

# An Intuitive Approach to Steady-State Kinetics

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Deriving the exact rate equation for even the simplest of kinetic systems can be a formidable task. If the steady-state approximation is applicable, however, an accurate rate equation can readily be obtained using linear algebra (1-3), partition analysis (4), or graph theory (5, 6). Several previous contributors to *this Journal* have discussed the validity of the steady-state approximation and have applied it to various kinetic systems (7-13). In this article, we attempt to provide an intuitive understanding of steady-state kinetics by considering some unusual, we hope enlightening, aspects of this concept.

## The Meaning of "Steady State"

Let us begin by defining "steady state". An intermediate is in steady state if its concentration does not change with time. Consider the reaction



where C is formed irreversibly from A via an intermediate, B. In eq 1, the rate constant  $k_1$  refers to the conversion of A to B, and the rate constants  $k_{-1}$  and  $k_2$  relate to the conversion of B to A and C, respectively.

Intermediate B is in steady state if its concentration is constant. Mathematically, the change in the concentration of B with time ( $d[B]/dt$ ) is given by

$$d[B]/dt = k_1[A] - (k_{-1} + k_2)[B] \quad (2)$$

where the first term on the right-hand side describes the rate at which B is formed and the second term describes the rate at which B breaks down. If the concentration of B is constant, then  $d[B]/dt = 0$ , and

$$k_1[A] = (k_{-1} + k_2)[B] \quad (3)$$

Although eq 3 is exactly true for merely an instant during the reaction of eq 1, it can often be a good *approximation* throughout the time course of the reaction (7, 10). Specifically, since the reaction begins in the absence of B, the concentration of B will be most nearly constant if it remains small throughout the time course of the reaction. According to eq 3, the concentration of B is small (i.e.,  $[B] \ll [A]$ ) if

$$k_{-1} + k_2 \gg k_1 \quad (4)$$

where again the rate constants  $k_{-1}$  and  $k_2$  refer to the breakdown of B, while  $k_1$  relates to the formation of B. The steady-state approximation is therefore valid throughout the time course of the reaction if the breakdown of the intermediate is much faster than its formation.

## Applying Free-Energy Profiles

A free-energy profile is a simple way to illustrate complex kinetic and thermodynamic relationships, such as those discussed above (14). Notional free-energy profiles depicting the six possible relationships between the rate constants of eq 1 are shown in Figure 1. In each of these profiles, the abscissa serves merely to depict the temporal mechanism of the reaction, showing here that A is converted to B, and then B is converted irreversibly to C.<sup>1</sup> The horizontal segments plotted on the ordinate are the relative free energies of the ground states of species A and species B, and of the transition states for the conversions of A to B and B to C.<sup>2</sup> In which

