

# *Lecture 22*

## *Ligand interactions*

Reading for today: Chapter 12 Section A

Reading for Monday: Chapter 12 Section B

## *Today's Goals*

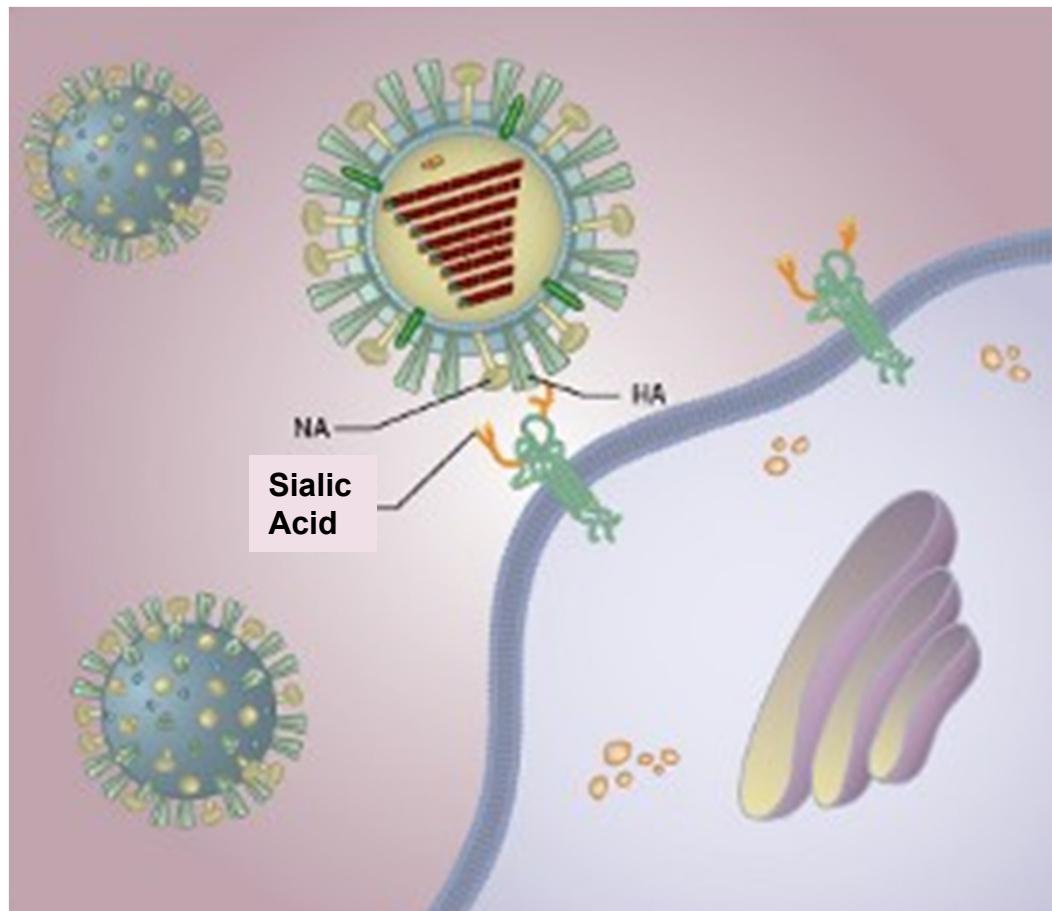
- Be able to apply concepts of equilibrium and thermodynamics to ligand-receptor interactions
  - Definitions
  - Relevant mathematical tools
  - Methodology
- Monday: some examples

# *Molecular recognition is ubiquitous in biology*

- Examples of molecular recognition events

## *Hemagglutinin ligand binds to a sialic acid receptor*

- The terms “receptor” and “ligand” are somewhat interchangeable, depending on the perspective.

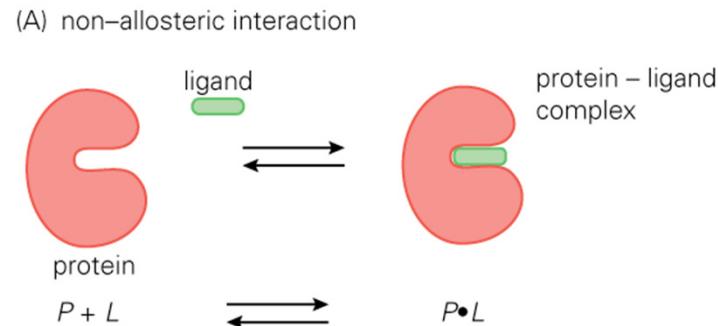


## *Affinity and specificity are key elements of molecular recognition*

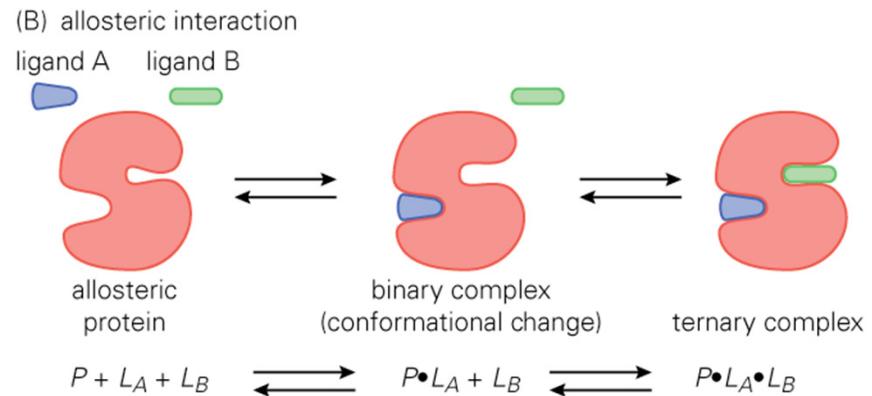
- We'll use the concept of equilibrium constant to look at the binding of one molecule to another
  - Molecular recognition through non-covalent interactions
- Two important parameters
  - *Affinity*: strength of the interaction – measured by the corresponding decrease in free energy upon binding
  - *Specificity*: relative strength of interaction for a cognate and non-cognate receptor-ligand complex

# *There are two basic kinds of interactions: Simple binding and allosteric interactions*

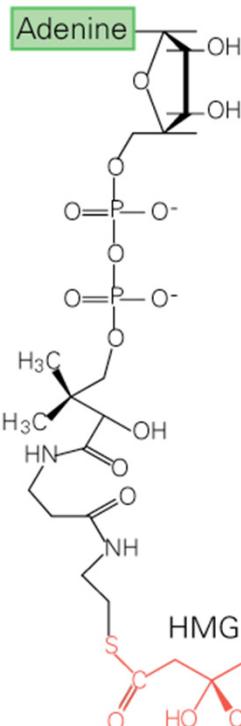
- Some binding reactions are “simple” equilibria – each encounter is independent



- Others are more complex, involving allostery, where binding of one ligand alters the affinity for another ligand



## Example: Statins



- Inhibitors that bind to the enzyme HMG-CoA reductase, the first step in the cholesterol synthesis pathway

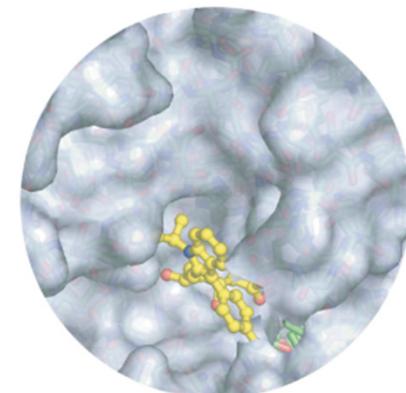
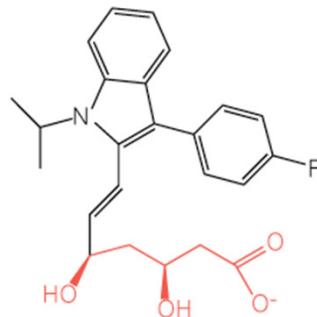
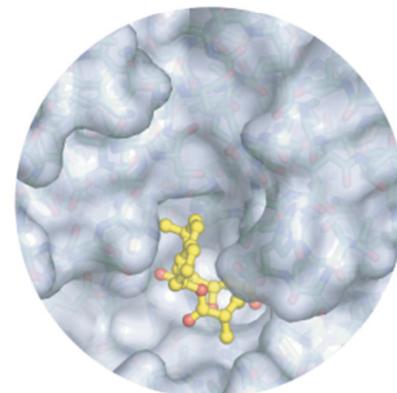
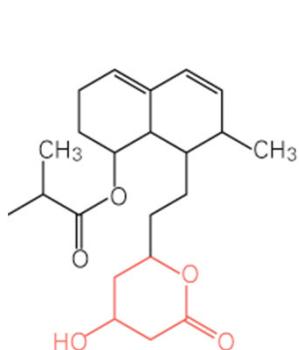
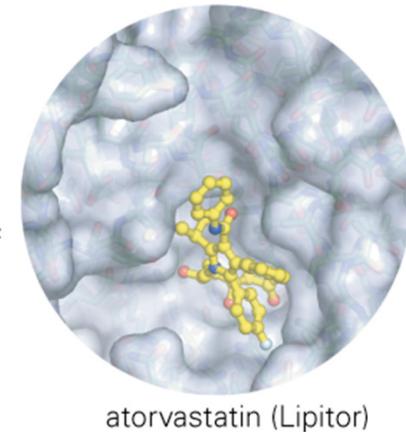
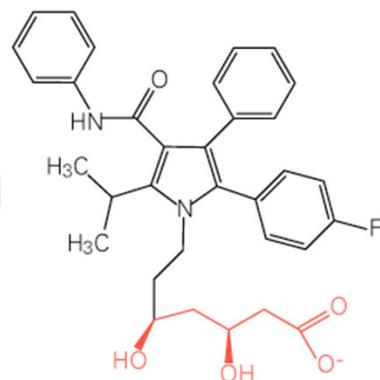
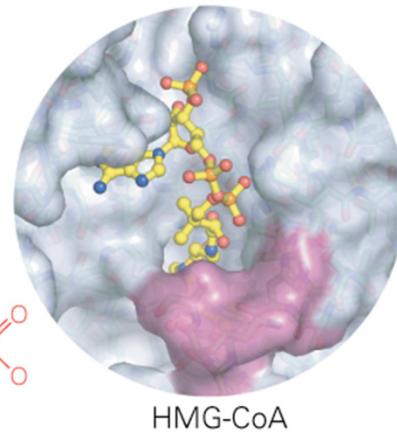
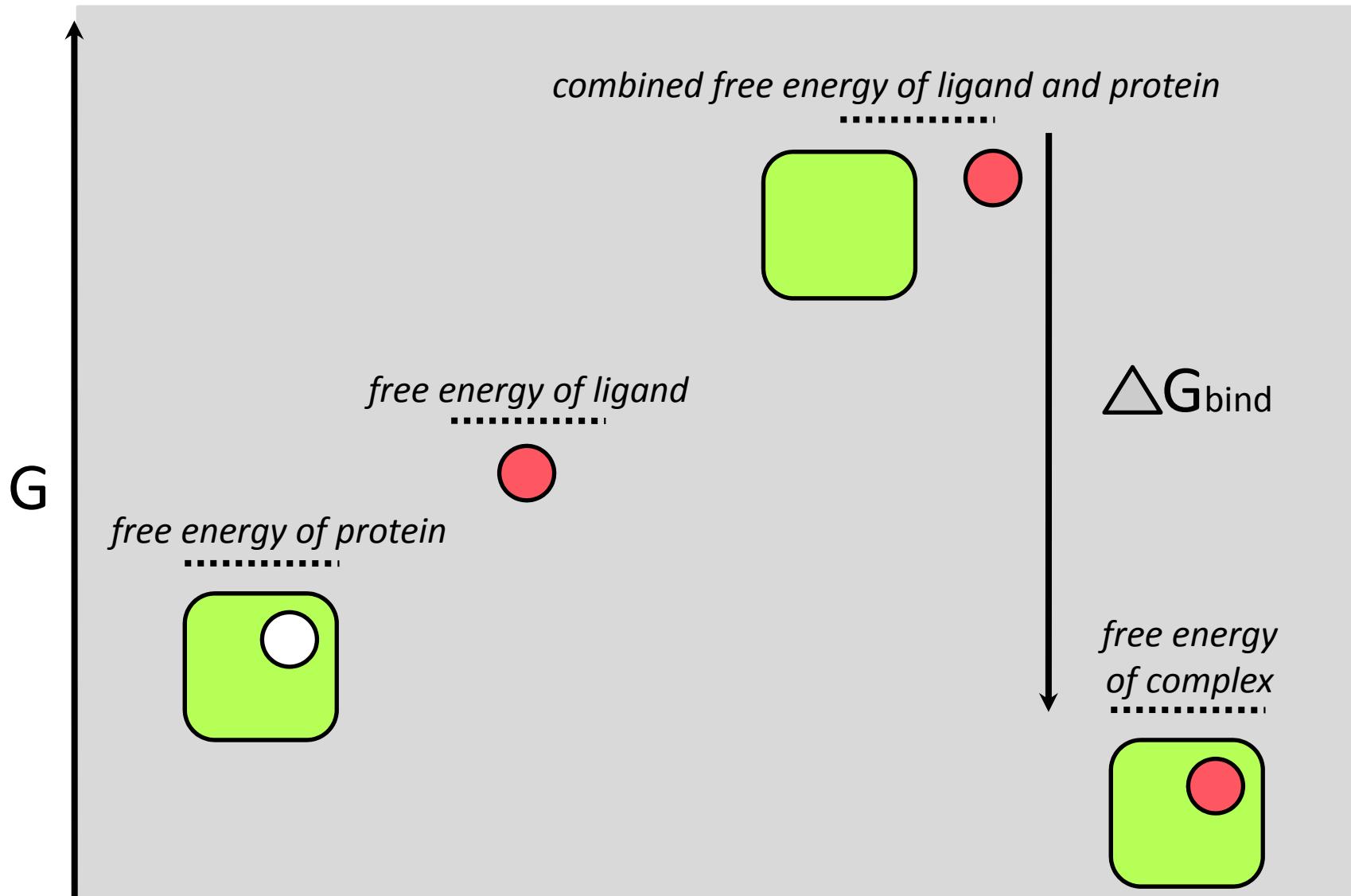


