1) a) There are 2 given plots for

Mos → MEK1

Mos → ERK2 (overall)

from observation of the plots and given fits we can get the $\frac{1}{2}$ max constant from the given Data fits. I also used Excell (in excell file) to replicate and find all steps.

BY OBSERVATION

Mos → MEK1 step (green line)

$K^{\frac{1}{2}} \approx 50$ nM

$n \approx 1.7$

- replicated the Data via linear regression in Excell

my fit to Data

$K^{\frac{1}{2}} \approx 60$ nM

$n \approx 1.95$

- So I'm in rough agreement with the Data given

Repeat for

Mos → ERK2 (overall)

$K^{\frac{1}{2}} \approx 36$ nM

$X = 4.9$

from plot

$K^{\frac{1}{2}} \approx 42$ nM

$X \approx 3.56$

from Excell replication

⇒

1) a) cont.

for MEK1 → ERK2 we cannot use the given plot for values so this was done only in linear regression

$$\theta = \frac{L^n}{a + L^n} \Rightarrow \frac{1}{\frac{a}{L^n} + 1} = \theta$$

$$= \frac{1}{\theta} = \frac{a}{L^n} + 1$$

$$= \frac{1}{\theta} - 1 = \frac{a}{L^n} \Rightarrow \ln\left(\frac{1}{\theta} - 1\right) = \ln\left(\frac{a}{L^n}\right)$$

$$\ln\left(\frac{\theta}{1-\theta}\right) = n\ln(L) - \ln(a)$$

linearized fit of Hill Equation

where θ = response (ERK2)

L = substrate (MEK1)

n = Hill coefficient

a = constant $= (k_{\frac{1}{2}})^n$

using this fit into Excell (See excell)

$k_{\frac{1}{2}} \approx 0,256$

$m \approx 3,119$

for MEK1 → ERK2

⇒

- See excell for Detailed solve of each linearization.

1) b) as can be seen from my fits in Excell

The sensitivity is NOT ultrasensitive in Mos → MEK1, and is NOT very ultrasensitive in MEK1 → ERK2.

However the overall step (Mos → ERK2) is very ultrasensitive

This means the ultrasensitivity of the entire cascade is much more than each individual step.

c) using our linearization form from before

$$ERK2 = \frac{Mos^X}{(K_{1/2})^X + Mos^X} \Rightarrow \ln\left(\frac{ERK2}{1-ERK2}\right) = -X\ln(K_{1/2}) + X\ln(Mos)$$

↑ $\frac{1}{2}$ max constant

Rearrange by Dividing out X

$$X = \frac{\ln\left(\frac{ERK2}{1-ERK2}\right)}{\ln(Mos) - \ln(K_{1/2})}$$

where

$$MEK1 = \frac{Mos^n}{a + Mos^n} \qquad ERK2 = \frac{MEK1^m}{b + MEK1^m}$$

Substituting it all in ⇒

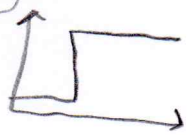
1) c)

$$X = \frac{\ln\left[\dfrac{\left(\dfrac{MEK1^{M}}{b + MEK1^{M}}\right)}{1 - \left(\dfrac{MEK1^{M}}{b + MEK1^{M}}\right)}\right]}{\ln(MOS) - \ln(K_2)}$$

$$X = \frac{\ln\left[\dfrac{\left(\dfrac{MOS^{n}}{a + MOS^{n}}\right)^{M}}{b + \left(\dfrac{MOS^{n}}{a + MOS^{n}}\right)^{M}}\right]}{\ln(MOS) - \ln(K_2)}$$

Using Excell and obsrvation of X, if $MOS \approx K_{\frac{1}{2}}$, X will be maximized, and tuning n and M such, X can get to Asmtope to close to ∞

So a maximal [X value is $+\infty$] which corresponds to a perfect step function



from the EXCell obsrvation Tuning n to increase, and M to Decrase, while making $MOS \approx K_{\frac{1}{2}}$ we can get the maximal X value,

i.e

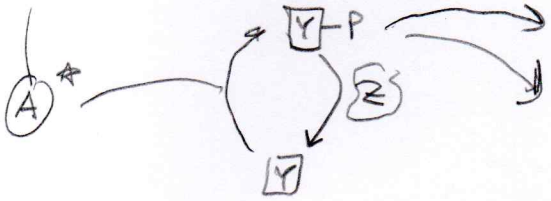
$n \uparrow$, $M \downarrow$, $MOS \approx K_{\frac{1}{2}} \Rightarrow$ X is maximized

