CONCEPT: PROTEIN-LIGAND FRACTIONAL SATURATION

Fraction of Ligand-Binding-Sites Occupied by Ligand (θ)

●*Fractional saturation* (θ or Y): *fraction of* _______________ (or *saturated*) ligand-binding-sites in a protein sample.

●θ: ratio of *occupied proteins* to *total protein*; reveals _______________ (%) of *occupied* ligand-binding-sites on a protein.

- □ Values of θ range from ____ (when *no* **L** is bound) to ____ (when *all* binding sites are bound by **L**).
- □ Recall: K_d = [**L**] when θ = ______, or ______% of all the available ligand-binding-sites are *occupied* by ligand.
- □ *Saturation curves* or *Protein-ligand-binding graphs* plot _____ on the y-axis and [**L**] on the x-axis.

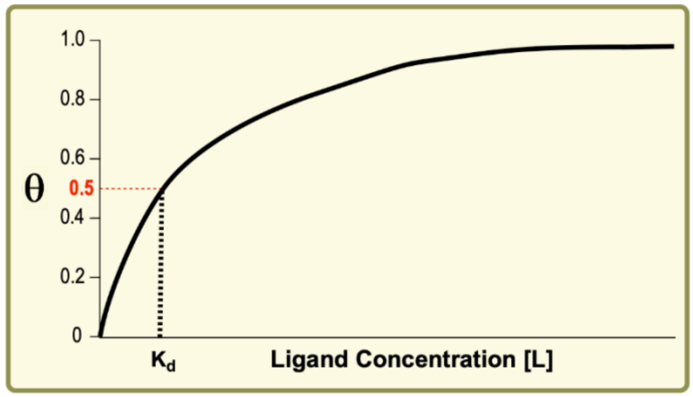
EXAMPLE: Protein-Ligand-Binding Plot.

$$\theta = Y = \frac{\text{Protein Binding Sites bound by Ligand}}{\text{Total Protein Binding Sites}} = \frac{[PL]}{[PL] + [P]}$$

Recall:

Rectangular Hyperbola Equation:

$$y = \frac{aX}{X + b}$$



Max θ

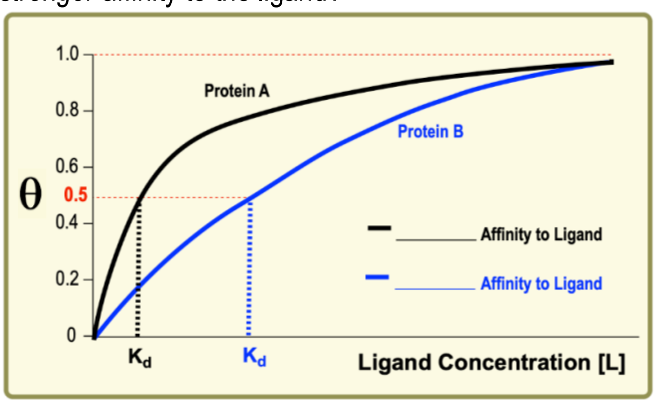
●For *ALL* protein-ligand interactions, the equivalent of the "V_{max}" is 100% **L**-binding (max θ = ___).

- □ Recall: V_{max} is subject to change with different enzymes; HOWEVER, max θ is *always* ____.
- □ Recall: K_m is similar to K_d, so the __________ the K_d value, the *stronger* the protein's affinity for that ligand.

EXAMPLE: Which protein has a *stronger* affinity to the ligand?

a) Protein A.

b) Protein B.



●Through *algebraic rearrangements* & *substitutions* of previous equations, θ can also defined in another way:

- □ This mathematically relates θ to _____ AND it *resembles* the Michaelis-Menten Equation.

$$\theta = Y = \frac{[PL]}{[PL] + [P]} = \frac{(\quad)[L]}{[L] + K_d}$$

$$\text{"}V_{max}\text{"} = \text{Max } \theta = 1$$

Michaelis-Menten Equation:

$$V_0 = \frac{V_{max}[S]}{[S] + K_m}$$

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EXAMPLE: If an antibody binds to an antigen (ligand) with a K_d of 5 x 10^{-8} M, what concentration of antigen will θ = 0.2?

a) 1.25 x 10^{-8} M. c) 3.8 x 10^{4} M.

b) 1.25 x 10^{-6} M. d) 2.1 x 10^{-2} M.

PRACTICE: Which of the following statements about protein-ligand binding is correct?