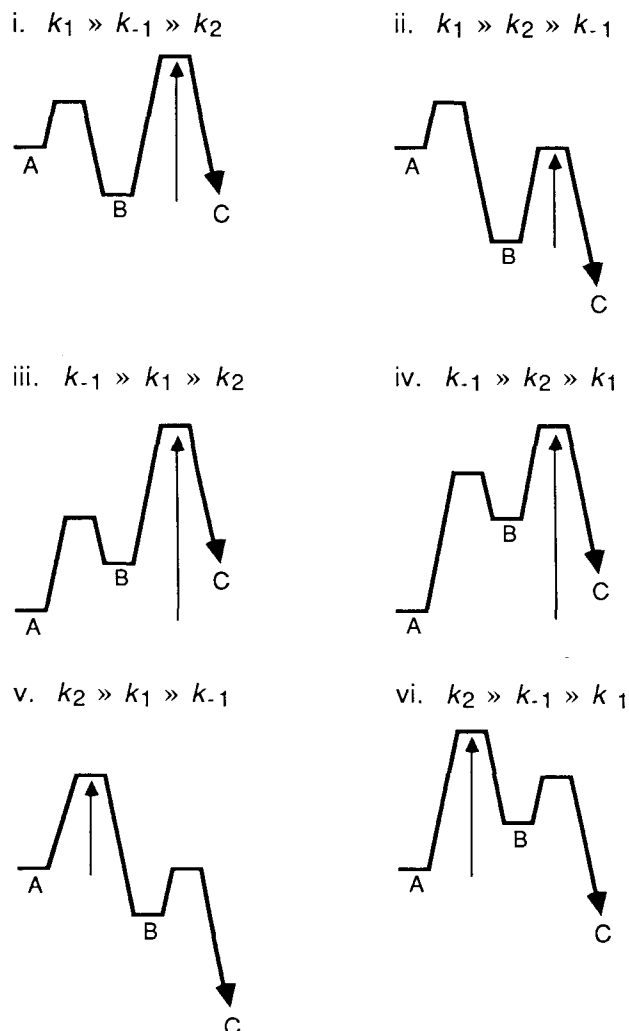


Figure 1. Notional free-energy profiles illustrating the six possible relationships between the rate constants of eq 1. The vertical arrows indicate the largest total free energy barrier that must be traversed in each profile.

<sup>1</sup> It has been said that time is what keeps everything from happening all at once. The abscissae of the free energy profiles in Figure 1 serve a similar purpose.

<sup>2</sup> The rate constant,  $k_r$ , for each step is related to the free energy difference,  $\Delta G^\ddagger$ , between the ground state and the transition state of that step according to the expression,  $\Delta G^\ddagger = -RT \ln(k_r h/k_B T)$ , where  $k_B$  is the Boltzmann constant and  $h$  is the Planck constant.

of the profiles in Figure 1 is intermediate B in steady state? Starting material A is more stable than B in profiles iii, iv, and vi. The steady-state approximation is assuredly valid for these profiles because the concentration of B will always be less than that of A. On the other hand, B is more stable than A in profiles i, ii, and v. Is the steady-state approximation necessarily invalid for these profiles? We have demonstrated that B is in steady state if eq 4 is obeyed. Although B is more stable than A in profile v (i.e.,  $k_1 \gg k_{-1}$ ), B does not accumulate in this profile because it is converted to C faster than it is formed from A (i.e.,  $k_2 \gg k_1$ ). Thus profile v illustrates an important distinction: steady state is a kinetic, not a thermodynamic, phenomenon. In profiles i and ii, however, B does accumulate because its formation is faster than its breakdown. The steady-state approximation is invalid for the energetics depicted in these two profiles.

### Illustrative Examples

We have discussed above the constraints on the concentration of intermediate B for the steady-state approximation to be valid for the reaction of eq 1. To illustrate these constraints, we now introduce several perturbations to eq 1 that could potentially affect the concentration of B.

First, let us consider the influence of the isotopic composition of intermediate B on the rate equation of eq 1. Suppose that an isotope (as indicated by a \* superscript) is introduced into the starting material of eq 1 such that



where the interconversion of  $A^*$  and  $B^*$  is unaffected by the isotopic substitution, but the conversion of isotopically labeled  $B^*$  to C is intrinsically more difficult than the conversion of unlabeled B to C (i.e.,  $k_2^* < k_2$ ).<sup>3</sup> In which of the profiles of Figure 1 will the isotopic substitution affect the rate equation? For profiles i and ii, the answer depends on how we monitor the reaction—by the disappearance of A, or by the appearance of C. Since B accumulates in profiles i and ii, the two processes are not equivalent, and the disappearance of A is initially much faster than the appearance of C. There is no isotope effect on the disappearance of A because the isotopic substitution does not affect the conversion of A to B. There is an isotope effect on the appearance of C, however, because the isotopically sensitive transition state is at the top of the largest free-energy barrier in the two profiles, as indicated by the vertical arrows of Figure 1.<sup>4</sup>

Intermediate B does not accumulate in profiles iii–vi, and the disappearance of A coincides with the appearance of C. In which of these four profiles does the isotopic substitution affect the rate equation? In profiles iii and iv, the isotopically sensitive transition state is at the top of the largest free-energy barrier. The isotopic substitution therefore alters the rate equation in these two profiles, and the nature of the steady-state intermediate, even though it does not accumulate, is important. In profiles v and vi, however, the largest barrier on the pathway does not include the isotopically sensitive transition state. Still, we must be careful because  $k_2^* < k_2$ , and the isotopic substitution increases the steady-state concentration of  $B^*$ . Nevertheless, as long as the condition for steady state maintains (now,  $k_{-1} + k_2^* \gg k_1$ ),  $B^*$  does not accumulate and the isotopic substitution does not significantly alter the rate equations of profiles v and vi. By this measure, the isotopic composition of B is irrelevant in the energetics depicted by these two profiles.

