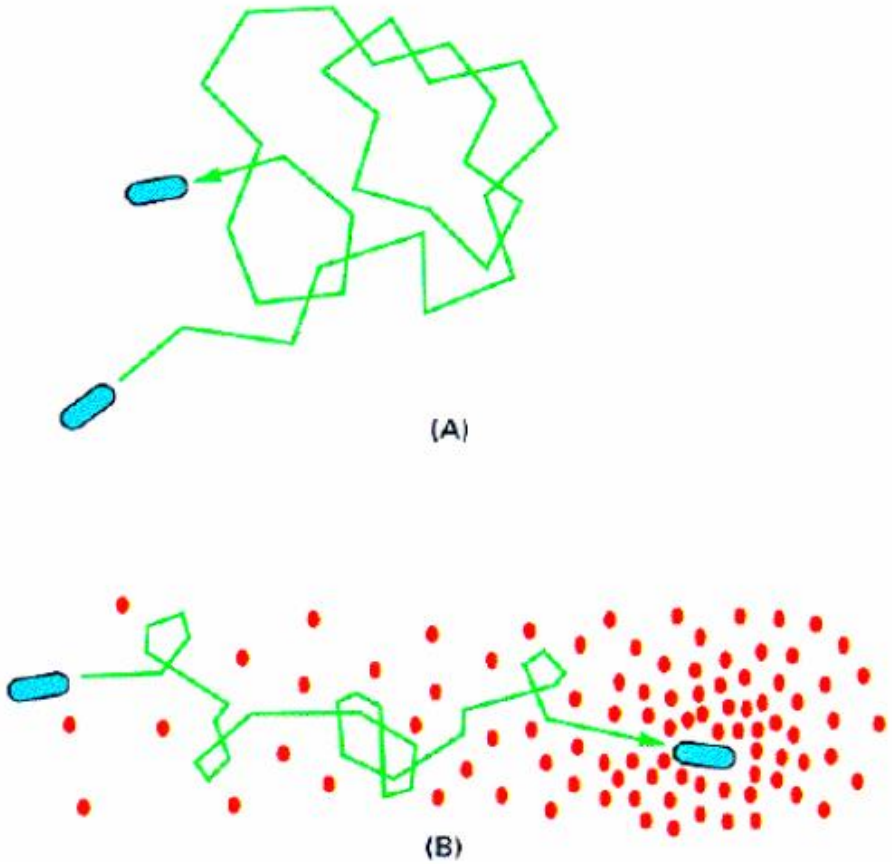
- **Bacteria sense nutrient gradients over four orders of magnidute of absolute concentration**
- **Detect relative changes of 2 %**

Chemotaxis: One of the best investigated biological systems

Bacterial Chemotaxis – The Strategy

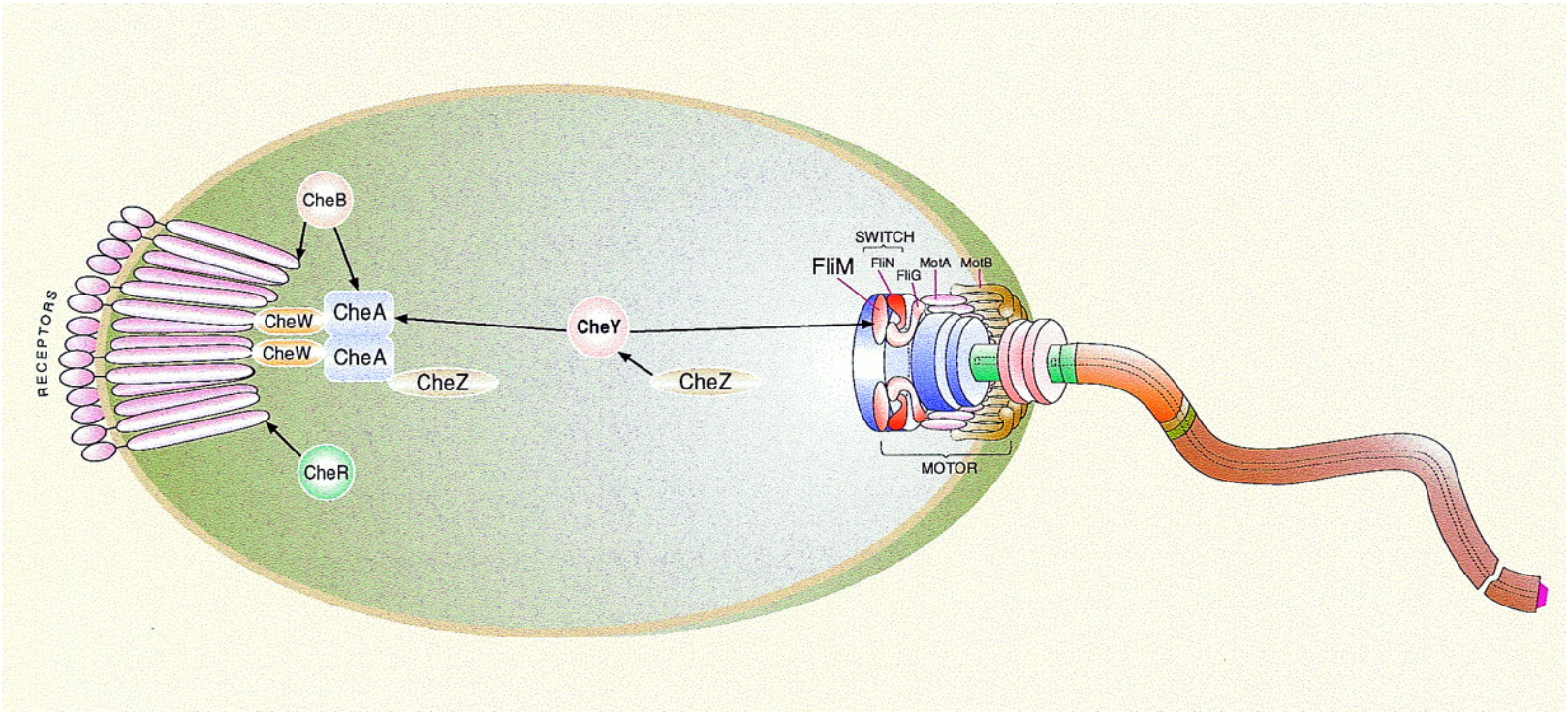
- Bacteria too small to compare front to end
- Strategy:
  - Change direction from time to time (tumble)
  - If concentration increases: reduce tumbling frequency
  - If concentration decrease: increase tumbling frequency
- Sense spatial gradients by temporal changes

