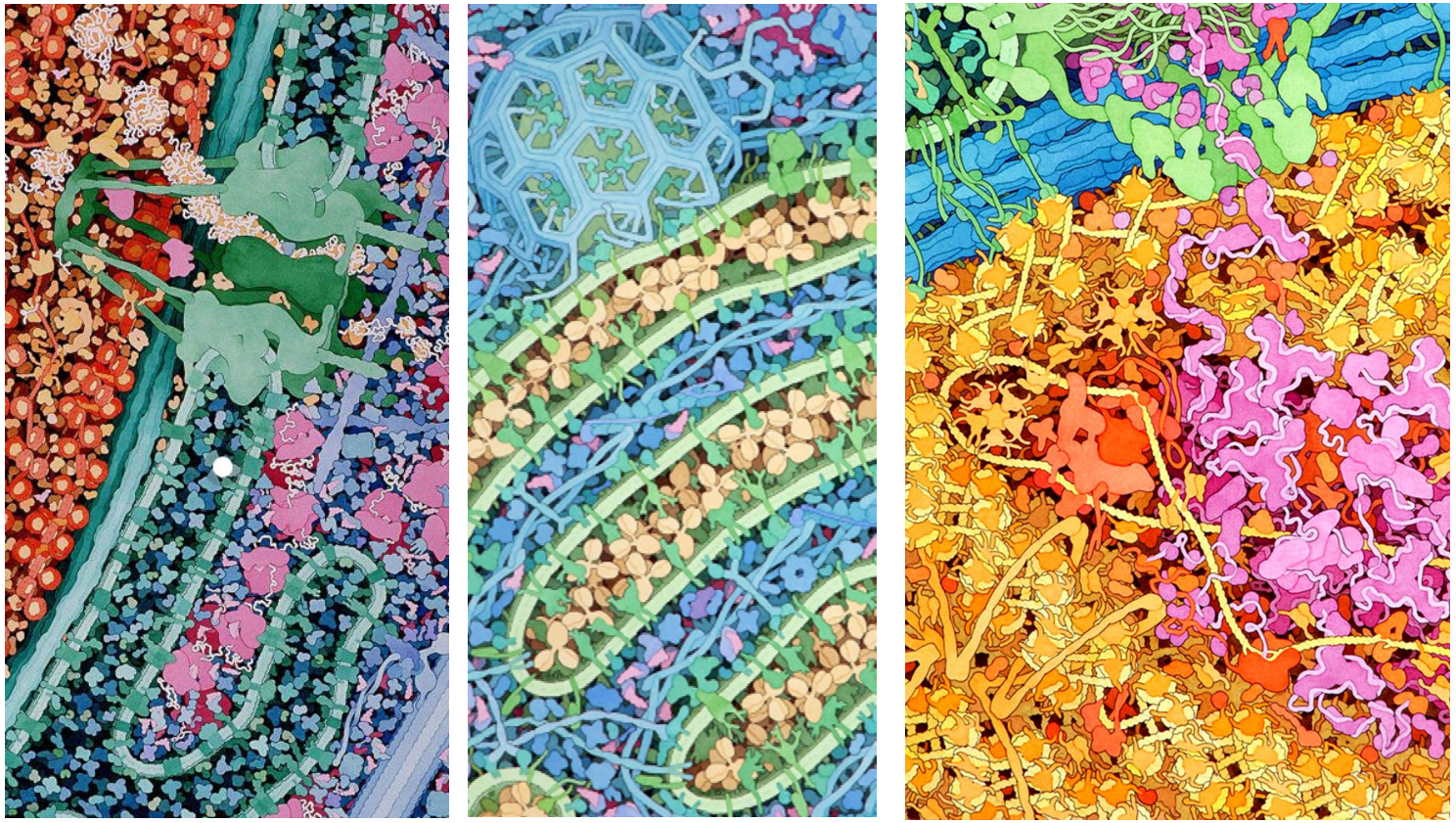
Binding interactions are the foundation of biology



David Goodsell

Cellular signaling – Receptor ligand interactions

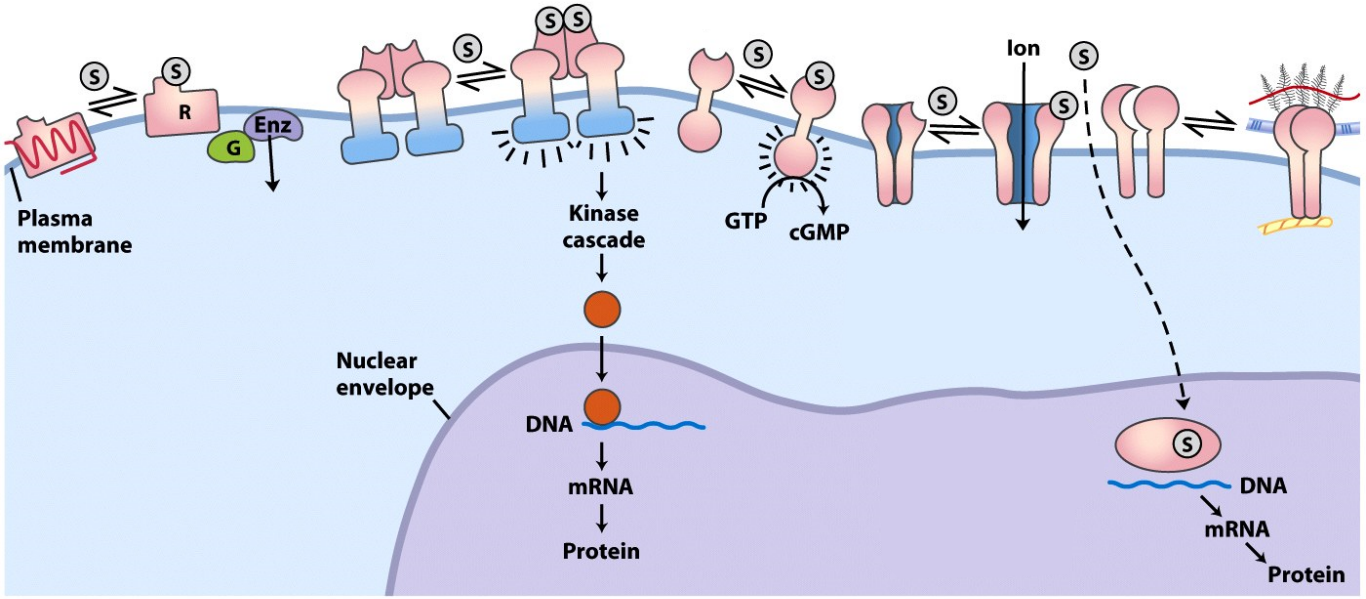
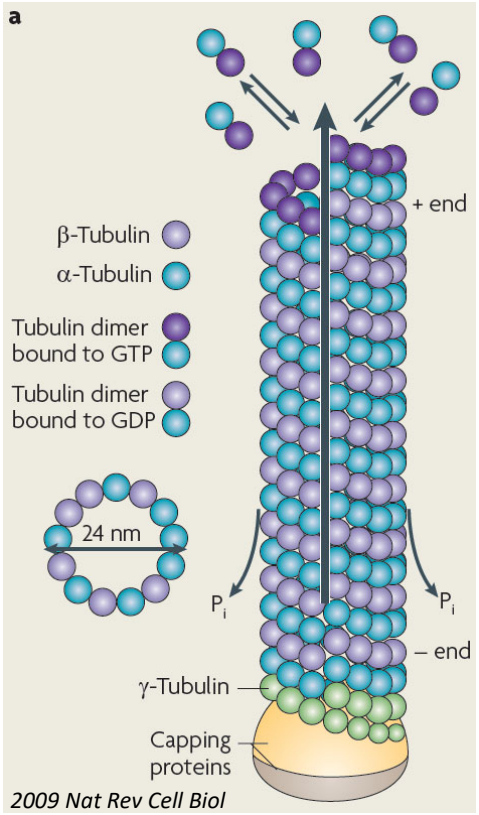
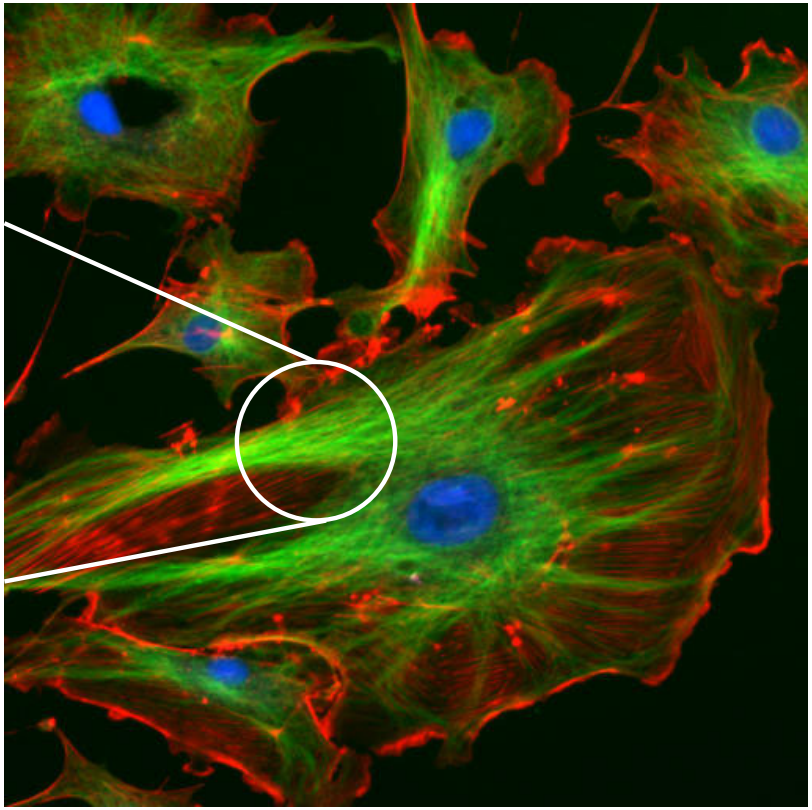


Figure 12-2
Lehninger Principles of Biochemistry, Fifth Edition

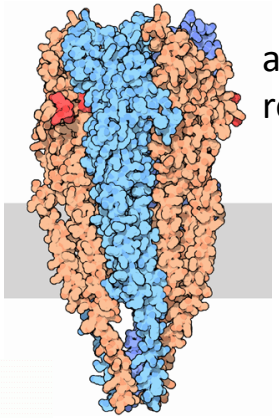
Protein – protein association: Large structures



