A structured approach …
Part III
Biological applications

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MAPK Pathway

- Responds to wide range of stimuli: cytokines, growth factors, neurotransmitters, cellular stress and cell adherence,…

- Pivotal role in many key cellular processes:
  - growth control in all its variations,
  - cell differentiation and survival
  - cellular adaptation to chemical and physical stress.

- Deregulated in various diseases: cancer; immunological, inflammatory and degenerative syndromes,

- Represents an important drug target.
MA1: Mass action for enzymatic reaction - phosphorylation

\[
E + A \xrightleftharpoons[k_2]{k_1} E \mid A \xrightarrow{k_3} E + B
\]

- A: substrate
- B: product (phosphorylated A)
- E: enzyme (kinase)
- E|A substrate-enzyme complex

Biological Applications
Differential equations

Enzymatic reaction

\[ A + E \xrightleftharpoons[k_2]{k_1} A \mid E \xrightarrow{k_3} B + E \]

\[
\begin{align*}
\frac{d[A]}{dt} &= -k_1 \times [A] \times [E] + k_2 \times [A \mid E] \\
\frac{d[A \mid E]}{dt} &= +k_1 \times [A] \times [E] - k_2 \times [A \mid E] - k_3 \times [A \mid E] \\
\frac{d[B]}{dt} &= + k_3 \times [A \mid E] \\
\frac{d[E]}{dt} &= -k_1 \times [A] \times [E] + k_2 \times [A \mid E] + k_3 \times [A \mid E]
\end{align*}
\]
MA2 model

\[ A + E \xrightleftharpoons[k_2]{} A \mid E \xrightarrow[k_1]{} B \mid E \xrightarrow[k_3]{} B \mid E \xrightarrow[k_4]{} B + E \]
MA3 model

\[
A + E \xrightarrow{k_1} A | E \xrightarrow{k_3} B | E \xrightarrow{k_4} B + E
\]
Multiple substrates

\[ A_1 + A_2 + E \xrightarrow[k_{2A_1}^{A_1}]{} A_1|E + A_2 \xrightarrow[k_{2A_2}^{A_2}]{k_{1A_1}+k_{1A_2}} A_1|A_2|E \xrightarrow[k_{3}]{} B_1 + B_2 + E \]
Metabolic pathways vs Signalling Pathways
(Petri Nets)
Mass action for enzymatic reaction - phosphorylation

- \( R \): substrate,
- \( R_p \): product (phosphorylated R)
- \( S_1 \): enzyme (kinase)
- \( R|S_1 \) substrate-enzyme complex

\[
R + S_1 \xrightleftharpoons[k_2]{k_1} R|S_1 \xrightarrow[k_3]{k_3} R_p + S_1
\]
Phosphorylation - dephosphorylation step
Mass action model 1

- R: unphosphorylated form
- \( R_p \): phosphorylated form
- S: kinase
- P: phosphotase
- \( R|S \): unphosphorylated + kinase complex
- \( R|P \): unphosphorylated + phosphotase complex

\[
R + S \xrightleftharpoons[k_2]{k_1} R|S \xrightarrow{k_3} R_p + S \\
R + P \xleftarrow{kr_3} R_p | P \xrightarrow{kr_1} R_p + P
\]
Phosphorylation - dephosphorylation loop
Mass action model 2

- $R$: unphosphorylated form
- $R_p$: phosphorylated form
- $S_1$: kinase
- $S_2$: phosphotase
- $R|S_1$: unphosphorylated+kinase complex
- $R_p|S_1$: phosphorylated+kinase complex
- $R|S_2$: unphosphorylated+phosphotase complex
- $R_p|S_2$: phosphorylated+phosphotase complex

\[ R + S_1 \xrightleftharpoons[k_2]{k_1} R|S_1 \xrightarrow[k_3]{k_4} R_p|S_1 \xrightleftharpoons[k_5]{k_1} R_p + S_1 \]

\[ R + S_2 \xrightleftharpoons[k_{5r}]{k_{4r}} R|S_2 \xrightarrow[k_{3r}]{k_{2r}} R_p|S_2 \xrightleftharpoons[k_{2r}]{k_{1r}} R_p + S_2 \]
Phosphorylation - dephosphorylation step
Mass action (all singing/dancing)

