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**

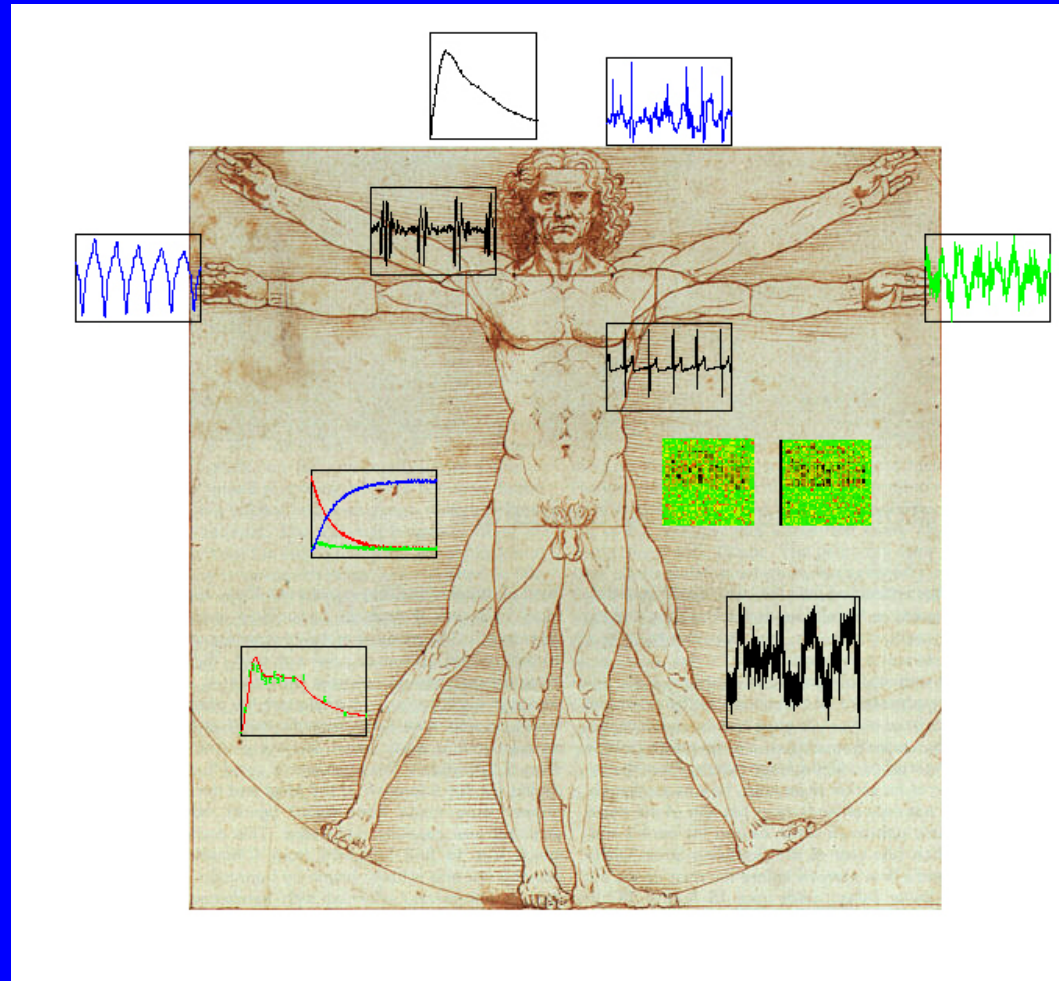# Enlarging Physics, Math, Engineering

- **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

(A)

