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Diffusional correlations among multiple active sites in a single enzyme

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Simulations of the enzymatic dynamics of a model enzyme containing multiple substrate binding sites indicate the existence of diffusional correlations in the chemical reactivity of the active sites. A coarsegrain, particle-based, mesoscopic description of the system, comprising the enzyme, the substrate, the product and solvent, is constructed to study these effects. The reactive and non-reactive dynamics is followed using a hybrid scheme that combines molecular dynamics for the enzyme, substrate and product molecules with multiparticle collision dynamics for the solvent. It is found that the reactivity of an individual active site in the multiple-active-site enzyme is reduced substantially, and this effect is analyzed and attributed to diffusive competition for the substrate among the different active sites in the enzyme.

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1 Introduction

The fact that the catalytic activity of enzymes underlies most of the biochemistry in the cell has provided the stimulus for research on the mechanisms and dynamics of enzymatic reactions. Enzymatic reactions are often strongly influenced by diffusion, and diffusion can give rise to power-law time dependence of rate coefficients and concentrations, and lead to modifications of classical mean field Michaelis-Menten (MM) kinetics. An extensive literature exists on this topic (see, for example, ref. 1-7). In systems containing many catalytic particles it is also known that diffusion can give rise to correlations in reactive events that cause reaction rates to depend on the volume fraction of catalytic particles.8 Similarly, simulations of systems containing a high volume fraction of enzymes have shown that diffusive competition among the different enzymes for the substrate can lead to correlations.⁹ If a single enzyme contains multiple active sites it is possible that diffusive coupling among sites could lead to correlations that may cause MM kinetics to break down. The possibility that such correlations could sometimes play a role in enzyme kinetics is the subject of this investigation.

Investigations of enzyme kinetics have been carried out at various levels of description, ranging from very detailed fully-atomistic treatments of the enzyme to simple models where the entire enzyme is represented by a structureless spherical particle. Coarse grain models which are not as detailed as fully atomistic descriptions of an enzyme but still account for the gross structure of the enzyme have also been constructed and studied.^{10–16} The simulations of competition among active sites in a single enzyme described in this paper employ a mesoscopic particle-based description of the enzymatic system. The enzyme is modeled as an elastic network of beads.¹⁷ The substrate, product and solvent molecules are explicitly included in the description, also at a coarse grain level. Consequently, a variety of effects, including structural correlations, fluctuations, diffusion, hydrodynamic interactions and cooperative binding, can be investigated within the context of the model.

The outline of the paper is as follows: in Section 2 we present the mesoscopic model for the entire enzymatic system, the enzyme with multiple active sites, the substrate, the product and solvent, and describe the dynamics that governs evolution of the entire system. Section 3 presents the results of simulations of the dynamics, gives evidence for the existence of correlations in the chemical reactivity, and discusses the applicability of Michaelis–Menten kinetics for this system. The conclusions of the study are given in Section 4.

2 Model for the enzymatic system

Mesoscopic models of an enzymatic system, where the enzyme was represented as a network of beads, the substrate and product molecules were structureless particles that interact with the enzyme through intermolecular potentials, and the substrate, product and chemically inert solvent molecules interacted among themselves by multiparticle collision (MPC) dynamics,^{18,19} have been described earlier.^{14,15} In this investigation we employ a variant of such models where the substrate



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The structural features of our model enzyme are based roughly on 4-oxalocrotonate tautomerase (4-OT), an enzyme which contains multiple binding sites and catalyzes the isomerization of unsaturated ketones.²⁰ This enzyme is a hexamer which is composed of three dimers;²¹ each monomeric enzyme in a dimer has 62 amino acid residues. The entire hexamer has six active sites where catalytic reactions can take place. In our model the 372 amino acid residues in the enzyme are represented by beads connected by elastic network interactions,¹⁷

$$V_{\rm EN}(\mathbf{R}) = \sum_{n=1}^{n_{\rm L}} \frac{1}{2} k \left(R_n - R_n^0 \right)^2, \tag{1}$$

where the sum is over all links in the network, *k* is the common force constant, R_n is the distance between beads in link n, R_n^0 is the equilibrium distance of this link, and n_L is the number of links. Here **R** is the set of all coordinates of the beads in the network. To build the network model, the coordinates **R**⁰ of the α -carbons, taken from the crystal structure of the enzyme,^{22,23} are assigned to the beads in the network. It is then assumed that two beads in the network are connected by a link *n* if their separation $R_n \leq R_{\text{max}}$, with $R_{\text{max}} = 10$ Å. Fig. 1 is a picture of the network model for the enzyme.

