Design Principles of a Bacterial Signalling Network

Why chemotaxis is more complicated than needed

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Outline

- Introduction
- Chemotaxis
- Barkai/Leibler Model
- Fluctuations, Cell-to-Cell Variability
- Design Principles of Robustness

Enlarging Physics, Math, Engineering

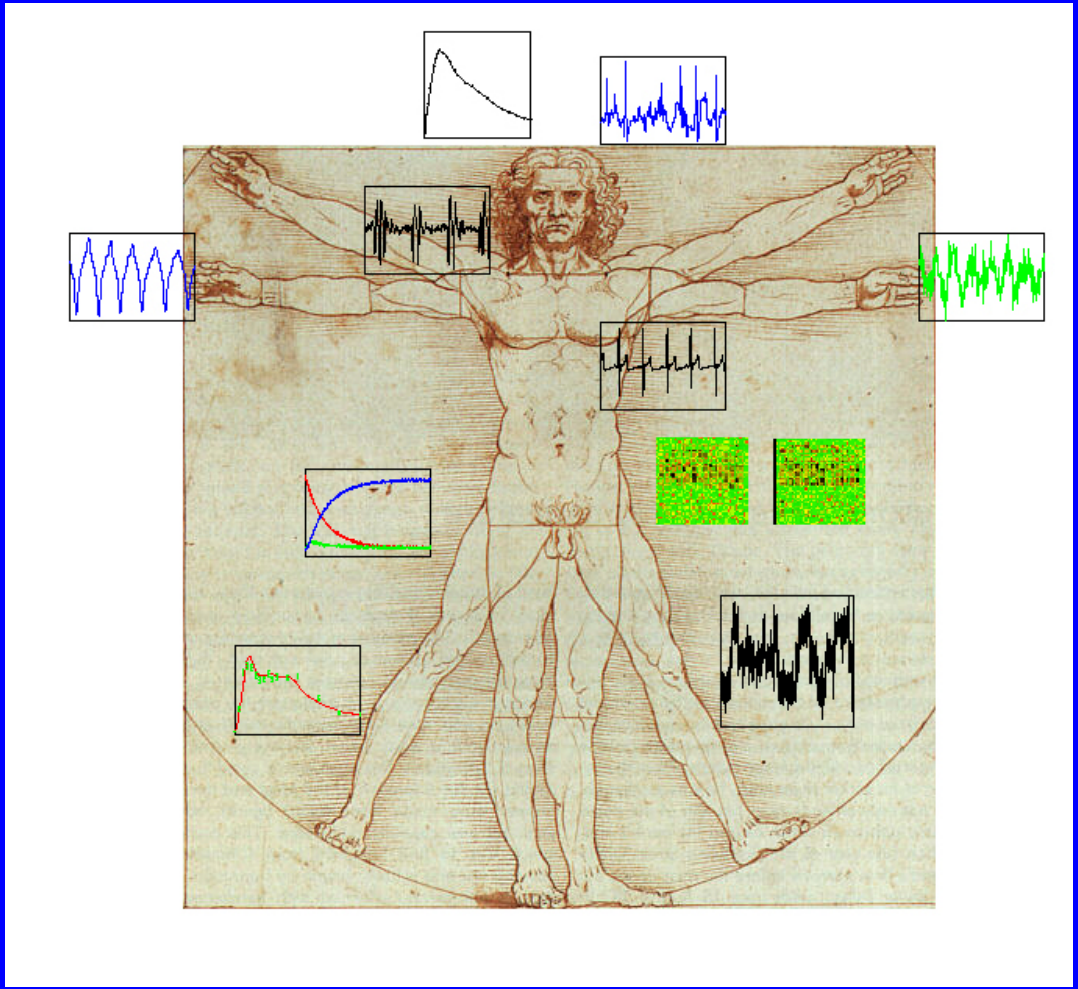
- **Since Newton:**

  Mathematization of inanimate nature

- **21st century:**

  Additionally: Mathematization of animate nature

Man : A Dynamical System



Diseases caused or expressed by malfunction of dynamical processes

Systems Biology

Understanding biomedical systems by data-based mathematical modelling of their dynamical behavior

Based on but more than ...

- **... Mathematical Biology: Data-based**
- **... Bioinformatics: Dynamics**
- **... o.p./g. – o.p.: System**
- **... another omics: Mathematics**

Why Modelling in Cell Biology?

- **Basic Research**
  - Genomes are sequenced, but ...
  - ... function determined by regulation
  - Regulation = Interaction & Dynamics
  - Function: Property of dynamic network
  - "Systems Biology"
- **Application**
  - Drug development takes 10 years and 1 bn $/€
  - Reduce effort by understanding systems

Two Differences between Physics and Biology

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

Physics in biology:

Apply mathematics to understand function

Bacterial Chemotaxis – The Phenomenon

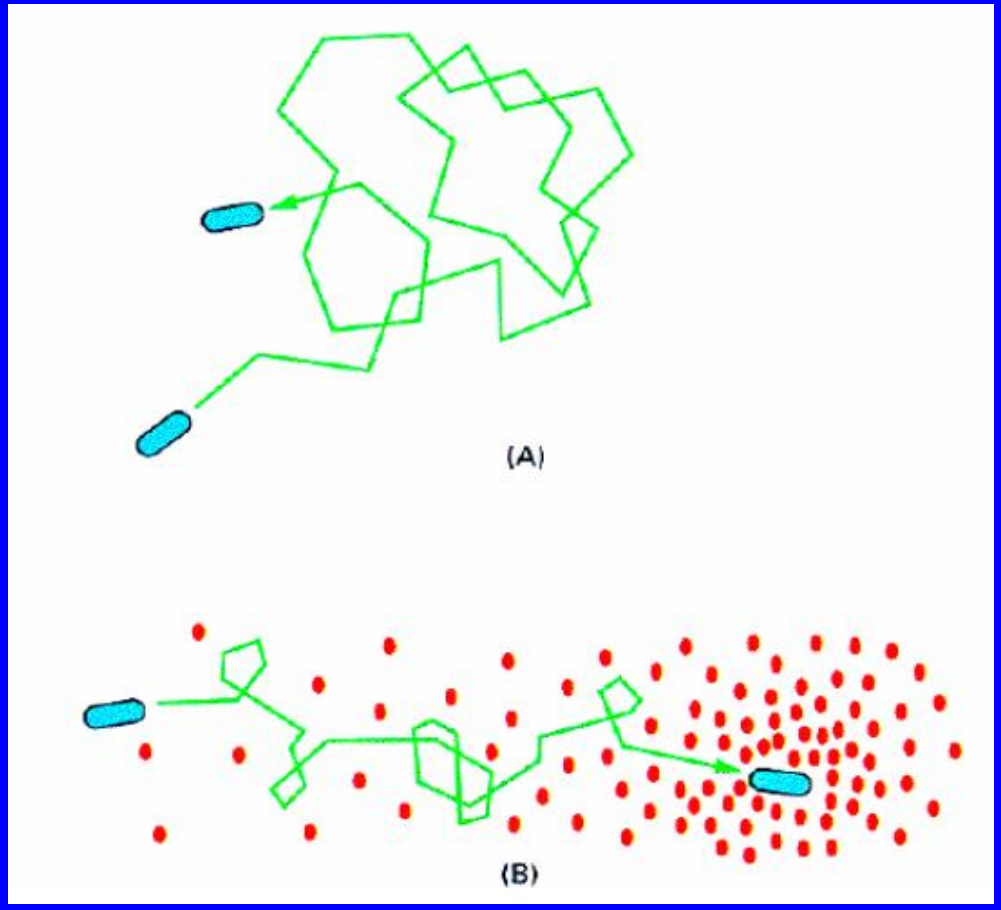
- Bacteria sense nutrient gradients over four orders of magnidute of absolute concentration
- Detect relative changes of 2 %
- Robust against pertubations