(B)
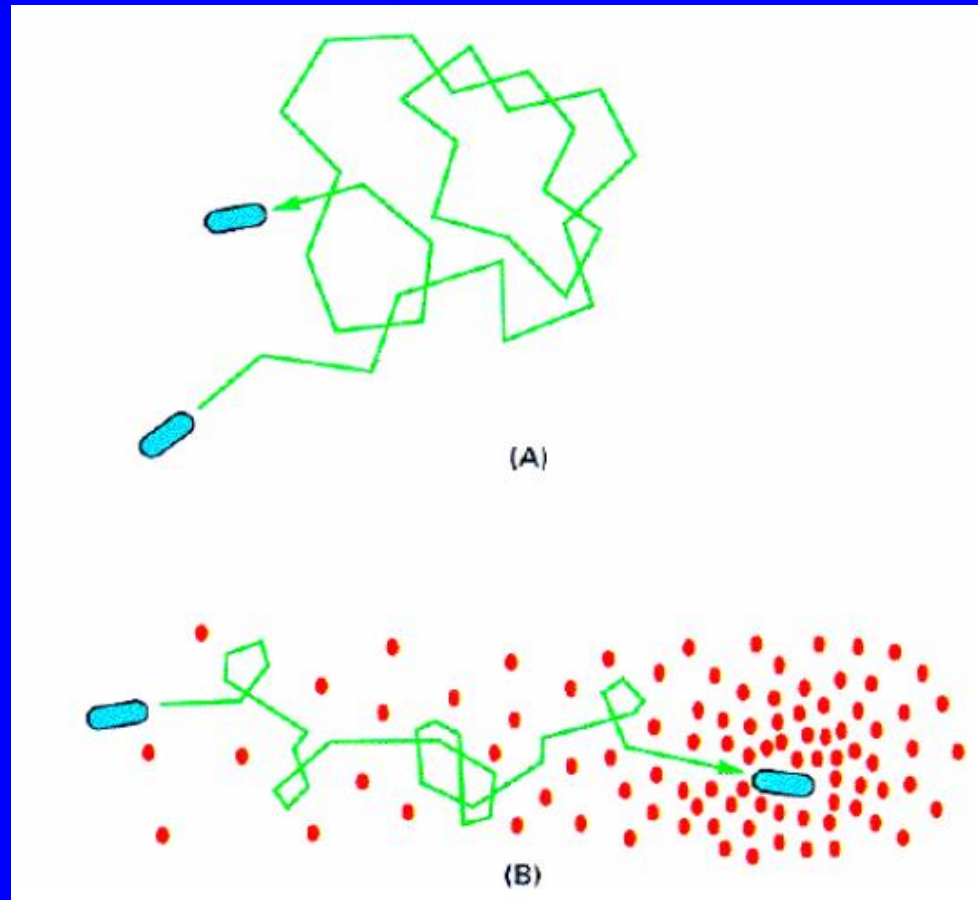
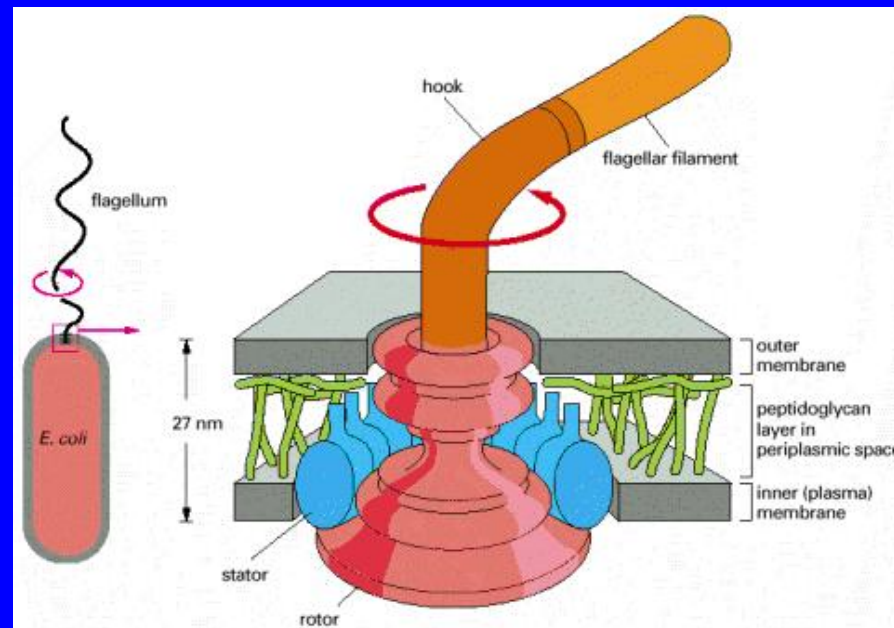**Random walk vs. biased random walk**
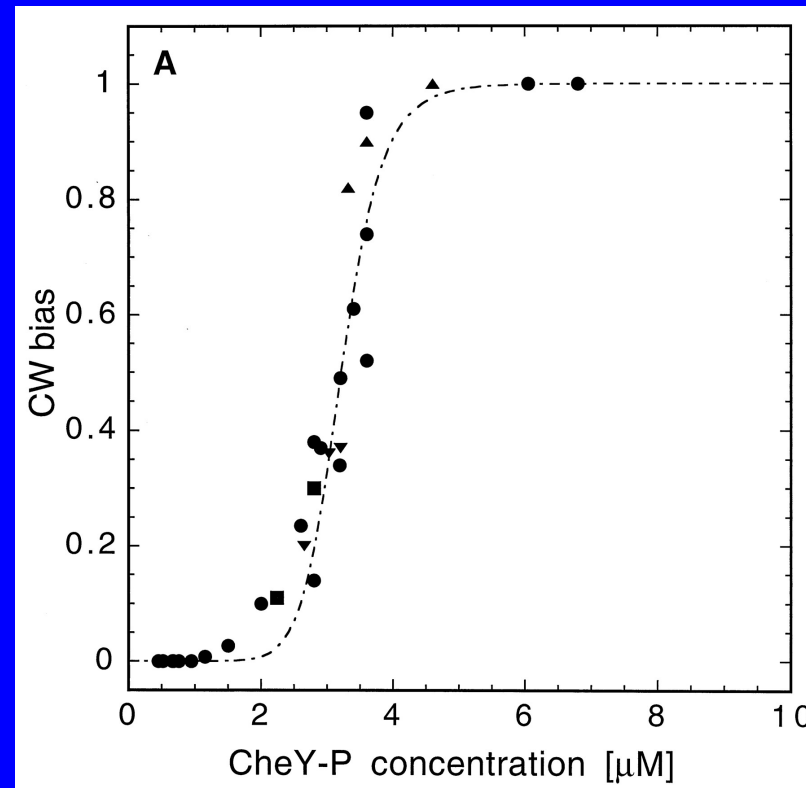
Chemotaxis in E. coli
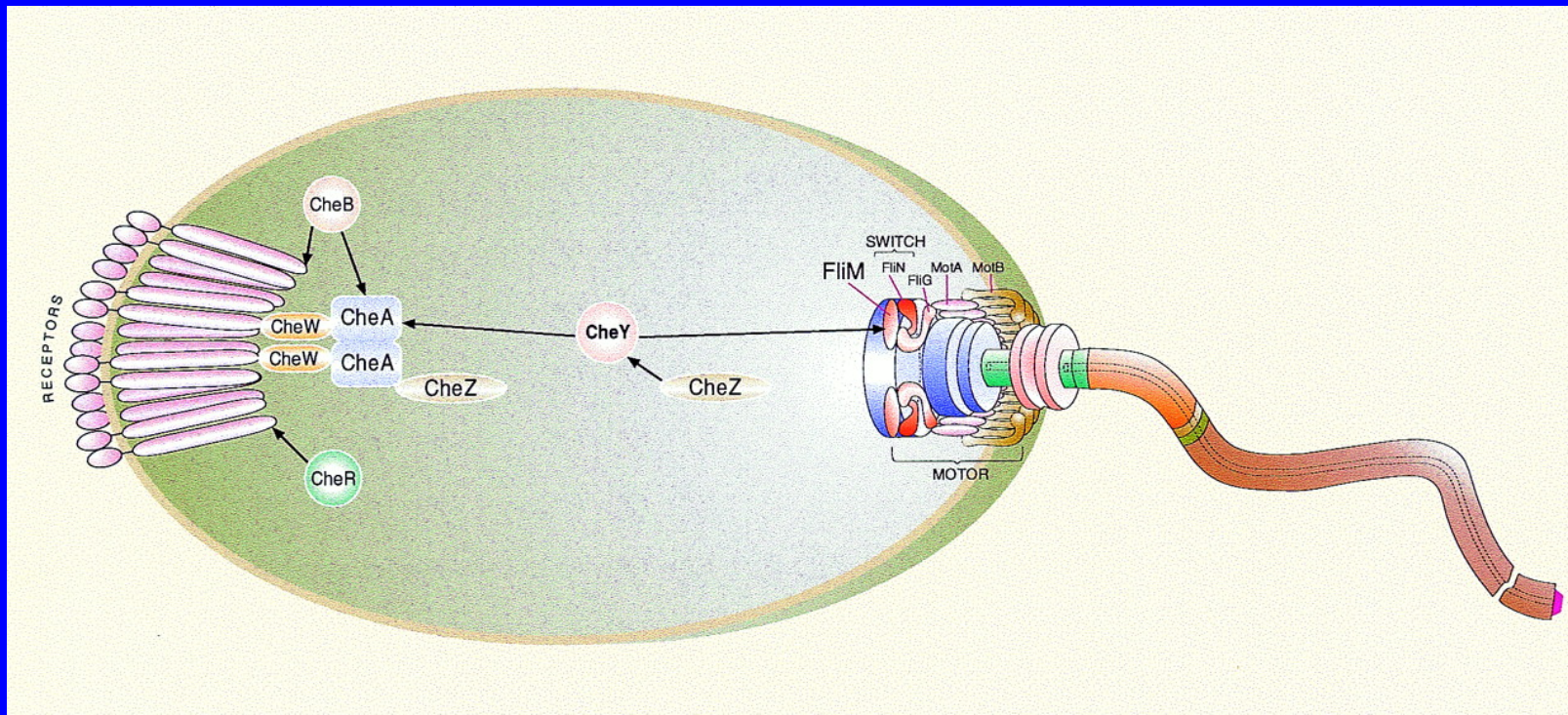
Chemotaxis – Flagella

