Chemotaxis, Thermotaxis, Or How Organisms Choose Where To Go

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Princeton University

PNAS 103, 1786 (2006)

PNAS 103, 13040 (2006)

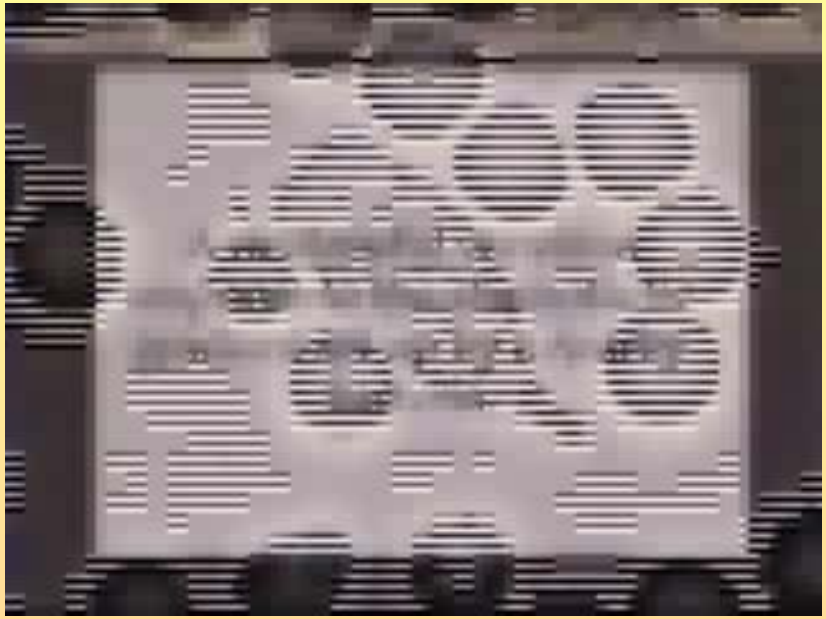
Biophys J. 90, 4317 (2006)

Experiments: Victor Sourjik, Howard Berg

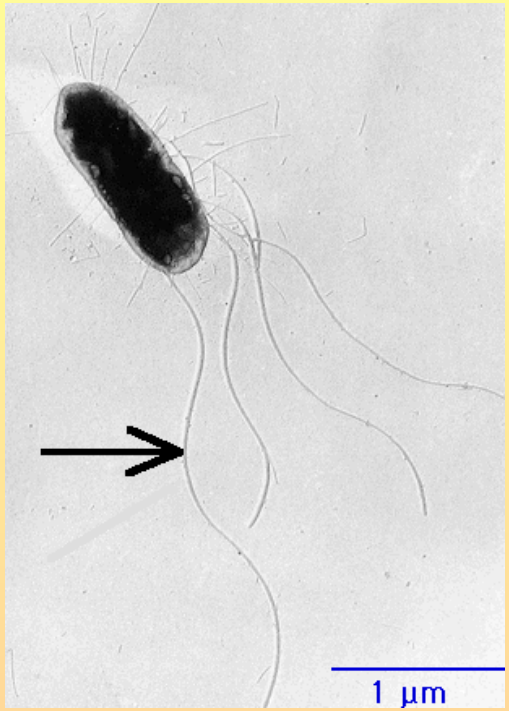
Harvard University

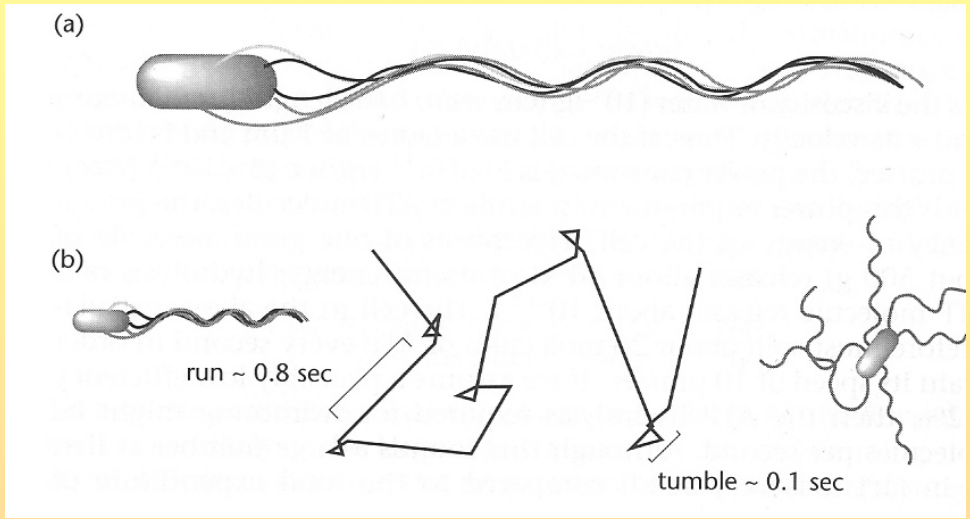
Ruprecht-Karls-Universität Heidelberg

cells can sense subtle chemical gradients and act upon it within seconds



E-Coli: the hero of the story:

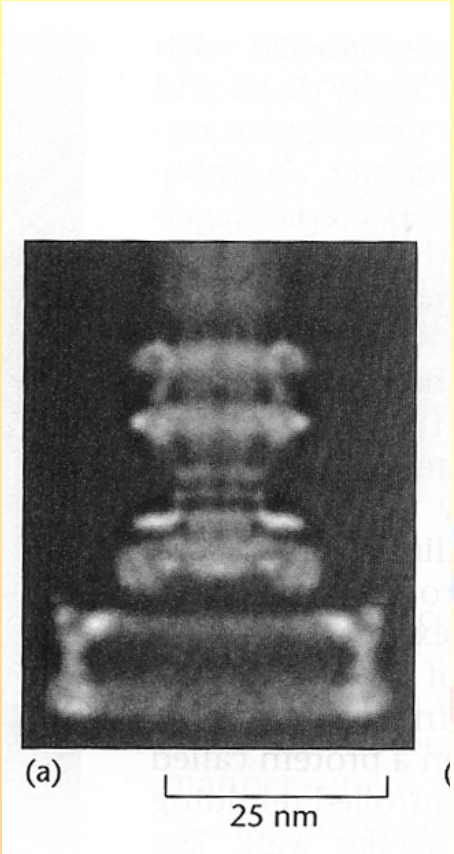


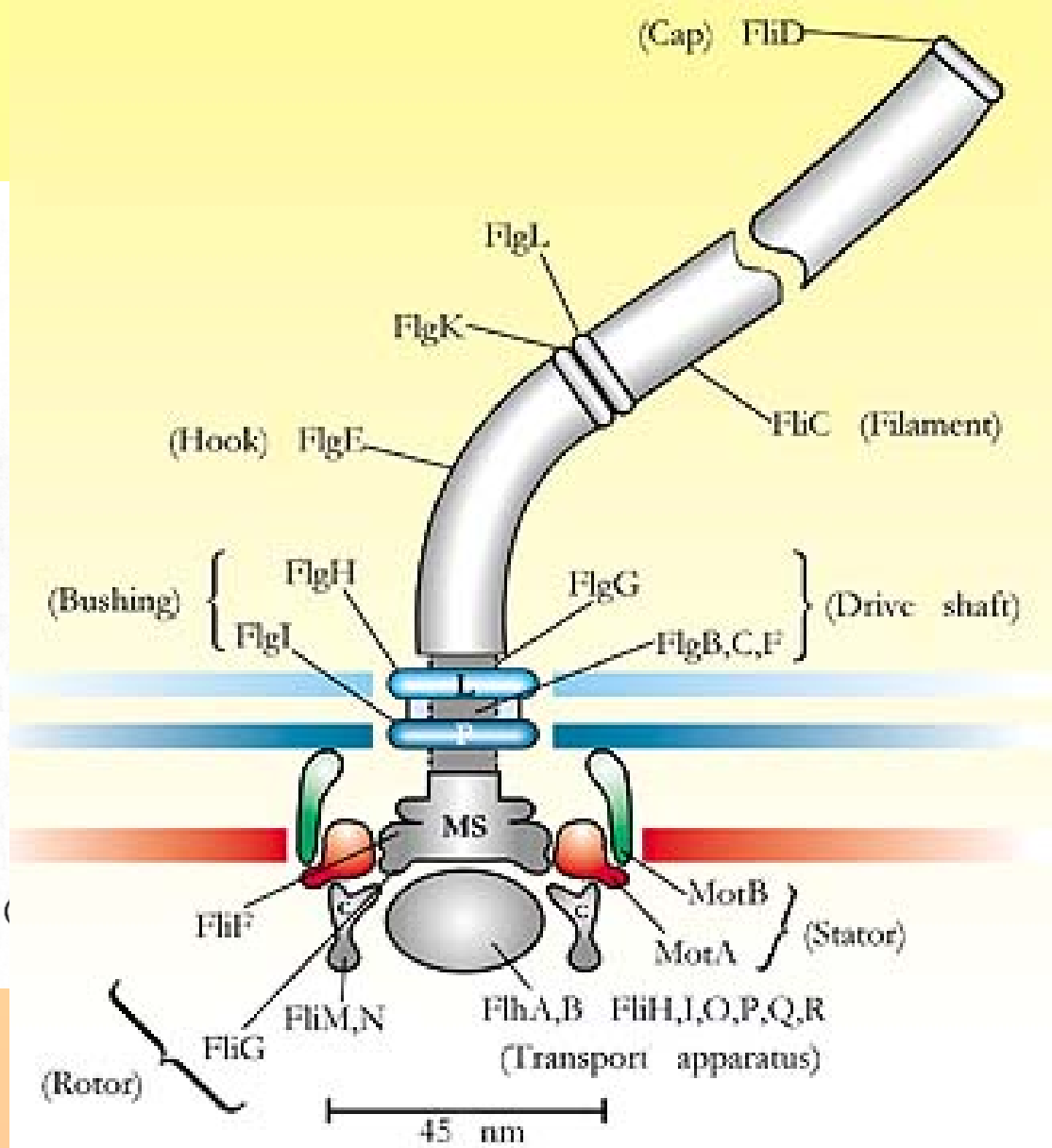


Run

Run, Tumble

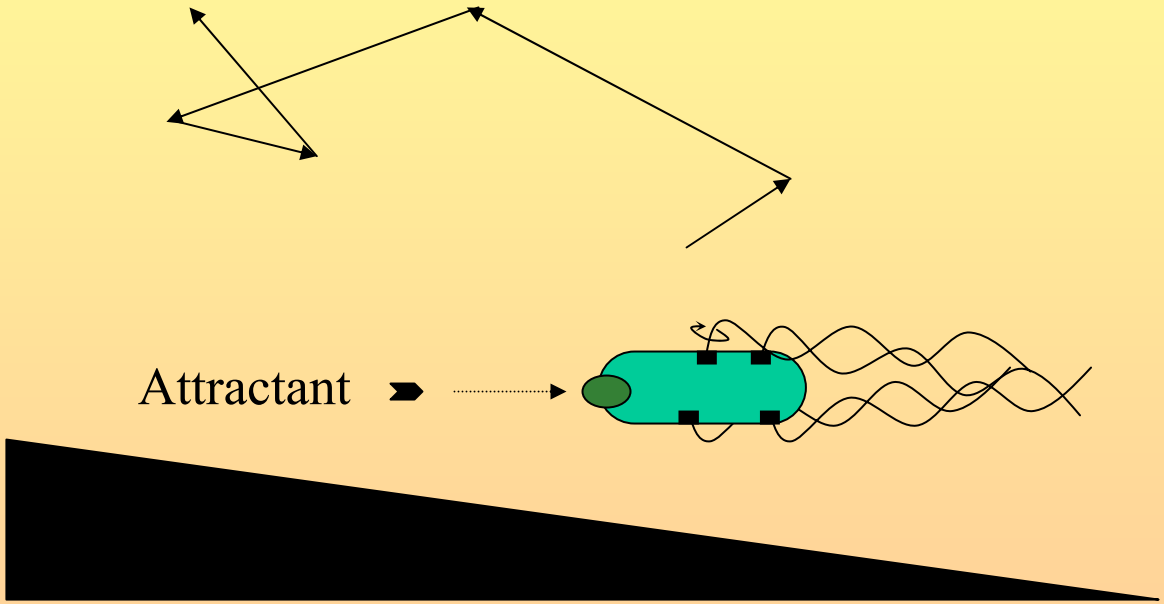
the bacterial motor:





Tethered cells

Bacterial strategy for chemotaxis: biased random walk

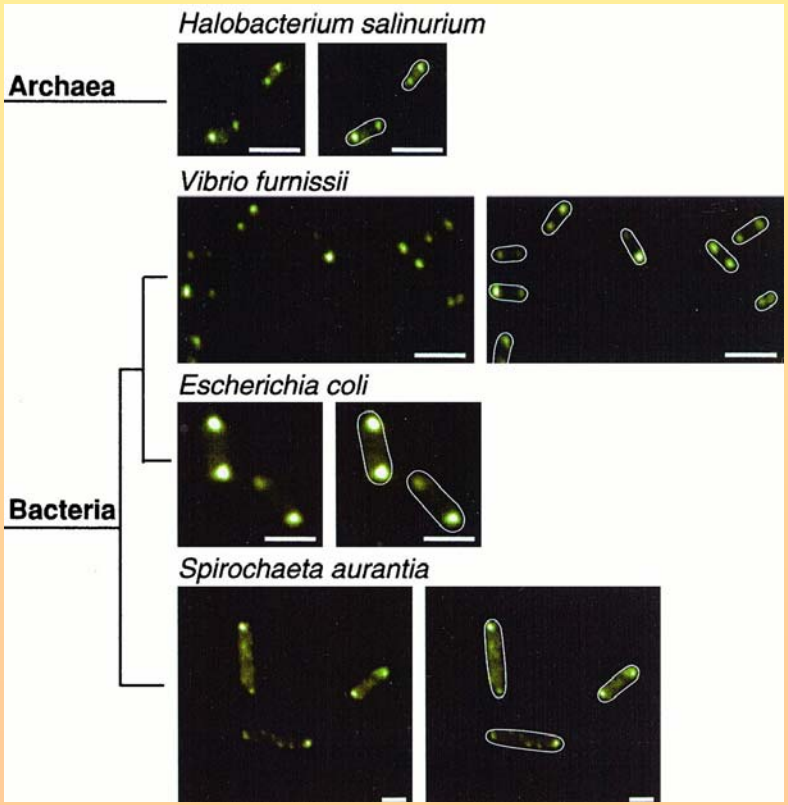


Temporal (not spatial) comparisons

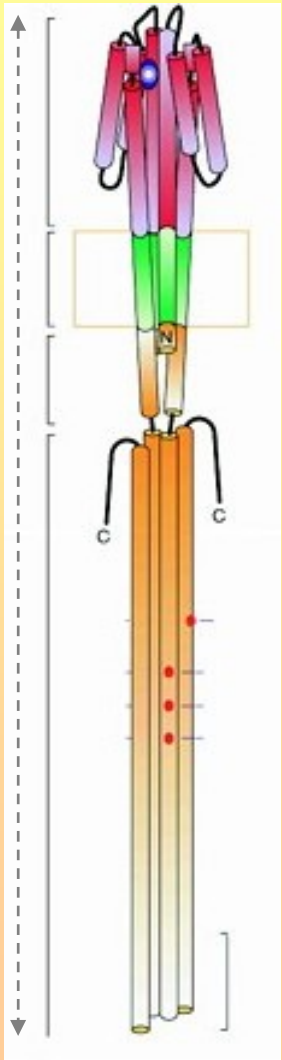
Chemo-receptors

5 different types: Tar (~900 copies), **Tsr** (~1600), **Trg** (~150), **Tap** (~150), **Aer** (150?)

Receptor clustering



Dimer



Sensor

Linker region

380 A

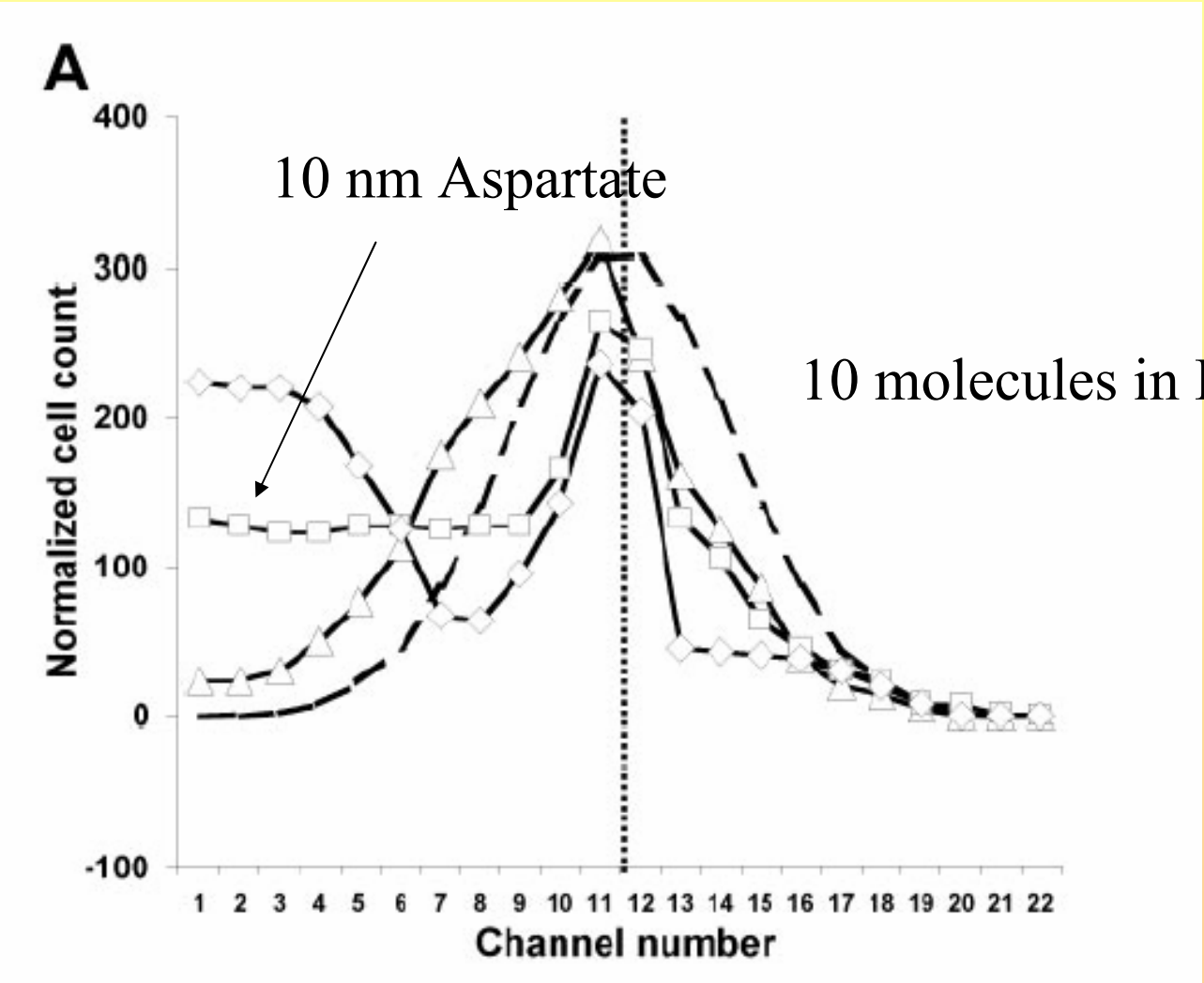
Cytoplasmic domain

Methyl binding sites CheB, CheR

CheA / CheW binding region

Stock (2000)

Extremely high sensitivity



10 molecules in E-Coli volume !

Mao et al. (2003)

Amplification

Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 8987–8991, December 1986
Biophysics

Temporal comparisons in bacterial chemotaxis

(impulse response/step response/adaptation/gain)

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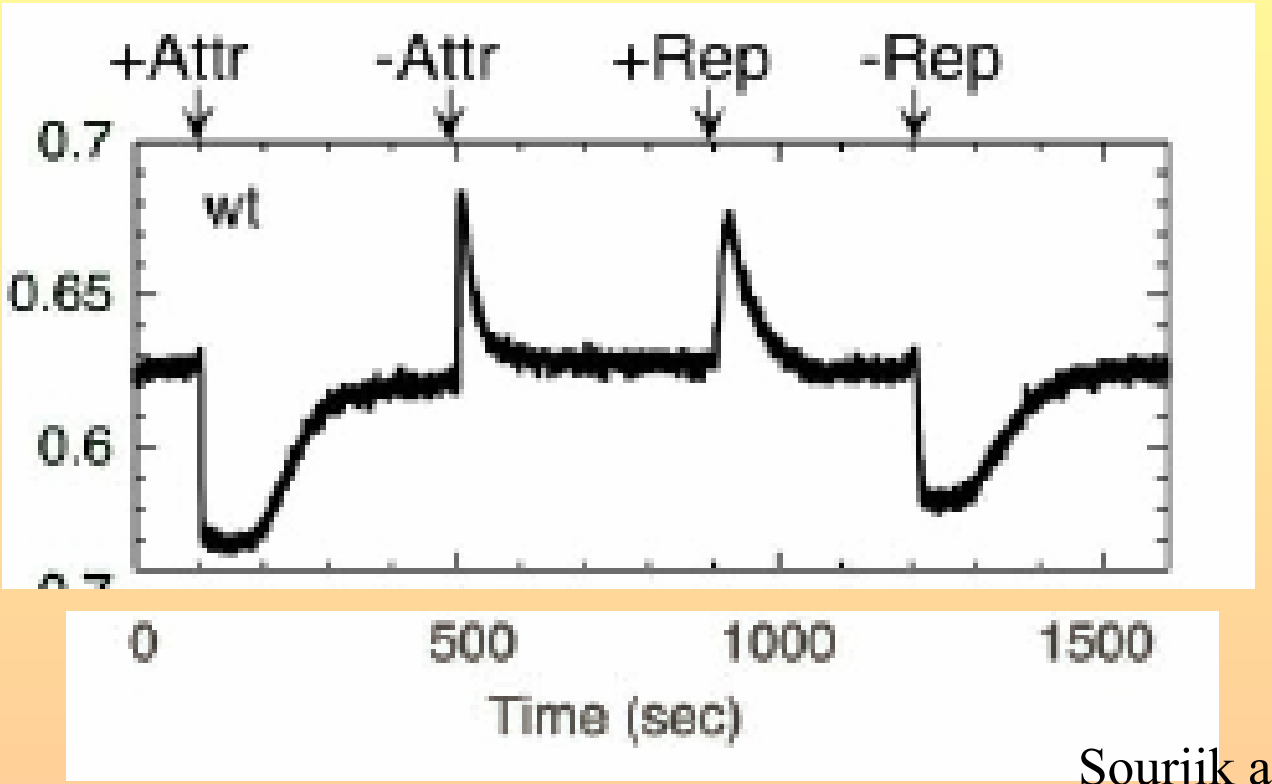
Contributed by Howard C. Berg, August 18, 1986

