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Zero-order ultrasensitivity: A study of criticality and fluctuations under the total quasi-steady state approximation in the linear noise regime

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HIGHLIGHTS

- A push–pull enzyme–substrate system is ultrasensitive under enzyme saturation.
- Deterministic chemical rate equations are inadequate for small substrate populations.
- We adopt a probabilistic approach, starting from master equation.
- Fluctuations are estimated within the linear noise approximation.
- Analytical results are supported by stochastic simulations.

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ABSTRACT

Zero-order ultrasensitivity (ZOU) is a long known and interesting phenomenon in enzyme networks. Here, a substrate is reversibly modified by two antagonistic enzymes (a 'push–pull' system) and the fraction in modified state undergoes a sharp switching from near-zero to near-unity at a critical value of the ratio of the enzyme concentrations, under saturation conditions. ZOU and its extensions have been studied for several decades now, ever since the seminal paper of Goldbeter and Koshland (1981); however, a complete probabilistic treatment, important for the study of fluctuations in finite populations, is still lacking. In this paper, we study ZOU using a modular approach, akin to the total quasi-steady state approximation (tQSSA). This approach leads to a set of Fokker–Planck (drift–diffusion) equations for the probability distributions of the intermediate enzyme-bound complexes, as well as the modified/unmodified fractions of substrate molecules. We obtain explicit expressions for various average fractions and their fluctuations in the linear noise approximation (LNA). The emergence of a 'critical point' for the switching transition is rigorously established. New analytical results are derived for the average and variance of the fractional substrate concentration in various chemical states in the near-critical regime. For the total fraction in the modified state, the variance is shown to be a maximum near the critical point and decays algebraically away from it, similar to a second-order phase transition. The new analytical results are compared with existing ones as well as detailed numerical simulations using a Gillespie algorithm.

1. Introduction

Goldbeter and Koshland (1981), hereafter referred to as GK first showed that reversible covalent modification (e.g. phosphorylation or methylation) of a protein (substrate), catalyzed by two enzymes, contains within it a mechanism equivalent to a molecular switch. This switch-like behavior emerges in the limit where the substrate concentration far exceeds the enzyme concentrations as well as their individual Michaelis constants, as a consequence of which the enzymes work in the 'zero-order' regime. In this regime, the net modification and de-modification rates, predicted by standard Michaelis–Menten kinetic equations, become effectively independent of concentration (hence called 'zero-order', as opposed to the first order regime, where the rates depend linearly on concentrations). The chemical equilibrium condition (which translates to a quadratic equation for the modified fraction when intermediates are neglected, and a cubic equation when they are not) predicts that the fraction of substrate in modified state is either none or all, in the limit of large substrate concentrations. Specifically, the solution of this equation displays the switch-like behavior described above as a function of the ratio $\alpha \equiv \nu_r R_0/\nu_b B_0$, where R_0 and B_0 are the enzyme concentrations and ν_r and ν_b their conversion rates. The 'critical point' of this transition was shown to be at $\alpha = 1$, (hereafter referred to as the GK point) independent of the ratio of the Michaelis constants of the enzymes. (Note: Throughout this paper, we shall use the words

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critical point and criticality in connection with ZOU although, despite many similarities, it is not a thermodynamic phase transition in the strict sense).

The GK switch was studied in more detail by some authors (e.g., Berg et al., 2000; Qian, 2003; Elf and Ehrenberg, 2003; Blüthgen et al., 2006; Ciliberto et al., 2007; Gomez-Uribe et al., 2007; Ge and Qian, 2008; Pedersen and Bersani, 2010; Xu and Gunawardena, 2012) and also extended in scope by others (Ortega et al., 2002; Samoilov et al., 2005; van Albada and ten Wolde, 2007; Szomolay and Shahrezaei, 2012) in more recent times. Notably, Berg et al. (2000) and later, Elf and Ehrenberg (2003) studied the fluctuations in the ultrasensitive module within some approximations (see discussions later) while Qian (2003), and later Ge and Qian (2008), identified ZOU as a temporal cooperativity phenomenon, mathematically similar to the better known allosteric cooperativity. In connection with ZOU, Blüthgen et al. (2006), Ciliberto et al. (2007) and Pedersen and Bersani (2010) showed that the total quasi-steady state approximation (tQSSA), introduced by Borghans et al. (1996) and studied further by Tzafriri and Edelman (2004) is superior to the Briggs–Haldane standard quasi-steady state approximation (sQSSA) when enzyme and substrate concentrations are comparable, or when the former actually exceeds the latter (whereas in sQSSA, the free substrate concentration is the slow variable, tQSSA replaces it with sum of the concentrations of the free substrate and the intermediate complex).

