PS #1 due today before 3PM
return in class, or Rm. 68-371B

Tomorrow W 10/03/07 Recitation topic
'Stability analysis and eigenvalues'

R 10/04/07 No lecture

T 10/09/07 Columbus day vacation

PS #2 was posted, due 10/18/07

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Mathematical basis of stability analysis

\[ \dot{x} = f(x, y) \] \quad \text{system of two coupled differential equations}

\[ \dot{y} = g(x, y) \]

**step 1** find nullclines and fixed point(s)

\[ \dot{x} = 0 \rightarrow f(x, y) = 0 \]

\[ \dot{y} = 0 \rightarrow g(x, y) = 0 \]

**step 2** consider small deviation from fixed point

\[ \tilde{x} = x - x_0 \]

\[ \tilde{y} = y - y_0 \]

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**step 3** consider small deviation from fixed point

\[ \tilde{x} \equiv x - x_0 \]

\[ \tilde{y} \equiv y - y_0 \]

**step 4** linearize around fixed point(s)

\[ \dot{\tilde{x}} = \tilde{x} \left. \frac{\partial f}{\partial x} \right|_{(x_0, y_0)} + \tilde{y} \left. \frac{\partial f}{\partial y} \right|_{(x_0, y_0)} = a\tilde{x} + b\tilde{y} \]

\[ \dot{\tilde{y}} = \tilde{x} \left. \frac{\partial g}{\partial x} \right|_{(x_0, y_0)} + \tilde{y} \left. \frac{\partial g}{\partial y} \right|_{(x_0, y_0)} = c\tilde{x} + d\tilde{y} \]

**step 5** determine matrix \( A \)

\[ A = \begin{bmatrix} a & b \\ c & d \end{bmatrix} \]

**step 6** determine stability of fixed point

\[ \tau = \text{trace}(A) = a + d \]

\[ \Delta = \det(A) = ad - bc \]

only if \( \tau < 0 \) and \( \Delta > 0 \), \((x_0, y_0)\) is a stable fixed point

!!! be careful: only valid for 2 dimensional systems !!!
**Last lectures: Genetic Switches**

L3-6: Naturally occurring: lambda lysis-lysogeny decision
lactose operon in *E. coli*

L7   : Engineered: genetic toggle switch

Switches are necessary for making 'decisions':
- development & differentiation (e.g. stem cells) *what to be?*
- metabolism *what to eat?*
- molecule synthesis (e.g amino acids) *what to produce?*

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**Box 1: The toggle model**

The behaviour of the toggle switch and the conditions for bistability can be understood using the following dimensionless model for the network:

\[
\frac{du}{dr} = \frac{\alpha_1}{1 + u^\beta} - u
\]  
\[
\frac{dv}{dr} = \frac{\alpha_2}{1 + v^\gamma} - v
\]

where \(u\) is the concentration of repressor 1, \(v\) is the concentration of repressor 2, \(\alpha_1\) is the effective rate of synthesis of repressor 1, \(\alpha_2\) is the effective rate of synthesis of repressor 2 in the presence of...

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**How to obtain this stability diagram?**

![Stability Diagram](chart.png)

**time scales for genetic regulation to react to extracellular stimulus ~ several hours**
What if faster response is needed?
- finding food
- chasing bait
- signal transduction

genetics is too slow!

Protein switches (active/inactive states)
(total amount active + inactive is constant,
ignore gene expression)
timescales 1 ms - minutes

Introducing the H atom for signal transduction:

chemotaxis of *Escherichia coli*

MBotC, Chapter 13

cell length ~ 1-2 µm, diameter ~ 0.5 µm
The Flagellum

Absence of chemical attractant

Presence of chemical attractant

chemical gradient sensed in a temporal manner

Berg & Turner
Proteins in the chemotactic network can be modified in different ways:

I  Phosphorylation (CheA, CheY, CheB)
II  Methylation (Tar receptor)

