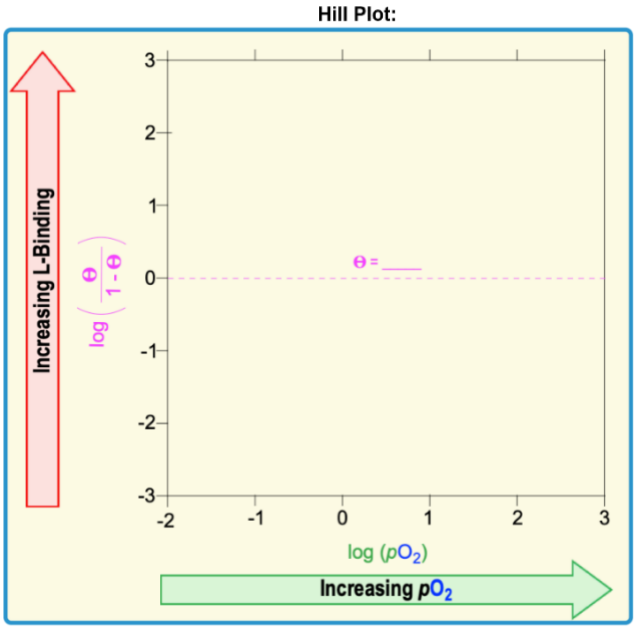
CONCEPT: HILL PLOT

- The *Hill Plot* is a _____ graph plotting the y-value & x-value of the *Hill Equation* respectively on the y-axis & x-axis.
- Slope of the line on a Hill Plot (slope = _____) denotes the Hill constant (n_H) & the degree of **L**-binding-site *interactions*.
  - □ Recall: for both Mb & Hb, **L** = O_2, and $[O_2]$ can be expressed with _____ (which means: log**[L]** → $\log(pO_2)$).

Equation of a Line: $y = mX + b$

Hill Equation: $\log\left(\frac{\theta}{1-\theta}\right) = n_H \log([L]) - n_H \log(K_d)$

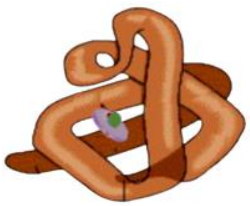


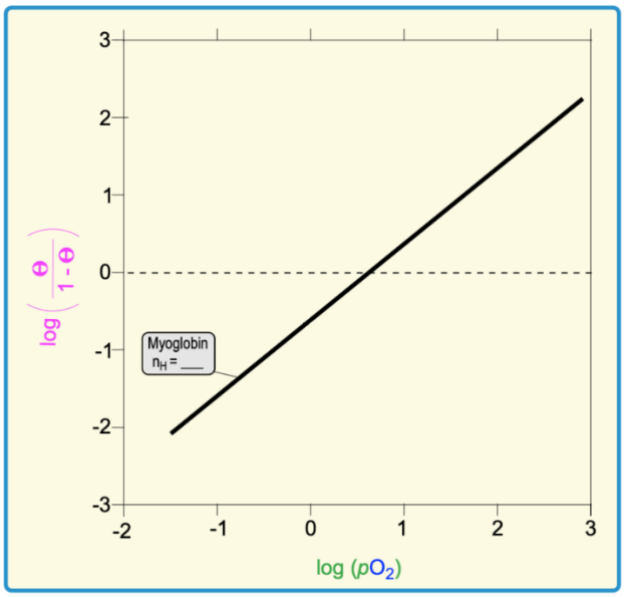
- On a Hill Plot, the ____-intercept reveals when θ = _____.
  - □ Recall: x-intercept is *ALWAYS* the x-value when y = ____.
  - □ *Note*: y-value of *Hill Equation* ($\log\left(\frac{\theta}{1-\theta}\right)$) will equal *zero* when the value of θ = _____.

Mb's Hill Plot

- Since myoglobin (Mb) only has 1-subunit, it is NOT an allosteric protein & has _____ cooperativity.
  - □ Recall: n_H = slope = ____ when there is *no* cooperativity.
  - □ Since x-intercept indicates the [L] where θ = _____, x-intercept also indicates K_d (protein affinity for ligand).