ZOU has been shown to be relevant in a number of systems (LaPorte and Koshland, 1983; Meinke et al., 1986; Cimino and Hervagault, 1987; Casati et al., 1999; Melen et al., 2005; Kim and Ferrell, 2007). Theoretical studies of the ZOU have, by and large, followed a chemical rate equation based approach, which is of a purely "mean-field" nature, and most authors have ignored fluctuations altogether. However, given the similarity of ZOU to a thermodynamic phase transition, it is natural to expect that biochemical fluctuations will be large near the transition point, an issue addressed in detail by Berg et al. (2000). In their model, a finite number N of substrate molecules were considered, out of which, say, n are in modified state at any given point of time, the probability for which was denoted P_n. The transition rates for the processes $n \leftrightarrow n \pm 1$ were assumed to be of the standard Michaelis–Menten type (derived under the sQSSA), and this, in our opinion, is a weakness of the model. The analytical calculations were restricted to the extreme case of an infinitely large substrate concentration. More recently, Elf and Ehrenberg (2003) obtained estimates for fluctuations in ZOU under the LNA; however, similar to Berg et al.(2000), macroscopic rates derived under sQSSA were used in their calculations. We shall attempt to show, in this paper, how these limitations can be overcome by using a different approach with a more controlled limiting procedure. Wherever relevant, we will also provide comparisons of our results with the older ones.

The principal objective of this work is the construction of a fully stochastic formulation of a two-state covalent modification system showing ZOU, with a complete treatment of fluctuations. For this purpose, we consider the system as consisting of two weakly connected modules, each populated by unmodified and modified substrate molecules respectively, in the spirit of tQSSA (Borghans et al., 1996). Discrete master equations are constructed to describe the dynamics in each, which are then converted to continuum Fokker–Planck equations by second-order truncation of the respective Kramers–Moyal expansions (Gardiner, 2004). A set of well-defined approximations, valid within the assumptions of tQSSA and the requirements of ZOU, then leads to an effective one-dimensional Fokker–Planck equation for the modified substrate fraction (the total population in the second module). Rigorous and elegant mathematical expressions for the averages and fluctuations in the steady state follow in a straightforward manner, which are shown to compare well with the results of detailed numerical simulations, done using a Gillespie algorithm (Gillespie, 1977) The present formalism can be potentially extended to more complex systems like a many-state reversible modification network (e.g., receptor methylation and demethylation in *Escherichia coli*).

2. Model and methods

2.1. Fokker–Planck equations from the master equation

We consider a cell of volume V, which contains N substrate molecules A at total concentration A_0 and two enzymes (which we shall call R and B) with total concentrations R_0 and B_0 respectively. The enzyme R binds to A with association rate k_+ and reversibly converts it to the intermediate state $\tilde{A}$; the backward transition $\tilde{A} \to A + R$ occurs at rate k_-, while $\tilde{A}$ is irreversibly converted to the product (modified form of A, which we denoteA^*) at rate ν_r. Similarly, A^* reversibly binds to B with rate k'_+ forming the second intermediate complex $\tilde{A}^*$, which dissociates to A^* and B at rate k'_-. The complex $\tilde{A}^*$ is converted back to the original, non-modified form A at a rate ν_b. The reaction scheme is illustrated in Fig. 1. We further define the following dissociation constants for the enzyme–substrate binding: $K_r = k_-/k_+$; $K_b = k'_-/k'_+$. This is the original model studied by Goldbeter and Koshland (1981).

In the limit where the rates ν_r and ν_b are small in comparison with the rates of enzyme binding and dissociation, the above system functions as a combination of two weakly coupled modules, the $A-\tilde{A}$ system (module 1) with $M_1 \equiv N(1-\xi)$ substrate molecules and the $A^*-\tilde{A}^*$ system (module 2) with $M_2 \equiv N\xi$ molecules, where ξ is the total fraction of substrate molecules in module 2. In the limit where the turnover rates ν_r and ν_b are sufficiently small (see more discussions later in Section 3.4), the internal dynamics of these modules occur on a timescale much smaller than the one involving changes in ξ itself; hence we may assume the two modules to be always in their steady states for each ξ. This is the essence of the tQSSA. The regime of validity of this scheme for irreversible Michaelis–Menten kinetics is discussed by Borghans et al. (1996) and its extension to reversible Michaelis–Menten kinetics was carried out by Tzafriri and Edelman (2004).

In module 1, the probability, $P_{M_1}(m_1)$, for m_1 number of molecules to be in $\tilde{A}$ state, satisfies the master equation:

$$\frac{\partial P_{M_1}(m_1,t)}{\partial t} = \omega_+(m_1-1)P_{M_1}(m_1-1) + \omega_-(m_1+1)P_{M_1}(m_1+1) - [\omega_+(m_1)+\omega_-(m_1)]P_{M_1}(m_1), \quad (1)$$

with rates ω_+ and ω_- defined as below:

$$\omega_+(m_1) = (M_1 - m_1)k_+ R_f(m_1); \quad \omega_-(m_1) = m_1 k_-, \quad (2)$$

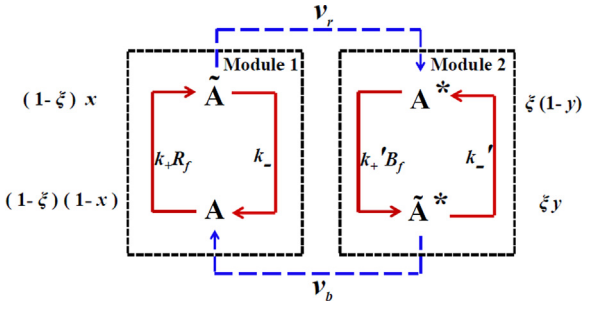

Fig. 1. A schematic diagram depicting the various dynamical processes, as well as the modules in our system. The expressions on the left and right sides denote the fractional concentrations of the corresponding species.