Mesoscopic statistical properties of multistep enzyme-mediated reactions

Andrew Mugler[1], Bryan C. Daniels[2], Wiet H. de Ronde[3], Nikolai A. Sinitsyn[4], and Ilya Nemenman[5]

Short Abstract — We demonstrate how enzymatic reactions with different intermediate reaction schemes can be distinguished on the basis of mesoscopic measurements alone (i.e. measurements of the mean and fluctuations of product molecules). We devise a perturbation theory (analogous to that used in quantum mechanics) for computing arbitrary cumulants of the distribution of product molecules as a function of the substrate concentration and the kinetic rates of the intermediate processes. We apply the theory to four example reaction schemes and outline how qualitative and quantitative differences among results suggest mesoscopic measurements that could distinguish among schemes.

Keywords — enzyme-mediated reactions, master equation, intrinsic noise, perturbation theory.

I. PURPOSE

Although enzyme-mediated reactions are traditionally described as two-step Michaelis-Menten processes, many involve intermediate steps (such as transitions among conformational states, transitions between active and inactive states, etc.). While intermediate states are directly detectable with single-molecule experiments [1], signatures of intermediate states are often present in simpler mesoscopic measurements, such as measurements of the mean flux or fluctuations in the flux of product molecules. Prediction of these signatures requires solving the master equation for each enzymatic reaction scheme. Here we present a perturbative method, analogous to that used in quantum mechanics, for computing from the master equation arbitrary cumulants of the distribution of product molecules. By applying the method to several examples of multistep enzymatic reactions, we demonstrate several qualitative and quantitative ways by which the internal details can be distinguished on the basis of mesoscopic measurements alone.

II. METHOD

We solve the master equation for each reaction scheme by employing the generating function [2]. Since cumulants are obtained by evaluating derivatives of the generating function at vanishing argument, we treat its argument as a small parameter in a perturbation theory analogous to that used in quantum mechanics. The first and second cumulants are then obtained from the first- and second-order eigenvalue corrections under the perturbation theory. The cumulants yield expressions in terms of the substrate concentration and kinetic rates for quantities that are experimentally measurable [3], such as the mean response and Fano factor. The method is extendible to arbitrary higher cumulants and applicable to any multistep enzymatic reaction scheme.

III. FINDINGS

We apply the method to four enzyme-mediated reactions: a simple Michaelis-Menten reaction, a reaction with one intermediate step (such as a conformational change), and two different reactions in which the enzyme switches between an active and an inactive state. We show that while the mean response of product to substrate has the same functional form for these four schemes, distinctions are possible based on measurements of the Fano factor, and we outline qualitative and quantitative features of the predicted expressions that would allow such distinctions to be made experimentally. Additionally, we show that measuring key features of the mean response and Fano factor curves allows one to solve for all three kinetic rates in a Michaelis-Menten process, which suggests an analogous procedure for more complex reactions. We view the presented method as a useful analytical tool for experimentalists wishing to uncover and quantify internal reaction details from bulk measurements.

REFERENCES

[1] English B, Min W, Van Oijen A, Lee KT, Luo G, Sun H, Cherayil B, Kou S, Xie XS (2006) Ever-fluctuating single enzyme molecules: Michaelis-Menten equation revisited. *Nat. Chem. Biol.* **2**, 87–94.

[2] van Kampen NG (1992) Stochastic processes in physics and chemistry. Amsterdam: North-Holland.

[3] Golding I, Paulsson J, Zawilski S, Cox E (2005) Real-time kinetics of gene activity in individual bacteria. *Cell* **123**, 1025-36.