Chemotaxis: One of the best investigated biological systems

Bacterial Chemotaxis – The Strategy

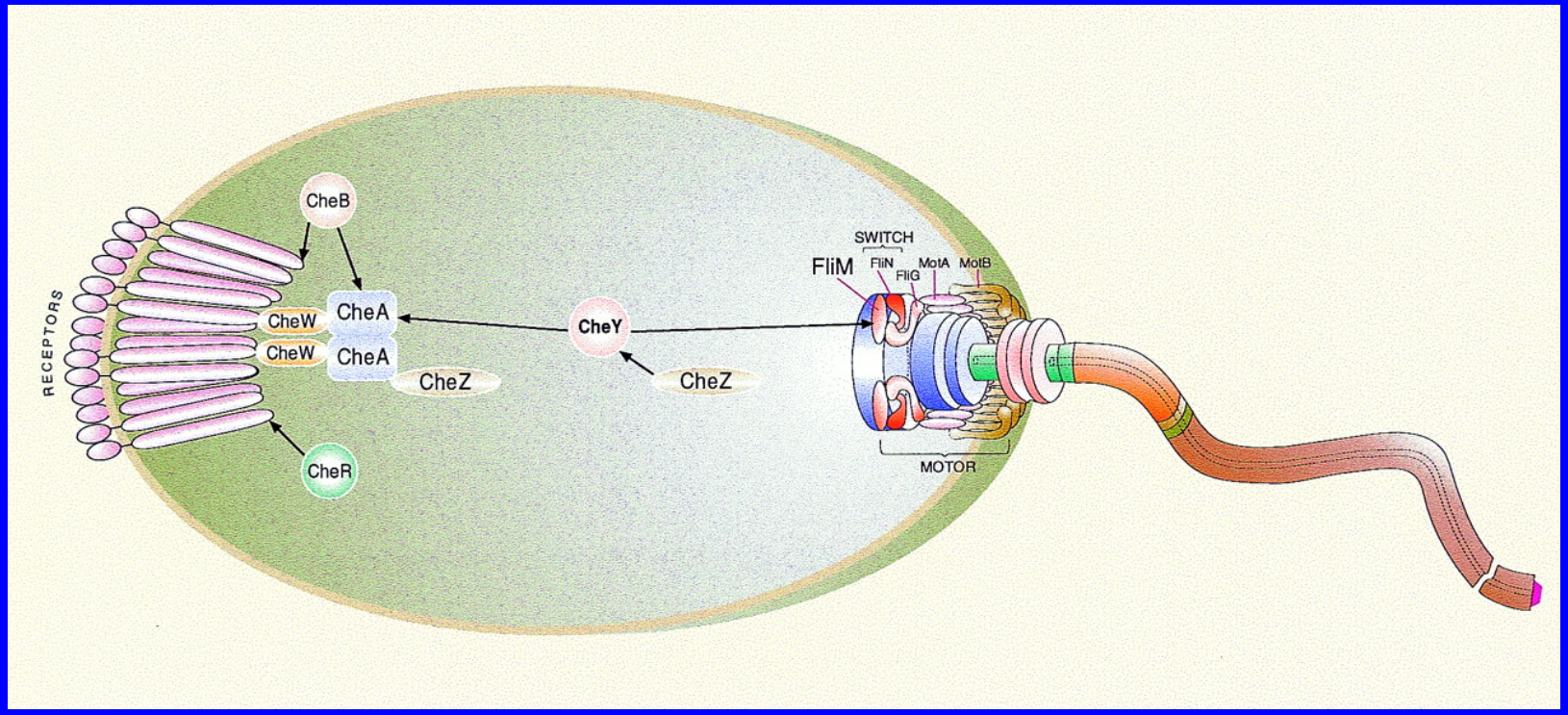
- Bacteria too small to compare front to end
- Strategy:
  - Change direction from time to time (tumble)
  - If concentration increases: reduce tumbling frequency
  - If concentration decrease: increase tumbling frequency
- Sense spatial gradients by temporal changes