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

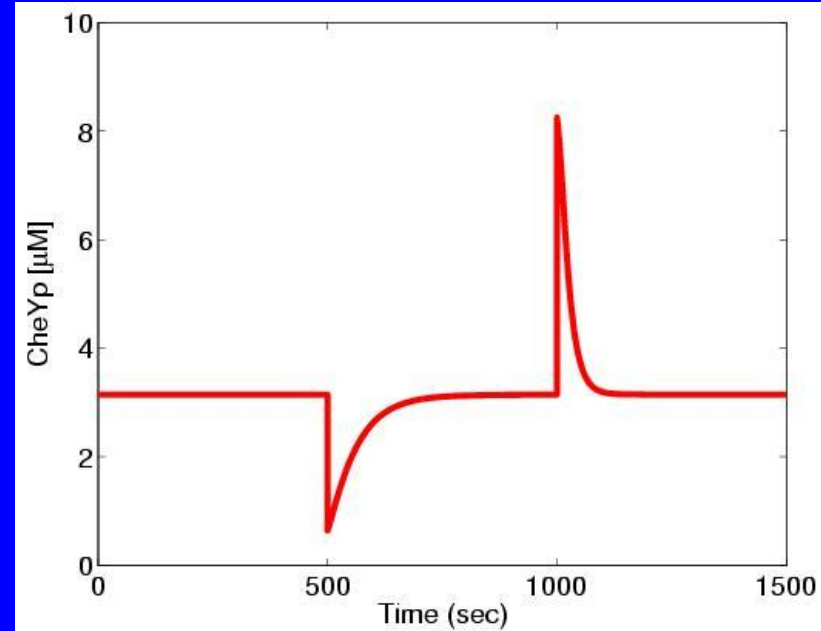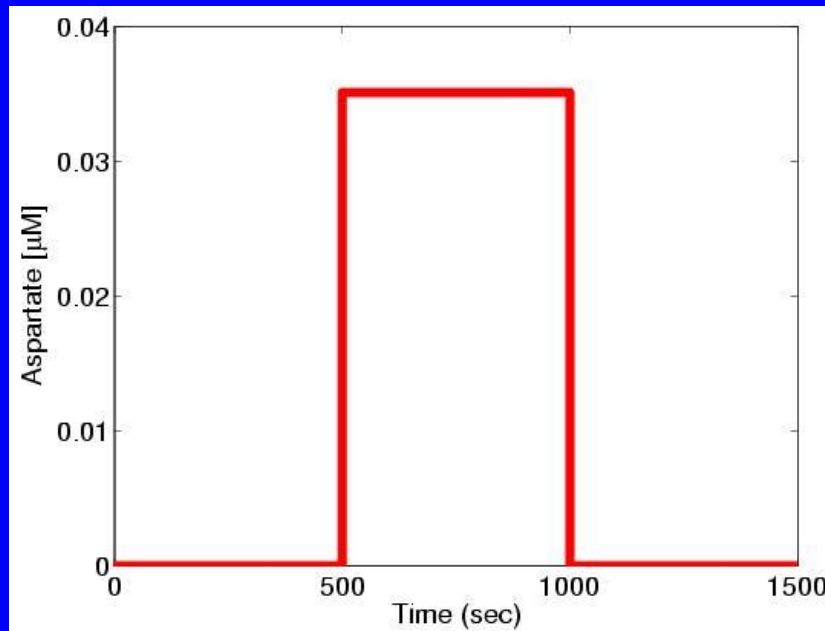**Important: A small working range**

