S1: Concentration and Time Scales in the Enzyme-Substrate Reaction

\[ E + S \xrightarrow{k_f} C \xrightarrow{k_{cat}} E + P \]

SFigure 1: The enzyme-substrate reaction written in canonical form

The minimal set of ODEs that describe the enzyme-substrate dynamics is given the following two equations:

\[
\frac{dC(t)}{dt} = k_f \cdot (E_0 - C(t)) \cdot S(t) - (k_r + k_{cat}) C(t) \quad (1)
\]

\[
\frac{dS(t)}{dt} = -k_f \cdot (E_0 - C(t)) \cdot S(t) + (k_r) C(t) \quad (2)
\]

The first step in analyzing the two ODEs is to rescale the dynamical variables for complex and substrate in a way such that they range from 0 to 1. This is done by substituting a rescaled variable that is the original variable divided by its maximal value. For substrate the rescaling is trivial as the maximal value is the initial amount of substrate. For complex the rescaling constant is slightly more difficult to find, as it starts at 0 and then ends at 0. However, we can find its rescaling constant by estimating the maximal value achieved by complex over the course of the reaction. This can be found at the point where the derivative of the complex with respect to time is zero (i.e. when the concentration of complex switches from being a rising value to a falling one). We denote the maximal concentration of complex to be \( \bar{C} \).

\[
0 \equiv k_f \cdot (E_0 - \bar{C}) \cdot S(t_{\text{max}}) - (k_r + k_{cat}) \bar{C} \quad (3)
\]

Solving for \( \bar{C} \) yields the following:

\[
\bar{C} = \frac{E_0 S(t_{\text{max}})}{S(t_{\text{max}}) + B} \quad (4)
\]

\[
B = \frac{k_r + k_{cat}}{k_f} \quad (5)
\]

\( S(t_{\text{max}}) \) is the concentration of the substrate at the time when the complex is at its maximal concentration. However, based on simulation results, the concentration of the substrate at the point where the concentration of complex is maximal is very close to \( S_0 \). Therefore a good approximation of the maximal concentration of the complex is given by this expression:

\[
\bar{C} = \frac{E_0 S_0}{S_0 + B} \quad (6)
\]

Here we recognize that the constant \( B \) is in fact the Michaelis-Menten constant \( K_M \), and emerged naturally from the non-dimensionalization process. The two rescaling constants \( S_0 \) and \( \bar{C} \) allow us to redefine new dynamical variables:
The form of the ODEs after non-dimensionalization for concentration is the following:

\[
\begin{align*}
C(t) & \rightarrow \tilde{c}(t) \cdot \bar{C} \\
S(t) & \rightarrow \tilde{s}(t) \cdot S_0
\end{align*}
\] (7)

\[
\begin{align*}
\frac{d\tilde{c}(t)}{dt} & = k_f \cdot (K_M + S_0) \tilde{s}(t) - k_f S_0 \tilde{c}(t) \tilde{s}(t) - (k_r + k_{cat}) \tilde{c}(t) \\
\frac{d\tilde{s}(t)}{dt} & = -k_f E_0 \tilde{s} + \frac{k_f E_0 S_0}{K_M + S_0} \tilde{c}(t) \tilde{s}(t) + \frac{k_r E_0}{K_M + S_0} \tilde{c}(t)
\end{align*}
\] (9)

An important but subtle point to note is that the rate constants have been altered in using rescaled dynamical variables. For example, consider the first term on the right-hand-side of the original ODE for \( \frac{dC(t)}{dt} \) which is \( k_f \cdot E_0 \cdot S(t) = 10^{-3} M^{-1} s^{-1} \cdot S(t) \). After rescaling, this term is transformed into \( k_f \cdot (K_M + S_0) \cdot \tilde{s}(t) = 2.1 \cdot 10^{-1} s^{-1} \tilde{s}(t) \). These changes are not merely cosmetic, as they now allow a direct comparison of rates of substrate and complex formation. Let us substitute the rescaled equations with numbers to show explicitly that one variable has much faster rates than the other:

\[
\begin{align*}
\frac{d\tilde{c}(t)}{d\tau} & = 20.0 \ s^{-1} \cdot \tilde{s}(t) - 10.0 \ s^{-1} \cdot \tilde{c}(t) \tilde{s}(t) - 11.0 \ s^{-1} \cdot \tilde{c}(t) \\
\frac{d\tilde{s}(t)}{d\tau} & = -0.1 \ s^{-1} \cdot \tilde{s}(t) + 0.048 \ s^{-1} \cdot \tilde{c}(t) \tilde{s}(t) + 0.048 \ s^{-1} \cdot \tilde{c}(t)
\end{align*}
\] (11)

The units for the rates no longer contain concentration units but still contain time. To proceed, we non-dimensionalize time using a fast time-scale. The reason for doing so is that the non-dimensionalization and rescaling brings the fast rates into the foreground by setting them close to 1, so that they may be compared against other rates. The comparison helps us in our subsequent approximation decisions by identifying which rates are slower, and how much slower they are. Later we will repeat the process for slower time scales to bring those into the foreground. In fact, the key point of examining dynamics at different time-scales and building different approximations for each scale is at the heart of singular perturbation theory. A number of choices are possible, but here we pick \( \tilde{t} \equiv (k_f S_0)^{-1} \). The equations for the early phase after non-dimensionalization with the fast time-scale are given as the following:

\[
\begin{align*}
\tau & \rightarrow t/\tilde{t} \\
\frac{d\tilde{c}(t)}{d\tau} & = \frac{K_M + S_0}{S_0} \tilde{s}(t) - \tilde{c}(t) \tilde{s}(t) - \frac{K_M}{S_0} \tilde{c}(t) \\
\frac{d\tilde{s}(t)}{d\tau} & = -E_0 \tilde{s}(t) + \frac{E_0}{K_M + S_0} \tilde{c}(t) \tilde{s}(t) + \frac{k_r E_0}{k_f S_0 (K_M + S_0)} \tilde{c}(t)
\end{align*}
\] (13-15)

For completeness, we explicitly show the values for these equations. The units for the rates are now entirely dimensionless:

\[
\begin{align*}
\frac{d\tilde{c}(t)}{d\tau} & = 2.0 \cdot \tilde{s}(t) - 1.0 \cdot \tilde{c}(t) \tilde{s}(t) - 1.1 \cdot \tilde{c}(t) \\
\frac{d\tilde{s}(t)}{d\tau} & = -0.01 \cdot \tilde{s}(t) + 0.0048 \cdot \tilde{c}(t) \tilde{s}(t) + 0.0048 \cdot \tilde{c}(t)
\end{align*}
\] (16-17)

Nothing has really changed except for the fact that the rates for complex are clearly on the order of 1, and the rates for substrate are two orders of magnitude slower. The 100-fold difference in rates allows us to...
apply what is called 0th order singular perturbation. Essentially, it means that in the early phase, as the concentration of complex changes, changes in concentration of substrate are so slow that we can effectively set \( dS(t)/dt \) to be 0. Thus in the early phase, the original dynamics from Equations 1-2 can be approximated by the following expressions:

\[
\begin{align*}
\frac{d\tilde{c}(\tau)}{d\tau} &= \frac{K_M + S_0}{S_0}(1 - \tilde{c}(\tau)) \\
\frac{d\tilde{s}(\tau)}{d\tau} &= 0 \\
\tilde{c}(0) &= 0 \\
\tilde{s}(0) &= 1
\end{align*}
\]

Because the rescaled substrate is effectively constant, its solution is trivial to obtain, reducing the system from a pair of coupled, nonlinear differential equations to a single, linear differential equation. The early phase solution to the reduced system is given as follows, from which the dynamics for the original non-scaled variables may be obtained. We call this solution the “inner” solution for reasons to be explained later.