Chemotaxis – Tumble and Swim



Random walk vs. biased random walk

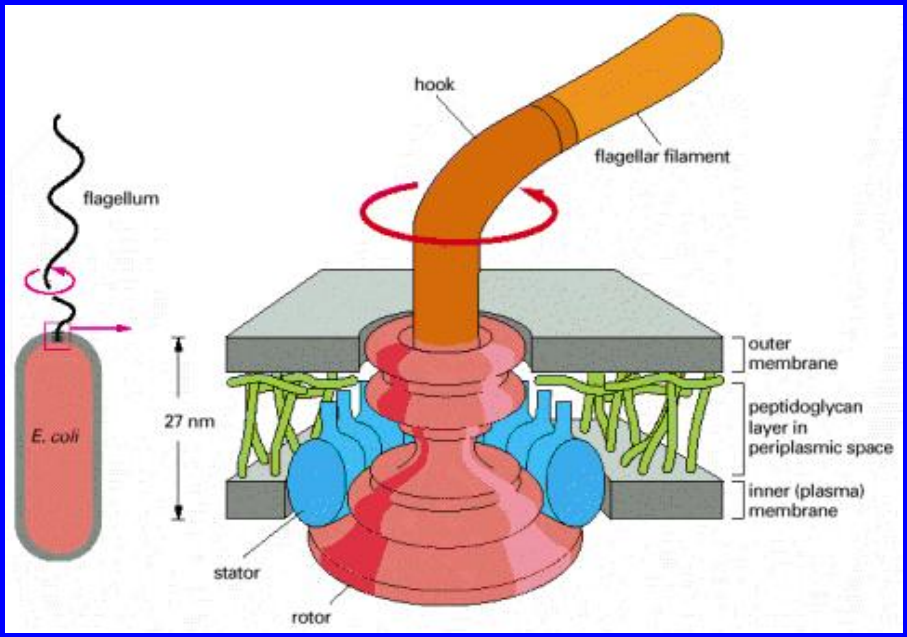
Chemotaxis in E. coli



Chemotaxis – Flagella

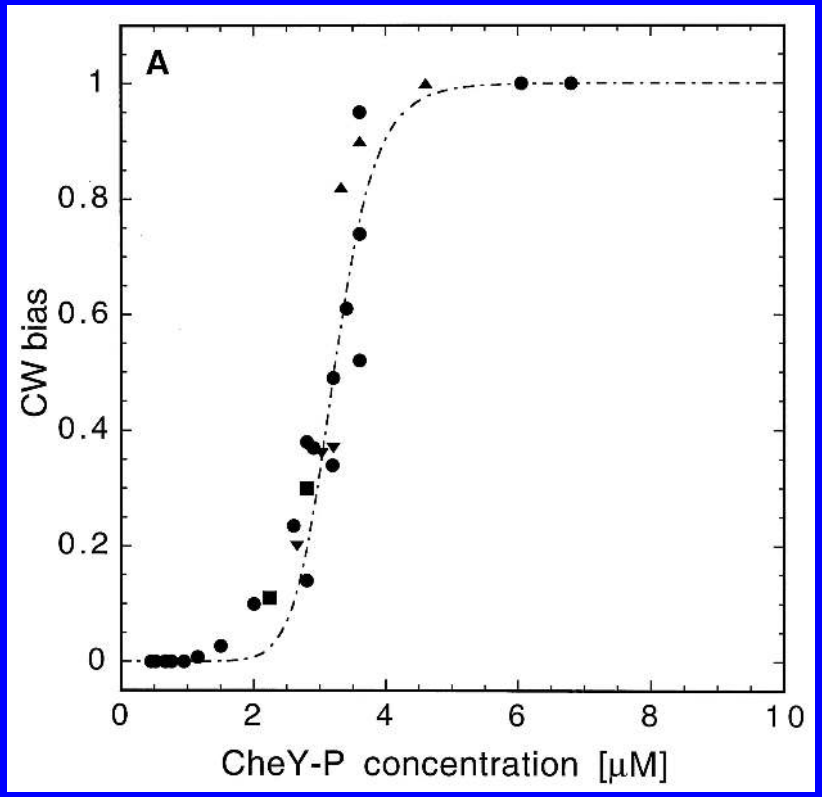
Movement by rotating corkscrew-flagella

- **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

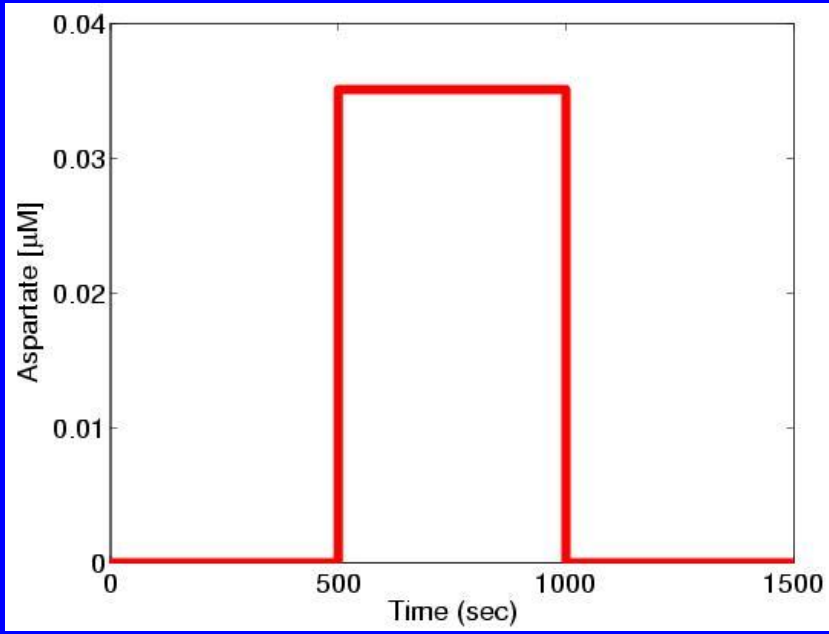
- Motor has a small range of sensitivity
- Cell is chemotactic for a large range of concentrations

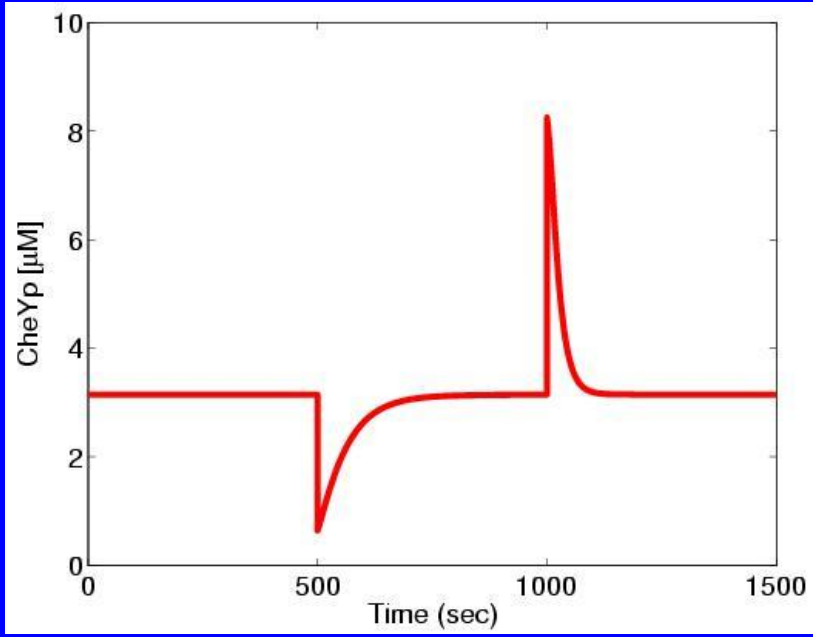
$\Longrightarrow$ System has to be adaptive:

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

Chemotaxis – The Task

Input: Nutrient concentration Output: Tumbling frequency





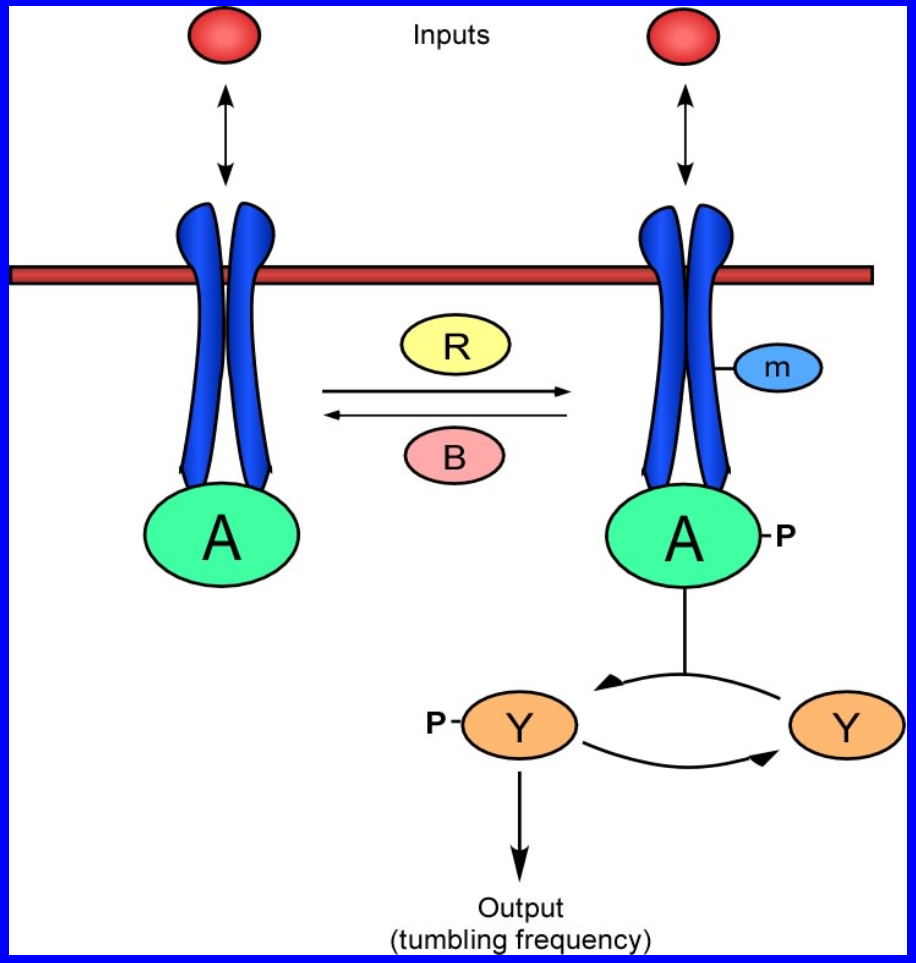
System performs a kind of differentiation

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

Phosporylation, Methylation = Chance of state

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 Ap:

$$\dot{A}p = k_A(A_{tot} - Ap)T_a - k_Y Ap(Y_{tot} - Yp)$$

Dynamics of Yp:

$$\dot{Y}p = k_Y Ap(Y_{tot} - Yp) - \gamma_Y Y_p$$

Perfect Adaptation

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 Yp**

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**

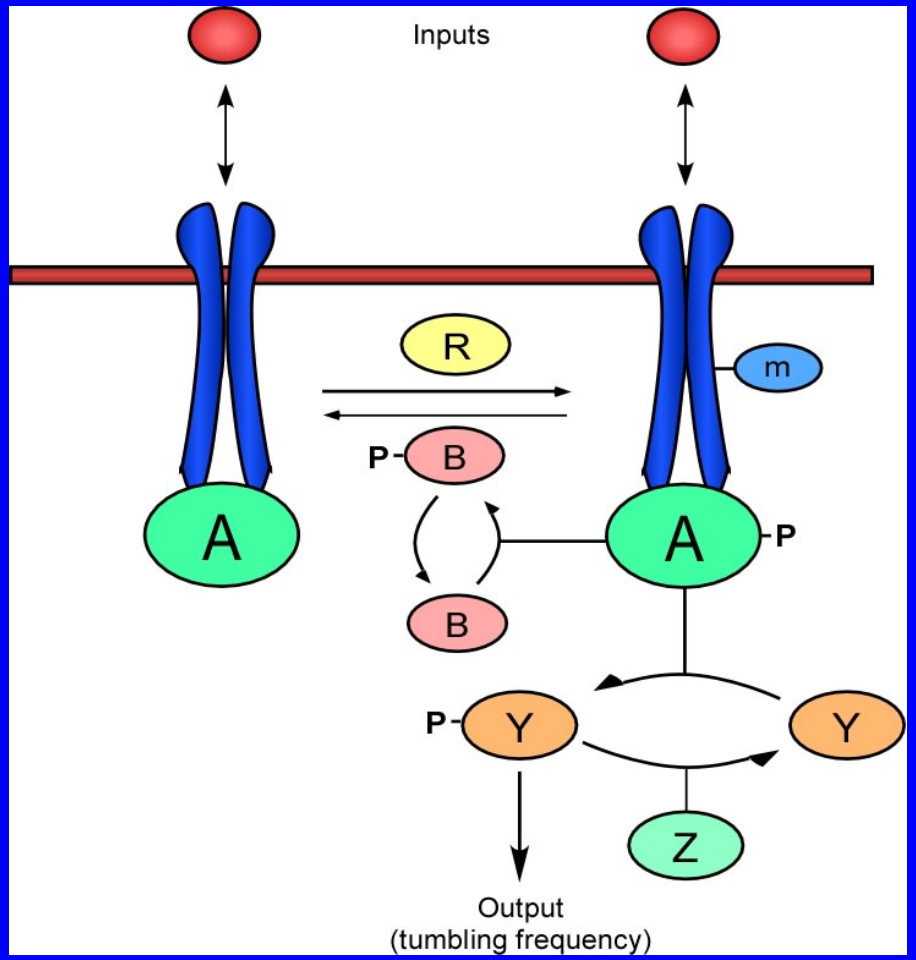
In words:

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**

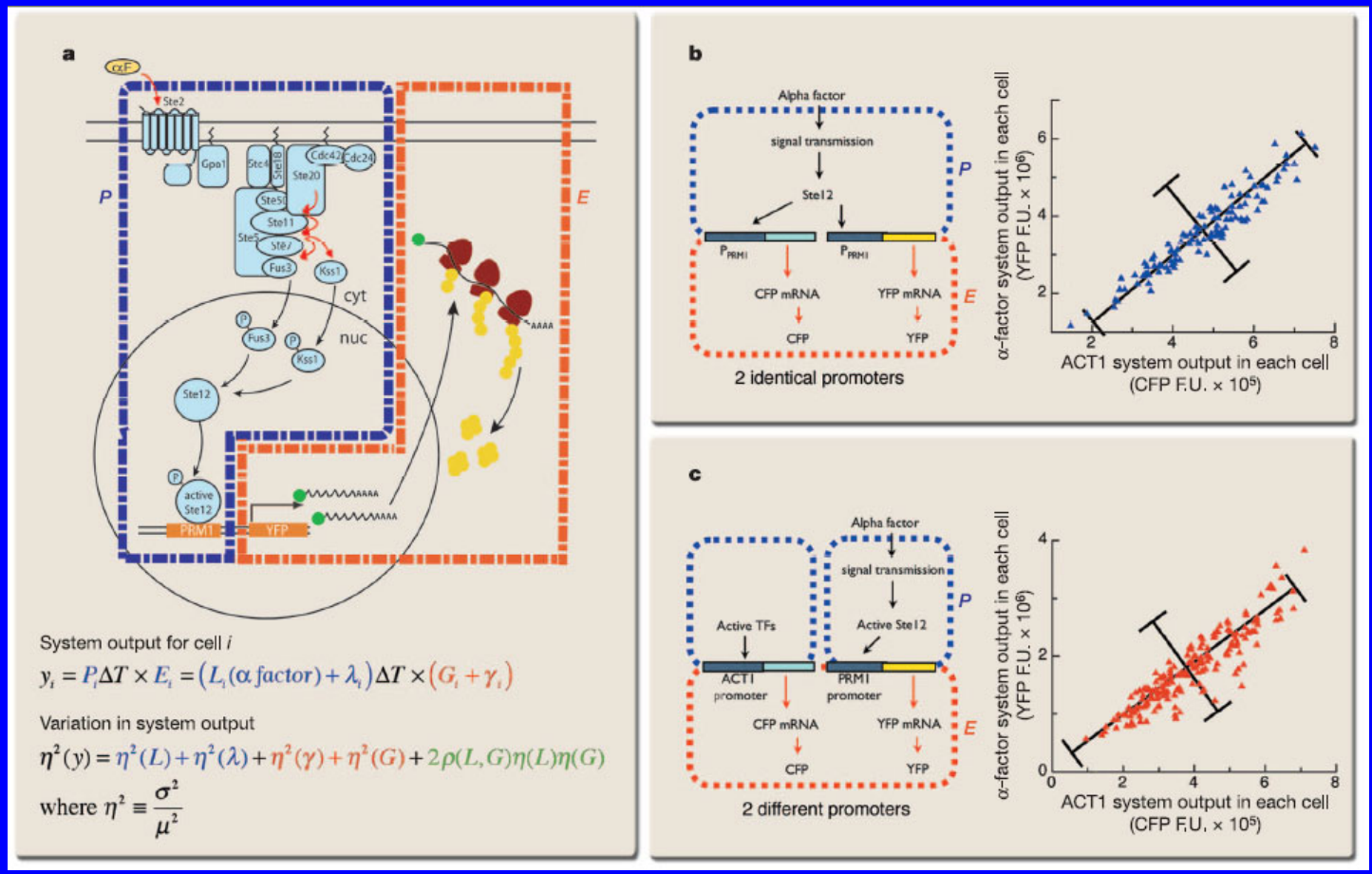
- **Extrinsic noise**

  Differences between identical reporters in different cells

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

  Cell-to-cell variability

Quantification of Variability



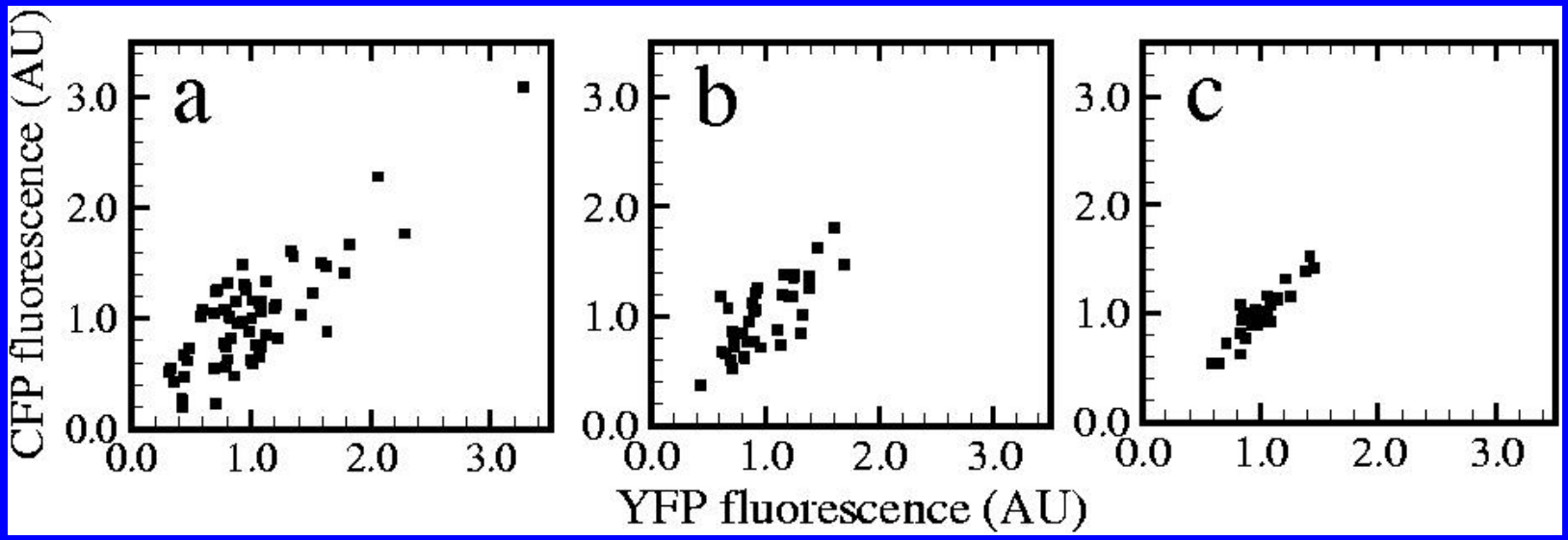
Colman-Lerner et al. Nature 437:699, 2005

