The Role of Dynamic Stimulation Pattern in the Analysis of Bistable Intracellular Networks

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Bistable intracellular networks play an important role in the functioning of living cells. Besides the well-established steady-state properties, some important characteristics of bistable systems arise from their dynamics. We demonstrate that:

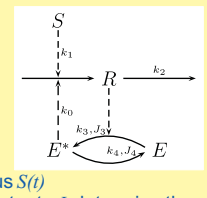
- a supercritical stimulus amplitude is not sufficient to move the system from the lower to the higher branch for either a step or a pulse input
- a switching surface can be identified for the system as a function of the initial condition, input pulse amplitude and duration (a supercritical signal)
- a minimal signal power is necessary to change the steady state of a bistable system
- we investigate and characterize the role of the duration of the stimulus

These results are relevant for the design of experiments, where it is often difficult to create a defined pattern for the stimulus.

Model System

- Mutually-activated system [1]
- Linear system coupled with a sigmoidal system through a positive feedback loop
- Mathematical representation

$$\begin{aligned}\frac{dR}{dt} &= k_0\,E^*(R) + k_1\,S - k_2\,R\\ E^*(R) &= G(k_3R, k_4, J_3, J_4)\end{aligned}$$



- Response component *R(t)* and external stimulus *S(t)*
- Kinetic constants k_i and Michaelis-Menten constants J_i determine the chemical properties of the involved species
- Modified form of the enzyme *E*(R)* facilitates the production of *R*
- (De)modification of enzyme *E* is assumed to be at steady state which is described by the Goldbeter-Koshland function *G(•)* [1,2]

$$G(k_3R, k_4, J_3, J_4) = \frac{2k_3RJ_4}{\mathcal{X} + \sqrt{\mathcal{X}^2 - 4(k_4 - k_3R)k_3RJ_4}}$$

$$\mathcal{X} = k_4 - k_3R + k_4J_3 + k_3RJ_4$$

- Typical sigmoidal shape *E*(R)*, Fig. 1 (left)
- High nonlinear behaviour can cause multiple steady states
- Positive feedback loop regulates the production rate of R in an autocatalytic fashion

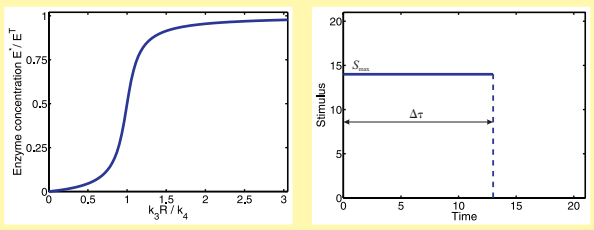


Fig. 1: Left - Concentration of the modified enzyme *E** as a function of the response component *R;* Right - External stimulus with amplitude S_{max} and duration $\Delta\tau$ which is used for the following investigations.

- Constant stimulus with amplitude S_{max} and duration $\Delta\tau$, Fig. 1 (right)

$$S = S_{\max}\,[\mathbf{u}(t) - \mathbf{u}(t - \Delta\tau)]$$

- Step function *u(t)* [3]
- Pulse if $\Delta\tau$ is finite
- Stepwise if $\Delta\tau = \infty$

Steady-State Properties

- Steady state with step input
- Determined by the balance equation

$$k_0\,E^*(R_{SS}) + k_1\,S_{\max} - k_2\,R_{SS} = 0$$

- Balance equation of a bistable system has at least three bio-physically realizable solutions
- Two stable branches, R_{SU} (upper) and R_{SL} (lower), are separated by an unstable one, R_{UN}, Fig. 5

$$R_{SS} \in \{R_{SU}, R_{UN}, R_{SL}\}$$

- Unstable and unphysical states define a forbidden region, Fig. 2
- Depending on the feedback strength, the network can be bistable in a reversible or irreversible manner [1] or monostable

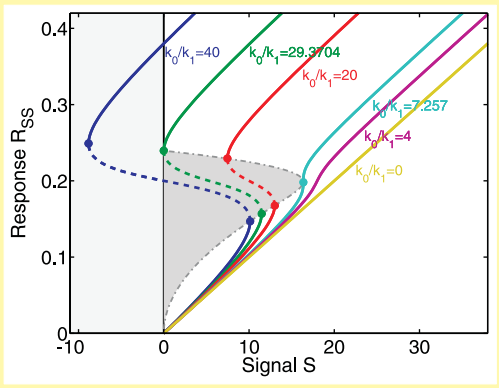


Fig. 2: Stimulus-Response curve for varying feedback strength. The system changes its behaviour from irreversible to reversible bistability and finally to monostability. The branches are separated by critical points (circles).

- Stability can be determined by the Jacobian of the system

$$\begin{aligned}J &= \frac{\partial}{\partial R}\frac{dR}{dt} = k_0\frac{\partial E^*(R)}{\partial R} - k_2,\\ &= \begin{cases}\text{negative for: } R_{SU} \text{ and } R_{SL}\ \text{(stable)}\\ \text{positive for: } R_{UN}\ \text{(unstable)}\end{cases}\end{aligned}$$

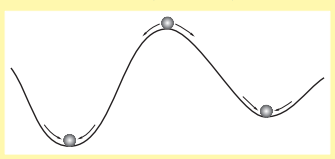


Fig. 3: Schematic representation of the stability of steady states. The system returns to the same stable steady state after a small pertubation and moves to a stable state if the instable state is pertubated.

