Penn Medicine

Ostap Laboratory

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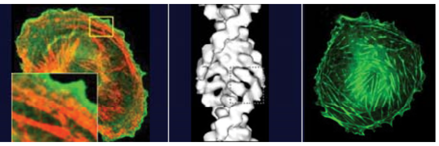

Equations for Steady-State Equilibrium Binding

(What equation do I use to calculate the Kd?)

If you have a binding reaction that is in equilibrium:

(1) $A + B \leftrightarrow AB$

then the dissociation constant (K_D) is defined as:

(2) $K_D = \frac{[A][B]}{[AB]}$

where [A], [B], and [AB] are the concentrations of the reactants at equilibrium. The total concentrations of the reactants (A_T and B_T , which are the concentrations you added to the “test tube”) are as follows:

(3) $[A_T] = [A] + [AB]$ which can be rearranged as $[A] = [A_T] - [AB]$

(4) $[B_T] = [B] + [AB]$ which can be rearranged as $[B] = [B_T] - [AB]$

Experimental Condition #1: If the concentration of $A_T >> B_T$ then one can make the approximation that free A = A_T, which makes the math easy.

Substitute eq. 4 into eq. 2:

(5) $K_D = \frac{[A]([B_T]-[AB])}{[AB]}$

rearrange:

(6) $[AB] = \frac{[B_T]\cdot[A]}{(K_D+[A])}$

Since A = A_T , we can write the equation as follows:

(7) $[AB] = \frac{[B_T]\cdot[A_T]}{(K_D+[A_T])}$

You can now write the equation in terms of the fraction (f_B) of B_T bound in the AB complex:

(8) $f_B = \frac{[AB]}{[B_T]} = \frac{[A_T]}{(K_D+[A_T])}$

This is the equation for a hyperbola. Remember, A_T must be >> B_T in your experiment too!!

Experimental Condition #2: Determine the K_D if you know $[A_T]$, $[B_T]$, and [AB]. This case is more general than Condition #1, but the math is more complicated. The goal is to solve the equation for [AB].

Substitute eq. 3 and eq. 4 into eq. 2:

(9) $$K_D = \frac{([A_T]-[AB])([B_T]-[AB])}{[AB]}$$

Rearrange the equation:

(10) $$K_D[AB] = ([A_T] - [AB])([B_T] - [AB])$$

Multiply it out and rearrange (concentration brackets are removed for clarity):

(11) $$AB^2 - (A_T + B_T + K_D)(AB) + (A_T B_T) = 0$$

into the form

(12) $$ax^2 + bx + c = 0$$

where,

(14) a = 1
(15) $b = -(A_T + B_T + K_D)$
(16) $c = (A_T B_T)$

which allows for solving via the quadratic equation:

(16) $$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

(17) $$AB = \frac{(A_T + B_T + K_D) - \sqrt{(A_T + B_T + K_D)^2 - 4(A_T B_T)}}{2}$$

This equation also is valid for “Experimental Condition #1.”