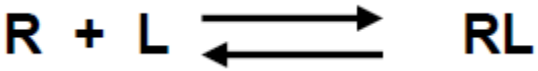
Interactions with ligands

C6215 - Lecture 8

Igor Kučera

Receptor-ligand equilibrium



R (receptor) could be enzyme, transporter, carrier protein, receptor, etc.
L (ligand) could be substrate, cofactor, inhibitor, hormone, DNA/RNA, another protein, etc.

$$K_{association} = K_a = \frac{[RL]}{[R]\cdot[L]} \qquad K_{dissociation} = K_d = \frac{[R]\cdot[L]}{[RL]} \qquad (1)$$

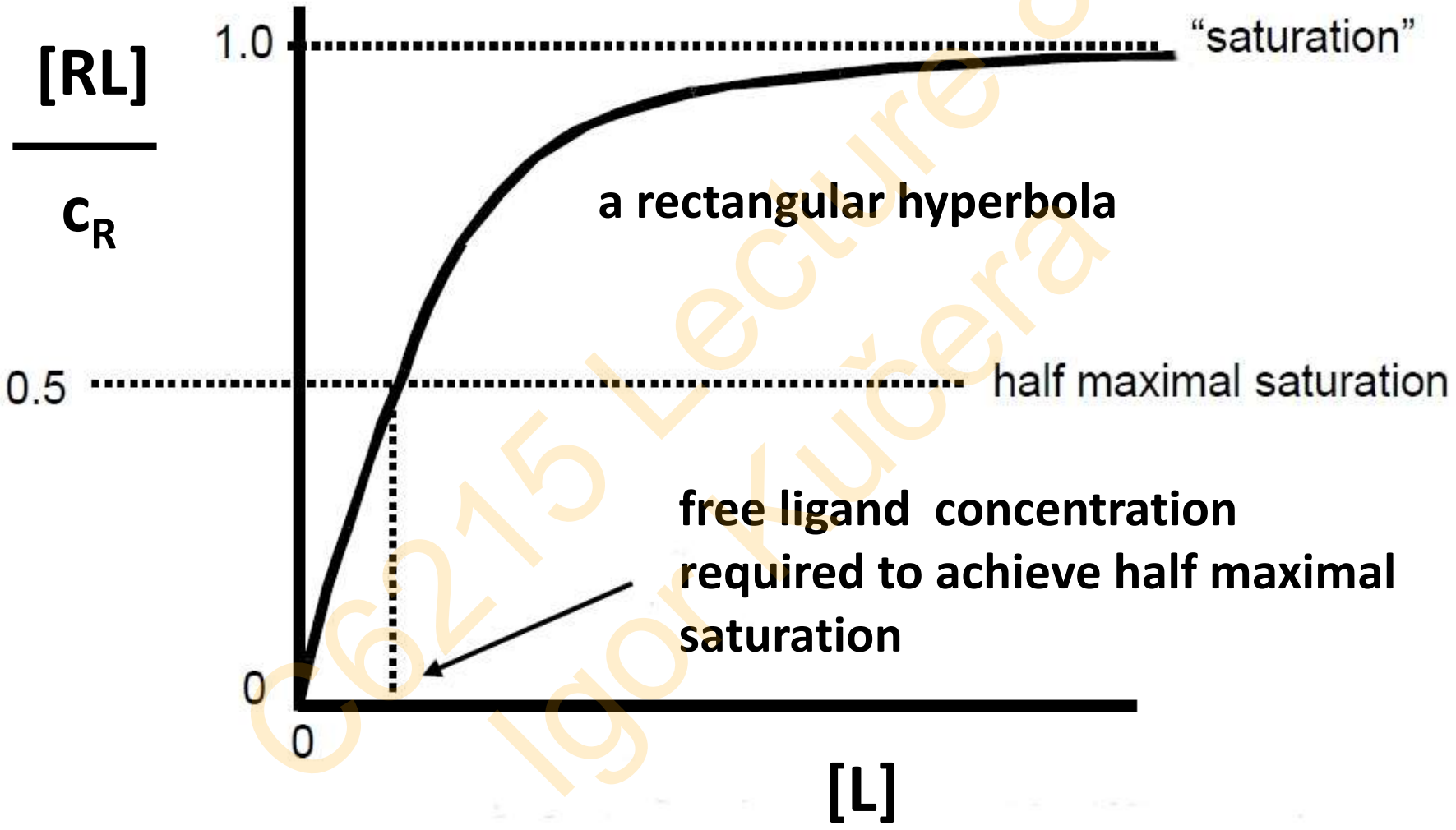
[R], [L], and [RL] are the concentrations of free R, free L and the complex at equilibrium.
Mass balance equations for the total concentration of R and L (c_R and c_L) can be written as

