The chemotaxis network of *E. coli*

Ned Wingreen

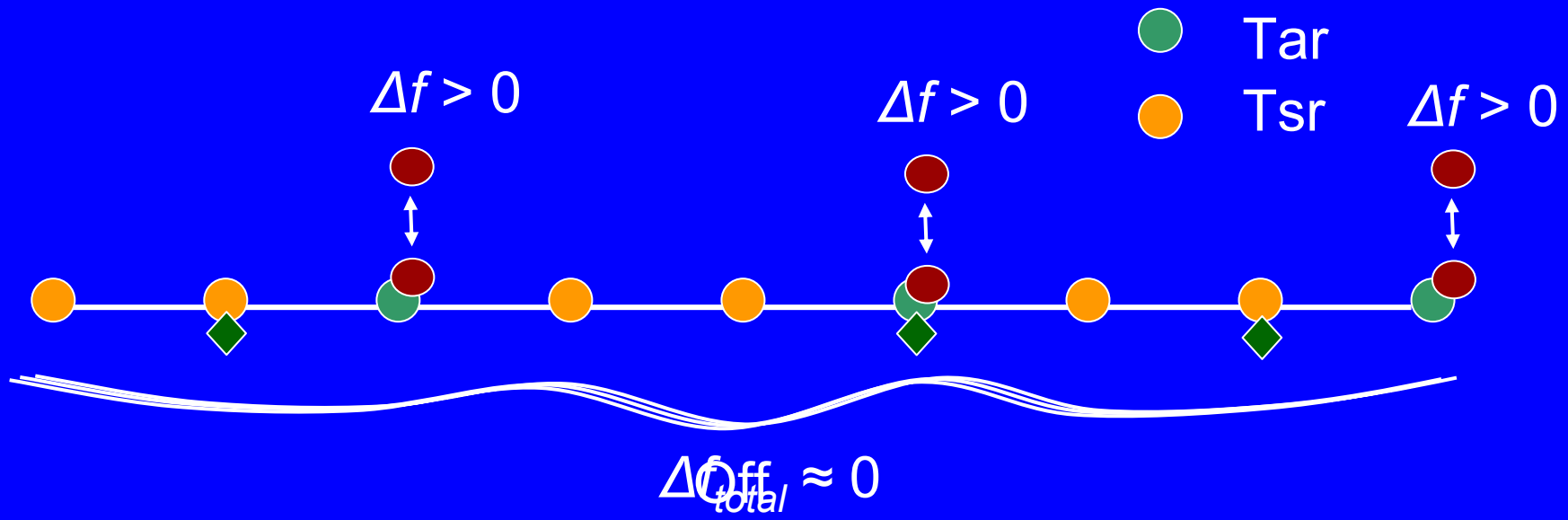
Boulder Summer School 2007

Thanks to: Robert Endres, Clinton Hansen, Juan Keymer, Yigal Meir, Monica Skoge, and Victor Sourjik

Support from HFSP

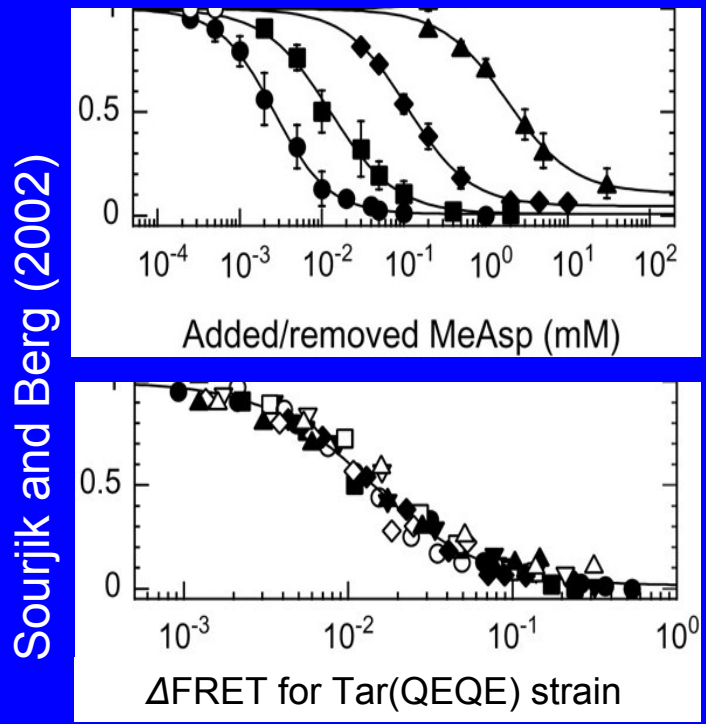
Adaptation

Adaptation uses methylation to adjust $\Delta f_{total} \approx 0$, and thereby enhances sensitivity.



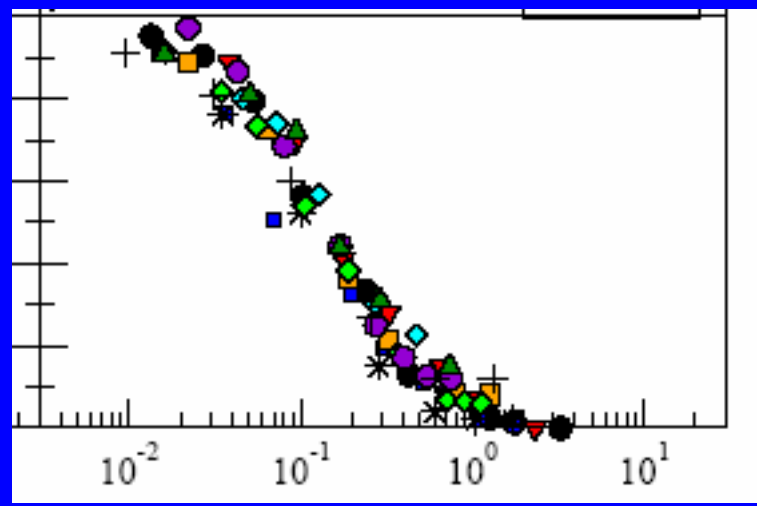
Scaling of wild-type adapted response

Sourjik and Berg: Δ[MeAsp] → ΔFRET{Tar(QEQE)}



Doesn't collapse zero-ambient data.

"Free energy" scaling: Δ[MeAsp] → $\Delta(F_{on} - F_{off})$

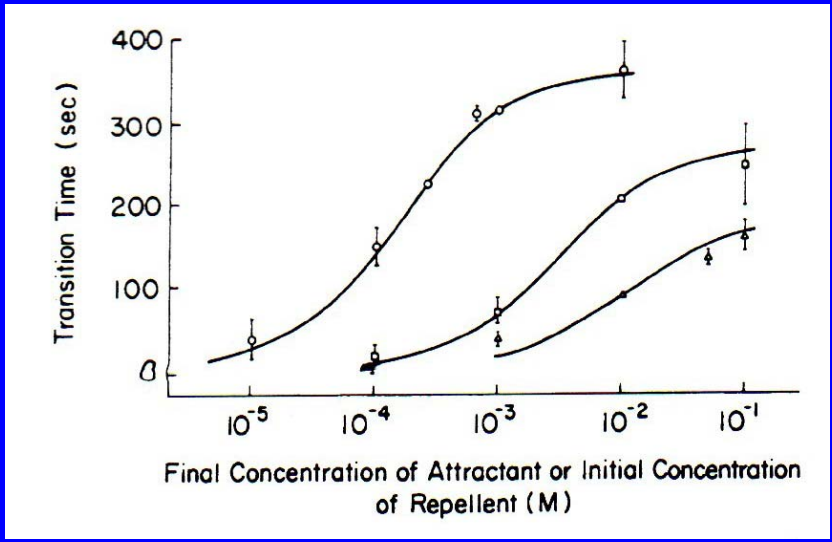


Includes zero-ambient data!