The internal structure of the substrate molecules is neglected and they are modeled as structureless particles (beads) which interact with the beads in the enzyme elastic network and with each other through effective intermolecular potentials, which we take to be repulsive Lennard-Jones (LJ) potentials:

$$V_{\rm LJ}(r) = 4\tilde{\varepsilon} \left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^6 + \left(\frac{1}{4}\right) \right] \theta \left(2^{1/6}\sigma - r\right).$$
(2)

Here $\theta(\mathbf{r})$ is the Heaviside function, σ is the bead diameter and $\mathbf{r} = |\mathbf{r}_i - \mathbf{r}_j|$ is the distance between the *i*th and *j*th beads. The energy parameter $\tilde{\varepsilon}$ takes the value ε for substrate–substrate interactions and $\varepsilon_{\rm bs}$ for enzyme bead–substrate interactions.

(If the enzyme bead is a member of an active site, eqn (4) below applies.)

Each active site in the enzyme is defined in terms of four beads²⁴ and intermolecular potentials that control the binding and unbinding of substrates, and release of products can then be constructed to correspond to the standard kinetic scheme:

$$\mathbf{E} + \mathbf{S} \xrightarrow[k_{-1}]{k_{-1}} \mathbf{C} \xrightarrow{k_2} \mathbf{E} + \mathbf{P}, \tag{3}$$

at each active site. Here E, S, C and P denote the enzyme, the substrate, the enzyme–substrate complex and the product, respectively. The rate coefficients are determined by the active site-substrate and product interactions, and the diffusion coefficients of these species.

We may construct a simple binding model for this multipleactive-site enzyme as follows: the four beads comprising an active site in the enzyme have center of mass position \mathbf{r}_{cm} . Letting \mathbf{r}_i denote the position of bead *i* in the active site, its distance from the center of mass is $R_{cm}^{(i)} = |\mathbf{r}_i - \mathbf{r}_{cm}|$. If $\mathbf{r} = \mathbf{r}_S - \mathbf{r}_i$ is the vector distance between a substrate molecule at \mathbf{r}_S and bead *i*, the substrate molecule interacts with active bead *i* through the potential

$$V_{\rm R}(r) = \begin{cases} 4\varepsilon_{\rm bs} \left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^6 + \frac{1}{4} \right], & r \le R_{\rm cm} \\ 0, & R_{\rm cm} < r < R_{\rm e} , \\ \frac{1}{2}k_{\rm C}(r - R_{\rm e})^2, & r \ge R_{\rm e} \end{cases}$$
(4)

where $k_{\rm C}$ is a force constant, $\varepsilon_{\rm bs}$ is the energy parameter introduced above, and the distance $R_{\rm e} = (R_{\rm cm} + d_{\rm R})$. (We drop the superscript (*i*) for notational simplicity.) The parameter $d_{\rm R}$ is used to define a spherical region of radius $d_{\rm R}$ about the center of mass of an active site, which, in turn, is used to specify a criterion for a reaction to take place. In particular, when a substrate molecule arrives within a distance $R_{\rm e}$ of all beads in an active site (see Fig. 2), the harmonic potential in eqn (4) turns on and the substrate binds to the active site. This reaction



Fig. 1 Network model of the enzyme. The reaction volumes of the six active sites, with radius $d_{\rm R} = 0.5$, are shown as large transparent yellow spheres. Amino acid residues are depicted as beads whose colors indicate whether the bead participates in the binding of substrate molecules (pink) or not (cyan).



Fig. 2 Schematic diagram showing four residues or active beads (pink spheres) that define an active site. Spheres with radius d_R surround each of these residues and their overlap is denoted by the region shaded by many small spheres. The center of mass of the four residues is denoted by a yellow sphere and is contained in the shaded region. When a substrate molecule enters this shaded region, reaction to form an enzyme–substrate complex is possible.

criterion leads to binding of the substrate in the vicinity of the center of mass of the four active beads in an active site as shown in the figure.

Fig. 1 also shows the six binding regions where an enzymesubstrate complex may form. The large transparent yellow spheres with radius $d_{\rm R}$ indicate the reaction volumes of the six active sites in the enzyme. The structure of these reaction volumes is shown in more detail in Fig. 2. Binding to the enzyme occurs when the dynamics controlled by enzyme-substrate interactions causes the substrate to reside in the region (R_{cm}, R_e) defined in eqn (4). Once binding to an active site leads to the formation of an enzyme-substrate complex configuration, the substrate may then be released with probability per unit time $p_{\rm B}$. or be converted to the product with probability per unit time $p_{\rm P}$. In the simulations reported below, the reaction parameter $d_{\rm R}$ = 0.5 and the reaction probabilities per unit time are $p_{\rm R} = 0.005$ and $p_{\rm P} = 0.005$. In keeping with our coarse grain description, and consistent with our focus on long scale diffusion effects, this model does not consider the details of the mechanism leading to product formation or substrate release but simply encodes the dynamics of substrate or product release in effective reaction probabilities.

