

Notes on the program

The program was written as an aid to exploration of the thermodynamic and kinetic parameters underlying the reaction at the Q_o-site of the bc₁ complex. Values for parameters known from experiment (E_m values of reactants, activation energy, driving force, distances for electron transfer obtained from structural models, etc.) are input through use of interactive controls. The program generates Marcus curves showing rate constant on a log scale ($\log_{10}k$) as a function of driving force (ΔG) for the two 1-electron partial reactions, using the input parameters to calculate these values, and to keep track of thermodynamic balance. The general idea is to move the curves around the plot area until they intercept particular values for $\log_{10}k$ and driving force appropriate to the system under study. Default values are appropriate for the bc₁ complex from wild-type *Rhodobacter sphaeroides*.

The program can be run by double-clicking the icon for Marcus_Bronsted.exe, or by selecting that file using Start... Run... from the desktop. The program starts with a blank plot area. Curves using the default parameters can be generated by clicking on the blank area. In the program, the curve of $\log_{10}k_{\text{lim}} \nu. \Delta G$ is plotted using values input by the user for the critical parameters γ , R , λ , and the two pK s. These make it possible to move the curve around the plot area so as to match experimental values for k and reaction driving force (ΔG_e^o). The program keeps track of the First Law interdependence of thermodynamic parameters for partitioning of the activation barrier (see **Marcus equations** below), and those for transfer of the first and second electrons based on the nature of the bifurcated reaction, as detailed in (1), and as summarized below.

Overall driving force for the bifurcated reaction (oxidation of QH₂ at the Q_o-site):

$$\Delta G^{o'} = -F \{ (E_{ISP}^{o'} + E_{b_L}^{o'}) - 2E_{Q/QH_2}^{o'} \}$$

Values for E_{ISP} and E_{b_L} on the right side of the equation are input via adjustable scroll bars (see below).

For the reaction from the ES-complex, the driving force is determined by the E_m of the bound Q/QH₂ couple:

$$\Delta G_{bound}^{o'} = -F \{ (E_{ISP}^{o'} + E_{b_L}^{o'}) - 2E_{Q/QH_2,bound}^{o'} \}$$

Values for E_{Q/QH_2} and $E_{Q/QH_2,bound}$ are assumed to be 90 mV and 130 mV respectively. These values can be changed by use of the Change hidden parameters dropdown list (see below).

For the semiquinone couples:

$$E_{SQ/QH_2}^{o'} = E_{ISP}^{o'} - \Delta E_m$$

(where ΔE_m is the redox driving force for the first electron transfer. $E_{ISP}^{o'}$ and ΔE_m are both input via scroll bars in the program)

$$E_{Q/SQ}^{o'} = 2E_{Q/QH_2,bound}^{o'} - E_{SQ/QH_2}^{o'}$$

Stability constant for semiquinone formation:

$$E_{Q/SQ}^{o'} - E_{SQ/QH_2}^{o'} = \frac{RT}{F} \ln K_s$$

Driving force for the second electron:

$$\Delta E_{2ndelectron}^{o'} = (E_{b_L}^{o'} - E_{Q'/SQ}^{o'}) = -\frac{\Delta G_{bound}^{o'}}{F} - \Delta E_m$$

Marcus equations

For each of the two electron transfer reactions at the Q_o-site, the program plots log₁₀(*k*) as a function of Δ*G* using the form of the Marcus equation selected by clicking on one of the four options buttons provided. These are, from left to right:

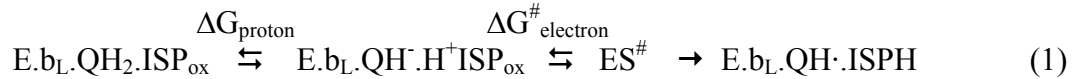
(i) **Moser-Dutton**. The equation derived by Moser et al. (2-4) for the dependence of rate constant on distance, Δ*G*, and λ, using the set of relationships from Marcus theory, detailed in (4).

$$\log_{10} k_{lim} = 15 - 0.6R - \gamma \frac{(\Delta G_e^o + \lambda)^2}{\lambda}$$

The term γ has the Moser et al. value of 3.1, which was chosen to provide a fit to experimental data, and justified by including quantum mechanical contributions to deal with tunneling effects that contribute to the overall rate (4, 6).