2)

a) amplification is required in chemotaxis (such as bacteria) to allow bacteria to sense food or signals even when they are very dilute or small, so it can get a full signal to run even from a single food molecule

adaptation is important to get the cell back to steady state after the impulse of signal, which is important in allowing it to reenter the tumbling "search" mode after its found food at the same frequency/strength as before the impulse of signal

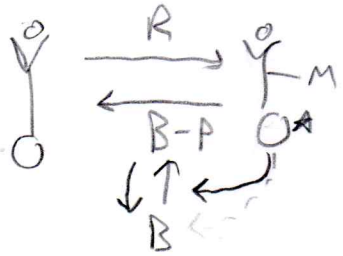
b) The mechanism for amplification in Box I is the phosphorylation of CheY from active A



where 1 A* signal can trigger several Y-P phosphorylation events

c) The adaptation is the methylations of the receptors, and subsequently the phosphorylation of B to drive demethylation

This to drive the cell back to steady state after the signal has occured,



d, e

2) d) The robustness - They are concerned with is The insensitivity of The Desired property to specific values of parameters like a ligand, Specifically because Bacteria need to retain Their Sensitivity to The ligand across a wide range of attractants concentration. ie The robustness is The return to a SS That will allow exact robustness and it can respond sensitivity wise to 1 nM of ligand or 100 nM of ligand.

e) The papers suggest That The lack of robustness may be important to allow a diverse array of response via Tumbling frequency in genetically identical Bacteria. The framework remains robust, but There is a lack of robustness That allows The Bacteria to explore Different rates of Tumbling between Bacteria in a colony, likely to increase The likelyhood a Bacteria will pick up on a signal The other Bacteria couldnt Due to Tumbling frequency.

3) a) original list

from paper

$\star$ = ACTIVE

$$E_M^\star \underset{a_M^+(L)}{\overset{a_M^-(L)}{\rightleftharpoons}} E_M$$

gives

2 methylation states

$M = 0, 1$

$a_0^+ = 0$

methylation is zeroth order

$$E_M^\star + B_p \underset{d_{bp}}{\overset{a_{bp}}{\rightleftharpoons}} \{E_M^\star B_p\} \xrightarrow{k_{bp}} E_{M-1}^\star + B_p$$

$$E_M^\star + B \underset{d_b}{\overset{a_b}{\rightleftharpoons}} \{E_M^\star B\} \xrightarrow{k_b} E_{M-1}^\star + B$$

$$E_M + R \underset{d_r}{\overset{a_r}{\rightleftharpoons}} \{E_M R\} \xrightarrow{k_r} E_{M+1} + R$$

R - methylases

B - Demethylases

$$\{E_M^\star B\} \xrightarrow{\beta_M(L)} E_M + B$$

$$\{E_M^\star B_p\} \xrightarrow{\beta_M(L)} E_M + B_p$$

$$B \underset{k_-}{\overset{k_+ A}{\rightleftharpoons}} B_p$$

$\Rightarrow$

$$A = \sum_M E_M^\star = E_0^\star + E_1^\star = A$$

now put in $M = 1, 0$

$\Rightarrow$

for $M=0$

$E_0^* \rightleftharpoons E_0$ ($a_0^-(L)$, $a_0^+(L)$)

$E_0^* + B_p \rightleftharpoons \{E_0^* B_p\} \xrightarrow{k_{bp}} E_{-1}^* + B_p$ (a_{bp}, d_{bp})

$E_0^* + B \rightleftharpoons \{E_0^* B\} \xrightarrow{k_b} E_{-1}^* + B$ (a_b, d_b)

$E_0 + R \rightleftharpoons \{E_0 R\} \xrightarrow{k_r} E_1 + R$ (a_r, d_r)

$\{E_0^* B\} \xrightarrow{\beta_0(L)} E_0 + B$

$\{E_0^* B_p\} \xrightarrow{\beta_0(L)} E_0 + B_p$

$B \underset{k_-}{\overset{k_+ A}{\rightleftharpoons}} B_p$

$\Rightarrow$ So a lot of reactions are crossed out. Because we are told only E_1 can be activated to E_1^*, so E_0^* cannot exist. Thus all reactions that require it are removed.