Chemotaxis – Tumble and Swim



Random walk vs. biased random walk

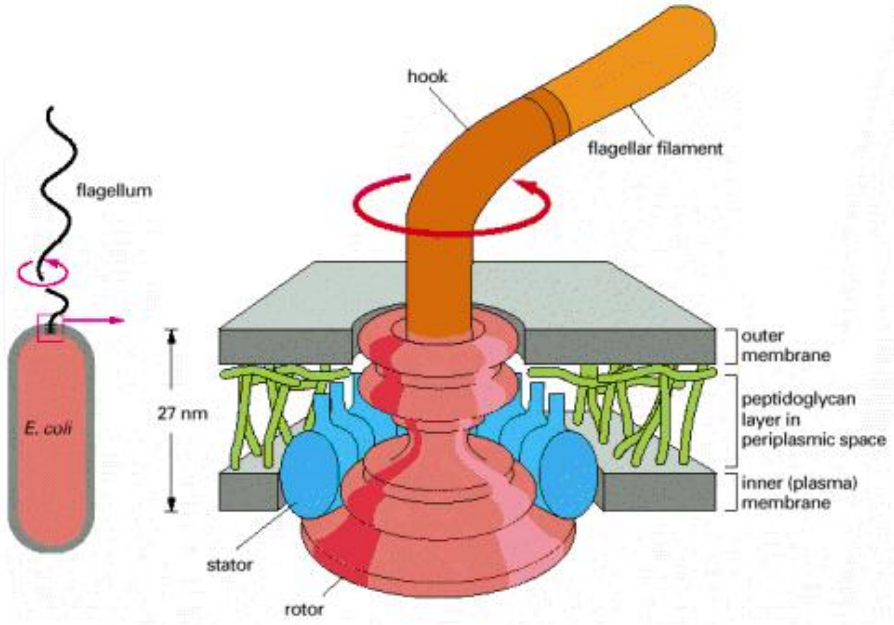
Chemotaxis in E. coli



Chemotaxis – Flagella

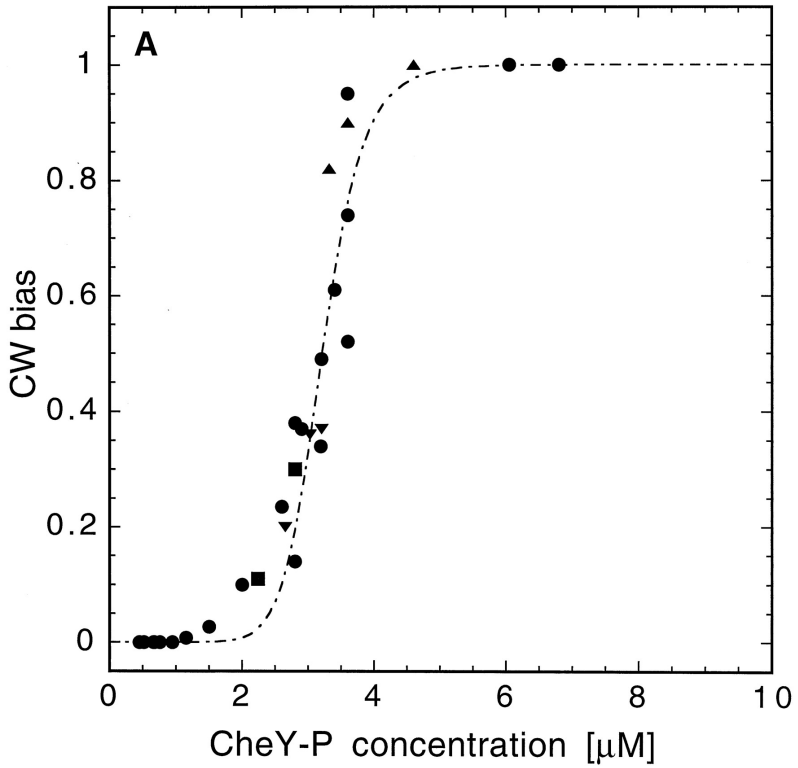
Movement by rotating corkscrew-flagella

- **counter-clockwise: form bundle: swim by marine propeller**
- **clockwise: rotate radially: tumble**



Chemotaxis – The Task

Tumbling/Swimming depends on phosphorylated CheY



Important: A small working range

Chemotaxis – Adaptation

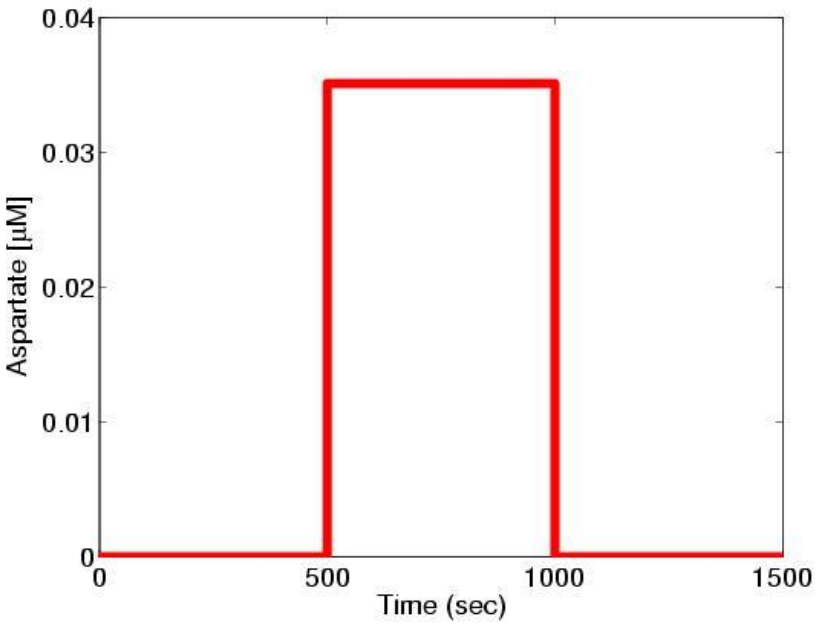
- **Motor has a small range of sensitivity**
- **Cell is chemotactic for a large range of concentrations**