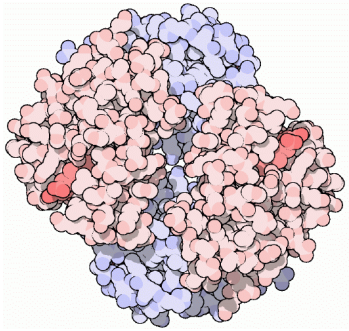


Types of association reactions

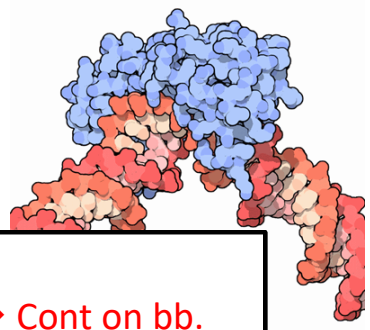
- **Protein – protein binding (homo-oligomers, hetero-oligomers)**
  - oligomeric enzymes
    - metabolic enzymes
    - ribosomes
    - channel proteins and pores
    - molecular machines
  - structural proteins
    - cytoskeleton
    - extracellular proteins
- **Receptor – ligand interactions**
  - signaling and import
  - cell surface receptors
  - intracellular receptor
- **Protein – DNA binding**
  - transcription factors
  - chromatin
  - DNA enzymes



acetylcholine receptor



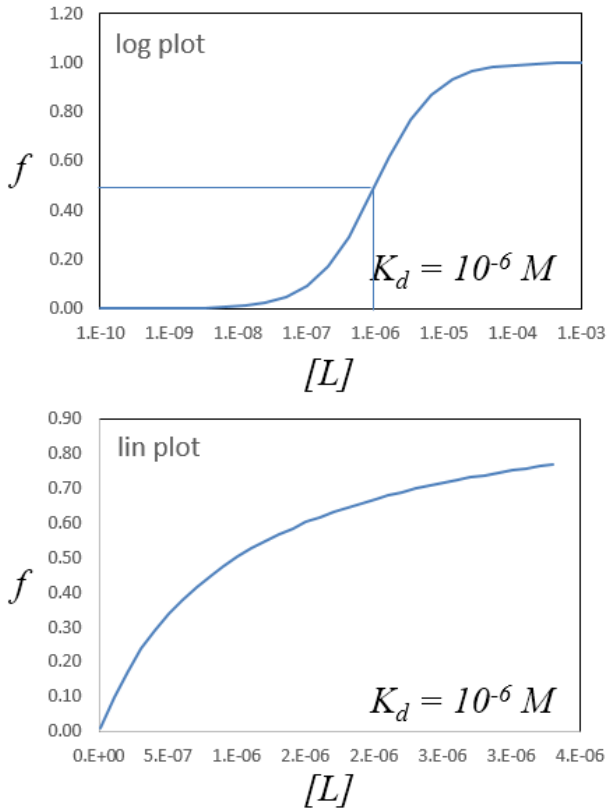
hemoglobine



TATA-bin
pro

→ Cont on bb.

Binding curves for one site binding



$$R + L \rightleftarrows RL$$

sigmoidal binding curve

advantage of semilogarithmic plot:
both baselines are available

The free energy of binding

The energy of a ligand-receptor interaction is determined by ΔG :

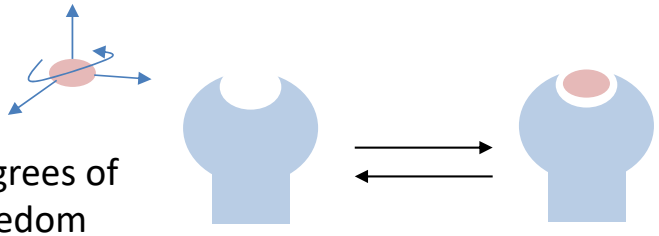
$$\Delta G = RT \ln K_D$$

... whereas ΔG itself can be separated into enthalpy and entropy

$$\Delta G = \Delta H - T\Delta S$$

+ ionic
+ vdW
+ H-bonds

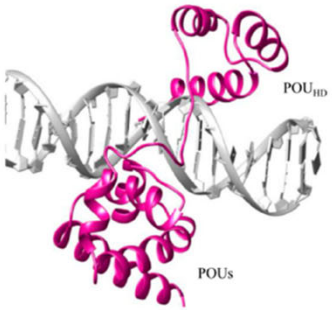
+ hydrophobic effect
- loss of ligand entropy!



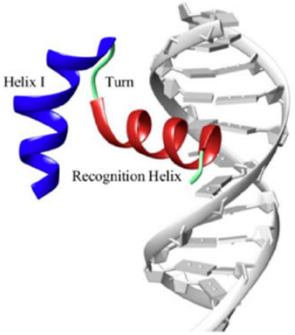
degrees of freedom

degrees of freedom fixed!

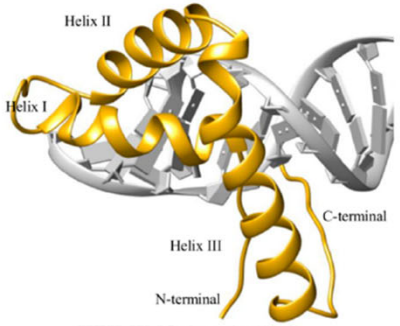
Calculation example



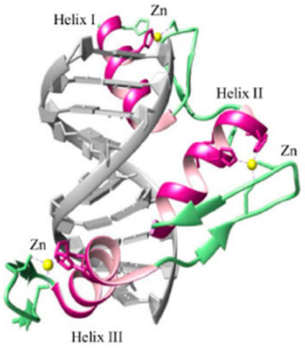
HTH-HD domain (3l1p)



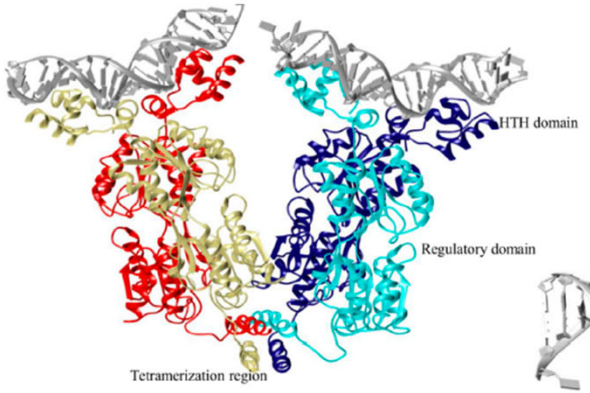
HTH domain (5d5v)



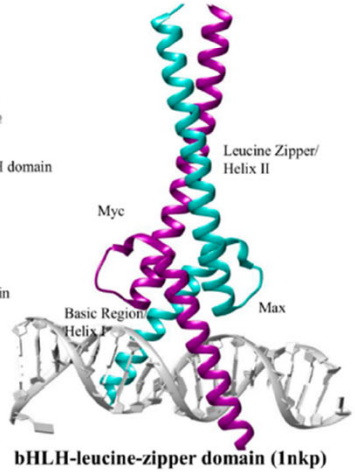
HTH-HMG domain (1gt0)



Zn finger domain (4m9e)



Lac repressor-DNA complex (1lbg)



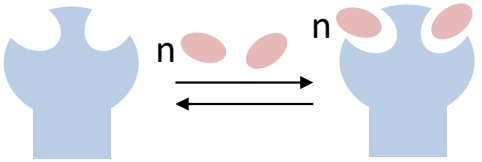
bHLH-leucine-zipper domain (1nkp)

https://doi.org/10.3390/genes8080192

- You have a protein (transcription factor) binding to a particular DNA sequence with a dissociation constant of 10^{-9} M.
- Now, you test a different sequence and find that the protein binds tenfold (10x) better
- what is the **free energy difference** between the two interactions?
- Could you suggest what molecular interaction could account for that difference?

Binding to multiple non-interacting sites

$$R + nL \rightleftarrows \rightleftarrows \ldots \rightleftarrows RL_n$$



for a single site: $L + s \rightleftarrows L_b$ $\quad K_d = \dfrac{[s][L]}{[L_b]}$

s: free binding site

L_b: bound ligand

$$f = \frac{RL + 2RL_2 + 3RL_3 + \ldots}{R + RL + RL_2 + \ldots} = \frac{\sum_{i=1}^{n} iRL_i}{\sum_{i=0}^{n} RL_i}$$

fraction of ligand bound per macromolecule

$$f = \frac{\sum_{i=1}^{n} i[R][L]^i / K'_i}{\sum_{i=0}^{n} [R][L]^i / K'_i} = \frac{\sum_{i=1}^{n} i[L]^i / K'_i}{\sum_{i=0}^{n} [L]^i / K'_i}$$

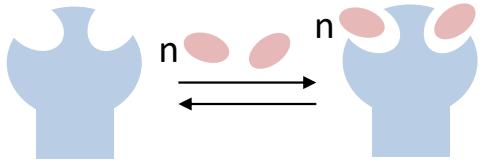
$$\boxed{f = \frac{n[L]}{[L] + K_d}}$$

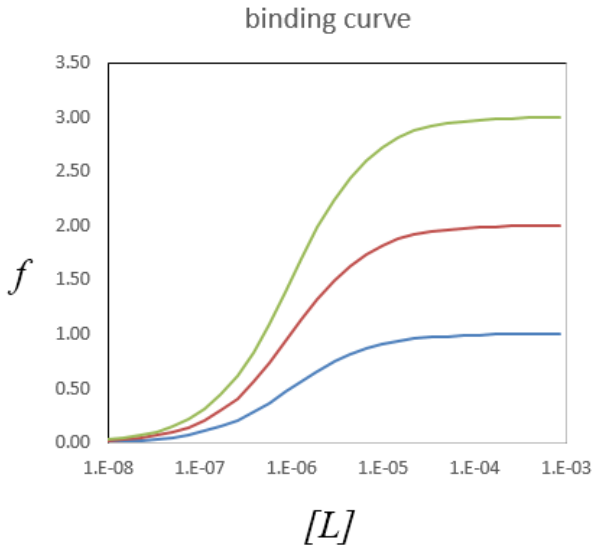
Binding isotherm for multi-site binding

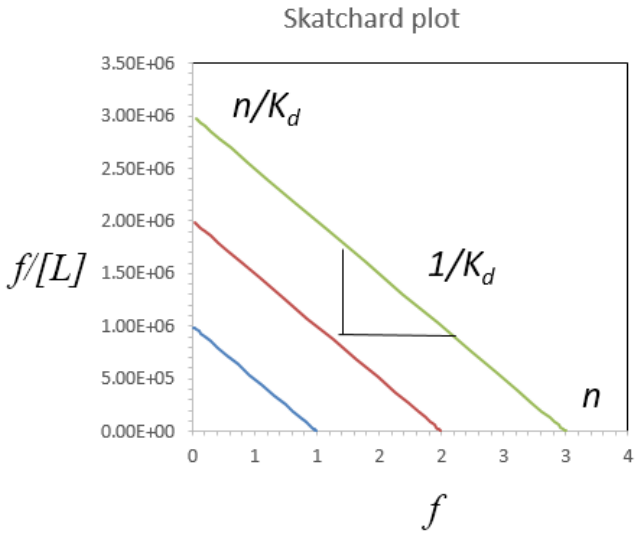
$$K'_i = K_1 \cdot K_2 \cdot K_3 \cdot \ldots K_i = \prod_i K_i$$