now E_1 $\Longrightarrow$

$$E_1^* \underset{a_1^+(L)}{\overset{a_1^-(L)}{\rightleftharpoons}} E_1$$

$M = 1$

$$E_1^* + B_p \underset{d_{bp}}{\overset{a_{bp}}{\rightleftharpoons}} \{E_1^* B_p\} \xrightarrow{k_{bp}} E_0^* + B_p$$

$$E_1^* + B \underset{d_b}{\overset{a_b}{\rightleftharpoons}} \{E_1^* B\} \xrightarrow{k_b} E_0^* + B$$

$$E_1 + R \underset{d_r}{\overset{a_r}{\rightleftharpoons}} \{E_1 R\} \xrightarrow{k_r} E_2 + R$$

only $M = 1, 2$

$$\{E_1^* B\} \xrightarrow{\beta_1(L)} E_1 + B$$

$$\{E_1^* B_p\} \xrightarrow{\beta_1(L)} E_1 + B_p$$

$$B \underset{k_-}{\overset{k_+ A}{\rightleftharpoons}} B_p$$

- I made 2 simplifying assumptions.
  - one is that if E_0^* cant exist, it is immediately turned into E_0, so I replaced E_0^* with E_0
  - E_2 cannot exist as only 1 methylation site exists on the receptor, so E_1 cannot undergo methylation

$\Rightarrow$

3) a) Thus the overall galacis are

$$E_1^* \underset{a_1^+}{\overset{a_1^-}{\rightleftharpoons}} E_1 \qquad [1]$$

$$E_1^* + B_p \underset{d_{bp}}{\overset{a_{bp}}{\rightleftharpoons}} \{E_1^* B_p\} \xrightarrow{k_{bp}} E_0 + B_p \qquad [2]$$

$$E_1^* + B \underset{d_b}{\overset{a_b}{\rightleftharpoons}} \{E_1^* B\} \xrightarrow{k_b} E_0 + B \qquad [3]$$

$$E_0 + R \underset{d_r}{\overset{a_r}{\rightleftharpoons}} \{E_0 R\} \xrightarrow{k_r} E_1 + R \qquad [4^*]$$

Assuming zero order this equation simplifies to

$$E_0 + R \xrightarrow{V_R} E_1 + R \qquad [4]$$

$$\{E_1^* B\} \xrightarrow{\beta_1} E_1 + B \qquad [5]$$

$$\{E_1^* B_p\} \xrightarrow{\beta_1} E_1 + B_p \qquad [6]$$

$$A = E_1^*$$

$$B \underset{k_-}{\overset{k_+ A}{\rightleftharpoons}} B_p \qquad [7]$$

final equations ↑

b, c, d ⟹

3) b) expressed as ODE's.

NP

$$\frac{dE_0}{dt} = \underbrace{d_r\{E_0R\} - a_r[E_0][R]}_{=V^R_{max}\ \text{DE Methylation}} + \underbrace{k_{bp}\{E_1^*B_p\}}_{\text{formerly } E_0^* \xrightarrow{\text{IMD}} E_0} + k_b\{E_1^*B\}$$

$$\frac{dE_1}{dt} = \underbrace{\{E_0R\}k_r}_{\text{DE-M}=V^R_{max}} + a_1^-E_1^* - a_1^+E_1 + \beta_1\{E_1^*B\} + \beta_1\{E_1^*B_p\}$$

$$\frac{dE_1^*}{dt} = d_1^+E_1 + a_1^-E_1^* + d_{bp}\{E_1^*B_p\} + d_b\{E_1^*B\} - a_{bp}[E_1^*][B_p] - a_b[E_1^*][B]$$

$$\frac{dB}{dt} = d_b\{E_1^*B\} - a_b[E_1^*][B] + \beta_1\{E_1^*B\} + k_b\{E_1^*B_p\} + k_-(B_p) - k_+A[B]$$

$$\frac{dB_p}{dt} = d_{bp}\{E_1^*B_p\} - a_{bp}[E_1^*][B_p] + \beta_1\{E_1^*B_p\} + k_{bp}\{E_1^*B_p\} + k_+A[B] - k_-[B_p]$$

$$A = \sum_m E_m^* = E_1^* = A$$

$$\frac{d\{E_1^*B\}}{dt} = -d_b\{E_1^*B\} + a_b[E_1^*][B] - \beta_1\{E_1^*B\} - k_b\{E_1^*B\}$$

$$\frac{d\{E_1^*B_p\}}{dt} = -d_{bp}\{E_1^*B_p\} + a_{bp}[E_1^*][B_p] - \beta_1\{E_1^*B_p\} - k_{bp}\{E_1^*B_p\}$$

3C) looking at given parameters

$E_m = 10\,\mu M$ $R = .2\,\mu M$ from paper

$\Rightarrow \frac{10}{.2} = 50$ so the reactant is in 50 times excess of the enzyme R that catalyzes the methylation

So the zeroth order approximation holds, i.e. that the rate does not depend on the concentration of reactants E_m if E is in large excess relative to R