\[
\begin{align*}
\tilde{c}_{inner}(\tau) &= 1 - \exp\left(-\frac{S_0 + K_M}{S_0} \tau\right) \\
\tilde{s}_{inner}(\tau) &= 1 \\
C_{inner}(t) &= \frac{E_0 S_0}{S_0 + K_M} (1 - \exp (-k_f(S_0 + K_M)t)) \\
S_{inner}(t) &= S_0
\end{align*}
\]

What do the dynamics of this solution look like? As the rescaled time \( \tau \) approaches infinity, \( \tilde{c}(\tau) \) approaches 1, and \( \tilde{s}(\tau) \) stays at 1. However this cannot be the whole story, because from a physical point of view, we know both substrate and complex should decay to 0 in the long run as they are converted into product. This is because the “inner” solution holds only for the early phase (up to \( t = 30s \). Thus, when we take the limit of \( \tau \to \infty \), we mean a “long time” for the early phase, but not so long that we enter the regime of the late phase. To understand the dynamics of the late phase, we must rescale time again so that the rates for the substrate are effectively 1. To do so, we make the substitution \( \tilde{\tau} = \tau / \left(\frac{K_M + S_0}{E_0}\right) \).

\[
\begin{align*}
\frac{d\tilde{c}(\tilde{\tau})}{d\tilde{\tau}} &= \frac{K_M + S_0}{E_0} \left(\frac{K_M + S_0}{S_0} \tilde{s}(\tilde{\tau}) - \tilde{c}(\tilde{\tau}) \tilde{s}(\tilde{\tau}) - \frac{K_M}{S_0} \tilde{c}(\tilde{\tau})\right) \\
\frac{d\tilde{s}(\tilde{\tau})}{d\tilde{\tau}} &= \frac{K_M + S_0}{S_0} \tilde{s}(\tilde{\tau}) + \tilde{c}(\tilde{\tau}) \tilde{s}(\tilde{\tau}) + \frac{k_r}{k_f S_0} \tilde{c}(\tilde{\tau})
\end{align*}
\]

Again, for completeness, we explicitly show the effective rates that result from this transformation.

\[
\begin{align*}
\frac{d\tilde{c}(t)}{d\tau} &= 420.0 \cdot \tilde{s}(t) - 210.0 \cdot \tilde{c}(t) \tilde{s}(t) - 462.0 \cdot \tilde{c}(t) \\
\frac{d\tilde{s}(t)}{d\tau} &= -2.1 \cdot \tilde{s}(t) + 2.016 \cdot \tilde{c}(t) \tilde{s}(t) + 2.016 \cdot \tilde{c}(t)
\end{align*}
\]

As before, we may apply 0th order singular perturbation theory. The dynamics in the late phase is slightly different from those in the early phase. In the early phase the rate for change in concentration of complex is near 1, and for substrate much slower. In the late phase the rate for change in concentration of substrate is near 1, and for complex much faster. Because complex is fast, it can be considered to be in
a quasi-equilibrium condition. Mathematically this means that the left-hand-side can be set to zero in the dynamical equation for complex.

\[
\frac{d\tilde{c}(\tau)}{d\tilde{\tau}} = 0 = \frac{K_M + S_0}{S_0} \tilde{s}(\tau) - \tilde{c}(\tau) \tilde{s}(\tau) - \frac{K_M}{S_0} \tilde{c}(\tau) \tag{30}
\]

\[
\frac{d\tilde{s}(\tau)}{d\tilde{\tau}} = \frac{K_M + S_0}{S_0} \tilde{s}(\tau) + \tilde{c}(\tau) \tilde{s}(\tau) + \frac{k_r}{k_f S_0} \tilde{c}(\tau) \tag{31}
\]

\[
\tilde{c}(0) = 1 \tag{32}
\]

\[
\tilde{s}(0) = 1 \tag{33}
\]

Although it is not apparent, the resulting pair of ODEs has again been reduced to a single variable system. Using the first equation, complex can be solved in terms of substrate. The expression can then be substituted into the second equation, rendering it dependent only on substrate. We leave the derivation as an exercise for the reader and present the so-called “outer” solution here.

