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Modeling an enzyme's response to an exothermic catalytic reaction

Part 1: Introduction

We consider how a protein compresses neighboring solvent as a result of a turnover event. In general the catalytic site within a protein is off center. We assume that all of the energy released during the course of the catalytic reaction is used to deform the protein; this is a clear upper bound.

The energy released by the turnover event propagates outward from the reaction site through the protein bonds. As a result of this deformation wave, the protein expands.

For simplicity, we assume the wave propagates radially from the reaction site, see Figure S6. The protein deformation compresses the solvent, which responds by pushing back as dictated by Newton's Third Law. A net reactive force is now imparted on the protein along the catalytic site to the center of mass axis in the direction of the center of mass. The protein center of mass is then translated by this reactive force, see Figure S7.

The protein radius is roughly 4nm and the time it takes for the deformation wave to cover the 8nm protein diameter is 2.7ps. This time is obtained from the speed of sound in an elastic solid, $v_p = \sqrt{K/\rho}$, where K is the protein bulk modulus and ρ the protein density. Using the bulk modulus for hemoglobin[1] and density as found for a spherical particle of radius 4nm and molecular weight equal to that of catalase, we get a speed of sound value of about 3nm/ps.

Part 2: Calculation of the pressure due to solvent compression

We first compute the pressure imparted on the solvent as the solvent compresses due to a protein deformation. From this pressure we will subsequently compute the directional force on the protein center of mass. For simplicity, we will assume that the deformation occurs only on the protein hemisphere in which the catalytic site is located; see Figure S6.

We begin by considering pressure volume changes in a solvent related by the compressibility, β, defined as follows

$$\beta = -\frac{1}{V}\frac{\partial V}{\partial p} \quad (1)$$

We define $\hat{n}$ as the direction from the solvent to the protein center. Since for liquids and solids adiabatic and isothermal compressibilities are approximately equal, we have not specified which variable to hold fixed in our definition for the solvent compressibility. Therefore we write $d\boldsymbol{p} = -\frac{dV}{\beta V}\hat{n}$.

We next consider the z-component for dp, dp_z, in spherical coordinates where $\hat{z}\cdot\hat{n} = cos\,\theta$, θ, the polar angle, is the angle between the r- and z directions, and r is the radial distance from the protein center:

$$dp_z(r,\theta,\varphi) = -\frac{1}{\beta V} r^2 cos\,\theta\, sin\,\theta\, dr\, d\varphi\, d\theta. \quad (2)$$

Integrating over φ from 0 to 2π and θ from 0 to π /2 yields:

$$dp_z(r) = \frac{\pi}{\beta V} r^2 dr = \frac{3}{2\beta R^3} r^2 dr. \quad (3)$$

V was set to the volume of a hemisphere of radius R. Finally integrating from R to R + ΔR, where ΔR is the radial deformation, we obtain:

$$p_z = \frac{1}{4\beta}\left[\left(\frac{\Delta R}{R}+1\right)^3 - 1\right] \approx 532\ pN/nm^2 \quad (4)$$

where we set β = 4.475 x10^{-10} Pa^{-1} at 25 degrees Celsius[2] and ΔR = 1nm. This ΔR is an estimate from height fluctuations of the AFM tip placed atop lysozyme while it is catalyzing reactions.[3] Ultimately, we will predict a ΔR from the catalytic heat evolved in the next section. In this way we will link the mechanical description to a thermodynamic picture.

To summarize, we have made the following assumptions: a) the protein is spherical with uniform ρ, b) only one hemisphere of the protein deforms and c) the protein deformation height is uniform.

