**Lecture 14: Analysis of Cooperative Binding**

**Goals:**

* Generate Hill Plot,
* Obtain KD and nh from Hill plot, correctly
* Interpret nh in terms of type of cooperativity.

**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).

**Characterization of Degree of Cooperativity:**

**Distribution of Ligands (two binding sites):**

|  |  |  |  |
| --- | --- | --- | --- |
| i) Non-cooperative. The binding constant remains the same for both binding events. @Y=0.5  **nH = 1.0** | ii) Negative cooperativity if KD2 > KD1 (or KA2 < KA1) i.e. the second binding is lower in [affinity.@Y=0.5](mailto:affinity.@Y=0.5)  **nH < 1.0** | iii) Positive cooperativity if KD2 < KD1 (or KA2 > KA1) i.e. the second binding is higher in affinity. @Y=0.5  **nH >1.0** | iv) Infinite pos cooperativity. The binding of the first ligand greatly increases the affinity for the next.  **nH = n (# sites)** |
|  |  |  |  |

**Binding of N-Ligands:**

**Non-cooperative Binding (any number of sites):**



**Infinitely Positive Cooperativity, n-sites.**



**Cooperative systems – General equation** (Hill Equation):



* In all cases, when [L]=KD, Y=0.5
* Thermodynamic information can only be obtained from the KD for non-cooperative binding. “KD-Ave = *f*(KD1, KD2, ….)

**Hill 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]

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

* 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. This is the ligand concentration to ½ saturate the binding sites.

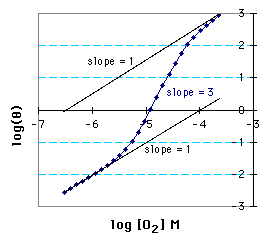
**Non-Cooperative Systems** *(n =1):*

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This is a straight line with a unit slope.

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



**Cooperative Systems.**

**Intermediate Ligand Concentration**@Y=0.5.:

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

**Intercept**: Ligand concentration to give Y=0.5 = KDAve.

**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.



**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.



Example: Rate the cooperativity of each of the following binding curves.