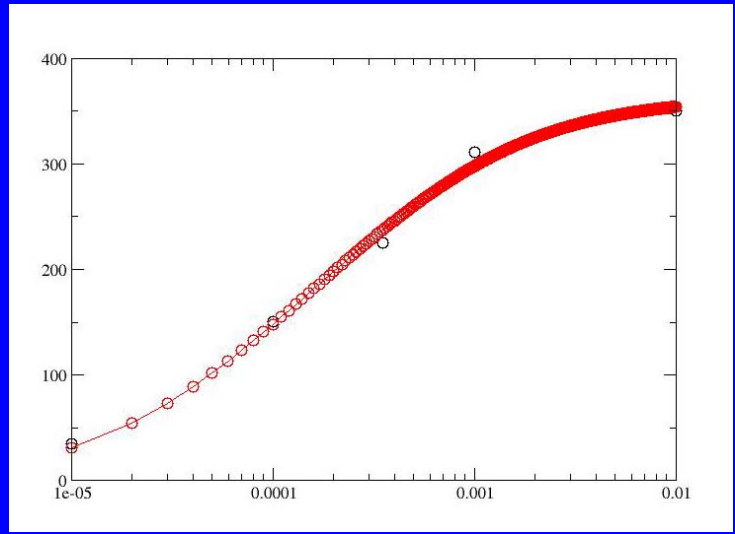
And yields K_Ds:

K_D^{off} = 25 μM, $K_D^{on} \approx$ 0.5 mM

Motor output also yields K_Ds



Berg and Tedesco (1975)



$$t \sim \Delta f \sim \log\left(\frac{1 + C / K_D^{off}}{1 + C / K_D^{on}}\right)$$

→ K_D^{off} = 27 μM, $K_D^{on} \approx$ 0.9 mM

2-state receptor model

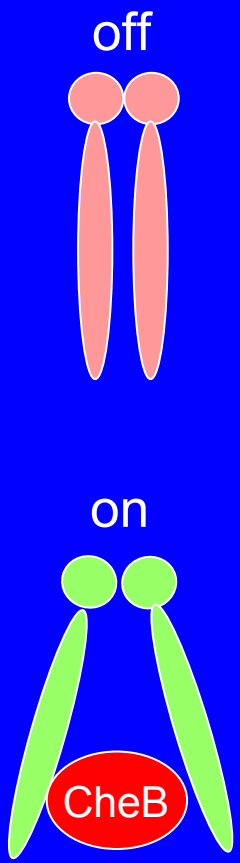
- Originally proposed by Asakura and Honda (1984).
- Modified by Barkai and Leibler (1998) to explain precise and robust adaptation:
  - Receptor complex has 2 states: “off”, i.e. inactive as kinase, and “on”, i.e. active as kinase.
  - Demethylation only occurs in “on” state,

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

  - Therefore, at steady state,

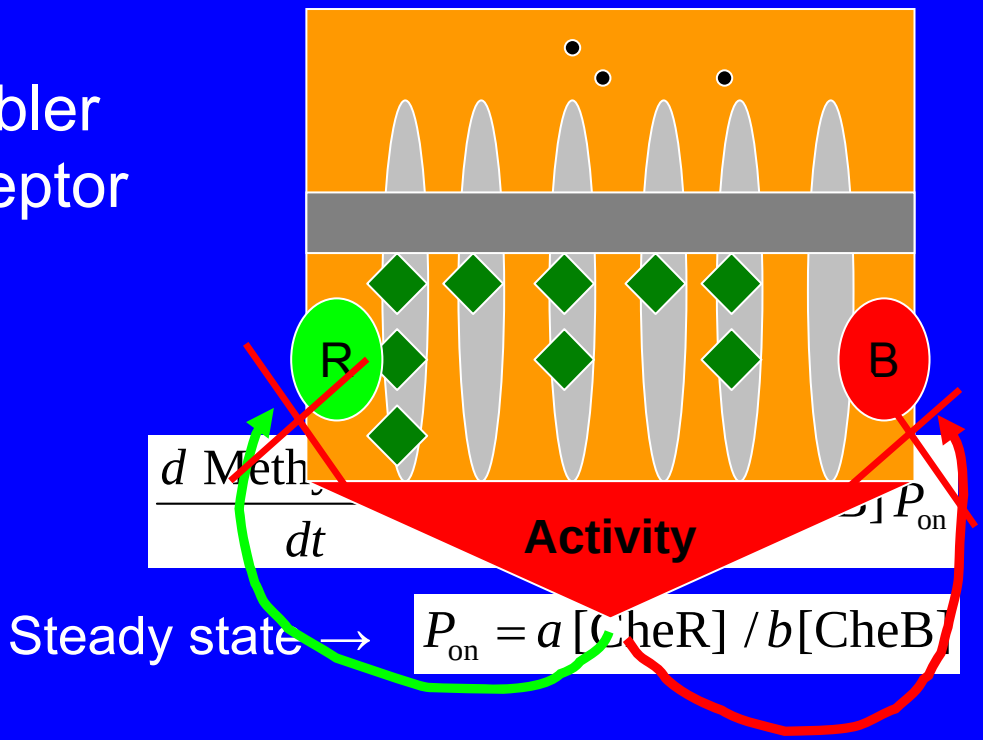
$$P_{\text{on}} = a\,[\text{CheR}]\ /\ b[\text{CheB}]$$

  - Which implies precise and robust adaptation of each receptor complex to a fixed activity.

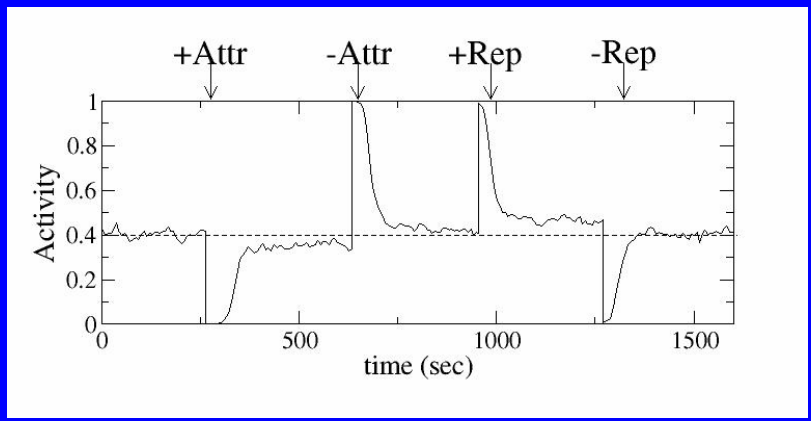


Failure of precise adaptation?

