KINETIC MODEL

Time of addition experiments involve the addition of HIV viral entry inhibitors at fixed times after temperature jump. Maraviroc, an antagonist of CCR5, and T20, a fusion inhibitor is typically administered in excess after a fixed time $T_{\rm i}$ after temperature jump.

For the CCR5 antagonist, we must model the kinetics of CCR5 binding to the gp120 glycoprotein subunit. For a single complex, typically a trimer of heterodimers of gp120 and gp41, we assume each gp120 can bind to CCR5. Only after a requisite number of CCR5 bind can fusion of a single virus particle occur.

Define the probabilities that any CD4-gp160 complex (or the fraction of complexes) has i CCR5 molecules bound as P_i. The rate equations are

$$\begin{aligned}
\frac{{\rm d}P_0(t)}{{\rm d}t} =& - p_0 P_0 + q_1 P_1 \\
\frac{{\rm d}P_1(t)}{{\rm d}t} =& p_0 P_0 - p_1 P_1 + q_2 P_2 \\
\frac{{\rm d}P_2(t)}{{\rm d}t} =& p_1 P_1 - p_2 P_2 + q_3 P_3 \\
& \cdots \\
\frac{{\rm d}P_N(t)}{{\rm d}t} =& p_{N-1} P_{N-1} - k_{\rm f} P_N \\
\frac{{\rm d}Q(t)}{{\rm d}t} =& k_{\rm f} P_N
\end{aligned} \tag{1}$$

where $p_n \propto c(t)$ is the binding rate of CCR5, which depends on the concentration $c(t)$ CCR5 molecules on the cell surface that are unbound to Maraviroc. The constant rate of detachment of CCR5 from a complex with n CCR5's bound are defined by q_n. The fusion rate is defined as $k_{\rm f}$. The free CCR5 and Maraviroc kinetics are described by

$$\frac{{\rm d}c(t)}{{\rm d}t} = -k_{\rm a} m(t) c(t) + k_{\rm d}(c_0 - c(t)), \tag{2}$$

where $c(t)$ is the surface concentration of CCR5 and $m(t)$ describes the depth-average Maraviroc concentration with units of number/area. The initial free CCR5 and effective 2D Maraviroc concentration are c_0 and m_0, respectively. Note that $m_0 > c_0$ m,eans as excess of Maraviroc is administered. From conservation, $m(t) = m_0 - c_0 + c(t)$. Solving Eq. 2, we find

$$\frac{\mathrm{d}c(t)}{\mathrm{d}t} = -k_{\mathrm{a}}c(t) + k_{\mathrm{d}}(c_0 - c(t)), \tag{3}$$

where c_0 is the initial concentration of CCR5 before administration of antagonist. The solution for the free CCR5 concentration after administration of inhibitor at time T_{i} is

$$c(t;T_{\mathrm{i}}) = c_0\left[1-\Theta(t-T_{\mathrm{i}})\right] + c_0\left[\left(1-\frac{k_{\mathrm{d}}}{K}\right)e^{-K(t-T_{\mathrm{i}})} + \frac{k_{\mathrm{d}}}{K}\right]\Theta(t-T_{\mathrm{i}}), \tag{4}$$

where $K \equiv k_{\mathrm{a}} + k_{\mathrm{d}}$. The model above assumes that no reaction is diffusion limited, either in bulk of on the cell surface.

The total probability of a single virus entering up to the measurement time T_{m} (typically 48 hours) is simply $Q(T_{\mathrm{m}})$. Given rates q_n and using rates of the form $p_n = k_n c(t;T_{\mathrm{i}}) \equiv (N-n)k_{\mathrm{b}}c(t;T_{\mathrm{i}})$, where k_{b} is an intrinisic rate per concentration of inhibitor binding to CCR5, we can solve Eqs. 1 numerically and find $Q(T_{\mathrm{m}})$ for various T_{i}. For a small MOI and a collection of cells, the fraction of cells infected after time T_{m} is simply $Q(T_{\mathrm{m}})$.