- R: unphosphorylated form
- \( R_p \): phosphorylated form
- S: kinase
- P: phosphotase
- \( R|S \): unphosphorylated + kinase complex
- \( R|P \): unphosphorylated + phosphotase complex

\[
R + S \xrightarrow{k_1} R|S \xrightarrow{k_3} R_p | S \xrightarrow{k_4} R_p + S \\
R + P \xleftarrow{kr_4} R|P \xleftarrow{kr_3} R_p | P \xrightarrow{kr_1} R_p + P
\]
Michaelis-Menten equation for phosphorylation-dephosphorylation

\[ V = k_3 \times [S] \times \frac{[R]}{(K_{m1} + [R])} - k_3' \times \frac{[R_p]}{(K_{m2} + [R_p])} \]

- Assumptions:
  1. No product reverts to initial substrate
  2. MM Equation holds at initial stage of reaction before concentration of product is appreciable
  3. \([\text{Enzyme}] \ll [\text{Substrate}]\)
- \(K_m\) is \([\text{Substrate}]\) at which the reaction rate is half its maximum value
- \(dR_p/dt = \text{reaction rate } V\)
- \(k_3 \times S = V_{\text{max}}\) for the forward reaction
- \(k_3' = V_{\text{max}}\) for the reverse reaction (Phosphotase is ignored)
- \(K_{m1} = (k_2+k_3)/k_1\) (k’s from mass-action 1)
Questions

• Is Michaelis-Menten adequate for phosphorylation pathways?
• Is Mass Action sufficient/correct for these pathways?
• What is the effect of negative feedback?
• Can we confirm the ‘negative feedback amplifier’ behaviour in both MM and MA models
• Can oscillators be built?
• Overall, what are the rules for component-based construction?
Biological Applications
Composition
Vertical & horizontal

2-stage cascade

1-stage cascade
double phosphorylation step
Composition
Vertical & horizontal

(a) S → Rp → P
    R → Rp

(b) S1 → Rp
    R → Rp
    P1

(c) S → Rp → Rp
    R → Rp
    P
Two stage, double phosphorylation
Phosphorylation cascade: 2-stage, Mass Action model 1

\[ R + S_1 \xrightarrow{k_1} R \mid S_1 \xrightarrow{k_3} R_p + S_1 \]

\[ R + P_1 \xleftarrow{k_3'} R \mid P_1 \xrightarrow{k_{1'}} R_p + P_1 \]

\[ RR + R_p \xleftarrow{kk_1} RR \mid R_p \xrightarrow{kk_3} RR_p + R_p \]

\[ RR + P_2 \xleftarrow{kk_3'} RR \mid P_2 \xrightarrow{k_{1'}} RR_p + P_2 \]
Phosphorylation cascade: 2-stage, Michaelis-Menten

\[
\frac{dR_p}{dt} = \frac{k_3 \times S_1 \times R}{K_{m1} + R} - \frac{k_3' \times R_p}{K_{m2} + R_p}
\]

\[
\frac{dRR_p}{dt} = \frac{kk_3 \times R_p \times RR}{KK_{m1} + RR} - \frac{kk_3' \times RR_p}{KK_{m2} + RR_p}
\]
3-stage Phosphorylation cascade (Mass Action)

\[ R + S_1 \xrightleftharpoons[k_2]{k_1} R \mid S_1 \xrightarrow{k_3} R_p + S_1 \]

\[ R + P_1 \xrightleftharpoons[k'_3]{k_3'} R_p \mid P_1 \xrightarrow[k'_2]{k_2} R_p + P_1 \]

\[ RR + R_p \xrightarrow[kk_3]{kk_1} RR \mid R_p \xrightarrow[kk_3]{kk_1} RR_p + R_p \]

\[ RR + P_2 \xrightleftharpoons[kk'_3]{kk'_1} RR \mid P_2 \xrightarrow[kk'_2]{kk'_1} RR_p + P_2 \]

\[ RRR + RR \xrightarrow[kk_3]{kk_1} RRR \mid RR \xrightarrow[kk_3]{kk_1} RRR_p + RRR \]

\[ RRR + P_3 \xrightleftharpoons[kk_3]{kk_1} RRR \mid P_3 \xrightarrow[kk_2]{kk_1} RRR_p + P_3 \]
Phosphorylation cascade: 3-stage, Michaelis-Menten