Chemotaxis − Adaptation

- **Motor has a small range of sensitivity**

- **Cell is chemotactic for a large range of concentrations**

$\Longrightarrow$ **System has to be** <u>**adaptive**</u>**:**

Steady state of CheYp must be independent from

absolute concentration of ligand

Chemotaxis – The Task

Input: Nutrient concentration Output: Tumbling frequency

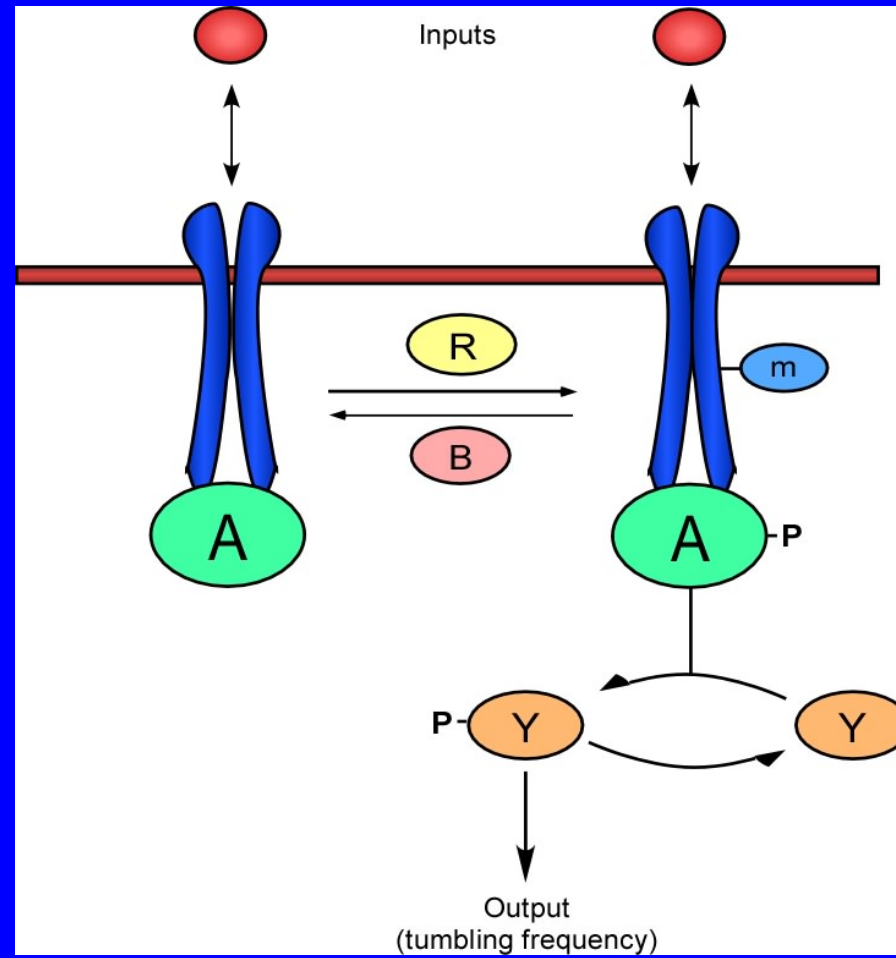**System performs a kind of differentiation**

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 $Y p$**

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**
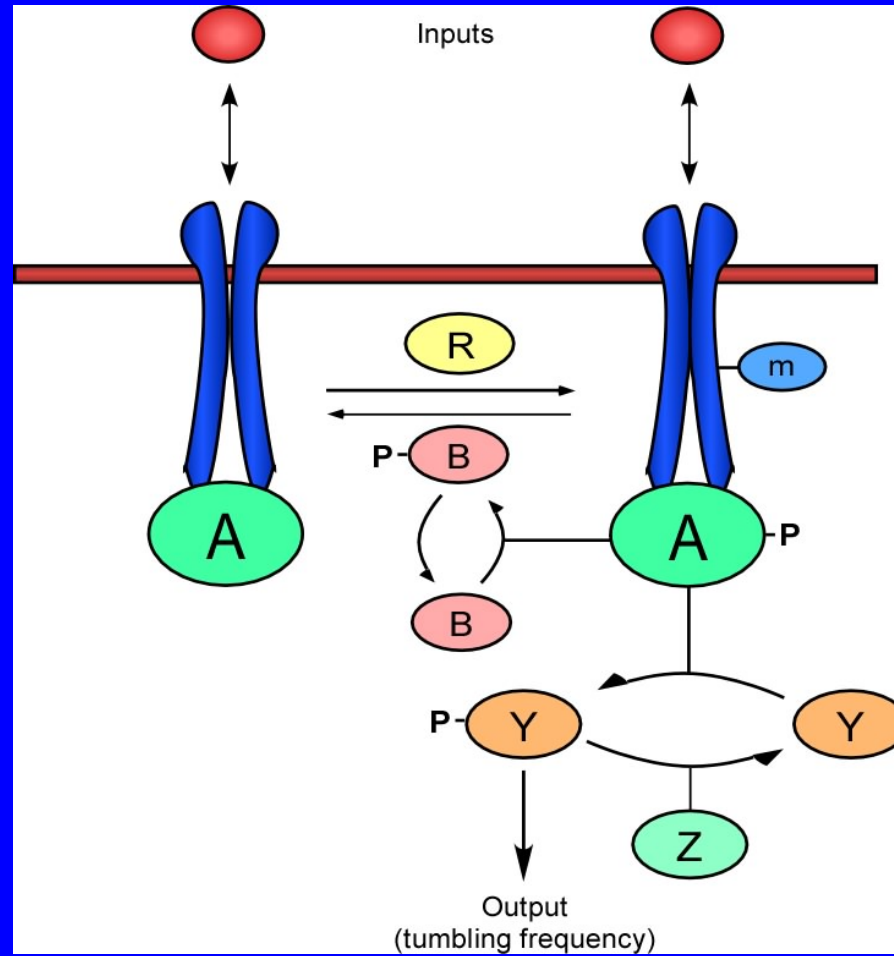
- **Integral negative feedback control**

