A Derivation of the gRNA:anti-gRNA model

In our approach to modeling molecular titration using anti-gRNA, we are interested in finding an equation that describes the equilibrium state of the system described by the following reactions:

$$\mathrm{gRNA} + \mathrm{anti{-}gRNA} \rightleftharpoons \mathrm{gRNA} : \mathrm{anti{-}gRNA} \tag{A.1}$$

$$\mathrm{gRNA} + \mathrm{Cas9} \rightleftharpoons \mathrm{gRNA} : \mathrm{Cas9} \tag{A.2}$$

gRNA:anti-gRNA serves as the inactive complex sink for the repressors of this system and gRNA:Cas9 is the active repressor complex. We would like to derive an expression for the equilibrium concentration of gRNA:Cas9. Once we have this expression, we will be able to see how this varies with changing parameters of the system. Such an expression can be found as the solution to a system of equations of the three relevant mass balance equations and the equilibrium expressions for the above two reactions. For the sake of readability, let A = [gRNA], B = [anti-gRNA], C = [Cas9], AB = [gRNA:anti-gRNA], and AC = [gRNA:Cas9]. k_1 and k_2 are the dissociation constants for (A.1) and (A.2) respectively, and A_T, B_T, and C_T, are parameters that describe the total concentrations in the system of gRNA, anti-gRNA, and Cas9 respectively. Then the system of equations is as follows:

$$A_T = A + AB + AC \tag{A.3}$$

$$B_T = B + AB \tag{A.4}$$

$$C_T = C + AC \tag{A.5}$$

$$k_1 = \frac{A \cdot B}{AB} \tag{A.6}$$

$$k_2 = \frac{A \cdot C}{AC} \tag{A.7}$$

To solve this system, we first write (A.4) as $B = B_T - AB$ and substitute that into (A.6):

$$\begin{aligned} k_1 &= \frac{A(B_T - AB)}{AB} \\ &= \frac{A \cdot B_T - A \cdot AB}{AB} \\ k_1 \cdot AB &= A \cdot B_T - A \cdot AB \\ (k_1 + A)AB &= A \cdot B_T \end{aligned} \tag{A.8}$$

Next, we write (A.3) as $AB = A_T - A - AC$ and substitute into (A.8) to get:

$$\begin{aligned} (k_1 + A)(A_T - A - AC) &= A \cdot B_T \\ k_1 \cdot A_T - k_1 \cdot AC - k_1 \cdot A + A \cdot A_T - A \cdot AC - A^2 &= A \cdot B_T \\ -A^2 - (k_1 + AC + B_T - A_T)A + K_1 \cdot A_T - k_1 \cdot AC &= 0 \end{aligned} \tag{A.9}$$

We are left with a system of 3 equations consisting of (A.5), (A.9), (A.7). We can write (A.5) as $C = C_T - AC$ and substitute that into (A.7) to get

$$\begin{aligned} k_2 &= \frac{A \cdot (C_T - AC)}{AC} \\ k_2 \cdot AC &= (A \cdot (C_T - AC) \\ A &= \frac{k_2 \cdot AC}{C_T - AC} \end{aligned} \tag{A.10}$$

We can then substitute (A.10) into (A.9), term by term:

$$-A^2 = -\frac{(k_2AC)^2}{(C_T - AC)^2} \tag{A.11}$$

$$\begin{aligned}-(k_1 + AC + B_T - A_T)A &= -\frac{(k_1 + AC + B_T - A_T)(k_2AC)}{C_T - AC} \\ &= -\frac{(k_1 + B_T - A_T)(k_2AC)}{C_T - AC} - \frac{k_2AC^2}{C_T - AC}\end{aligned} \tag{A.12}$$

$$K_1A_T - k_1AC = K_1A_T - k_1AC \tag{A.13}$$

Now we simplify (A.9) as the sum of (A.11), (A.12), and (A.13), and we have:

$$\begin{aligned}-\frac{(k_2AC)^2}{(C_T - AC)^2} - \frac{(k_1 + B_T - A_T)(k_2AC)}{C_T - AC} - \frac{k_2AC^2}{C_T - AC} + K_1A_T - k_1AC = 0 \\ -k_2^2AC^2 - (k_1 + B_T - A_T)(k_2AC)(C_T - AC) \\ -k_2AC^2(C_T - AC) + k_1A_T(C_T - AC)^2 - k_1AC(C_T - AC)^2 = 0\end{aligned} \tag{A.14}$$

Expanding and combining like terms yields our desired polynomial in AC:

$$\begin{aligned}(k_2 - k_1)AC^3 + (2k_1C_T + k_1A_T - k_2C_T + k_2(k_1 + B_T - A_T) - k_2^2)AC^2 \\ +(-k_1C_T^2 - 2k_1A_TC_T - k_2C_T(k_1 + B_T - A_T))AC + k_1A_TC_T^2 = 0\end{aligned} \tag{A.15}$$

B Derivation of the decoy binding sites model

The modeling of molecular titration using decoy binding sites is very similar to the modeling using anti-gRNA. Unlike anti-gRNA, the decoy binding sites will serve as a buffer for gRNA:Cas9 as opposed to naked gRNA, but gRNA:Cas9 remains as the active repressor complex, as seen in the following reactions:

$$\text{gRNA} + \text{Cas9} \rightleftharpoons \text{gRNA : Cas9} \tag{B.1}$$

$$\text{gRNA : Cas9 + decoyDNA} \rightleftharpoons \text{gRNA : Cas9 : decoyDNA} \tag{B.2}$$