\[
\begin{align*}
\frac{dR_p}{dt} &= \frac{k_3 \times S_1 \times R}{K_{m1} + R} - \frac{k_3' \times R_p}{K_{m2} + R_p} \\
\frac{dRR_p}{dt} &= \frac{kk_3 \times R_p \times RR}{KK_{m1} + RR} - \frac{kk_k' \times RR_p}{KK_{m2} + RR_p} \\
\frac{dRRR_p}{dt} &= \frac{kkk_3 \times RR_p \times RRR}{KKK_{m1} + RRR} - \frac{kkk_3' \times RRR_p}{KKK_{m2} + RRR_p}
\end{align*}
\]
3-stage
Phosphorylation cascade + feedback

\[ R_{R_p} + S_1 \rightarrow R_{R_p} | S_1 \]

\[ R + R_{R_p} | S_1 \rightarrow R | R_{R_p} | S_1 \rightarrow R_{R_p} | S_1 \]

\[ R_{R_p} + P_1 \rightarrow R_{R_p} | P_1 \]

\[ R_{R_p} + P_1 \rightarrow R_{R_p} | P_1 \]

\[ R_{R_p} + P_1 \rightarrow R_{R_p} | P_1 \]

Biological Applications
Phosphorylation cascade + negative feedback: 2-stage, Mass Action model 1

\[ RR_p + S_1 \xrightleftharpoons{ki}{ki'} \rightarrow RR_p | S_1 \]

\[ R + S_1 \xrightleftharpoons{k_1}{k_2} \rightarrow R | S_1 \xrightarrow{k_3} R_p + S_1 \]

\[ R + P_1 \xrightleftharpoons{k_3'}{k_2'} \rightarrow R | P_1 \xrightleftharpoons{k_1'}{k_2'} \rightarrow R_p + P_1 \]

\[ RR + R_p \xrightleftharpoons{kk_1}{kk_2} \rightarrow RR | R_p \xrightarrow{kk_3} RR_p + R_p \]

\[ RR + P_2 \xrightleftharpoons{kk_3'}{kk_2'} \rightarrow RR | P_2 \xrightleftharpoons{kk_1'}{kk_2'} \rightarrow RR_p + P_2 \]
Phosphorylation cascade + negative feedback: 2-stage, Michaelis-Menten

\[
\frac{dR_p}{dt} = \frac{k_3 \times S_1 \times R}{K_{m1} \times \left(1 + \frac{RR_p}{K_i}\right)} + R - \frac{k_3' \times R_p}{K_{m2} + R_p}
\]

\[
\frac{dRR_p}{dt} = \frac{kk_3 \times R_p \times RR}{KK_{m1} + RR} - \frac{kk_3' \times RR_p}{KK_{m2} + RR_p}
\]

- Using Competitive Inhibition
- \(K_i\) is the dissociation constant for the SI complex

\[
V = V_{max} \times \frac{[S]}{[S] + K_m \times \left(1 + \frac{[I]}{K_i}\right)}
\]
Phosphorylation cascade + negative feedback: 3-stage, Mass Action, model 1

\[ RRR_p + S_1 \xrightarrow{k_l} RRR_p \mid S_1 \]

\[ R + S_1 \xrightarrow{k_1} R \mid S_1 \xrightarrow{k_3} R_p + S_1 \]

\[ R + P_1 \xleftarrow{k_3} R_p \mid P_1 \xleftarrow{k_3} R_p + P_1 \]

\[ RR + R_p \xleftarrow{k_2} RR \mid R_p \xrightarrow{k_{kk_3}} RRR + R_p \]

\[ RR + R_p \xleftarrow{k_2} RR \mid R_p \xrightarrow{k_{kk_3}} RRR + R_p \]

\[ RRR + RRR_p \xleftarrow{k_{kk_2}} RRR \mid RRR_p \xrightarrow{k_{kk_3}} RRRR + RRR_p \]

\[ RRR + RRR_p \xleftarrow{k_{kk_2}} RRR \mid RRR_p \xrightarrow{k_{kk_3}} RRRR + RRR_p \]

\[ RRR + RRR_p \xleftarrow{k_{kk_2}} RRR \mid RRR_p \xrightarrow{k_{kk_3}} RRRR + RRR_p \]

\[ RRR + RRR_p \xleftarrow{k_{kk_2}} RRR \mid RRR_p \xrightarrow{k_{kk_3}} RRRR + RRR_p \]
Phosphorylation cascade + negative feedback: 3-stage, Michaelis-Menten

\[
\frac{dR_p}{dt} = \frac{k_3 \times S_1 \times R}{K_{m1} \times \left(1 + \frac{RRR_p}{K_i}\right)} + R - \frac{k_3' \times R_p}{K_{m2} + R_p}
\]

\[
\frac{dRR_p}{dt} = \frac{kk_3 \times R_p \times RR}{KK_{m1} + RR} - \frac{kk_3' \times RRR_p}{KK_{m2} + RRR_p}
\]

\[
\frac{dRRR_p}{dt} = \frac{kkk_3 \times RRR_p \times RRR}{KKK_{m1} + RRR} - \frac{kkk_3' \times RRR_p}{KKK_{m2} + RRR_p}
\]