Mb = ___ subunit = _____ cooperativity (slope = n_H = 1)





CONCEPT: HILL PLOT

Hb's Hill Plot

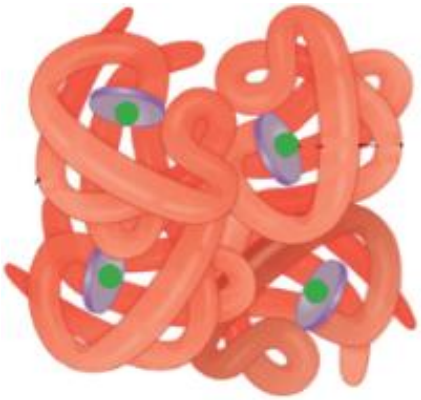
●Unlike Mb (which forms a single line), Hb's PL-data seems to form _____ identifiable lines when plotted on a Hill Plot.

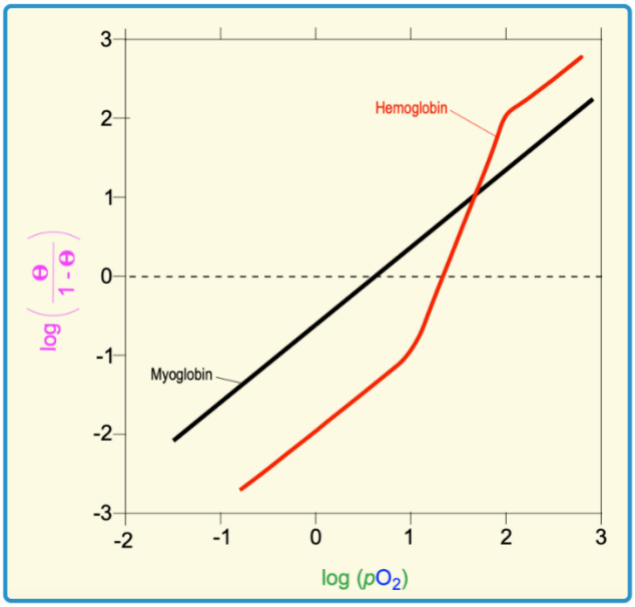
□ *Two* of Hb's lines are _____ to Mb's line, meaning they have the *same* slope (slope = ____ = 1).

□ Recall: a slope or n_H of 1 means _____ cooperativity.

□ Hb binds its _____ and _____ O_2 non-cooperatively.

●HOWEVER, Hb's 3rd line has a *different/greater* slope (slope = n_H = ____), suggesting _____ cooperativity.





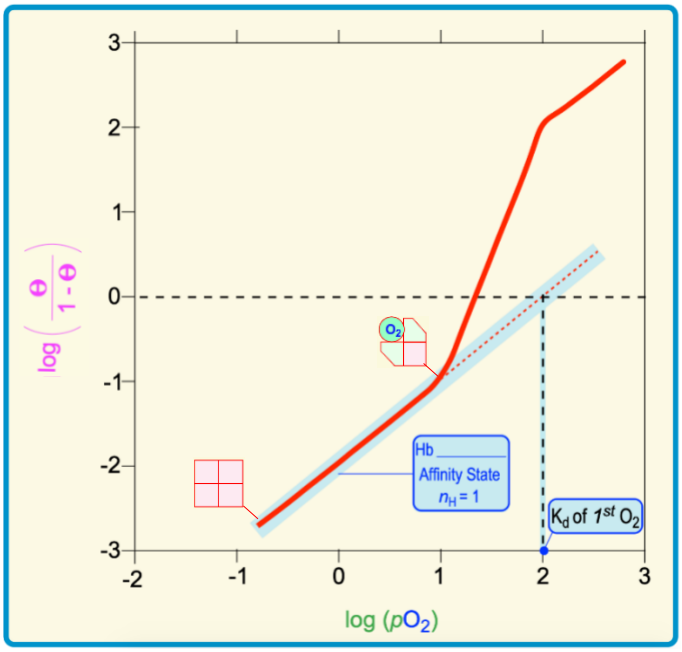
Hb's Lowest O_2-Affinity State

●Recall: Hb is NOT always displaying cooperativity; Hb binds its _____ & _____ O_2 *non*-cooperatively (slope = n_H = 1).