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
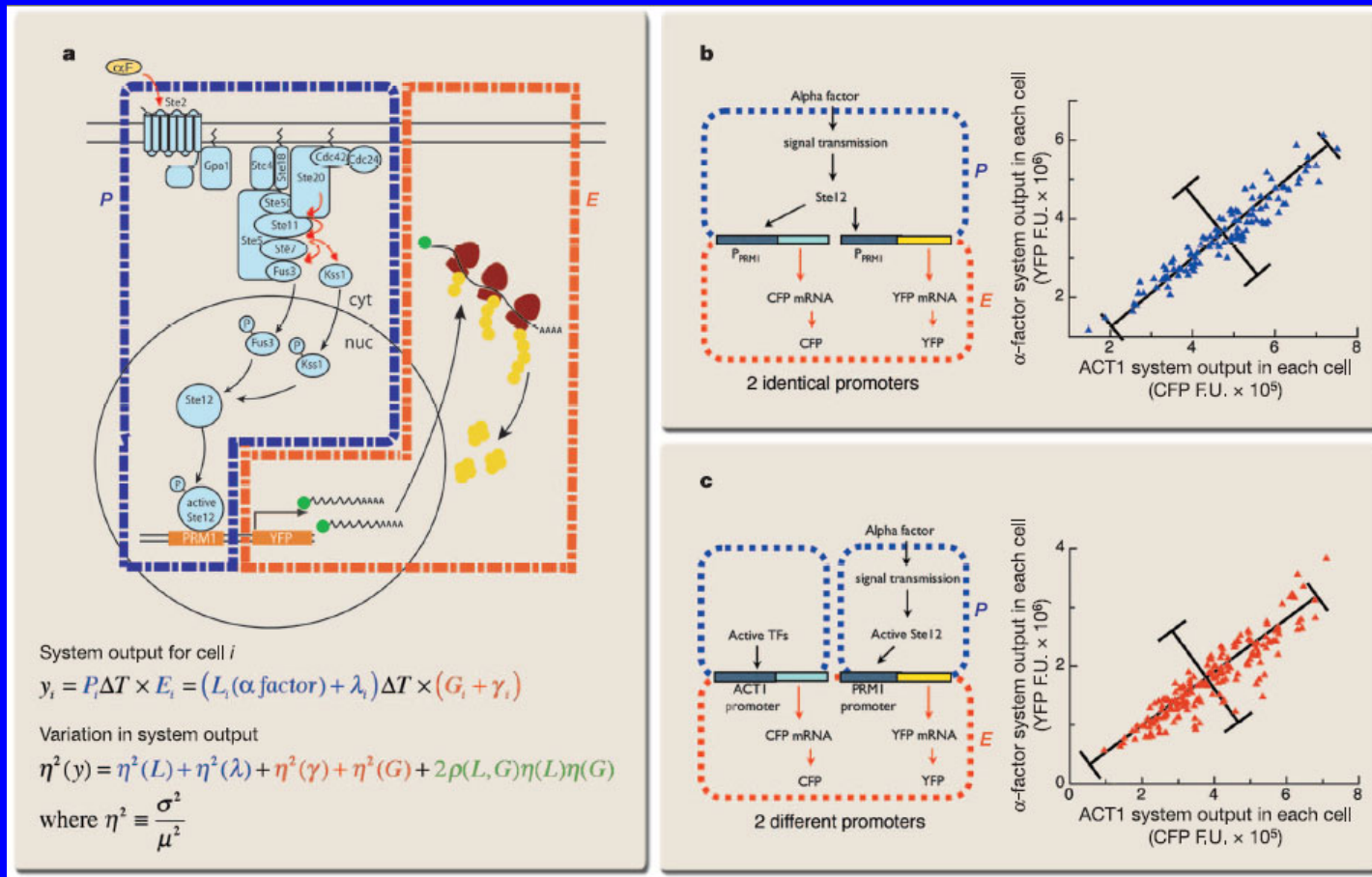
  – **Stochasticity of reactions**

- **Extrinsic noise**

  Differences between identical reporters in different cells

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

  Cell-to-cell variability

Quantification of Variability

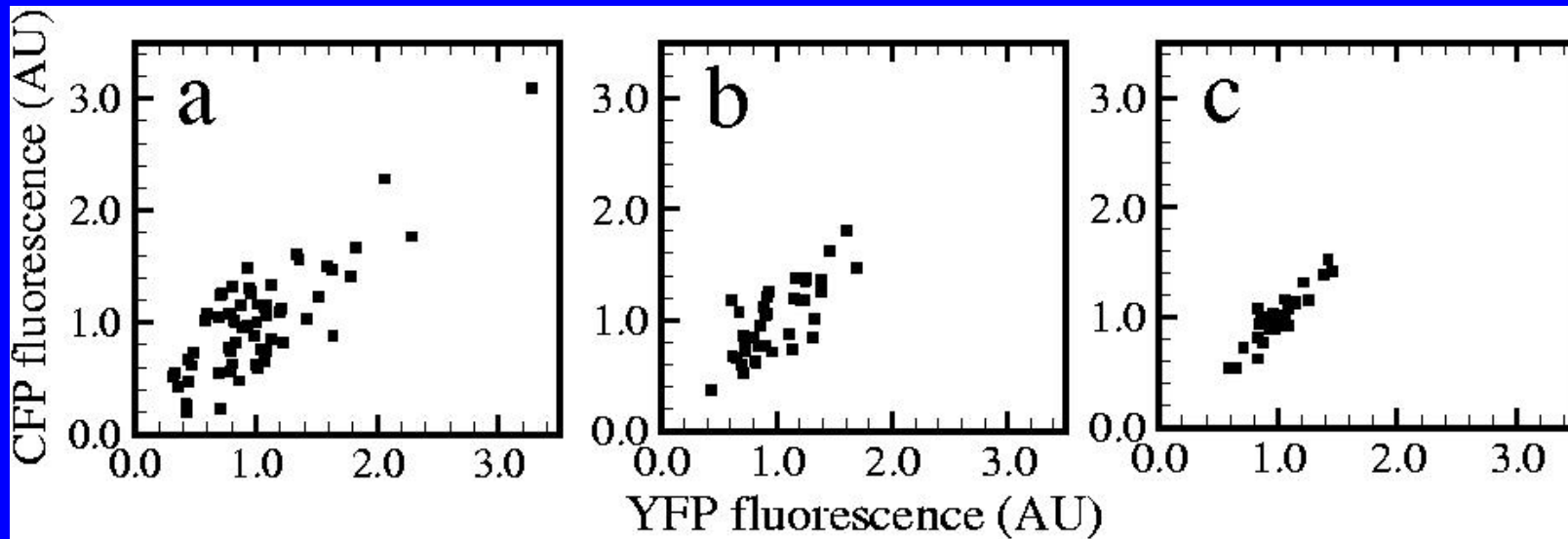Colman-Lerner et al. Nature 437:699, 2005