Scatchard plot for multisite binding

Linearization method







Plot of the ratio of bound ligand, L_b and unbound ligand, L vs. L_b

Cooperative binding

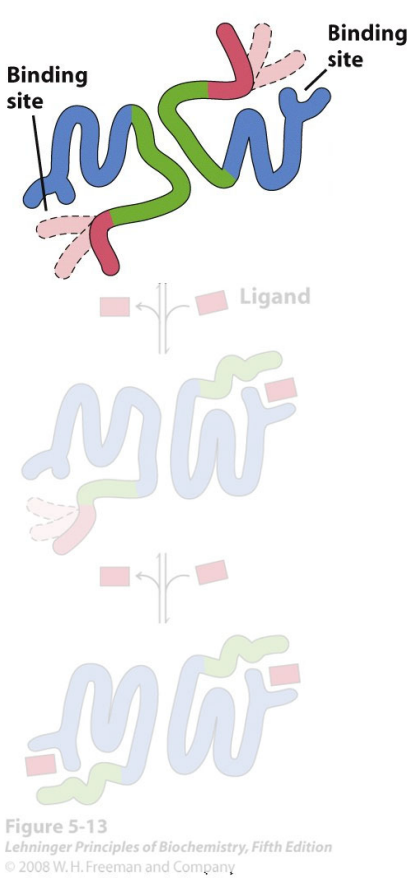


Figure 5-13
Lehninger Principles of Biochemistry, Fifth Edition

no ligand, flexible regions at binding site, interior low stability regions

one site bound
protomer converted into high affinity conformation
second binding site stabilized

second ligand bound with higher affinity
→ positive cooperativity

Cooperativity: Hemoglobin

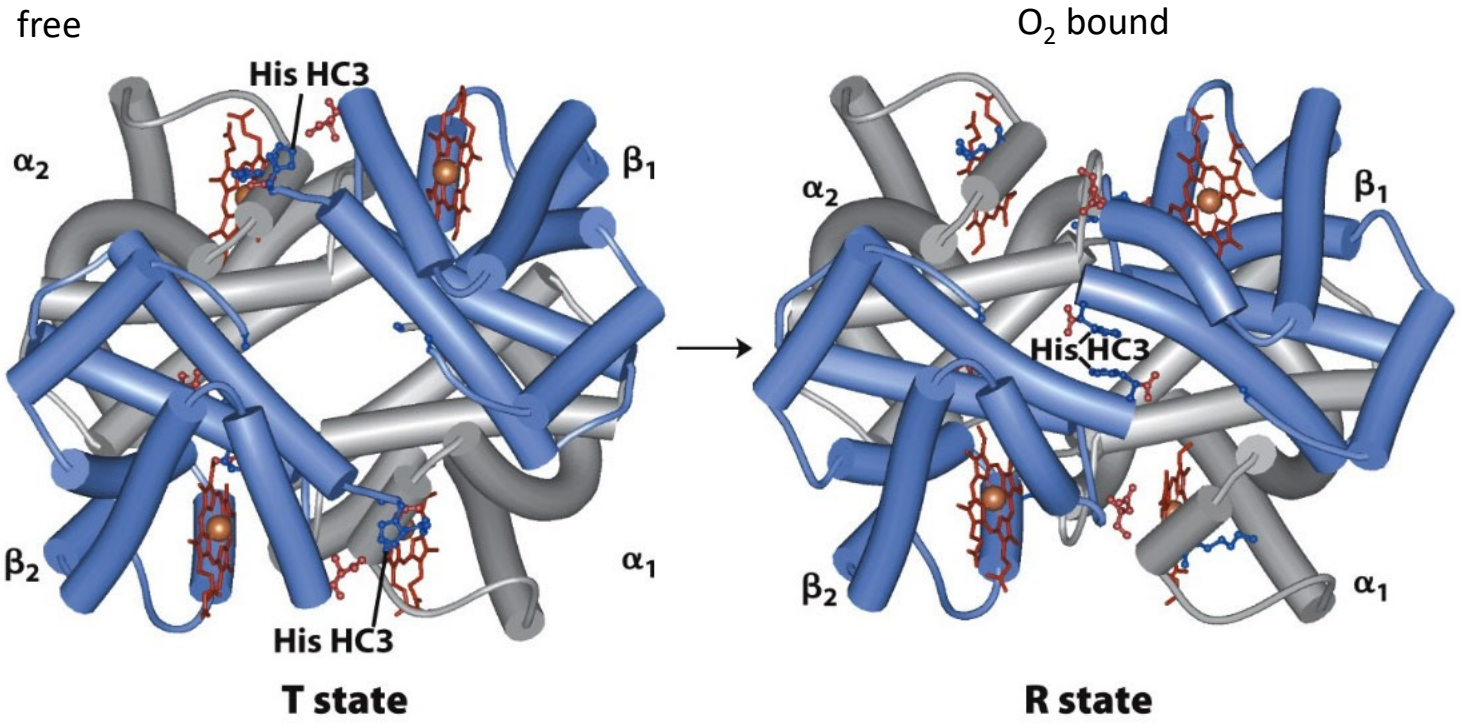
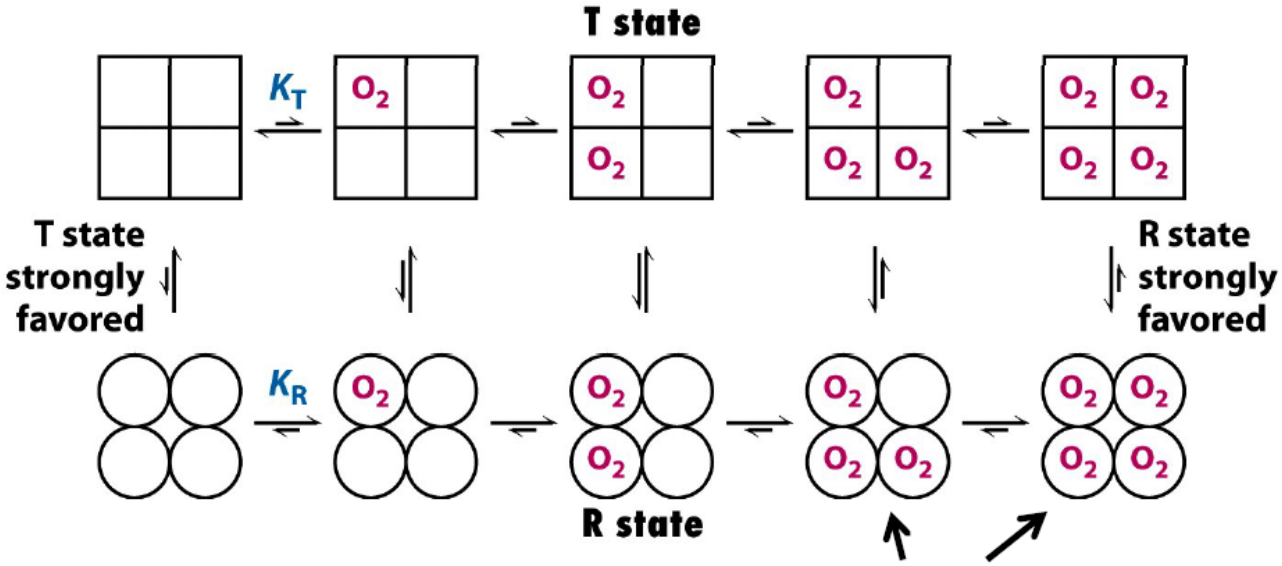


Figure 5-10
Lehninger Principles of Biochemistry, Fifth Edition

Fun fact: 750 g hemoglobin per adult, 10^{22} molecules (10^{8} molecules per blood cell).

Cooperativity: Hemoglobin



Cooperativity: Hemoglobin

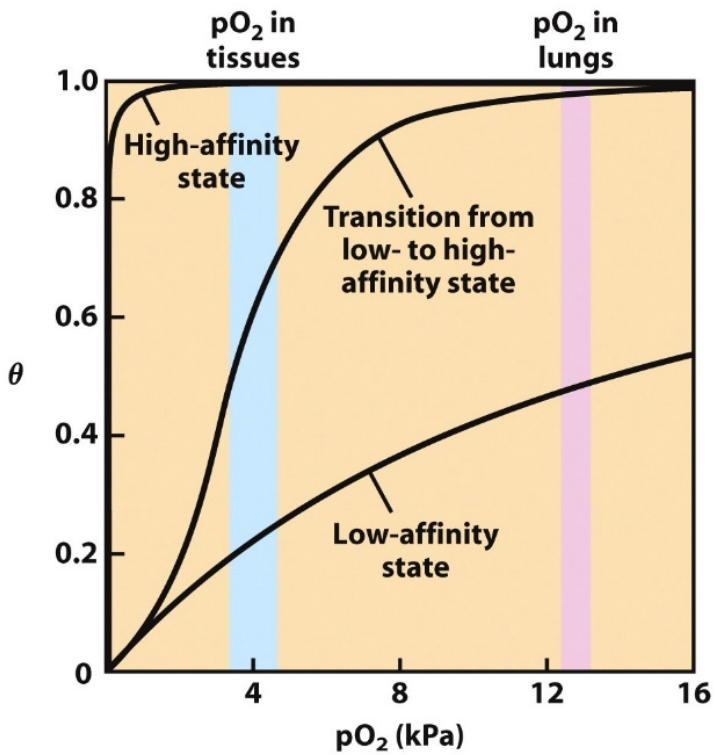


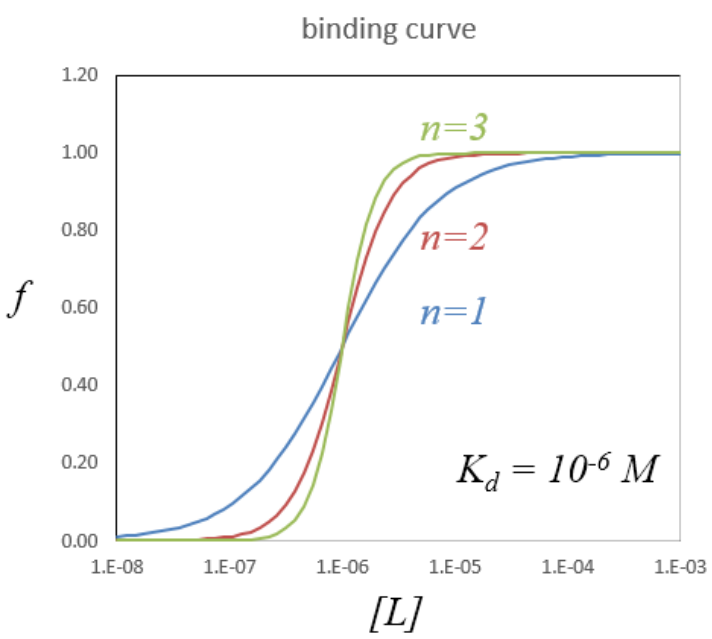
Figure 5-12
Lehninger Principles of Biochemistry, Fifth Edition