□ Hb subunits *equally* & _____ compete for O_2-binding (*without* cooperativity) *until* 1st O_2 binds.

□ This line represents Hb's _____ O_2-affinity.

□ *Lowest* O_2-affinity means _____ K_d for 1st O_2.



CONCEPT: HILL PLOT

Hb's Cooperative State

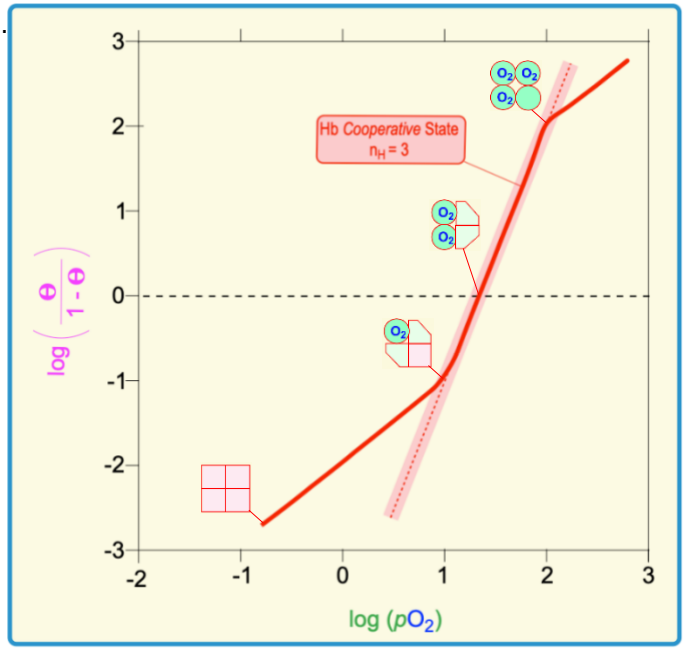
●*After* the 1st O_2 binds to Hb, Hb subunits begin to display _____ *cooperativity* (slope = n_H = 3).

□ Recall: Hb's O_2-binding behavior explained via a _____ of *concerted* & *sequential* models ($n_H \neq n$).

□ Hb displays *positive* cooperativity from the 1st bound O_2 up *until* the 3rd O_2 binds.

□ Hb subunits are NOT equally competing for O_2-binding.

= *Increased* O_2-affinity = Positive cooperativity



Hb's Highest O_2-Affinity State

●*After* the 3rd O_2 binds to Hb, the 4th and final O_2 binds _____-cooperatively, just like the 1st O_2 did (slope = n_H = 1).

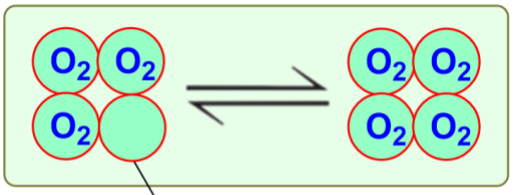
□ Once again, Hb subunits *equally* & *independently* compete for binding to the *last* O_2 (_____ cooperativity).

□ Last unoccupied Hb subunit is in the full R-state experiencing _____ model features (symmetry rule).