- Using Competitive Inhibition
- Ki is the dissociation constant for the SI complex

\[
V = V_{max} \times \frac{[S]}{[S] + K_m \times \left(1 + \frac{[I]}{[K_i]}\right)}
\]
3-stage, negative feedback
Phosphorylation cascade + negative feedback: 3-stage, Inhibitor on 2nd stage, Mass Action

\[ RRR_p + S_{1} \xrightarrow{\kappa_1} RRR_p \mid S_1 \]

\[ R + S_{1} \xrightarrow{\kappa_2} R \mid S_1 \xrightarrow{k_1} R_p + S_1 \]

\[ R + P_1 \xleftarrow{k'_1} R_p \mid P_1 \xrightarrow{\kappa_2} R_p + P_1 \]

\[ RR + R_p \xrightarrow{\kappa_1} RR \mid R_p \xrightarrow{\kappa_2} RR_p + R_p \]

\[ RR + P_2 \xleftarrow{\kappa_2} RR_p \mid P_2 \xrightarrow{\kappa_1} RR_p + P_2 \]

\[ RRR + RR \xrightarrow{\kappa_1} RRR \mid RR \xrightarrow{\kappa_2} RRR_p + RRR_p \]

\[ RRR + R_p \xrightarrow{\kappa_1} RRR_p \mid RRR_p \xrightarrow{\kappa_2} RRR_p + RRR_p \]

\[ U + RR \xrightarrow{\kappa_1} U \mid RR \]

\[ U + RRR_p \xrightarrow{\kappa_2} U \mid RRR_p \]

\[ U \mid RR + R_p \xrightarrow{\kappa_1} U \mid RR \mid R_p \xrightarrow{\kappa_2} U \mid RR_p + R_p \]

\[ U \mid RR + P_2 \xleftarrow{\kappa_2} U \mid RR_p \mid P_2 \xrightarrow{\kappa_1} U \mid RR_p + P_2 \]
3-stage, negative feedback + inhibitor
‘Real cascade & feedback’

\[
\begin{align*}
\text{Ras} & \quad \downarrow \\
\text{Raf} & \quad \downarrow \\
\text{MEK} & \quad \downarrow \\
\text{ERK} &
\end{align*}
\]

\text{U0126} \quad \rightarrow \quad \text{MEK}
Is the ERK pathway a negative feedback amplifier?

Sauro HM, Kholodenko BN.
Quantitative analysis of signaling networks.
Negative Feedback Amplifier

• A negative feedback amplifier stems from the field of electronics and consists of an amplifier with a negative feedback loop from the output of the amplifier to its input.

• The negative feedback loop results in a system that is much more robust to disturbances in the amplifier.

• The negative feedback amplifier was invented in 1927 by Harold Black of Western Electric and was originally used for reducing distortion in long distance telephone lines.

• The negative feedback amplifier is now a key electrical component used in a wide variety of applications

![Diagram of Negative Feedback Amplifier]
Negative Feedback Amplifier

Steady State Equation

\[ y = \frac{Au}{1 + AF} \]

Biological Applications

\[ y = Ae \]
\[ e = u - Fy \]
\[ y = A (u - Fy) \]
\[ y + AFy = Au \]
\[ y (1 + AF) = Au \]
The negative feedback imparts signalling robustness

A large change in amplifier gain leads to a small change in output \( y \)
Feedback

Increasing Feedback

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Biological Applications
The negative feedback imparts signalling robustness

Standard Amplifier

\[ y = A \cdot u \]

Negative Feedback Amplifier

\[ y = A \cdot u / (1 + A \cdot F) \]

Sudden drop in Amplifier (A) gain

\[ \Delta y \text{ Output} \]

Sudden drop in Amplifier (A) gain

\[ \Delta y \text{ Output} \]
Application to Biology

- The ERK cascade is a well known biological amplifier and contains numerous negative feedback loops.