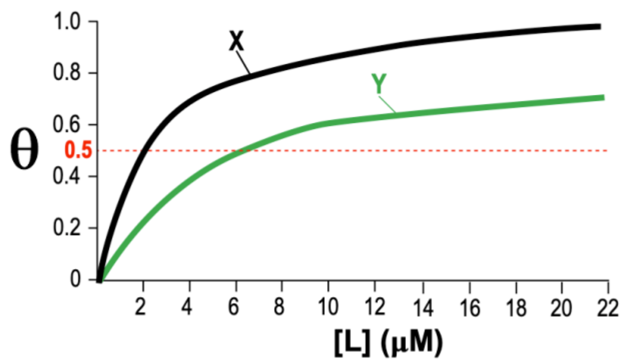
a) The K_a is equal to the concentration of ligand when all the binding sites are occupied.

b) The larger the K_a, the stronger the affinity a protein has for its ligand.

c) The larger the K_a, the faster the binding.

d) The K_a is independent of conditions including salt concentrations and pH.

PRACTICE: Consider the following graph for parts A-C.



A) What is the protein-ligand dissociation constant (K_d) for protein X?

a) 2 µM. b) 4 µM. c) 6 µM. d) 8 µM.

B) What is the protein-ligand dissociation constant (K_d) for protein Y?

a) 2 µM. b) 4 µM. c) 6 µM. d) 8 µM.

C) Which protein has a greater affinity for ligand A?

a) Protein X. b) Protein Y.

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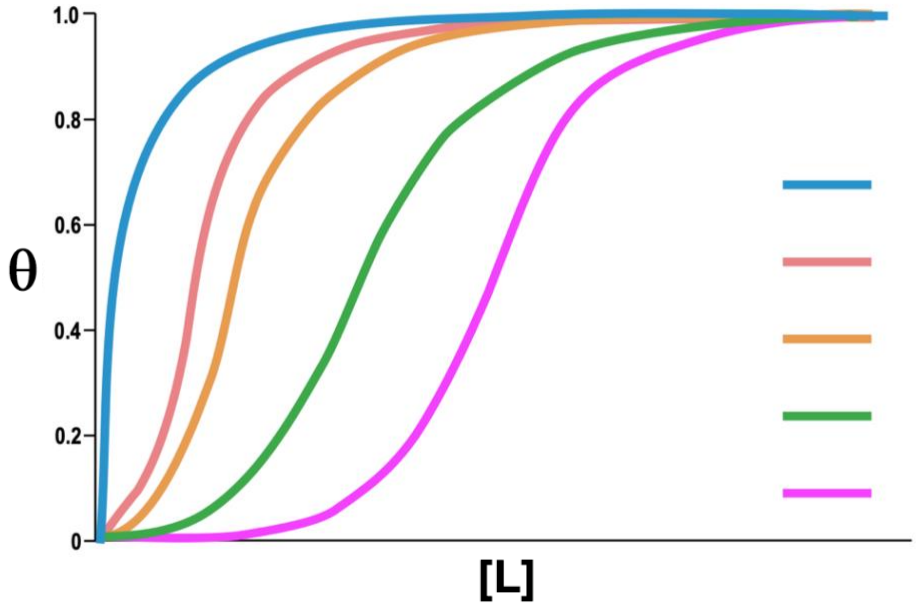
PRACTICE: Match the dissociation constants in the table below to the appropriate curves on the graph.

Protein Name	K_d (M)
A	2×10^{-6}
B	1×10^{-7}
C	1×10^{-6}
D	4×10^{-8}
E	9×10^{-7}



PRACTICE: Use the table below to answer questions A, B & C below.

[Ligand] (nM)	q of Protein 1	q of Protein 2
0.5	0.2	0.05
1	0.5	0.2
2	0.8	0.5
3	0.9	0.8

A) Which protein has a greater affinity for their ligand?

a) Protein 1.

b) Protein 2.

B) According to the data in the table, what is the dissociation constant (K_d) for Protein 1?

Protein 1 K_d = ________

C) According to the data in the table, what is the association constant (K_a) for Protein 2?

Protein 2 K_a = ________

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PRACTICE: A sample of cells has a total protein-receptor concentration of 10 mM. 25% of the protein-receptors are occupied with ligand when the concentration of free ligand is 15 mM. Calculate the K_d for the receptor-ligand interaction.

a) 5 mM.

b) 67 mM.

c) 45 mM.

d) 7.5 mM.

e) 2.5 mM.