(ii) **MD-Brønsted**. The Moser et al. equation, with the Arrhenius exponential function for the first electron transfer step split into two contributions (7, 13). The transfer of the first electron is treated as a proton-coupled electron transfer, in which the proton transfer step is unfavorable, and contributes to the activation barrier. This gives separate contributions from the proton transfer step and the electron transfer step. The contribution of the proton transfer step is calculated using the Brønsted equation, based on the following assumptions:

a). The electron transfer can occur only when the proton configuration is favorable. This requires that the proton be transferred through the H-bond before electron transfer can occur.



b). The value for Δ*G*_{proton} is given by the Brønsted relationship (8, 9), which describes the equilibrium distribution of the H⁺ along a H-bond in terms of the p*K* values of the H-bond donor (p*K*_D) and acceptor (p*K*_A):

$$\Delta G_{proton} = 2.303RT(pK_D - pK_A) = 2.303RT(pK_{QH2} - pK_{ISPox})$$

c) Rates of H⁺ transfer through H-bonds are inherently rapid (~2.10¹¹ s⁻¹), ~1000 faster than the maximal electron transfer rate at this distance (8, 9). To a close approximation, the proton transfer contribution can therefore be treated as a separate probability function given by the Brønsted term. This allows for a great simplification in thermodynamic treatment.

$$\begin{aligned} k_{lim} &= k_o \exp\{-(\Delta G_{electron}^\# + \Delta G_{proton})F/RT\} \\ &= k_o \exp\{-\Delta G_{electron}^\# F/RT\} \exp\{-2.303\Delta pK\} \end{aligned}$$

Expanding the terms, using the Moser et al. pre-exponential factor for k_o , the Moser et al. term for the electron transfer contribution, $\Delta G^{\#}_{\text{electron}}$ (with $\gamma=3.1$), and the Brønsted term for ΔG_{proton} , we get:

$$\log_{10} k_{\text{lim}} = 15 - 0.6R - \gamma \frac{(\Delta G_e^o + \lambda)^2}{\lambda} - (pK_{QH_2} - pK_{ISP_{ox}})$$

(iii) **Marcus.** The Moser et al. equation, with the parameter 3.1 replaced by the classical Marcus parameter. The term γ is $F/(4 \times 2.303RT)$, and has a value of 4.22 at 298 K.

$$\log_{10} k_{\text{lim}} = 13 - \frac{\beta}{2.303}(R - 3.6) - \gamma \frac{(\Delta G_e^o + \lambda)^2}{\lambda}$$

(iv) **Marcus-Bronsted.** Equation (iii) with the exponential function split as in (ii)

$$\log_{10} k_{\text{lim}} = 13 - \frac{\beta}{2.303}(R - 3.6) - \gamma \frac{(\Delta G_e^o + \lambda)^2}{\lambda} - (pK_{QH_2} - pK_{ISP_{ox}})$$

Values for ΔG_e and λ are in eV, and the sign of ΔG is that of classical thermodynamics (the value is negative for a spontaneous exergonic process). The factor F in γ converts from electrical to chemical units, and the disappearance of an equivalent factor in the Brønsted term comes from cancellation.

Moving the curves around

Before examining the curves, it is worth noting some properties of the equations and the resulting curves. The inverted parabola has a width determined by γ (the lower value resulting from the Moser et al. (3, 4) treatment gives a wider parabola, and consequently a shallower slope at any particular value for $\log_{10}k$), and by λ (larger values give wider parabolas), and is offset vertically by changing the distance, R , and Brønsted terms (pK values). These latter do not modify the shape of the curve since their value in the equation is independent of ΔG , the dependent variable. Changing λ also shifts the curve horizontally so that the peak position (when $\lambda = -\Delta G$) is at higher values of ΔG for lower values of λ .

A satisfactory fit is found when the Marcus curve intersects a horizontal line corresponding to a measured rate constant, and a vertical line corresponding to a measured driving force. Since the first electron transfer is rate determining, this condition is usually most important for the corresponding values for this reaction. The horizontal dashed line is appropriate for the observed rate of the wild-type reaction (the overall rate, but limited by the first electron transfer). The program generates vertical lines corresponding to values for driving forces for first, second and overall reactions.

Treatment of endergonic reactions

It has been suggested in (2) that an alternative form of the Marcus equation should be used for treatment of endergonic processes. This alternative form was not used in the program because it is unnecessary, and, in the form recommended, is wrong. However, application of the conventional treatment to the endergonic first electron transfer at the Q_o -site (13) has been criticized, and it therefore seems necessary to justify it more fully.