$$c_R = [R] + [RL] \qquad (2)$$
$$c_L = [L] + [RL] \qquad (3)$$

By elimination of [R] from equations (1) and (2) we obtain

$$[RL] = c_R \frac{K_a[L]}{K_a[L] + 1} = c_R \frac{[L]}{[L] + K_d} \qquad (4)$$

The graphical representation of equation (4) is:



The problem is that we often do not know [L] but only the total ligand concentration c_L.

Elimination of both [R] and [L] from equations (1) - (3) leads to a quadratic equation in [RL]

$$[RL]^2 - (c_L + c_R + K_d)\,[RL] + c_L\,c_R = 0$$

The only acceptable root is

$$[RL] = \frac{(c_L + c_R + K_d) - \sqrt{(c_L + c_R + K_d)^2 - 4c_L c_R}}{2} \qquad (5)$$

Using the well-known identity $(a-b)(a+b)=a^2-b^2$, Eq. (5) can be put in the form

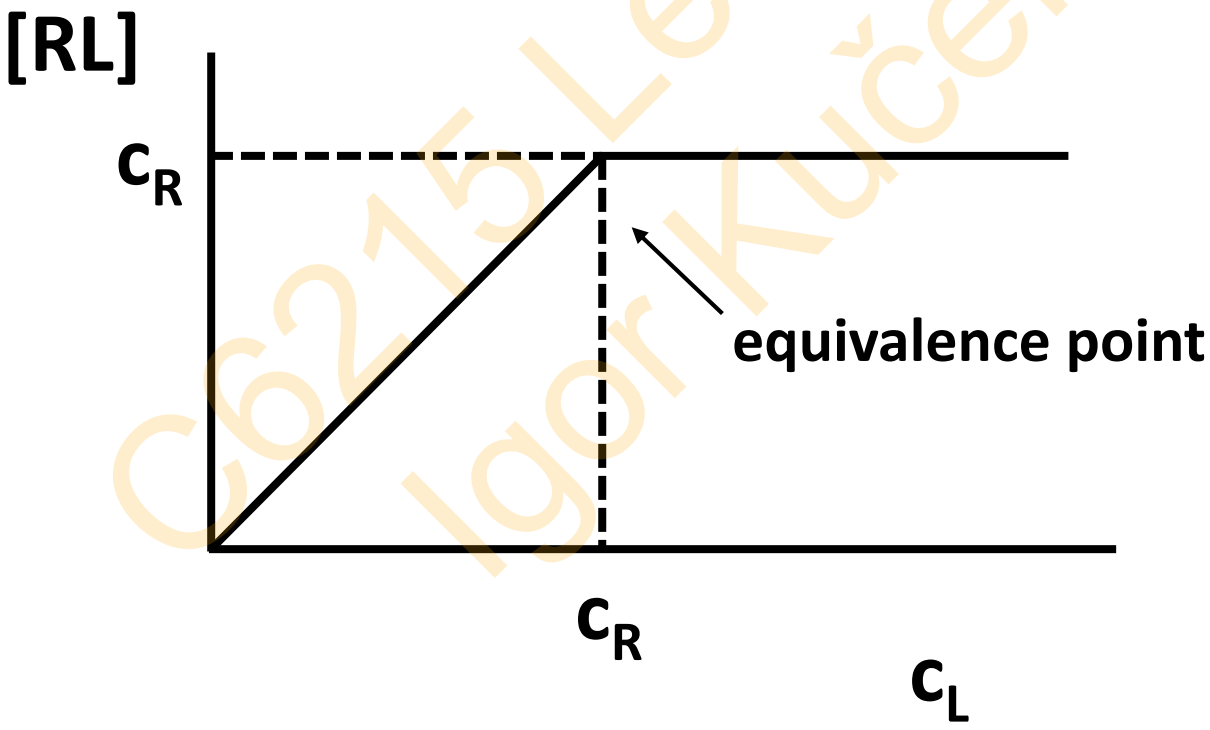
$$[RL] = \frac{2c_R}{1 + \frac{c_R}{c_L} + \frac{K_d}{c_L} + \sqrt{\left(1 + \frac{c_R}{c_L} + \frac{K_d}{c_L}\right)^2 - 4\frac{c_R}{c_L}}}$$