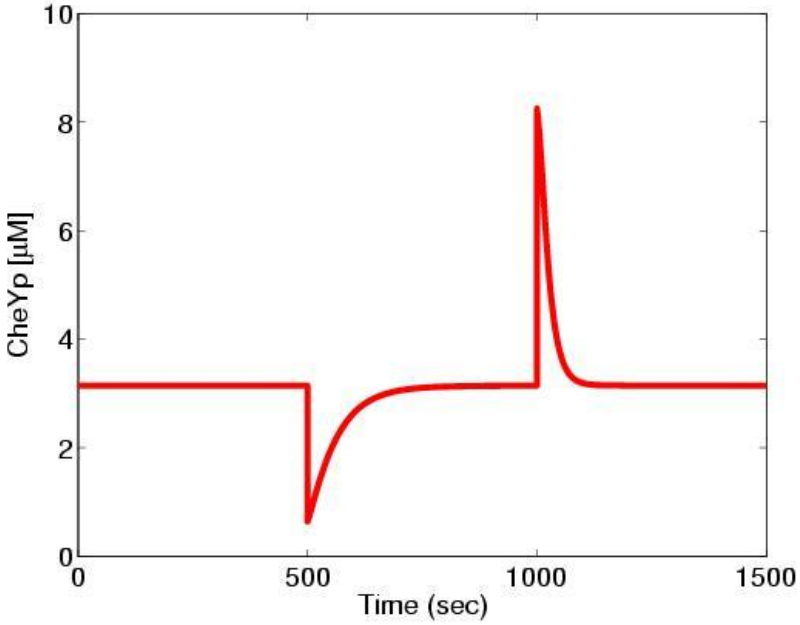
$\Longrightarrow$ **System has to be adaptive:**

Steady state of CheYp must be independent from absolute concentration of ligand

Chemotaxis – The Task

Input: Nutrient concentration Output: Tumbling frequency



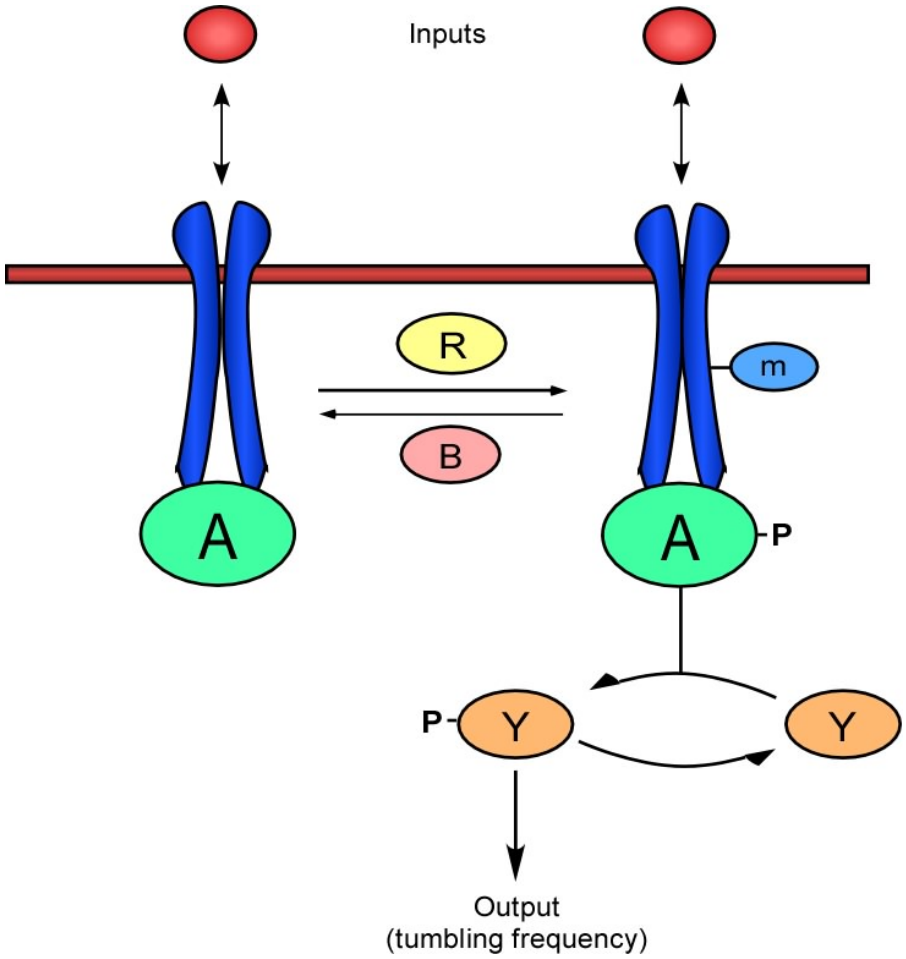


System performs a kind of differentiation

The Players and their Roles

- **T: Receptors**
- **CheR: Methyltransferase, adds CH_3**
- **CheB: Methylesterase, removes CH_3**
- **CheA: Kinase, adds PO_4**
- **CheZ: Phosphatase, removes PO_4**
- **CheY: Signaling protein**

Barkai/Leibler Model – Graphical Version



Barkai/Leibler Model – Mathematical Version

Probability for activating methylated receptor by ligand L:

$$p = \left(1 - \frac{L}{K_L + L}\right)$$

Concentration of activated receptors T_a :

$$T_a = p\, T_m$$

Methylation/demethylation dynamics of receptors:

$$\dot{T}_m = k_R R - k_B B\, \frac{T_a}{K_B + T_a}$$

Dynamics of A_p:

$$\dot{A}_p = k_A(A_{tot} - A_p)T_a - k_Y A_p(Y_{tot} - Y_p)$$

Dynamics of Y_p:

$$\dot{Y}_p = k_Y A_p(Y_{tot} - Y_p) - \gamma_Y Y_p$$

Perfect Adaptation by $T_a = p(L)\, T_m(T_a)$

Steady state of T_a from

$$\dot{T}_m = k_R R - k_B B \, \frac{T_a}{K_B + T_a} = 0$$

yields

$$T_a^{ss} = K_B \, \frac{k_R R}{k_B B - k_R R}$$

- **Independent from ligand concentration L**
- **Steady state is stable**
- **The same holds for Y_p**

Barkai & Leibler, Nature 387:913, 1997

The Mechanism: $T_a = p(L)\, T_m(T_a)$

- **Increasing L leads to fast decrease of T_a**
- **Ap & Yp are fastly dephosphorylated**
- **T_m is slowly increased**
- **Turns T_a and Ap & Yp back to steady state**
- **Integral negative feedback control**

