Stability and noise in biochemical switches

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Abstract

Many processes in biology, from the regulation of gene expression in bacteria to memory in the brain, involve switches constructed from networks of biochemical reactions. Crucial molecules are present in small numbers, raising questions about noise and stability. Analysis of noise in simple reaction schemes indicates that switches stable for years and switchable in milliseconds can be built from fewer than one hundred molecules. Prospects for direct tests of this prediction, as well as implications, are discussed.

1 Introduction

The problem of building a reliable switch arises in several different biological contexts. The classical example is the switching on and off of gene expression during development [1], or in simpler systems such as phage λ [2]. It is likely that the cell cycle should also be viewed as a sequence of switching events among discrete states, rather than as a continuously running clock [3]. The stable switching of a specific class of kinase molecules between active and inactive states is believed to play a role in synaptic plasticity, and by implication in the maintenance of stored memories [4]. Although many details of mechanism remain to be discovered, these systems seem to have several common features. First, the stable states of the switches are dissipative, so that they reflect a balance among competing biochemical reactions. Second, the total number of molecules involved in the construction of the switch is not large. Finally, the switch, once flipped, must be stable for a time long compared to the switching time, perhaps—for development and for memory—even for a time comparable to the life of the organism. Intuitively we might expect that systems with small numbers of molecules would be subject to noise and instability [5], and while this is true we shall see that extremely stable biochemical switches can in fact be built from a few tens of molecules. This has interesting implications for how we think about several cellular processes, and should be testable directly.

Many biological molecules can exist in multiple states, and biochemical switches use this molecular multistability so that the state of the switch can be 'read out' by sampling the states (or enzymatic activities) of individual molecules. Nonetheless, these biochemical switches are based on a network of reactions, with stable states that are collective properties of the network dynamics and not of any individual molecule. Most previous work on the properties of biochemical reaction networks has involved detailed simulation of particular kinetic schemes [6], for example in discussing the kinase switch that is involved in synaptic plasticity [7]. Even the problem of noise has been discussed heuristically in this context [8]. The goal in

the present analysis is to separate the problem of noise and stability from other issues, and to see if it is possible to make some general statements about the limits to stability in switches built from a small number of molecules. This effort should be seen as being in the same spirit as recent work on bacterial chemotaxis, where the goal was to understand how certain features of the computations involved in signal processing can emerge robustly from the network of biochemical reactions, independent of kinetic details [9].

2 Stochastic kinetic equations

Imagine that we write down the kinetic equations for some set of biochemical reactions which describe the putative switch. Now let us assume that most of the reactions are fast, so that there is a single molecular species whose concentration varies more slowly than all the others. Then the dynamics of the switch essentially are one dimensional, and this simplification allows a complete discussion using standard analytical methods. In particular, in this limit there are general bounds on the stability of switches, and these bounds are independent of (incompletely known) details in the biochemical kinetics. It should be possible to make progress on multidimensional versions of the problem, but the point here is to show that there exists a limit in which stable switches can be built from small numbers of molecules.

Let the number of molecules of the 'slow species' be n. All the different reactions can be broken into two classes: the synthesis of the slow species at a rate $f(n)$ molecules per second, and its degradation at a rate $g(n)$ molecules per second; the dependencies on n can be complicated because they include the effects of all other species in the system. Then, if we could neglect fluctuations, we would write the effective kinetic equation

$$\frac{dn}{dt} = f(n) - g(n). \tag{1}$$

If the system is to function as a switch, then the stationarity condition $f(n) = g(n)$ must have multiple solutions with appropriate local stability properties.

The fact that molecules are discrete units means that we need to give the chemical kinetic Eq. (1) another interpretation. It is the mean field approximation to a stochastic process in which there is a probability per unit time $f(n)$ of making the transition $n \to n+1$, and a probability per unit time $g(n)$ of the opposite transition $n \to n-1$. Thus if we consider the probability $P(n,t)$ for there being n molecules at time t, this distribution obeys the evolution (or 'master') equation

$$\frac{\partial P(n,t)}{\partial t} = f(n-1)P(n-1,t) + g(n+1)P(n+1,t) - [f(n)+g(n)]P(n,t), \tag{2}$$

with obvious corrections for $n = 0, 1$. We are interested in the effects of stochasticity for n not too small. Then 1 is small compared with typical values of n, and we can approximate $P(n,t)$ as being a smooth function of n. We can expand Eq. (2) in derivatives of the distribution, and keep the leading terms:

$$\frac{\partial P(n,t)}{\partial t} = \frac{\partial}{\partial n} \left\{ [g(n) - f(n)]P(n,t) + \frac{1}{2}\frac{\partial}{\partial n}[f(n) + g(n)]P(n,t) \right\}. \tag{3}$$

This is analogous to the diffusion equation for a particle moving in a potential, but this analogy works only if allow the effective temperature to vary with the position of the particle.