from which it follows that $[RL] \rightarrow c_R$, and hence also $[RL]/c_R \rightarrow 1$, when $c_L \rightarrow \infty$. Contrary to the dependence of [RL] on [L] (Eq. (4)), the [RL] vs. c_L dependence (Eq. (5)) is generally not hyperbolic, although it displays a saturation behavior.

In the limiting case where ligand binding is extremely tight ($K_a \rightarrow \infty$, $K_d \rightarrow 0$), Eq. (5) simplifies to

$$[\mathbf{RL}] = \frac{\mathbf{c_L} + \mathbf{c_R} - |\mathbf{c_L} - \mathbf{c_R}|}{\mathbf{2}}$$

The amount of receptor-ligand complex increases equimolarly with the amount of ligand added and the receptor becomes saturated at $c_L = c_R$.



Since for small x it holds $\sqrt{1-x} \approx 1 - \frac{x}{2}$

after dividing both numerator and denominator of Eq. (5) by $c_L + c_R + K_d$ we obtain

$$[RL] = \frac{1 - \sqrt{1 - \frac{4c_L c_R}{(c_L + c_R + K_d)^2}}}{\frac{2}{c_L + c_R + K_d}} \approx \frac{1 - \left(1 - \frac{2c_L c_R}{(c_L + c_R + K_d)^2}\right)}{\frac{2}{c_L + c_R + K_d}} = \frac{c_L c_R}{c_L + c_R + K_d}$$

In case of $c_R << K_d$, it simplifies further

$$[RL] = \frac{c_L c_R}{c_L + c_R + K_d} \approx c_R \frac{c_L}{c_L + K_d}$$

Thus, when the condition $c_R << K_d$ is fulfilled, the dependence of [RL] on c_L is hyperbolic and half maximal saturation of the receptor occurs at the total ligand concentration equal to K_d.

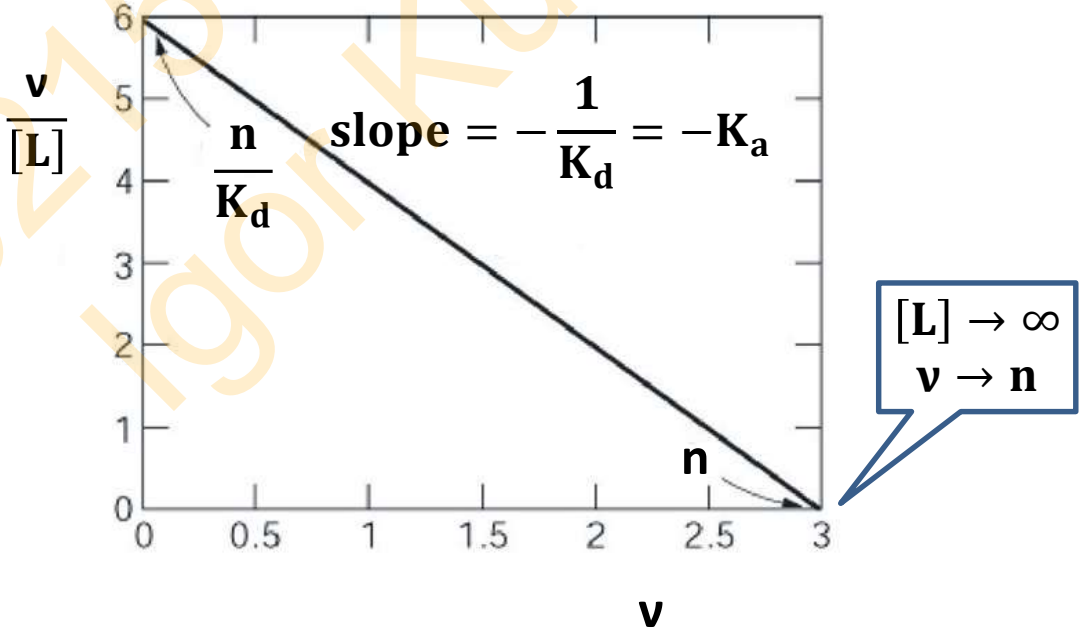
Scatchard plot

George Scatchard
(1892-1973)