\[
\tilde{c}_{\text{outer}}(\tilde{\tau}) = \frac{K_M + S_0}{S_0} \tilde{s}_{\text{outer}}(\tilde{\tau}) \tag{34}
\]

\[
\tilde{s}_{\text{outer}}(\tilde{\tau}) - \tilde{s}_0 + \frac{K_M}{S_0} = -\frac{K_M + S_0}{k_f S_0} \tilde{c}_{\text{outer}} \tilde{\tau} \tag{35}
\]

\[
C_{\text{outer}}(t) = \frac{E_0 \tilde{s}_{\text{outer}}(t)}{\tilde{s}_{\text{outer}}(t) + K_M} \tag{36}
\]

\[
S_{\text{outer}}(t) - S_0 + K_M \log \frac{\tilde{s}_{\text{outer}}(t)}{S_0} = -k_{\text{cat}} E_0 t \tag{37}
\]

Plotting the solution for both complex and substrate now shows the correct behavior: both start at 1 (the maximal values respectively) and decay to 0. However, the astute reader will recognize that something seems wrong because at time 0, there is no complex, yet the outer solution tells us that complex starts at 1. The reason is that just as the inner solution is incorrect at infinite time, the outer solution is incorrect at time 0. The outer solution is only valid starting at some time \( \tilde{t} \) as defined previously.

The two solutions need to be stitched together for a complete solution that spans all time-scales. This is done by first confirming that the behavior of the inner and outer solutions match at the boundary conditions. That is, the long-term behavior of the early phase should match up with the initial behavior of the late phase. Mathematically, this is expressed as the requirement that limit of the inner solution as \( \tau \to \infty \) is equivalent to the limit of the outer solution as \( \tilde{\tau} \to 0 \). The matching of boundary conditions underlies the concept of “matched asymptotic expansions” when using singular perturbation theory to solve differential equations.

For the rescaled concentration of substrate, \( \tilde{s}_{\text{inner}}(\tau) \to 1 \) as \( \tau \to \infty \), and \( \tilde{s}_{\text{outer}}(\tilde{\tau}) \to 1 \) as \( \tilde{\tau} \to 0 \). Similarly for the rescaled concentration of complex, \( \tilde{c}_{\text{inner}}(\tau) \to 1 \) as \( \tau \to \infty \), and \( \tilde{c}_{\text{outer}}(\tilde{\tau}) \to 1 \) as \( \tilde{\tau} \to 0 \).

Thus, the complete solution in rescaled units is given by the sum of the inner and outer solutions, but with the boundary value subtracted out:

\[
\tilde{s}(t) = \tilde{s}_{\text{inner}}(t) + \tilde{s}_{\text{outer}}(t) - 1 + \epsilon \tag{38}
\]

\[
\tilde{c}(t) = \tilde{c}_{\text{inner}}(t) + \tilde{c}_{\text{outer}}(t) - 1 + \epsilon \tag{39}
\]

The \( \epsilon \) indicates an error that must accompany any inexact approximation. Removing the rescaling yields the full solution for substrate and complex in units of moles.

\[
\hat{S}(t) \approx \hat{S}_{\text{inner}}(t) + \hat{S}_{\text{outer}}(t) - S_0 \tag{40}
\]

\[
\hat{C}(t) \approx \hat{C}_{\text{inner}}(t) + \hat{C}_{\text{outer}}(t) - \frac{E_0 S_0}{K_M + S_0} \tag{41}
\]
S2: Nonclassical Regime for the Enzyme-Substrate Reaction

The classical Michaelis-Menten presentation of the enzyme-substrate reaction (SFig. 1) is valid for a particular set of enzyme and substrate conditions, drawn from a detailed consideration of metabolic pathways. In the treatment published by Briggs and Haldane, this was noted as the condition $E_0 \ll S_0$, i.e. concentration of the catalytic enzyme is much lower than concentration of metabolic substrate. Later analysis shows that the more complete condition for validity of classical analysis is given by $E_0 \ll (S_0 + K_M)$. On the other hand, there is not much analysis in literature of the enzyme-substrate reaction when such conditions are violated. Here we give a full treatment of the enzyme-substrate reaction for a case in which the classical condition is violated, $E_0 \approx S_0 + K_M$, i.e. the enzyme concentration $E_0$ is on the same order of magnitude as the sum $K_M + S_0$.