Results

E. coli and yeast:

- **Extrinsic noise is larger than intrinsic noise**

- **Protein concentrations fluctuate in a <u>correlated</u> 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]**
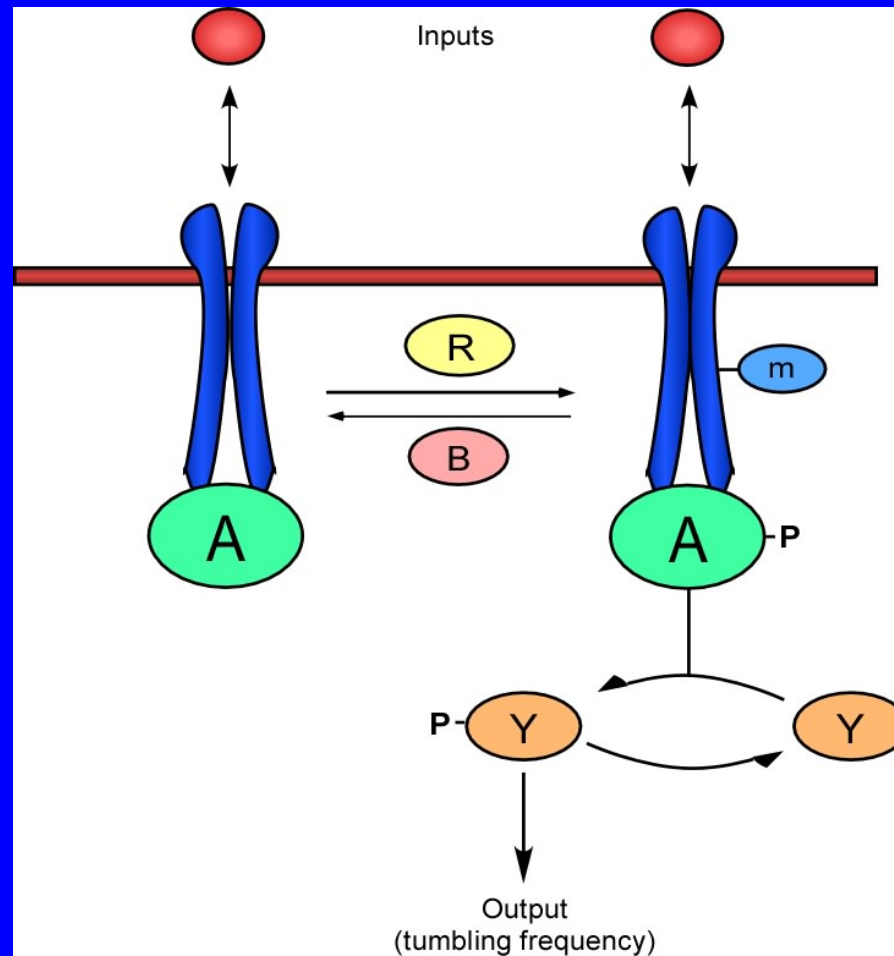
- **For correlated fluctuation:**

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

  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):

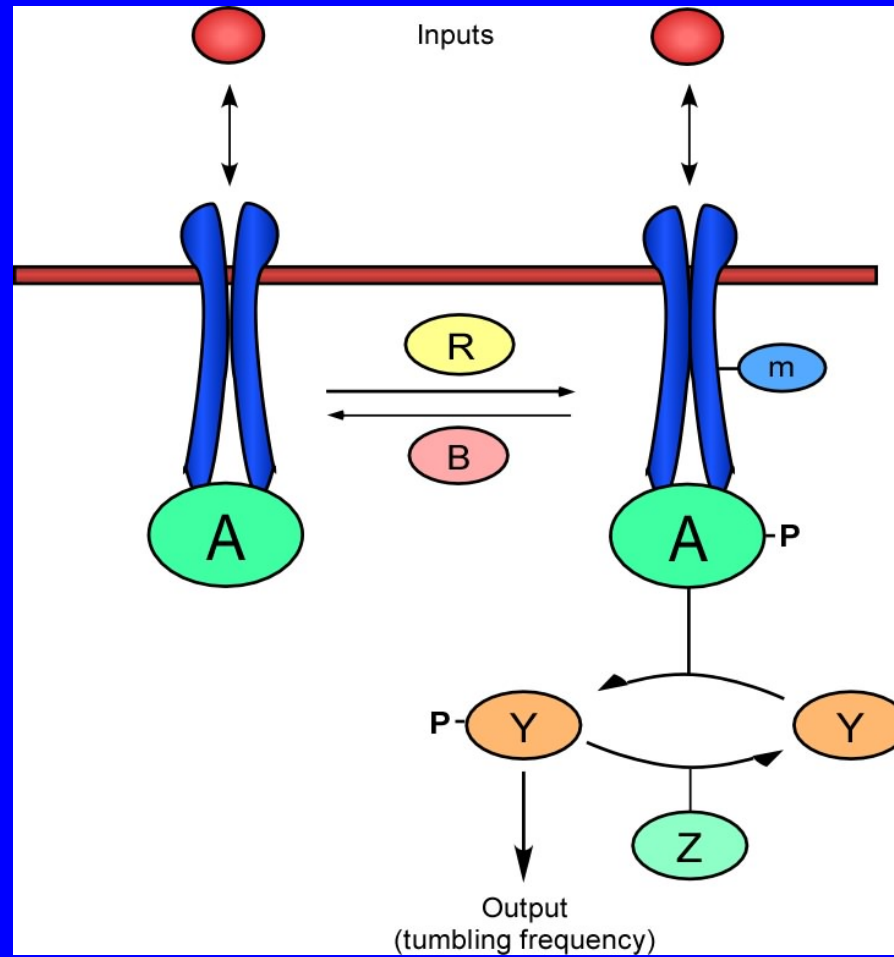$$T_a^{ss} = K_B \frac{k_R R}{k_B B - k_R R} \qquad \textbf{o.k.}$$

