

Linear robustness analysis of non-linear biomolecular networks with kinetic perturbations

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Informal definition of robustness

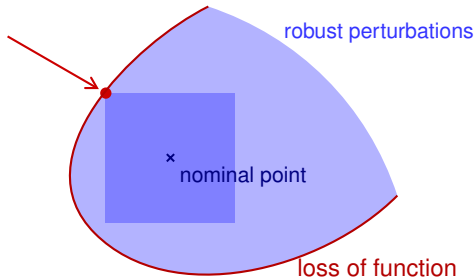
Robustness is a property that allows a **system** to **maintain** its **function** in the presence of internal and external **perturbations**.

H. Kitano, 2004

- **System / Model**: $\dot{x} = Sv(x)$
 - $x \in \mathbb{R}^n$: amounts of signalling molecules
 - $v(x) \in \mathbb{R}^m$: reaction rates
 - $S \in \mathbb{R}^{n \times m}$: stoichiometric matrix
- **Perturbations**: **kinetic perturbations** (changes in reaction kinetics v)
- **Function**: Qualitative dynamical behaviour (stability, oscillations, ...)



Smallest non-robust
perturbation in ∞ -
norm



- **Non-robust perturbation** = any deviation from the nominal point for which the system **loses functionality**
- **Robustness measure** = **smallest** norm of non-robust perturbations



Robustness analysis for model evaluation

- Biological systems are robust $\leftrightarrow$ models should be robust!
- Find parts of the system which need to be modelled carefully
- Evaluate effects of uncertainty on model predictions

Robustness analysis for system understanding

- Understand mechanisms which confer robustness to biological systems
- Detect fragile points, e.g. for medical intervention
- Guide system design in bioengineering and synthetic biology



- 1 Theory of kinetic perturbations
- 2 Robustness analysis with kinetic perturbations
- 3 Adaptation with kinetic perturbations

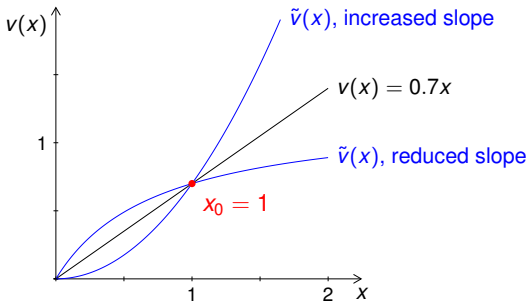


- 1 Theory of kinetic perturbations
 - Definition of kinetic perturbations
 - Mapping kinetic perturbations to parameter variations
- 2 Robustness analysis with kinetic perturbations
- 3 Adaptation with kinetic perturbations



Kinetic perturbations = reaction rate perturbations

- nominal rates $v(x)$ to perturbed rates $\tilde{v}(x)$
- ⇒ nominal model $\dot{x} = Sv(x)$ to perturbed model $\dot{x} = S\tilde{v}(x)$
- Consider nominal steady state x_0 : $Sv(x_0) = 0$
- Kinetic perturbation, if $\tilde{v}(x_0) = v(x_0)$
- Reaction rates in steady state unperturbed, but reaction rate slopes may vary:





Definition

The network $\dot{x} = S\tilde{v}(x)$ is said to be a *kinetic perturbation* of the nominal network $\dot{x} = Sv(x)$ at $x_0 \in \mathbb{R}^n$ with $Sv(x_0) = 0$, if $\tilde{v}(x_0) = v(x_0) =: v_0$.

- Consider the reaction rate Jacobian $V = \frac{\partial v}{\partial x}$
- For the perturbed network, we have

$$\tilde{V}(x_0) = \frac{\partial \tilde{v}}{\partial x}(x_0) = V(x_0) + \bar{\Delta}$$

- Local effects of the kinetic perturbation are determined by $\bar{\Delta}$
 - Scaled perturbation $\Delta_{i\ell} = \frac{x_{0,i}}{v_{0,i}} \bar{\Delta}_{i\ell}$
 - $\|\Delta\|$ is a measure for the perturbation size

- Steady state concentration x_0 and flux v_0 are unperturbed
- Steady state reaction slopes $V(x_0)$ are perturbed by $\bar{\Delta}$