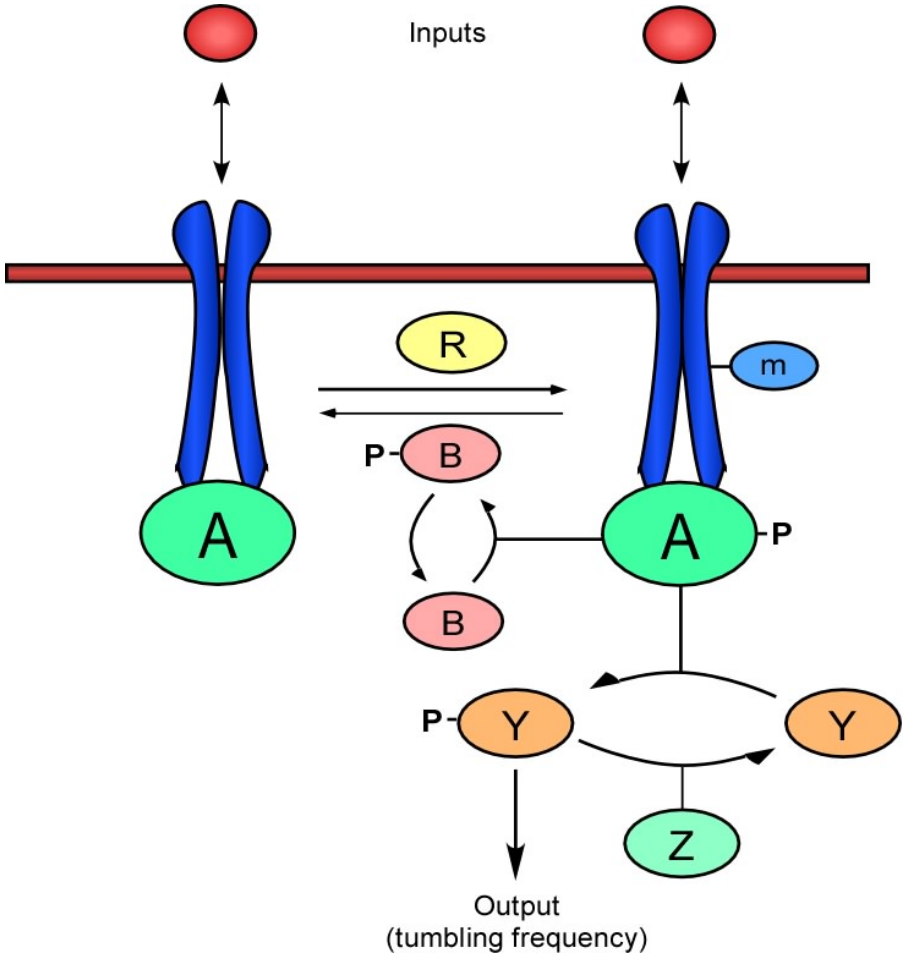
In words:

Degree of methylation compensates/remembers absolute concentration of ligand

But ...