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## *Thermodynamics provide insights into molecular interactions*

- Using thermodynamics, binding reactions can be studied to provide insights into
  - Affinity and specificity
  - Contribution of enthalpy and entropy
  - Dependence on temperature
  - Contribution of chemical groups on the ligand and/or receptor to these various parameters
- This information can in turn be used to understand the system and alter the system (e.g. drug design)

## *Graphical representation of ligand binding free energy*



## Ligand binding and association constant

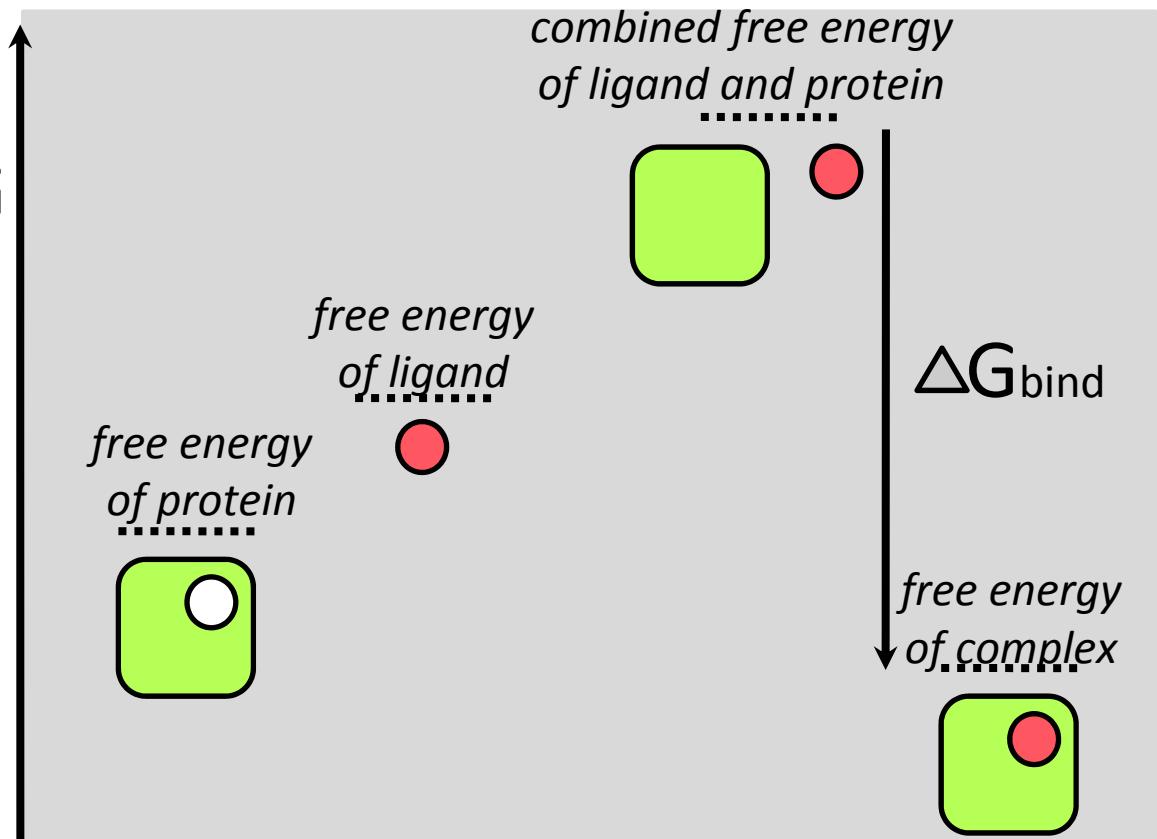


- Equilibrium constant:

$$K_A = \frac{[R \cdot L]}{[R][L]}$$

- Free energy change  
(binding free energy):

$$\Delta G_{bind}^{\circ} = -RT \ln K_A$$



# Ligand binding and dissociation constant

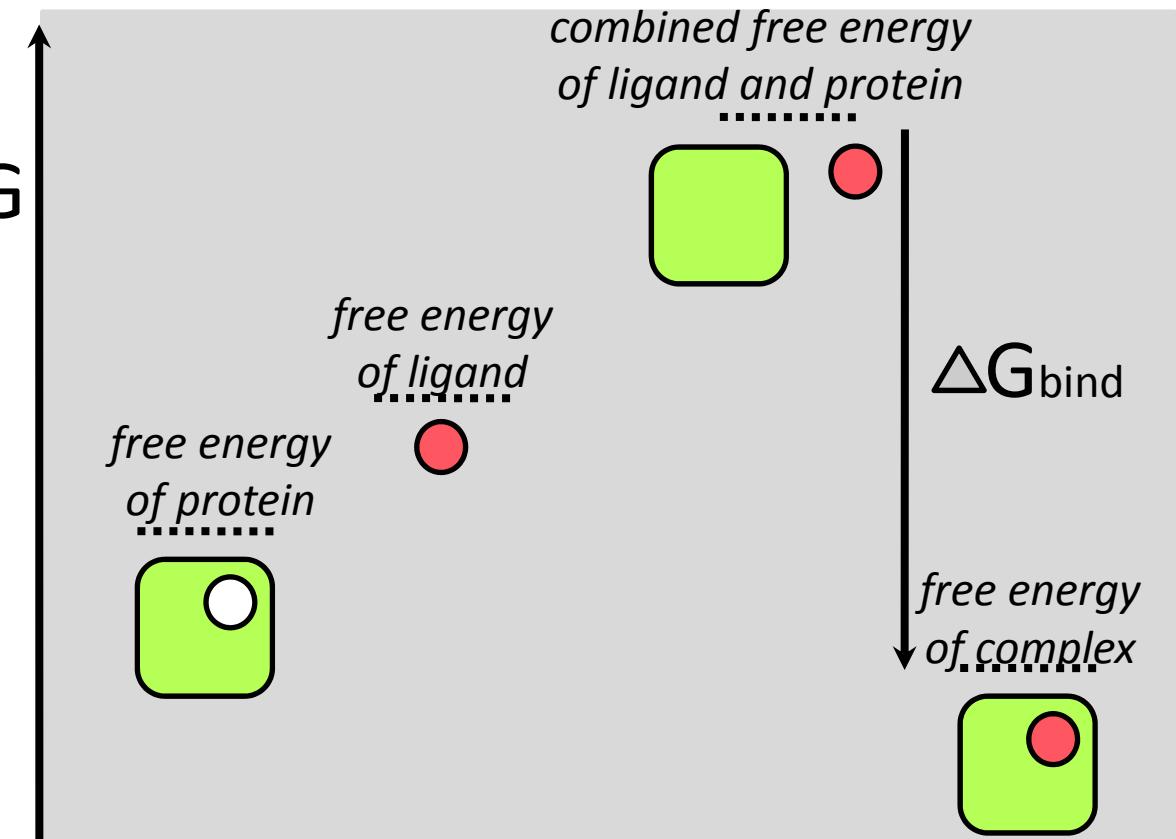


- Equilibrium constant:

$$K_D = \frac{[R][L]}{[R \cdot L]} = \frac{1}{K_A}$$

- Free energy change (binding free energy):

$$\Delta G_{bind}^o = +RT \ln K_D$$



Units for  $K_A$  and  $K_D$ ?

Why do we typically use the dissociation constant?

## *Why do we use the dissociation constant?*

- We will show that the  $K_D$  is equal in magnitude to the concentration of ligand at which half the receptor is occupied at equilibrium:

- Fractional occupancy (saturation):  $f = \frac{[R \cdot L]}{[R] + [R \cdot L]}$

$$K_D = \frac{[R][L]}{[R \cdot L]} \text{ rearranges to } [R \cdot L] = \frac{[R][L]}{K_D}$$

Substituting:

$$f = \frac{\cancel{[R][L]} / K_D}{\cancel{1} \cancel{[R]} + \cancel{[R][L]} / K_D} = \frac{[L] / K_D}{1 + [L] / K_D}$$

Multiplying by  $K_D$  on top and bottom:  $f = \frac{[L]}{K_D + [L]}$

## *Why do we use the dissociation constant?*

- So we see that:



$$f = \frac{[L]}{K_D + [L]}$$

- When  $[L] = K_D$ :  
$$f = \frac{K_D}{K_D + K_D} = \frac{1}{2}$$
- The  $K_D$  is equal in magnitude to the concentration of ligand at which half the receptor is occupied at equilibrium
- Although  $K_D$  is a dimensionless number, it is often expressed in molar units to reflect its significance