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:

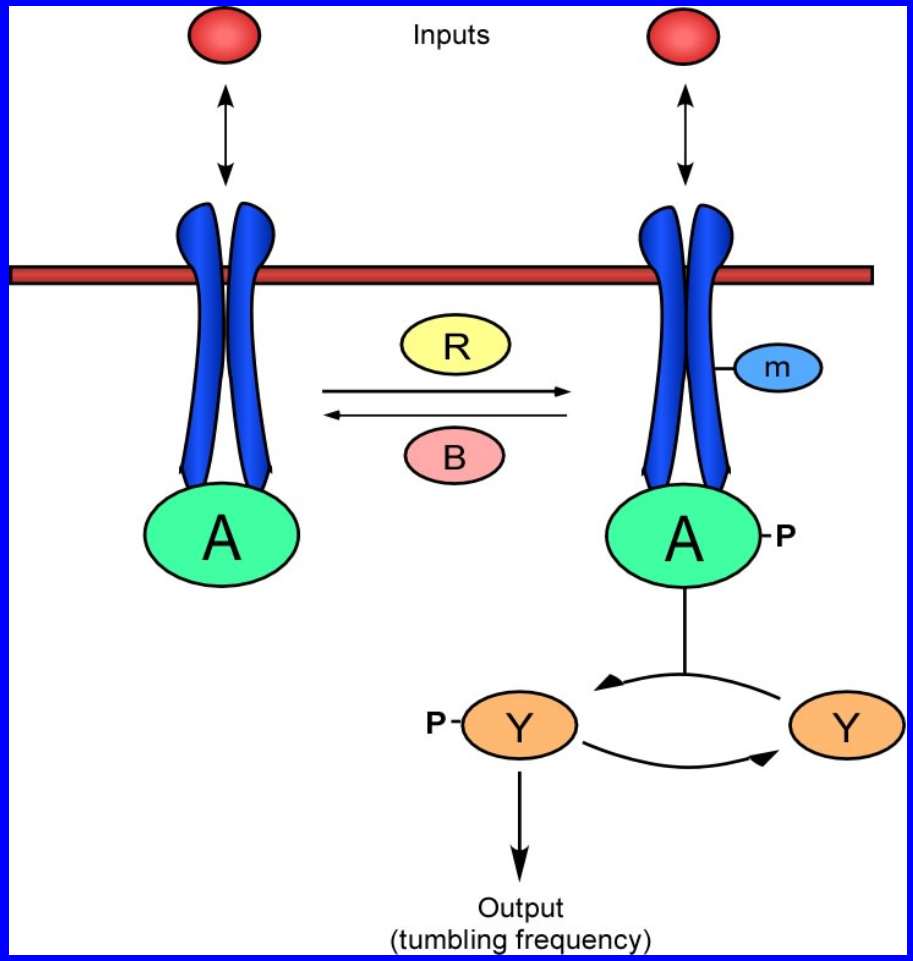
- Steady state level Yp in [2.2 μM, 4.3 μM]
- For correlated fluctuation:

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

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

  Use feedback-loops to attenuate noise

Application to Barkai/Leibler Model



Robustness of Barkai/Leibler Model

Steady states (with some approximations):

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

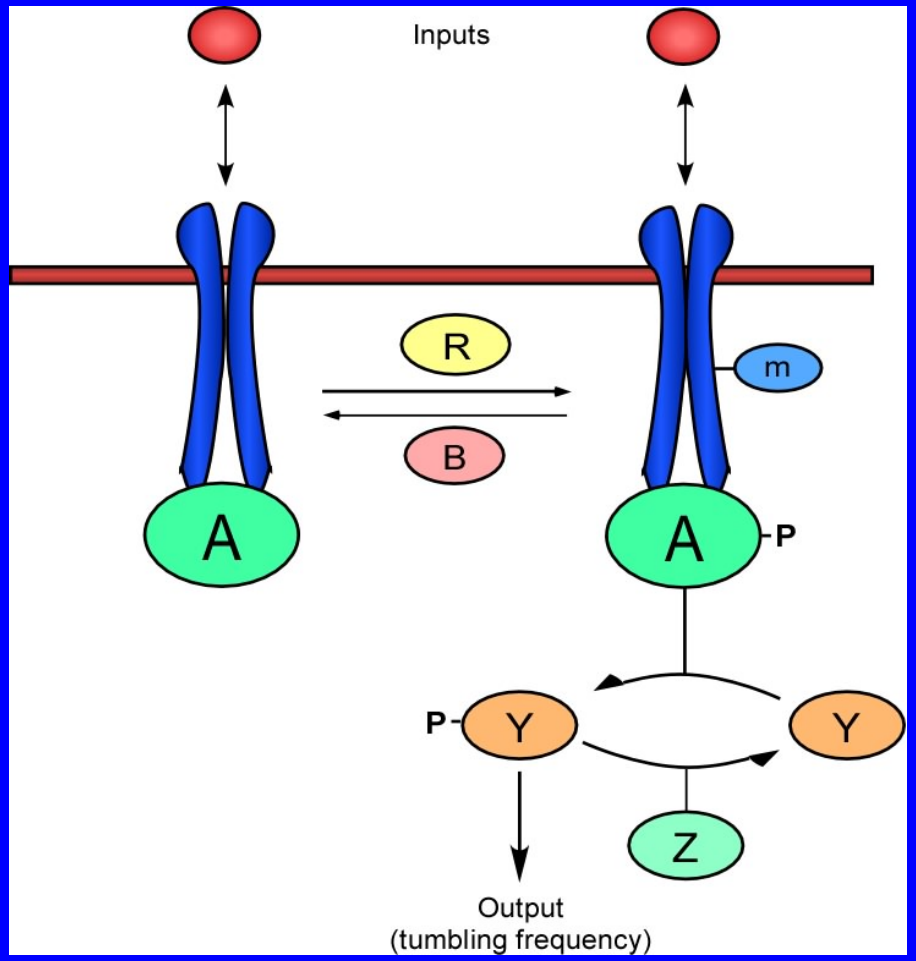
$$Ap^{ss} \approx \frac{k_A T_a^{ss}}{k_Y} \frac{A_{tot}}{Y_{tot}} \qquad \text{o.k.}$$

$$Yp^{ss} = \frac{k_y Ap^{ss}}{k_Y Ap^{ss} + \gamma_Y} Y_{tot} \qquad \text{not o.k.}$$