As with diffusion or Brownian motion, there is an alternative to the diffusion equation for $P(n,t)$ and this is to write an equation of motion for $n(t)$ which supplements

Eq. (1) by the addition of a random or Langevin force $\xi(t)$:

$$\frac{dn}{dt} = f(n) - g(n) + \xi(t),\tag{4}$$

$$\langle \xi(t)\xi(t') \rangle = [f(n) + g(n)]\delta(t - t').\tag{5}$$

From the Langevin equation we can also develop the distribution functional for the probability of trajectories $n(t)$. It should be emphasized that all of these approaches are equivalent provided that we are careful to treat the spatial variations of the effective temperature [10].[1] In one dimension this complication does not impede solving the problem. For any particular kinetic scheme we can compute the effective potential and temperature, and kinetic schemes with multiple stable states correspond to potential functions with multiple minima.

3 Noise induced switching rates

We want to know how the noise term destabilizes the distinct stable states of the switch. If the noise is small, then by analogy with thermal noise we expect that there will be some small jitter around the stable states, but also some rate of spontaneous jumping between the states, analogous to thermal activation over an energy barrier as in a chemical reaction. This jumping rate should be the product of an "attempt frequency"—of order the relaxation rate in the neighborhood of one stable state—and a "Boltzmann factor" that expresses the exponentially small probability of going over the barrier. For ordinary chemical reactions this Boltzmann factor is just $\exp(-F^\dagger/k_BT)$, where $F^\dagger$ is the activation free energy. If we want to build a switch that can be stable for a time much longer than the switching time itself, then the Boltzmann factor has to provide this large ratio of time scales.

There are several ways to calculate the analog of the Boltzmann factor for the dynamics in Eq. (4). The first step is to make more explicit the analogy with Brownian motion and thermal activation. Recall that Brownian motion of an over-damped particle is described by the Langevin equation

$$\gamma\frac{dx}{dt} = -V'(x) + \eta(t),\tag{6}$$

where γ is drag coefficient of the particle, $V(x)$ is the potential, and the noise force has correlations $\langle \eta(t)\eta(t') \rangle = 2\gamma T\delta(t - t')$, where T is the absolute temperature measured in energy units so that Boltzmann's constant is equal to one. Comparing with Eq. (4), we see that our problem is equivalent to a particle with $\gamma = 1$ in an effective potential $V_{\mathrm{eff}}(n)$ such that $V'_{\mathrm{eff}}(n) = g(n) - f(n)$, at an effective temperature $T_{\mathrm{eff}}(n) = [f(n) + g(n)]/2$.

If the temperature were uniform then the equilibrium distribution of n would be $P_{\mathrm{eq}}(n) \propto \exp[-V_{\mathrm{eff}}(n)/T_{\mathrm{eff}}]$. With nonuniform temperature the result is (up to

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[1]In a review written for a biological audience, McAdams and Arkin [11] state that Langevin methods are unsound and can yield invalid predictions precisely for the case of bistable reaction systems which interests us here; this is part of their argument for the necessity of stochastic simulation methods as opposed to analytic approaches. Their reference for the failure of Langevin methods [12], however, seems to consider only Langevin terms with constant spectral density, thus ignoring (in the present language) the spatial variations of effective temperature. For the present problem this would mean replacing the noise correlation function $[f(n) + g(n)]\delta(t - t')$ in Eq. (5) by $Q\delta(t - t')$ where Q is a constant. This indeed is wrong, and is not equivalent to the master equation. On the other hand, if the arguments of Refs. [11, 12] were generally correct, they would imply that Langevin methods could not used for the description of Brownian motion with a spatially varying temperature, and this would be quite a surprise.

weakly varying prefactors)

$$P_{\text{eq}}(n) \quad \propto \quad \exp[-U(n)] \tag{7}$$

$$U(n) \quad = \quad \int_0^n dy \frac{V'_{\text{eff}}(y)}{T_{\text{eff}}(y)}. \tag{8}$$

One way to identify the Boltzmann factor for spontaneous switching is then to compute the relative equilibrium occupancy of the stable states (n_0 and n_1) and the unstable "transition state" at n_*. The result is that the effective activation energy for transitions from a stable state at $n = n_0$ to the stable state at $n = n_1 > n_0$ is

$$F^\dagger(n_0 \to n_1) = 2k_B T \int_{n_0}^{n_*} dn \frac{g(n) - f(n)}{g(n) + f(n)}, \tag{9}$$

where n_* is the unstable point, and similarly for the reverse transition,

$$F^\dagger(n_1 \to n_0) = 2k_B T \int_{n_*}^{n_1} dn \frac{f(n) - g(n)}{g(n) + f(n)}. \tag{10}$$