- At first sight, it has the correct structure to be a negative feedback amplifier.

- If the ERK cascade is a negative feedback amplifier it should be robust to disturbances within the cascade.

- From a biological point of view, these disturbances could be caused by drugs, such as U0126, aimed at decreasing the activity of the ERK cascade.

- This suggests that these drugs will be relatively ineffective.

- In fact, current drugs aimed at decreasing the activity of the MAPK pathway have proved less efficient in in vivo applications than anticipated from in vitro inhibition assays.

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Sauro & Kholodenko (2004)
Raf/MEK/ERK amplifies the signal

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How to test if the ERK pathway is a NFA?

Generate input:
Stimulate with GF

“Disturb the Amplifier”: Use a MEK inhibitor, such as U0126

Remove negative feedback

Measure signal output:
 i.e. ERK phosphorylation
Hypothesis: Braking the feedback should sensitise the ERK pathway to MEK-inhibitor

Feedback intact

Feedback removed

Ras-GTP

Raf-1

MEK1/2

ERK1/2

MEK inhibitor

phospho-ERK

U0126

Biological Applications
How to test if the ERK pathway is a NFA?

**Strategy**

- In vivo system that allows us to compare feedback broken to feedback intact model.
- Computational Model of ERK pathway with/without feedback
Computational Modeling 1: Build the model

- Non-linear ordinary differential equations (ODE's).
- ODE's were solved using Math Lab and Gepasi.
- Models are based on the Schoeberl et al. (2002) model.
- Kinetic parameters are from literature, previous models and "guesstimates".

Schoeberl et al. (2002), Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors, Nature Biotechnology 20, 370-375
Amplifier / negative feedback

- Model amplifier strength by
  - Adding inhibitor to 2nd stage
  - Modifying kk3, kkk3 [i.e. modifying rate of production of RRP, RRRP]
  - Add/remove cascade elements
- Then plot amp strength versus output, e.g. [U] vs [RRRP]
- Model feedback strength by
  - Leaving out feedback loop
  - Varying ki, and plot ki vs [RRRP]
- Notes: avoid saturation; use signal in linear range; ? model degradation in S1 signal?
Computational Modeling 2:

Results

Prediction: Braking the feedback modulates drug response
Sensitivity of kinetic parameters is decreased due to Negative Feedback
The experimental systems

Negative feedback loops intact

One feedback loop eliminated by constitutively active RasV12 mutant

Both feedback loops eliminated by BXB-ER (4-OHT regulatable Raf-1 mutant)

Biological Applications
Breaking the ERK feedback with BXBER

Raf-1

- Regulatory Domain
- Kinase Domain

BXB-ER

- CR3
- ER hormone binding

ERK feedback phosphorylation sites

BXB-ER stimulated with 4-OHT
(4-Hydroxy Tamoxifen, a synthetic estrogen)

Raf-1 stimulated with EGF

ppERK levels

ppERK levels

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51
Ablation of feedback by BXBER decreases robustness to MEK-inhibitor U0126

Computer Simulation

Feedback Broken

Initial [RasGTP] (decreasing) representing Initial [4557W] (increasing) or Initial [U0126] (increasing)

Biological Applications
Ablation of feedback by BXBER decreases robustness to MEK-inhibitor U0126
Signal recovery after MEK inhibition

Simulation

Experiment

U0126 added

pERK1/2, +EGF
pERK1/2, + BXBER/4HT

0 10 20 40 80 min stimulation
Oscillations! Phosphorylation cascade + negative feedback: 3-stage, Inhibitor on 2nd stage, Mass Action

Conditions
S1=3
Inhibitor=0.5
Combination of positive & negative feedback
Mathematical Model

Modeling and Analysis of Two Feedback Loop Dynamics in Ras/Raf-1/MEK/ERK Signaling Pathway
Kwang-Hyun Cho, Sung-Young Shin, Walter Kolch, Olaf Wolkenhauer. ICSB 2004

Biological Applications
Combination of positive & negative feedback: Simulation

No Feedback

Positive Feedback

Negative Feedback

Positive & Negative Feedback

Biological Applications
Combination of positive & negative feedback: Simulation vs. Experimental Data

Western blots COS1 cell lysates

Comparison of experimental data and simulation result

Experiment
Simulation