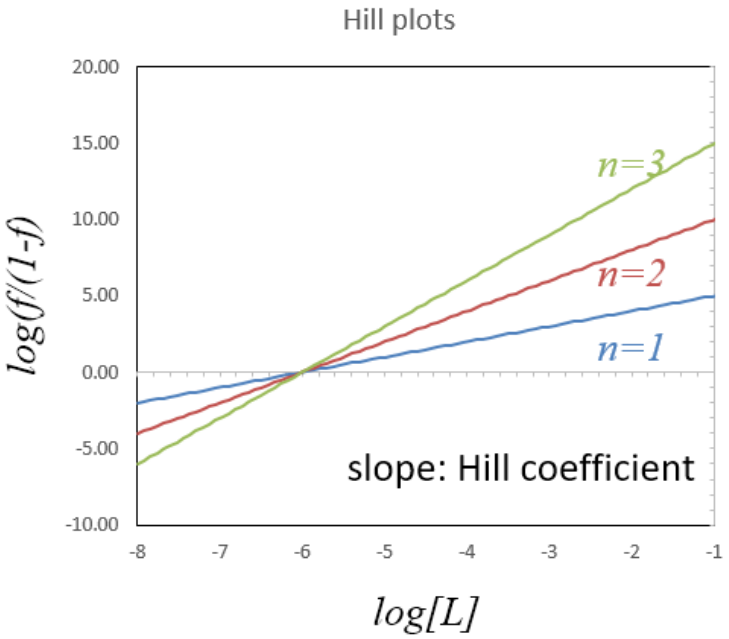
Finely tuned transition between high- to low affinity state

- transport function of hemoglobin
- transfer to myoglobin (only high affinity state)
- blood O2 saturation: critical parameter

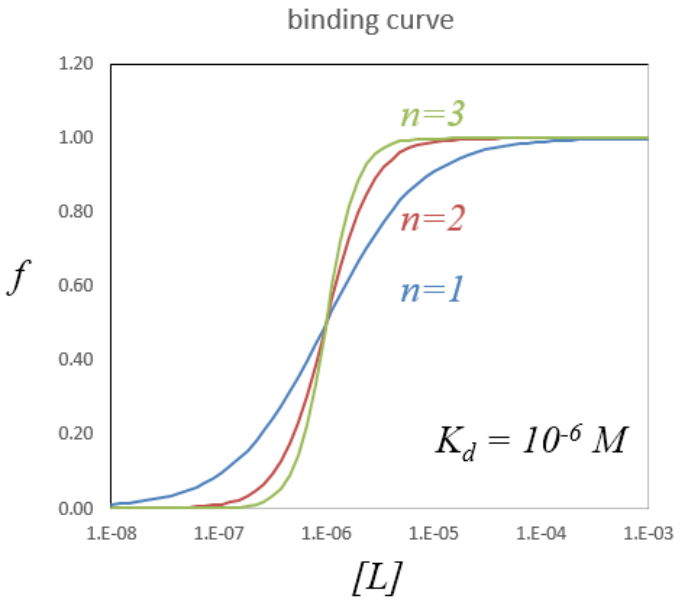
discussion on bb

The Hill plot





Interpretation of the Hill coefficient



For a receptor / protein with x binding sites:

Total cooperativity (all-or-none transition): $n = x$

No cooperativitiy: $n = 1$

All other cases: $1 < n < x$

Empirical description of the behavior

Effect of the receptor concentration

$$R + L \underset{K_d}{\rightleftharpoons} RL$$

Up to this point, we have assumed that [R] is much lower than the K_d

discussion on bb

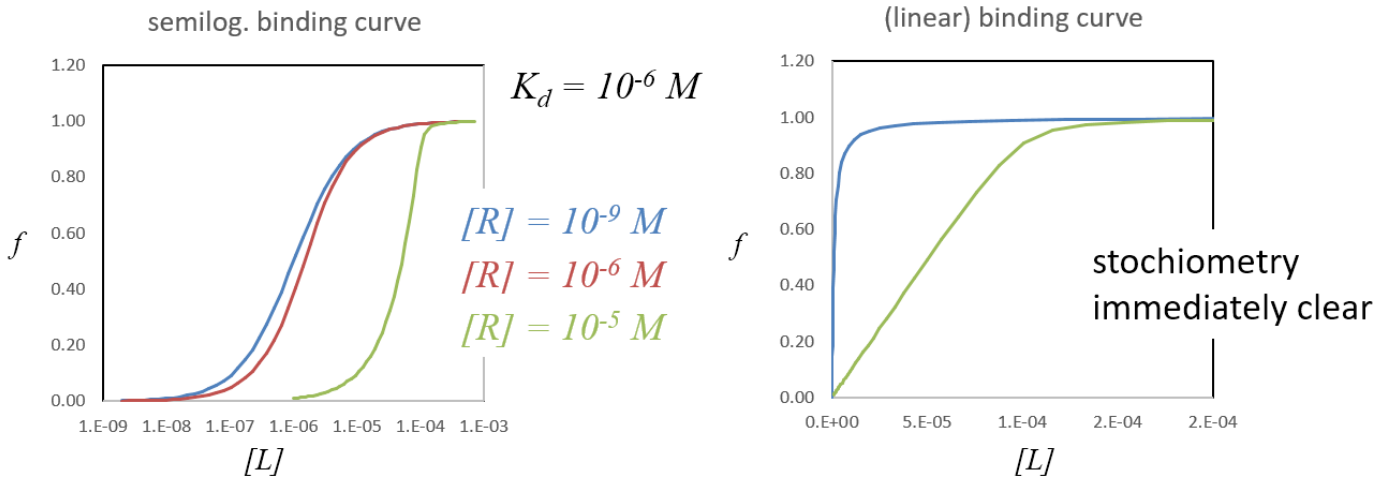
Effect of the receptor concentration

$$R + L \underset{K_d}{\rightleftharpoons} RL$$

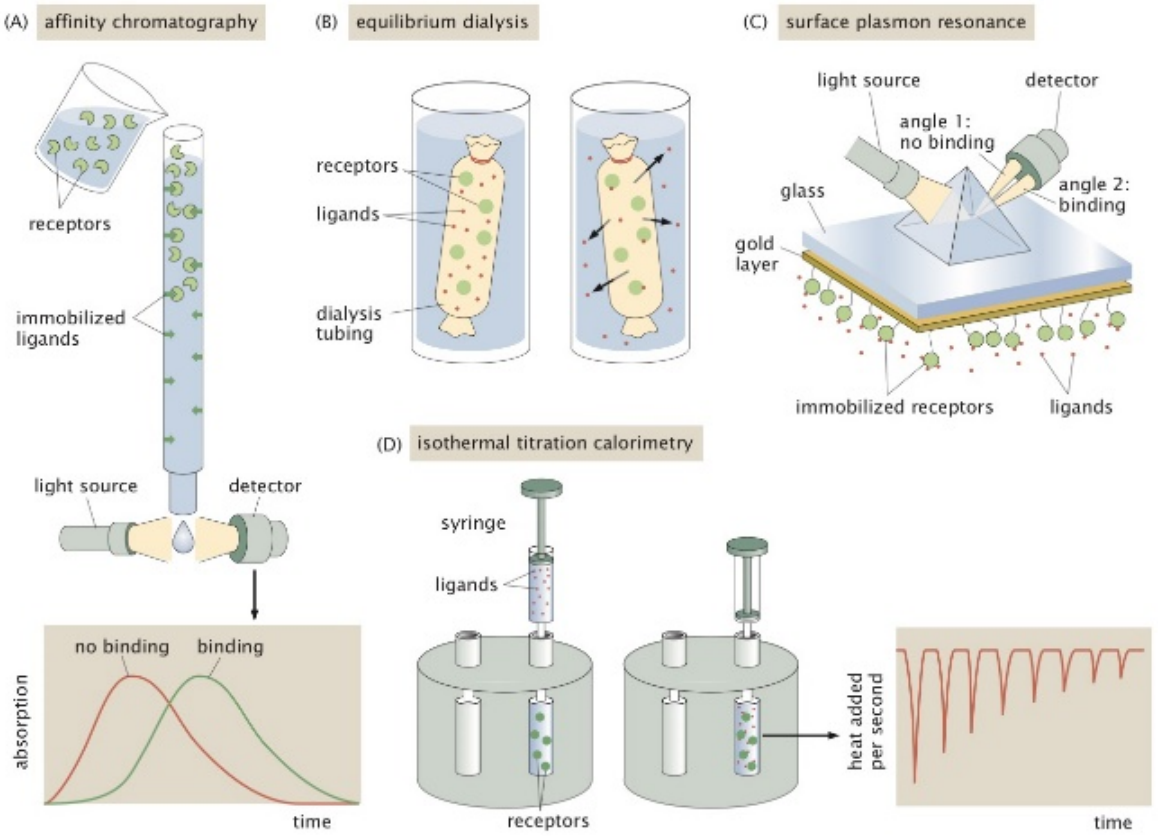
Up to this point, we have assumed that [R] is much lower than the K_d

if this is not the case the fraction bound ligand is expressed as:

$$\frac{[RL]}{[R_{tot}]} = \frac{K_d + [R_{tot}] + [L_{tot}]}{2} - \frac{\sqrt{(K_d + [R_{tot}] + [L_{tot}])^2 - 4[R_{tot}][L_{tot}]}}{2}$$



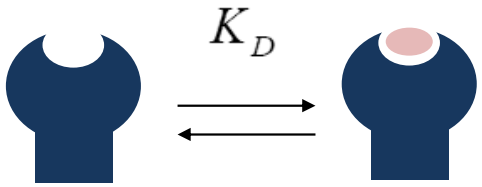
Measuring binding interactions



Using calorimetry to determine binding interactions

The energy of a ligand-receptor interaction is determined by ΔG :

$$\Delta G = RT \ln K_D$$



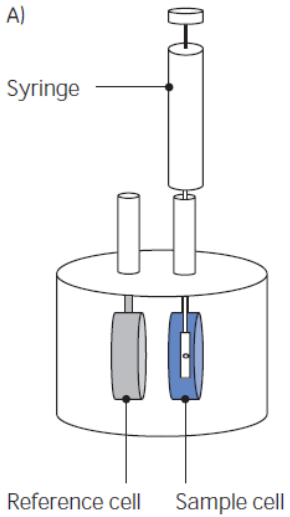
degrees of freedom fixed!

