

# Non-Boltzmann Rate Distributions in Stochastically Gated Reactions

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Recently, a new mechanism for reaction selectivity, arising from conformational gating of the reactions, has been reported in the acetylcholinesterase system. Fluctuations in the enzyme are thought to greatly slow the access of molecules larger than the normal substrate to the active-site region. By assuming the gate fluctuations occur as a Brownian process in a harmonic well, it is possible to approximate the reaction rates for various limiting cases of substrate size. However, it is not possible to simplify the rates into a ratio which is equivalent to the Boltzmann distribution of states for the gate fluctuations.

Many important reactions in chemistry and biochemistry that would otherwise obey diffusion-limited kinetics are modulated by gating effects due to conformational changes in the reactants.<sup>1–4</sup> Recently, Zhou et al. reported a new mechanism for binding selectivity arising from such conformational gating in acetylcholinesterase (AChE).<sup>4</sup> AChE hydrolyzes acetylcholine (ACh) with an extraordinarily high catalytic efficiency ( $k_{\text{cat}}/K_m \sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) despite the location of the catalytic triad at the bottom of a narrow, 20 Å long gorge.<sup>5–8</sup> Furthermore, X-ray crystallography studies show this gorge to be constricted by several aromatic residues to a diameter too small for ACh to pass.<sup>8</sup> A molecular dynamics simulation of AChE showed that this constriction opened wide enough to admit an ACh molecule only infrequently.<sup>9</sup>

To determine the impact of such a fluctuating constriction on the catalytic activity, Zhou et al.<sup>4</sup> applied the theory developed for stochastically gated reactions<sup>2,10</sup> to obtain an expression for the rate of ACh hydrolysis. Assuming the gate fluctuations behaved in a Brownian fashion under the influence of a harmonic potential, they were able to fit the molecular dynamics data to a model which captured the dynamic nature of the active-site accessibility. Using this model, Zhou et al. found that the reaction rate was merely decreased by a factor of 2 relative to the rate for AChE with its gate fixed open, when gating was considered for substrates of ACh size (2.4 Å radius), while the rate was reduced by three orders of magnitude for only slightly larger substrates (2.8 Å). Such a dramatic selectivity between substrates of marginally different sizes raises the question of how the reaction rates are distributed for substrates of arbitrary diameters. Specifically, we are interested in determining if the rates of the gated reactions obey the Boltzmann distribution characteristic of the underlying gate fluctuations.

To address this question, we consider the system as a reactive sink screened from an initial distribution of substrates by a gate.

When the gate is open, substrates can diffuse past and be absorbed into the sink, while a closed gate acts as a reflective barrier. This gate changes states in a Brownian fashion, subject to a harmonic potential. In the steady state, the probability distribution of the gate is Gaussian, with mean effective radius  $x_m$  and standard deviation  $\sigma = (2/\kappa\beta)^{1/2}$ .<sup>11</sup> Here,  $\beta^{-1}$  is the Boltzmann constant multiplied by the absolute temperature and  $\kappa$  is the force constant of the harmonic potential. Obviously, the definition of the gate as “open” or “closed” depends on the size of the substrate attempting to pass. The relative gate radius required to admit a substrate of radius  $s$  is  $\eta \equiv (s - x_m)/(2^{1/2}\sigma)$ .<sup>4</sup> The quantity  $\eta^2$  is then just the energy of the system scaled by  $\beta$ , and the Gaussian distribution  $e^{-\eta^2}$  is proportional to the Boltzmann probability distribution of states in the harmonic well. Therefore, the probability  $p_o$  of an open gate is simply the probability the gate has a coordinate larger than some  $\eta$  or the area under the Gaussian curve beyond some threshold value  $\eta$ :  $p_o = \text{erfc}(\eta)/2$ .

Following the work of Zhou,<sup>10</sup> the rate was taken to be

$$\frac{1}{k_g} = \frac{1}{k} + \frac{w_o}{w_c \hat{w}(w)} \quad (1)$$

Here  $k$  is the rate constant of the reaction if the gate is fixed open,  $k_g$  is that for the gated reaction,  $w_o$  and  $w_c$  are the switching rates between the open and closed states of the gate, and  $\hat{w}(w)$  is related to the time-dependent rate coefficient of the ungated system. Letting  $w \equiv w_o + w_c$ ,  $\hat{w}(w)$  was found to be  $(Dw)^{1/2} A \langle e^{-\beta U} \rangle / 2$ .<sup>10</sup>  $A$  is the substrate accessible area of a gate with maximum aperture  $r_{as}$ ,  $D$  is the substrate diffusion constant, and  $\langle e^{-\beta U} \rangle$  is the average Boltzmann factor for substrate across the gate opening. Using the definition of Zhou et al.,<sup>4</sup> the accessible area is given by  $A \equiv \pi(r_{as} - s)^2$ . In a medium of viscosity  $\nu$ ,  $D$  can be related to the substrate size by the Stokes-Einstein relation,  $D = (6\pi s \nu \beta)^{-1}$ . The probabilities of open and closed gates are  $p_o = w_c/w$  and  $p_c = w_o/w$ . Furthermore, the result of Zhou et al.<sup>4</sup> can be used to give an additional expression