The two approaches, if used with care, are equivalent. However, the equation below, suggested in (2), although derived through a sensible route, is wrong.

$$\log_{10} k_{et}^{end} = 13.0 - 0.6(R - 3.6) - 3.1(-\Delta G + \lambda)^2 / \lambda - \Delta G / 0.06$$

In addition to what appears to be a conventional Marcus term, this equation seems to contain an extra Boltzmann term contributing to the energy barrier (the right-most term). This is not an “additional term”, however, but simply arises when estimating the activation barrier and rate-constant in the endergonic direction (which cannot be measured easily) using the reaction properties measured in the exergonic direction. It can be derived in its simplest form as follows (using electrical units for ΔG):

$$A \xrightleftharpoons[k_b^{end}]{k_f^{end}} B ; \quad K_{end} = k_f^{end}/k_b^{end} = k_f^{end}/k_f^{ex}$$

$$k_f^{end} = k_f^{ex} K_{end}$$

$$\log_{10} k_f^{end} = \log_{10} k_f^{ex} + \log_{10} K_{end} = \log_{10} k_f^{ex} - \Delta G_{end}^0 F/2.303RT$$

This expression can be extended using classical Marcus treatment (4, 5), and substitution using the Arrhenius expression, $k_f^{ex} = k_0 \exp(-\Delta G_{ex}^\ddagger \cdot F/RT)$, the Marcus term, $\Delta G_{end}^\ddagger = (\Delta G_{end}^0 + \lambda)^2/4\lambda$, for which $\gamma = 4.23$ at 298 K is appropriate, and the Moser et al. (3) expression for k_0 in terms of distance, R .

$$\log_{10} k_f^{end} = 13.0 - 0.6(R - 3.6) - \gamma(\Delta G_{ex}^0 + \lambda_{ex})^2 / \lambda_{ex} - \Delta G_{end}^0 F/2.303RT$$

The form of the equation given above is correct. The λ used here is that for the exergonic reaction; the value is the same in both directions if the parabolas have the same shape (the same “spring constant”), but not otherwise.

In the Moser-Dutton (3, 4) formulation, in which quantum-mechanical corrections modify the pre-exponential factor, γ has a value of 3.1 instead of 4.23. The Page et al. (2) version (below) is obtained by substituting $-\Delta G_{end}^0$ for ΔG_{ex}^0 , and ignoring any possible difference between λ_{ex} and λ_{end} .

$$\log_{10} k_{et}^{end} = 13.0 - 0.6(R - 3.6) - 3.1(-\Delta G + \lambda)^2 / \lambda - \Delta G / 0.06$$

The error comes from use of the quantum mechanical modification of the value for γ , which is applied to only one of the two terms making up the Marcus expression for ΔG_{end}^\ddagger .

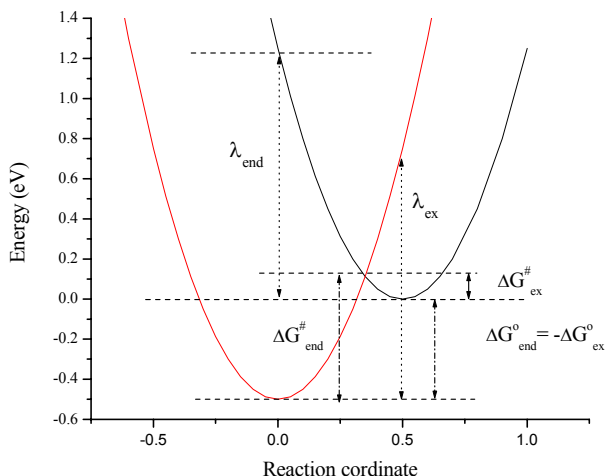


Fig. 1. Marcus parabolas for an endergonic process, labeled to show the terms discussed in the text.

An alternative treatment can be framed in terms of a set of Marcus parabolas (Fig. 1) (5, 6). The endergonic energy barrier, ΔG_{end}^\ddagger , is calculated by adding ΔG_{ex}^\ddagger , the energy barrier in the exergonic direction, to the energy difference in the endergonic direction (the Boltzmann term in question). ΔG_{ex}^\ddagger is estimated using the Marcus expression, with ΔG_{ex}^0 for the

exergonic reaction direction, and a value for λ , λ_{ex} , obtained from measurement of the exergonic rate. The tricky factor is again the replacement of ΔG_{ex}^o by $-\Delta G_{end}^o$, its numerical equivalent. The underlying rate equation in the endergonic direction has the same conventional Arrhenius form as that in the exergonic direction.