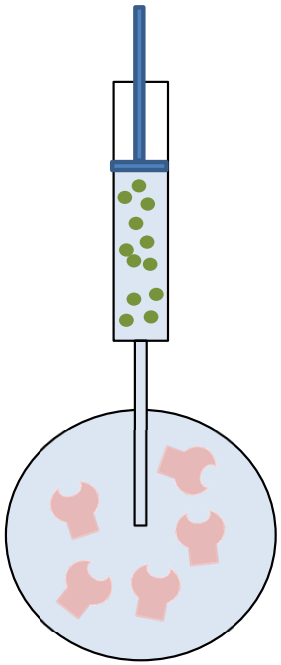
... whereas ΔG itself can be separated into enthalpy and entropy

$$\Delta G = \Delta H - T\Delta S$$

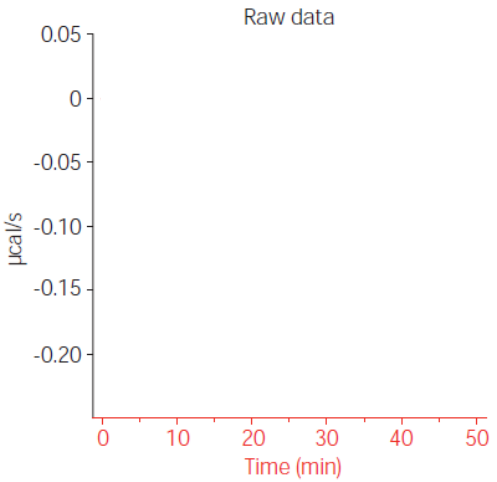
Calorimetry directly informs on thermodynamic parameters ΔH and ΔS

Using calorimetry to determine binding interactions: Isothermal titration calorimetry ITC



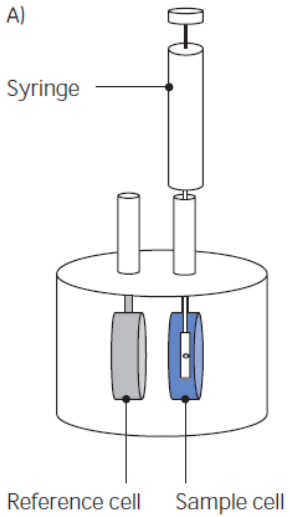


$$\Delta G = RT \ln K_D$$
$$\Delta G = \Delta H - T\Delta S$$

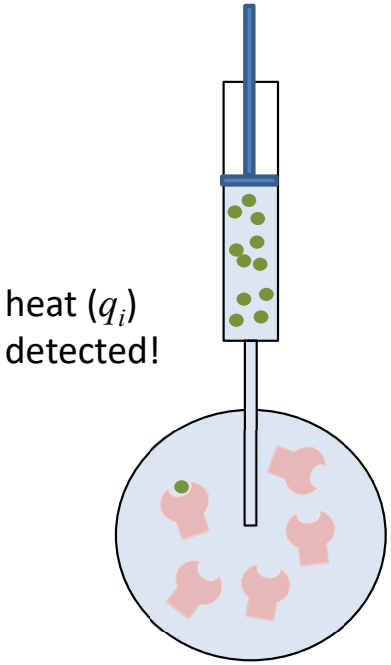


injection of ligand solution into receptor solution

Isothermal titration calorimetry

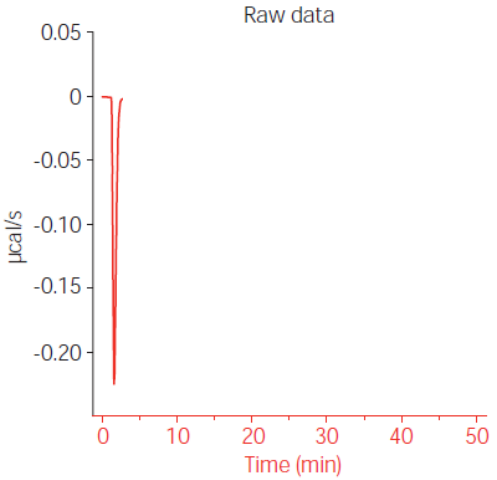


injection of ligand solution into receptor solution



heat (q_i) detected!

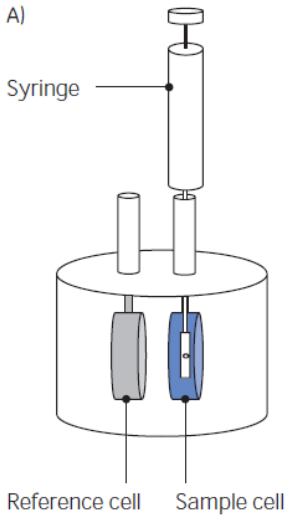
$$\Delta G = RT \ln K_D$$
$$\Delta G = \Delta H - T\Delta S$$



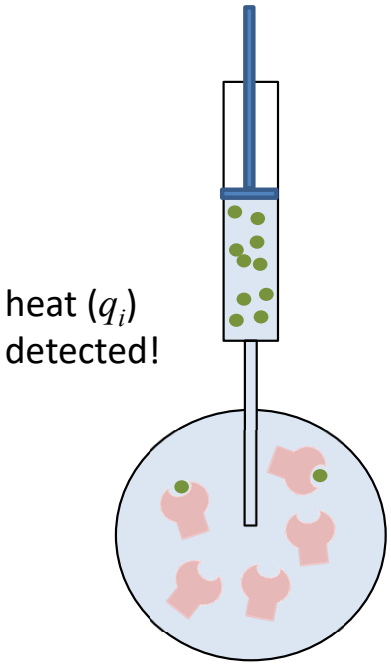
all injected ligand is bound

binding energy is released and is measured as heat

Isothermal titration calorimetry



injection of ligand solution into receptor solution



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$$\Delta G = RT \ln K_D$$
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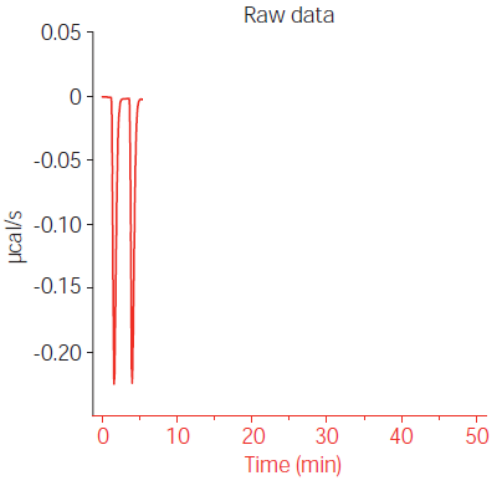

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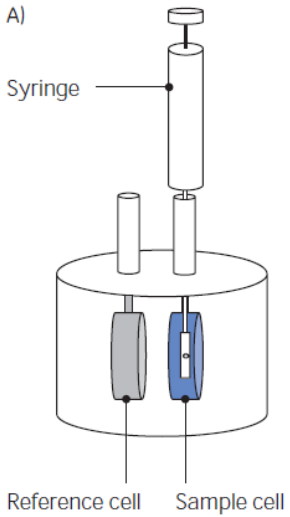
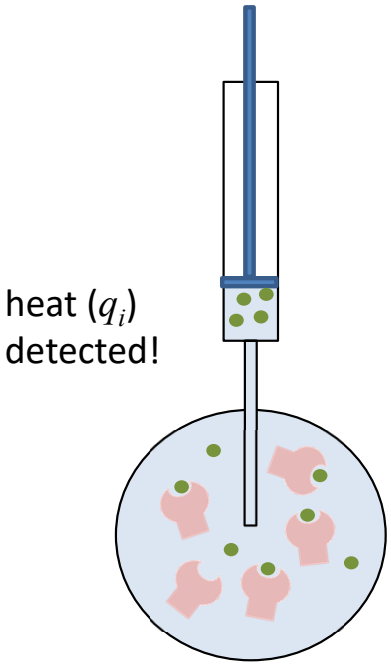
Isothermal titration calorimetry

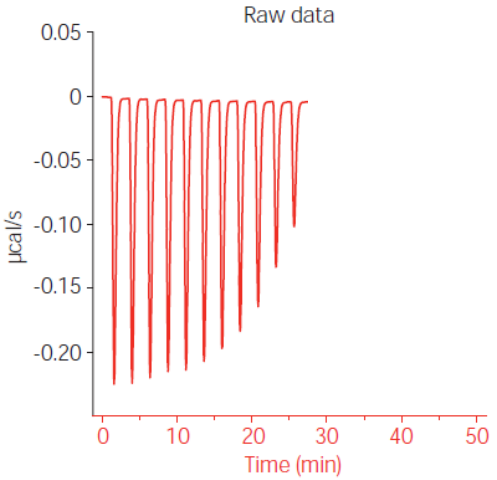


injection of ligand solution into receptor solution



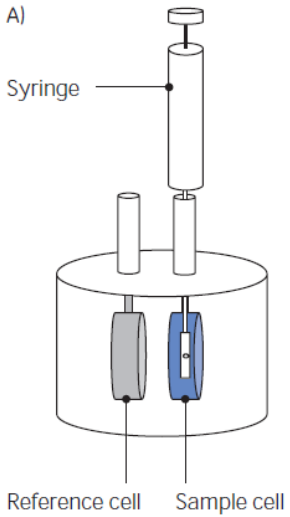
heat (q_i) detected!

$$\Delta G = RT \ln K_D$$
$$\Delta G = \Delta H - T\Delta S$$



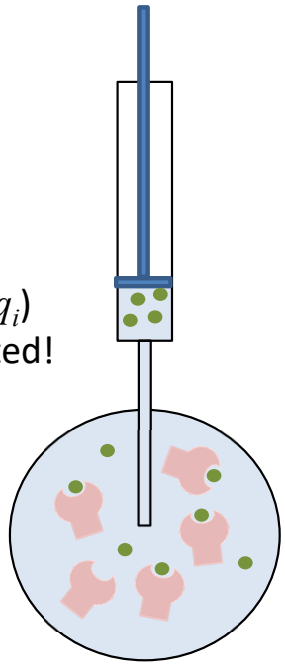
around the Kd, no longer all ligand is bound, less heat is released

Isothermal titration calorimetry

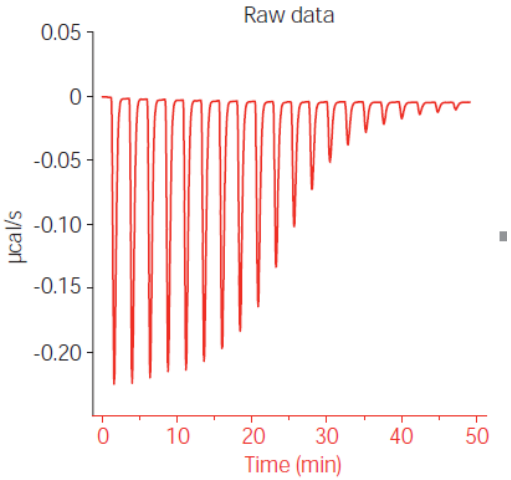


injection of ligand solution into receptor solution

heat (q_i) detected!