There are no solvent-solvent and bead-solvent interaction potentials. Instead, the effects of these interactions are accounted for by multparticle collisions.^{18,19} In MPC dynamics. particles stream and undergo effective collisions at discrete time intervals τ , accounting for the effects of many real collisions during this time interval. The collisions are carried out by dividing the system into a grid of cells with volumes $\mathscr{V}_{\xi} = a^3$ and assigning rotation operators $\hat{\omega}_{\xi}$, chosen from some sets of rotation operators, to each cell of the system at the time of collision. Particles within each cell "collide" with each other and the post-collision velocity of particle *i* in a cell ξ is given by $\mathbf{v}_i' =$ $\mathbf{V}_{\varepsilon} + \hat{\omega}_{\varepsilon}(\mathbf{v}_i - \mathbf{V}_{\varepsilon})$, where \mathbf{V}_{ε} is the center of mass velocity of particles in the cell and $\hat{\omega}_{\xi}$ is the rotation operator of the cell ξ .²⁵ The MD-MPC dynamics satisfies mass, momentum, and energy conservation laws.^{26,27} This model accounts for thermal fluctuations and the conservation laws guarantee that hydrodynamic flow effects are correctly captured by the dynamics.

Finally, in order to maintain the system in a nonequilibrium state, product molecules are removed from the system and the substrate is added when product molecules reach a distance of L/4 from the center of mass of the enzyme, where *L* is the length of simulation box. This condition is equivalent to the addition of the reaction $P \xrightarrow{k_3} S$ at the spherical boundary with radius L/4 and implies that $[S_0] = [S] + [P]$ in the simulation volume.

3 Simulation of enzymatic dynamics

The simulations of the enzymatic reaction dynamics were carried out in a cubic box with linear dimension L = 30 containing a single enzyme, 280 substrate molecules (initial number density $n_s = [S_0] = 0.01$) and approximately 297 000 solvent molecules (average number density of $n_s = 11$). The MPC time is $\tau = 0.1$ in all simulations. Periodic boundary conditions

were employed in the simulations. Results are reported in dimensionless units. $^{\rm 28}$

Based on eqn (3), the rate of production of the product is $v(t) = d[P](t)/dt = k_2[C]$, and the maximum production rate is $v_{\text{max}} = k_2[E_0]$, where $[E_0]$ is the initial concentration of enzyme. Thus, since there is one enzyme in the system, the ratio $v(t)/v_{\text{max}} = \langle N_C(t) \rangle$ is the average number of enzyme–substrate complexes in the system at time *t*, where the angle brackets signify an average over realizations.

Fig. 3 (top) plots $\bar{N}_{\rm C}(t) = \langle N_{\rm C}(t) \rangle / n_{\rm A}$ versus time, where $n_{\rm A}$ is the number of active sites in the enzyme, for two cases: only one catalytic site in the enzyme is active, and all six catalytic sites are active. The two curves are not the same and the fact that they do not superpose is indicative of correlations in the enzymatic activity. If only one catalytic site in the enzyme is active, it has approximately 1.25 times the catalytic activity of a site in the model hexameric enzyme. Since our model for substrate binding does not include conformational changes upon binding, which could influence binding at nearby active sites, the correlations we observe are most likely due to diffusive competition for the available substrate when all six sites are active. Binding at one active site reduces the number of available



Fig. 3 Plots of the average number of enzyme–substrate complexes per enzyme active site, $\bar{N}_{\rm C}(t) = \langle N_{\rm C}(t) \rangle / n_{\rm A}$ versus time. The two curves in the plots are: single active catalytic site ($n_{\rm A} = 1$; black lines) and six active catalytic sites ($n_{\rm A} = 6$; blue lines). The top panel is for a system with enzyme–substrate interactions, while the bottom panel is for a system with no enzyme–substrate interactions. The results were computed from averages over 1200 realizations. The dashed line is a fit of eqn (5) to the simulation data for a single active site using k_1^0 as the only fitting parameter.

substrate molecules in its vicinity and this will affect binding to nearby active sites.