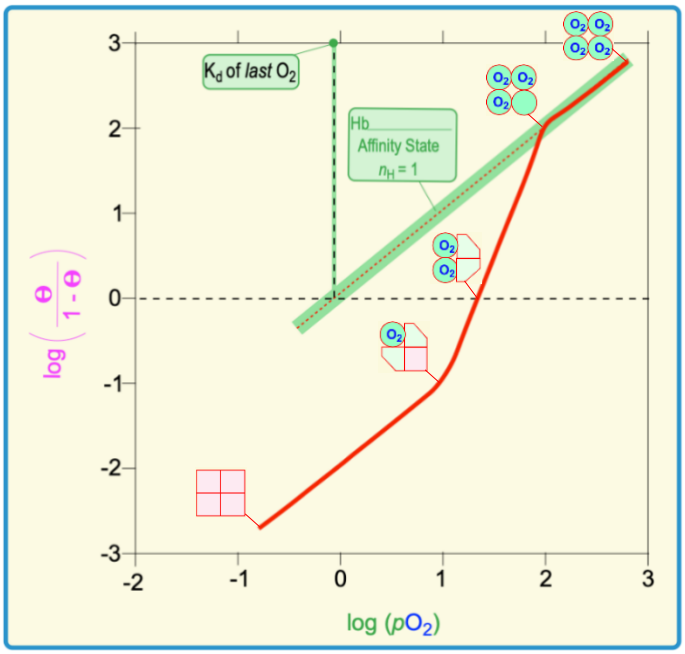
□ This line represents Hb's _____ O_2-affinity.

□ *Highest* O_2-affinity means _____ K_d for 4th O_2.

Feature of Concerted Model:



Last unbound subunit in full R-state *equally* competes for O_2-binding, *independently* of other subunits.



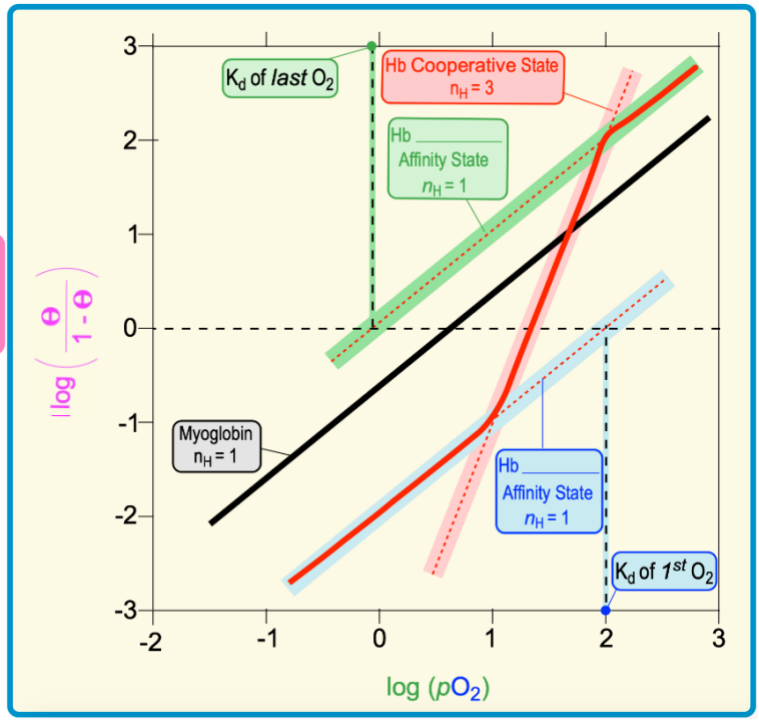
CONCEPT: HILL PLOT

Hill Plot Breakdown

●Hill plots *visually* display **PL**-affinities (K_d) and the degree of _____ (n_H) in a PL-interaction.

Hill Equation:

$$\log\left(\frac{\theta}{1-\theta}\right) = n_H \log([L]) - n_H \log(K_d)$$



"Velocity" & "Acceleration"