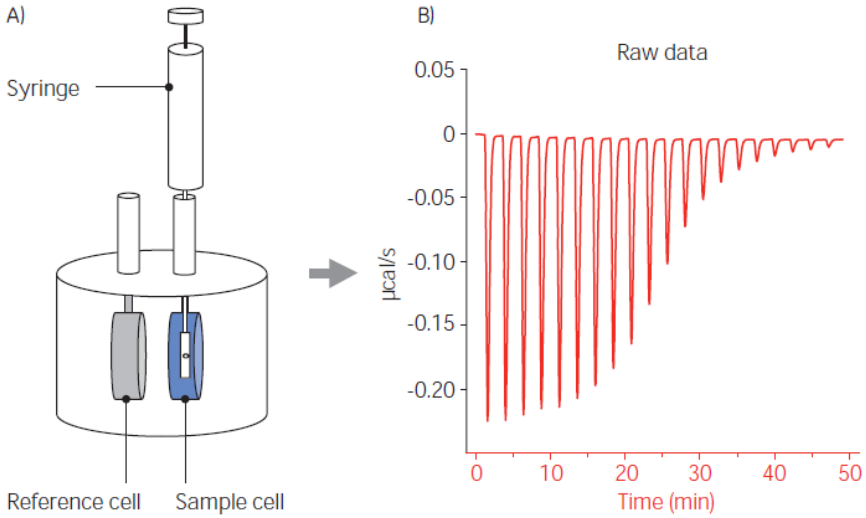


$$\Delta G = RT \ln K_D$$
$$\Delta G = \Delta H - T\Delta S$$



around the Kd, no longer all ligand is bound, less heat is released

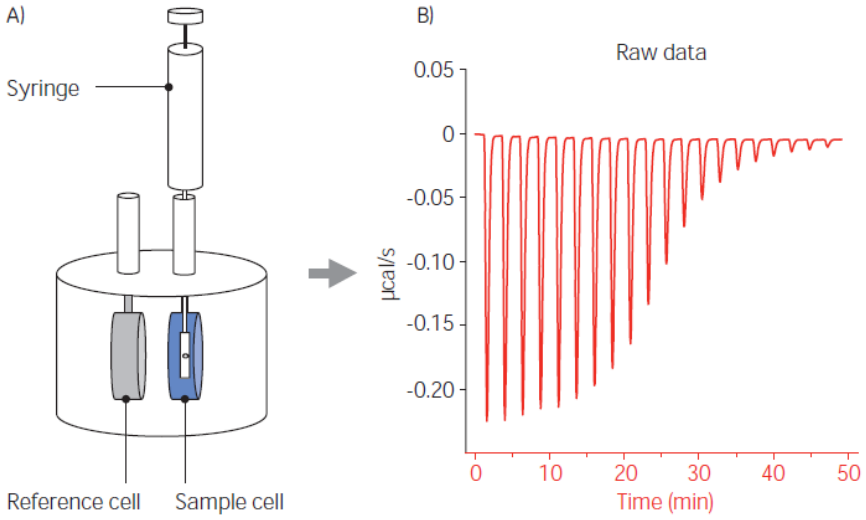
Isothermal titration calorimetry



injection of ligand solution into receptor solution

measurement of heat of binding

Isothermal titration calorimetry



injection of ligand solution into receptor solution

measurement of heat of binding

Analysis:

heat released (absorbed) for each injection

$$q_i = \Delta H_{app} \cdot V_C \left([RL]_{b,i} - [RL]_{b,i-1} \right)$$

Vc : volume of the cell

using binding isotherm for multisite binding:

$$f = \frac{\mathrm{n}[L]}{[L] + K_d} = \frac{[RL]}{[R_{tot}]}$$

$$q_i = \Delta H_{app} \cdot V_C \left(f_i [R_{tot}]_i - f_{i-1} [R_{tot}]_{i-1} \right)$$

ITC: Binding to n independent, equal sites

total heat released
(complete integral of curve):

$$Q = \sum_{i=1}^{N} q_i = \Delta H_{app} \cdot V_C \cdot [RL] = \Delta H_{app} \cdot V_C \cdot [R_{tot}] \cdot f$$

using the general solution, as shown before:

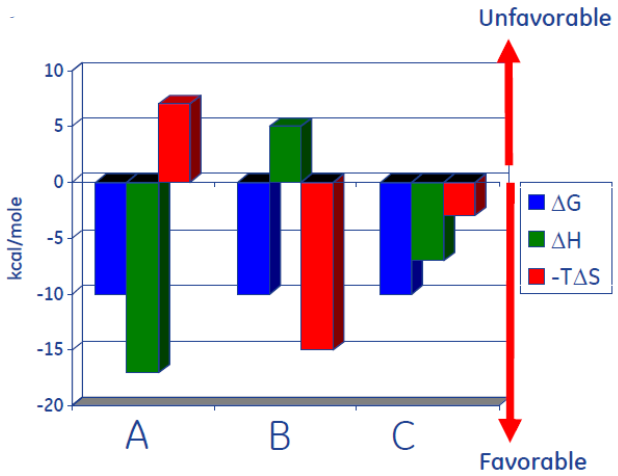
$$Q = \frac{\Delta H_{app} \cdot V_C}{2} \left([L_{tot}] + n[R_{tot}] + K_D - \sqrt{([L_{tot}] + n[R_{tot}] + K_D)^2 - 4n[R_{tot}][L_{tot}]} \right)$$

differentiate for $[L_{tot}]$:

$$\frac{dQ}{d[L_{tot}]} = \frac{\Delta H_{app} \cdot V_C}{2} \left(1 - \frac{[L_{tot}] + K_D - n[R_{tot}]}{\sqrt{([L_{tot}] + n[R_{tot}] + K_D)^2 - 4n[R_{tot}][L_{tot}]}} \right)$$

Simulated ITC traces

ITC can give informations about binding mechanisms



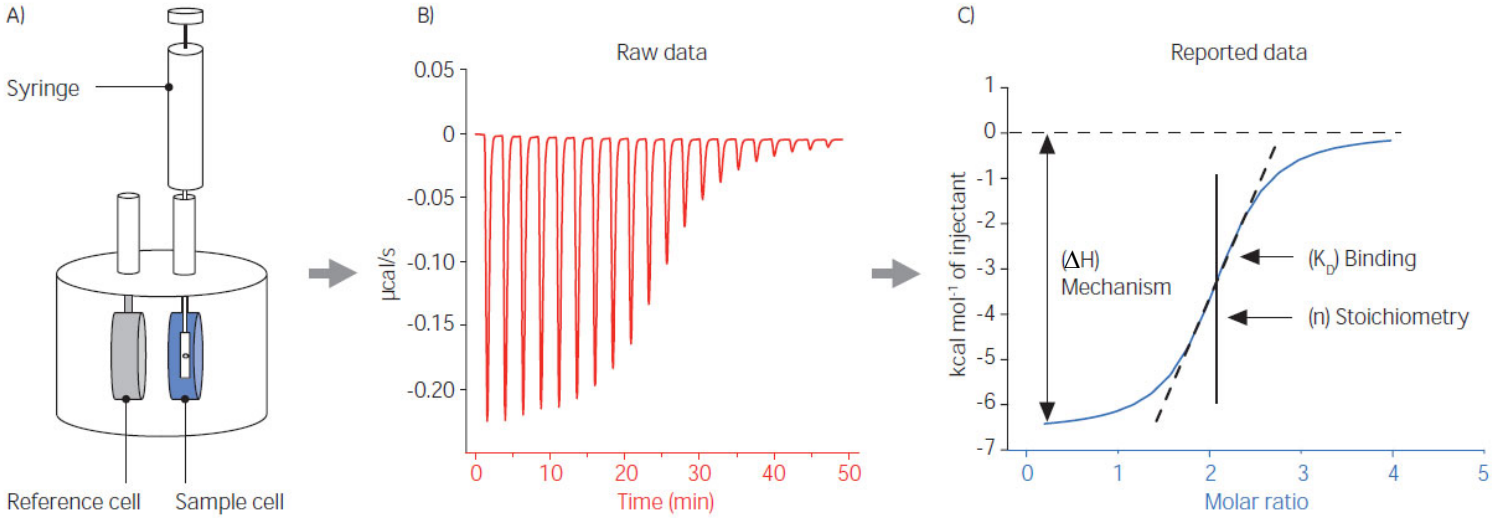
…all three binding reactions have the same ΔG.

A) good hydrogen bonding (enthalpy) and unfavorable conformational changes.

B) Hydrophobic interactions drive binding

C) both favorable enthalpic interactions and hydrophobic interactions

Isothermal titration calorimetry



injection of ligand solution into receptor solution

measurement of heat of binding

Fitting to **appropriate model:** determination of all parameters of a binding reaction

for useful traces, $[R_{tot}]/K_D * n$ = 5 to 500 (10 to 100 ideal)

Fluorescence anisotropy: Transition dipole moment

Interaction with light:
Incident light ***E*** induces a dipole μ_{ind}:

$$\mu_{ind} = \alpha \bullet E$$

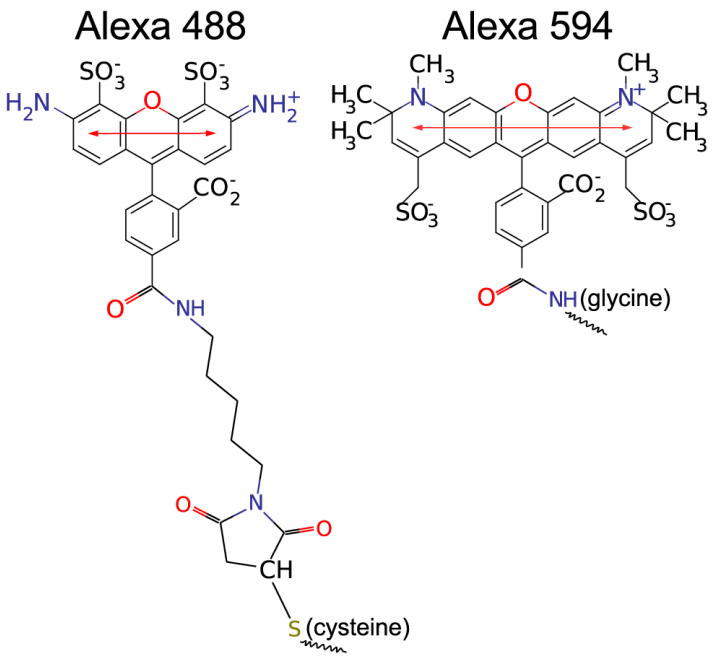
α : polarisability

Transition dipole moment:

Ground state wave funct.: ψ_a
Excited state wave funct.: ψ_b

$$\left\langle \psi_b \left| \underline{\boldsymbol{\mu}} \right| \psi_a \right\rangle$$

can be interpreted as a vector



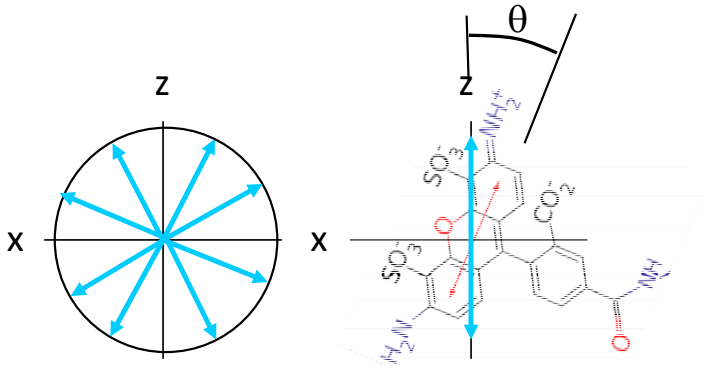
Hoefling et al. PLOS ONE 2011

Excitation of chromophore subpopulation

Conditions:
Immobile chromophores (e.g. embedded in a glass)

Excitation light **vertically polarized**

Probability of absorption: $p \sim cos^2\theta$



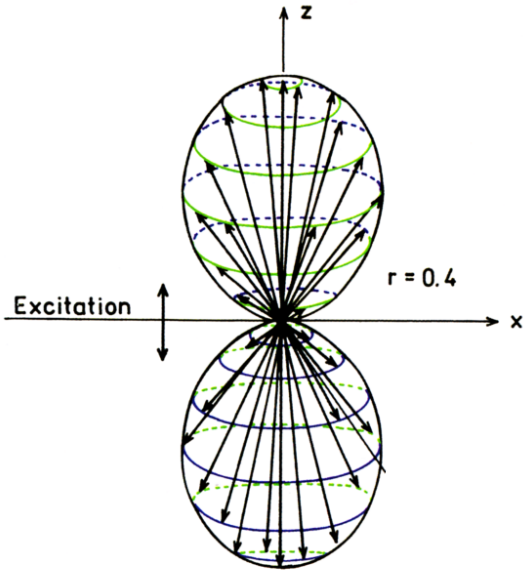


Figure 10.6. Excited-state distribution for immobile fluorophores with $r_0 = 0.4$.