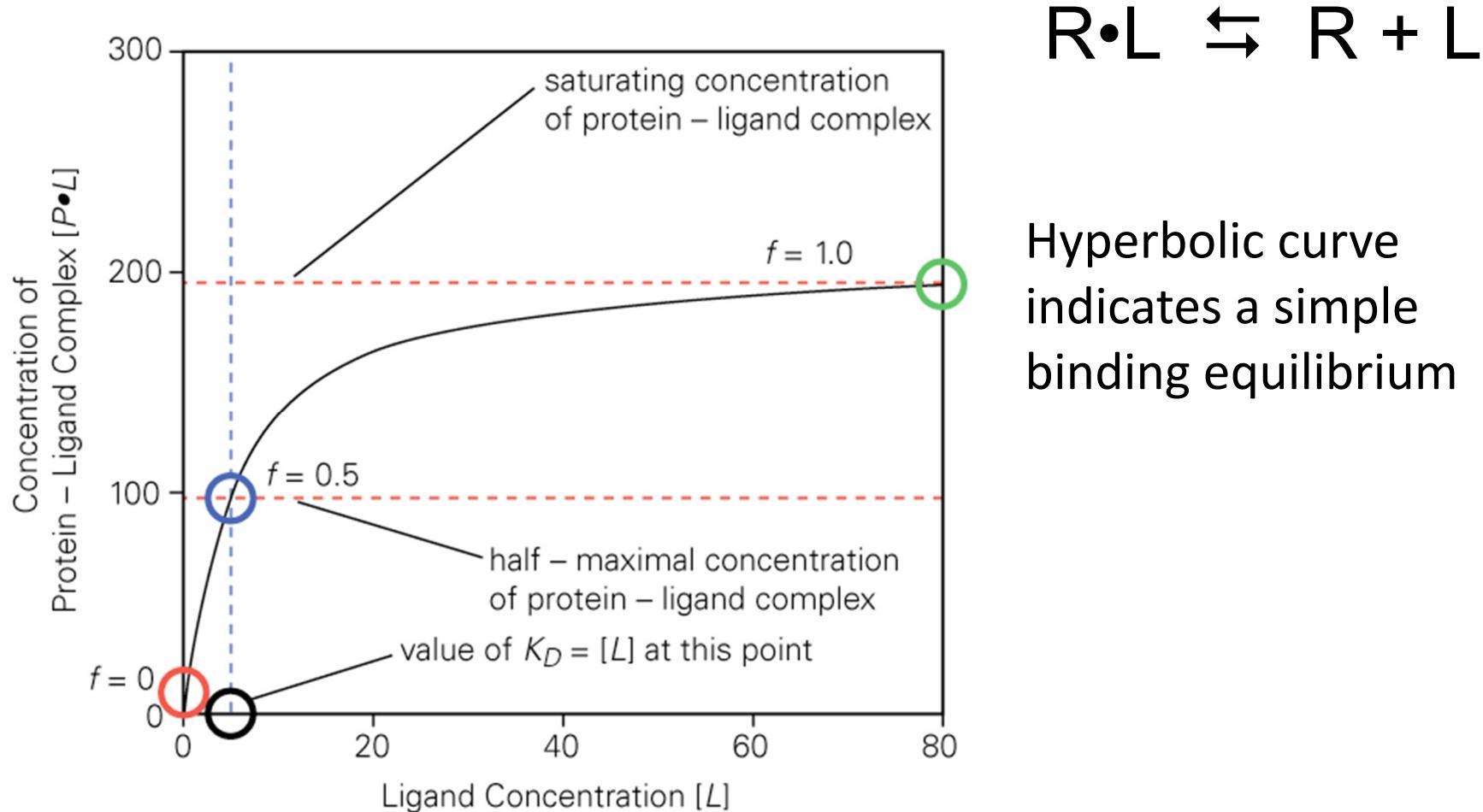
## *Typical $K_D$ values in biology*

- Wide range of  $K_D$  values reflect different biological functions

Type	$K_D$	$\Delta G_{bind}$
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# *Binding isotherm describes fraction bound as a function of ligand concentration*

- Binding curve or isotherm plots  $[R \cdot L]$  vs  $[L]$ :



Hyperbolic curve indicates a simple binding equilibrium

# *Binding isotherm tends to 1 at high ligand concentrations*

- Binding curve or isotherm plots  $[R \cdot L]$  vs  $[L]$ :

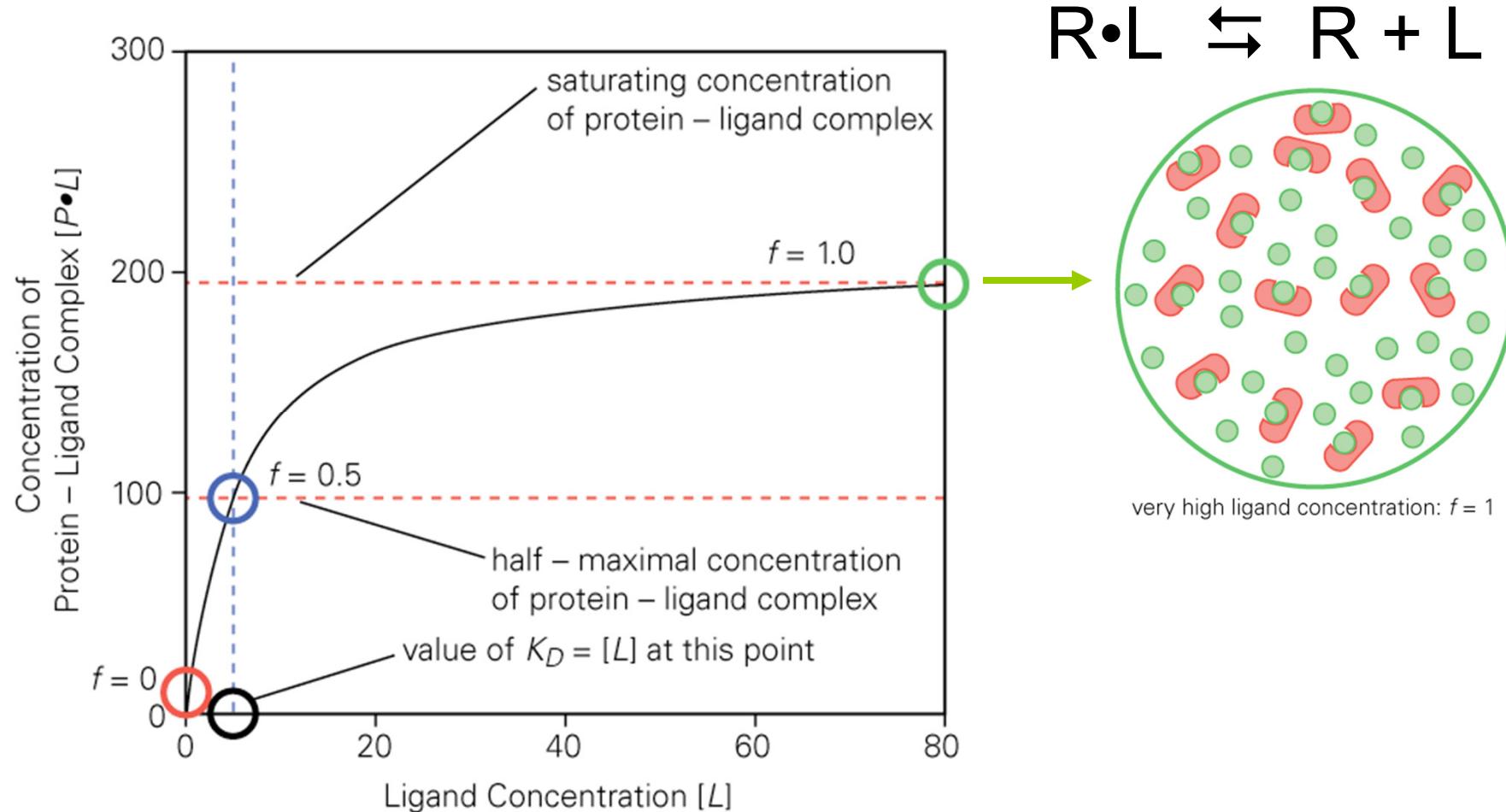


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# *Binding isotherm tends to 0 at low ligand concentrations*

- Binding curve or isotherm plots  $[R \cdot L]$  vs  $[L]$ :

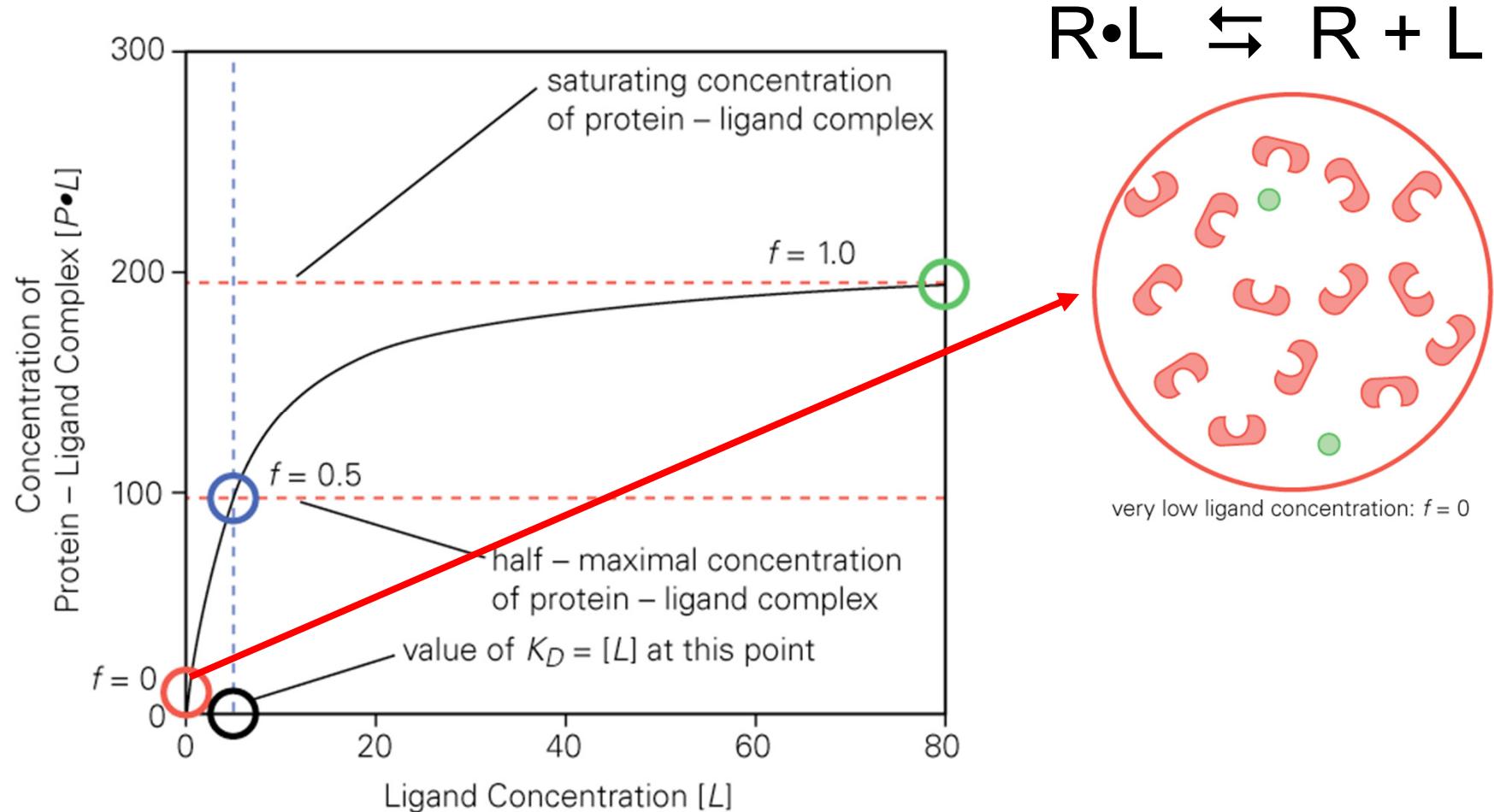


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## *Binding isotherm is half maximal at $[L] = K_D$*

- Binding curve or isotherm plots  $[R \cdot L]$  vs  $[L]$ :