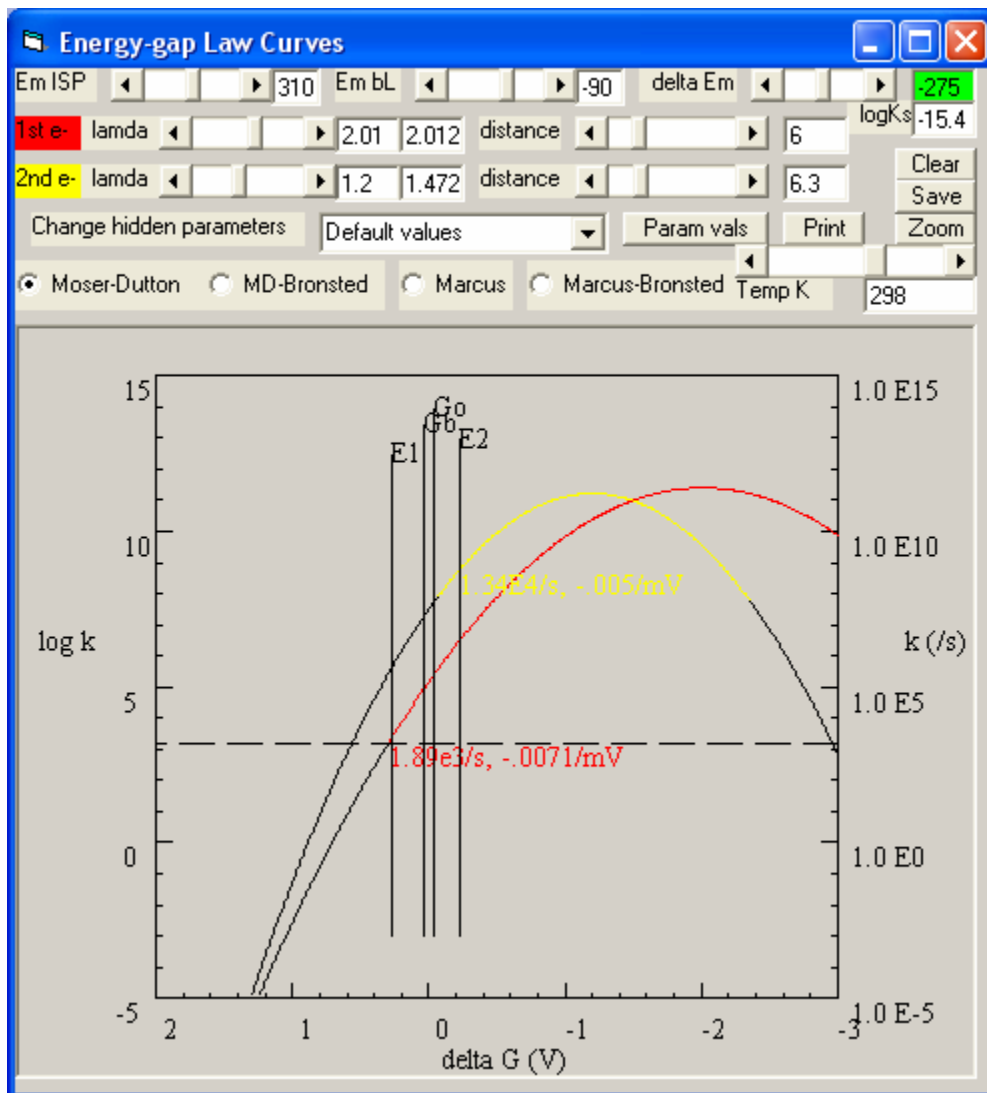
$$k_{cat} = k_0 \exp(-\Delta G_{end}^{\#} / RT) = k_0 \exp(-(\Delta G_{ex}^{\#} + \Delta G_{end}^o) / RT)$$

Derivation of the Marcus term for $\Delta G^{\#}$ from Hooke's Law, using parabolas for either exergonic or endergonic reactions, gives the same conventional Marcus equation.