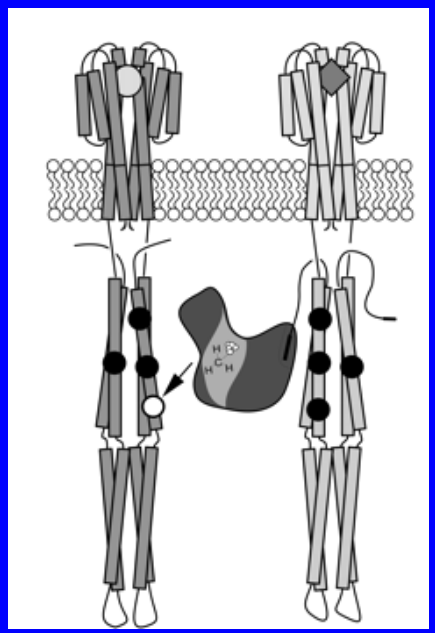
Barkai-Leibler single-receptor adaptation model:



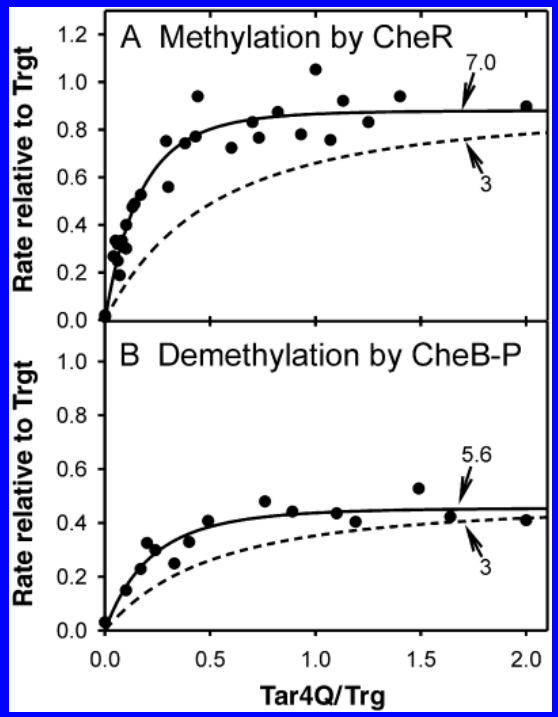
Yields imprecise adaptation of receptor clusters:



Help from “assistance neighborhoods”



Antommattei *et al.* (2004)

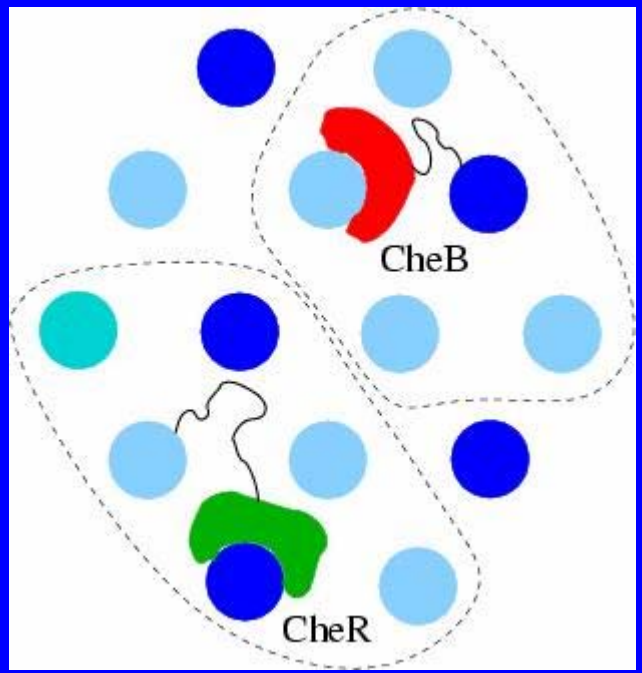


Li and Hazelbauer (2005)

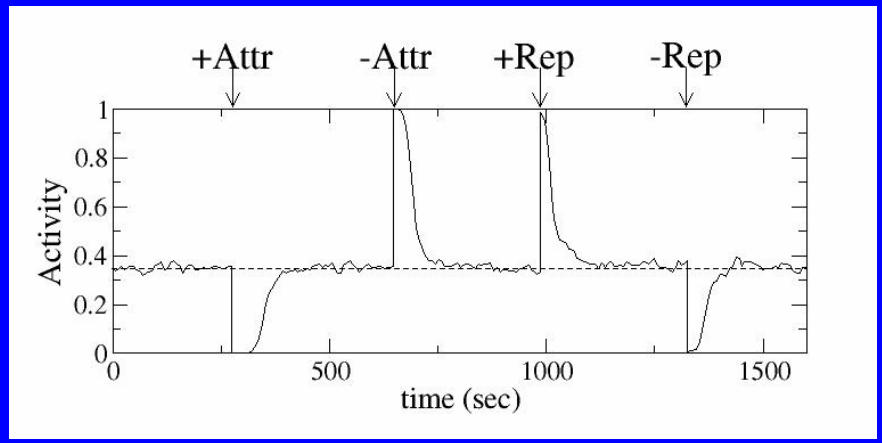
Tethered CheR/CheB act on neighborhood of 5-7 receptors.

Precise adaptation saved!

Assistance-neighborhood model

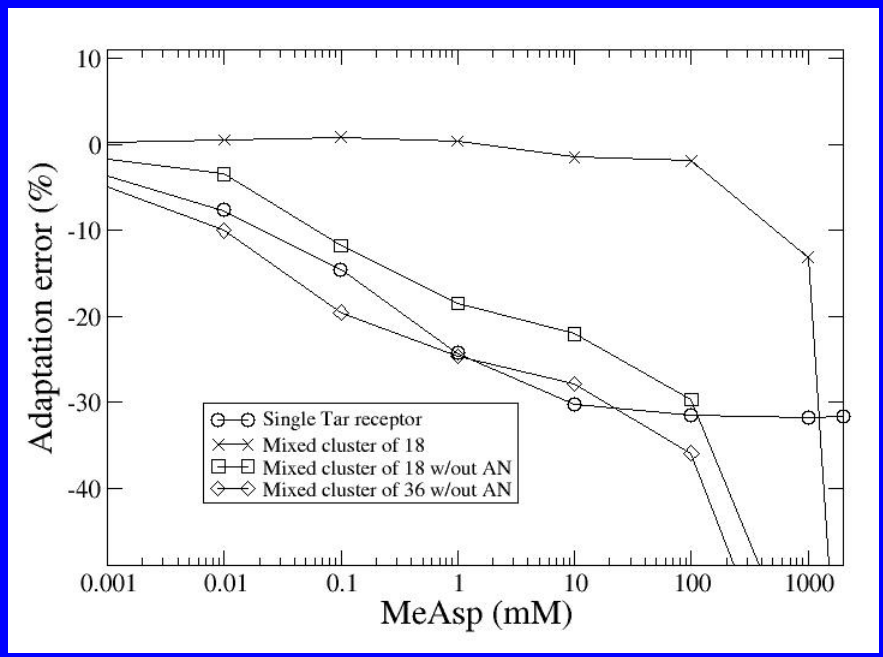


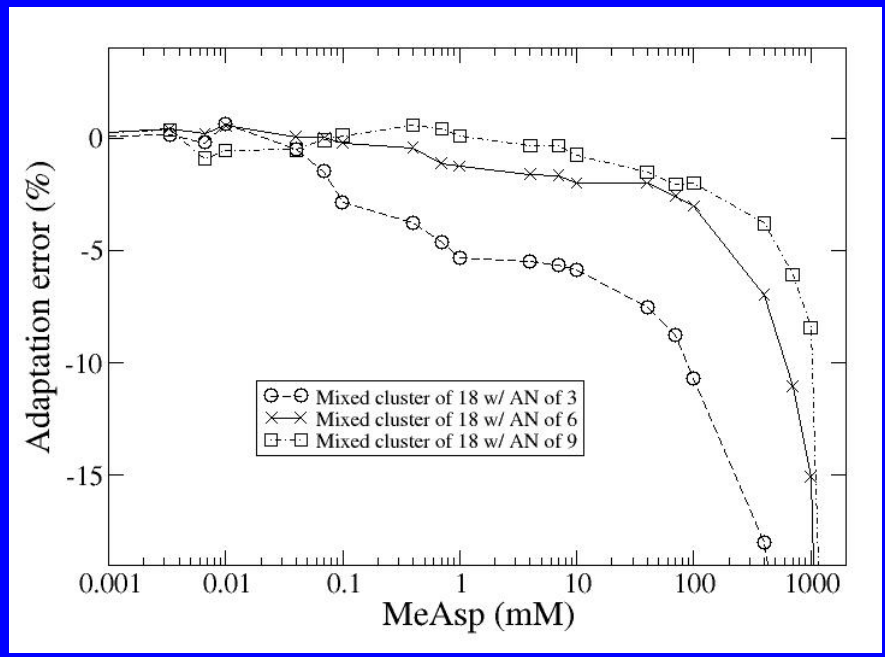
Barkai-Leibler + assistance neighborhoods = precise adaptation:



Precision of adaptation with assistance neighborhoods

Assistance neighborhood of ~ 6 receptors sufficient for precise adaptation:

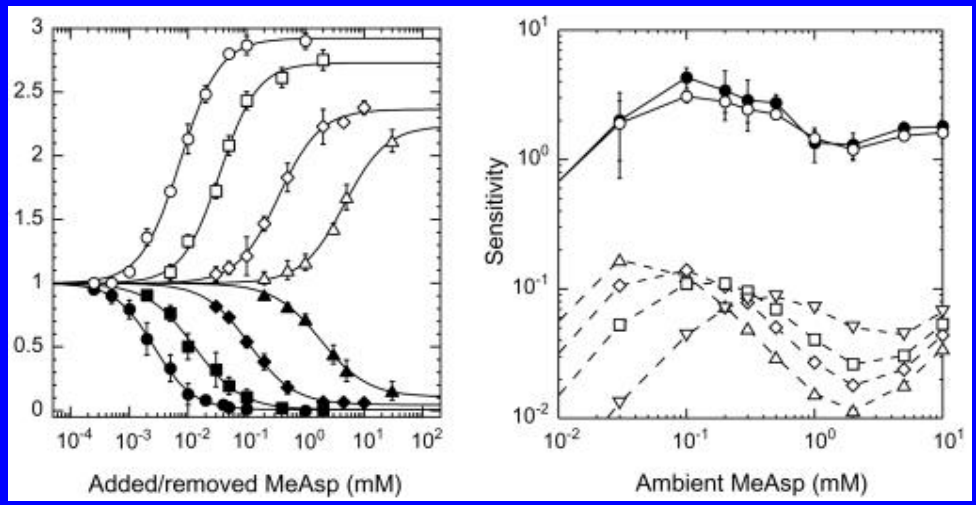




Adaptation error: $\frac{A([L]) - A(0)}{A(0)}$

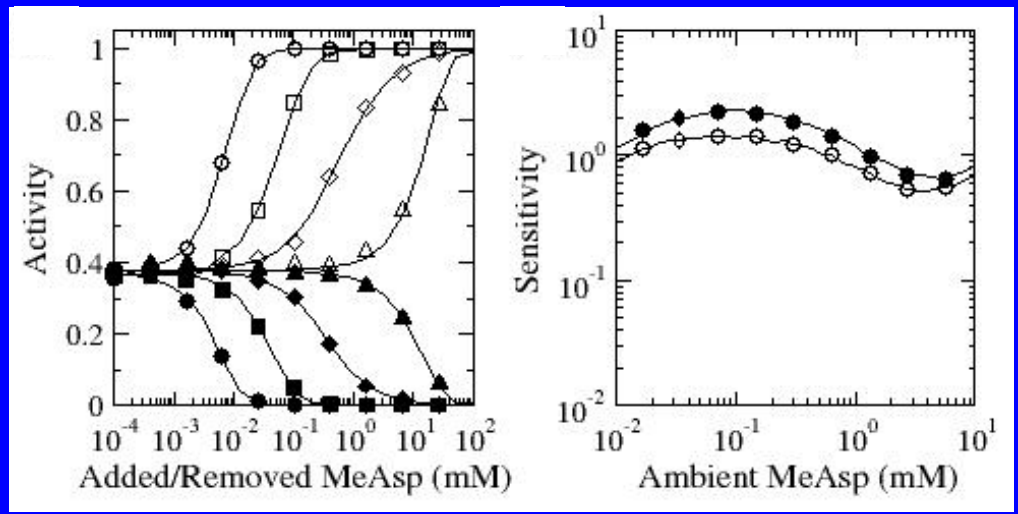
Initial response and sensitivity of adapted receptors

Experiment



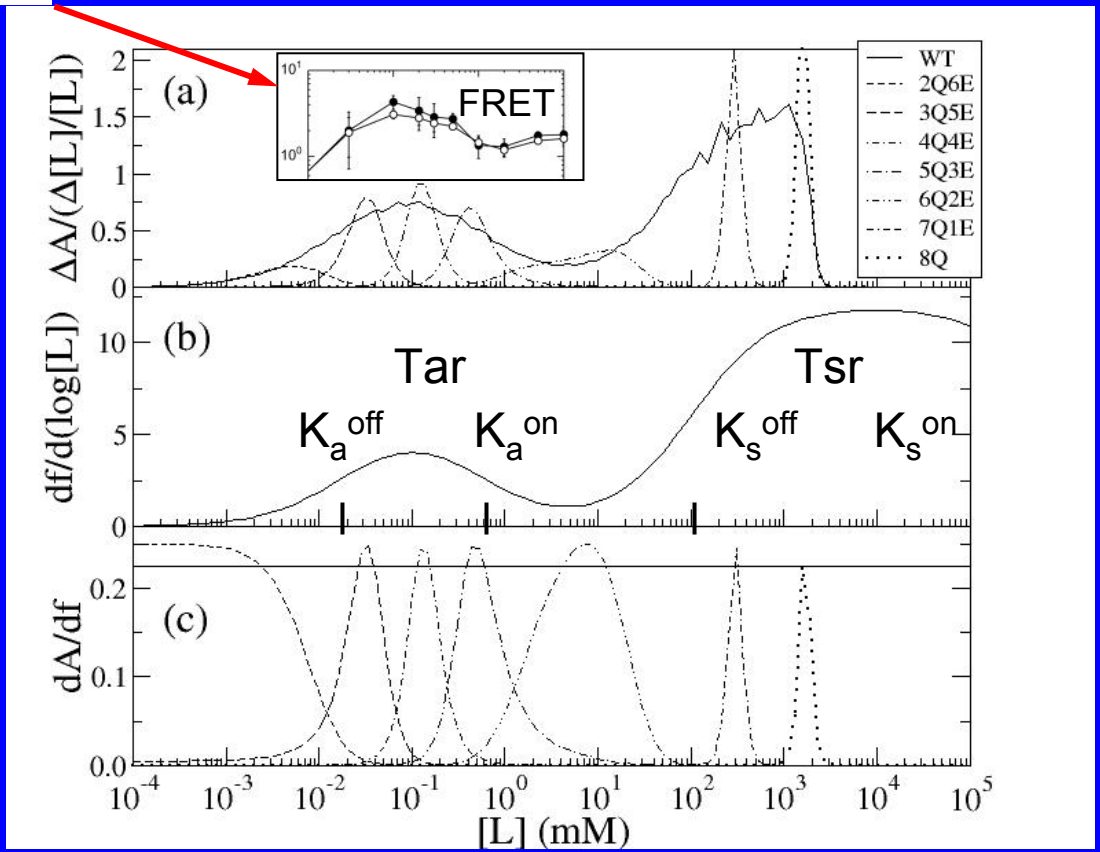
Sourjik and Berg (2002)