Let's assume a simplification for which we can find analytic or semi-analytic solutions to make the comparison with data more efficient. First, if the binding of CCR5 is strong, $q_n \approx 0$ and we can set $k_{\mathrm{f}} = 0$ and approximate $Q(T_{\mathrm{m}}) \approx P_N(\infty)$. We can define a new time variable

$$\tau(t) = \int_0^t c(t';T_{\mathrm{i}})\mathrm{d}t' \tag{5}$$

and write the rate equations as

$$\begin{aligned}
\frac{\mathrm{d}P_0(\tau)}{\mathrm{d}\tau} &= -k_0P_0 \\
\frac{\mathrm{d}P_1(\tau)}{\mathrm{d}\tau} &= k_0P_0 - k_1P_1 \\
\frac{\mathrm{d}P_2(\tau)}{\mathrm{d}\tau} &= k_1P_1 - k_2P_2 \\
&\cdots \\
\frac{\mathrm{d}P_N(\tau)}{\mathrm{d}\tau} &= k_{N-1}P_{N-1}
\end{aligned} \tag{6}$$

Upon using Laplace transforms, we solve for $\tilde{P}_N(s)$ to find

$$\tilde{P}_N(s) = \frac{1}{s}\prod_{n=0}^{N-1}\frac{k_n}{(s+k_n)}, \tag{7}$$

and the τ-dependent solution

$$P_N(\tau) = \left(1 - e^{-k_\mathrm{b}\tau(t)}\right)^N. \tag{8}$$

For the fusion inhibitor experiment (T20), we can still use Eqs. 1 but since the inhibitor does not bind with CCR5, the rates $p_n = k_n c_0$ are constant in time. Rather, the fusion inhibitor peptide binds to the pre-fusion complex and prevents the last step described by rate k_f. The probability that the pre-fusion complex (or fraction of complexes) is not bound to the inhibitor is denoted $P_\mathrm{u}(t)$. Therefore, the rate of fusion is $k_\mathrm{f} P_\mathrm{u}(t)$, where

$$\frac{\mathrm{d}P_\mathrm{u}}{\mathrm{d}t} = -k_\mathrm{a} P_\mathrm{u}(t) + k_\mathrm{d}(1 - P_\mathrm{u}(t)), \tag{9}$$

leading to

$$P_\mathrm{u}(t; T_\mathrm{i}) = [1 - \Theta(t - T_\mathrm{i})] + \left[\left(1 - \frac{k_\mathrm{d}}{K}\right) e^{-K(t-T_\mathrm{i})} + \frac{k_\mathrm{d}}{K}\right] \Theta(t - T_\mathrm{i}), \tag{10}$$

SIMPLEST MODEL INCLUDING BOTH CCR5 AND FUSION INHIBITORS

Consider 5 species, P_0, the CD4-attached complex, P_1, the activated CD4-attached complex, P_2, the CD4-CCR5 complex, P_3, the activated CD4-CCR5 complex, and Q, the viruses that have entered.

$$\begin{aligned}
\frac{\mathrm{d}P_0(t)}{\mathrm{d}t} &= -\,p_0 P_0 + q_1 P_1 \\
\frac{\mathrm{d}P_1(t)}{\mathrm{d}t} &= p_0 P_0 - (p_1 + q_1) P_1 + q_2 P_2 \\
\frac{\mathrm{d}P_2(t)}{\mathrm{d}t} &= p_1 P_1 - (p_2 + q_3) P_2 + q_3 P_3 \\
\frac{\mathrm{d}P_3(t)}{\mathrm{d}t} &= p_2 P_2 - (p_3 + k_\mathrm{f}) P_3 \\
\frac{\mathrm{d}Q(t)}{\mathrm{d}t} &= k_\mathrm{f} P_3
\end{aligned} \tag{11}$$

In the above, the rate p_2 might depend on CCR5 (and hence the antagonist) concentration while p_3 and/or k_f might depend on the T20 concentration.

