Mesoscopic statistical properties of multistep enzyme-mediated reactions

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Abstract

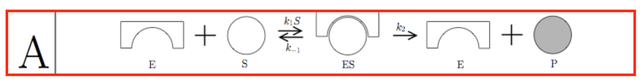
We demonstrate how enzymatic reactions with different intermediate reaction schemes can be distinguished on the basis of mesoscopic measurements alone (i.e. measurements of the mean and fluctuations of product molecules). We devise a perturbation theory (analogous to that used in quantum mechanics) for 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.

Motivation

- Many enzyme-mediated reactions have intermediate steps (e.g. conformational states or active/inactive states).
- While intermediate steps are directly detectable with single-molecule experiments, signatures are often present in simpler mesoscopic measurements (e.g. mean flux or fluctuations in the flux of product molecules).
- We extract such signatures by using a perturbative method to compute from the master equation the mean, variance, and arbitrarily higher cumulants of the product molecule distribution.

Method

We demonstrate the method on the Michaelis-Menten reaction (A).



Master equation (where n is the number of product molecules):

$$\begin{aligned}\dot{P}_n^E &= -k_1 S P_n^E + k_{-1} P_n^{ES} + k_2 P_{n-1}^{ES} \\ \dot{P}_n^{ES} &= k_1 S P_n^E - (k_{-1}+k_2) P_n^{ES}\end{aligned}$$

Or, for generating function $G^z(\chi) = \sum_{n=0}^{\infty} P_n^z e^{i\chi n} \quad (z \in \{E, ES\})$,

$$|\dot{G}\rangle = \hat{H}|G\rangle, \qquad \hat{H} = \begin{pmatrix} -k_1 S & k_{-1} + k_2 e^{i\chi} \\ k_1 S & -(k_{-1}+k_2) \end{pmatrix}$$

For t much longer than enzyme-turnover time,

$$\ln G(\chi) = \ln\langle \hat{1}|G\rangle \approx \lambda_0(\chi) t$$

where λ_0 is the least negative eigenvalue of H.

Expanding $\lambda_0(\chi) = \sum_{m=0}^{\infty} \lambda_0^{(m)} \frac{(i\chi)^m}{m!}$, the first two cumulants are

1. mean number of product molecules:
$$\langle n \rangle = \left.\frac{d(\ln G)}{d(i\chi)}\right|_{\chi=0} = \lambda_0^{(1)} t$$

2. variance of product molecule distribution:
$$\sigma^2 = \left.\frac{d^2(\ln G)}{d(i\chi)^2}\right|_{\chi=0} = \lambda_0^{(2)} t$$

Perturb $\hat{H} = \hat{H}^{(0)} + \hat{H}^{(1)} \sum_{m=1}^{\infty} (i\chi)^m / m!$ in (vanishing) χ,

$$\hat{H}^{(0)} = \begin{pmatrix} -k_1 S & k_{-1}+k_2 \\ k_1 S & -(k_{-1}+k_2) \end{pmatrix}, \quad \hat{H}^{(1)} = k_2 \begin{pmatrix} 0 & 1 \\ 0 & 0 \end{pmatrix}$$

to find $\lambda_0^{(1)}, \lambda_0^{(2)}$, as in quantum mechanics:

$$\begin{aligned}\lambda_0^{(1)} &= \langle u_0^{(0)}|\hat{H}^{(1)}|u_0^{(0)}\rangle, \\ \lambda_0^{(2)} &= \lambda_0^{(1)} - 2\sum_{j\neq 0} \frac{1}{\lambda_j^{(0)}} |\langle u_j^{(0)}|\hat{H}^{(1)}|u_0^{(0)}\rangle|^2\end{aligned}$$

where $|u_j^{(0)}\rangle$ are the eigenfunctions of $H^{(0)}$.