RESULTS

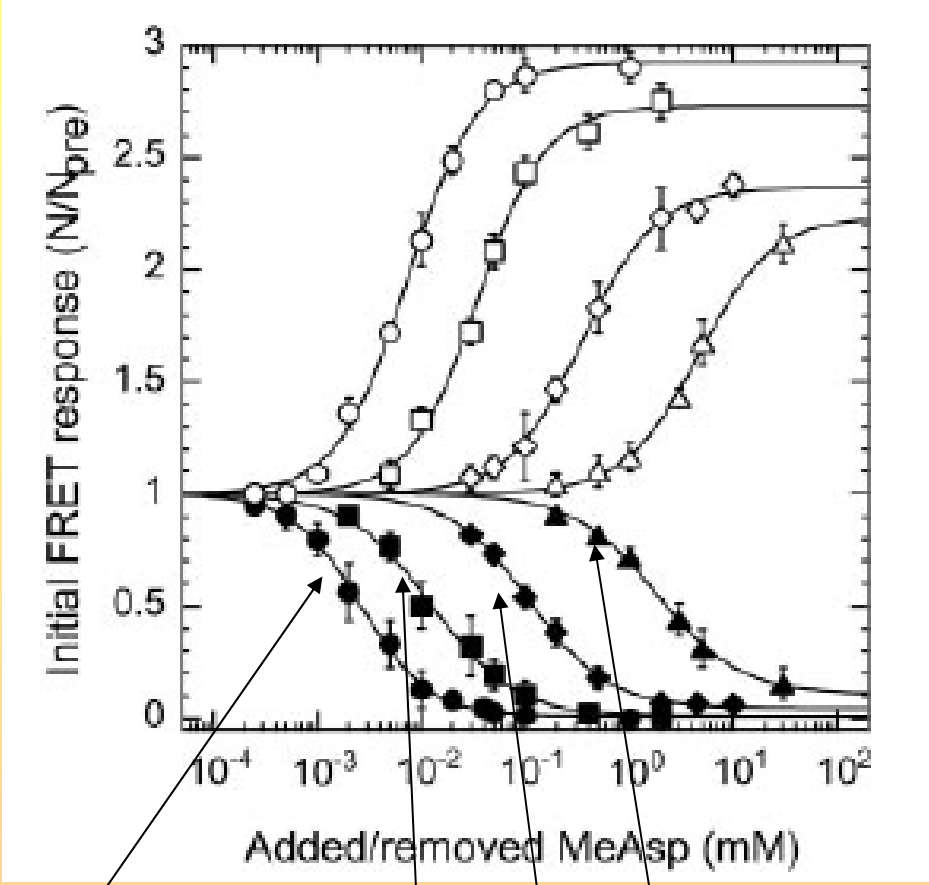
Calibration of the Impulse Response. Given the impulse response of Fig. 1 (induced by pulses of small but unknown amplitude), one can predict the time course of the response to an arbitrary stimulus; however, the amplitude of this response is unknown up to a constant scaling factor. To predict both the amplitude and the time course of a response, this scaling factor must be determined. First, we measured the rate at which attractant was released from a particular set of pipettes by exposing cells 5 μm away to a large step in current (−100 nA) and recording their recovery times: this works because the steady-state concentration of attractant a fixed distance away from the tip of a pipette is proportional to the rate of release (p. 23 of ref. 17), and the recovery time is proportional to the net change in receptor occupancy (cf. table 1 of ref. 16). Next, we measured the amplitude of the response of the same cells to a smaller step in current (−3 to −10 nA). Assuming that the rate of release varies linearly with current, the change in concentration generated by the smaller step was determined. The type of response generated by the smaller steps is shown in Fig. 2. Note that the response is not saturated. For the subset of cells used in the calibration (those exposed to α-methyl-DL-aspartate; see figure legend) a change in bias of 0.23 occurred for an estimated change in fraction of receptor bound of 0.0042. Finally, we calibrated the impulse response by subtracting the baseline and scaling its integral to the change in bias of the calibrated step response. We found that a response of the amplitude shown in Fig. 1 would be generated by a pulse that increased the receptor occupancy by 0.19 for a period of 20 msec (the approximate width of the shortest pulse used in our experiments).

Precise adaptation



Sourjik and Berg (2001)

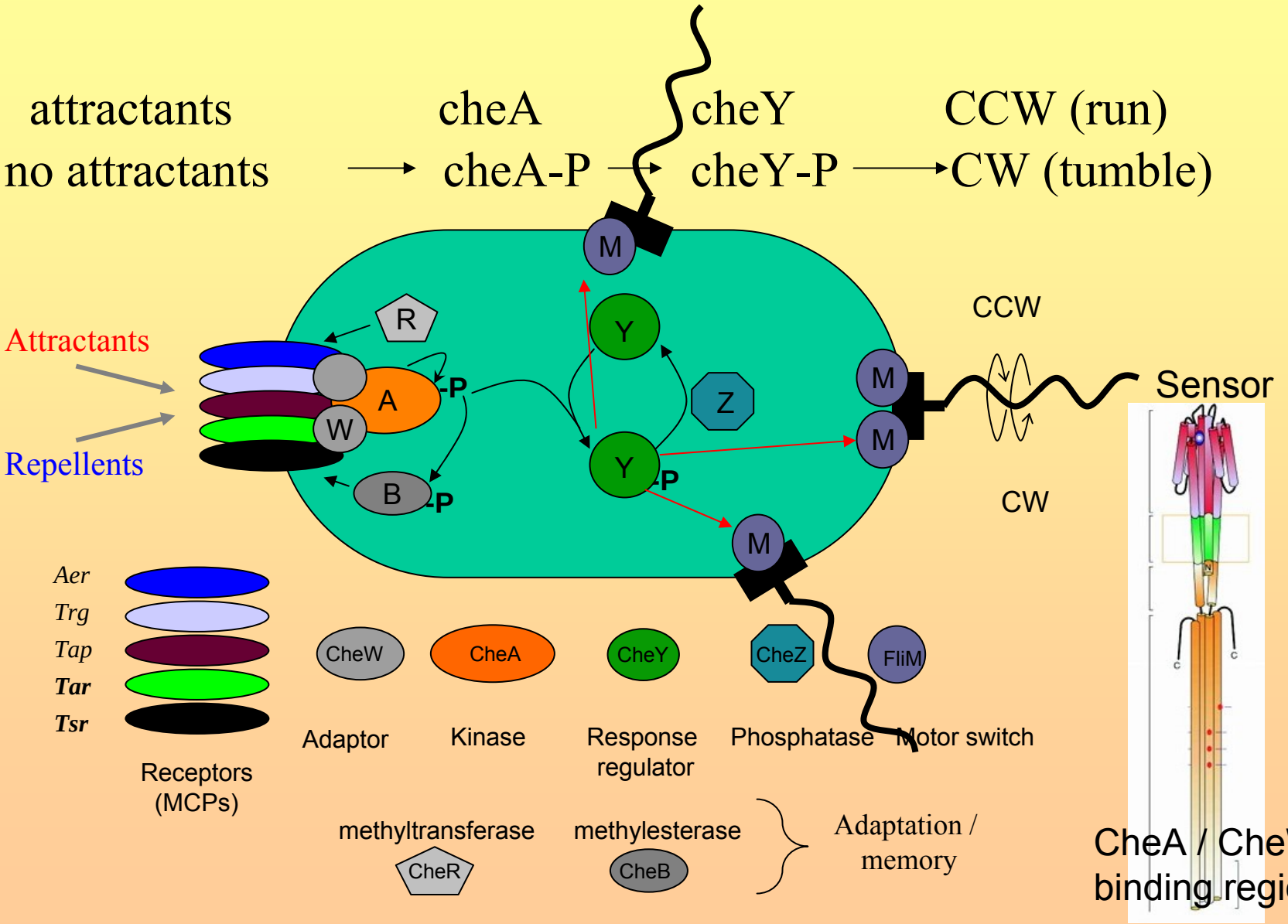
Wide dynamic range



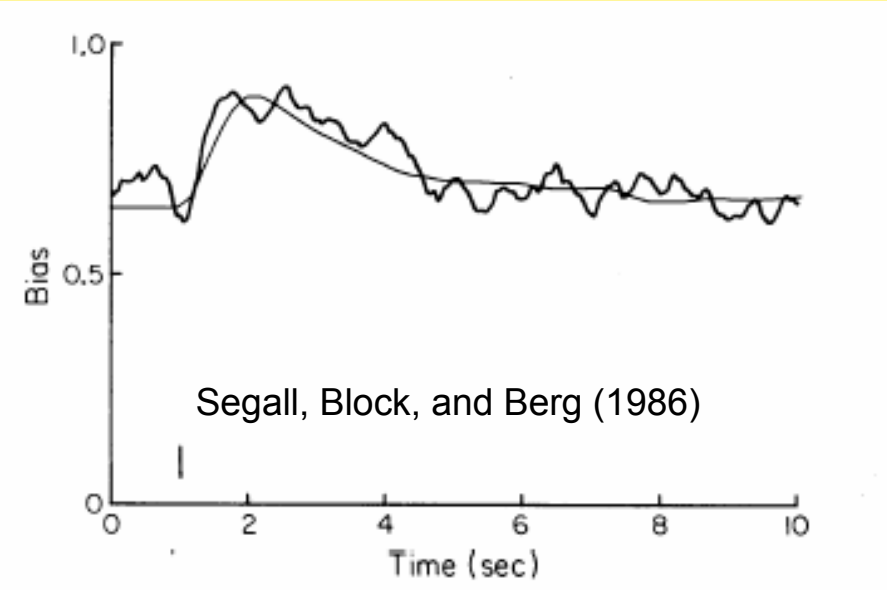
0 0.1 0.5 5 mM

Sourjik and Berg (2001)

Signalling pathway in chemotaxis of *E. coli*



Signaling properties of the chemotaxis network



CCW vs CW bias for tethered cells in response to step in attractant

- "Robust and precise adaptation": range of 3-4 orders of magnitude of attractant
- "Signal integration": multiple attractants
- "Sensitivity": amplification
- Wide dynamical range

Receptors translate amount of attractants to activity (cheA to cheA-P)

