**Lecture 14: Analysis of Cooperative Binding**

**Key Terms:**

* Hill Plot: log(Y/(1 - Y)) versus log[L]
* x-intercept = logKD
* Hill coefficient, *nh*
1. Positive cooperativity: *nh* >1
2. Negative cooperativity: *nh* < 1
3. Non-cooperative: *nh* = 1
* A homotropic ***positively*** cooperative system will show a \_\_\_\_\_\_\_\_\_\_\_\_\_ in affinity as ligand binds, due to the stabilization of the \_\_\_\_\_\_\_ state.
* A homotropic ***negatively*** cooperative system will show a \_\_\_\_\_\_\_\_\_\_\_\_ in affinity as ligand binds, due to the stabilization of the \_\_\_\_\_\_\_ state.

**Review of Types of Binding:**

**Non-cooperative:**  No interaction between sites. A protein with a single site must show non-cooperative binding.

**Homotropic positive cooperativity:** Multiple interacting ligand binding sites required, binding at one increases affinity at another by increasing R state.

**Homotropic negative cooperativity:** Multiple interacting ligand binding sites required, binding at one decreases affinity at another by increasing T state.

**Allosteric control - non-cooperative binding:**

* Heterotropic activator increases R-state. Binding affinity of ligand for one or more non-interacting sites increases.
* Heterotropic inhibitor increases T-state. Binding affinity of ligand for one or more non-interacting sites decreases.

**Allosteric control with cooperative binding:** Heterotropic activator increases R-state, increasing average affinity. Heterotropic inhibitor increases T-state, reducing average affinity. Ligand binds to multiple interacting states (homotropic) with some form of cooperative binding, neg or positive (positive cooperativity for the **ligand** is shown here).



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| **Hill Coefficient** | **Interpretation** |
| < 1 | Negative cooperativity. |
| =1 | Non-cooperative |
| >1 | Positive cooperativity. |
| = n, number of binding sites. | Infinitely strong positive cooperativity. |

It is possible to quantify the degree of cooperativity by analysis of the binding data using a Hill plot. The outcome of this analysis is the Hill coefficient, which has the characteristics summarized to the right:

**Behavior of Cooperative Systems:**

**Single Site Binding - Non-cooperative:**

 When Y = 0.5 what is [L]?

Consider a **two step** binding:



  

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| i) Non-cooperative. The binding constant remains the same for both binding events. | ii) Positive cooperativity if KD2 < KD1 (or KA2 > KA1) i.e. the second binding is higher in affinity.  | iii) Negative cooperativity if KD2 > KD1 (or KA2 < KA1) i.e. the second binding is lower in affinity. |
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**Two Binding Sites - Infinitely Positive Cooperativity:**

Consider an *infinitely* positive cooperative system such that the second dissociation constant (KD2) is much lower than the dissociation constant for binding the first ligand (KD1). Then the only species present in solution are [M] and [ML2]. The fractional saturation under these conditions is:



When Y = 0.5 what is [L]?

**Generalize to N-Ligands - Infinitely Positive Cooperativity:**



When Y = 0.5 what is [L]?

**N-Ligands – General Cooperative Binding:**

 For less cooperative systems, the fractional saturation can be *approximated* by:



* Where *nh is* the Hill coefficient. This is a measure of the *degree* of cooperativity.
* KD-AVE is the "average" KD; when [L]=KDAVE, Y=1/2.
* The KD-AVE is the root of a polynomial of the individual dissociation constants (Note: for two binding sites KD-AVE=$\sqrt[2]{K\_{D1}K\_{D2}}$ , regardless of the degree of cooperativity.)

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| **Single Site - Non-cooperative** |  | Y=0.5 when  |  |
| **Two sites - infinitely positive cooperative** |  | Y=0.5 when  | **[ML] = 0**[ML2] > 0 |
| **N-sites - infinitely positive cooperative:** |  | Y=0.5 when  | **[ML]…[MLN-1] = 0**[MLN] > 0 |
| **N-sites - just plain pos. cooperative:** |  | Y=0.5 when  | [ML]…[MLN-1] > 0[MLN] > 0 |

**Hill Equation and Plot:** The Hill coefficient, and the "average" KD can be obtained from a Hill Plot. The Hill plot is based on the following transformation of the above binding equation:

* Plot of *log* [Y/(1 - Y)] versus *log*[L]
* The Hill coefficient, *nh*, is the slope as the line crosses the *x*-axis.
* The *log*KD-aveis the intersection of the Hill curve with the *x*-axis.

**Non-Cooperative Systems** *(n =1):*

****This is a straight line with a unit slope.

* Intersection with *x*-axis (Y = 0.5) gives the true KD.

**Cooperative Systems.**

**Low ligand:** At very low ligand concentration, the binding *appears* non-cooperative because most of the macromolecule is in the [M] form. Therefore the Hill plot is initially linear, with a slope =1, intersecting x-axis at logKD1.

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**High ligand:** At very high ligand concentration, the binding also *appears* non-cooperative because most of the macromolecule is in the [MLn] form. Therefore the Hill plot is again linear, with a slope = 1, intersecting the x-axis at log KDn.

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**Intermediate Ligand Concentration** (@Y=0.5):

**Slope:** Hill coefficient (0 ↔ 1 ↔ *n*)

**Intercept**: Ligand concentration to give Y=0.5 (True KD for non-cooperative binding.)

**Microscopic and Macroscopic Binding constants:**

Cooperativity requires a change in the affinity of the binding site.

* In the case of positive cooperativity the affinity increases (KA increases, KD decreases).
* In the case of negative cooperativity the affinity decreases (KA decreases, KD increases).
* For proteins that bind multiple ligands to identical sites the experimentally determined affinity constants will change even if there is no cooperativity. For a two binding sites the affinity for the second site will be lower by a factor of two. This difference is due entirely to statistical factors – there are no differences between the molecular interactions in the two sites.

**Microscopic KA:** This is the association constant for a *single site*, and is just the ratio of the on- and off-rates: KA=*kON/kOFF*. It reflects the intrinsic affinity between the protein and the ligand: **ΔGo = -RT ln KA**

**Macroscopic KA:** This is the *observed* KA based on the concentrations of the various species, i.e. KA1=[ML]/[M][L], i.e. what you would typically consider the equilibrium constant.

**One-binding site:** The macroscopic and microscopic are the same.

**Two binding sites:** For the first binding event this is 2×KA since there are two ways to form the [ML] species, i.e. KA1=*2kON/kOff*. For the second binding event there are two ways for the ligand to leave, so KA2=*kON/2kOff.*

**Three binding sites**

