Mathematical basis of stability analysis

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

system of two coupled differential equations

step 1
find nullclines and fixed point(s)

\[ \dot{x} = 0 \rightarrow f(x_0, y_0) = 0 \]
\[ \dot{y} = 0 \rightarrow g(x_0, y_0) = 0 \]

step 2
consider small deviation from fixed point

\[ \tilde{x} \equiv x - x_0 \]
\[ \tilde{y} \equiv y - y_0 \]
\[ \tilde{x} \equiv x - x_o \]
\[ \tilde{y} \equiv y - y_o \]

Consider small deviation from fixed point.

**Step 3**

Linearize around fixed point(s).

\[ \dot{x} \approx \tilde{x} \frac{\partial f}{\partial x} \bigg|_{(x_o, y_o)} + \tilde{y} \frac{\partial f}{\partial y} \bigg|_{(x_o, y_o)} \equiv a\tilde{x} + b\tilde{y} \]

\[ \dot{y} \approx \tilde{x} \frac{\partial g}{\partial x} \bigg|_{(x_o, y_o)} + \tilde{y} \frac{\partial g}{\partial y} \bigg|_{(x_o, y_o)} \equiv c\tilde{x} + d\tilde{y} \]

**Step 4**

Determine matrix A.

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

determine matrix A

determine trace and determinant of A:

\[ \tau = \text{trace}(A) = a + d \]
\[ \Delta = \text{det}(A) = ad - bc \]

step 6

determine stability of fixed point

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**

L3-4: Naturally occurring: lysis-lysogeny decision

L5-6: 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?*
time scales for genetic regulation \(~ 10 \text{ min - hours}\)

Images removed to due copyright considerations.
What if faster response is needed?

- finding food
- chasing bait
- signal transduction

Image removed due to copyright considerations.

*genetics is too slow!*

Protein switches (active/inactive states)
(tot al amount active + inactive is constant, ignore gene expression)
timescales 1 ms - minutes
Introducing the H atom for signal transduction:

chemotaxis of *Escherichia coli*

Image removed due to copyright considerations.
Figure 1A in Mittal, N., E. O. Budrene, M. P. Brenner, and A. Van Oudenaarden. "Motility of Escherichia coli cells in clusters formed by chemotactic aggregation." Proc Natl Acad Sci U S A. 100, no. 23 (Nov 11, 2003): 13259-63.

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

Images removed due to copyright considerations.
The Flagellum

Image removed due to copyright considerations.
Absence of chemical attractant

Image by MIT OCW.
Presence of chemical attractant

Chemical Gradient Sensed in a Temporal Manner

Image by MIT OCW.
Chemotactic pathway in *E. coli*
*Towards more complex system networks.*

Image removed due to copyright considerations.
Proteins in the chemotactic network can be modified in different ways:

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

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

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

**CheA** \((\text{CheA}_p)\) 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)

Courtesy of Annual Review of Cell and Developmental Biology. Used with permission.
I  Phosphorylation (CheA, CheY, CheB)

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

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

CheY\text{p} binds to the motor (FliM),
motor rotates CW (= tumbles)

logic:

high levels of CheA -> high levels of CheY\text{p}  
(lots of tumbles)

low levels of CheA -> low levels of CheY\text{p}  
(straight swimming)
CheZ dephosphorylates CheY$_p$
(opposite function as CheA)

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

logic:
high levels of CheZ -> low levels of CheY$_p$

(straight swimming)
II Methylation (tar receptor)

*CheR* adds methyl group

*CheB<sub>p</sub>* 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.

Image removed due to copyright considerations.
Chemotactic Pathway in E. coli.

why is this all so complex ?
methylolation is important for adaptation
(∼ background subtraction)

*E. coli* can sense aspartate from 10 nM - 1 mM and sense changes as small as 0.1%
Before starting with the modeling, first let’s look at some recent experiments

Sourjik et al., PNAS 99, 123 (2002)

Remember scientists have been working on \textit{E. coli} chemotaxis for about 100 years now

Single cell chemotactic analysis
correlation CW bias & CheY-P gene expression

cells have plasmids with CheY-GFP under inducible promoter

assumption: all CheY is phosphorylated

strain: CheY-, CheZ-, CheB-

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FRET (fluorescence resonant transfer)


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CheY-YFP (yellow)  
CheZ-CFP (blue)  
CheZ binds only to CheYp!!  

adding attractant  
leads to immediate  
lower concentration of CheY$_p$-CheZ complex,  
lower [CheYp],  
less tumbling

Figures 1A and 1B in Sourjik, V., and Berg HC.  
"Receptor sensitivity in bacterial chemotaxis."  
*Proc Natl Acad Sci U S A* 99, no. 1  

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Figure 2 in Sourjik, V., and Berg HC. "Receptor sensitivity in bacterial chemotaxis." Proc Natl Acad Sci U S A 99, no. 1 (Jan 8, 2002): 123-7.

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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

Images removed due to copyright considerations. See Figure 2 in Alon, U., M. G. Surette, N. Barkai, and S. Leibler. "Robustness in bacterial chemotaxis." Nature 397, no. 6715 (Jan 14, 1999): 168-71.

not all parameters are robust !
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

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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\textsubscript{p} 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.

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Ligand bound states generally have lower autophosphorylation rates
CheR methylates ligand-bound states more rapidly

Consider step in aspartate concentration time ~ 1 ms, increase in ligand bound complex.

time ~ 5 s, total # of phosphorylated complexes decreases gradually because ligand bound complexes do not autophosphorylate very well also: CheB$_p$ decreases low CheA$_p$, low CheY$_p$, tumble suppression

time \sim 50 \text{s}, slowly the unbound complex methylate. Note that demethylation is switched because of low levels of CheAp (low CheBp).

Higher methylation states autophosphorylate easier, so slowly CheA_p adapts to its initial level.

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