... this model is not realised by nature

Nature's E. Coli



Sources of Variability

- **Intrinsic noise**

  Differences between identical reporters within one cell

  – **Stochasticity of reactions**

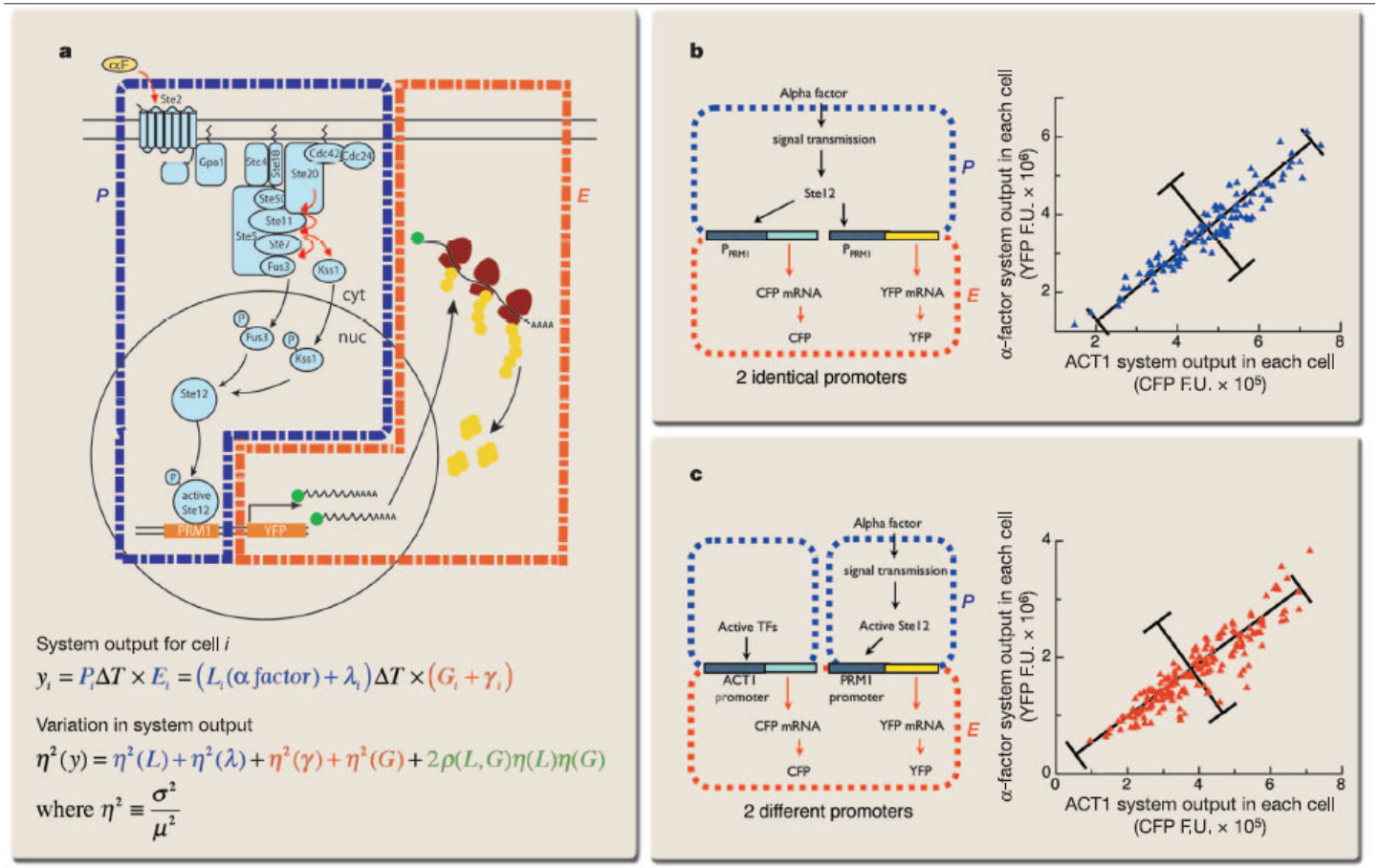
- **Extrinsic noise**

  Differences between identical reporters in different cells

  – **Expression level of signaling proteins**
  – **Number of ribosomes**

  Cell-to-cell variability

Quantification of Variability



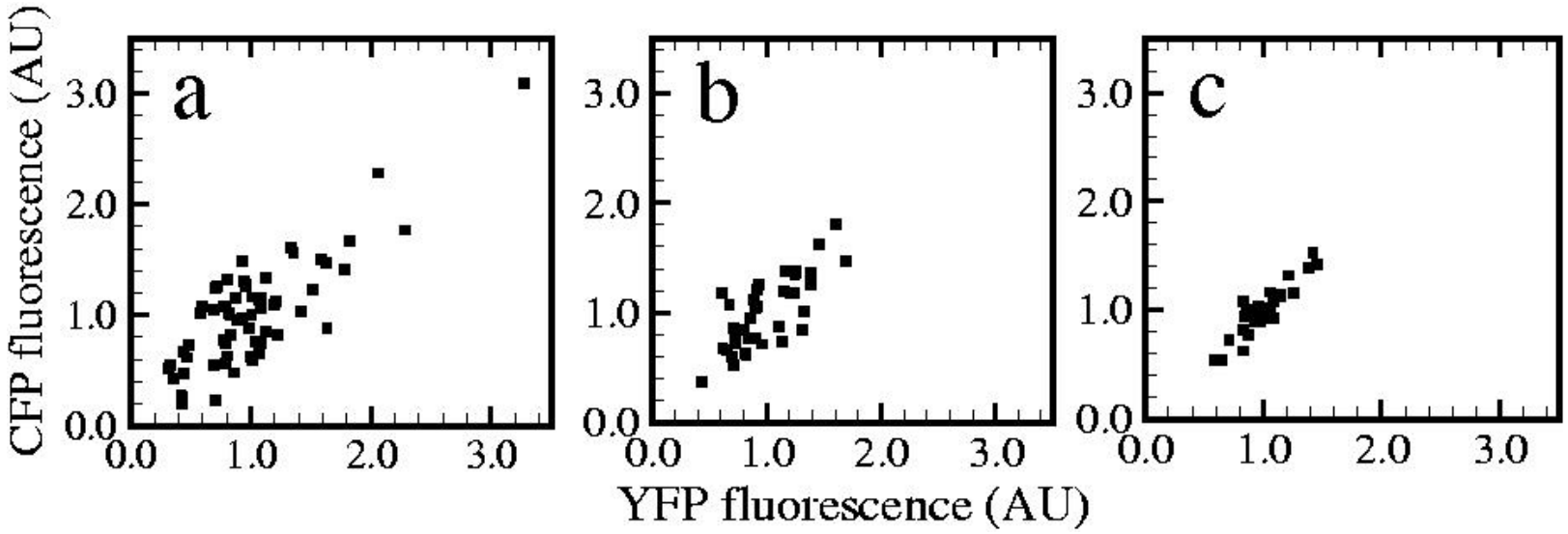
Colman-Lerner et al. Nature 437:699, 2005

Results

E. coli and yeast:

- **Extrinsic noise is larger than intrinsic noise**
- **Protein concentrations fluctuate in a correlated manner**

Fluctuations and Chemotaxis



- Cell-to-cell fluctuations up to factor of ten
- Correlated fluctuations are dominant

A Robustness Principle

The functionality of a pathway must be robust against fluctuations of protein levels.

For chemotaxis:

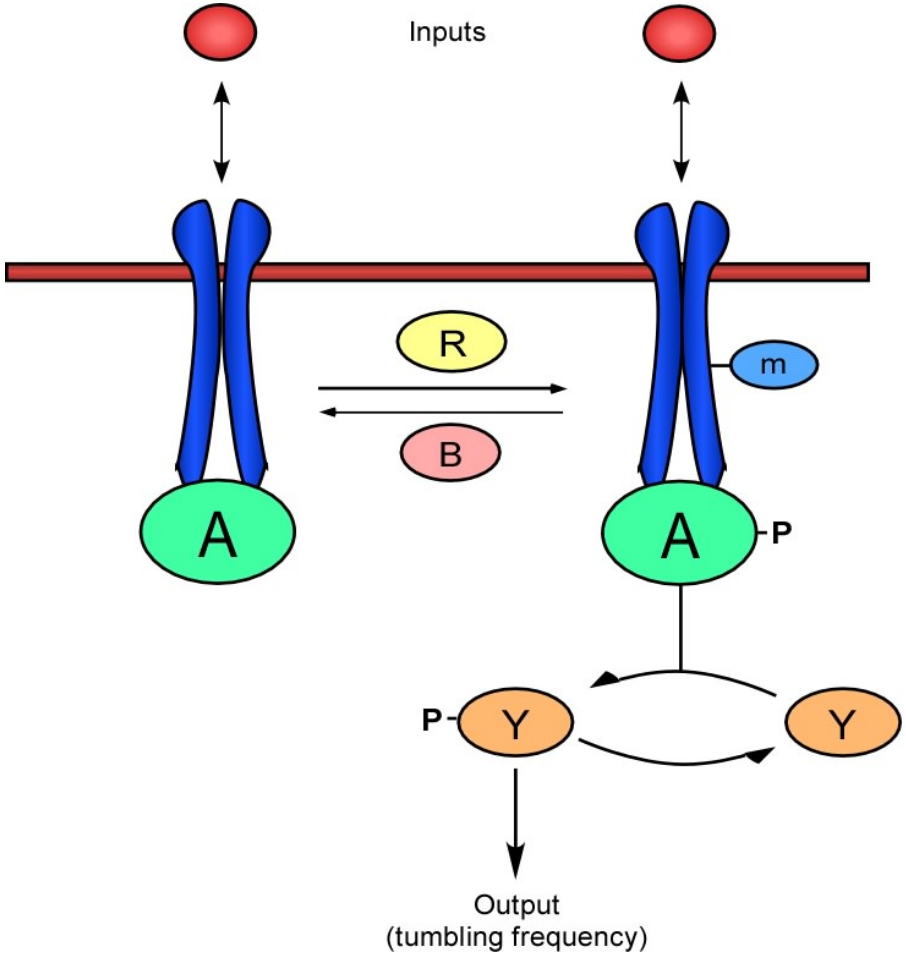
- **Steady state level Y_p in [2.2 μM, 4.3 μM]**
- **For correlated fluctuation:**