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

$$Yp^{ss} = \frac{k_y Ap^{ss}}{k_Z} \frac{Y_{tot}}{Z_{tot}} \qquad \text{o.k.}$$

Extension of the Model



Robustness Against Correlated Fluctuations

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

Robustness Against Uncorrelated Fluctuations

Diminish uncorrelated noise by a classical feedback

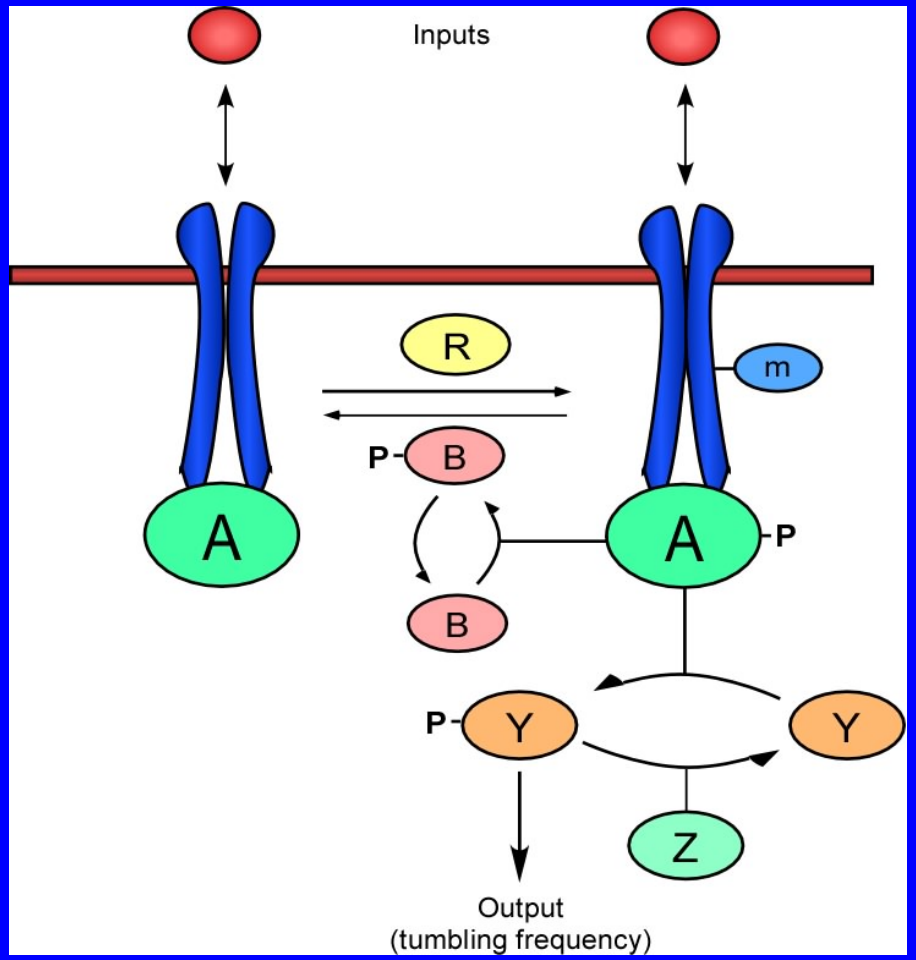
- **Methylesterase B can be phoshorylated by Ap**
- **Only Bp can demethylate receptors**