Input: amount of attractants

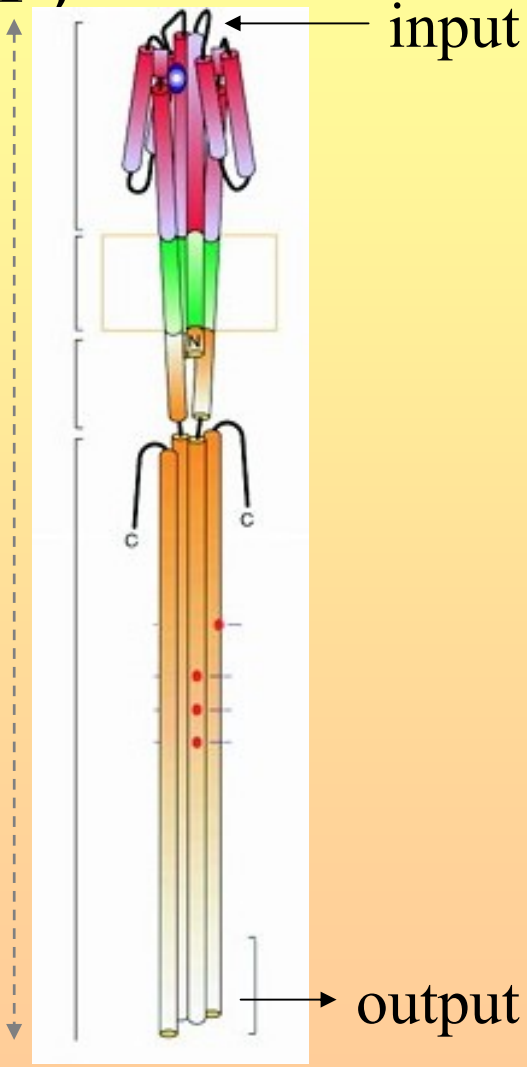
Output: activity

Attractant binding inhibits phosphorylation of CheA

[C] ↗ ⟶ P_{on} ↘

$$P_{on}[c] = \frac{e^{-F^{on}/kT}}{e^{-F^{on}/kT} + e^{-F^{off}/kT}} = \frac{1}{1+e^{-\Delta f[c]}}$$

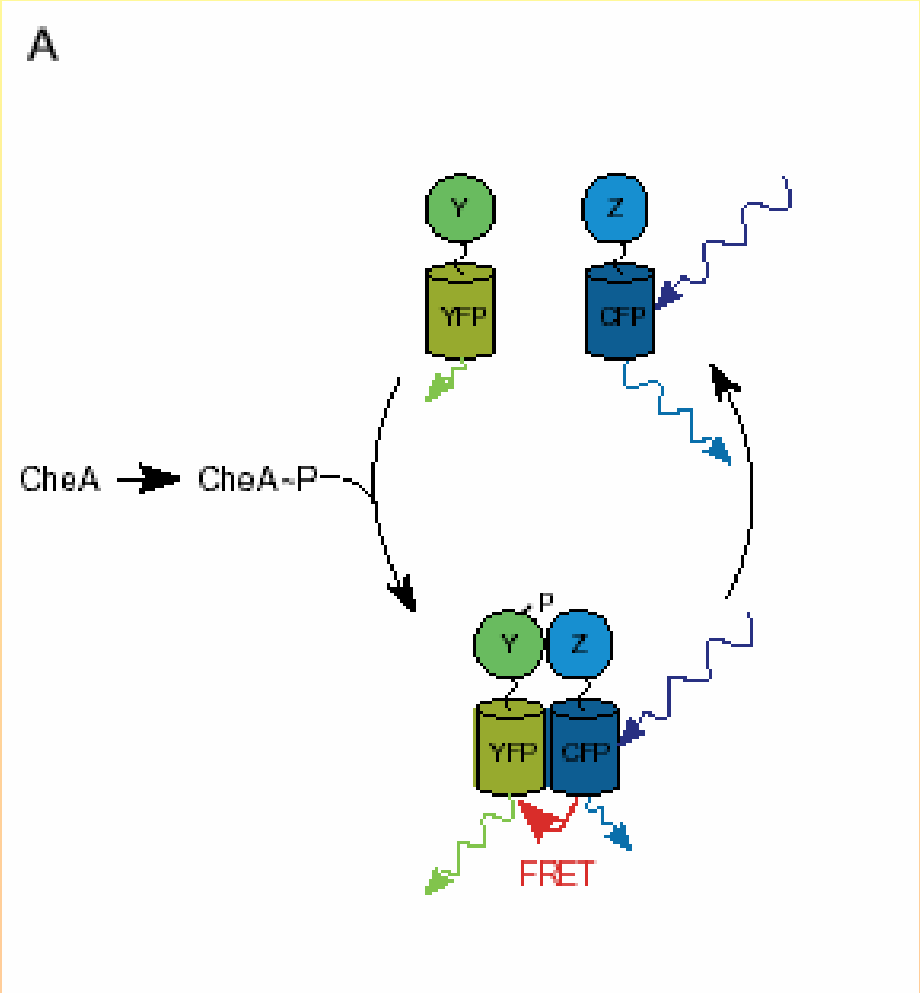
$$\Delta F = \Delta\varepsilon(c=0) + kT\sum_i \log(1 + C_i / K_i)$$

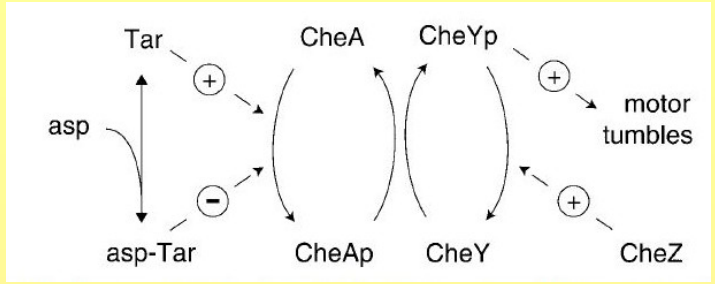


How can one measure activity (P_{on}) ?

FRET (Sourjik and Berg)

Fluorescence resonance energy transfer



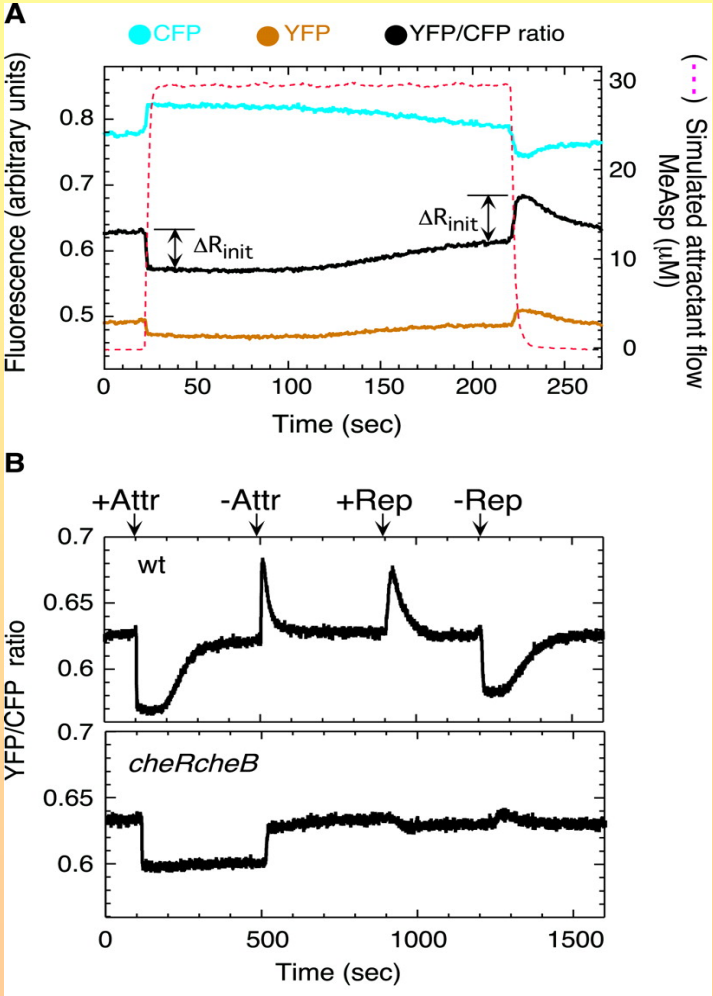


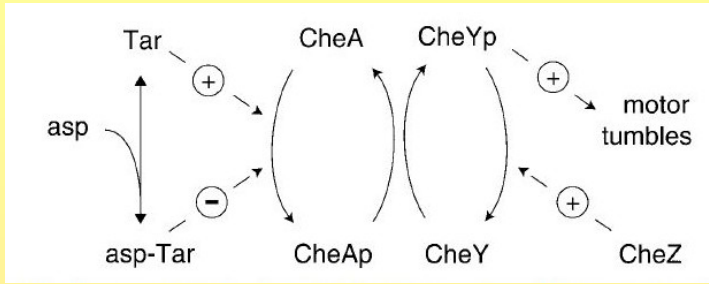
Conditions for FRET:

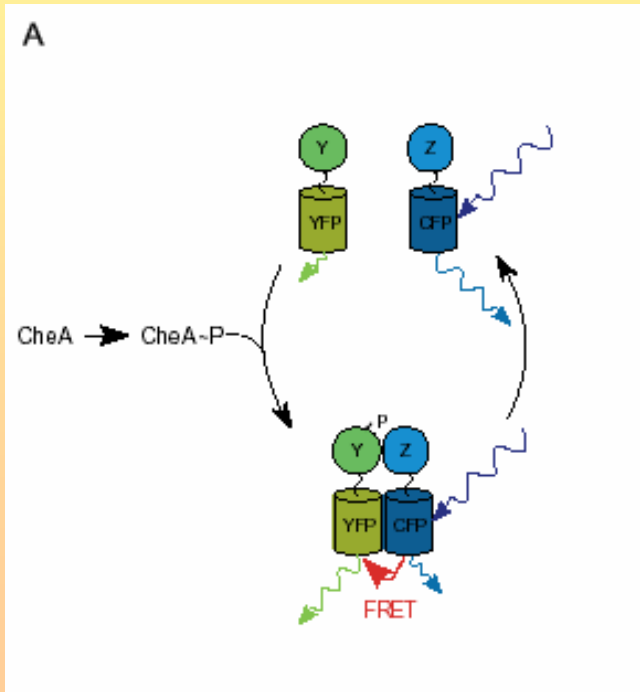
Overlap between emission and excitation spectra of the donor and excitation pair.

Pairs must be within about a “Forster radius.”
~ 3-5 nm

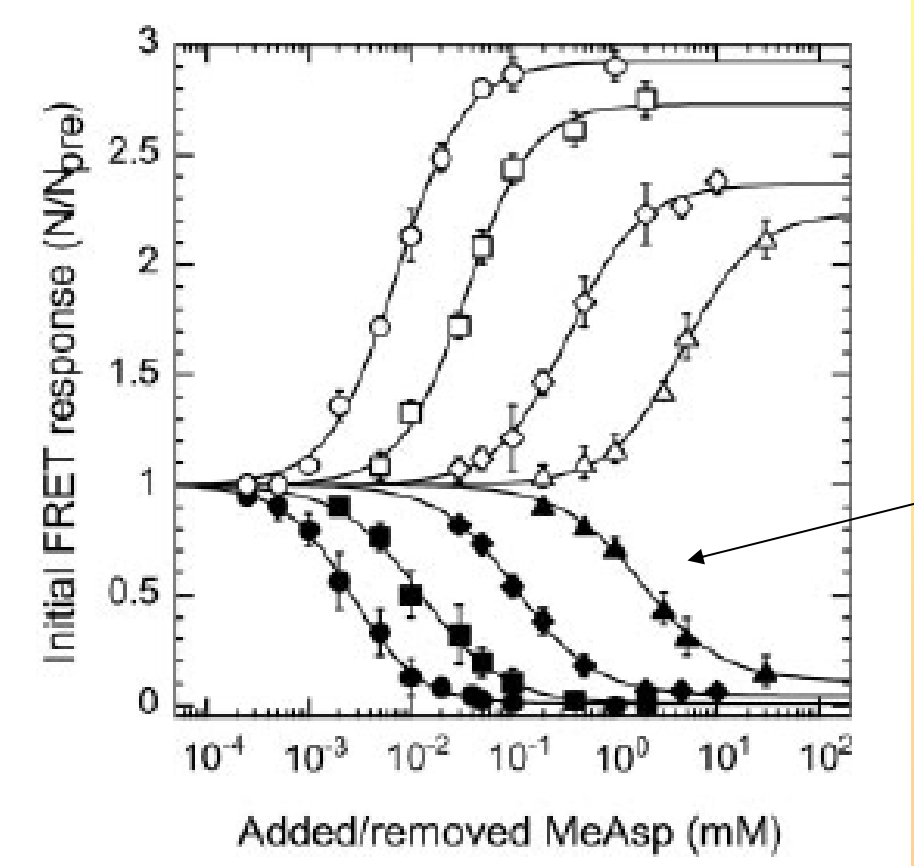
FRET (cont.)