Mapping kinetic perturbations to parameter changes

- Considering generalised mass action (GMA) networks
- GMA reaction rate: $v_i(x) = k_i \prod_{\ell=1}^n x_{\ell}^{\alpha_{i\ell}}$
 - $\alpha_{i\ell} > 0$: x_{ℓ} is a substrate for / activates v_i
 - $\alpha_{i\ell} < 0$: x_{ℓ} inhibits v_i
- Non-integer kinetic orders $\alpha_{i\ell}$
 - Effect of constrained diffusion (fractal reaction kinetics)
 - Result of model simplifications (as in S-systems, Savageau *et al.*)
- Perturbed reaction rate $\tilde{v}_i(x) = \tilde{k}_i \prod_{\ell=1}^n x_{\ell}^{\tilde{\alpha}_{i\ell}}$

Kinetic perturbation

$$\frac{\partial \tilde{v}_i}{\partial x_{\ell}} = \frac{\partial v_i}{\partial x_{\ell}} + \frac{v_{0,i}}{x_{0,\ell}} \Delta_{i\ell}$$

$\Leftrightarrow$

Parameter change

$$\tilde{\alpha}_{i\ell} = \alpha_{i\ell} + \Delta_{i\ell}$$

$$\tilde{k}_i = k_i \prod_{\ell=1}^n x_{0,\ell}^{-\Delta_{i\ell}}$$



Are kinetic perturbations biochemically plausible?

Yes, because kinetic perturbations map to parameter variations in reasonable model classes for intracellular networks such as

- GMA networks
- enzymatic networks (Michaelis-Menten or Hill reaction kinetics)
- genetic networks (sigmoidal activation & inhibition functions)

Biochemical interpretation

- Positive $\Delta_{i\ell}$: more cooperativity in reaction i with respect to species ℓ
- Negative $\Delta_{i\ell}$: increase saturation in reaction i with respect to species ℓ

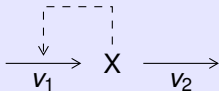


- 1 Theory of kinetic perturbations
- 2 Robustness analysis with kinetic perturbations
 - Kinetic perturbations affect dynamical properties
 - Robustness analysis via the structured singular value
 - Example: Oscillations in the MAPK cascade
- 3 Adaptation with kinetic perturbations

Inducing bifurcations by kinetic perturbations



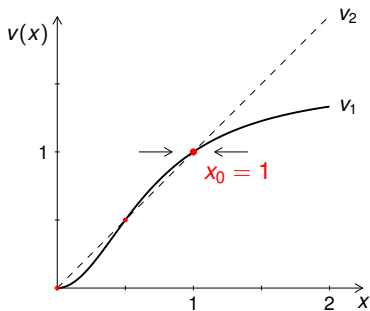
Example “network”



$$v_1 = \frac{kx^2}{1+Mx^2}$$

$$\dot{x} = v_1 - v_2$$

$$v_2 = x$$



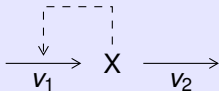
- $\frac{\partial \tilde{v}_1}{\partial x}(1) = \frac{\partial v_1}{\partial x}(1) + \bar{\Delta}$
- $\bar{\Delta} = 0$: x_0 stable
- $\bar{\Delta} = \bar{\Delta}^*$: transcritical bifurcation at x_0
- $\bar{\Delta} > \bar{\Delta}^*$: x_0 unstable

Loss of bistability by a kinetic perturbation

Inducing bifurcations by kinetic perturbations



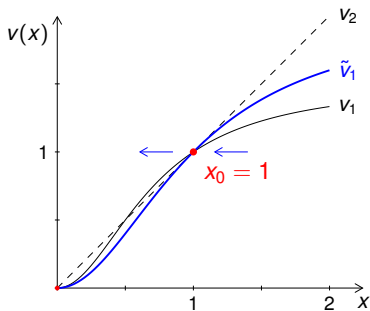
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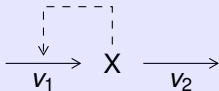
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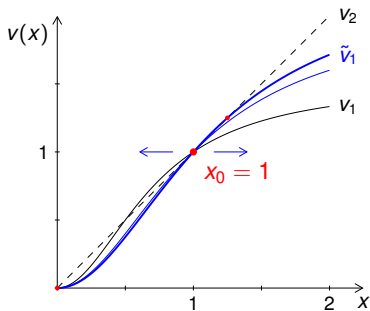
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Loss of bistability by a kinetic perturbation