Lakowicz, Principles of fluorescence spectroscopy

Fluorescence emission anisotropy

Conditions:

Emission dipole colinear absorption dipole

No molecular motion

Emission **is polarized**

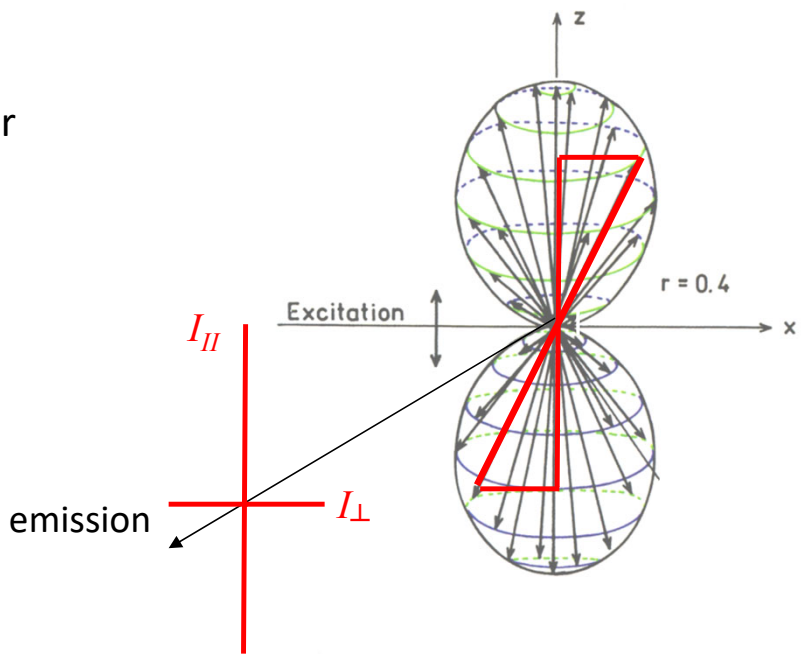
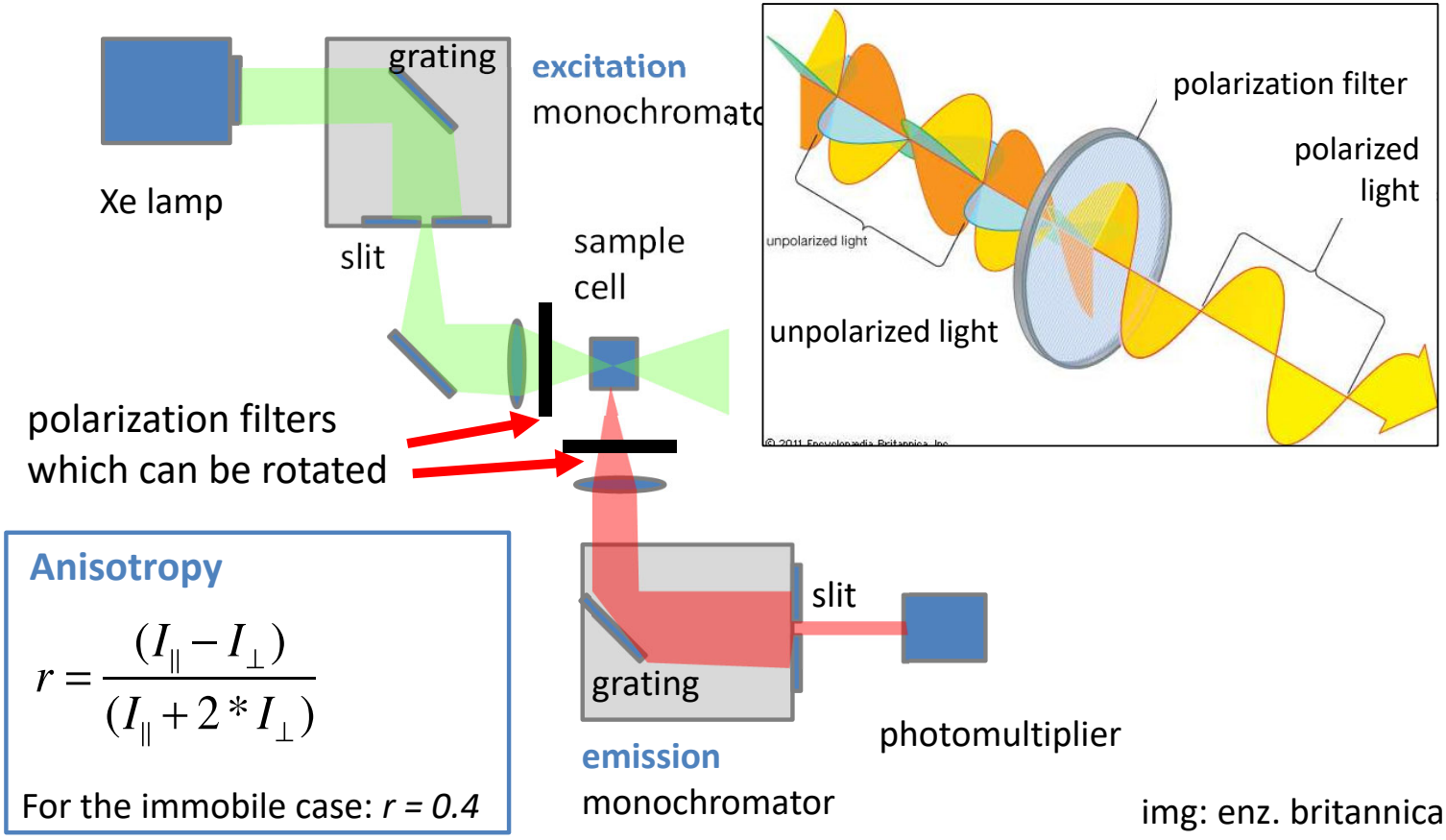


Figure 10.6. Excited-state distribution for immobile fluorophores with $r_0 = 0.4$.

Lakowicz, Principles of fluorescence spectroscopy

Measuring fluorescence anisotropy



Anisotropy

$$r = \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + 2 * I_{\perp})}$$

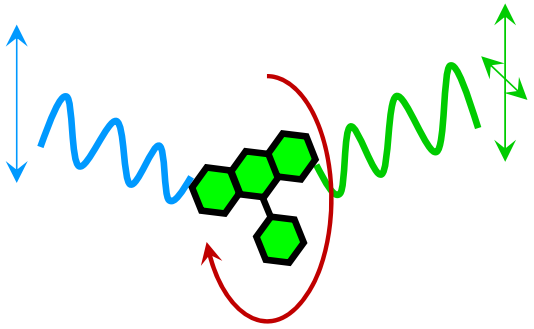
For the immobile case: *r = 0.4*

img: enz. britannica

Loss of fluorescence anisotropy

Anisotropy

$$r = \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + 2 * I_{\perp})}$$



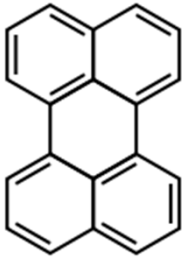
Rotational diffusion:

The Perrin equation

$$\frac{r_o}{r} = 1 + \frac{\tau}{\theta} = 1 + 6D\tau$$

r_0: anisotropy in the absence of motion
τ : fluorescence lifetime
θ : rotation correlation time
D : rotational diffusion coefficient

e.g.: Perylene



r_0: 0.36
τ : 6 ns
Anisotropy in solution (EtOH): 0.005

Loss of fluorescence anisotropy: Proteins

Rotational diffusion:

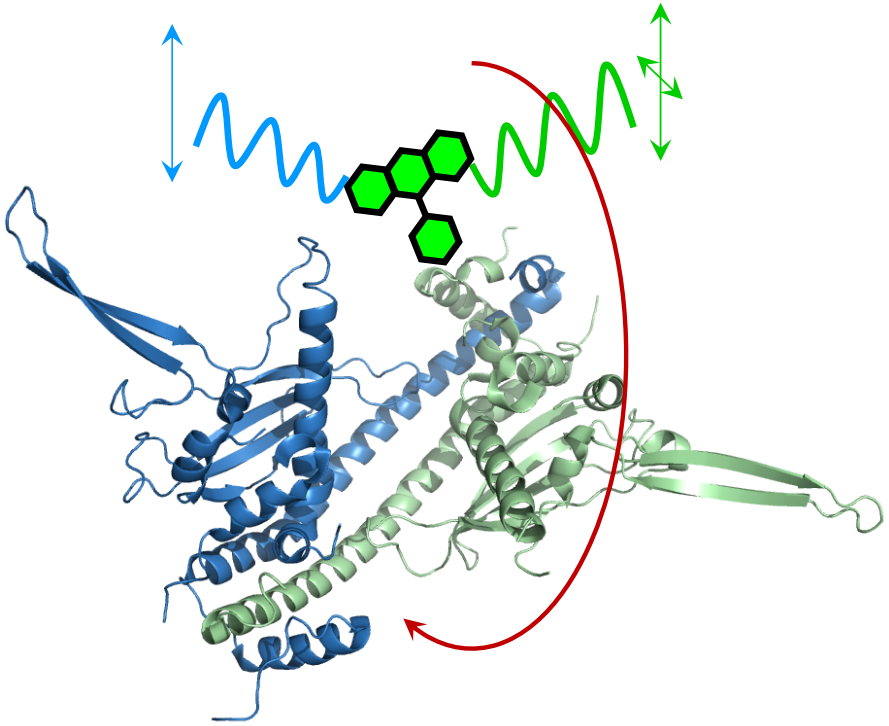
The Perrin equation

$$\frac{r_o}{r} = 1 + \frac{\tau}{\theta} = 1 + 6D\tau$$

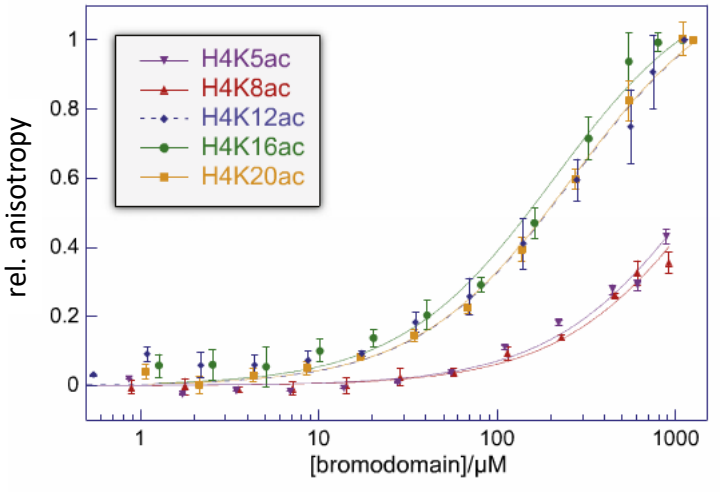
For a 50 kDa protein the rotation correlation time θ = 14 ns

$$\theta = \frac{\eta V}{RT} = \frac{\eta}{RT} \cdot M(\bar{v} + h)$$

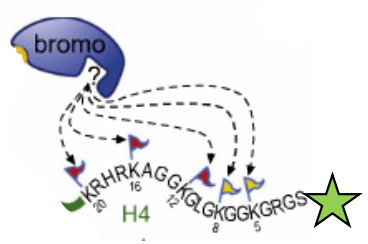
η = viscosity
V = volume
M = molecular weight
v = specific volume protein
h = hydration (g/g protein)