A
CFP
YFP
YFP/CFP ratio
Fluorescence (arbitrary units)
Simulated attractant flow MeAsp (μM)
ΔR_init
ΔR_init
Time (sec)
B
+Attr
-Attr
+Rep
-Rep
wt
cheRcheB
YFP/CFP ratio
Time (sec)


Tar
CheA
CheYp
asp
motor
tumbles
asp-Tar
CheAp
CheY
CheZ


A
Y
Z
YFP
CFP
CheA
CheA~P
P
FRET

FRET results

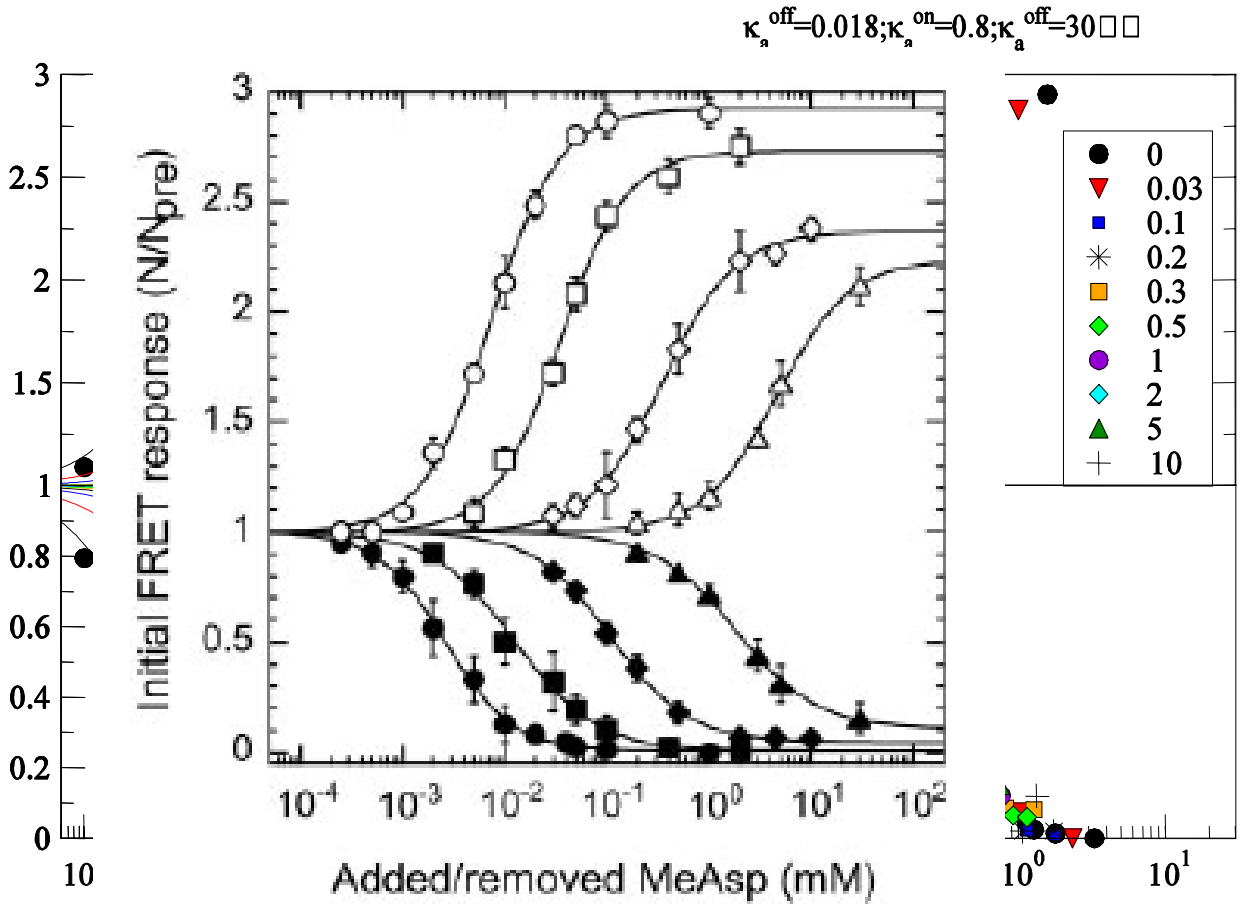


$$P_{on} = \frac{1}{1 + \left(\frac{[c]}{\kappa_d}\right)^n}$$

n – Hill coefficient

Fig. 3. Response of wild-type cells to steps of MeAsp at different ambient concentrations, measured with the CheY/CheZ FRET pair. (*A*) Initial response amplitudes as a function of the magnitude of the step change in concentration of MeAsp after complete adaptation to ambient concentrations 0 (●), 0.1 (■), 0.5 (◆), and 5 mM (▲). Additional MeAsp was added (closed symbols) and then removed (open symbols) in a sequence of steps of increasing size (as in Fig. 2).

If everything is only a function of free energy difference, all curves can be collapsed by scaling



Receptors: activity function depends on methylation

- Adaptation – how much activity occurs for the same amount of attractant binding.

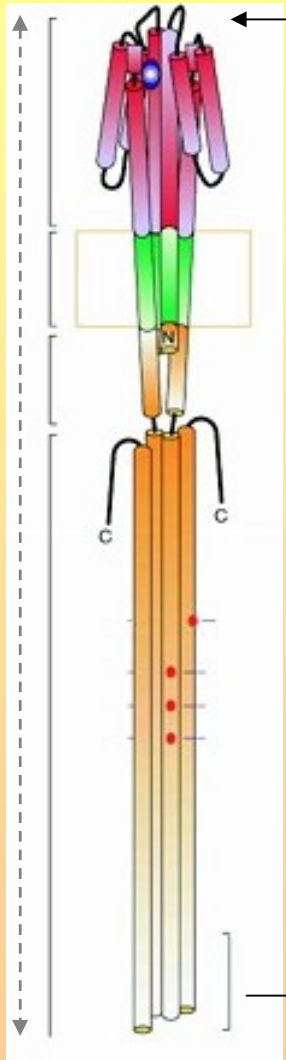
  More attractant → increased methylation by CheR → increased activity

  Less attractant → increased demethylation by CheB → decreased actvity

Control of the function $P_{on}([C])$

$$P_{\text{on}}[C,m] = \frac{1}{1+e^{-\Delta f[C,m]}}$$

$$\Delta F(C,m) = \Delta\varepsilon(m) + kT\sum_i \log(1 + C_i / K_i)$$



Adaptation: P_{on}

$$P_{\text{on}} = \frac{e^{-F^{on}/kT}}{e^{-F^{\text{on}}/kT} + e^{-F^{\text{off}}/kT}} = \frac{1}{1+e^{-\Delta F/kT}}$$

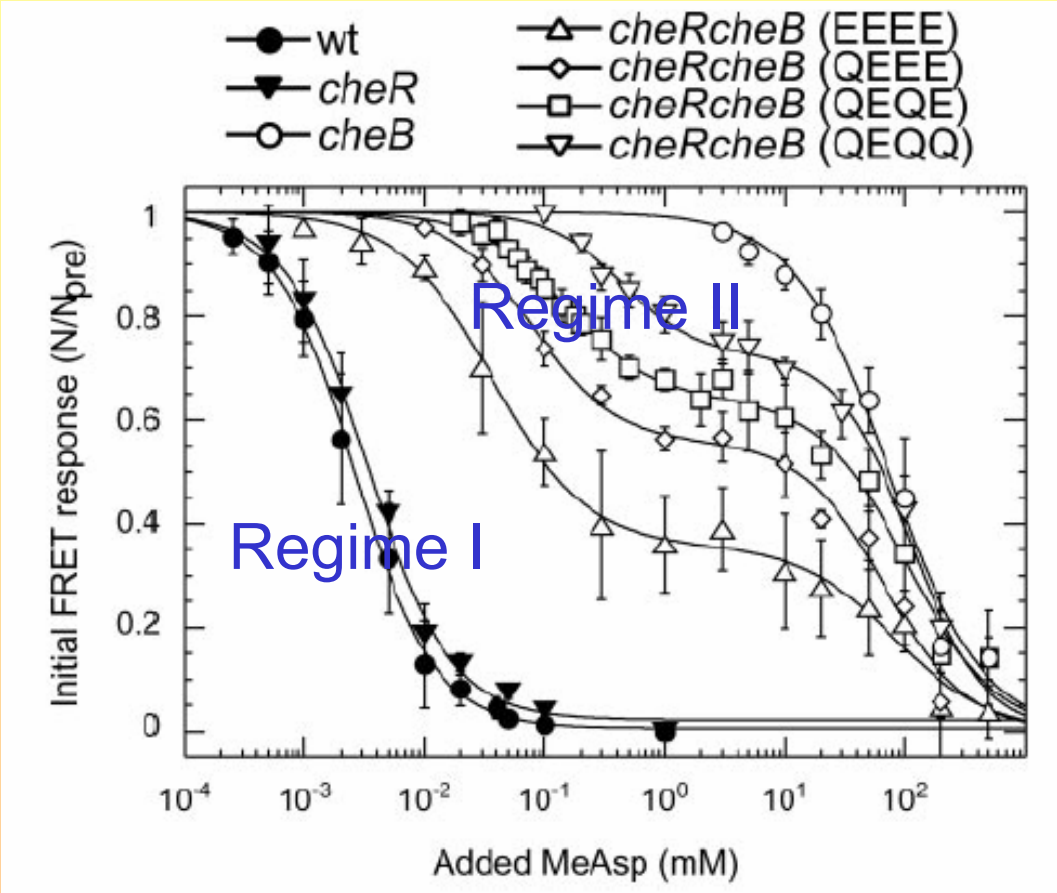
Allowing for attractants/repellents and methylation:

$$\Delta F = \Delta\varepsilon(m) + kT\sum_i \log(1 + C_i / K_i)$$

To adapt, the methylation changes to achieve $\Delta F = const$

FRET data: two regimes of activity

Sourjik and Berg (2002)