Perturbed Jacobian

$$\tilde{A}(\Delta) = A + S(\text{diag } v_0)\Delta(\text{diag } x_0)^{-1}$$

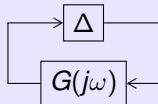
Robustness radius for kinetic perturbations

- Robustness measure = size of smallest perturbation such that $\tilde{A}(\Delta)$ has an eigenvalue on the imaginary axis

$$\psi = \inf \{ \|\Delta\| \mid \det(j\omega I - \tilde{A}(\Delta)) = 0 \}$$

- Can be formulated as structured singular value (μ) problem with $G(j\omega) = (\text{diag } x_0)^{-1}(j\omega I - A)^{-1}S(\text{diag } v_0)$

Result:
$$\psi = \left(\sup_{\omega} \mu_{\Delta} G(j\omega) \right)^{-1}$$



Robustness problem can be solved with standard μ analysis tools.

Robustness radius for scalar perturbations



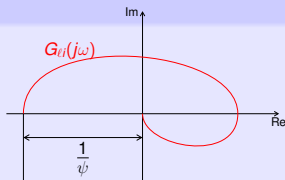
- Perturbation of a single interaction: Species ℓ on reaction i

$$\bar{\Delta} = \begin{pmatrix} 0 \\ \vdots \\ 1 \\ \vdots \\ 0 \end{pmatrix} \bar{\Delta}_{i\ell(0 \dots 1 \dots 0)} = \mathbf{e}_i \bar{\Delta}_{i\ell} \mathbf{e}_\ell^T$$

- Perturbed Jacobian $\tilde{A}(\Delta_{i\ell}) = A + S \mathbf{e}_i v_{0,i} \Delta_{i\ell} x_{0,\ell}^{-1} \mathbf{e}_\ell^T$

Robustness radius

$$\begin{aligned} \psi &= \{|\Delta_{i\ell}| \mid \det(j\omega I - \tilde{A}(\Delta_{i\ell})) = 0\} \\ &= \left(\sup_{\omega \in \mathcal{R}(G_{\ell i})} |G_{\ell i}(j\omega)| \right)^{-1} \end{aligned}$$



- μ -problem with $G_{\ell i}(j\omega) = \mathbf{e}_\ell^T x_{0,\ell}^{-1} (j\omega I - A)^{-1} S v_{0,i} \mathbf{e}_i$

- Explicit formula for the robustness radius
- Allows to detect fragile interactions in the network



- Perturbation of several interactions either
 - from one species ℓ to several reactions $E_i v$ or
 - from several species $E_\ell x$ to one reaction i .

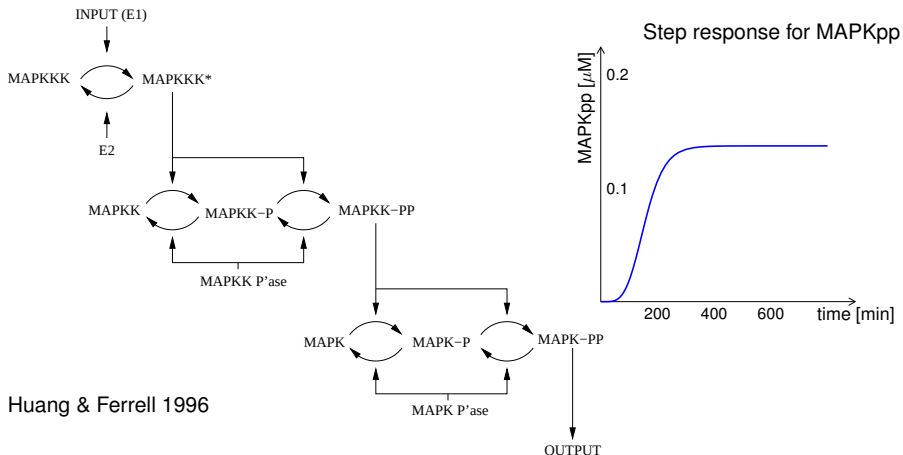
$$\bar{\Delta} = E_i \bar{\Delta}_{i\ell} e_\ell^T$$

- Perturbed Jacobian $\tilde{A}(\Delta_{i\ell}) = A + SE_i \text{diag}(E_i v_0) \Delta_{i\ell} x_{0,\ell}^{-1} e_\ell^T$

Robustness analysis in the vector case

- Results depend on the norm that is chosen:
 - 1-norm or ∞ -norm: robustness radius from a linear program
 - 2-norm: explicit formula for robustness radius
- Also requires to compute a supremum over ω .
- Non-robust perturbation is the solution of an affine equation $M\bar{\Delta} = b$.