References

[1] J.J. Tyson *et al.*, Curr. Opin. Cell Biol. 15, 221 (2003)
[2] A. Goldbeter *et al.*, Proc. Natl. Acad. Sci USA 78, 6840 (1981)
[3] Bracewell, R., *The Fourier Transform and ist Applications*, McGraw Hill (1999)
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Dynamic Behaviour

- Transient dynamics of the irreversible bistable network for different initial states R_0 and different supercritical stimulus pulses of different durations $\Delta\tau$
- Supercritical constant stimulus $S_{max} > S_{crit}$ always switches the system to the upper stable branch
- Systems behaviour for subcritical stimulus $S_{max} < S_{crit}$ depends on R_0, Fig. 4

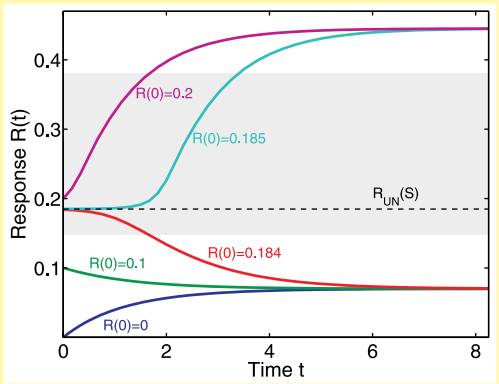

Fig. 4: Relaxation of the system for a constant subcritical stimulus $S_{max} < S_{crit}$ for different initial states. Depending on the initial state R_0 the bistable network approaches the lower or upper branch.

- Unstable branch R_{UN} acts as separatrix for subcritical signals
- Upper branch is approached for $R_0 > R_{UN}$, otherwise the lower branch, Fig. 5

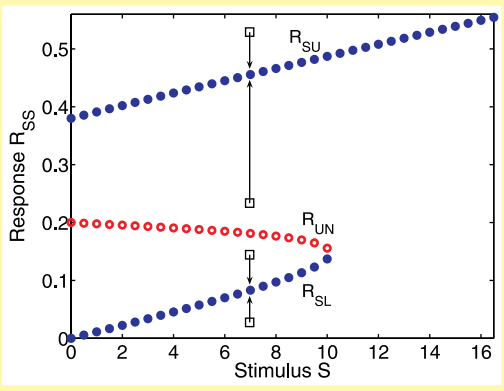

Fig. 5: Relationship between initial conditions R_0 (squares), stable steady states (R_{SU}, R_{SL}), unstable steady state (R_{UN}) and the corresponding critical value of step amplitude S_{crit}.

- Response to a supercritical pulse depends on the duration $\Delta\tau$, Fig. 6
- A critical duration $\Delta\tau_{min}$ is necessary in order to switch the system
- Transient response has to exceed the unstable branch before the stimulus becomes subcritical

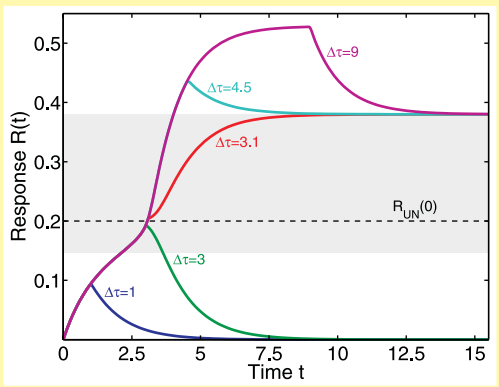

Fig. 6: Transient pulse response to supercritical stimulus $S_{max} > S_{crit}$ as a function of the duration $\Delta\tau$ from the origin. If R exceeds the unstable state during this time it approaches the upper branch after the pulse is switched off. Otherwise the response returns to the lower branch.

Switching Surface

- Signal amplitude, signal duration, and initial state can be combined in a three dimensional plot, Fig. 7
- Switching surface separates the parameter combinations which switch the irreversible bistable network to the upper stable branch and which not
- Different characteristic behaviour are identified

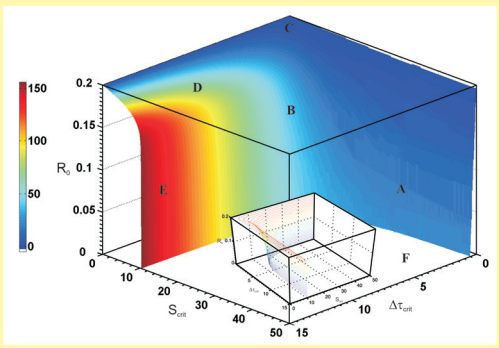

Fig. 7: Switching surface of the bistable network as response to the pulse. Regions A-F represent characteristics of the surface. Heatmap color refers to the critical power [4].