Simulation (with assistance neighborhoods)



Two peaks of sensitivity

$$\frac{\Delta A / A}{[\Delta L]/[L]} \approx \frac{d(\log A)}{d(\log[L])}$$

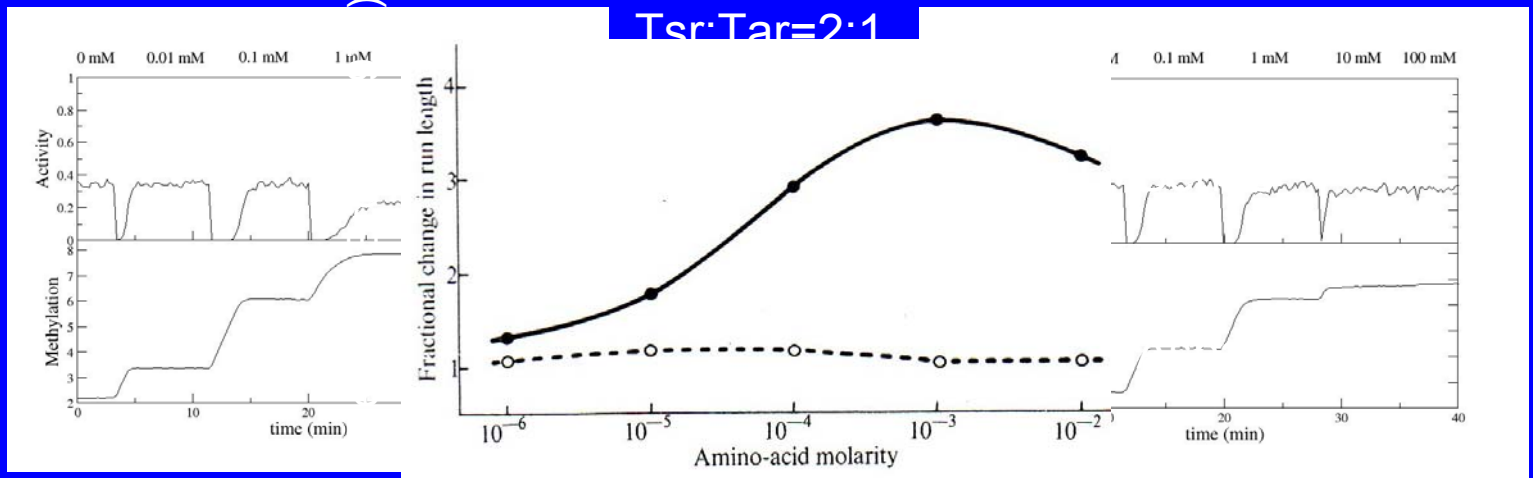


Simulation

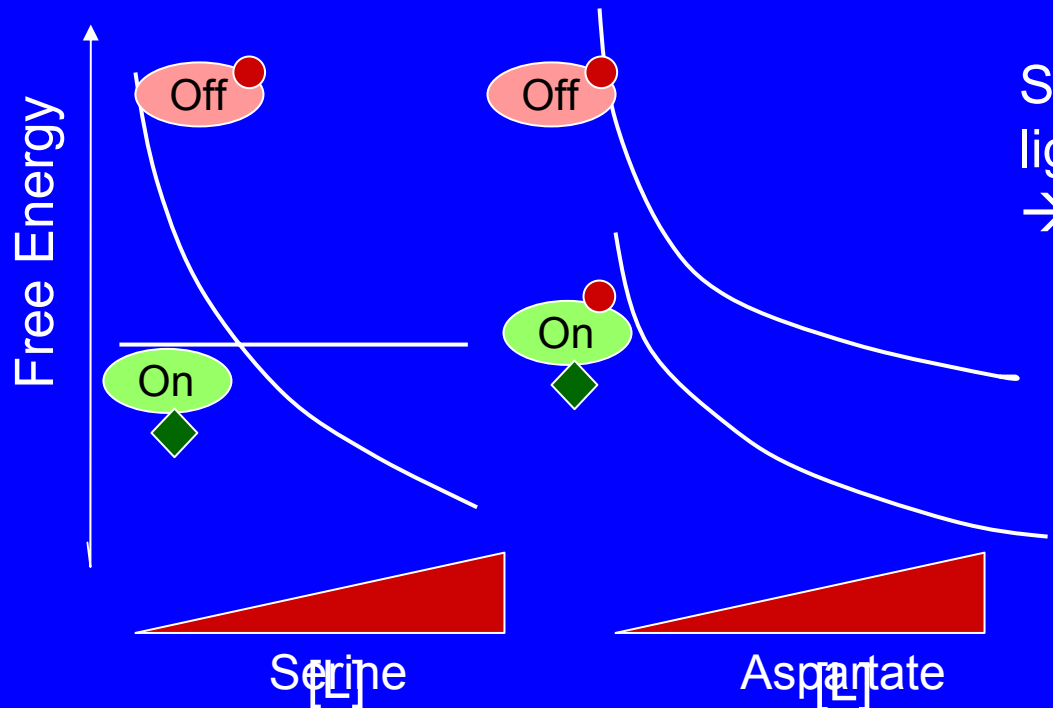
Analytic result for single cluster

$$\frac{dA}{d(\log[L])} = \frac{dA}{df} \cdot \frac{df}{d(\log[L])}$$

Prediction: Two limits of adaptation



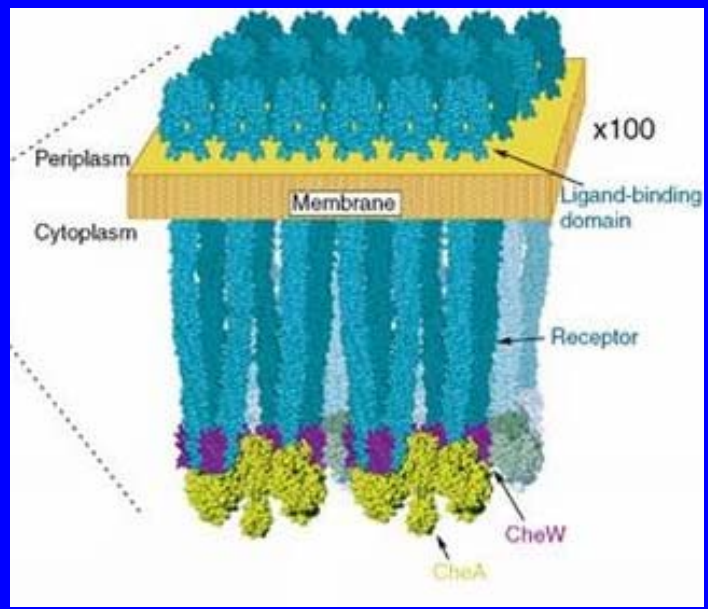
Full methylation before saturation → adaptation stops



Saturation by ligand → no response for [L] > K_D^{on}

Open questions

- What determines cluster size and what is the mechanism of receptor-receptor coupling?
- Two limits of adaptation?
- What is being optimized?