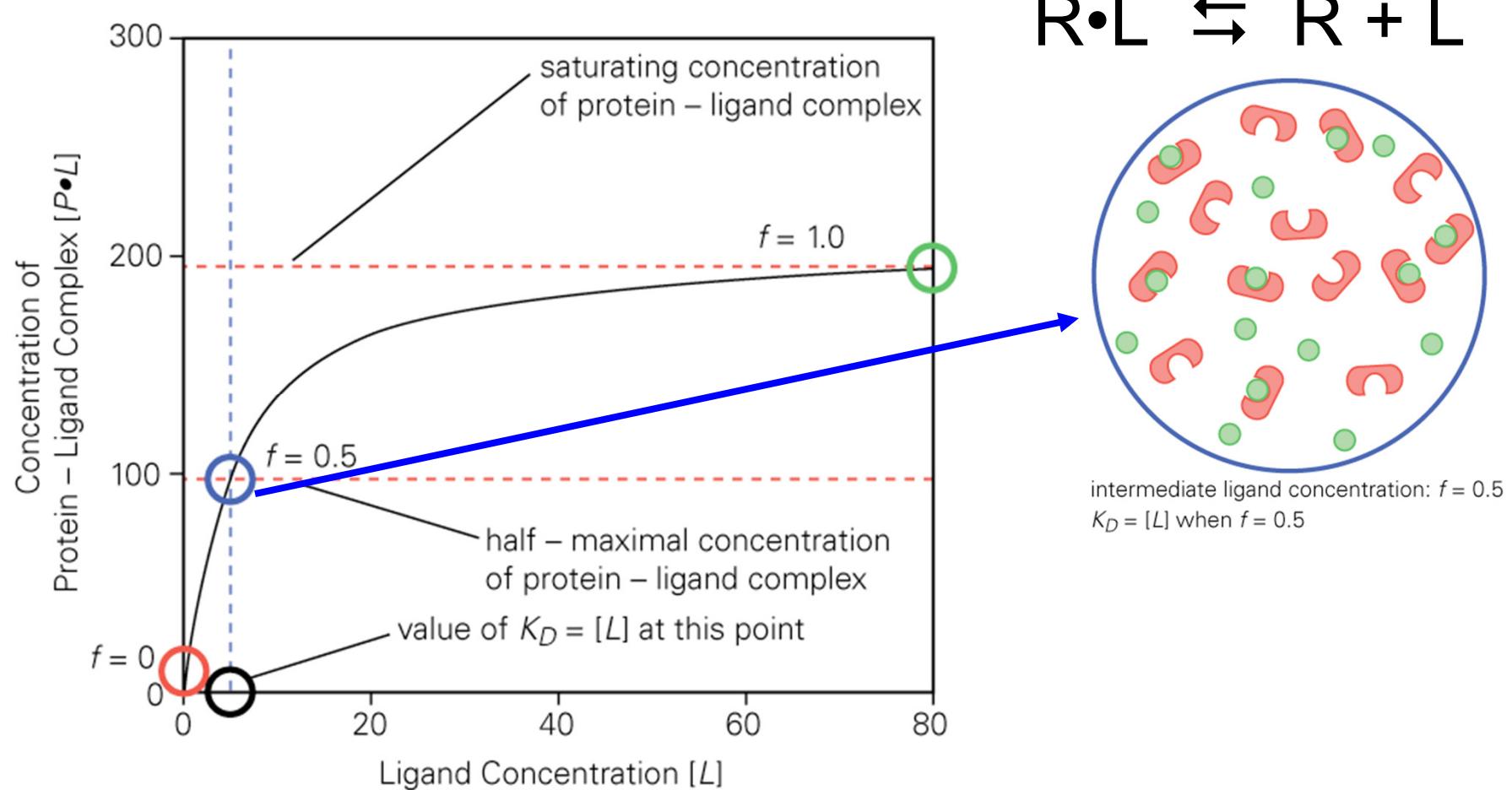


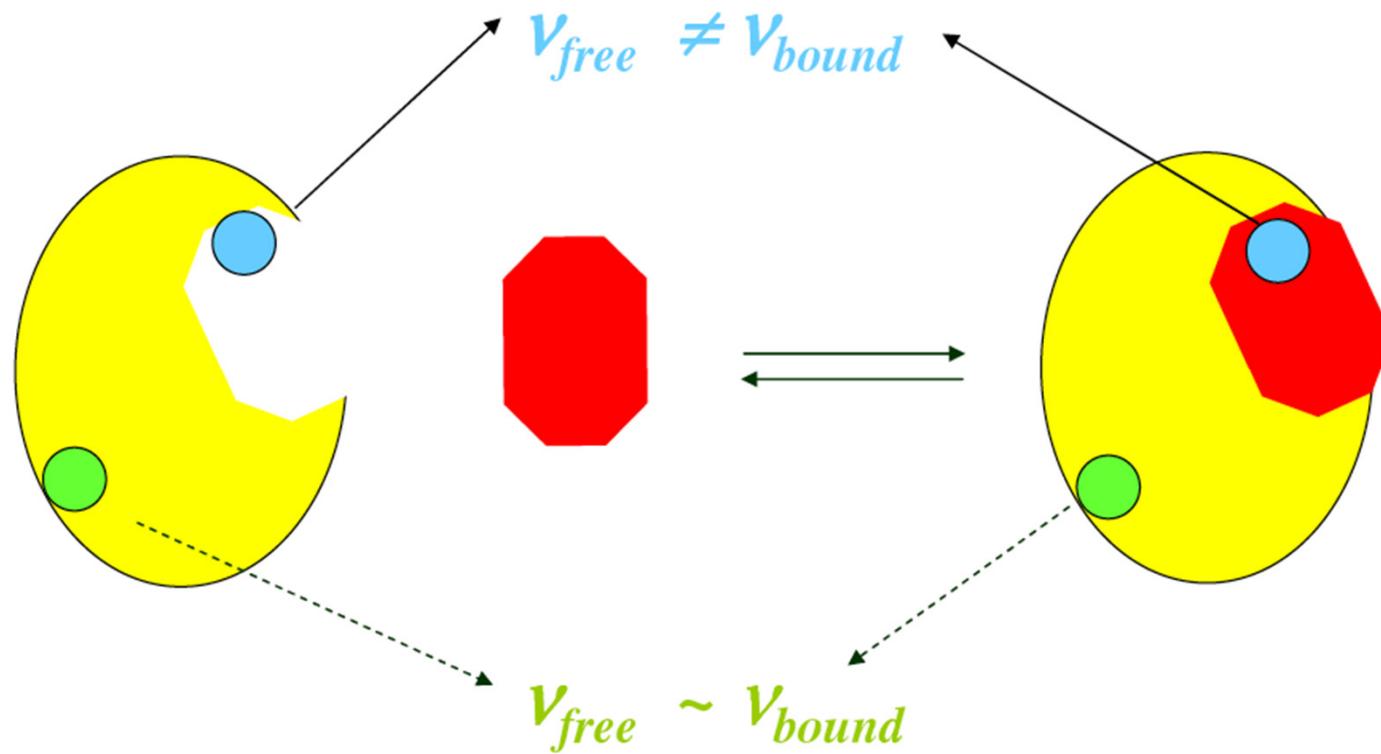
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# *Experimental determination of $K_D$*

- Need to measure the fraction of bound ligand
- There are many kinds of binding assays, for example:
  - Separating bound from unbound
    - Pulldown assay
    - Electrophoretic mobility shift assay (EMSA)
  - Homogeneous assay
    - NMR or other spectroscopic methods

## *NMR can be used to study ligand binding*

- A ligand will change the chemical shifts locally – in the vicinity of the binding site



- This can be used to identify the ligand binding site

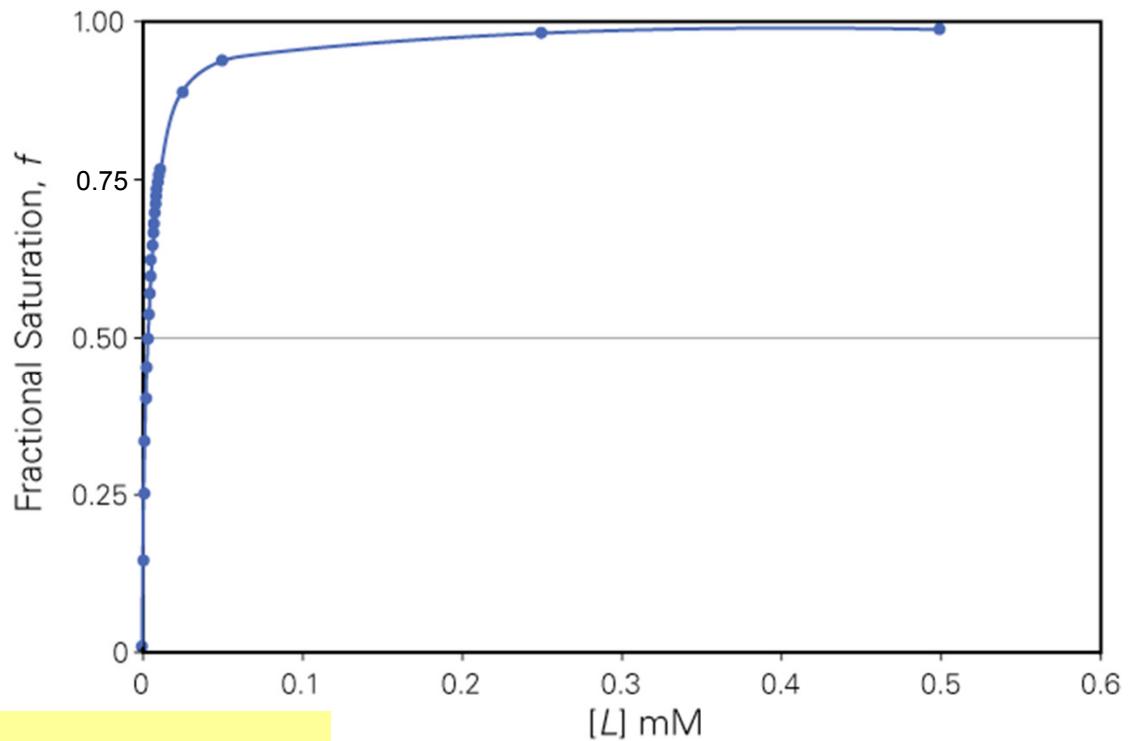
# *Measuring SH2 binding to peptide ligand by NMR*

## *Binding isotherm can be difficult to interpret*

- $K_D$  is difficult to determine visually and the most interesting part of the curve is condensed at low concentration

- From:

$$f = \frac{[L]}{K_D + [L]}$$



- We get:

$$\log\left(\frac{f}{1-f}\right) = \log[L] - \log K_D$$

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## *Deriving the log-log plot equation*