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for the rates

$$\frac{1}{w} = \frac{\pi^{1/2}\sigma^2}{2D_g p_o} \int_{\eta}^{\infty} \text{erfc}^2(y) e^{y^2} dy \equiv \frac{\pi^{1/2}\sigma^2}{2D_g p_o} I(\eta) \quad (2)$$

where  $D_g$  is an effective diffusion constant for the gate fluctuations and the integral is denoted by  $I(\eta)$ . Using all this information gives a final expression for the rate

$$\frac{1}{k_g} = \frac{1}{k} + \frac{2 - \text{erfc}(\eta)}{\alpha(r_{as} - x_m - 2^{1/2}\sigma\eta)^2 \text{erfc}(\eta)} \left[ \frac{(x_m + 2^{1/2}\sigma\eta)I(\eta)}{\text{erfc}(\eta)} \right]^{1/2} \quad (3)$$

where  $\alpha$  represents constants independent of substrate size.

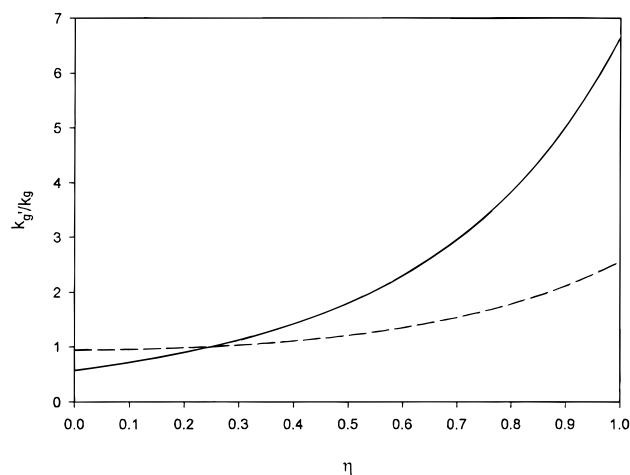
To express a ratio of reaction rates for substrates of different size in simple terms, it is necessary to simplify eq 3. Since the integral  $I(\eta)$  cannot be expressed in closed form, some approximation must be made for its dependence on  $\eta$ . Numerical integration shows the function  $[\text{erfc}(\eta)/I(\eta)]^{1/2}$  is linear at all but very small values of  $\eta$ .<sup>12</sup> Furthermore, the second derivative of this function decreases exponentially from a maximum value of approximately 0.41 at  $\eta = 0$ . Therefore,  $I(\eta)$  can be fit to a straight line with very little error. For  $I(\eta) \approx b_0 + b_1\eta$ , linear regression over the interval  $\eta \in (0.0, 5.0)$  gave  $b_0 = 1.29$  and  $b_1 = 1.662$  with  $r^2 = 0.998$ .<sup>12</sup>

To further reduce eq 3, it will be assumed the ungated reaction rate is large enough that its reciprocal is negligible with respect to the second term, representing gate fluctuations, in eq 3. While this is not true for all possible  $w_o$ ,  $w_c$ , and  $w_h(w)$ , it is informative to examine the form of the ratio where this assumption is valid. For the AChE example investigated by Zhou et al.,<sup>4</sup> this assumption fails for  $s = 2.4$  Å but very quickly becomes acceptable with increasing  $s$ . Additional simplification of eq 3 can be accomplished by considering two different regimes of  $\eta$ . When the system is such that the gate is open relatively often,  $\eta, \eta' \ll 1$ , the radical, linear, and quadratic terms can be neglected and the ratio can be expressed as

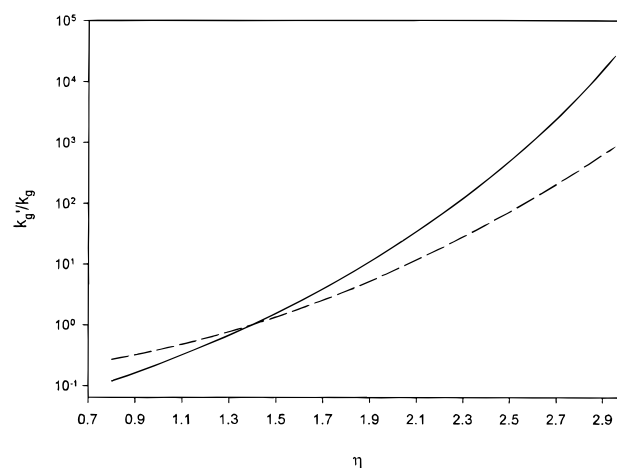
$$\frac{k'_g}{k_g} \approx \frac{[2 - \text{erfc}(\eta)] \text{erfc}(\eta')}{[2 - \text{erfc}(\eta')] \text{erfc}(\eta)} \quad \eta, \eta' \ll 1 \quad (4)$$