Assume that R has n independent binding sites for L. The number of occupied sites (v) can be expressed as

$$\nu = \frac{[L_{bound}]}{c_R} = n \cdot \frac{[L]}{[L] + K_d}$$

$$\boxed{\frac{\nu}{[L]} = -\frac{1}{K_d} \cdot \nu + \frac{n}{K_d}}$$



Techniques to measure binding constants

SEPARATION-BASED

❑ heterogeneous
- dialysis
- chromatography
- surface plasmon resonance

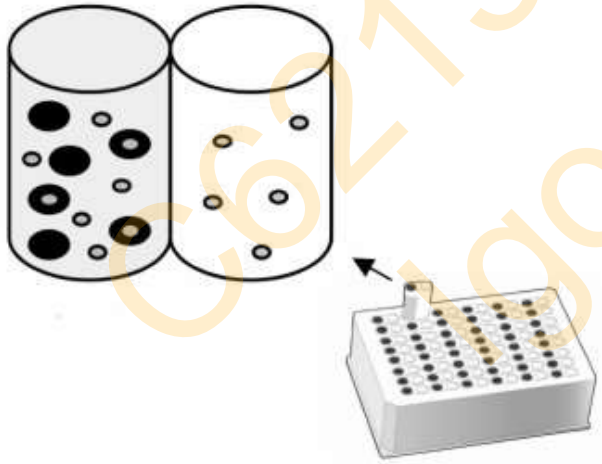
❑ homogeneous
- affinity capillary electrophoresis
- centrifugation
- electrospray ionization mass spectrometry

NON-SEPARATION-BASED

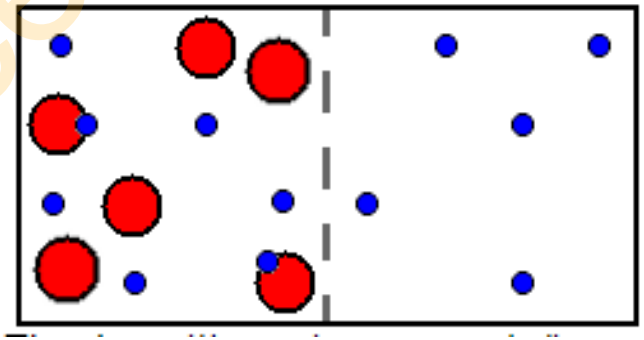
- spectroscopy (UV-Vis, IR)
- fluorescence
- isothermal titration calorimetry
- nuclear magnetic resonance

Equilibrium dialysis

Equilibrium dialysis uses a two-chambered device with the chambers separated by a semipermeable membrane. The protein solution containing ligand is placed in one chamber while buffer is placed in the opposing chamber. When equilibrium is reached, the unbound ligand will be at equal concentrations on both sides of the membrane while the bound ligand will remain in the protein chamber. The total ligand concentration is sampled from the protein side while the free ligand concentration is sampled from the buffer side. The bound ligand is then calculated from these measurements.



A schematic illustration of the calculation of the dissociation constant from the equilibrium dialysis data