The existence of correlations in the enzymatic activity of the catalytic sites can be seen in the structure of the probability distributions $P_s(t_L)$ and $P_d(t_L)$ of the lifetimes t_L for a substrate which is released from an active site in the enzyme–substrate complex to rebind to the same and different active sites, respectively, in the enzyme. Fig. 4 plots histograms corresponding to $P_s(t_L)$ (upper panel) and $P_d(t_L)$ (lower panel). Once released from an active site the probability of binding to a different active site is substantial and, as expected, at short times, decays more slowly than that for rebinding to the same active site. In addition both $P_s(t_L)$ and $P_d(t_L)$ decay as $t_L^{-3/2}$ at very long times as expected for a diffusive process.^{29,30} Thus, long time scale correlations exist in the substrate binding events on the various active sites in the enzyme.

Interactions of the enzyme with substrate and product molecules can modify the environment around the active sites. Some of these structures are evident in the radial function distributions, $g^{\mathbb{R}}(r)$ and $g^{\mathbb{P}}(r)$, where *r* is the radial distance from the center of mass of the enzyme to substrate (R) and product (P) molecules, respectively, which are shown in Fig. 5. Results are presented for situations where all six binding sites in the enzyme are active (blue lines) and also when only a single



Fig. 4 Histogram plots of the (unnormalized) probability distributions $P_s(t_L)$ and $P_d(t_L)$ of times taken for a substrate released from an active site in the enzyme-substrate complex to rebind to same (top panel) and different (bottom panel) active sites in the enzyme. The insets present log-log plots showing the long-time power-law behavior. The results were computed from averages over 1200 realizations.



Fig. 5 Radial distribution functions, $g^{R}(r)$ and $g^{P}(r)$, as function of the radial distance *r* from the center of mass of protein to substrate (R) or product (P) molecules, respectively, for enzymes with $n_{A} = 6$ (solid blue lines) and $n_{A} = 1$ (solid black lines). For comparison, these radial distribution functions are also shown for systems with no protein bead–substrate interactions (dashed lines). The results were computed from averages over 1200 realizations.

binding site is active (black lines). As expected, interactions of the substrate or product molecules with enzyme beads hinder penetration into the interior of the enzyme. The sharp fall in substrate density at $r \approx 2.2$ can serve to define an effective radius $R_{\rm p}$ of the enzyme. The peak at $r \approx 1.35$ corresponds to binding of the substrate at the active sites, which are located at approximately this distance from the center of mass of the enzyme. The increase in substrate and product density at small values of r reflects the fact that the hollow channel region in the interior of the barrel-shaped enzyme (see Fig. 1) contains these species. When only a single binding site is active, there is a large concentration of substrate in this channel; by contrast, when all six binding sites are active there is a strong depletion of the substrate and the channel contains a high concentration of product. From the structural information on g(r) we can conclude that trajectories leading to substrate binding will experience a highly heterogeneous environment. This feature precludes a simple analysis of trajectories leading to binding: substrate binding is influenced by enzyme structural effects and diffusion is a strong function of the environment since trajectories explore the bulk solution as well as the enzyme interior.

Insight into the correlations among active site reactivity can be gained by comparison of the above results with those for a modification of the model where the enzyme–substrate interactions are removed, while still retaining binding interactions with the active site beads. The plots of $g^{\rm R}(r)$ and $g^{\rm P}(r)$ for this case are given in Fig. 5 and the lack of structure is evident. For this model the substrate molecules can freely diffuse through the enzyme to reach the active sites. Fig. 3 (bottom) plots $\bar{N}_{\rm C}(t) =$ $\langle N_{\rm C}(t) \rangle / n_{\rm A}$ versus time, for the same two cases as in Fig. 3 (top), except now without enzyme–substrate interactions. Once again, even without such interactions, clear evidence of correlations is seen since the curves do not superpose.

If only a single catalytic site is active, the simple model without interactions reduces to that of a roughly spherical enzyme with a radius approximately equal to $d_{\rm R}$, the size of an active site. In such a situation MM kinetics (modified to account for diffusion-influenced reaction rates³¹) should provide an adequate description of the time evolution of the concentrations, apart from long-time power-law behavior arising from the diffusion of substrate molecules. Solution of the mean field rate law corresponding to the MM kinetic scheme, using a steady state approximation on the rate of complex formation and the assumption³² that $[S] \approx [S_0] \gg [E_0]$, yields the familiar expression for $\bar{N}_{\rm C}(t)$:

$$\bar{N}_{\rm C}(t) = (N_{\rm C})_{\rm ss} (1 - e^{-(k_1 [S_0] + k_{-1} + k_2)t}).$$
(5)