Part 3: Calculation of the maximum pressure of an acoustic wave generated by a turnover event

In the previous section, we had assumed that the protein deformation did not generate an acoustic wave in the solvent. Here we will assume that the protein deforms and that its shape is not restored, all of the energy dissipating as solvent acoustic waves. We now ask what is the pressure of this acoustic wave? This pressure should give us an alternative estimate to an upper bound on the pressure the solvent can reflect back on the protein. Here we will not arbitrarily assume a 1nm deformation (see Figure S8). We begin by defining the power per unit area, I, pumped by the protein into the solvent following a catalytic event, as follows[4]:

$$I = \frac{P}{A} = \frac{p^2}{v_s \rho_s} \tag{5}$$

where p is the pressure of the outgoing wave in the solvent, v_s is the speed of sound in the solvent, and ρ_s is the density of the solvent. In writing Eq. 5 we assume no dissipation of the acoustic wave.

The pressure from Eq. 5 is the following:

$$p = \sqrt{\frac{E v_s \rho_s}{tA}} = \sqrt{\frac{40 k_B T v_s \rho_s}{2\pi R^2 t}} \tag{6}$$

where we have set the power, P, equal to the energy $E = Q = 40 k_B T$ per time t. Here Q is the heat generated by a turnover event, while A was set to the area of the protein hemisphere. Since there is no dissipation of the acoustic wave, we have assumed that the energy flux at an infinitesimal distance from the protein surface is the same as it would be at its surface. Also, t is the time required for the deformation of the protein hemisphere.

This amount of time, t, is the amount of time it takes for the deformation wave to travel from the catalytic site, which, for simplicity, we assume to be at the protein's north pole, to the protein's equator, and can be calculated as $t = R\sqrt{2}/v_p \approx 2ps$ where v_p = 3nm/ps is the previously calculated speed of the deformation wave within the protein. To clarify, this is not the time for heat dissipation, which can take place on a longer time scale. Using the following quantities: R = 4nm, the density of water ρ_s = 1000kg/m^3, the speed of sound in water at 25 degrees Celsius $v_s \approx 1.5$ x10^3 m/s, R = 4nm and T = 300K, we find p ≈ 51pN/nm^2. In the negative z-direction, this corresponds to $p_z \approx 25$pN/nm^2. Using Eq. 4, 51pN/nm^2 corresponds to a radial deformation of 0.06nm, or 5% of the protein volume.

To summarize, we have made the following assumptions: a) the protein is spherical with uniform ρ, b) only one hemisphere of the protein deforms and c) all the energy is transferred in 2 ps into the solvent as an unattenuated acoustic wave.

Finally, we would like to stress that local heating of the solvent surrounding the enzyme cannot account for the observed diffusion increase. We calculate the local solvent temperature increase assuming all the reaction energy is poured into a 1 nm thick layer of solvent surrounding catalase (which we assume is a sphere with a radius of approximately 4 nm). We then use the total reaction heat for catalase of 40 k_BT, the shell volume and outer area, the volumetric heat capacity of water C = 4.18 J/Kcm^3, and the definition of volumetric heat capacity C = Q/VΔT to find that the solvent shell temperature increases by at most 0.15 K. This solvent heating does not account for the observed diffusion coefficient increase of the enzymes.

A stochastic theory for protein diffusion

We consider the evolution of the center of mass of the protein, **x**(t), which diffuses according to two diffusion coefficients: D_0 and D_1. D_0 denotes the diffusion coefficient in the absence of a chemical reaction and D_1 denotes the diffusion coefficient following a chemical reaction.

We will assume that the center of mass of the protein experiences enhanced diffusion during a time interval δt.

The reaction rate is V. Thus the probability of a reaction -and thus the probability of experiencing a diffusion coefficient rise within δt- is Vδt. For simplicity of calculation only, we will assume that Vδt << 1. This is a reasonable assumption given that V can be 5 x10^4 s^{-1} at large substrate concentration for catalase, while we will find that a typical value of δt can be of the order of ns.