  Steady state invariant under transformation: $X_i \to \lambda X_i$

  Important quantities may only depend on ratios of concentrations
- **For uncorrelated fluctuations:**

  Use negative feedback-loop to attenuate noise

Application to Barkai/Leibler Model



Robustness of Barkai/Leibler Model

Steady states:

$$T_a^{ss} = K_B \frac{k_R R}{k_B B - k_R R} \qquad \textbf{o.k.}$$

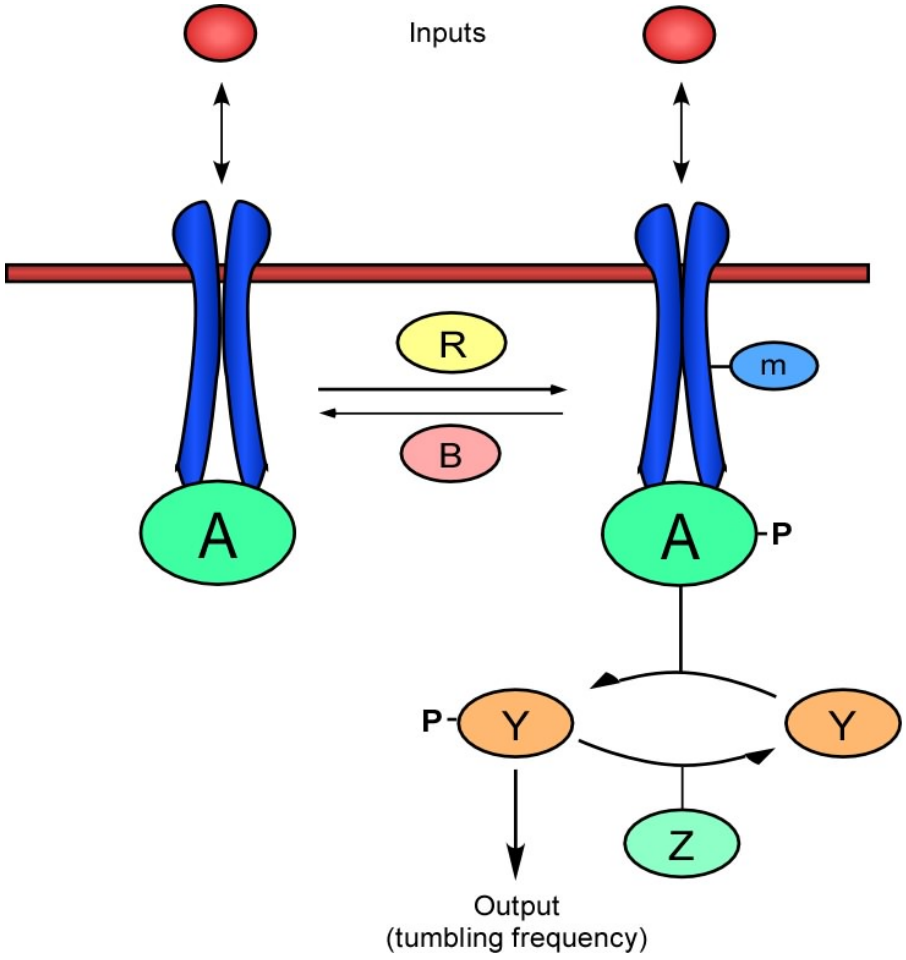
$$A_p^{ss} \approx \frac{k_A T_a^{ss}}{k_Y} \frac{A_{tot}}{Y_{tot}} \qquad \textbf{o.k. (w.o.a)}$$

$$Y_p^{ss} = \frac{k_y A_p^{ss}}{k_Y A_p^{ss} + \gamma_Y} Y_{tot} \qquad \textbf{not o.k.}$$

Cure: Y_p must have a phosphatase ($CheZ$)

$$Y_p^{ss} = \frac{k_y A_p^{ss}}{k_Z} \frac{Y_{tot}}{Z_{tot}} \qquad \textbf{o.k.}$$

Extension of the Model



Robustness Against Correlated Fluctuations

- Y_p **must have a phosphatase (**$CheZ$**)**
- **Methyltransferase** $CheR$ **has to work at saturation**
- **The pathway must be weakly activated,** $X_p \ll X_{tot}$

Robustness Against Uncorrelated Fluctuations

Diminish uncorrelated noise by a classical negative feedback

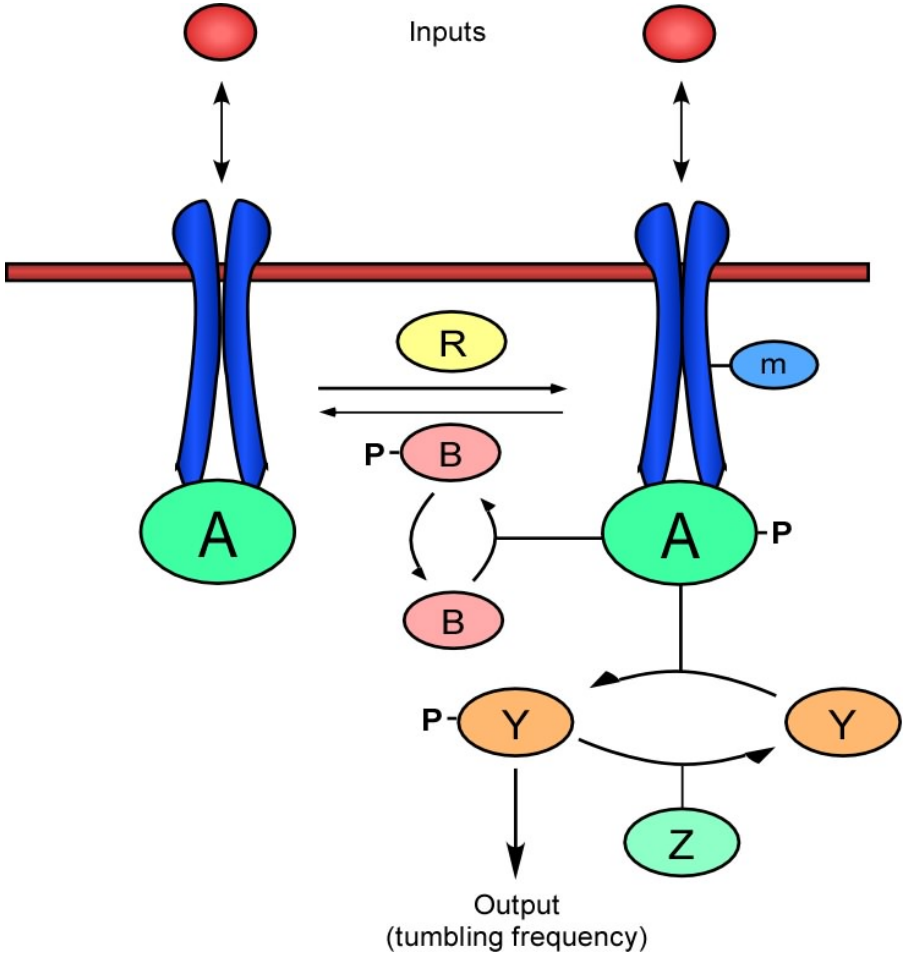
- **Methylesterase B can be phoshorylated by A_p**
- **Only B_p can demethylate receptors**