Stock (2000)

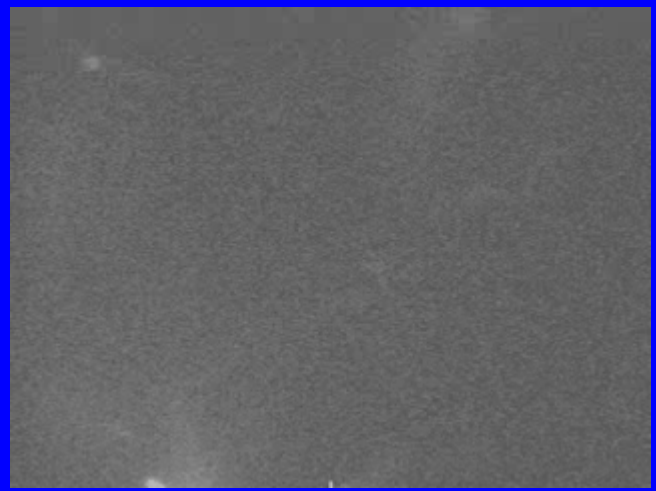
Conclusions

- *E. coli* chemotaxis network remarkable for:
  - precise and robust adaptation
  - signal integration
  - sensitivity
- FRET studies reveal two regimes of receptor activity
- Model of mixed clusters of 2-state receptors accounts for network properties and for two regimes
- Precise adaptation of clusters requires assistance neighborhoods
- Prediction: two possible limits of adaptation

Outline

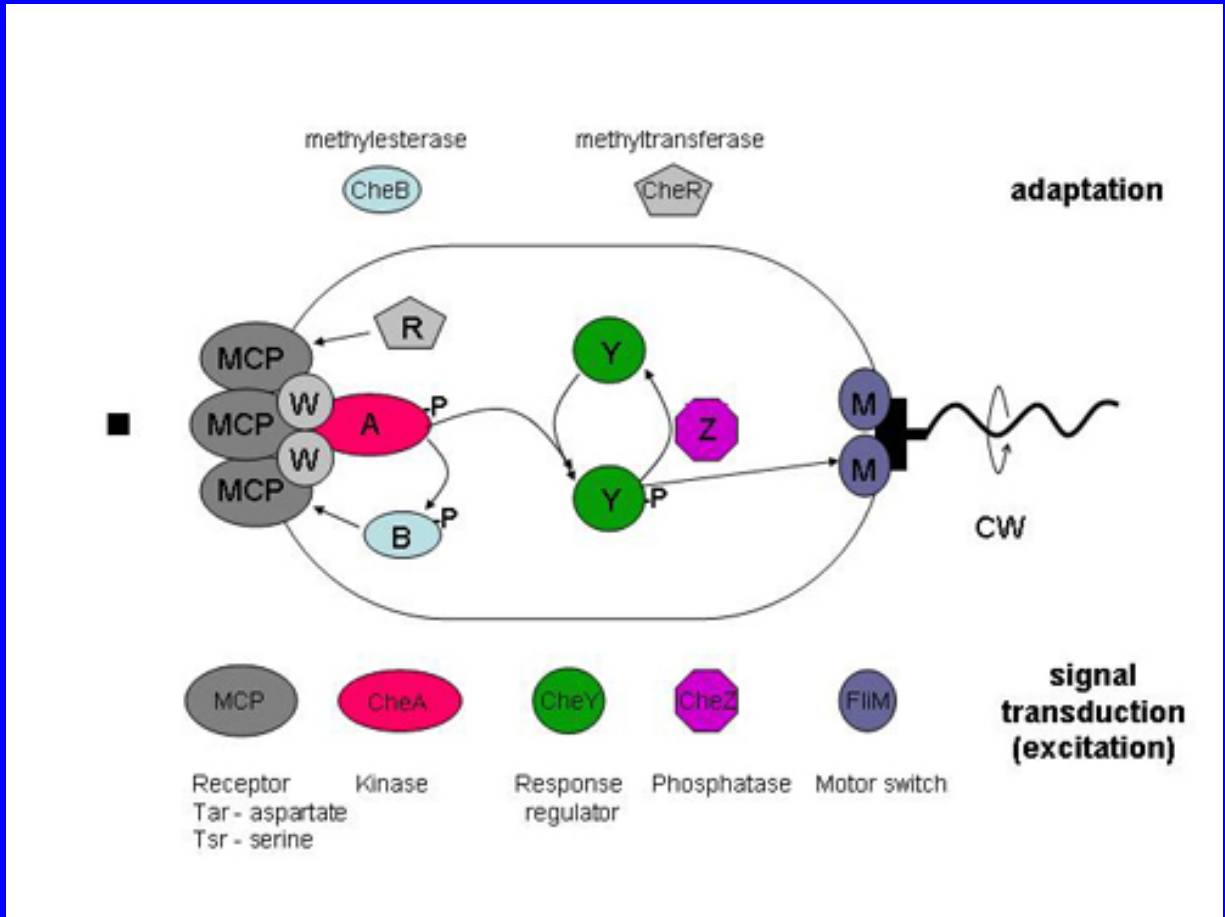
- Introduction to chemotaxis in *E. coli*
  - The chemotaxis network
  - Two regimes of activity
  - Receptors function collectively
- Modeling
  - Mixed clusters of receptors
  - Precise adaptation through “assistance neighborhoods”

E. coli chemotaxis: runs and tumbles



(Thanks to Howard Berg.)

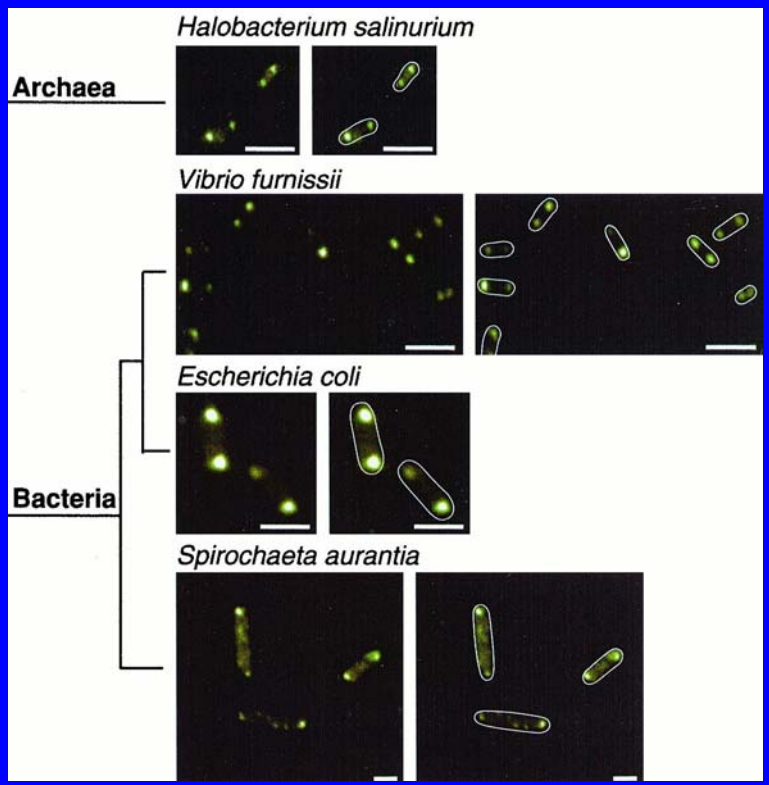
The chemotaxis network



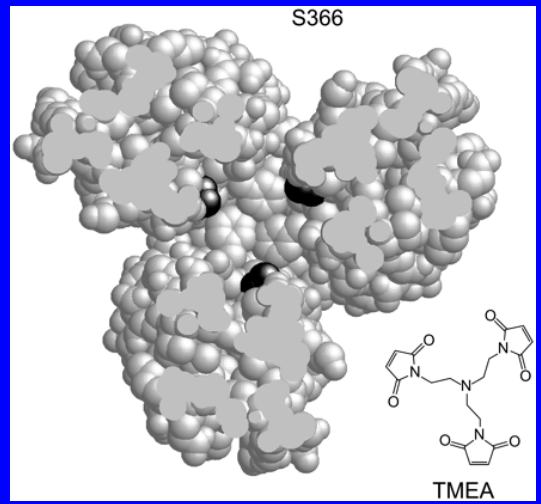
http://www.rowland.harvard.edu/labs/bacteria/projects_fret.html