- Fraction bound receptor:      Fraction free receptor:

$$f = \frac{[L]}{K_D + [L]}$$

$$1 - f = 1 - \frac{[L]}{K_D + [L]}$$

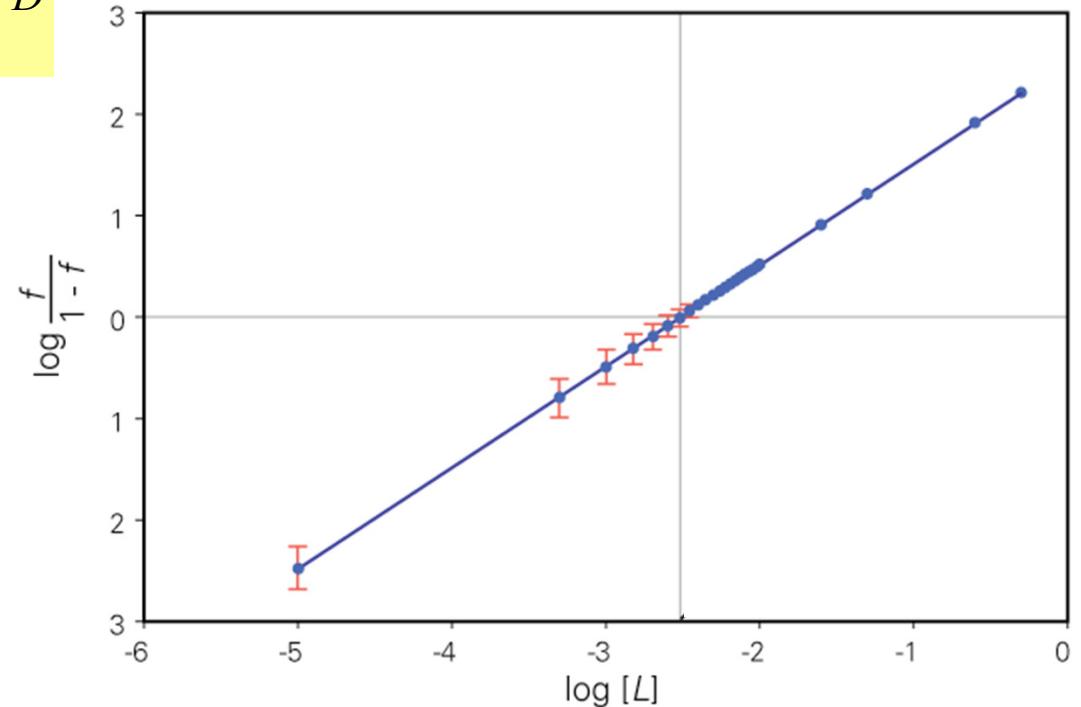
$$1 - f = \frac{K_D + [L] - [L]}{K_D + [L]} = \frac{K_D}{K_D + [L]}$$

- The ratio:  $\frac{f}{1-f} = \frac{\frac{[L]}{K_D + [L]}}{\frac{K_D}{K_D + [L]}} = \frac{[L]}{K_D}$
- Taking the log on both sides:

$$\log \frac{f}{1-f} = \log \frac{[L]}{K_D} = \log[L] - \log K_D$$

## *Linear log-log isotherm indicates a simple binding reaction*

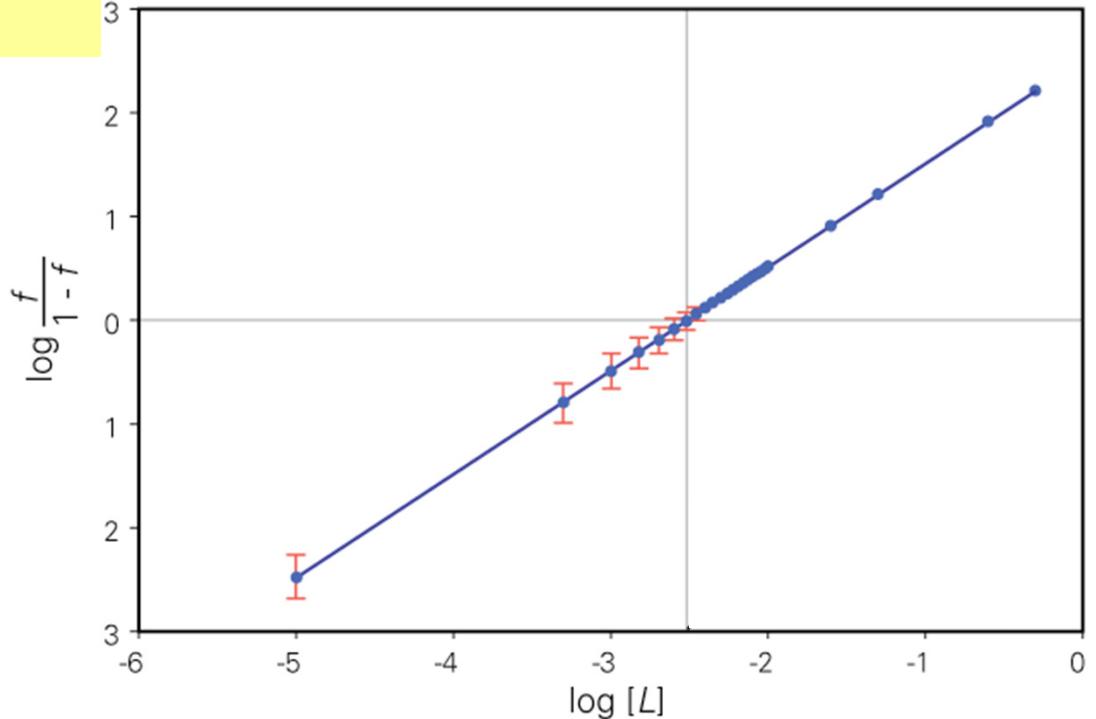
$$\log\left(\frac{f}{1-f}\right) = \log[L] - \log K_D$$



## *Log-log isotherm x-intercept provides the $K_D$*

$$\log\left(\frac{f}{1-f}\right) = \log[L] - \log K_D$$

- When  $\left(\frac{f}{1-f}\right) = 1$
- Then  $\log\left(\frac{f}{1-f}\right) = 0$
- And  $\log[L] = \log K_D$



# Measuring binding affinity using a pulldown assay

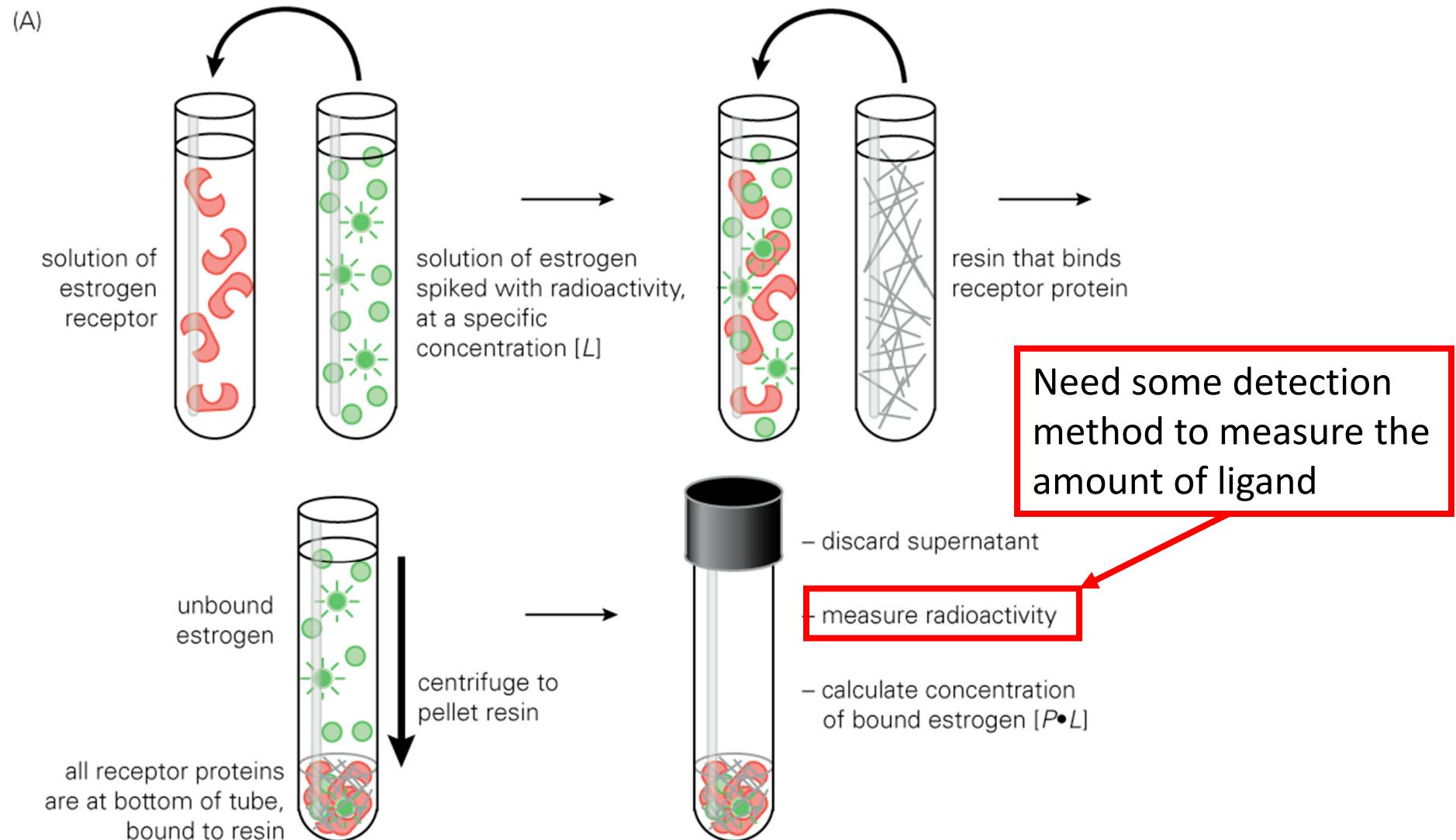
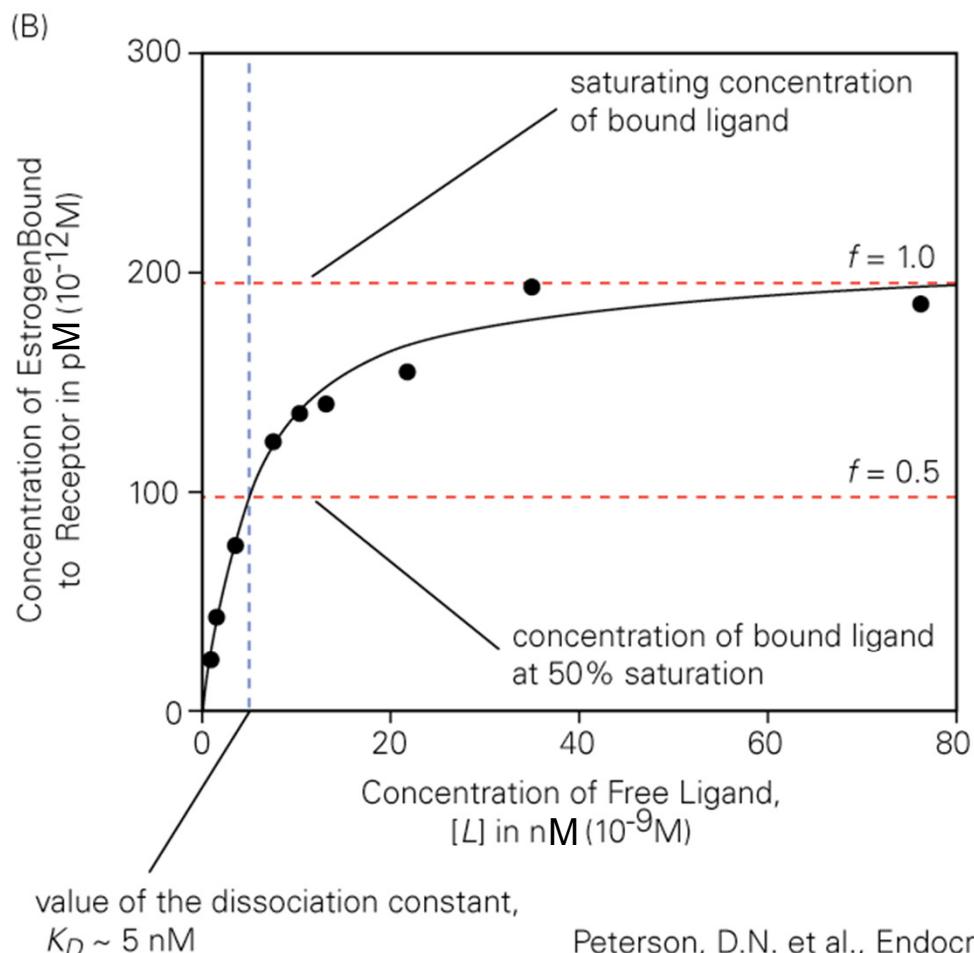


