DERIVING A MODEL FOR RADIOLIGAND-RECEPTOR COMPLEXES OVER TIME GIVEN THE PRESENCE OF A COMPETITOR, LAW OF MASS ACTION KINETICS, & MUTUALLY EXCLUSIVE BINDING

Consider a system where a radioligand and a competitor bind to a receptor population with specific rate constants and according to the law of mass action. Binding is reversible and mutually exclusive.

$$R + L \leftrightarrow RL$$

$$R + I \leftrightarrow RI$$

Where:

- *R* = Receptor
- *L* = Radioligand
- *I* = Competing drug (inhibitor)
- *RL* = Radioligand-receptor complex
- *RI* = Competitor-receptor complex
- k_1 = on-rate constant for *R* + *L* (min^{-1} M^{-1})
- k_2 = off-rate constant for *R* + *L* (min^{-1})
- k_3 = on-rate constant for *R* + *I* (min^{-1} M^{-1})
- k_4 = off-rate constant for *R* + *I* (min^{-1})

To simplify the differential equations, we again assume conditions where only a small fraction of the ligand or competitor (<10%) bind to the receptor, thus effectively treating the unbound radioligand and competitor as constants that are approximately equal to the total drug concentrations administered.

The binding reactions in conjunction with our assumptions lead us to the following rate equations:

$$\frac{d[RL]}{dt} = k_1[R][L] - k_2[RL]$$

$$\frac{d[RI]}{dt} = k_3[R][I] - k_4[RI]$$

$$[R] = N - [RL] - [RI]$$

Where *N* is the total concentration of receptors.

We begin by substituting the free receptor equation into both of the differential equations:

$$\frac{d[RL]}{dt} = k_1(N - [RL] - [RI])[L] - k_2[RL] = Nk_1[L] - [RL]k_1[L] - [RI]k_1[L] - k_2[RL]$$

$$\frac{d[RI]}{dt} = k_3(N - [RL] - [RI])[I] - k_4[RI] = Nk_3[I] - [RL]k_3[I] - [RI]k_3[I] - k_4[RI]$$

To simplify the notation, we will define y as *[RL]* and x as *[RI]*.

$$\frac{dy}{dt} = Nk_1[L] - k_1[L]y - k_1[L]x - k_2 y$$

$$\frac{dx}{dt} = Nk_3[I] - k_3[I]y - k_3[I]x - k_4 x$$

The initial conditions of the system are $[RL](0) = 0$ and $[RI](0) = 0$. Taking the Laplace transform of both equations yields:

$$s\hat{y} - y(0) = \frac{Nk_1[L]}{s} - k_1[L]\hat{y} - k_1[L]\hat{x} - k_2\hat{y}$$

$$s\hat{x} - x(0) = \frac{Nk_3[I]}{s} - k_3[I]\hat{y} - k_3[I]\hat{x} - k_4\hat{x}$$

Substituting in the initial conditions yields:

$$s\hat{y} = \frac{Nk_1[L]}{s} - k_1[L]\hat{y} - k_1[L]\hat{x} - k_2\hat{y}$$

$$s\hat{x} = \frac{Nk_3[I]}{s} - k_3[I]\hat{y} - k_3[I]\hat{x} - k_4\hat{x}$$

Solving the second equation for $\hat{x}$:

$$s\hat{x} + k_3[I]\hat{x} + k_4\hat{x} = \frac{Nk_3[I]}{s} - k_3[I]\hat{y}$$
$$\hat{x}(s + k_3[I] + k_4) = \frac{Nk_3[I]}{s} - k_3[I]\hat{y}$$
$$\hat{x} = \frac{\frac{Nk_3[I]}{s} - k_3[I]\hat{y}}{s + k_3[I] + k_4}$$

Now we plug this into our first equation and solve for $\hat{y}$:

$$s\hat{y} = \frac{Nk_1[L]}{s} - k_1[L]\hat{y} - k_1[L]\frac{\frac{Nk_3[I]}{s} - k_3[I]\hat{y}}{s + k_3[I] + k_4} - k_2\hat{y}$$

$$s\hat{y} + k_1[L]\hat{y} + k_1[L]\frac{\frac{Nk_3[I]}{s} - k_3[I]\hat{y}}{s + k_3[I] + k_4} + k_2\hat{y} = \frac{Nk_1[L]}{s}$$

$$s\hat{y} + k_1[L]\hat{y} - \frac{k_1[L]k_3[I]\hat{y}}{s + k_3[I] + k_4} + k_2\hat{y} = \frac{Nk_1[L]}{s} - \frac{\frac{k_1[L]Nk_3[I]}{s}}{s + k_3[I] + k_4}$$

$$\hat{y}\left(s + k_1[L] - \frac{k_1[L]k_3[I]}{s + k_3[I] + k_4} + k_2\right) = \frac{Nk_1[L]}{s} - \frac{\frac{k_1[L]Nk_3[I]}{s}}{s + k_3[I] + k_4}$$

$$\hat{y}\left(\frac{s(s + k_3[I] + k_4) + k_1[L](s + k_3[I] + k_4) - k_1[L]k_3[I] + k_2(s + k_3[I] + k_4)}{\cancel{s + k_3[I] + k_4}}\right) = \frac{Nk_1[L](s + k_3[I] + k_4) - k_1[L]Nk_3[I]}{s(\cancel{s + k_3[I] + k_4})}$$

$$\hat{y}\big(s(s + k_3[I] + k_4) + k_1[L](s + k_3[I] + k_4) - k_1[L]k_3[I] + k_2(s + k_3[I] + k_4)\big) = \frac{Nk_1[L](s + k_3[I] + k_4) - k_1[L]Nk_3[I]}{s}$$