The MAPK cascade



Huang & Ferrell 1996

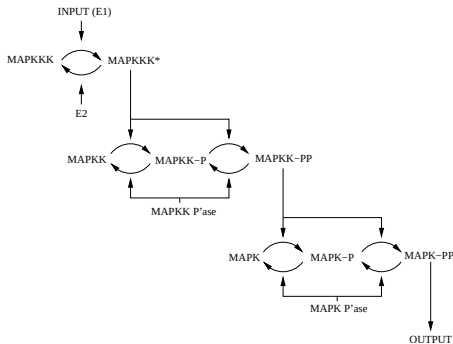
- Medium scale network with 22 species and 30 (irreversible) reactions

Where to perturb this network in order to get oscillations?

Single interaction analysis of the MAPK cascade



- Checking all 660 network interactions for a scalar kinetic perturbation to induce oscillations
- Found 8 fragile interactions ($|\Delta^*| < 1$), e.g.:

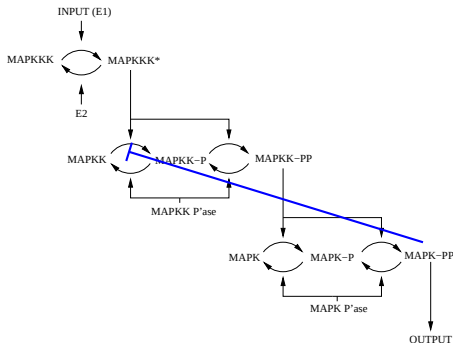


- Negative feedback from MAPKpp to MAPKK phosphorylation
- Positive feedback from MAPKpp to catalysis of MAPK / MAPKKpp complex
- Saturated catalysis of MAPKpp / Phosphatase complex

Single interaction analysis of the MAPK cascade



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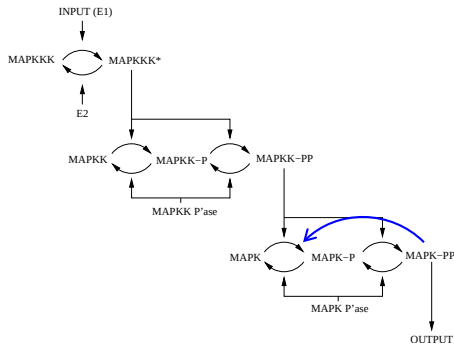


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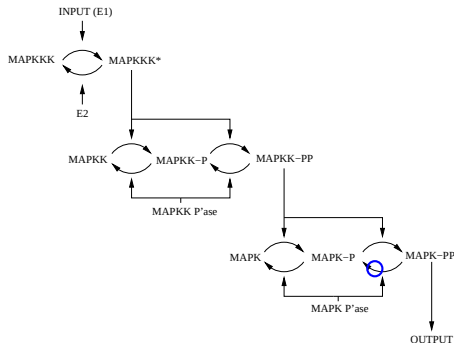


- Negative feedback from MAPK^* to MAPKK^*
- Positive feedback from MAPK^* to catalysis of $\text{MAPK} / \text{MAPKK}^*$ complex
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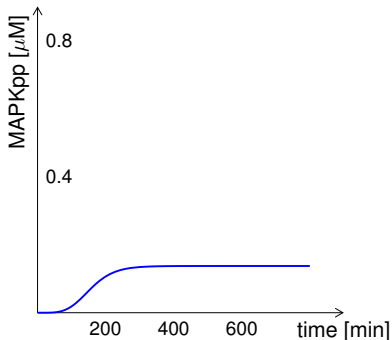
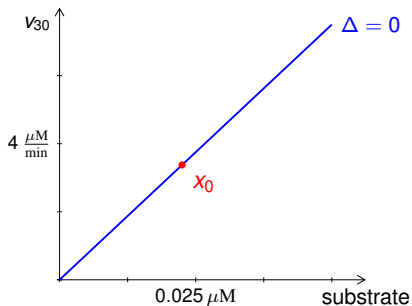