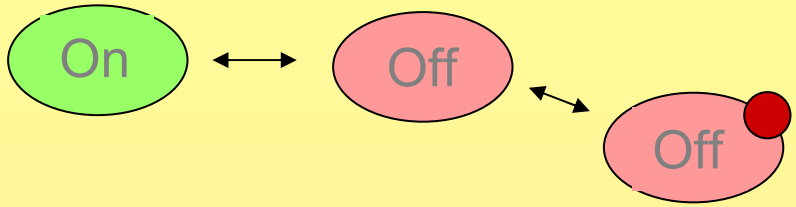


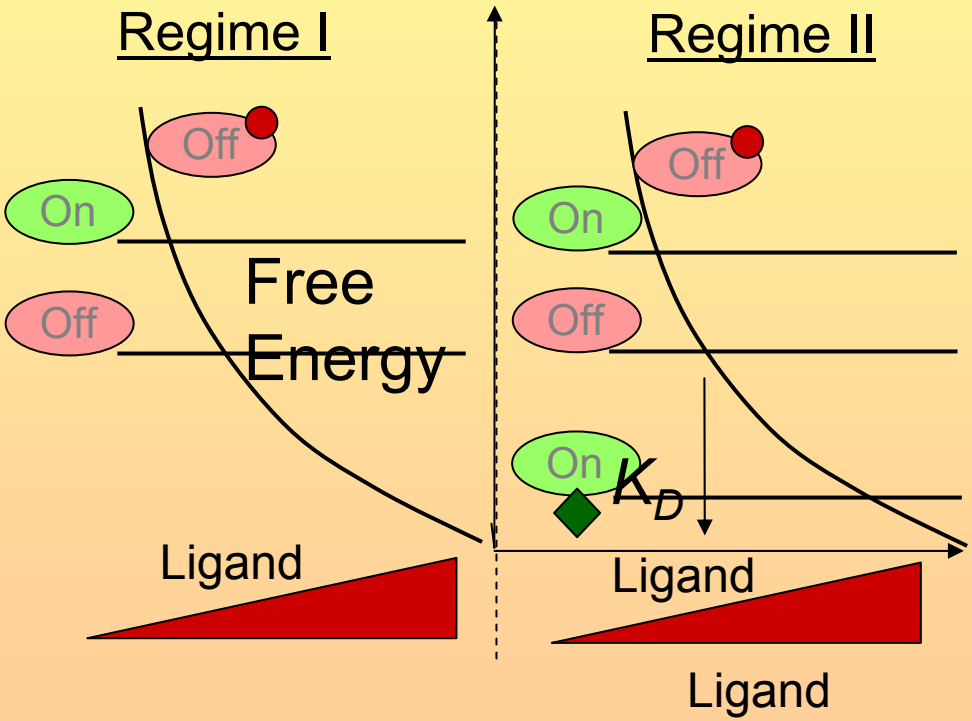
Regime I:
- Activity moderate to low at zero ambient MeAsp (0.06,1)
- K_D small and almost constant

Regime II:
- Activity high (saturated?) at zero ambient MeAsp (1.3-1.9)
- K_{D1} large and increasing with methylation
- Plateau in activity
- K_{D2} approximately constant

Two regimes of receptor activity consistent with 2-state receptor model.

Two regimes of a 2-state receptor





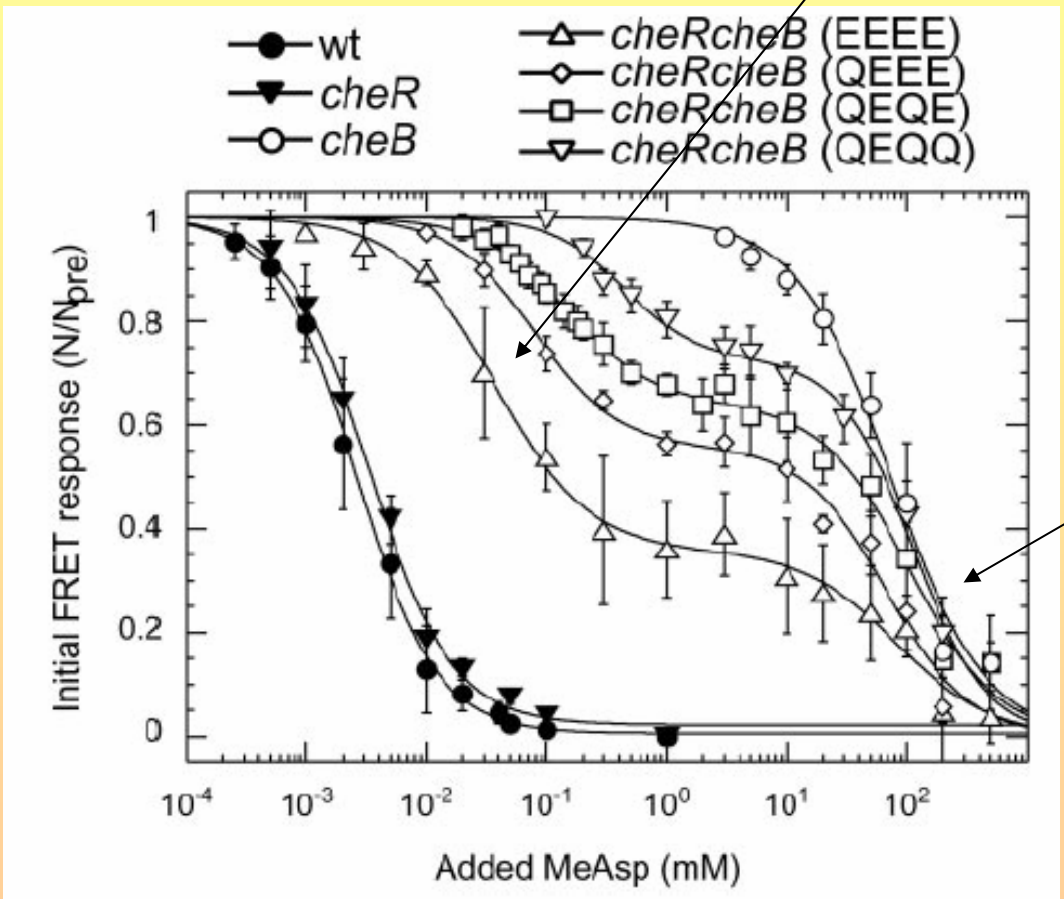
Regime I:
- Activity low to very low at zero ligand concentration
- $K_i = K_D^{off}$

Regime II:
- Activity high (saturated) at zero ligand concentration
- K_i increasing as ε^{on} ↓

However, single receptor does not account for low apparent K_i in Regime I.

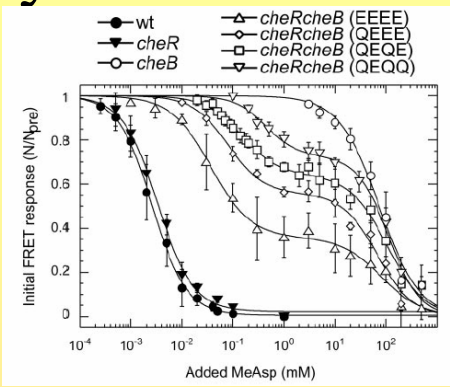
$$\text{Activity} = \frac{e^{-\varepsilon^{on}}}{e^{-\varepsilon^{on}} + e^{-\varepsilon^{off}} \frac{C}{K_D^{off}}}$$

Tars turn off



Tsrs turn off

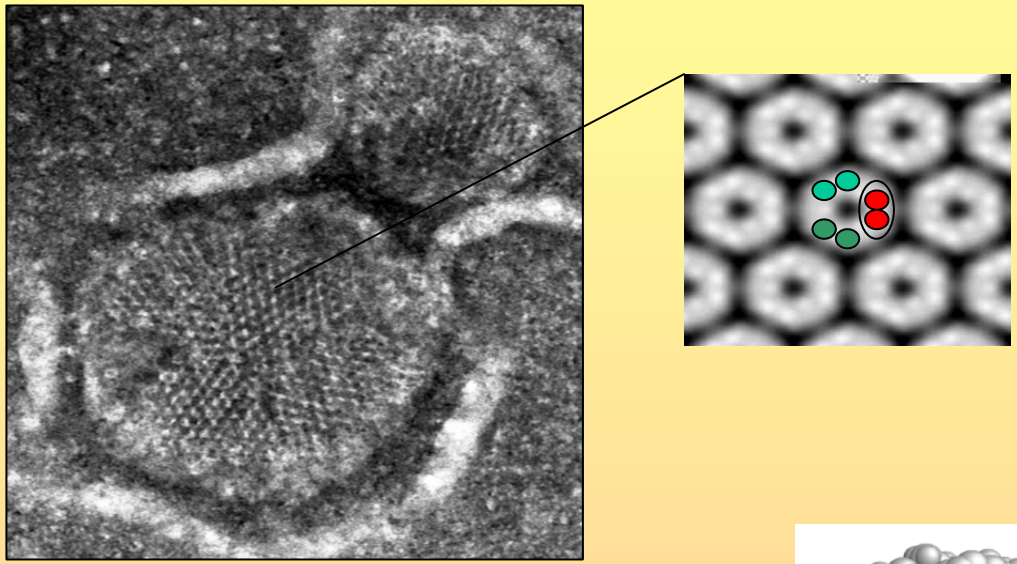
- Why all receptors turns off simultaneously in wild type ?
- Why is κ_d so small in wild type ?
- Why Hill coefficient larger than 1 ?
- Where does amplification comes from ?
- Why plateaus at different heights and different κ_ds ?
- How is the system sensitive to ligands attracted to minority receptor ?



→ Receptor – receptor coupling

Duke and Bray (1999)

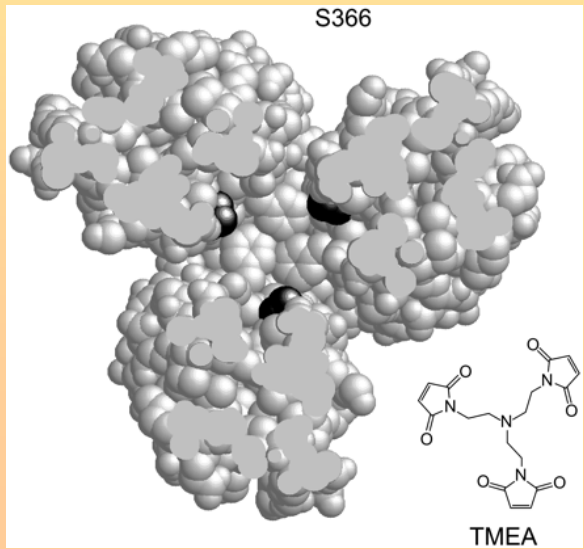
Receptors are clustered globally into a large array, and locally into trimers of dimers.