Chemoreceptor clustering

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



Gestwicki *et al.* (2000)



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

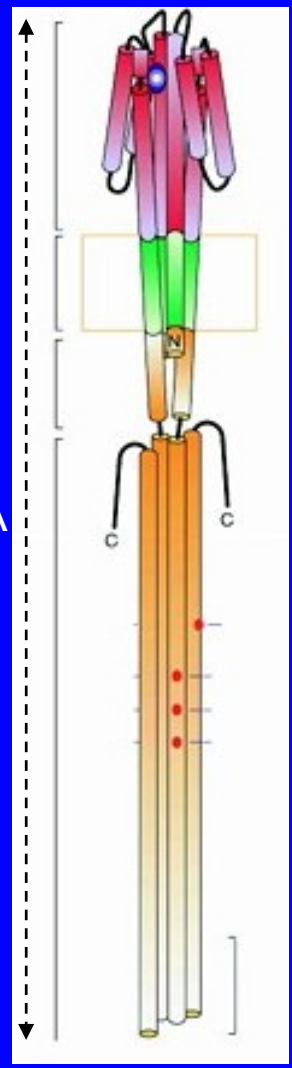
Chemoreceptors

Tar - aspartate, glutamate (~900 copies)
Tsr - serine (~1600)
Trg - ribose, galactose (~150)
Tap - dipeptides (~150)
(Aer - oxygen via FAD (150?))

•Attractant binding <u>inhibits</u>
phosphorylation of CheA

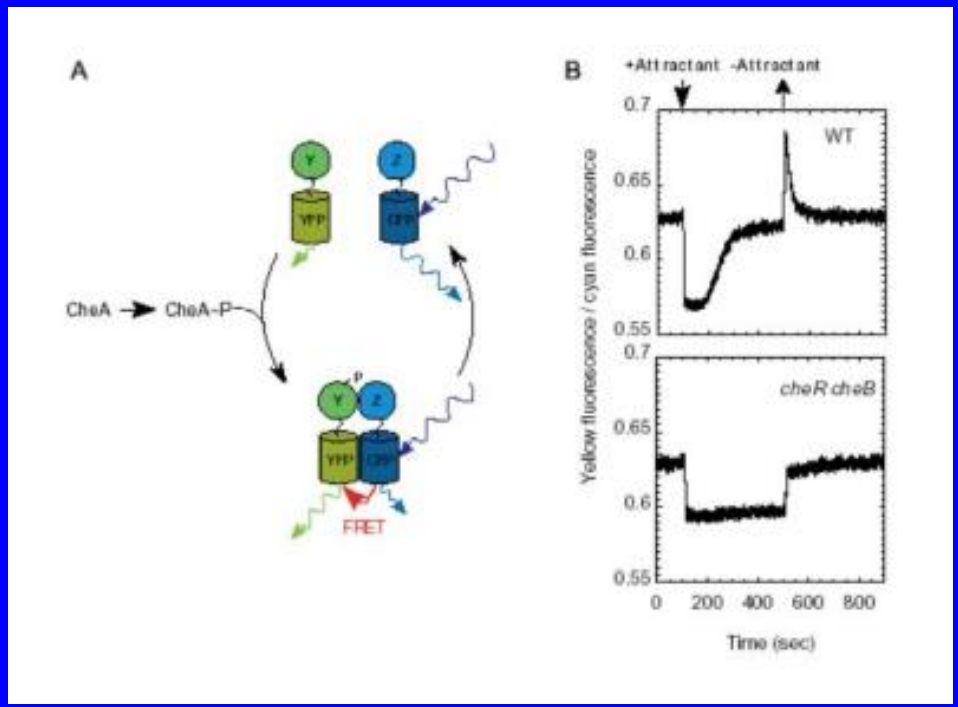
•Adaptation:
More attractant
→ increased methylation by CheR
→ faster phosphorylation of CheA

Less attractant
→ increased demethylation by CheB
→ slower phosphorylation of CheA



Stock (2000)

In vivo FRET studies of receptor activity

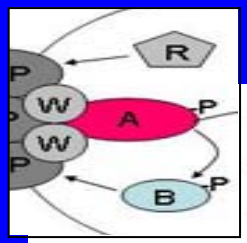


Sourjik and Berg (2002)

Real-time measurement of rate of phosphorylation of CheY.

(FRET also allows subcellular imaging, Vaknin and Berg (2004).)

FRET data: two regimes of activity



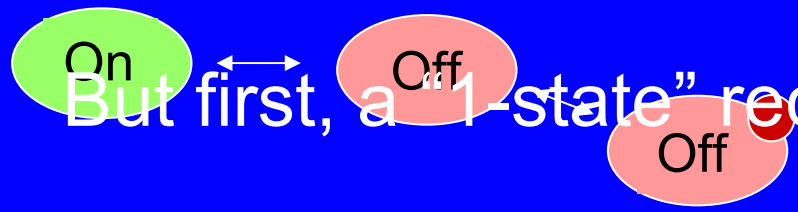
Sourjik and Berg (2002)

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

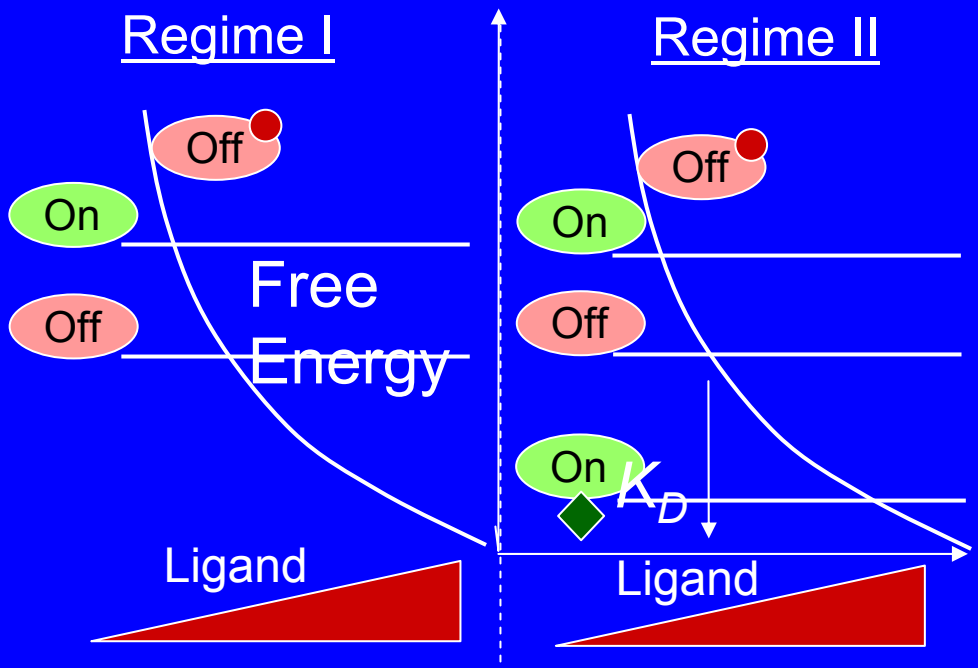
Regime II:
- Activity high (saturated) at zero ambient MeAsp (1.3-1.9)
- K_{i1} large and increasing with methylation
- Plateau in activity
- K_{i2} approximately constant