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

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

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

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

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

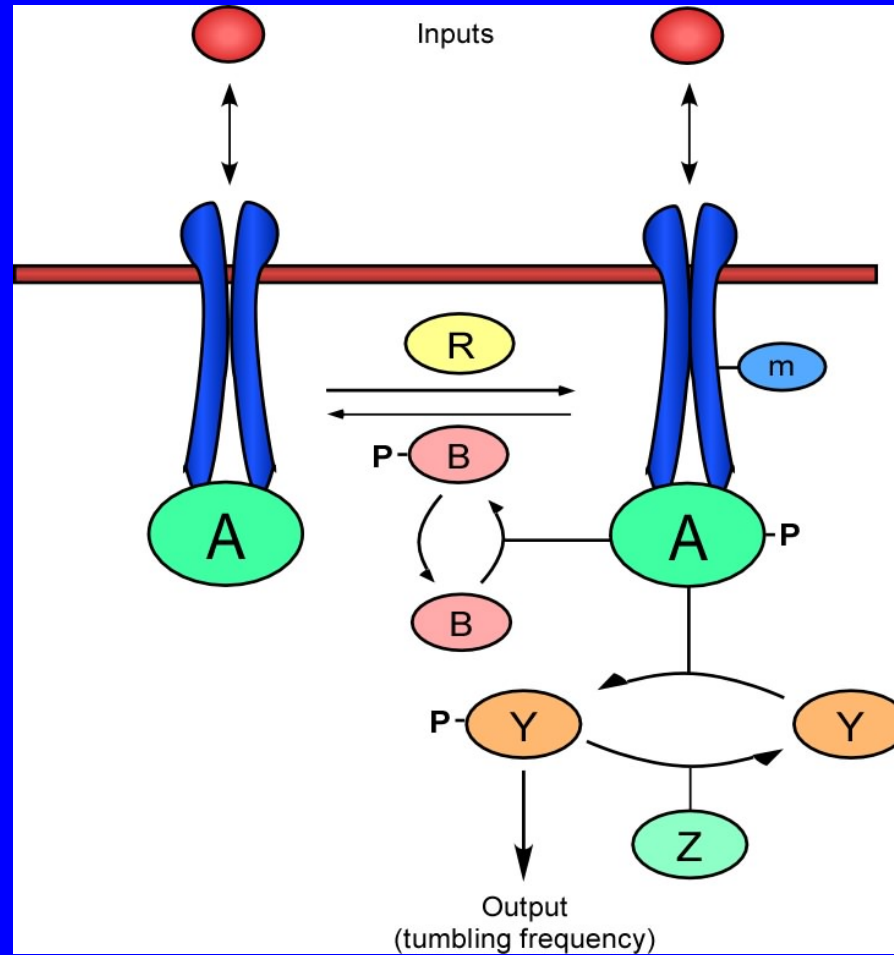**Diminish uncorrelated noise by a classical feedback**

- **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
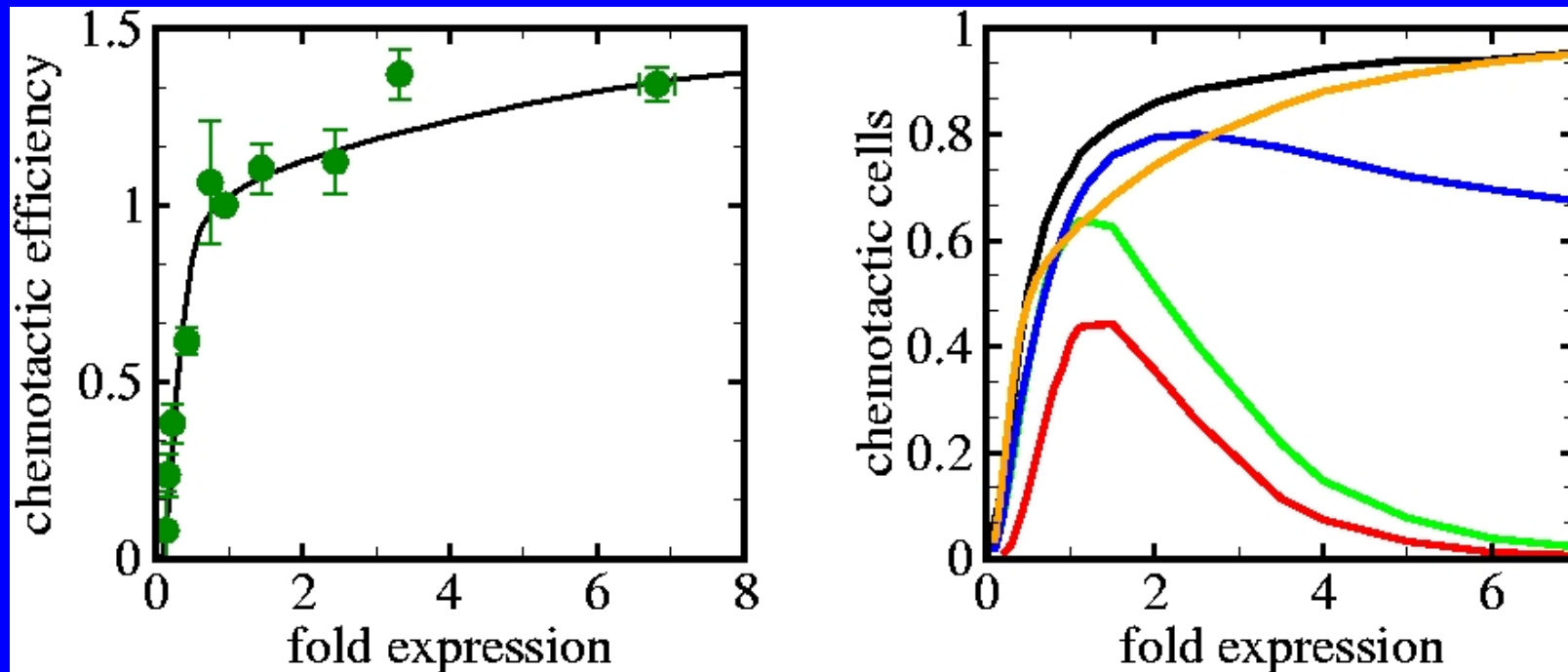
In silico **Biology**