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

- **Robustness against correlated fluctuations:**

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

Final Model



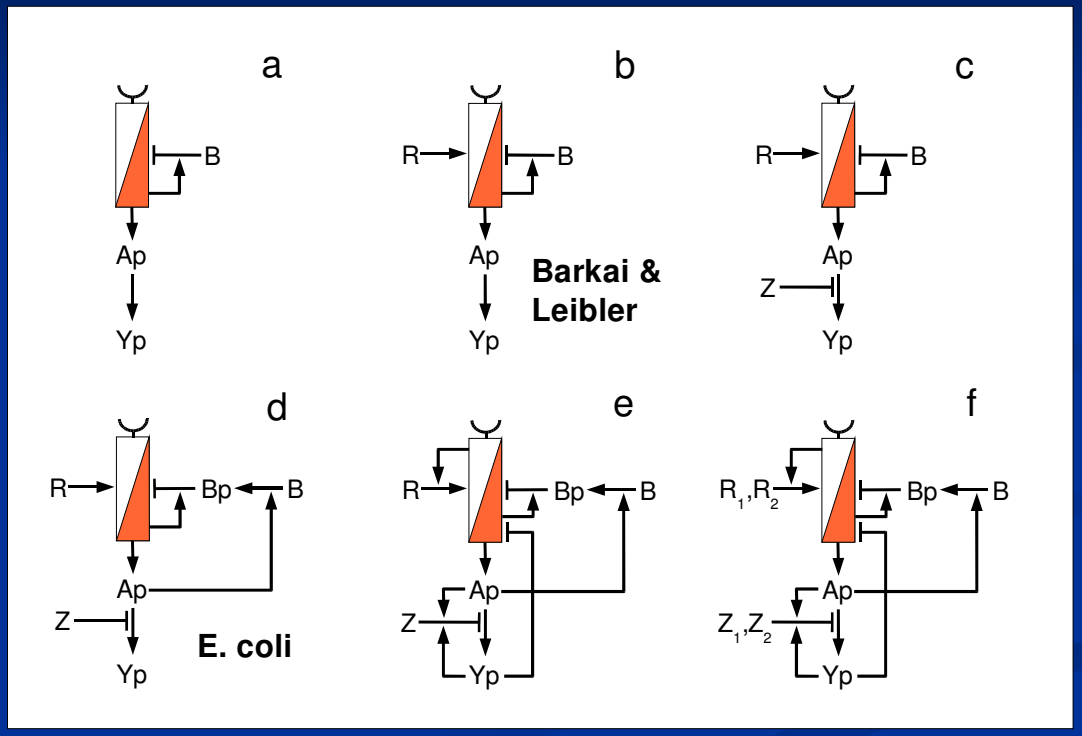
And this is how E. coli looks like

In silico Biology

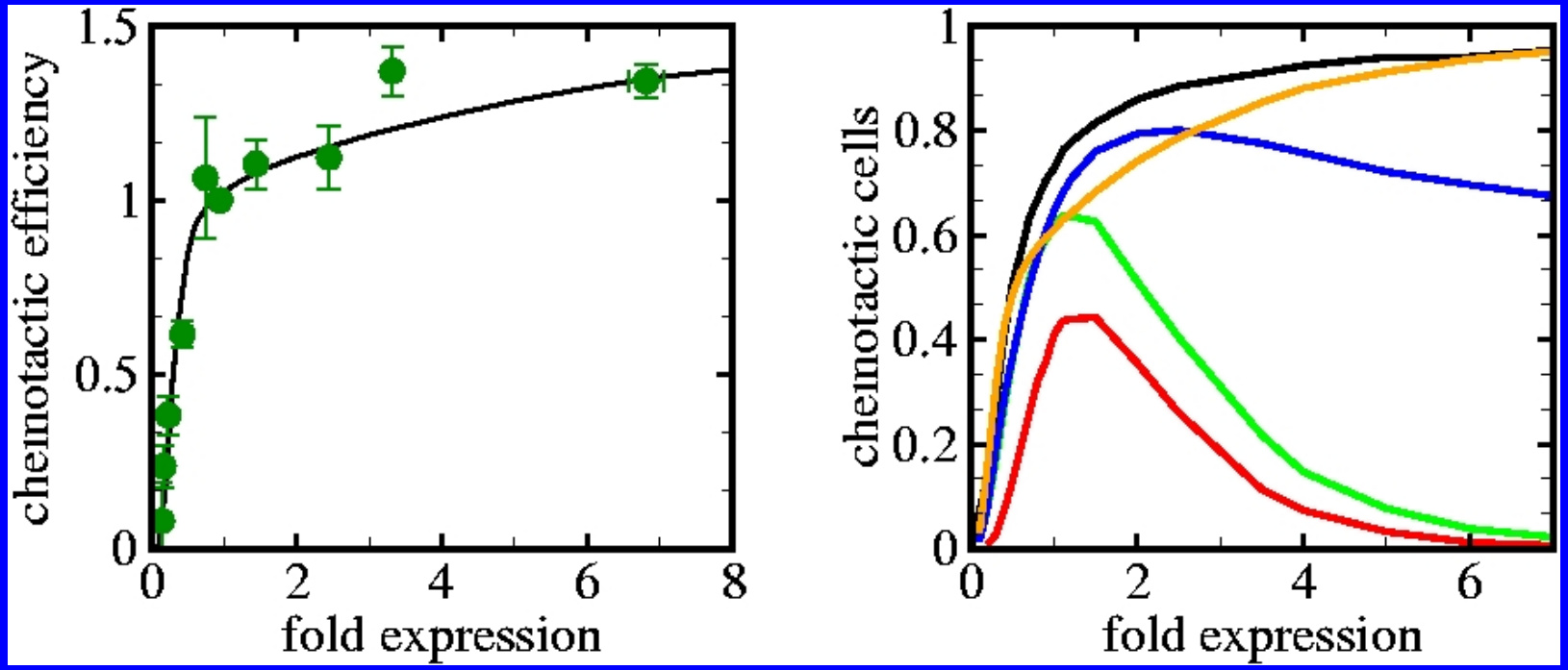
- **Choose different pathway topologies**
- **Parameters known experimentally**
- **Protein concentrations from experimental distributions**

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

Cartoons of Perfect Adaptive Pathways



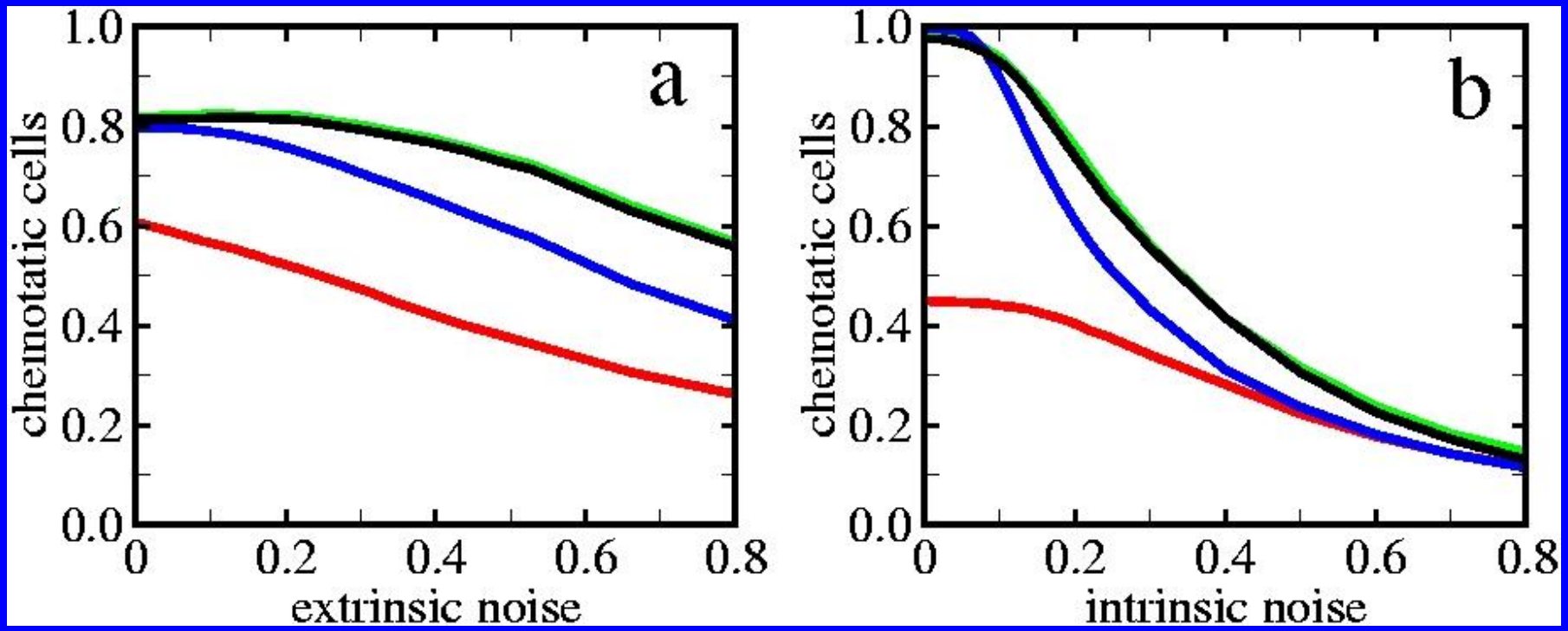
Results: in vivo vs. in silico



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
- E. coli is as complex as necessary but as simple as possible

Work done by

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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

Open Positions

- **BMBF Systems Biology of Hepatocytes** $HepatoSys$
- **DFG Graduate College 1305: Plant Signaling Systems**
- **BMBF Research Unit Systems Biology** $FRISYS$

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