The condition $E_0 \approx S_0 + K_M$ is in fact not unusual. For example, in certain cancer cell lines, the concentration of EGFR (the receptor that phosphorylates substrate proteins) is on the order of $10^{-6}M$, $K_M \approx 10^{-6}M$ and the concentration of substrate protein Shc (a target of EGFR) is $S_0 \approx 10^{-7}M$.

As in our analysis of the classical condition, we work in the reduced system of $C(t)$ and $S(t)$.

The minimal number of ODEs needed after eliminating variables with the help of the conservation conditions is two as before:

\[
\frac{dC(t)}{dt} = k_f \cdot (E_0 - C(t)) \cdot S(t) - (k_r + k_{cat}) C(t) \tag{42}
\]
\[
\frac{dS(t)}{dt} = -k_f \cdot (E_0 - C(t)) \cdot S(t) + (k_r) C(t) \tag{43}
\]

Here we assume classical rate constants and but non-classical concentrations of enzyme and substrate:

\[
k_f = 10^5 M^{-1}s^{-1} \tag{44}
\]
\[
k_r = 10^{-1} s^{-1} \tag{45}
\]
\[
k_{cat} = 10^{-2} s^{-1} \tag{46}
\]
\[
E_0 = 10^{-6} \tag{47}
\]
\[
S_0 = 10^{-7} \tag{48}
\]

The concentration is as before:

\[
\bar{C} = \frac{E_0S_0}{S_0 + K_M} \tag{49}
\]

\[
C(t) \rightarrow \bar{c}(t) \cdot \bar{C} \tag{50}
\]

\[
S(t) \rightarrow \bar{s}(t) \cdot S_0 \tag{51}
\]

Dynamics after concentration non-dimensionalization:

\[
\frac{d\bar{c}(t)}{dt} = k_f \cdot (K_M + S_0) \cdot \bar{s}(t) - k_fS_0\bar{c}(t)\bar{s}(t) - (k_r + k_{cat}) \bar{c}(t) \tag{52}
\]
\[
\frac{d\bar{s}(t)}{dt} = -k_fE_0\bar{s}(t) + \frac{k_fE_0S_0}{K_M + S_0} \cdot \bar{c}(t)\bar{s}(t) + \frac{k_rE_0}{K_M + S_0} \bar{c}(t) \tag{53}
\]
Examining the non-dimensionalized equations of motion reveal that the slow processes are very different for this condition versus the classical condition. This is clearer to see if values are substituted into all the rate constants and initial conditions.

\[
\frac{d\tilde{c}(t)}{dt} = -\frac{11}{100} \tilde{c}(t) + \frac{3}{25} \tilde{s}(t) - \frac{1}{100} \tilde{c}(t)\tilde{s}(t) \tag{54}
\]
\[
\frac{d\tilde{s}(t)}{dt} = \frac{1}{12} \tilde{c}(t) - \frac{1}{10} \tilde{s}(t) + \frac{1}{120} \tilde{c}(t)\tilde{s}(t) \tag{55}
\]

Whereas for the classical case, the non-dimensionalized rates for \( \tilde{c}(t) \) were uniformly much faster compared to \( \tilde{s}(t) \), the non-classical case is characterized by slow and fast rates in dynamics of both non-dimensionalized substrate and complex. To proceed, we remove the terms with the slowest rates containing the product \( \tilde{c}(t)\tilde{s}(t) \).