The protein center of mass diffusion is isotropic. For this reason, we consider the x-component of the center of mass of the protein which satisfies the following expression

$$x_{t+\delta t} = x_t + \sqrt{2D_0\delta t}\cdot\xi_t\cdot(1-\alpha_t) + \sqrt{2D_1\delta t}\cdot\xi_t\cdot\alpha_t \quad (1)$$

where we have introduced two random variables: α_t and ξ_t. α_t is a Bernoulli random variable. It has a mean of $\langle\alpha_t\rangle$ = Vδt and a variance $\langle\alpha_t^2\rangle$-$\langle\alpha_t\rangle^2$ = Vδt (1- Vδt) ~ Vδt + O($V^2\delta t^2$). ξ_t is a Gaussian random variable with mean 0 and variance 1.

To be clear, δt defines the rough timescale during which the center of mass experiences the enhanced diffusion coefficient.

We can use the center of mass of the protein to define a new effective diffusion coefficient as follows

$$D = \frac{\langle(x_{t+\delta t} - x_t)^2\rangle}{2\delta t} \quad (2)$$

where the x component of $\mathbf{x}_t$ is given by Eq. (1). We obtain

$$D = D_0(1 - V\,\delta t) + D_1 V\delta t \quad (3)$$

Eq. (3) is easy to understand. It is the equilibrium average of the two different diffusion coefficients in the limit that the time between reactions 1/V exceeds δt which, as noted earlier, is a reasonable assumption here.

We now express D_1 using measurable constants. We assume that the velocity, **v**(t), of the protein's center of mass following a catalytic event at the heart of the protein dissipates according to

$$m\frac{d\mathbf{v}(t)}{dt} = -\zeta\mathbf{v}(t) + \delta\mathbf{F}(t) \quad (4)$$

where m is the mass of the protein, δ**F** is an uncorrelated frictional force with zero mean and fixed variance, and ζ sets the effective relaxation time scale that can be thought of as an apparent friction coefficient. We reemphasize that the solvent surrounding the protein is not locally heated but that the protein center of mass displaces following a reaction because of the mechanistic interpretation given in the previous section.

The enzyme's trajectory over an interval δt is a segment whose jump size is either determined by D_0 or D_1 and is independent of all other segments.

Expressing the diffusion coefficient as a time integral over the velocity-time autocorrelation function we obtain[5]

$$D_1 = \frac{1}{3}\int_0^\infty dt\langle\mathbf{v}(t)\cdot\mathbf{v}(0)\rangle = \frac{m}{3\zeta}\langle\mathbf{v}(0)\cdot\mathbf{v}(0)\rangle = \frac{2}{3\zeta}\gamma Q \quad (5)$$

where in writing the final equality, we have assumed that the protein center of mass kinetic energy is γQ. Here Q is the total heat released by a single catalytic event and γ is the fraction of heat mechanically imparted to the protein's center of mass.

Inserting Eq. (5) into Eq. (3) we get

$$D = D_0 + \frac{2m}{3\zeta^2}\gamma Q V \quad (6)$$

where we have replaced δt with m/ζ. The second term on the right hand side is purely mechanical in origin and is easy to understand. Just like the regular diffusion coefficient is k_BT/ζ, here, D_1 is proportional to $\gamma Q/\zeta$.

Now, by assumption $0 < \gamma < 1$. As an upper bound, we take $\gamma = 1$ and ask what is ζ? We then use this ζ taken from Eq. 6 to estimate δt assuming a catalase mass of 240 kDa and a reaction heat of 40 kT and we find δt can be anywhere from 1 ns upwards.

Heme excitation experiment

Catalase's heme strongly absorbs light at 405 nm (Soret peak).[6] Most of the energy absorbed by the heme is released as heat[7] and the timescale has been investigated by both MD and ultrafast spectroscopy[8]. The details of this dissipation are tied to both protein topology and function[9,10]. In agreement with our heating hypothesis, we find that the diffusion coefficient of heme containing catalase increases with laser power. A quantitative comparison between the effect of the heat evolved by the catalytic reaction and the heat evolved by the de-excitation of the heme will depend on the dominant mechanisms of heat dissipation at the relevant timescale.

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