$$\hat{y} = \frac{Nk_1[L](s + k_3[I] + k_4) - k_1[L]Nk_3[I]}{s\big(s(s + k_3[I] + k_4) + k_1[L](s + k_3[I] + k_4) - k_1[L]k_3[I] + k_2(s + k_3[I] + k_4)\big)}$$

$$\hat{y} = \frac{Nk_1[L]s \cancel{+ Nk_1[L]k_3[I]} + Nk_1[L]k_4 \cancel{- k_1[L]Nk_3[I]}}{s(s^2 + k_3[I]s + k_4 s + k_1[L]s \cancel{+ k_1[L]k_3[I]} + k_1[L]k_4 \cancel{- k_1[L]k_3[I]} + k_2 s + k_2 k_3[I] + k_2 k_4)}$$

$$\hat{y} = \frac{Nk_1[L]s + Nk_1[L]k_4}{s(s^2 + k_3[I]s + k_4 s + k_1[L]s + k_1[L]k_4 + k_2 s + k_2 k_3[I] + k_2 k_4)}$$

$$\hat{y} = \frac{Nk_1[L](s + k_4)}{s\big(s^2 + (k_3[I] + k_1[L] + k_4 + k_2)s + (k_1[L]k_4 + k_2 k_3[I] + k_2 k_4)\big)}$$

At this point, we will apply the quadratic formula to the denominator's quadratic expression:

$$\frac{-(k_3[I] + k_1[L] + k_4 + k_2) \pm \sqrt{(k_3[I] + k_1[L] + k_4 + k_2)^2 - 4(1)(k_1[L]k_4 + k_2 k_3[I] + k_2 k_4)}}{2(1)}$$

We can define new variables, K_F and K_S:

$$K_F = 0.5\left[(k_3[I] + k_1[L] + k_4 + k_2) + \sqrt{(k_3[I] + k_1[L] + k_4 + k_2)^2 - 4(1)(k_1[L]k_4 + k_2 k_3[I] + k_2 k_4)}\right]$$
$$K_S = 0.5\left[(k_3[I] + k_1[L] + k_4 + k_2) - \sqrt{(k_3[I] + k_1[L] + k_4 + k_2)^2 - 4(1)(k_1[L]k_4 + k_2 k_3[I] + k_2 k_4)}\right]$$

Given these variables, we can rewrite $\hat{y}$ as:

$$\hat{y} = \frac{Nk_1[L](s + k_4)}{s(s + K_F)(s + K_S)} = \frac{Nk_1[L]s + Nk_1 k_4[L]}{s(s + K_F)(s + K_S)} = \frac{Nk_1[L]\cancel{s}}{\cancel{s}(s + K_F)(s + K_S)} + \frac{Nk_1 k_4[L]}{s(s + K_F)(s + K_S)}$$

$$= \frac{Nk_1[L]}{(s + K_F)(s + K_S)} + \frac{Nk_1 k_4[L]}{s(s + K_F)(s + K_S)}$$

Now we are at a point where we can inverse transform our function back to the time domain. A [table of LaPlace transforms](#) (page 2-61) shows that the form of the inverse we need is:

$$y(t) = \frac{A(e^{-bt} - e^{at})}{a - b} + \frac{B}{a - b}\left[\left(\frac{1 - e^{-bt}}{b}\right) - \left(\frac{1 - e^{-at}}{a}\right)\right]$$

Substituting in our like terms yields:

$$y(t) = \frac{Nk_1[L](e^{-K_S t} - e^{K_F t})}{K_F - K_S} + \frac{Nk_1 k_4[L]}{K_F - K_S}\left[\left(\frac{1 - e^{-K_S t}}{K_S}\right) - \left(\frac{1 - e^{-K_F t}}{K_F}\right)\right]$$

$$y(t) = \frac{Nk_1[L]}{K_F - K_S}\left[(e^{-K_S t} - e^{K_F t}) + \left[\left(\frac{k_4 - k_4 e^{-K_S t}}{K_S}\right) - \left(\frac{k_4 - k_4 e^{-K_F t}}{K_F}\right)\right]\right]$$

$$y(t) = \frac{Nk_1[L]}{K_F - K_S}\left[\frac{K_S K_F e^{-K_S t} - K_S K_F e^{-K_F t} + K_F k_4 - K_F k_4 e^{-K_S t} - K_S k_4 + K_S k_4 e^{-K_F t}}{K_S K_F}\right]$$

$$y(t) = \frac{Nk_1[L]}{K_F - K_S}\left[\frac{k_4(K_F - K_S)}{K_S K_F} + \frac{\cancel{K_S}k_4 e^{-K_F t} - \cancel{K_S}K_F e^{-K_F t}}{\cancel{K_S}K_F} + \frac{K_S\cancel{K_F}e^{-K_S t} - \cancel{K_F}k_4 e^{-K_S t}}{K_S\cancel{K_F}}\right]$$

$$y(t) = \frac{Nk_1[L]}{K_F - K_S}\left[\frac{k_4(K_F - K_S)}{K_S K_F} + \frac{(k_4 - K_F)e^{-K_F t}}{K_F} - \frac{(k_4 - K_S)e^{-K_S t}}{K_S}\right]$$

Substituting back in our original notation, the final result is:

$$[RL] = \frac{Nk_1[L]}{K_F - K_S}\left[\frac{k_4(K_F - K_S)}{K_S K_F} + \frac{(k_4 - K_F)e^{-K_F t}}{K_F} - \frac{(k_4 - K_S)e^{-K_S t}}{K_S}\right]$$

Motulsky, H. J. & Mahan, L. C. The kinetics of competitive radioligand binding predicted by the law of mass action. *Molecular pharmacology* **25**, 1-9 (1984).