**Summary of Ligand Binding:**

*Some Definitions:*

* Y = Fractional saturation. Fraction of binding sites occupied by ligand, varies from 0 to 1.
* Y = [ML]/([ML]+[M])
* *n* = Number of binding sites.
* KA = Association constant or equilibrium constant, KA=[ML]/[M][L].
* KD = Dissociation constant, KD=1/KA. Y = 0.5 when [L]=KD, *always*, regardless of cooperativity.
* *nh* = Hill coefficient, measure of degree of cooperativity.

**Non-Cooperative:**

*One Site (n=1)*



Binding curve:



*Two-sites (n=2)*

Binding equation is the same:Y=[L]/(KD+[L])

**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].

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.*

**Cooperative binding:** *Example* - Trimer *M+L → ML+L → ML2+L → ML3*



Non-cooperative pos/neg coop. Infinite positive coop.

**Hill Plot:** Plot log(Y/(1-Y)) *versus* log[L]



**KD-ave** : Ligand concentration when curve crosses x-axis (Y=0.5), depends on product of all individual KD values.

***nh***: Slope, Δ(log(Y/(1-Y))/Δlog([L]), *when curve crosses x-axis.*

**Type & degree of cooperativity:**

• nh=1 for non-cooperative binding: Binding of one ligand has *no* *effect* on the binding of others.

• nh>1 for positive cooperativity: Binding of the 1st ligand *enhances* the binding of additional ones.

• nh<1 for negative cooperativity: Binding of the 1st ligand *impairs* the binding of additional ones.

• The closer nh is to n, the stronger the cooperativity, maximum value is n, # of sites.

**Important parameters and how to obtain them:**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **One-site or *n*-sites non-cooperative** | **Cooperative** |
| **KD** (This is always [L] that gives Y=0.5) | 1. Binding Curve, When Y=0.5, [L] = KD  2. Scatchard plot, slope= -1/KD. | 1. Y = 0.5 on binding curve.  2. ***x***-intercept of Hill Plot |
| ***nh*** (Hill coefficient) | Not applicable | Slope of Hill plot at Y=0.5, [log(Y/(1-Y)]=0 |
| ΔGo= | ΔGo = -RT ln KA = -RT ln (1/KD) | **ΔGo ≠ -RT ln (1/KDAve)** |