Gestwicki *et al.* (2000)

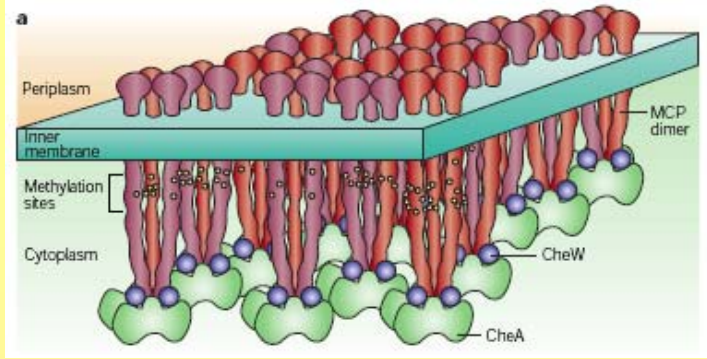
McAndrew *et al.* (2004, 2005)

cluster of
trimers of dimers



Kim *et al.* (1999); Studdert and Parkinson (2004)

Receptor-receptor coupling



- Each receptor can be either active (S=1/2) or inactive (S=-1/2)
- Increase in attractant concentration enhances the probability of being inactive (uniform magnetic field)
- Each (in)active receptor increase the probability of other receptors to be (in)active

$$F = \sum_i HS_i - \sum_{<ij>} J_{ij} S_i S_j$$

MWC model

N receptors are all "on" or all "off" together

$$P_{\text{on}} = \frac{1}{1 + e^{N\Delta\varepsilon}\left(1 + \frac{C}{K_D^{\text{off}}}\right)^N}, \quad \Delta\varepsilon = \varepsilon^{\text{on}} - \varepsilon^{\text{off}}$$

Regime II ($\Delta\varepsilon < 0$):

$$P_{\text{on}} = \frac{1}{e^{N\Delta\varepsilon}\left(1 + \frac{CN}{K_D^{\text{off}}}\right)^N}$$

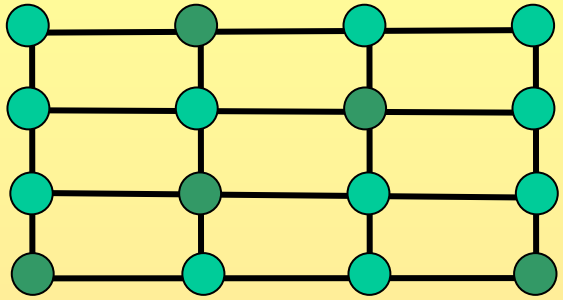
Receptor-receptor coupling gives enhanced sensitivity (low K_D) in Regime I, and enhanced cooperativity (high Hill coefficient) in Regime II.

K_D determined by

- Low activity: $\sim e^{-N\Delta\varepsilon}$ at zero ligand concentration ($C=0$)
- $P_{on}(K_D) = P_{on}(0)/2 \Rightarrow K_D = K_D^{\text{off}} e^{-\Delta\varepsilon}$ $K_D = K_D^{\text{off}}/N$ *(high sensitivity)*
- Hill coefficient = 1

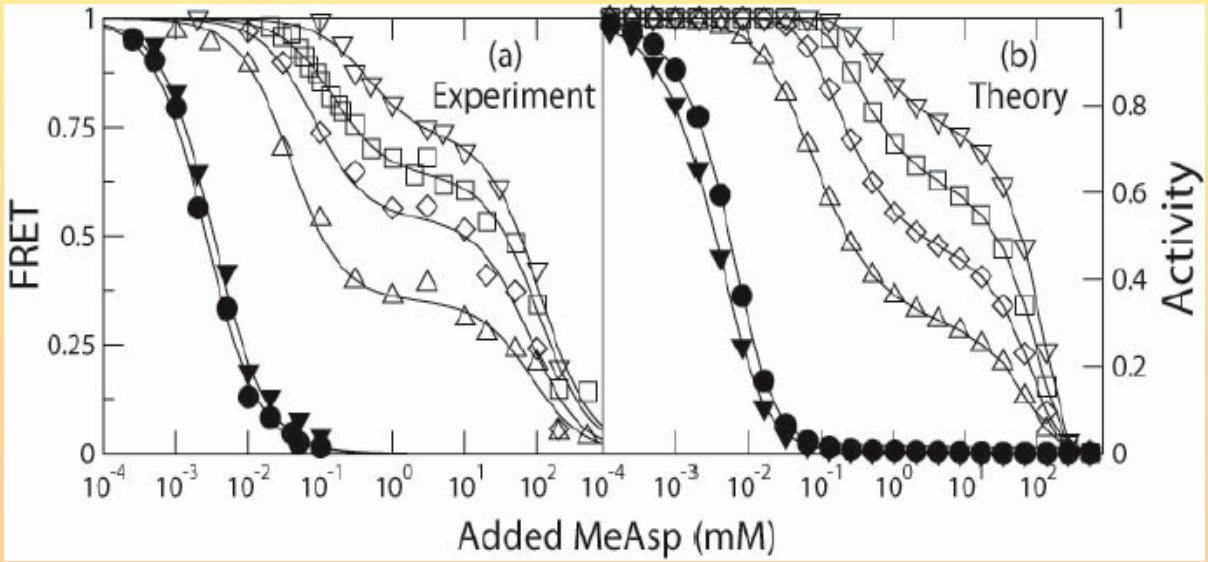
Hill coefficient = *N*

Mixed complex MWC model



Tar

Tsr



Regime I:

- $K_i = K_D^{off} / N$.

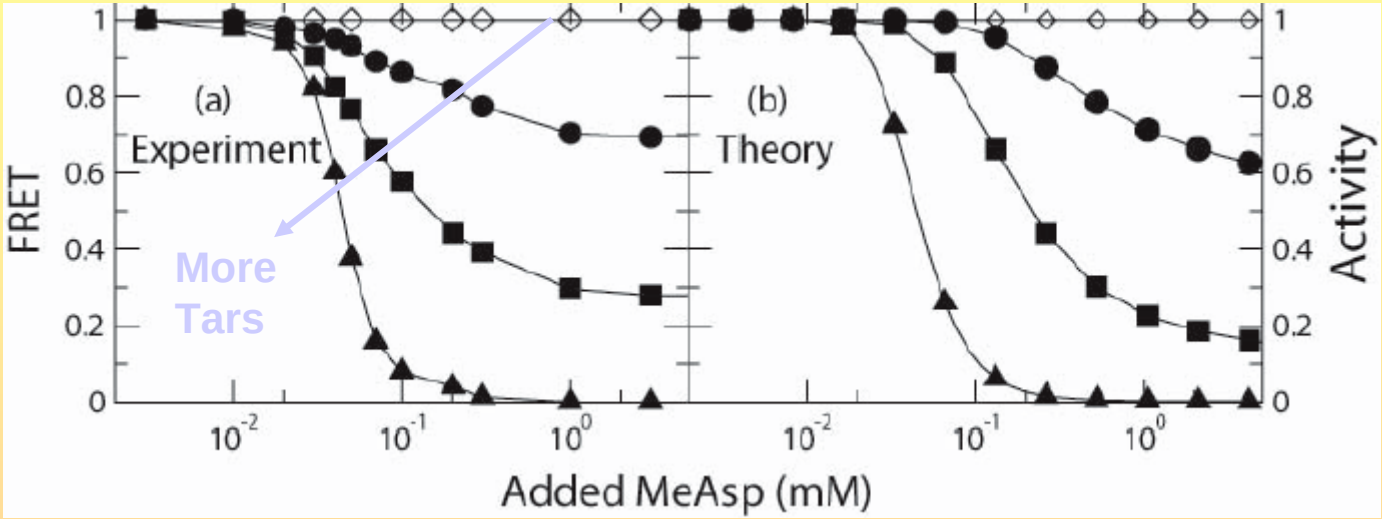
Regime II:

- Plateaus: some complexes “on”, some “off”.
- Hill coefficient ≈ 1.

Mixed complexes of size 14-16.

Each complex is an independent 2-state system.

Receptor homogeneity and cooperativity



Receptors are in Regime II:

• Hill coefficient increases with Tar homogeneity because more receptors bind ligand at transition.

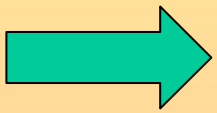
• K_i (or K_{i1}) decreases with Tar homogeneity because fewer Tsrs need to be switched off.

What about adaptation ?

Highest sensitivity for ΔF=0, this is what methylation does:

$$\Delta F(C,m) = \Delta\varepsilon(m) + \sum_i \log(1 + \frac{C_i}{K_i})$$

Barkai and Leibler: $\frac{d\ \text{Methylation}}{dt} = a[\text{CheR}] - b[\text{CheB}]\, P_{\text{on}}$



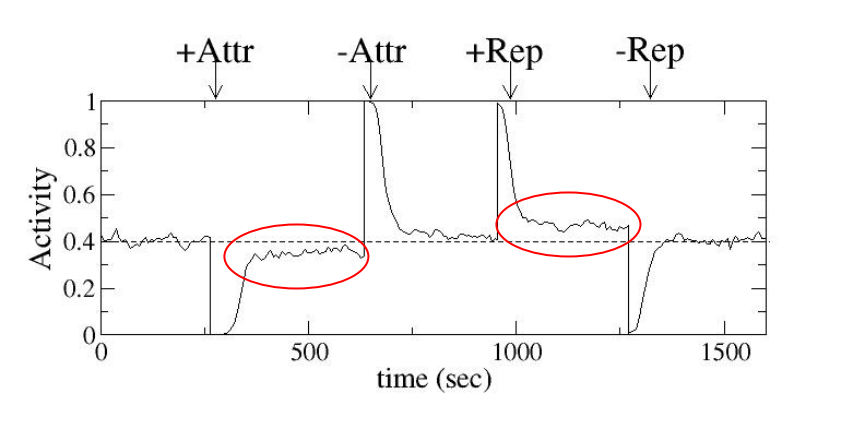
$P_{\text{on}} = a\,[\text{CheR}] \,/\, b[\text{CheB}]$

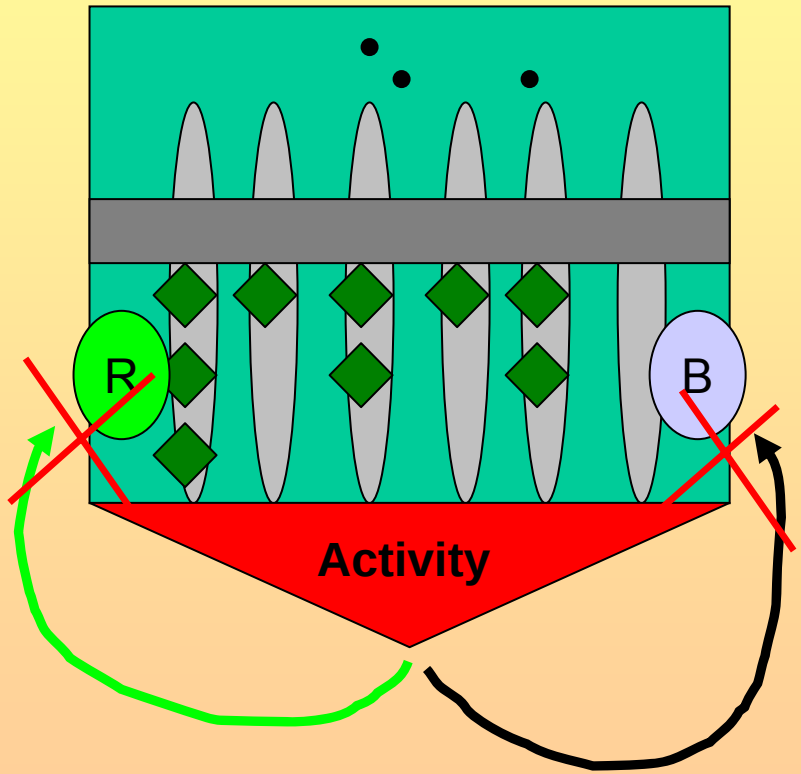
Assumes continuous level of methylation !

What happens if one takes into account discreteness of methylation levels ?