In the steady state the average number of complexes is $(N_{\rm C})_{\rm ss} = [S_0]/(K_{\rm M} + [S_0])$ with the diffusion-modified Michaelis constant³¹ $K_{\rm M} = K_{\rm M}^0 + k_2/k_{\rm D}$ where $K_{\rm M}^0 = (k_{-1}^0 + k_2)/k_1^0$. The rate constants k_1^0 and k_{-1}^0 are the intrinsic values of k_1 and k_{-1} , respectively, assuming that diffusion is very rapid. The rate constant expressions that account for the diffusive approach of the substrate and the enzyme into close configurations where reaction takes place can be expressed in terms of the intrinsic rate constants and the Smoluchowski rate constant $k_{\rm D} = 4\pi DR_0$, with D being the relative diffusion coefficient of the enzyme and the substrate and R_0 an effective radius for reaction: $k_1 =$ $k_1^0 k_D / (k_1^0 + k_D)$ and $k_{-1} = k_{-1}^0 k_D / (k_1^0 + k_D)$. The diffusion coefficient of the substrate molecules is known analytically for MPC dynamics^{26,27} and for our system parameters is given by D =0.048. (Direct simulation of this coefficient yields D = 0.052 in good agreement with the theoretical prediction.) Using $R_0 = d_{\rm R} = 0.5$ as the effective radius of the active site, we have $k_{\rm D}$ = 0.30. The values of $k_{-1}^0 = k_2 = 0.005$, [S₀] = 0.01 are given as input parameters. Only the intrinsic rate constant k_1^0 in eqn (5) is not explicitly known due to the complexity of our substrate binding model. Using k_1^0 as a single parameter to fit the simulation data we find $k_1^0 \approx 0.26$ and the dashed black curve in Fig. 3 (bottom) This plot confirms that MM kinetics provides an adequate description of the kinetics for this case. Since the enzyme active sites no longer act independently in the hexameric enzyme, the simple MM kinetic model breaks down and cannot be applied to the fully active enzyme, even when enzyme-substrate interactions are neglected.

Although $\bar{N}_{\rm C}(t)$ depends on enzyme-substrate interactions, the ratio $\bar{N}_{\rm C}^{(1)}(t)/\bar{N}_{\rm C}^{(6)}(t)$, where the superscripts (1) and (6) refer to one and six active sites, respectively, is the same within our statistical uncertainty for systems with and without

Finally we note that there are small effects due to the specific structure of our model enzyme. When all six binding sites are active, small oscillations can be observed in the plots of $\bar{N}_{c}(t)$ versus time (see Fig. 3 (top)). The simulations start with an unbound enzyme. After initial substrate binding, which occurs quickly, product molecules are formed and released into the system. Some are released in the hollow channel in the enzyme which, as a result, acquires a large amount of product. (See the plot of $g^{P}(r)$ in Fig. 5.) Since the channel confines this species, product molecules diffuse slowly out of the channel. Consequently, binding at the active sites from the channel is inhibited when a large number of product molecules are present there. A significant number of substrate molecules enter the channel only after the product has left. This can then again lead to a small burst of the product within the channel; very weak damped oscillations in $\bar{N}_{\rm C}(t)$ could occur as a result of these processes. When only a single binding site is active, the product concentration in the channel is not large and oscillations are not observed (see Fig. 3 (top)).

4 Conclusion

The results in this paper show that enzymes with multiple active sites can exhibit correlations in activity, even when binding at an active site is not accompanied by conformational changes that influence the activity of nearby active sites. These correlations were attributed to diffusive interactions among the active sites arising from local inhomogeneities in substrate concentration stemming from substrate binding. Such effects lead to reduction in the net activity of the enzyme in comparison to the activity of the same number of independent active sites. In our model enzyme large-scale conformational changes do not occur in the enzyme upon substrate binding and this feature has allowed us to focus on effects arising from diffusional correlations. Our coarse grain model is very simple and neglects many details of the binding process. However, the existence of diffusional correlations does not depend on fine details of the reaction mechanism; thus, many of the conclusions of our study should be generally applicable. Consequently, our study serves to point out that diffusional correlations could play a role in enzymatic kinetics when enzymes possess multiple binding sites. Although the intention of this study was to examine generic aspects of diffusional competition among multiple active active sites in a single enzyme, and our network model for the 4-OT enzyme provided an example where such competition could play a role, we note that the reaction probabilities and potential parameters can be tuned to model specific enzymatic systems at a mesoscopic level.

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Paper

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