\[
\frac{d\tilde{c}(t)}{dt} = k_f \cdot (K_M + S_0) \tilde{s}(t) - (k_r + k_{cat}) \tilde{c}(t) \tag{56}
\]
\[
\frac{d\tilde{s}(t)}{dt} = -k_f E_0 \tilde{s}(t) + \frac{k_r E_0}{K_M + S_0} \tilde{c}(t) \tag{57}
\]

In this approximation, the system is linear and can be trivially solved by diagonalizing the right-hand-side.

\[
\frac{d}{dt} \begin{pmatrix} \tilde{c}(t) \\ \tilde{s}(t) \end{pmatrix} = M \begin{pmatrix} \tilde{c}(t) \\ \tilde{s}(t) \end{pmatrix} \tag{58}
\]
\[
M = \begin{pmatrix} k_f \cdot (K_M + S_0) & -(k_r + k_{cat}) \\ -k_f E_0 & \frac{k_r E_0}{K_M + S_0} \end{pmatrix} \tag{59}
\]

The matrix \( M \) can be diagonalized to give dynamics of the following form:

\[
\frac{d}{dt} \begin{pmatrix} \xi_1(t) \\ \xi_2(t) \end{pmatrix} = \begin{pmatrix} -\lambda_1 & 0 \\ 0 & -\lambda_2 \end{pmatrix} \begin{pmatrix} \xi_1(t) \\ \xi_2(t) \end{pmatrix} \tag{60}
\]
\[
\begin{pmatrix} \xi_1^0 \\ \xi_2^0 \end{pmatrix} = P^{-1} \begin{pmatrix} 0 \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ \lambda_1 \lambda_2 \end{pmatrix} \tag{61}
\]
\[
\begin{pmatrix} \tilde{c}(t) \\ \tilde{s}(t) \end{pmatrix} = P \begin{pmatrix} \xi_1(t) \\ \xi_2(t) \end{pmatrix} \tag{62}
\]

The method for obtaining the solution is now straightforward as it is merely a linear ODE. The dynamics can be trivially solved for the linear system, which is an exponentially decaying function for both components \( \xi_1(t) \) and \( \xi_2(t) \). Using the last line one can transform the solution back into the variables \( \tilde{c}(t) \) and \( \tilde{s}(t) \). The algebra for the exact solution is tedious. Instead, here we identify the two salient aspects of the solution, which should suffice to yield a very accurate approximation of the dynamics for \( \tilde{c}(t) \) and \( \tilde{s}(t) \).

The first important detail to note is that the eigenvalues \( \lambda_1 \) and \( \lambda_2 \) constitute the two time-scales observed in the figure showing dynamics. These are rather simple to express in terms of the fundamental constants:

\[
\lambda_1 = -\frac{1}{2} \left[ k_{cat} + k_f E_0 + k_r + \sqrt{(k_f E_0 + k_r + k_{cat})^2 - 4k_f E_0 k_{cat}} \right] \tag{63}
\]
\[
\lambda_2 = -\frac{1}{2} \left[ k_{cat} + k_f E_0 + k_r - \sqrt{(k_f E_0 + k_r + k_{cat})^2 - 4k_f E_0 k_{cat}} \right] \tag{64}
\]
The second detail is that the early phase of the dynamics is characterized by the early rise of $\tilde{c}(t)$ and the early fall of $\tilde{s}(t)$ to intermediate values. The point at which they meet can be approximated by the condition when the eigenvector $\xi_1(t)$ has decayed to near 0.

$$\begin{pmatrix} \tilde{c}_{\text{int}} \\ \tilde{s}_{\text{int}} \end{pmatrix} = P \cdot \begin{pmatrix} 0 \\ (P^{-1})_{2,2} \end{pmatrix} \quad (66)$$

Therefore, the complete description of dynamics in this regime is as follows. The complex and substrate execute exponential rise and fall respectively with a fast rate ($t \approx \frac{1}{\lambda_1}$) until they reach an intermediate point $\begin{pmatrix} \tilde{c}_{\text{int}} \\ \tilde{s}_{\text{int}} \end{pmatrix}$, after which they both decay exponentially with a slow rate ($t \approx \frac{1}{\lambda_2}$).