The "additional Boltzmann term" of the Page et al. (2) equation is not an extra term contributing to the barrier. The treatment used in (13) and in this program, in which the endergonic region is treated using the standard Marcus term, is perfectly appropriate. This can most easily be demonstrated by numerical substitution. Identical Marcus curves are generated using the exergonic or endergonic forms of the Page-Moser-Dutton equation, as long as a simple Marcus treatment (giving γ of 4.23 at 298 K) is used. However, with the Page et al. (2) equation, although $\Delta G_{end}^{\#}$ and $(\Delta G_{ex}^{\#} + \Delta G_{end}^o)$ have the same numerical value, the quantum mechanical correction implicit in $\gamma = 3.1$ is applied only to the part of this value corresponding to $\Delta G_{ex}^{\#}$. As a consequence, different values are generated for k_f^{end} when using the two equations, and the Marcus curve resulting from the treatment recommended in (2) (in which the "endergonic" equation is used only for the endergonic part) shows a discontinuity in slope at the exergonic to endergonic transition. This is clearly wrong!

Using the program

Parameters for the equation used are set by changing input values through the adjustable scroll bars. These are described with reference to the image below.



Top row:

Em ISP: The midpoint redox potential of the iron sulfur protein ($E_m(\text{ISP})$). The value is reported in the adjacent text box.

Em bL: The midpoint redox potential of heme b_L ($E_m(\text{bL})$). The value is reported in the adjacent text box.

delta Em: The redox difference for the first electron transfer ($E_m(\text{ISP}) - E_m(\text{QH}^+/\text{QH}_2(\text{bound}))$). This is equal to $-\Delta G_1/F$, the value used in subsequent calculations. The difference can be changed without changing $E_m(\text{ISP})$ or $E_m(\text{bL})$, by changing $E_m(\text{QH}^+/\text{QH}_2(\text{bound}))$.

Two adjacent text boxes are affected by changes made using this scroll bar. The top one, colored light green above, reports the current value of $\Delta E_m^{(ISP-QH^+/QH_2)}$ ($= -\Delta G_1/F$). The text box below, labeled **logKs**, reports the current value for $\log_{10}(K_s)$, calculated from:

$$E_{m(Q/QH^+)(bound)} - E_{m(QH^+/QH_2)(bound)} = 2.303 RT/F \log_{10}(K_s)$$

Changes using **Em ISP** or **delta Em** affect the values reported in these two boxes, depending on user choice, indicated by their color. The default is to leave $-\Delta G_1/F$ constant when changing **Em ISP**, so that K_s varies. Alternatively, K_s can be held constant, so that $-\Delta G_1/F$ changes as **Em ISP** is varied. This choice is selected by clicking on the **logKs** text box (which changes to light green to show the selection). The other option is selected by clicking on the delta Em text box above. Changing **delta Em** with $E_{m(ISP)}$ fixed necessarily changes both $E_{m(Q/QH^+)(bound)}$ and K_s , and also the driving force for the second electron. The program takes care of the relationship between all these terms, and uses the new values for subsequent calculations, and the next displayed graph.

Second and third rows, - parameters for the first and second electron transfer reactions, as indicated.

The labels are colored red and yellow to reflect the change in color on the plotted curves, described below.

lamda: The value for reorganization energy, λ , is input by using the scroll bar. The current value is reported in the adjacent text box. The second text box shows the value for λ calculated using the Marcus relationship:

$$\Delta G^\# = (\lambda + \Delta G)^2 / 4\lambda$$

The value for λ is calculated internally using a crude iterative procedure, and might fail at extremes of the range, so use with caution. The reporter text box changes to light blue as a warning if the current value is larger than the Marcus value. Since the latter changes with other input parameters, this color change also occurs whenever the above condition is satisfied on changing other parameters.

distance: The distance between redox centers, assumed to be the distance between nearest atoms of the conjugated electronic system (2). The adjacent text box shows the current value.

Fourth row:

Change hidden parameters: Parameters not accessible through the scroll bars can be changed by clicking an item in the drop-down list. This brings up an Input box, allowing the parameter to be modified. The new value is then displayed in the drop-down list, and in any listing of parameters elsewhere in the program, and is used in calculation of other parameters.

Changing the activation energies changes the other parameters of the Marcus relationship, which are changed in the appropriate text boxes.