For the fully irreversible case, $q_i = 0$ and we find

$$\tilde{Q}(s) = \frac{p_0 p_2 p_2}{s(s+p_0)(s+p_1)(s+p_2)(s+k_{\rm f})}. \tag{12}$$

For constant rates (no inhibitor added), the solution is

$$Q(t) = 1 + k_{\rm f} p_0 p_1 p_2 \left[\frac{e^{-k_{\rm f} t}}{k_{\rm f}(k_{\rm f}-p_0)(k_{\rm f}-p_1)(k_{\rm f}-p_2)} + \frac{e^{-p_0 t}}{p_0(p_0-k_{\rm f})(p_0-p_1)(p_0-p_2)} + \frac{e^{-p_1 t}}{p_1(p_1-k_{\rm f})(p_1-p_0)(p_1-p_2)} + \frac{e^{-p_2 t}}{p_2(p_2-k_{\rm f})(p_2-p_0)(p_2-p_1)} \right].$$

where $t = 0$ corresponds to the time at which temperature is instantly raised. The total signal is thus $Q(T_{\rm m})$ where $T_{\rm m}$ is typically 48 hours.

Suppose we stop CCR5 binding at time T. Thus at time T, the rate p_1 is set to zero. For fusion inhibitors, we set either p_2 and/or $k_{\rm f}$ equal to zero at time T.

Late stage T20: $k_{\rm f} = 0$ **at time** T

In this case, the final fusion step is shut off at time T and the total fraction entered by $Q(T_{\rm m}) \approx Q(\infty)$ is simply $Q(T)$ given by Eq. 13 above.

Further approximations are easily derived. If $p_0 \gg p_1, p_2, k_{\rm f}$,

$$Q(T) = 1 - k_{\rm f} p_1 p_2 \left[\frac{e^{-k_{\rm f} T}}{k_{\rm f}(k_{\rm f}-p_1)(k_{\rm f}-p_2)} + \frac{e^{-p_1 T}}{p_1(p_1-k_{\rm f})(p_1-p_2)} + \frac{e^{-p_2 T}}{p_2(p_2-k_{\rm f})(p_2-p_1)} \right]. \tag{13}$$

If both $p_0, p_2 \gg p_1, k_{\rm f}$,

$$Q(T) = 1 - \left(\frac{k_{\rm f} e^{-p_1 T} - p_1 e^{-k_{\rm f} T}}{k_{\rm f} - p_1} \right). \tag{14}$$

Early stage T20: $p_2 = 0$ **at time** T

For this case, we find the total fraction of infections as

$$Q(T) + P_3(T) = 1 - \frac{p_0 p_2 e^{-p_1 T}}{(p_1-p_0)(p_1-p_2)} - \frac{p_1 p_2 e^{-p_0 T}}{(p_0-p_1)(p_0-p_2)} - \frac{p_0 p_1 e^{-p_2 T}}{(p_2-p_0)(p_2-p_1)}. \tag{15}$$

Further approximations are easily derived. If $p_0 \gg p_1, p_2$, we have

$$Q(T) = 1 - \left(\frac{p_2 e^{-p_1 T} - p_1 e^{-p_2 T}}{p_2 - p_1} \right). \tag{16}$$

Finally, if $p_2 \gg p_1$, $Q(T) = 1 - e^{-p_1 T}$.

CCR5 antagonist: $p_1 = 0$ at time T

$$Q(T) + P_3(T) + P_2(T) = 1 - \left(\frac{p_1 e^{-p_0 T} - p_0 e^{-p_0 T}}{p_1 - p_0} \right). \tag{17}$$

If we take $p_0 \gg p_1$, the result reduces to the $N = 1$ result from Eq. 8:

$$Q(T) + P_3(T) + P_2(T) \approx 1 - e^{-p_0 T}. \tag{18}$$

To include detachment ($q_i > 0$), we can solve for the full problem which is messy. Let us assume just $q_2 = 0$ (CCR5 does not unbind). In this case,

$$\tilde{Q}(s) = \frac{k_\mathrm{f} p_0 p_2 p_2}{s(p_0(s + p_1) + s(s + p_1 + q_1))(k_\mathrm{f}(s + p_2) + s(s + p_2 + q_3))}. \tag{19}$$

The poles are at $s = 0$ and $s = u_\pm$ and $s = v_\pm$ where

$$u_\pm = -\frac{(p_0 + p_1 + q_1)}{2} \pm \frac{1}{2}\sqrt{(p_0 + p_1 + q_1)^2 - 4p_0 p_1} \tag{20}$$

$$v_\pm = -\frac{(k_\mathrm{f} + p_2 + q_3)}{2} \pm \frac{1}{2}\sqrt{(k_\mathrm{f} + p_2 + q_3)^2 - 4k_\mathrm{f} p_2} \tag{21}$$