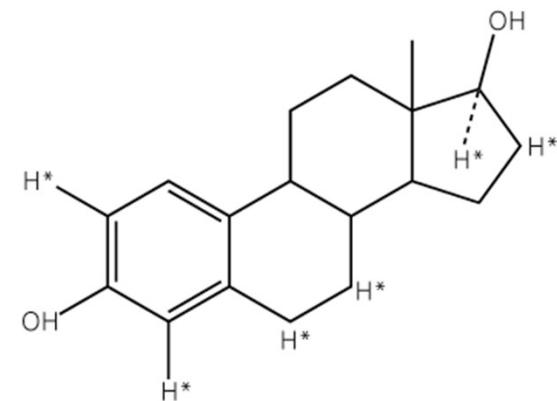
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## Experimental data

- Estrogen binding to the estrogen receptor, measured using a pulldown assay



(C) tritiated estrogen



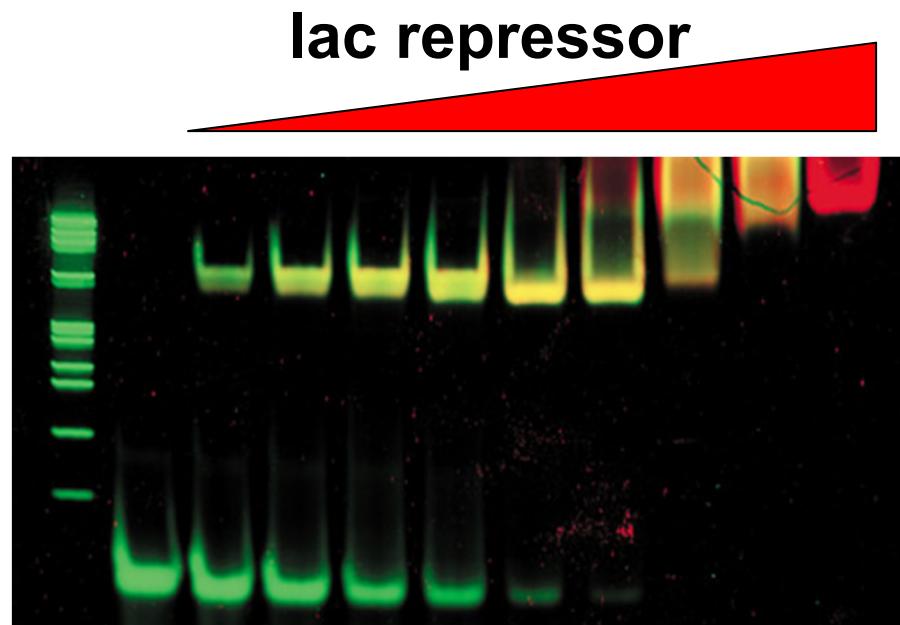
Saturation point of the curve is equated to  $f = 1$

Peterson, D.N. et al., Endocrinology, (1998),  
v. 139, 1082-1092. Figure 5

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## *Electrophoretic mobility shift assay*

- Titration of lac operator DNA with lac repressor protein



## *Ligand concentration*

- Note that all previous equations refer to  $[L]_{\text{free}}$ , which may be difficult to measure.

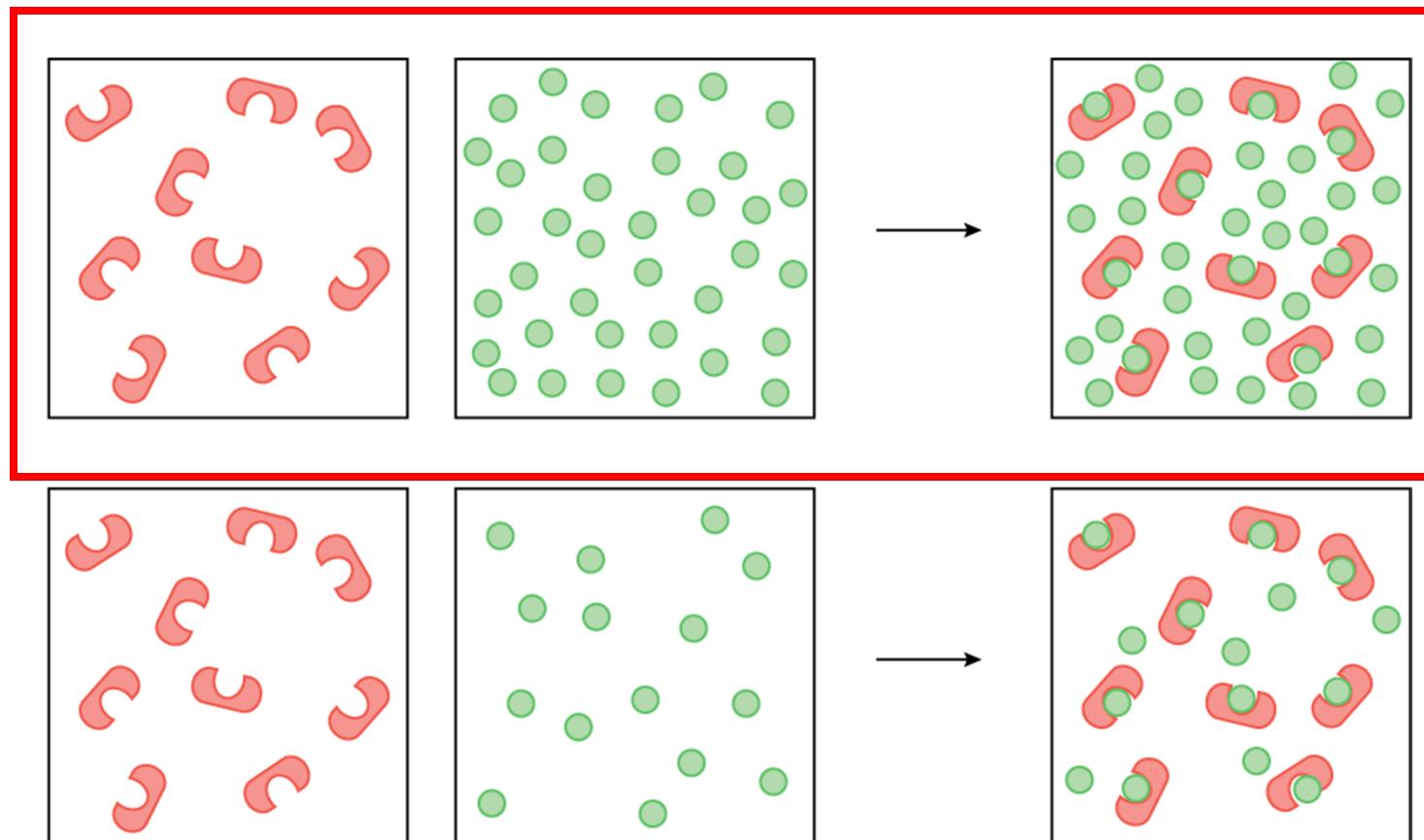


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# Scatchard analysis when [R] is unknown

- Often it is easier to measure the ligand concentration than the protein concentration
  - e.g. binding of ligand to receptors at the surface of cells or to receptors in whole cell lysates
- We can use the Scatchard analysis in such cases

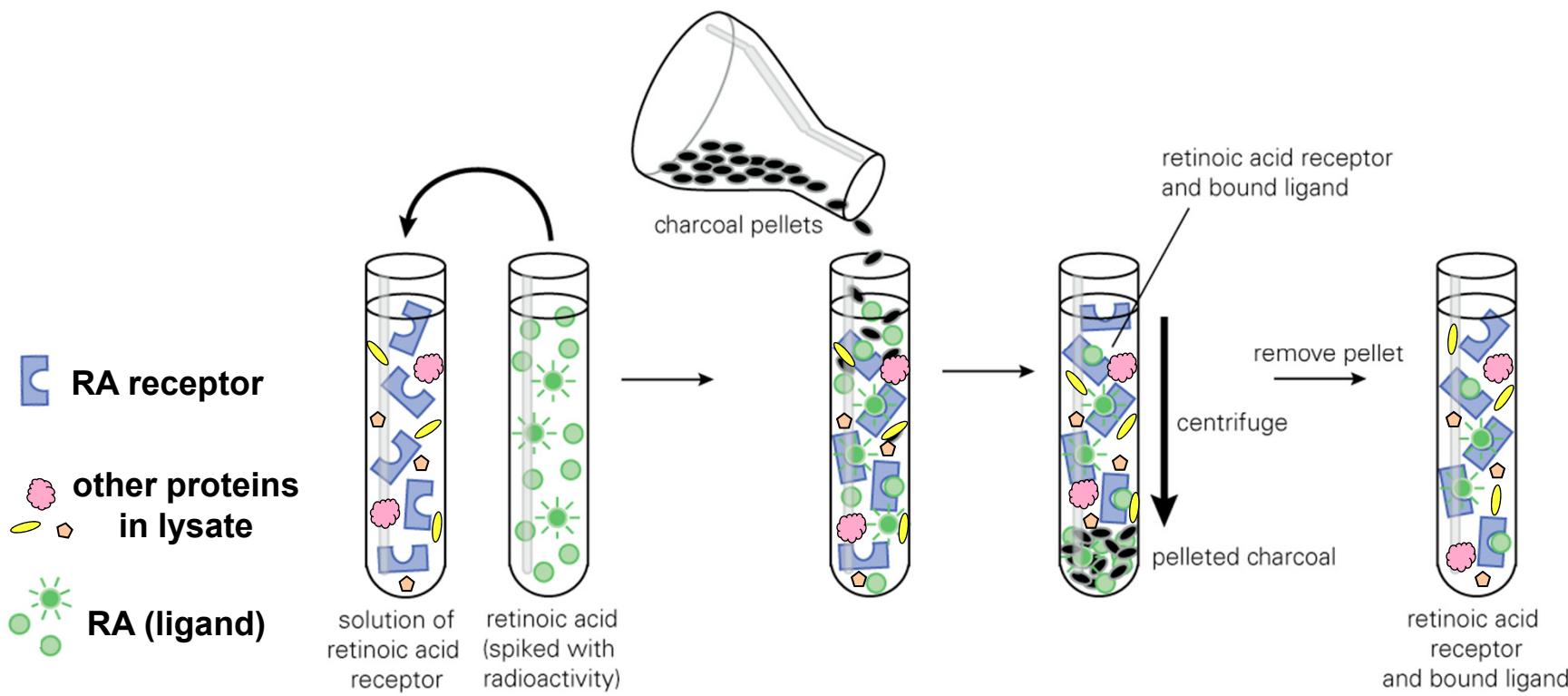
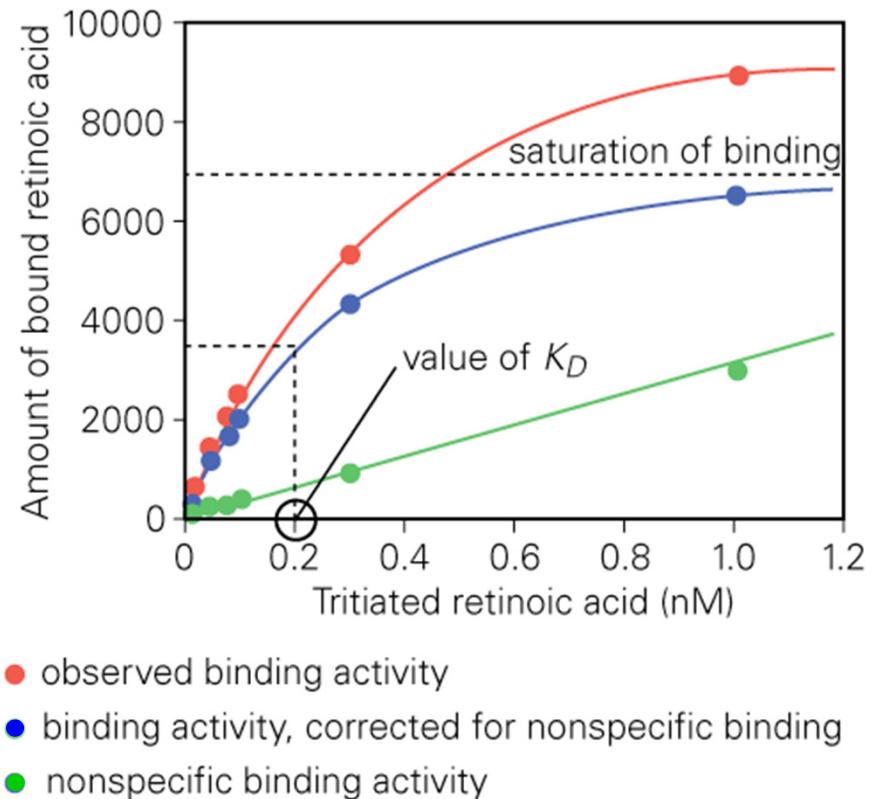