An alternative approach is to note that the distribution of trajectories $n(t)$ includes locally optimal paths that carry the system from each stable point up to the transition state; the effective activation free energy can then be written as an integral along these optimal paths. The use of optimal path ideas in chemical kinetics has a long history, going back at least to Onsager. A discussion in the spirit of the present one is Ref. [13]. For equations of the general form

$$\frac{dn}{dt} = -V'_{\text{eff}}(n) + \xi(t), \tag{11}$$

with $\langle \xi(t)\xi(t') \rangle = 2T_{\text{eff}}(t)\delta(t-t')$, the probability distribution for trajectories $P[n(t)]$ can be written as [10]

$$P[n(t)] \quad = \quad \exp\left(-S[n(t)]\right) \tag{12}$$

$$S[n(t)] \quad = \quad \frac{1}{4}\int dt \frac{1}{T_{\text{eff}}(t)}[\dot{n}(t) + V'_{\text{eff}}(n(t))]^2 - \frac{1}{2}\int dt V''_{\text{eff}}(n(t)). \tag{13}$$

If the temperature T_{eff} is small, then the trajectories that minimize the action should be determined primarily by minimizing the first term in Eq. (13), which is $\sim 1/T_{\text{eff}}$. Identifying the effective potential and temperature as above, the relevant term is

$$\frac{1}{2}\int dt \frac{[\dot{n} - f(n) + g(n)]^2}{f(n) + g(n)} \quad = \quad \frac{1}{2}\int dt \frac{\dot{n}^2}{f(n) + g(n)} + \frac{1}{2}\int dt \frac{[f(n) - g(n)]^2}{f(n) + g(n)}$$
$$- \int dt\dot{n}\frac{f(n) - g(n)}{f(n) + g(n)}. \tag{14}$$

We are searching for trajectories which take $n(t)$ from a stable point n_0 where $f(n_0) = g(n_0)$ through the unstable point n_* where f and g are again equal but the derivative of their difference (the curvature of the potential) has changed sign. For a discussion of the analogous quantum mechanical problem of tunneling in a double well, see Ref. [14]. First we note that along any trajectory from n_0 to n_* we can simplify the third term in Eq. (14):

$$\int dt\dot{n}\frac{f(n) - g(n)}{f(n) + g(n)} = \int_{n_0}^{n_*} dn \frac{f(n) - g(n)}{f(n) + g(n)}. \tag{15}$$

This term thus depends on the endpoints of the trajectory and not on the path, and therefore cannot contribute to the structure of the optimal path. In the analogy to mechanics, the first two terms are equivalent to the (Euclidean) action for a particle with position dependent mass in a potential; this means that along extremal trajectories there is a conserved energy

$$E = \frac{1}{2}\frac{\dot{n}^2}{f(n)+g(n)} - \frac{1}{2}\frac{[f(n)-g(n)]^2}{f(n)+g(n)}. \tag{16}$$

At the endpoints of the trajectory we have $\dot{n} = 0$ and $f(n) = g(n)$, and so we are looking for zero energy trajectories, along which

$$\dot{n}(t) = \pm[f(n(t)) - g(n(t))]. \tag{17}$$

Substituting back into Eq. (14), and being careful about the signs, we find once again Eq's. (9,10).

Both the 'transition state' and the optimal path method involve approximations, but if the noise is not too large the approximations are good and the results of the two methods agree. Yet another approach is to solve the master equation (2) directly, and again one gets the same answer for the switching rate when the noise is small, as expected since all the different approaches are all equivalent if we make consistent approximations. It is much more work to find the prefactors of the rates, but we are concerned here with orders of magnitude, and hence the prefactors aren't so important.

4   Interpretation

The crucial thing to notice in this calculation is that the integrands in Eq's. (9,10) are bounded by one, so the activation energy (in units of the thermal energy $k_B T$) is bounded by twice the change in the number of molecules. Translating back to the spontaneous switching rates, the result is that the noise driven switching time is longer than the relaxation time after switching by a factor that is bounded,

$$\frac{\text{spontaneous switching time}}{\text{relaxation time}} < \exp(\Delta n), \tag{18}$$

where Δn is the change in the number of molecules required to go from one stable 'switched' state to the other. Imagine that we have a reaction scheme in which the difference between the two stable states corresponds to roughly 25 molecules. Then it is possible to have a Boltzmann factor of up to $\exp(25) \sim 10^{10}$. Usually we think of this as a limit to stability: with 25 molecules we can have a Boltzmann factor of no more than $\sim 10^{10}$. But here I want to emphasize the positive statement that there exist kinetic schemes in which just 25 molecules would be sufficient to have this level of stability. This corresponds to years per millisecond: with twenty five molecules, a biochemical switch that can flip in milliseconds can be stable for years. Real chemical reaction schemes will not saturate this bound, but certainly such stability is possible with roughly 100 molecules. The genetic switch in λ phage operates with roughly 100 copies of the repressor molecules, and even in this simple system there is extreme stability: the genetic switch is flipped spontaneously only once in 10^5 generations of the host bacterium [2]. Kinetic schemes with greater cooperativity get closer to the bound, achieving greater stability for the same number of molecules.