In our next example, we consider a side reaction that diverts B from the main reaction path of eq 1, siphoning it off to form an otherwise inert species. For instance, suppose that B is a base that interconverts rapidly (relative to its conversion to both A and C) with a conjugate acid,  $BH^+$ . Eq 1 then becomes

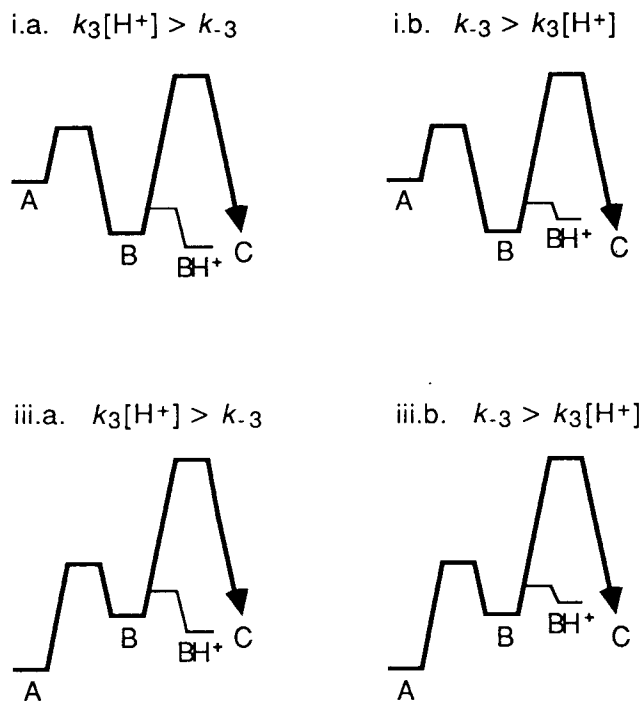


Figure 2. Expansion of free-energy profiles i and iii from Figure 1 to incorporate the rapid interconversion of B with a conjugate acid,  $BH^+$ , as in eq 6. The protonation of B is illustrated as an exothermic process (in i.a and iii.a) or an endothermic process (in i.b and iii.b).



Does the presence of  $BH^+$  alter the rate equation of eq 1? The change in the concentration of B with time is now given by

$$d[B]/dt = k_1[A] + k_{-3}[BH^+] - (k_{-1} + k_2 + k_3[H^+])[B] \quad (7)$$

Since we have defined the acid–base equilibrium between  $BH^+$  and B to be rapid,  $k_3[H^+][B] = k_{-3}[BH^+]$ , and eq 7 reduces to eq 2. The steady-state concentration of B is therefore identical for eq 1 and 6. Since the rate equation for the reactions of both eq 1 and 6 is always given by

$$d[C]/dt = k_2[B] \quad (8)$$

the presence of  $BH^+$  has no steady-state kinetic consequence.<sup>5</sup>

The formation of  $BH^+$  diverts B from the main reaction path in all of the profiles of Figure 1. This detour does not affect the steady-state kinetics of profiles iii–vi because

<sup>3</sup> For instance, an appropriate isotopic substitution will effectively alter only  $k_2$  in the Michaelis–Menten mechanism of enzymatic catalysis, in which  $k_1$  and  $k_{-1}$  relate to the reversible binding of a substrate to an enzyme, while  $k_2$  refers to an irreversible chemical conversion (15–17).

<sup>4</sup> An isotope effect results primarily from the difference in zero-point energies of the labeled and unlabeled ground states (18). For simplicity, however, a kinetic isotope effect is best illustrated on a free energy profile as a transition state effect.

<sup>5</sup> This result is reminiscent of the Curtin–Hammett principle (19–21), which states that the ratio of products formed from conformational isomers is independent of the conformer population ratio.