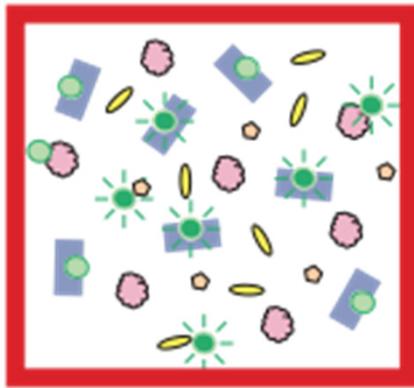
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## *Background subtraction*

- Some assays include background binding – unsaturatable
- Can be measured in the presence of excess unlabeled ligand and subtracted from the signals
- Saturatable -> specific
- Unsaturatable -> non-specific

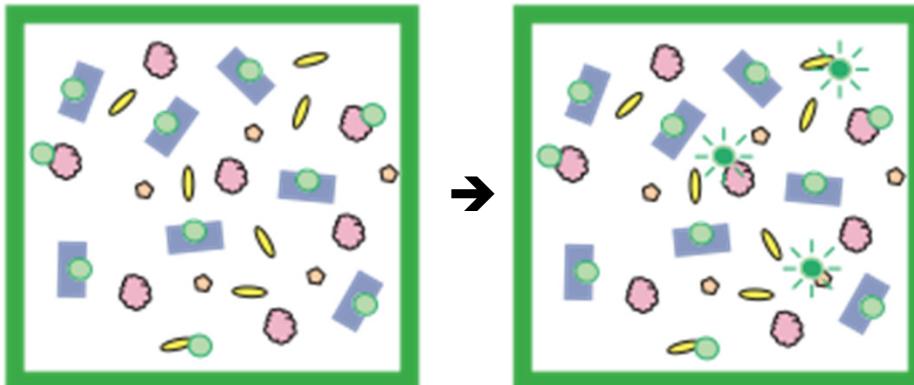


# Background subtraction

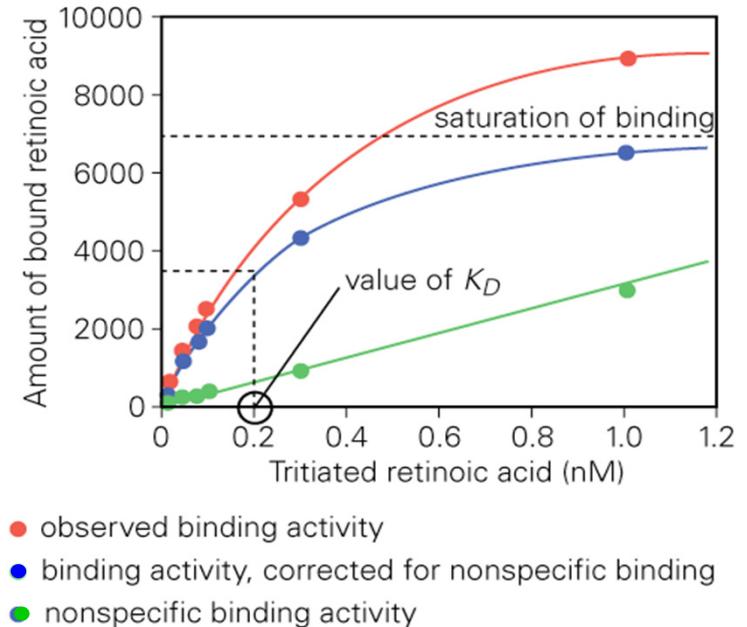


**Observed binding:** labeled ligand binds specifically to its receptor, as well as non-specifically to other molecules in the lysate

**Measuring background binding:** first saturate specific receptor using a high concentration of unlabeled ligand, then add labeled ligand



Any binding of labeled ligand is due to interaction of ligand with proteins *other than* the receptor



$$\begin{aligned} &\text{observed binding activity} \\ &- \text{background binding activity} \\ &= \text{corrected binding activity} \end{aligned}$$

## *Deriving the Scatchard plot equation*



- If the ligand doesn't bind to anything else, then  $[R \cdot L] = [L]_{\text{bound}}$

$$[R] = [R]_{\text{total}} - [R \cdot L] = [R]_{\text{total}} - [L]_{\text{bound}}$$

$$\frac{[R][L]}{[R \cdot L]} = K_D = \frac{([R]_{\text{total}} - [L]_{\text{bound}})[L]}{[L]_{\text{bound}}}$$

- Rearranging: 
$$\frac{[L]_{\text{bound}}}{[L]} = \frac{([R]_{\text{total}} - [L]_{\text{bound}})}{K_D}$$

- Scatchard equation: 
$$\frac{[L]_{\text{bound}}}{[L]} = -\frac{[L]_{\text{bound}}}{K_D} + \frac{[R]_{\text{total}}}{K_D}$$

## *Scatchard plot equation*

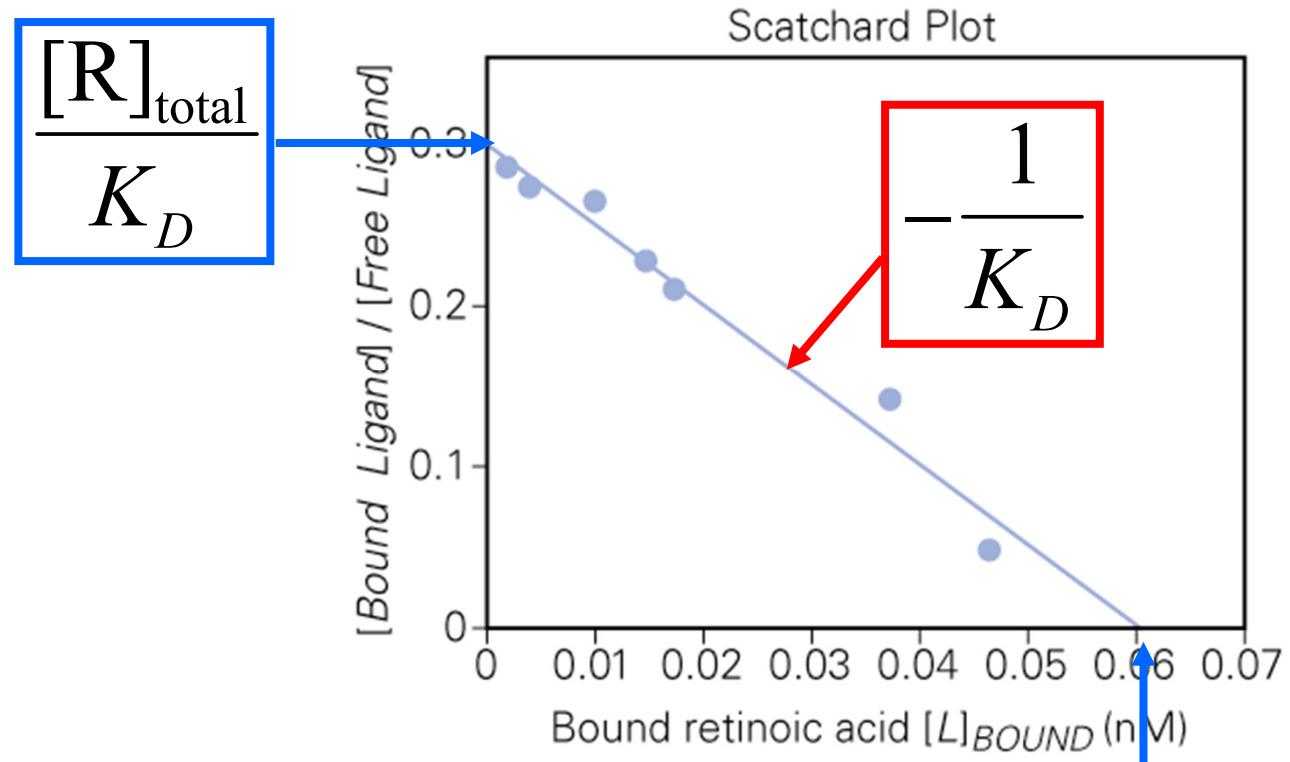


- Scatchard plot equation:

$$\frac{[L]_{\text{bound}}}{[L]} = -\frac{1}{K_D} [L]_{\text{bound}} + \frac{[R]_{\text{total}}}{K_D}$$

- Takes the form  $y = ax + b$
- Scatchard plot with  $[L]_{\text{bound}}/[L]$  vs.  $[L]_{\text{bound}}$

## Scatchard plot analysis



$$\frac{[L]_{bound}}{[L]} = \frac{1}{K_D}[L]_{bound} + \frac{[R]_{total}}{K_D}$$

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## *Relevant [L] range*

$$f = \frac{[L]}{K_D + [L]}$$

- Two orders of magnitude spanning the  $K_D$  spans the range from mostly free receptor to mostly bound receptor
  - $0.1K_D < [L] < 10K_D$  spans 9% to 91% bound