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Effect of a kinetic perturbation on the dynamics



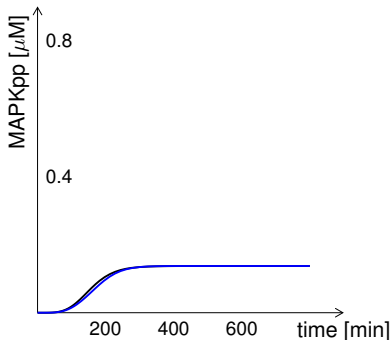
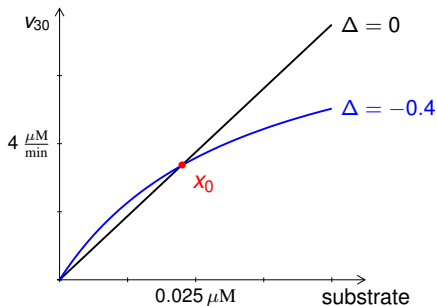
Critical perturbation $\Delta^* = -0.76$



Effect of a kinetic perturbation on the dynamics



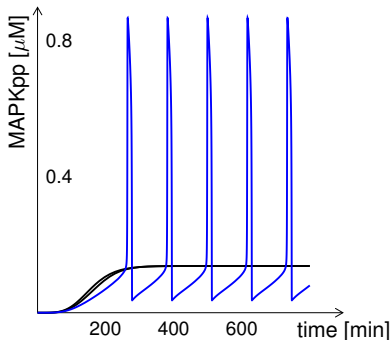
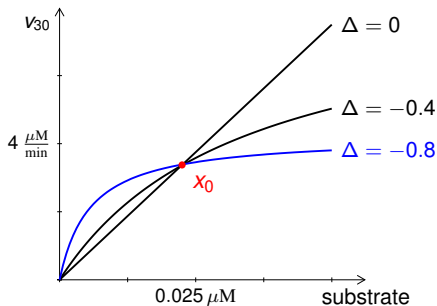
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Effect of a kinetic perturbation on the dynamics



Critical perturbation $\Delta^* = -0.76$



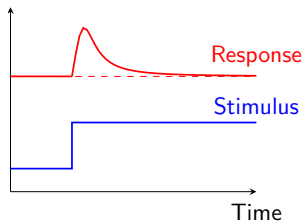


- 1 Theory of kinetic perturbations
- 2 Robustness analysis with kinetic perturbations
- 3 **Adaptation with kinetic perturbations**
 - Properties of biomolecular adaptation
 - Achieving adaptation with kinetic perturbations
 - Example: Adaptation in the MAPK cascade



1. Adaptation

A dynamical system $u \mapsto y$ is said to adapt, if persistent changes in u do lead to transient, but not persistent changes in y .



Local adaptation = consider only infinitesimally small changes in u

2. Local adaptation without transient response

The system $u \mapsto y$ is said to perfectly adapt locally at (u_0, y_0) , if

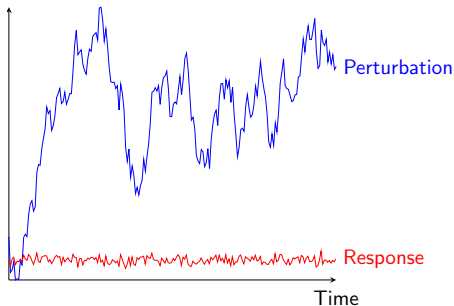
$$\lim_{u_s \rightarrow 0} \frac{\lim_{t \rightarrow \infty} (y(t, u_0 + u_s h(t)) - y_0)}{u_s} = 0.$$



Adaptation to a slowly changing
perturbation

=

Robustness of steady state
concentration to this perturbation



Slowly changing perturbations in biology

- Protein amounts due to stochastic gene expression
- Environmental conditions such as light, temperature, or nutrient availability



1. Linear approximation at steady state

$$\dot{x} = A x + B u$$

$$dy = C x,$$

$$\text{with: } A = S \frac{\partial v}{\partial x}(x_0, u_0)$$

$$B = S \frac{\partial v}{\partial u}(x_0, u_0)$$

2. Transformation to frequency domain

$$dy(s) = C(sI - A)^{-1} B du(s) = G(s) du(s).$$

3. Necessary and sufficient condition for local adaptation

By the final value theorem of the Laplace transformation:

$$\text{local adaptation} \Leftrightarrow G(0) = 0 \stackrel{A \text{ invertible}}{\Leftrightarrow} \det \begin{pmatrix} A & B \\ C & 0 \end{pmatrix} = 0$$

Last condition is affected by kinetic perturbations!