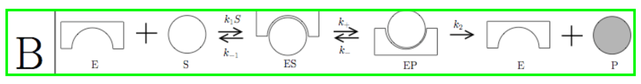
For Michaelis-Menten (A), the mean flux and Fano factor are

$$\begin{aligned}V_A &= \frac{d\langle n\rangle}{dt} = \lambda_0^{(1)} = V_A^{\max} \frac{S}{S+K_A} \\ F_A &= \frac{\sigma^2}{\langle n\rangle} = \frac{\lambda_0^{(2)}}{\lambda_0^{(1)}} = 1 - \alpha_A \frac{S}{(S+K_A)^2}\end{aligned}$$

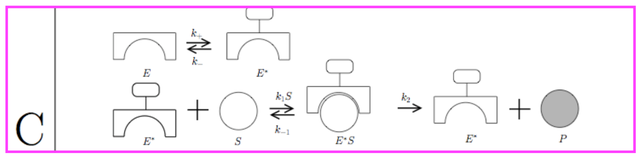
where $V_A^{\max} = k_2, K_A = (k_2+k_{-1})/k_1, \alpha_A = 2k_2/k_1.$

Results

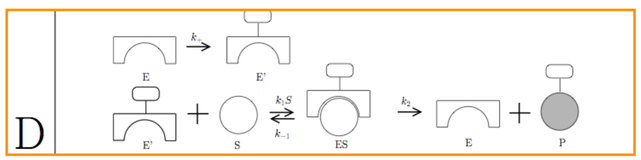
Reaction B: enzyme undergoes intermediate conformational change



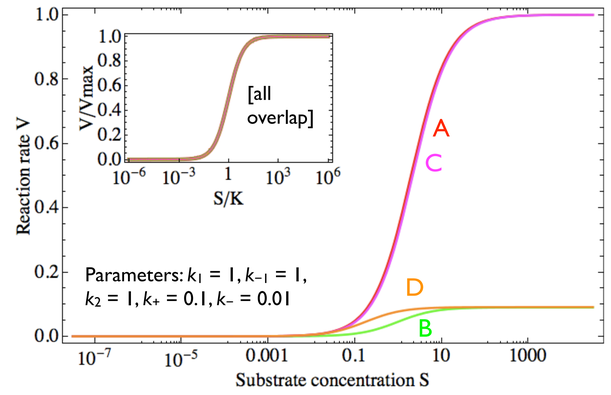
Reaction C: enzyme goes between active and inactive states



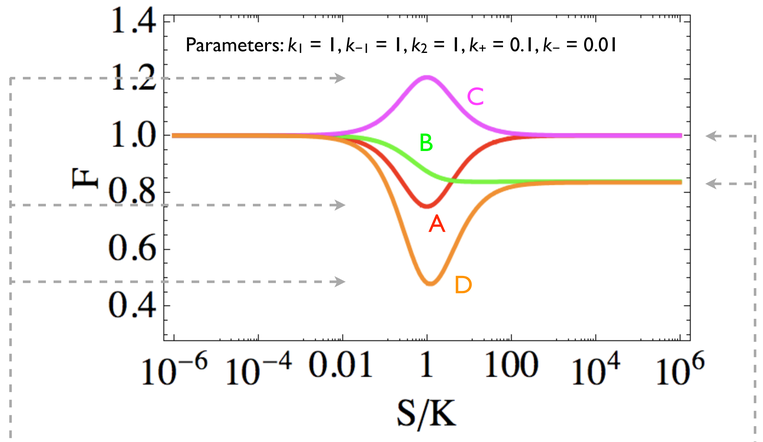
Reaction D: enzyme goes between active and inactive states; product formation returns enzyme to inactive state



All reactions A-D have the same functional form for mean flux V:



Distinctions are possible by measuring the Fano factor F:



Measurable features:

- Value of F at saturation
- Extreme value of F
- Location of extremum

Features are bounded by the reaction scheme:

	A	B	C	D
$F(S\to\infty)$	1	$[\frac{1}{2}, 1]$	1	$[\frac{1}{2}, 1]$
F^*	$[\frac{1}{2}, 1]$	$[\frac{1}{3}, 1]$	$[\frac{1}{2}, \infty)$	$[\frac{1}{3}, 1]$
S^*/K	1	$[1, \infty)$	1	$[1, \infty)$

More information:

Preprint: arxiv.org/abs/0811.3283
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