Imprecise adaptation of receptor clusters

Simulation

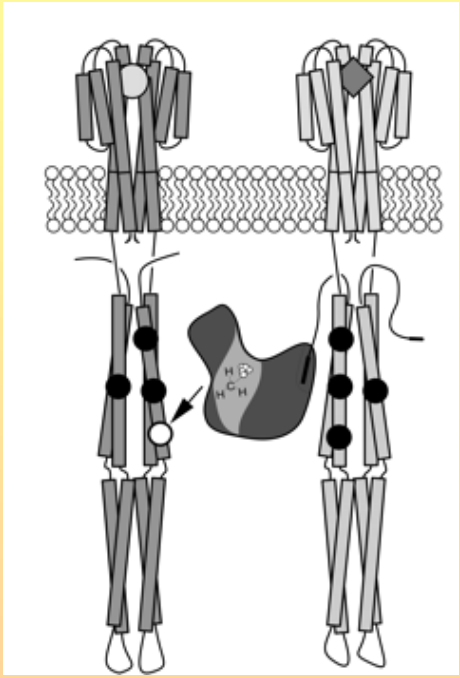




* Gillespie algorithm
  stochastic and exact
* Cluster size 18 receptors
* [Tar:Tsr]=[1:2]
* averages of 100 independent runs

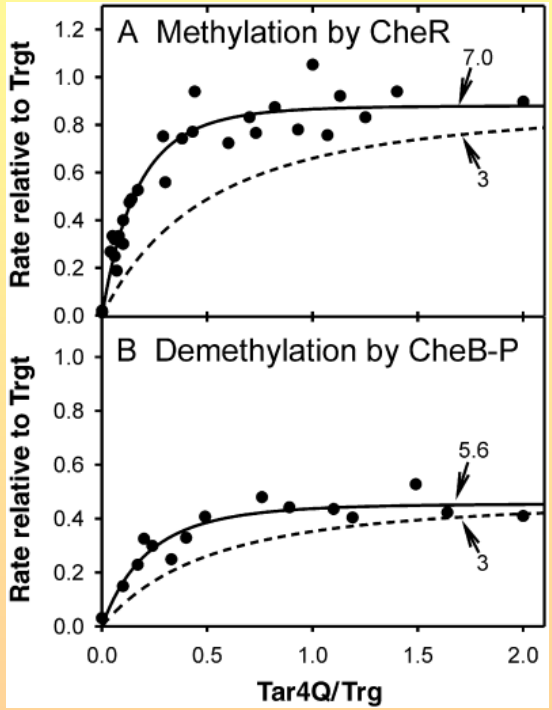
Adaptation via “assistance neighborhoods”

aspartate



Antommattei *et al.* (2004)

Trg(QQEQ) Tar (QQQQ)

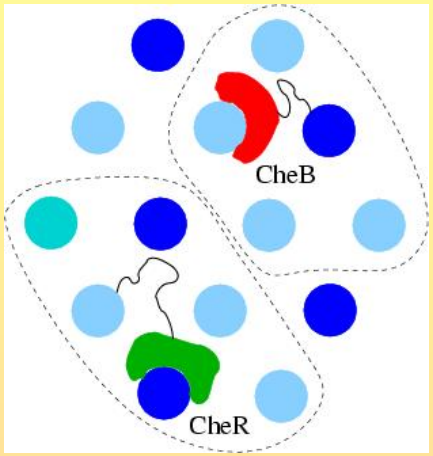


Li and Hazelbauer (2005)

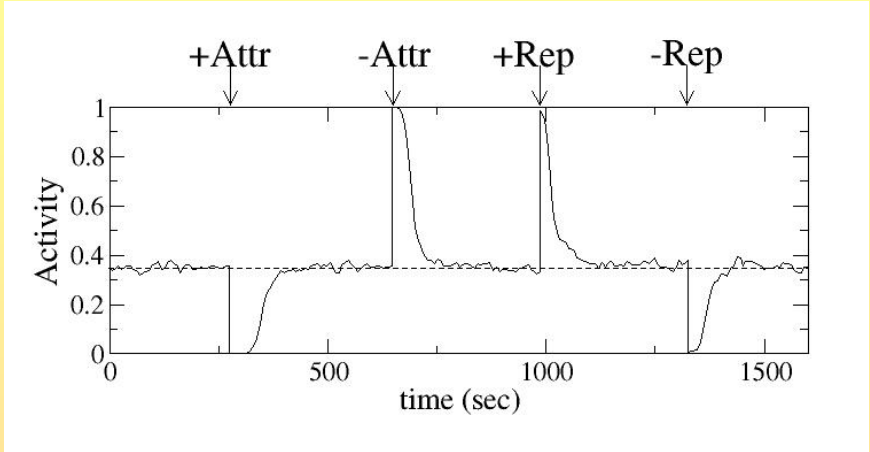
CheR and CheB do not act on single receptors, but on groups of receptors

Assistance neighborhoods restore precise adaptation

Model:



Simulation

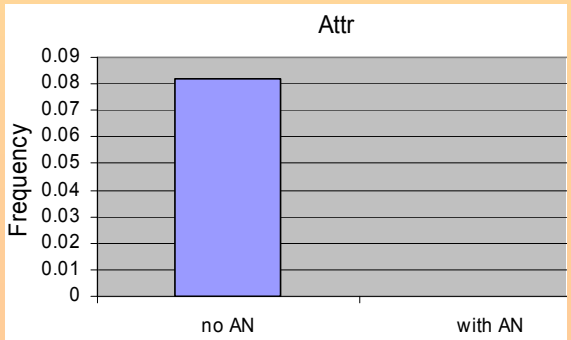


* stationary bound CheR and CheB

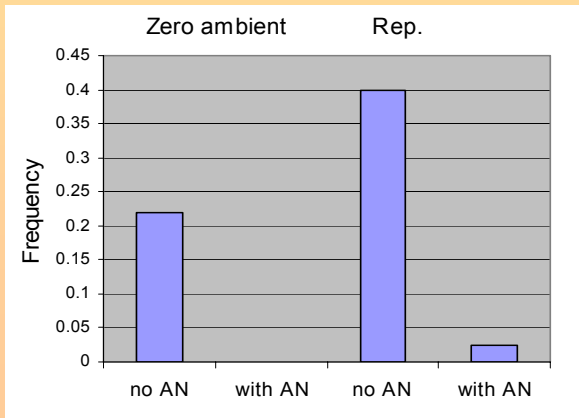
* fixed assistance neighborhoods

* each modification site accessed equally likely

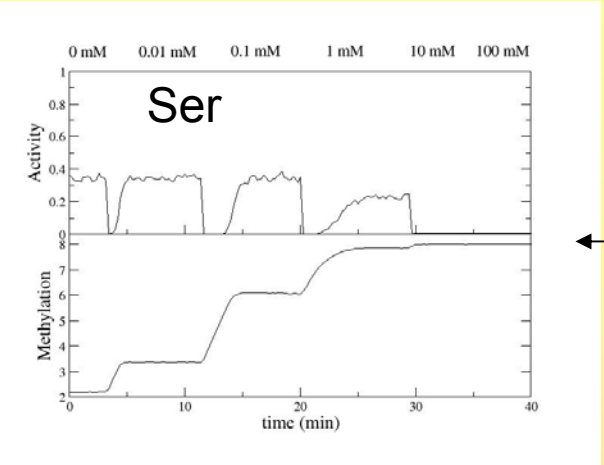
Abortive methylation attempts



Abortive demethylation attempts

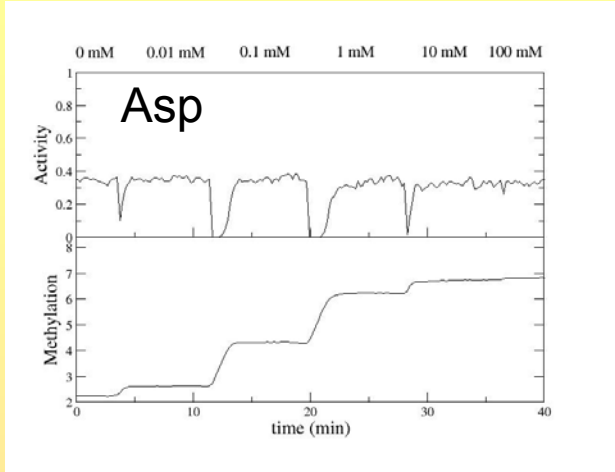


Prediction: Two limits of adaptation



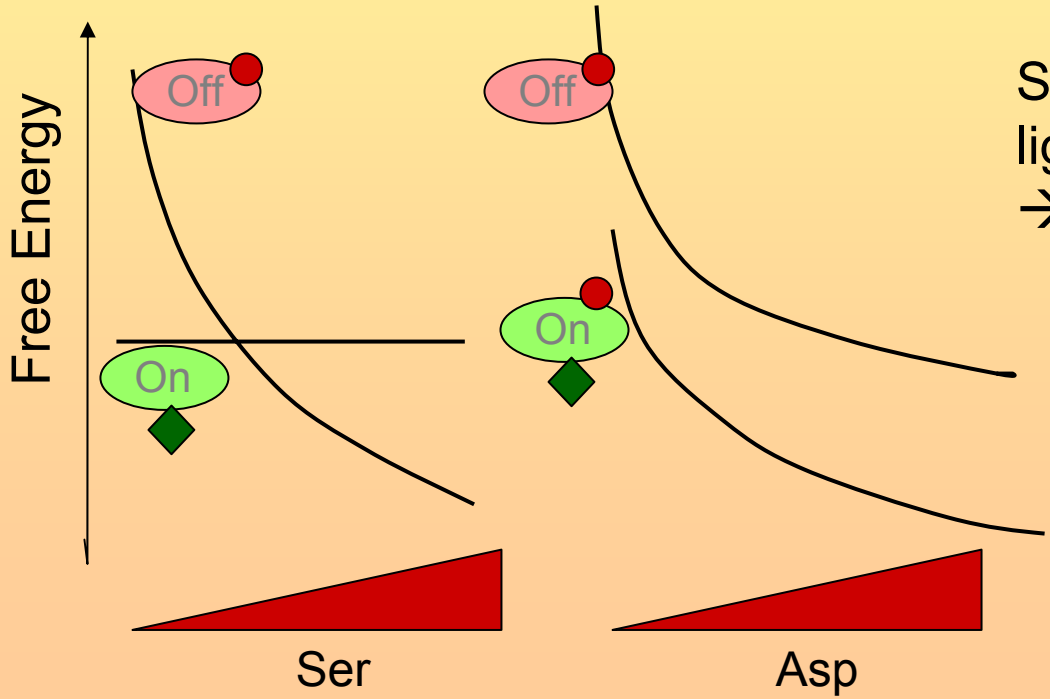
Tsr:Tar=2:1

fully methylated



Full methylation before saturation

→ adaptation stops



Saturation by ligand

→ no response for [L] > K_D^{on}

Conclusions

- Signaling properties of the chemotaxis network:
  - Precise and robust adaptation
  - Signal integration
  - Sensitivity
- FRET studies reveal two regimes of activity
  - Regime I: low activity and constant K_D
  - Regime II: high activity and increasing K_D
- Model of coupled 2-state receptors account for signaling properties, and for two regimes
  - Regime I ($\Delta\varepsilon > 0$): coupling → enhanced sensitivity
  - Regime II ($\Delta\varepsilon < 0$): coupling → enhanced cooperativity (but only for homogeneous clusters)
- Adaptation "homogenizes" receptors ($\Delta\varepsilon \approx 0$) for enhanced sensitivity