$$f(0.1K_D) = \frac{0.1K_D}{K_D + 0.1K_D} = \frac{0.1}{1.1} = 9\%$$

$$f(10K_D) = \frac{10K_D}{K_D + 10K_D} = \frac{10}{11} = 91\%$$

- This represents the relevant concentration range for regulatory interactions

## Semi-log plot of universal binding isotherm

- Semi-log plot illustrates the universal relevant concentration range

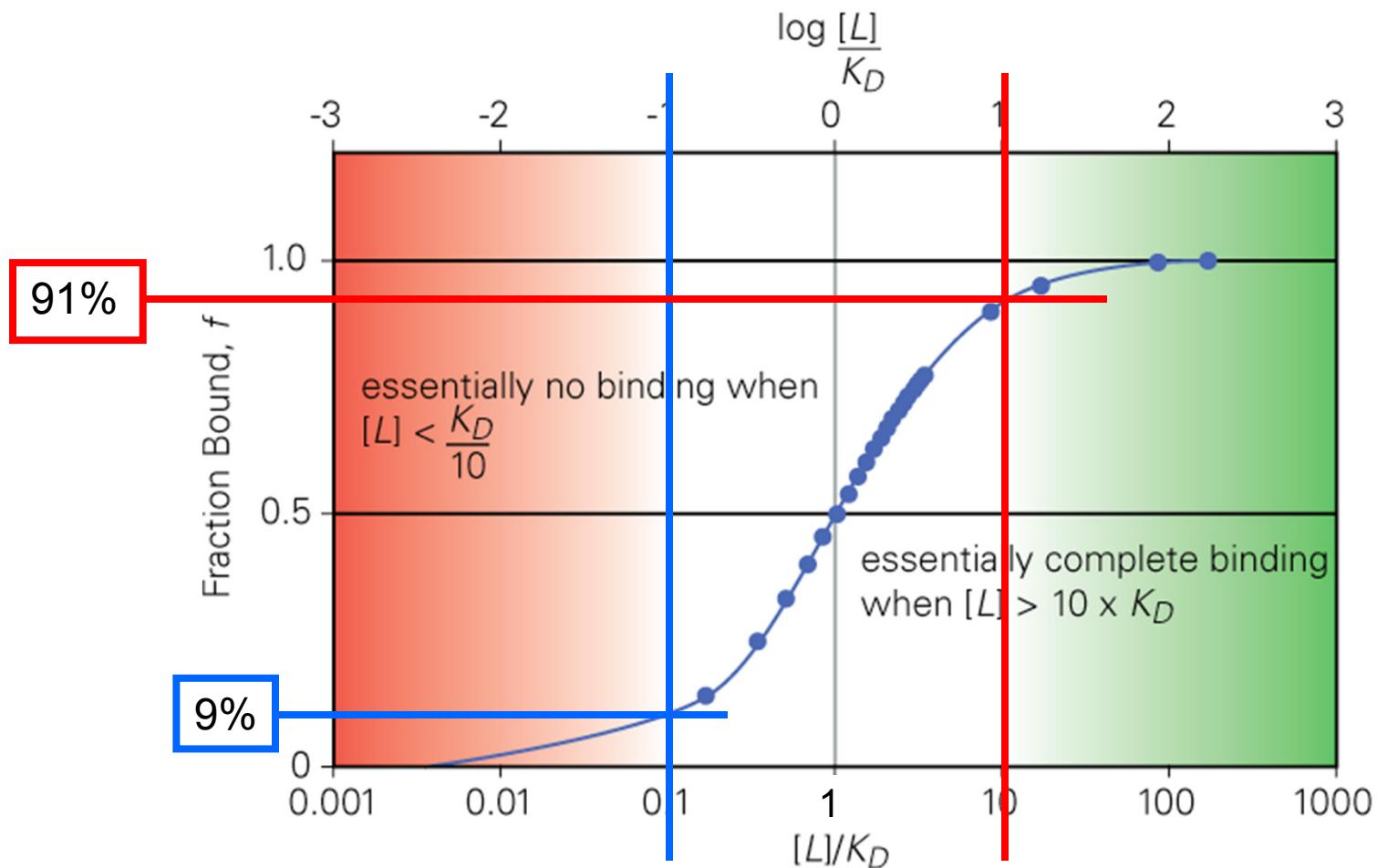
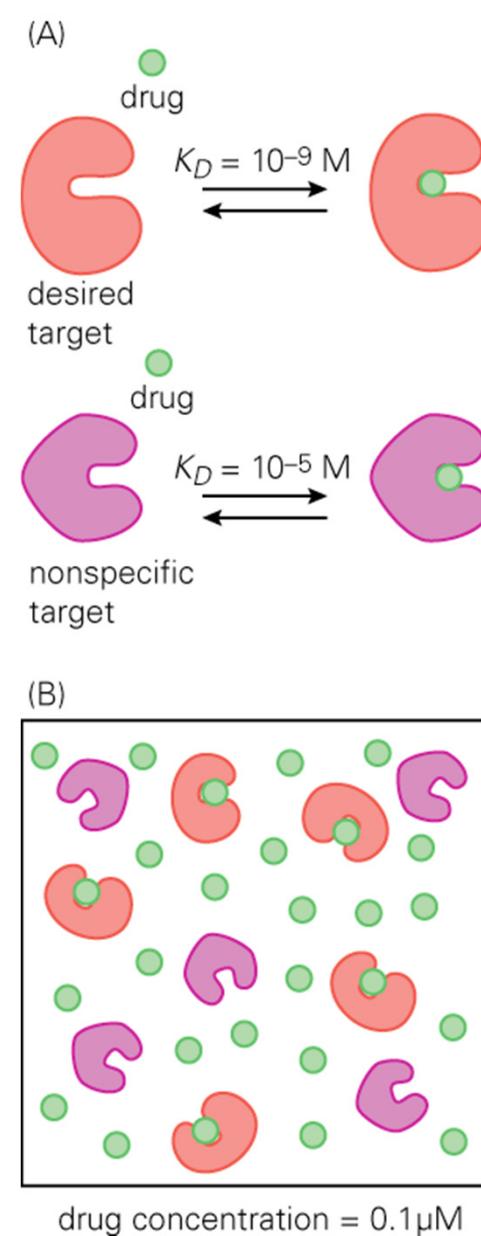


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# Specificity

- **Specificity:** relative strength of interaction for a cognate and non-cognate receptor-ligand complex
  - Depends on the ratio of the  $K_D$  values
  - Use the universal binding isotherm to determine the best ligand concentration to insure a specific interaction



# Semi-log plot of universal binding isotherm

- 4 orders of magnitude difference in  $K_D$  makes for a very specific interaction

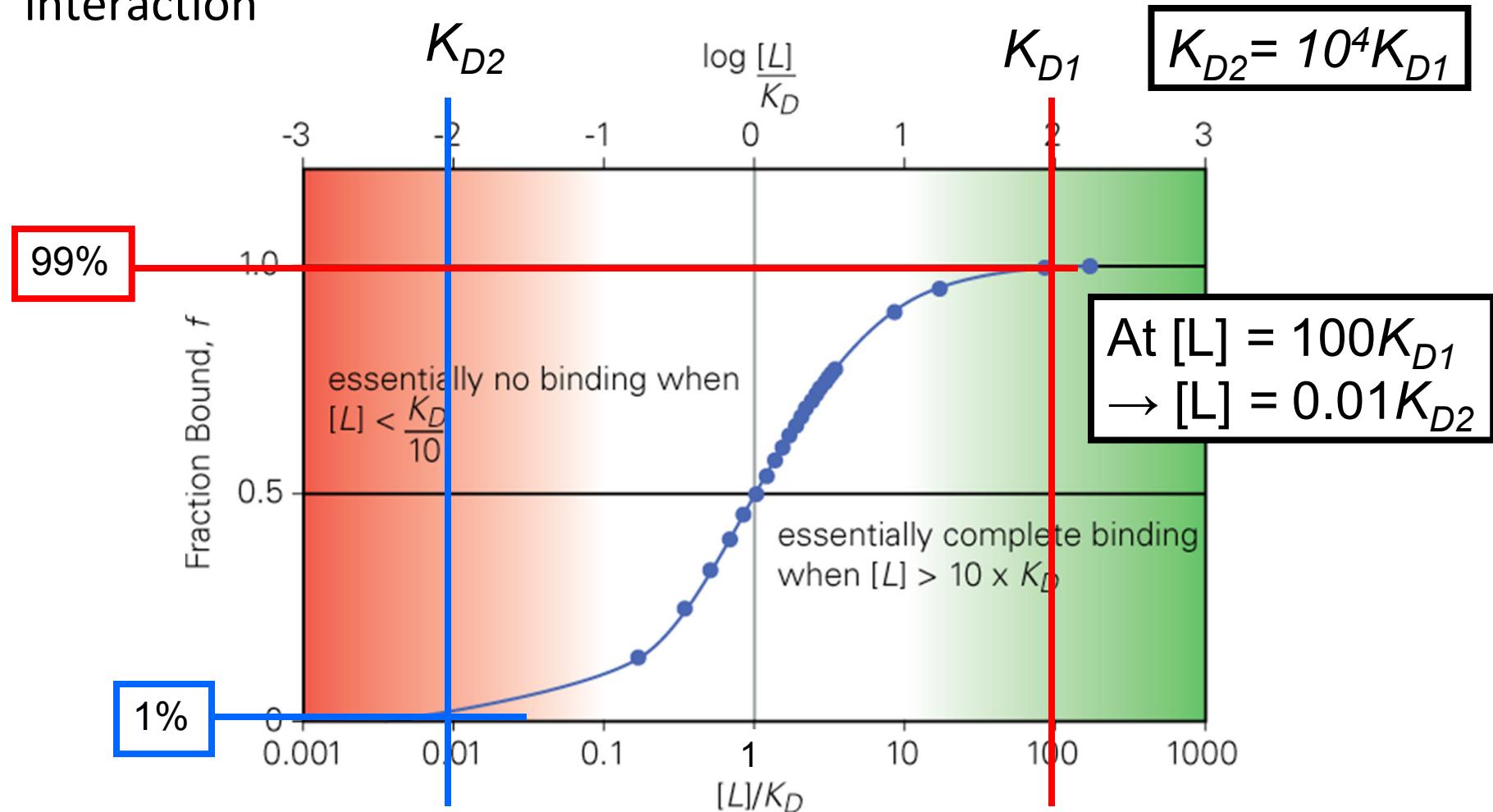


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# Specificity

- $K_D(\text{specific}) = 10^{-9} \text{ M}$
- $K_D(\text{nonspecific}) = 10^{-5} \text{ M}$
- At  $[L] = 10^{-7} \text{ M}$ 
  - 99% binding to desired target
  - 1% binding to nonspecific target
  - Good specificity and proper dosage limit undesirable side effects

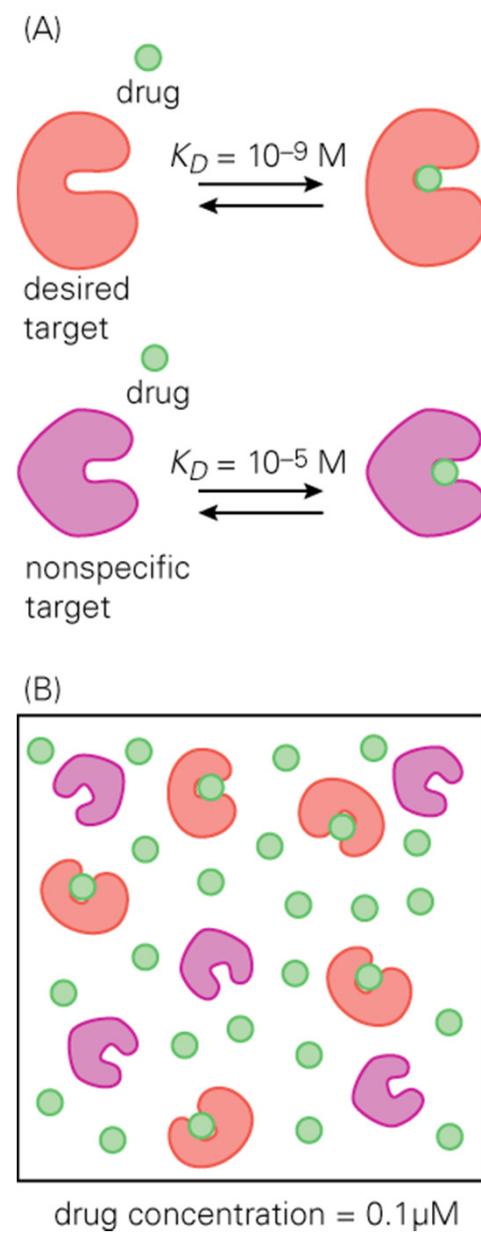


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## *Some concepts to remember*

- Binding assays measure the bound ligand
- The dissociation constant,  $K_D$ , corresponds to the ligand concentration at which half of the receptors are occupied
- Scatchard analysis can provide the  $K_D$  and the concentration of receptor
  - Linear Scatchard plot indicates a simple binding reaction
  - Both affinity and specificity are important