For a representative value of  $\eta' = 0.25$ , the mean relative error for this approximation on the interval (0.0, 1.0) is 9%. If the reaction rate ratio obeyed a Boltzmann distribution corresponding to energies of the underlying gate states, then two substrates requiring values of  $\eta$  and  $\eta'$  to pass by the gate would react with rates  $k_g$  and  $k'_g$  in a ratio  $k'_g/k_g = e^{-\eta'^2}/e^{-\eta^2}$ . Obviously, eq 4 does not obey such a distribution. A graph of the actual behavior of this ratio with respect to  $\eta$  at  $\eta' = 0.25$  is shown in Figure 1 for contrast with the corresponding Boltzmann distribution. On the basis of this graph, it can be seen that this limiting form of the ratio (eq 4) selects against large substrates much more strongly than the corresponding Boltzmann distribution.

When the system is such that the gate is rarely open,  $\eta/\eta' > 1$ . In this domain,  $\text{erfc}(\eta) \ll 2$ , simplifying the differences  $2 - \text{erfc}(\eta)$  appearing the ratio. Furthermore, the asymptotic form of this function  $\text{erfc}(\eta) \sim e^{-\eta^2}/(\pi^{1/2}\eta)$  can be used due to its rapidly converging behavior of  $O(e^{-\eta^2}/\eta^2)$ .<sup>13</sup> Finally, due to the dominant behavior of  $\text{erfc}$  in this function, more drastic approximations can be made with little error, such as  $(b_0 + b_1\eta)/\eta \approx b_1$  and  $(x_m + 2^{1/2}\sigma\eta)^{1/2}(x_m + 2^{1/2}\sigma\eta)^{-1/2} \approx (\eta/\eta')^{1/2}$ .



**Figure 1.** Reaction rate ratio is small  $\eta$  limit (eq 4, solid line), and Boltzmann distribution of reaction rates (dashed line). Both ratios are plotted with respect to  $\eta$ , holding  $\eta'$  constant at 0.25.



**Figure 2.** Reaction rate ratio in moderate to large  $\eta$  limit (eq 5, solid line), and Boltzmann distribution of reaction rates (dashed line). Both ratios are plotted with respect to  $\eta$ , holding  $\eta'$  constant at 1.4.

Using all these simplifications, the ratio of rates reduces to

$$\frac{k'_g}{k_g} \approx \frac{e^{-\eta'^2}(r_{as} - x_m - 2^{1/2}\sigma\eta')^2}{e^{-\eta^2}(r_{as} - x_m - 2^{1/2}\sigma\eta)^2} \left( \frac{\eta}{\eta'} \right)^{1/2} \quad \eta, \eta' \geq 1 \quad (5)$$

Using the values of  $x_m = 1.95$  Å,  $r_{as} = 3.0$  Å,  $\sigma = 0.22$  Å, and  $s = 2.4$  Å from the AChE study of Zhou et al.<sup>4</sup> gives  $\eta' \approx 1.4$ . At this value of  $\eta'$ , the mean relative error in this approximation is roughly 10% over  $\eta \in (1.0, 3.0)$ . Values of  $\eta > r_{as}$  are not physically meaningful, so for the AChE system, this represents a good estimate of the error in this approximation. The rate ratio in this moderate gate coordinate limit (eq 5) also does not follow the underlying Boltzmann distribution of the gate. Figure 2 compares eq 5 with the Boltzmann distribution using  $\eta' = 1.4$ . The agreement with the Boltzmann distribution at  $\eta \approx 1$  is deceptive because the  $1/k \approx 0$  approximation required to derive eq 5 fails for the AChE system in this range of  $\eta$ .

It is interesting to note the ratios of rates in eqs 4 and 5 contain only equilibrium quantities, much like the Boltzmann distribution. If  $1/k$  is not negligible, the constant  $\alpha$  remains in the ratio, which then includes terms involving dynamic properties. Another interesting result of these calculations is the similarity between eq 5 and the results for the mean first passage

time for a particle over a barrier undergoing rapid dichotomous fluctuations.<sup>14</sup> In the latter case, the investigators found rates distributed in qualitative agreement with eq 5 for rapidly fluctuating barriers. This agreement is not surprising as the fast fluctuation limit of their model essentially corresponds to the "white noise" implicit in the model used here.

In conclusion, we have shown that the ratio of rates for a stochastically gated diffusion-influenced reaction cannot be simply connected to the Boltzmann distribution of the gate. Although it is tempting to invoke Arrhenius-type models for diffusion over most barriers to reaction, it is, in general incorrect for a gate of this nature. Instead, the rates are distributed with a much more complicated behavior, which generally exhibits a much stronger increase with  $\eta$  than the simple  $e^{\eta^2}$  of activated processes over nonfluctuating barriers. This mechanism of conformational gating can not only provide very efficient substrate selection, but it also offers a means to tailor the rates of reactions of various substrates in a unique fashion.

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