Let A = [gRNA], B=[Cas9], C = [decoyDNA], AB = [gRNA:Cas9], and ABC = [gRNA:Cas9:decoyDNA]. k_1 and k_2 are the dissociation constants for (B.1) and (B.2) respectively, and A_T, B_T, and C_T are parameters that describe the total concentrations of gRNA, Cas9, and decoyDNA respectively. The system of equations that describe this system are the following 3 mass balance equations and 2 equilbrium expressions:

$$A_T = A + AB + ABC \tag{B.3}$$

$$B_T = B + AB + ABC \tag{B.4}$$

$$C_T = C + ABC \tag{B.5}$$

$$k_1 = \frac{A \cdot B}{AB} \tag{B.6}$$

$$k_2 = \frac{AB \cdot C}{ABC} \tag{B.7}$$

We wish to generate a polynomial in terms of AB so that we can solve for its equilibrium concentation in terms of the parameters of the system. To do this, we start off by writing (B.5) as $ABC = C_T - C$ and substituting into (B.3) and (B.4).

$$A_T = A + AB + C_T - C \tag{B.8}$$

$$B_T = B + AB + C_T - C \tag{B.9}$$

Next, we write (B.7) as $C = \dfrac{k_2 C_T}{k_2 + AB}$ and substitute it into (B.8) and(B.9), which yields

$$\begin{aligned} A_T =& A + AB + C_T - \frac{k_2 C_T}{k_2 + AB} \\ A =& A_T - AB - C_T + \frac{k_2 C_T}{k_2 + AB} \end{aligned} \tag{B.10}$$

$$\begin{aligned} B_T =& B + AB + C_T - \frac{k_2 C_T}{k_2 + AB} \\ B =& B_T - AB - C_T + \frac{k_2 C_T}{k_2 + AB} \end{aligned} \tag{B.11}$$

We rewrite (B.6) as $k_1 AB = A \cdot B$. We can then condense our system of equations into a single equation by substituting (B.10)and (B.11) into this expression as follows

$$\begin{aligned} k_1 AB =& A \cdot B \\ k_1 AB =& \left(A_T - AB - C_T + \frac{k_2 C_T}{k_2 + AB}\right)\left(B_T - AB - C_T + \frac{k_2 C_T}{k_2 + AB}\right) \\ k_1 AB(AB + k_2)^2 =& [(A_T - C_T - AB)(AB + k_2) + k_2 C_T][(B_T - C_T - AB)(AB + k_2) + k_2 C_T] \\ =& [(A_T - C_T - k_2) - AB^2 + k_2(A_T - C_T) + k_2 C_T][(B_T - C_T - k_2) - AB^2 \\ & + k_2(B_T - C_T) + k_2 C_T] \\ =& [(A_T - C_T - k_2)AB - AB^2 + k_2 A_T][(B_T - C_T - k_2)AB - AB^2 + k_2 B_T] \\ =& (A_T - C_T - k_2)(B_T - C_T - k_2)AB^2 - (A_T - C_T - k_2)AB^3 \\ & + k_2 B_T(A_T - C_T - k_2)AB - (B_T - C_T - k_2)AB^3 + AB^4 - k_2 B_T AB^2 \\ & + k_2 A_T(B_T - C_T - k_2)AB - k_2 A_T AB^2 + k_2^2 A_T B_T \\ =& AB^4 - [(A_T - C_T - k_2) + (B_T - C_T - k_2)]AB^3 + [(A_T - C_T - k_2)(B_T - C_T - k_2) \\ & - k_2 B_T - k_2 A_T]AB^2 + [k_2 B_T(A_T - C_T - k_2) + k_2 A_T(B_T - C_T - k_2)]AB + k_2^2 A_T B_T \end{aligned} \tag{B.12}$$

Expanding the left hand side, we have that:

$$\begin{aligned} k_1 AB(AB + k_2)^2 =& k_1 AB(AB^2 + 2k_2 AB + k_2^2) \\ =& k_1 AB^3 + 2k_1 k_2 AB^2 + k_1 k_2^2 AB \end{aligned} \tag{B.13}$$

Lastly, we can equate (B.12) and (B.13)

$$\begin{aligned} 0 =& AB^4 - [(A_T - C_T - k_2) + (B_T - C_T - k_2) + k_1]AB^3 + [(A_T - C_T - k_2)(B_T - C_T - k_2) - k_2 B_T \\ & - k_2 A_T - 2k_1 k_2]AB^2 + [k_2 B_T(A_T - C_T - k_2) + k_2 A_T(B_T - C_T - k_2) - k_1 k_2^2]AB + k_2^2 A_T B_T \end{aligned} \tag{B.14}$$

which can also be written as

$$\begin{aligned} AB^4 + (2k_2 + 2C_T - A_T - B_T - k_1)AB^3 + [(A_T - C_T - k_2)(B_T - C_T - k_2) - k_2(B_T + A_T + 2k_1)]AB^2 \\ + k_2[B_T(A_T - C_T - k_2) + A_T(B_T - C_T - k_2) - k_1 k_2]AB + k_2^2 A_T B_T = 0 \end{aligned} \tag{B.15}$$

which is our final polynomial in AB.