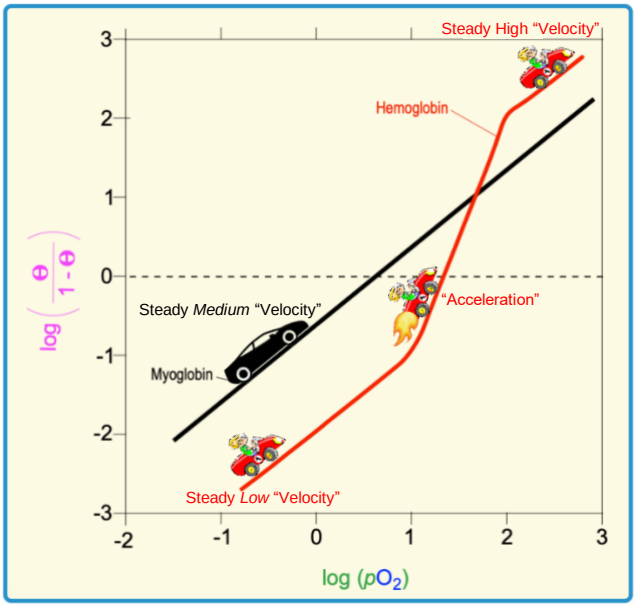
●Mb's & Hb's O_2-binding can be thought of as _____ (for *O_2-affinity*) and _____ (for *cooperativity*).

- □ Mb ALWAYS maintains the *same*, relatively high/medium velocity with _____ acceleration.
- □ Hb starts off with low velocity and does NOT begin accelerating *until* it binds it's 1st O_2.
- □ Once Hb binds 3 O_2, it reaches/maintains its max velocity & stops accelerating.

Steady = *No "acceleration" = No cooperativity*

"Velocity" = O_2-affinity

"Acceleration" = Positive Cooperativity



CONCEPT: HILL PLOT

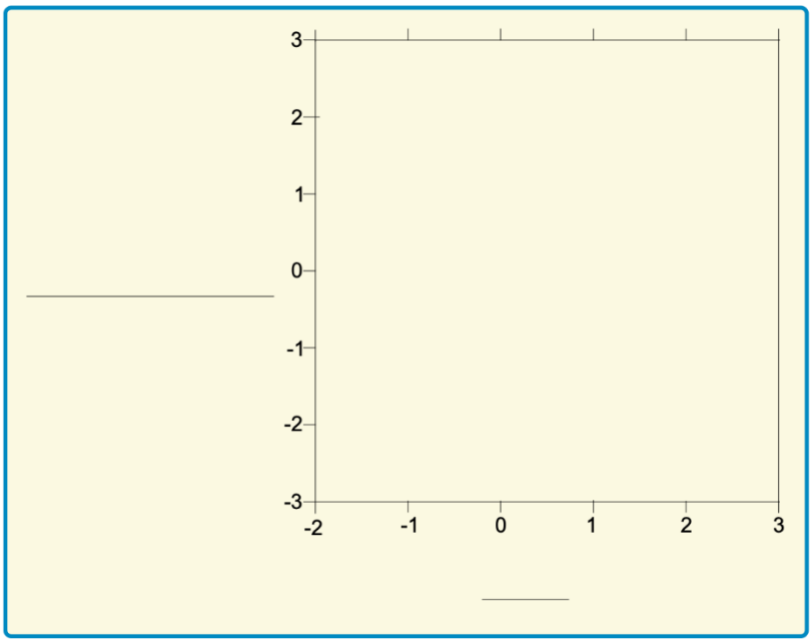
PRACTICE: Which of the following situations would produce a Hill plot with $n_H < 1.0$?

a) The protein is a single polypeptide with a single ligand binding site. As purified, the protein preparation is heterogeneous, containing some protein molecules that are partially denatured and having a lower binding affinity.

b) The protein is a single polypeptide with two ligand binding sites, each having a different affinity for the ligand.

c) The protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site decreases the binding affinity of other sites for the ligand.

PRACTICE: Label the axis of the Hill Plot below & fill-in the graph with Mb's & Hb's approximate O_2-binding data.



PRACTICE: The slope of a Hill plot for hemoglobin _____; whereas that for myoglobin _____.

a) is about 3 in its cooperative state; is 1.0.

b) decreases at low pO_2 ; is constant at all pO_2.

c) increases at high pO_2 ; curves upward for all pO_2.

d) Is 1.0 ; is about 4.

e) Both A and B.