Effect of kinetic perturbation on adaptation condition

- Perturbation of Jacobian due to a kinetic perturbation $\ell \rightarrow i$:

$$\tilde{A} = A + S e_i v_{0,i} \Delta_{i\ell}(x_{0,\ell})^{-1} e_\ell^T$$

- Adaptation as goal:

$$\det \begin{pmatrix} \tilde{A} & B \\ C & 0 \end{pmatrix} = \det \begin{pmatrix} A + S e_i v_{0,i} \Delta_{i\ell}(x_{0,\ell})^{-1} e_\ell^T & B \\ C & 0 \end{pmatrix} = 0.$$

Compute a critical kinetic perturbation

$$\Delta_{i\ell}^* = (-e_j^T M_{out} M_0^{-1} M_{in} e_\ell)^{-1} \frac{x_{0,\ell}}{v_{0,i}}$$

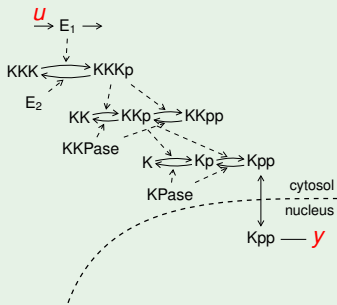
with

$$M_0 = \begin{pmatrix} A & B \\ C & 0 \end{pmatrix} \quad M_{in} = \begin{pmatrix} I \\ 0 \end{pmatrix} S \quad M_{out} = (I \quad 0)$$

Example: Adaptation in the MAPK cascade



Goal: achieve adaptation of nuclear MAPKpp



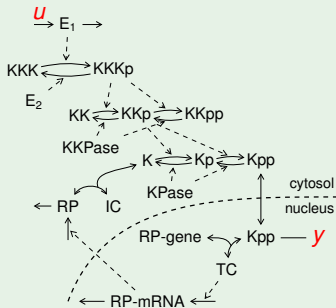
- Huang-Ferrell MAPK cascade model as before
- Added an input by controlling E_1 production

Result of analysis with kinetic perturbations

- No adaptation with transient response possible through kinetic perturbations
- Network lacks a feedback or feedforward loop

Adaptation in an extended MAPK cascade model

Model feedback extension

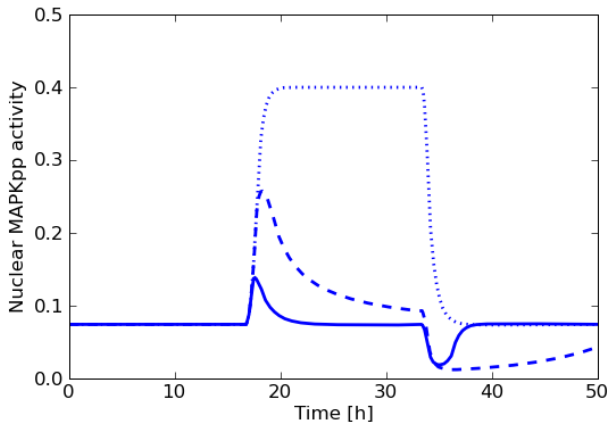


- Added a protein genetically regulated by MAPK
- Feedback by formation of inhibitory complex

Candidate interactions for adaptation

Found five candidate interactions in newly added feedback loop for adaptation via kinetic perturbations.

Response of original and perturbed networks



- Adaptation achieved even for large stimulus changes.
- Non-linear dynamics depend on which interaction is targeted.



Kinetic perturbations

- Perturbation class suited for biochemical network analysis
- Kinetic perturbations = reaction rate slope changes at steady state
- Biochemically plausible for GMA / enzymatic / genetic networks

Robustness analysis with kinetic perturbations

- Efficient robustness analysis via μ -analysis methods
- Results indicate fragile network interactions
- Non-robust perturbations are explicitly computed

Adaptation with kinetic perturbations

- Find network modifications to achieve perfect local adaptation
- Steady state is kept unperturbed intrinsically
- Adaptation even to large stimulus changes in many cases