$$\Delta Y_p = -\frac{\frac{\partial f}{\partial T_a}\frac{\partial T_a}{\partial R}}{\alpha + \beta \frac{\partial B_p}{\partial A_p}} \Delta R$$

- **Robustness against correlated fluctuations:**

  $\Longrightarrow$ **B_p must <u>not</u> have a phosphatase**

Final Model



And this is how E. coli looks like

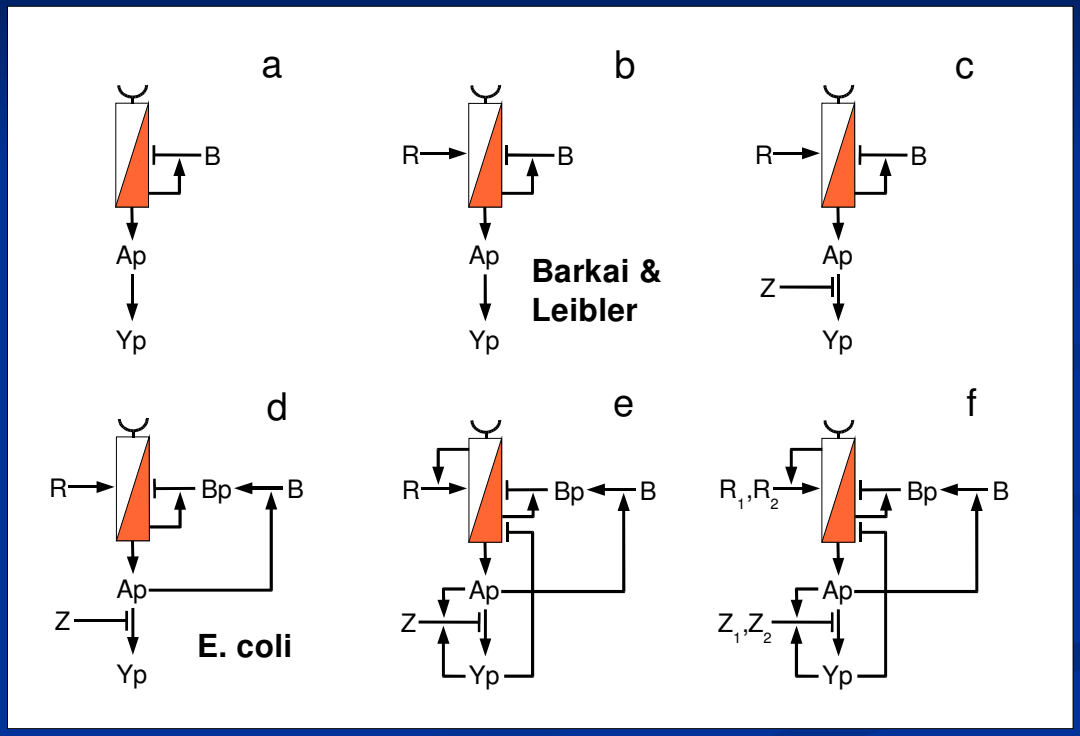
In silico Biology

Is nature's solution optimal ?

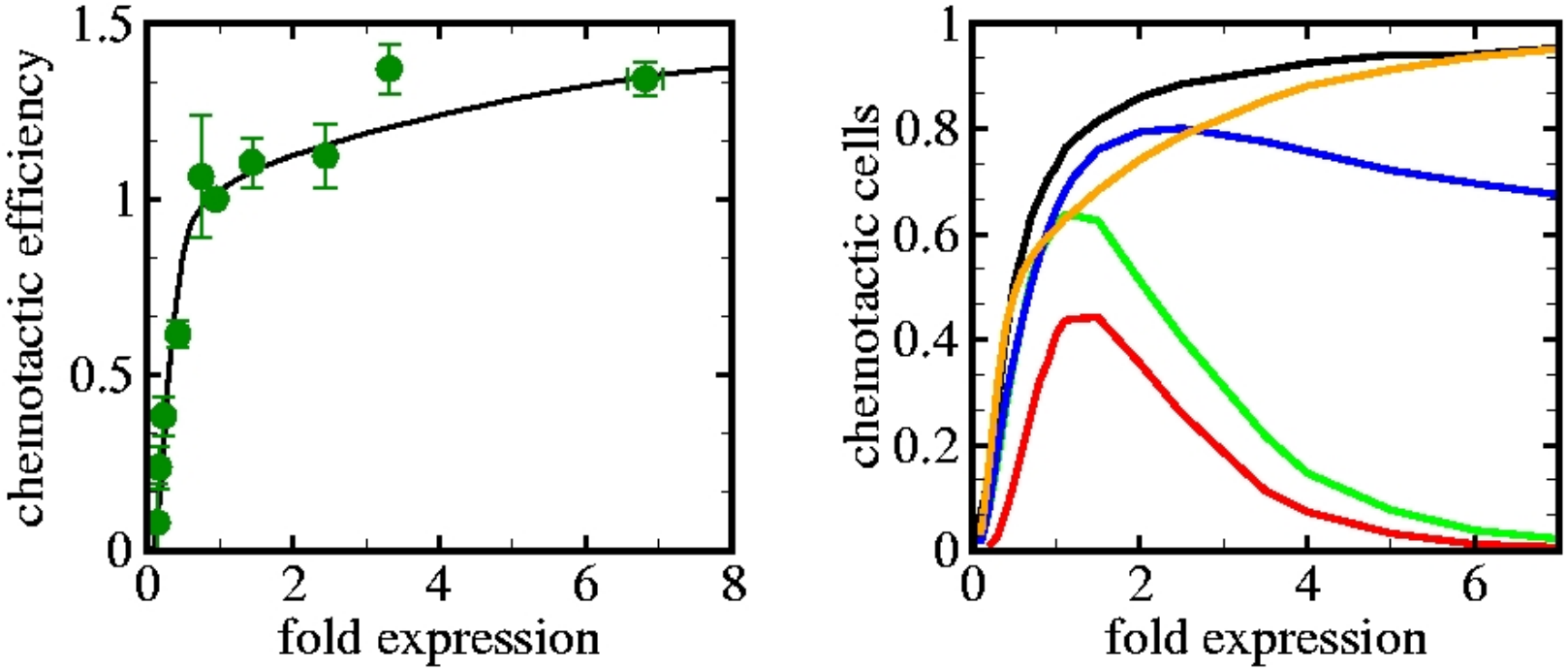
- **Choose different chemotactic pathway topologies**
- **Protein concentrations from experimental distributions**