The value **Em(Q/QH2)(bound)** reflects the differential binding of QH_2 over Q in formation of the ES-complex. It is assumed that the activation barrier reflects the reaction from

this state. Changing the value will change K_s , since values for the semiquinone couples are calculated using:

$$E_{m(Q/QH\cdot)(bound)} + E_{m(QH\cdot/QH2)(bound)} = 2E_{m(Q/QH2)(bound)}$$

The value for $E_{m(QH\cdot/QH2)(bound)}$ is set by **Em ISP** and **delta Em**.

Values **pKISPOx** and **pKQH2** are used only when the equations modified by the Brønsted term are used. The value are used to determine the Brønsted term, and their meaning is obvious.

Parameter values: Current values for all parameters are shown in the message box that pops up when this button is clicked.

Other buttons:

Clear: Clears the image area

Save: Saves the current parameters, and the curves, to a file. The file is stored in the local directory. If the default file name (Marcus_data.txt) is used, the save will overwrite any previous data. The curves are stored as X, Y pairs, suitable for import to graphics programs.

Zoom: Zooms in to the central area of the plot for a closer look.

Print: Prints the parameters, and the current curves, to the default system printer.

The display.

The curves calculated as above are displayed in the image area. The curves are updated by clicking on the image area. The area can be cleared to avoid clutter by clicking the **Clear** button (see above).

Also shown in the display are the following:

Go: A vertical bar at $\Delta G^{\circ}/F$ (to give the value in eV) for the overall reaction, using the relationship (10, and see above):

$$\Delta G^{\circ} = -\Sigma(zFE) = -F\{(E_{m(ISP)} + E_{m(bL)}) - 2E_{m(Q/QH2)(pool)}\}$$

Gb: A vertical bar at $\Delta G^{\circ}/F$ for the reaction from the ES-complex, using the relationship (1):

$$\Delta G^{\circ} = -\Sigma(zFE) = -F\{(E_{m(ISP)} + E_{m(bL)}) - 2E_{m(Q/QH2)(bound)}\}$$

E1: A vertical bar at $\Delta G_1/F$ for the first electron transfer, calculated from:

$$\Delta G_1/F = -(E_{m(ISP)} - E_{m(QH\cdot/QH2)(bound)})$$

E2: A vertical bar at $\Delta G_2/F$ for the second electron transfer, calculated from:

$$\Delta G_2/F = -(E_{m(bL)} - E_{m(Q/QH\cdot)(bound)})$$

When equations including the Brønsted term are used, the value ($pK_{QH2} - pK_{ISPOx}$) is subtracted from E1 to give the driving force for the electron transfer component.

The curves: The color of the curves changes (to red or yellow, for 1st. and 2nd. electron transfers, respectively) when the value for the rate of the reaction is in the range compatible with the experimental results. Rate is calculated from:

$$v = k(\text{occupancy})$$

A value of $k = 1.5 \cdot 10^3 \text{ s}^{-1}$ was used for the measured overall rate constant. An occupancy of $1 \text{ mol.}(\text{mol bc}_1)^{-1}$ for the ES-complex (saturating substrates) was assumed for the 1st. electron transfer. For the 2nd. electron transfer, the occupancy of the intermediate semiquinone was calculated using:

$$K_1 = \exp\{-\Delta G_1/RT\}$$

$$\text{Occupancy} = K_1 / (1 + K_1)$$

This ignores the contribution of dissociation to products to the ΔG for the first electron transfer, but simplifies the program.

Text values: At the intercept of the Marcus curve with the ΔG value for each reaction, the rate was calculated, and is displayed on the screen in red (1st. electron) or yellow (2nd. electron). Also displayed is the slope of the curve ($d(\log_{10}(k))/d(\Delta G/F)$) at this point, in units mV^{-1} . Note that the sign is determined by the sign for ΔG (see note above), and that the x-scale has ΔG with -ve values to the right.

Use of the program depends on the nature of the mechanistic model being tested. As a general rule, in a reaction showing the kinetic complexity (11) of the Q_o -site bifurcated reaction, one needs to identify the rate limiting partial process. For this process, a satisfactory fit of the plotted curve to the experimental values is found when the three lines, the Marcus curve, the observed k_{cat} , given by the horizontal line, and the driving force, represented by the vertical line appropriate to the partial process, intercept at a single point. The treatment here is based on the experimental evidence showing that the first electron transfer is limiting.

For further discussion, see references 12, 13.

References

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