To then compress

$$E_0 + R \underset{d_r}{\overset{a_r}{\rightleftharpoons}} \{E_0R\} \xrightarrow{K_r} E_1 + R \qquad [4^*]$$

into a single expression for the zeroth order rate

If its zeroth order, each $E_0 = E_1 \cdot Ct$ where C is some constant to make a straight line

To find the rate C that it goes,

$$\frac{d[E_1]}{dt} = \{E_0R\}K_r \qquad \frac{d[E_0]}{dt} = -a_r[E_0][R] + d_r\{E_0R\}$$

If zero order the rate of removal of E_0 will equal the rate of formation of E_1

ie $E_1 + R \xrightarrow{K} E_0 + R \qquad [4]$

So

$$\{E_0R\}K_r = -(-a_r[E_0][R] + d_r\{E_0R\}) \Rightarrow \{E_0R\} = \frac{a_r[R][E_0]}{(K_r + d_r)}$$

Zeroth order = 1

$$\frac{d[E_1]}{dt} \equiv \underbrace{\frac{K_r a_r}{(K_r + d_r)}}_{V_{max}}[R]\cancel{[E_0]} = \boxed{\frac{K_r a_r}{(K_r + d_r)}R = V_{max}}$$

$$\boxed{V_{max\ methylation}} = \frac{(.1)(.2)}{(.1+.1)}(.2)\frac{\mu M}{s} = \boxed{0.02\frac{\mu M}{s}}$$

d) from TU paper

$$A^{ST} = K_b \frac{V_{max}^R}{V_{max}^B - V_{max}^R} = K_b \frac{V_{max}^R}{V^B B_p - V_{max}^R}$$

which is from

$$\frac{dE_M}{dt} = 0 = \underbrace{V_{max}^R}_{\text{Methylation}} + - \underbrace{V_{max}^B \frac{A}{K_B + A}}_{\text{Demethylation}}$$

from before $V_{max}^R \approx 0.02$

so

$$A^{ST} = K_B \frac{0.02}{V^B B_p - 0.02}$$

(NOTE I got STUCK Here, The next stuff was a lot of ASSUMPTIONS to get a value rate)

Now looking at the Demethylation

$$E_1^* + B_p \underset{d_{pp}}{\overset{a_{bp}}{\rightleftharpoons}} \{E_1^* B_p\} \xrightarrow{K_{bp}} E_0 + B_p$$

Assume zero order from E_1^* at 10μM > B_p at 2μM

at V_B^{max} (maximum activity rate requires max E_1^*

performing the same operation as done on Methylation

$$\frac{dE_1^*}{dt} = \frac{K_{bp} a_{bp}}{(k_r + d_{bp})} [B_p][E_0]$$

"zero order" Demethylation of E_1^*

$$\approx \frac{(0.1)(0.1)}{(0.1 + 0.01)} (2) \approx 1.818 = V^B B_p = V_{max}$$

$$\text{So } A^{ST} = K_B \frac{0.02}{1.18 - 0.02} = .011 K_B$$

If A^{ST} is zero order in B and first order in A

$$A^{ST} = \frac{V^{R max}}{V^{B max}} = \frac{0.02}{1.818} \approx \boxed{0.011 = A^{ST}}$$

(way too low)

4) Used MATLAB To solve The system of equations from 3(a)

- See provided Code

a) generating responses (E^* vs TIME) like The paper we would see a Change in Activity with ligand, however ours caused activity To increase NOT Decrease, so a potential error here but if The Trend is reversed (Negative at our activity is The positive paper activity) we see a similar Dip early on.

what Difference we had is That as ligand was increased, The steady state would Decrease, so That tells us our 2-state model is NOT exact Robustness like The simplified or 3-state model in The paper. ⇒

b) Comparing A^{ST} To 3d prediction our steady state at similar values was around ≈ 0.4 at a ligand of 0.1, and under similar Conditions This is Different from 3d, when comparing it after removing The inhibition of B our new Steady state is lower at ≈ 0.125 which gets Closer (Due To removing The inhibition of B)

4c) in reality CheY provides Amplification of E_1^*, which meas it will be sensitive to changes in Ligand concentration, so specific parametrs such as Binding affinity of CheZ to the CheY complex, and kinetic constants would need to be made precice to cause higher sensitivity, so the Adaptation made it such that these parametrs did not need to be tuned to be precice, however in amplification (specifically for ultra sensitivity) the enzyme parametrs k, or hill coefficients would need to be maximized as in question 1c and 1d to increase sensitivity, and thus Amplification.