- A: Low values of R_0 and $\Delta\tau_{crit}$ require a high value of the pulse amplitude.
- B: With ever so slight increase in $\Delta\tau_{crit}$ and for the same low values of R_0, a much lower value of the pulse amplitude is needed for switching.
- C: At a fixed but high level of R_0 the switching is independent of critical pulse duration and only a low level of stimulus amplitude is needed.
- D: For amplitudes $10 > S_{max} > 8$ the switching surface slopes faster along the initial condition range R_0=[0.15,0.18).
- E: Between $10.4 > S_{max} > 10.1$, the switching surface rapidly falls indicating that the system can switch for smaller values of R_0 for given $\Delta\tau_{crit}$ and ever so small increase in S_{max}, finally having the ability to switch at $R_0 = 0$.
- F: This flat region indicates that for $R_0 = 0$, the system can switch for a wide range of an area spanned by $S_{max} \in [10.4, 50]$ and $\Delta\tau_{crit} \in [1,15]$.

Critical Signal Duration

- The original system of differential equations cannot be solved analytically due to the high nonlinear characteristics of the Goldbeter-Koshland function *G(•)*
- Taylor expansion to approximate the enzyme concentration *E(R)* [4]

$$\begin{aligned}E^*(R) &= \frac{J_4}{1+J_3}\frac{k_3}{k_4}R + \frac{1+J_3+J_3J_4}{(1+J_3)^3}\frac{k_3^2}{k_4^2}R^2 + \mathcal{O}(R^3)\\ &= C_1\,R + C_2\,R^2 + \mathcal{O}(R^3),\end{aligned}$$

- Linear approximation with respect to response component *R*
- Positive feedback decreases the degradation of *R*
- Linear differential equation

$$\frac{dR}{dt} = k_1\,S_{\max} - (k_2 - k_0\,C_1)\,R$$

- Analytical solution can be transformed with respect to critical signal duration [4]
- Decreased degradation rate constant k_2' and displacements between initial state R_0 and stable steady state R_{SS} and unstable state R_{UN}, respectively, determines the critical duration

$$\Delta\tau_{\min} = \frac{1}{k_2'}\ln\frac{R_{SS} - R_0}{R_{SS} - R_{UN}} \qquad k_2' = k_2 - k_0\,C_1$$
$$R_{SS} = k_1 S_{\max}/k_2'$$

- Good agreement for high stimuli, Fig. 8
- Deviations for small supercritical stimuli because of neglect of higher feedback terms in the above expansion
- Asymptote for strong stimuli $S_{max} \gg k_2'\,R_{UN}/k_1$

$$\Delta\tau_{\min} \approx \frac{R_{UN} - R_0}{k_1 S_{\max}}$$

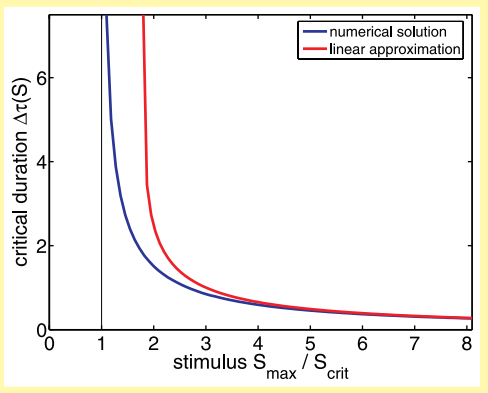

Fig. 8: Critical duration $\Delta\tau_{min}$ as a function of the stimulus amplitude S_{max}. Comparison of the results of a numerical calculation with the linear approximation.

Critical Signal Power

- Signal power (dosage) sums over the whole applied external stimulus

$$P = \int_0^\infty S(t)\,dt$$

- P_{crit} measures the dosage which is required to switch the system from a lower to a upper stable state applying a constant stimulus S_{max}

$$P_{crit} = S_{\max} \cdot \Delta\tau_{crit}$$

- Linear approximation and numerical results agree for high stimuli, Fig. 9
- Minimal dosage P_{min} is required for switching the system
- For strong stimuli it follows

$$P_{\min} = \frac{R_{UN} - R_0}{k_1}$$

  - independent from the signal strength
  - determined only by rate coefficient k_1, but independent from k_2' which describes the relaxation of the system

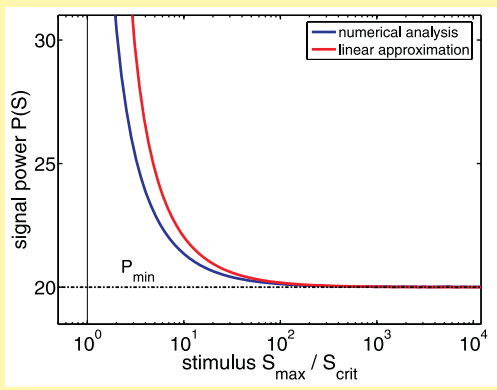

Fig. 9: Semilogarithmic plot of P_{crit} as a function of the stimulus amplitude S_{max} for the nonlinear system and the linear approximation. As pointed out in the text, there is a minimal signal power required to switch the system from the lower to the upper branch. Using the asymptotic behaviour for strong stimuli this limit can be evaluated. Interestingly it is independent from the feedback strength.

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