In electronics, the construction of digital elements provides insulation against fluctuations on a microscopic scale and allows a separation between the logical and physical design of a large system. We see that, once a cell has access to several tens of molecules, it is possible to construct 'digital' switch elements with dynamics that are no longer significantly affected by microscopic fluctuations. Furthermore, weak

interactions of these molecules with other cellular components cannot change the basic 'states' of the switch, although these interactions can couple state changes to other events.

The importance of this 'digitization' on the scale of $10 - 100$ molecules is illustrated by different models for pattern formation in development. In the classical model due to Turing, patterns are expressed by spatial variations in the concentration of different molecules, and patterns arise because uniform concentrations are rendered unstable through the combination of nonlinearities in the kinetics with the different diffusion constants of different substances. In this picture, the spatial structure of the pattern is linked directly to physical properties of the molecules. An alternative that each spatial location is labelled by a set of discrete possible states, and patterns evolve out of the 'automaton' rules by which each location changes state in relation to the neighboring states. In this picture states and rules are more abstract, and the dynamics of pattern formation is really at a different level of description from the molecular dynamics of chemical reactions and diffusion. Reliable implementations of automaton rules apparently are accessible as soon as the relevant chemical reactions involve a few dozen molecules.

Biochemical switches have been reconstituted in vitro, but I am not aware of any attempts to verify that stable switching is possible with small numbers of molecules. It would be most interesting to study model systems in which one could confine and monitor sufficiently few molecules that it becomes possible to observe spontaneous switching, that is the breakdown of stability. Although genetic switches have certain advantages, even the simplest systems would require full enzymatic apparatus for gene expression (but see Ref. [16] for recent progress on controllable in vitro expression systems).[2] Kinase switches are much simpler, since they can be constructed from just a few proteins and can be triggered by calcium; caged calcium allows for an optical pulse to serve as input.

At reasonable protein concentrations, $10 - 100$ molecules are found in a volume of roughly 1 $(\mu m)^3$. Thus it should be possible to fabricate an array of 'cells' with linear dimensions ranging from 100 nm to 10 μm, such that solutions of kinase and accessory proteins would switch stably in the larger cells but exhibit instability and spontaneous switching in the smaller cells. The state of the switch could be read out by including marker proteins that would serve as substrates of the kinase but have, for example, fluorescence lines that are shifted by phosphorylation, or by having fluorescent probes on the kinase itself; transitions of single enzyme molecules should be observable [15].

A related idea would be to construct vesicles containing ligand gate ion channels which can conduct calcium, and then have inside the vesicle enzymes for synthesis and degradation of the ligand which are calcium sensitive. The cGMP channels of rod photoreceptors are an example, and in rods the cyclase synthesizing cGMP is calcium sensitive, but the sign is wrong to make a switch [17]; presumably this could solved by appropriate mixing and matching of protein components from different cells. In such a vesicle the different stable states would be distinguished by different

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[2]Note also that reactions involving polymer synthesis (mRNA from DNA or protein from mRNA) are not 'elementary' reactions in the sense described by Eq. (2). Synthesis of a single mRNA molecule involves thousands of steps, each of which occurs (conditionally) at constant probability per unit time, and so the noise in the overall synthesis reaction is very different. If the synthesis enzymes are highly processive, so that the polymerization apparatus incoporates many monomers into the polymer before 'backing up' or falling off the template, then synthesis itself involves a delay but relatively little noise; the dominant source of noise becomes the assembly and disassembly of the polymerization complex. Thus there is some subtlety in trying to relate a simple model to the complex sequence of reactions involved in gene expression. On the other hand a detailed simulation is problematic, since there are so many different elementary steps with unknown rates. This combination of circumstances would make experiments on a minimal, in vitro genetic switch espcially interesting.

levels of internal calcium (as with adaptation states in the rod), and these could be read out optically using calcium indicators; caged calcium would again provide an optical input to flip the switch. Amusingly, a close packed array of such vesicles with ~ 100 nm dimension would provide an optically addressable and writable memory with storage density comparable to current RAM, albeit with much slower switching.

In summary, it should be possible to build stable biochemical switches from a few tens of molecules, and it seems likely that nature makes use of these. To test our understanding of stability we have to construct systems which cross the threshold for observable instabilities, and this seems accessible experimentally in several systems.

Acknowledgments

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