Compare chemotactic behaviour of *in silico* mutants to *in vivo* E. coli for different expression levels of proteins

Cartoons of Perfect Adaptive Pathways



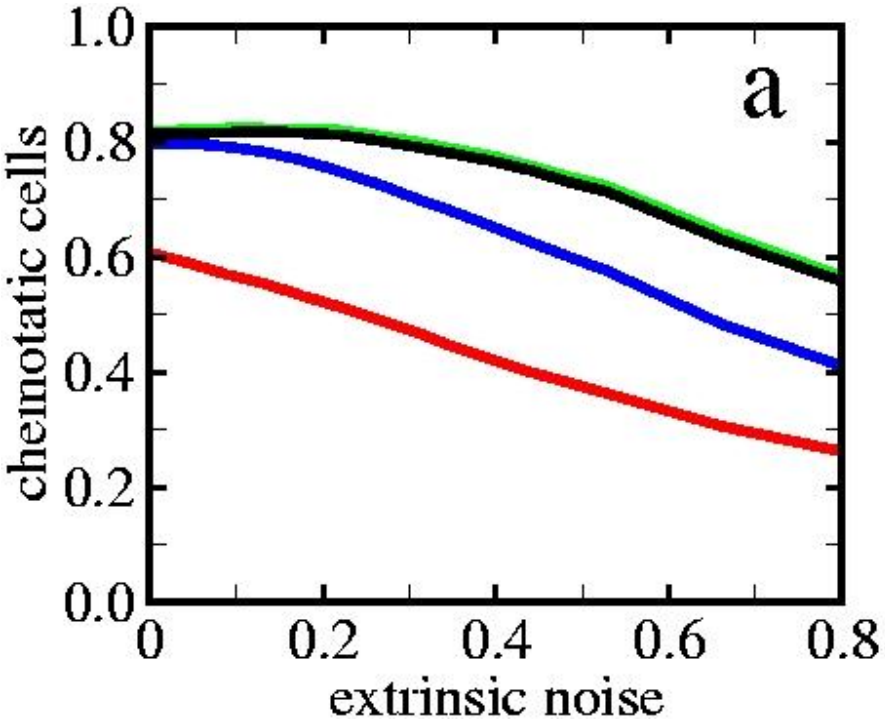
Results: in vivo vs. in silico

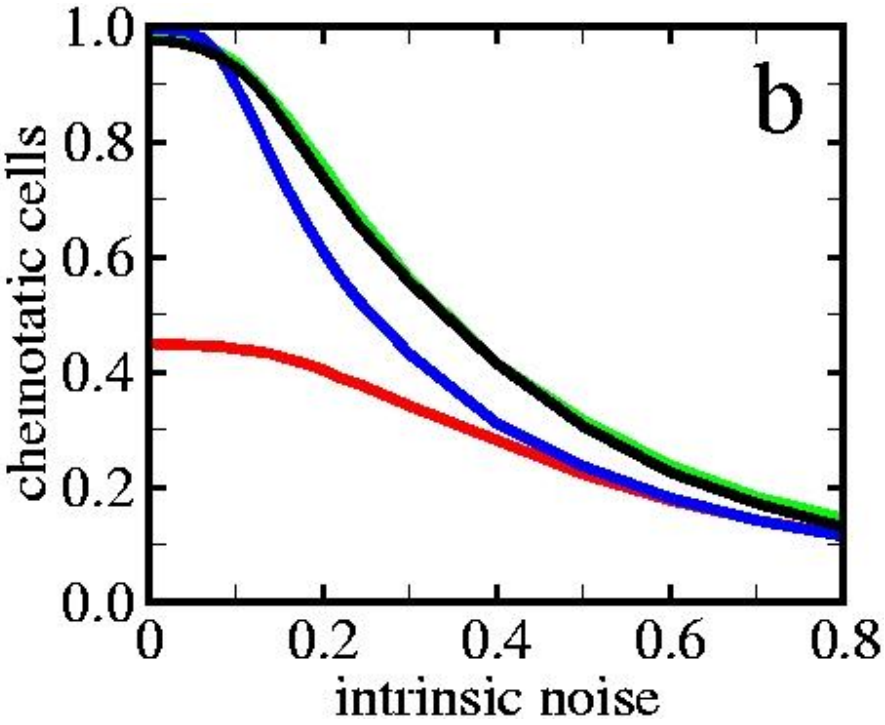


red: Barkai/Leibler, black: final model, cyan: without feedback

blue: CheR not in saturation, green: CheBp with phosphatase

Impossible Experiments





wild type: 0.4 **wild type: 0.2**

red: BL, black: fm, blue: w/out fb, green: mcm

Conclusions

- **E. coli has to be adaptive and robust**
- **E. coli seems to be optimised to deal with fluctuations:**
  - **Uncorrelated noise: Feedback control**
  - **Correlated noise: Phosphatase here, saturation there**
- **Deals with noise on protein level, not in expression process**
- **E. coli is as complex as necessary but as simple as possible**

Acknowledgements

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M. Kollmann, L. Lovdok, K. Bartholomé, J. Timmer, V. Sourjik.

Design principles of a bacterial signalling network, Nature 438:504, 2005

Number of Players per Cell

- **Receptors: 40.000**
- **CheB: 400**
- **CheR: 300**
- **CheY: 14000**
- **CheZ: 6000**