Measuring protein-protein interactions with anisotropy



Ruthenburg et al. Cell 2011



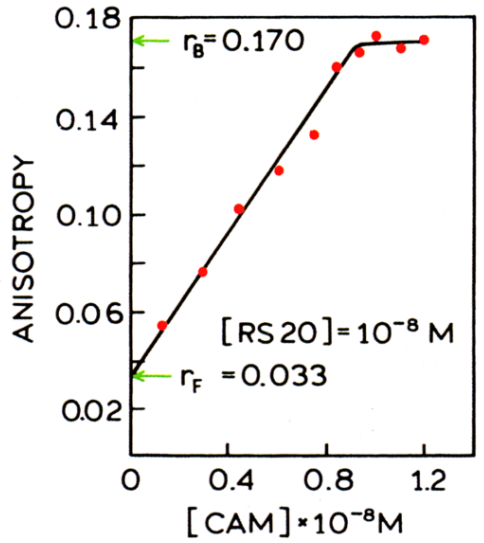
Protein domain (bromodomain) interacting with modified histone peptides

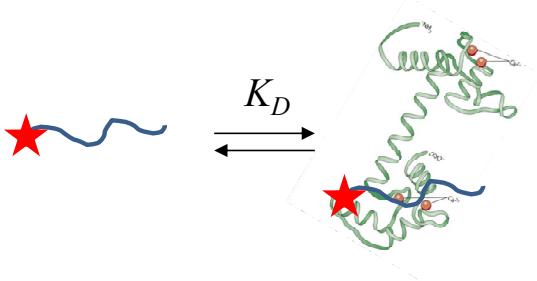
Peptides contain fluorophore, are kept at the same concentration

Protein is titrated and anisotropy is determiend for each concentration

→ Kd is obtained

Stochiometry determined by anisotropy measurement



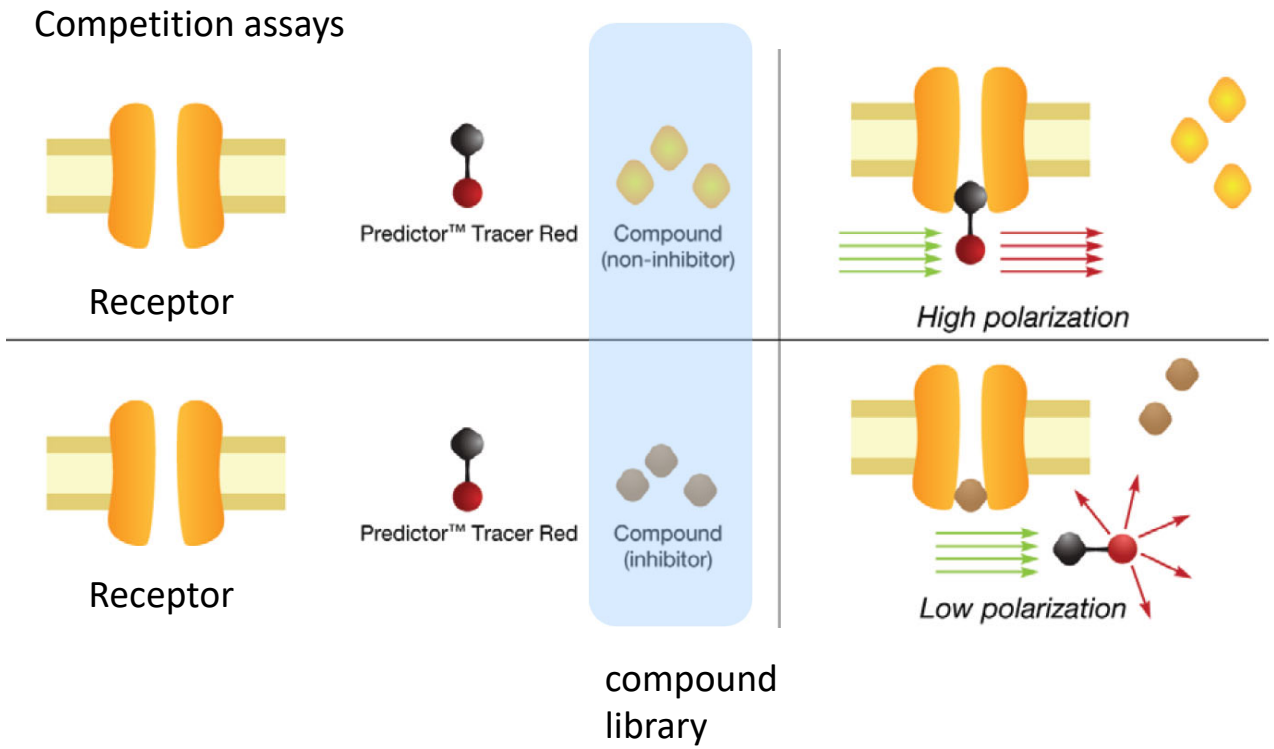


Fluorescence anisotropy measurements can be used to determine binding reactions

Example: MLCK peptide (RS20) binds calmodulin

Determining tryptophan **fluorescence anisotropy** in peptide, the binding constant can be determined in a **titration**.

Fluorescence anistropy in HTS

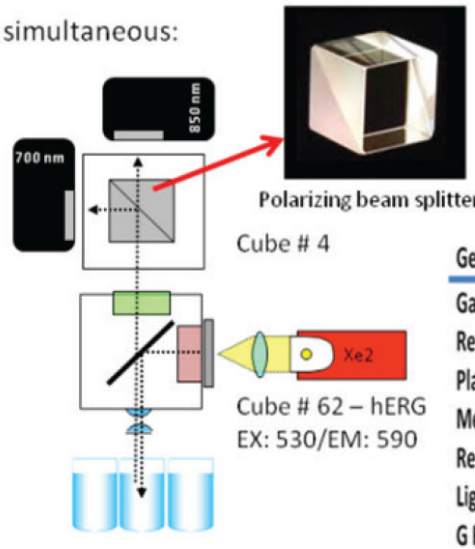


High throughput evaluation using plate readers

Fluorescence Polarization

- Specialized optics:
  - FP cube beam splitter
  - Non-standard high-transmission filters

Top simultaneous:



Polarizing beam splitter

Cube # 4

Cube # 62 – hERG
EX: 530/EM: 590

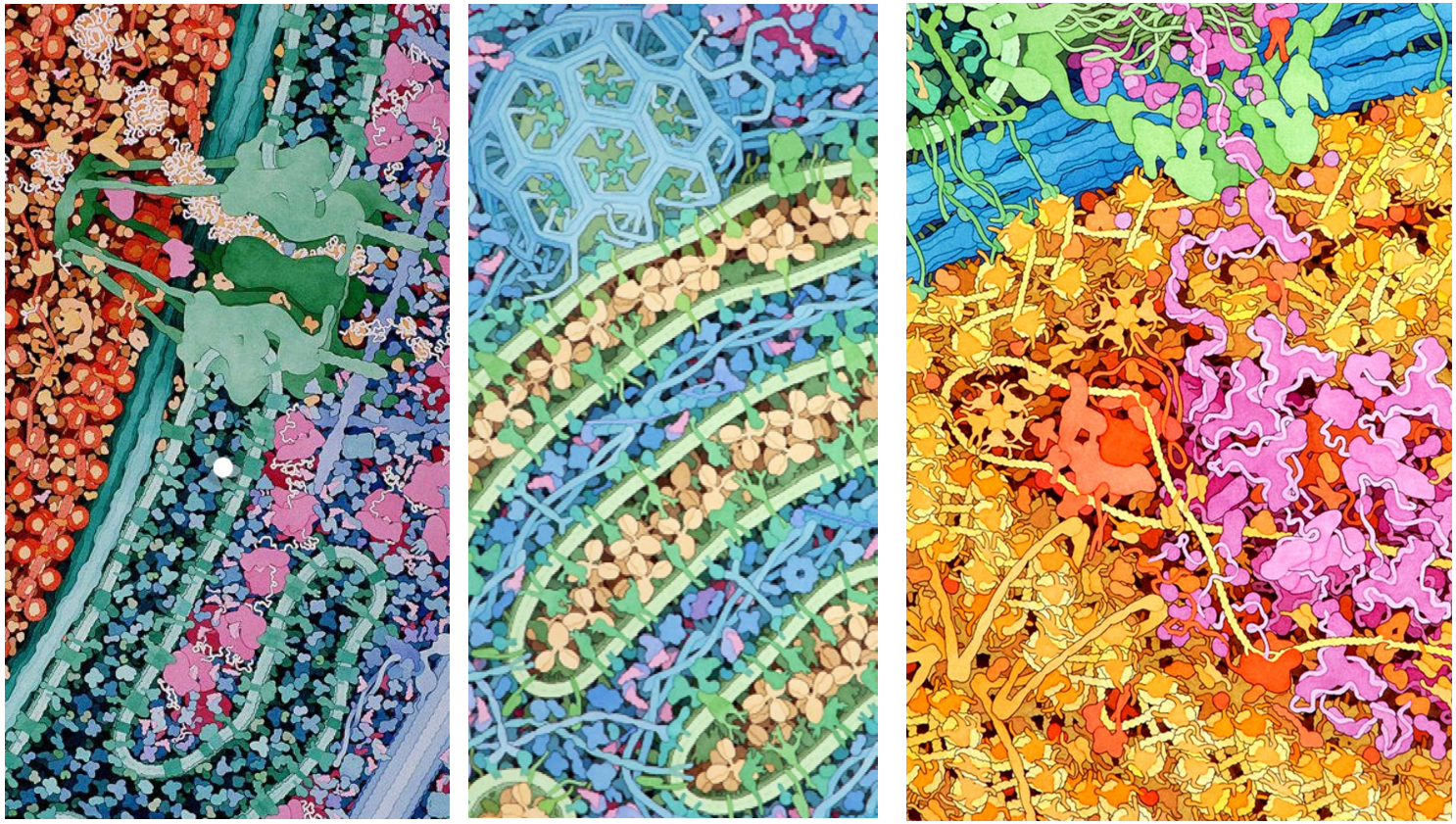
Gen5™ Protocol Parameters	Top PMT	Side PMT	
Gain*	97	119	–
Read Speed	–	–	Normal
Plate Movement Delay	–	–	0
Measurements/well**	–	–	10-200
Read Height (mm)	–	–	10.25
Light Source	–	–	Xenon, Low
G Factor	–	–	Fixed value: 1

** It is recommended Gain be determined for each reader.*

***Higher measurements/well can result in greater precision & specificity but lower read times*

Source: Biotek

In cells, interactions are managed by compartmentalization



David Goodsell