**Phosphorylation (CheA, CheY, CheB)**

CheA (protein kinase), uses ATP to phosphorylate one of its histidines.

\[
\text{CheA + ATP} \leftrightarrow \text{CheA}^p + \text{ADP}
\]

CheA (CheA_p) is bound to the Tar receptor through an adapter protein CheW. CheW is not known to have any enzymatic activity. (these proteins are sometimes called 'scaffolding protein')

CheA_p is unstable and transfers its phosphoryl group to CheY (highly soluble, diffuses through the cytoplasm)
I  Phosphorylation (CheA, CheY, CheB)

autophosphorylation: \[ \text{CheA} + \text{ATP} \leftrightarrow \text{CheA}_p + \text{ADP} \]

phosphoryltransfer: \[ \text{CheA}_p + \text{CheY} \leftrightarrow \text{CheA} + \text{CheY}_p \]

CheY$_p$ binds to the motor (FliM), motor rotates CW (= tumbles)

logic:
- high levels of CheA$_p$ -> high levels of CheY$_p$ (lots of tumbles)
- low levels of CheA$_p$ -> low levels of CheY$_p$ (straight swimming)

II  Methylation (tar receptor)

CheZ dephosphorylates CheY$_p$ (phosphatase, opposite function as CheA)

\[ \text{CheY}_p + \text{CheZ} \rightarrow \text{CheY} + \text{CheZ} \]

logic:
- high levels of CheZ -> low levels of CheY$_p$ (straight swimming)

CheR adds methyl group
CheB$_p$ removes methyl group

phosphorylation state of CheB is controlled by CheA
Methylation - Phosphorylation coupling

phosphorylation state of CheB is controlled by CheA

Role of ligand binding

The rate of CheA phosphorylation is stimulated by unoccupied receptors
why is this all so complex?

Before starting with the modeling, first let’s look at some recent experiments:


Remember scientists have been working on *E. coli* chemotaxis for about 100 years now.

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*E. coli* can sense aspartate from 10 nM - 1 mM and sense changes as small as 0.1%
Single cell chemotactic analysis

- Cells have plasmids with CheY-GFP under inducible promoter.
- Assumption: all CheY is phosphorylated.
- Strain: CheY-, CheZ-, CheB-.
- Hill #: ~10

**FRET (fluorescence resonant transfer)**

- Low YFP/CFP: unbound
- High YFP/CFP: bound
- CheY-YFP (yellow)
- CheZ-CFP (blue)
- CheZ binds only to CheYp!!
- Adding attractant leads to immediate lower concentration of CheYp-CheZ complex, lower [CheYp], less tumbling
amplification between receptors and CheYp: ~35
amplification between CheYp and motor: ~10
total amplification ~ 350
our models should reproduce this (hint: receptor clustering)

Models should also reproduce qualitative properties such as perfect adaptation
Perfect adaptation is robust against changes in Che-protein concentrations

Goal of next lecture is develop models that qualitatively and quantitative reproduce these phenomena, such as:

- huge gain
- sensitivity
- perfect adaptation

All these effects are ubiquitous in signal transduction pathways in general.

‘Fine tuned model for perfect adaptation’

Spiro et al. PNAS 94, 7263-7268 (1997)
A model of excitation and adaptation in bacterial chemotaxis

key player: Tar-CheA-CheW complex
assumptions:

1. Tar is only receptor type, CheW and CheA always bound to Tar
2. Methylation occurs in specific order
3. Consider only 3 highest methylation states
4. Only CheB demethylates
5. Phosphorylation of CheA does not affect ligand (un)binding
6. Tar-CheR binding does not affect ligand un(binding) and phosphorylation of CheA
7. CheZ is not regulated
8. Phosphotransfer from complex to CheY or CheB is not affected by occupancy or methylation state.

The key to adapting perfectly is to return the level of phosphorylated receptor to its pre-stimulus level, and this occurs because CheA autophosphorylates more rapidly the more highly methylated the receptor!