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

Two regimes of a 2-state receptor

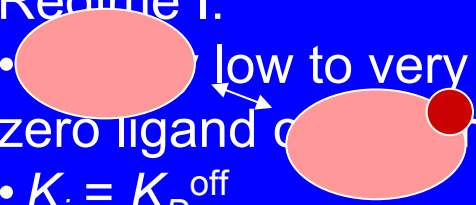


But first, a "1-state" receptor:



Regime I:

- low to very low at zero ligand c ration
- $K_i = K_D^{off}$



Regime II:

) at n

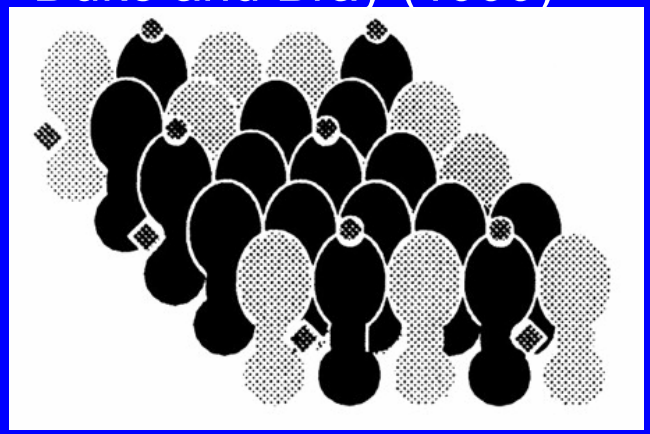
$$P_{\text{no ligand bound}} = \frac{1}{1+\frac{C}{K_D}}$$

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

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

Receptor-receptor coupling

Duke and Bray (1999)



Duke and Bray (1999) proposed that receptor-receptor coupling could enhance sensitivity to ligands.

MWC model: if *N* receptors are all "on" or all "off" together,

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

Regime I ($\Delta\varepsilon > 0$):
- Low activity ~ $e^{-N\Delta\varepsilon}$ at zero ligand concentration
- $K_i = K_D^{\text{off}} / N$
- Hill coefficient = 1

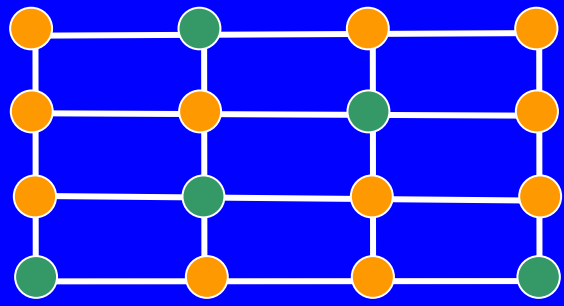
Regime II ($\Delta\varepsilon < 0$):
- $K_i = K_D^{\text{off}}\, e^{-\Delta\varepsilon}$
- Hill coefficient = N

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

Mixed cluster MWC model

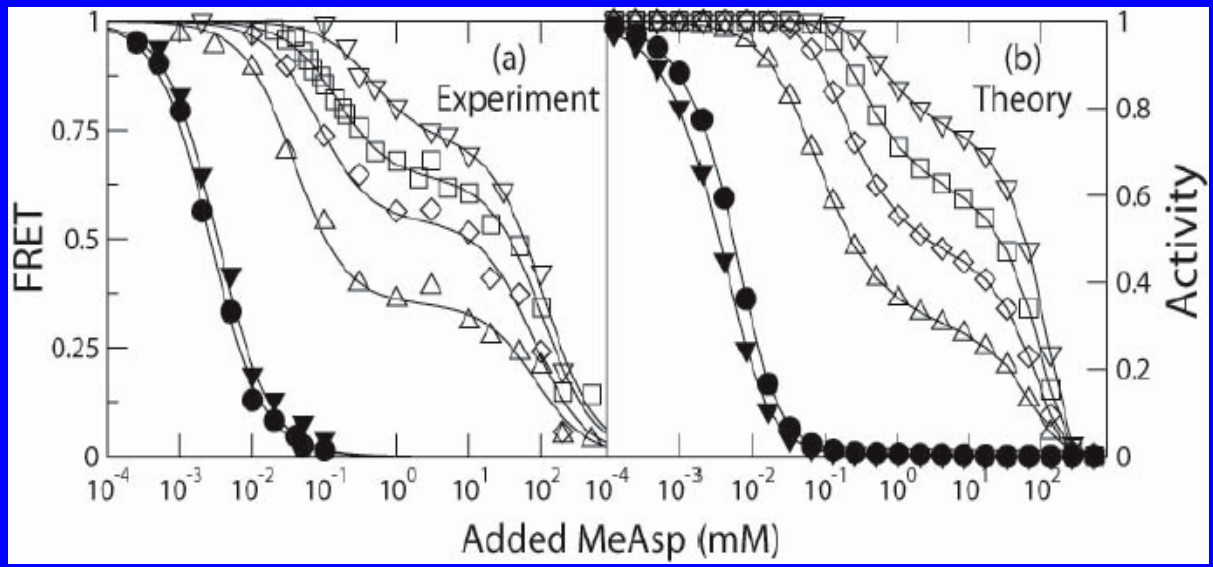
Mello and Tu (2005)

Keymer *et al.* (2006)



Tar

Tsr



Regime I:

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

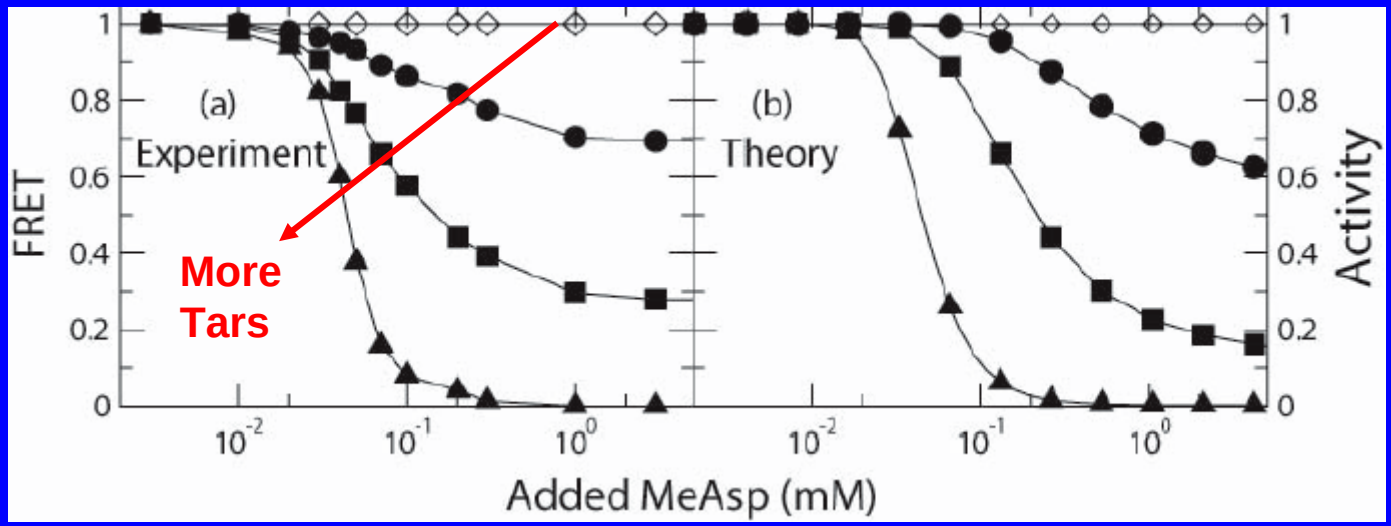
Regime II:

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

Mixed clusters of size 14-16.

Each cluster 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.