- **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**
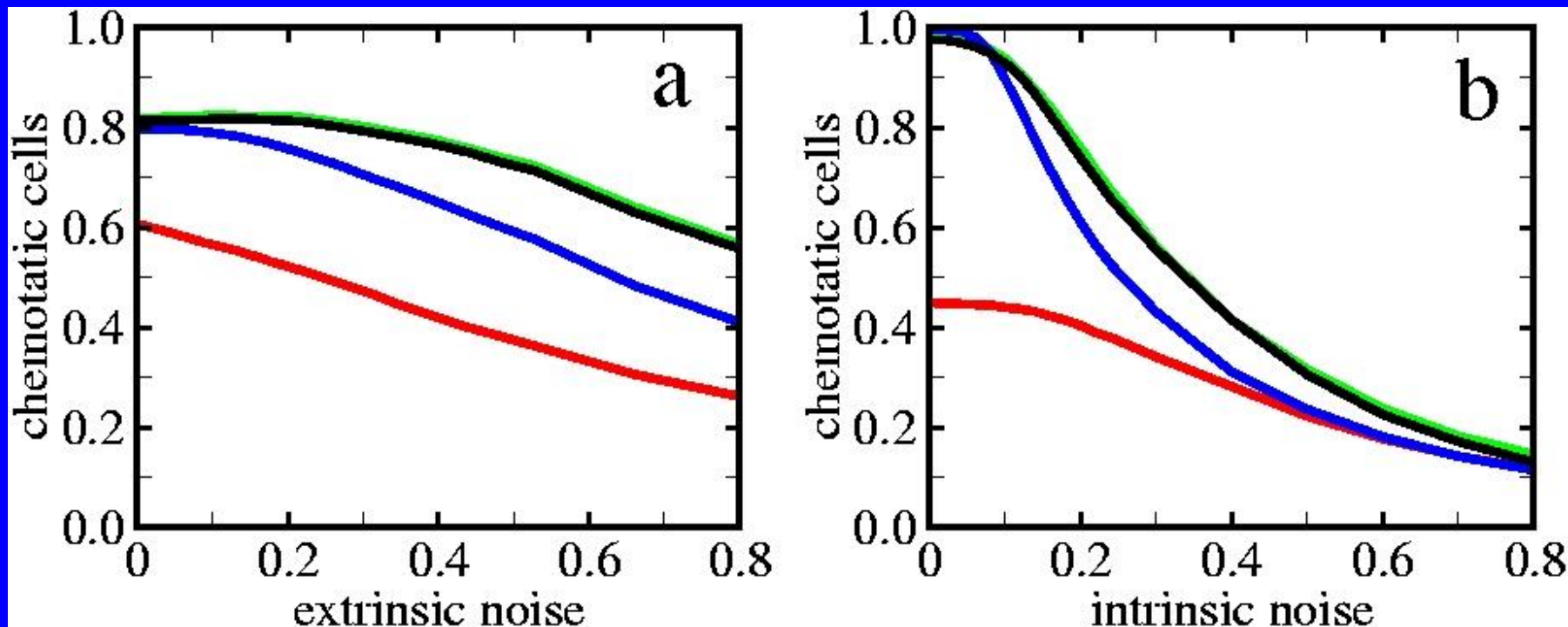
Cartoons of Perfect Adaptive Pathways

35
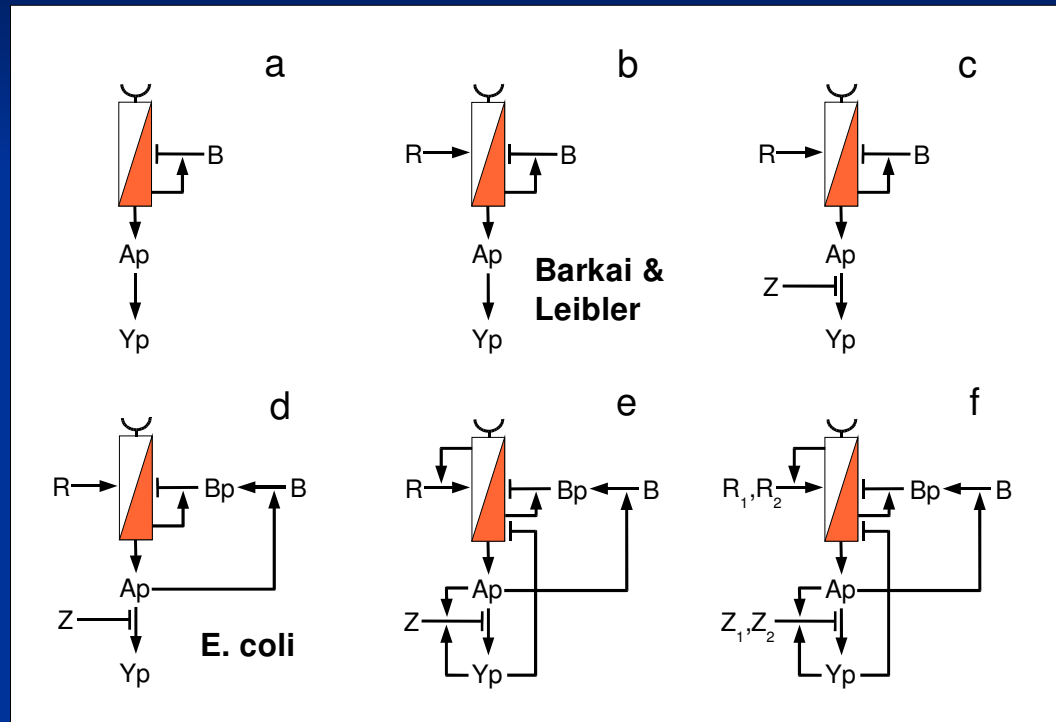
Results: in vivo vs. in silico

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 <u>and</u> 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

Physics Institute   **Centre for Molecular Biology**

University of Freiburg   **University of Heidelberg**

Markus Kollmann   **Victor Sourjik**

Kilian Bartholomé   **Linda Lovdok**

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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