$c_R = 6$

$c_L = 7$

$[L] = 5$

$[RL] = 7-5 = 2$

$[R] = 6-2 = 4$

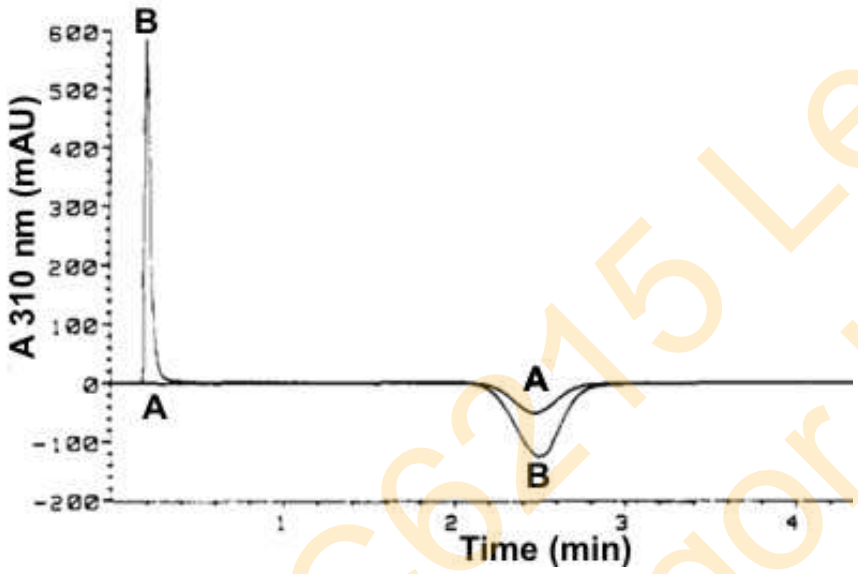
$c_L = [L] = 5$

$$K_d = \frac{4 \times 5}{2} = 10$$

Howard et al. Comb Chem High Throughput Screen. 2010, 13, 170

Hummel – Dryer method

A known quantity of receptor is injected on a size exclusion chromatography column and eluted with a buffer containing a constant concentration of ligand. An amount of ligand, determined by the dissociation constant(s) of the equilibrium and the free ligand concentration, binds to the receptor and migrates with it, while a trough in the ligand concentration, corresponding to the quantity withdrawn from the solvent, migrates at its proper rate.



Hummel-Dreyer elution profile (trace B) from the injection of 50 µl of 54 µM HSA into a mobile phase of 81.1 µM warfarin in 0.067 M phosphate buffer (pH = 7.4) flowing at 2.0 ml/min through a 5 cm × 4.6 mm ISRP column. Mobile phase response monitored at 310 nm with response zeroed at the beginning of the run. Trace A is from the injection of 50 µl of 0.067 M buffer blank.

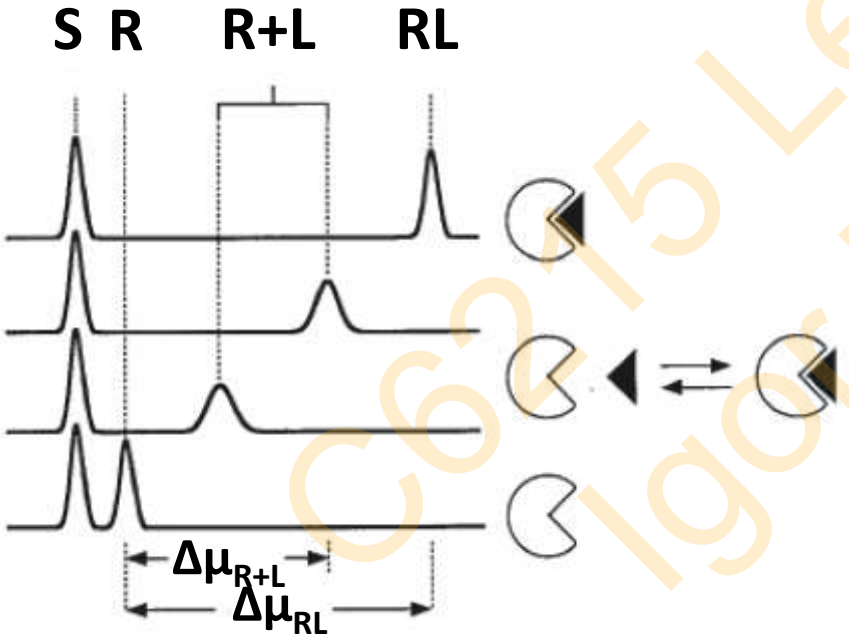
The bound ligand concentration is calculated as ligand concentration in the buffer times $(A_s - A_b)/A_b$ where A_s is area of the sample peak and A_b area of the buffer peak.

Hummel and Dryer, Biochim. Biophys. Acta 1962, 63, 530
Pinkerton and Koeplinger, Anal. Chem. 1990, 62, 2114
Berger and Girault, J. Chromatogr. B 2003, 797, 51

Affinity capillary electrophoresis

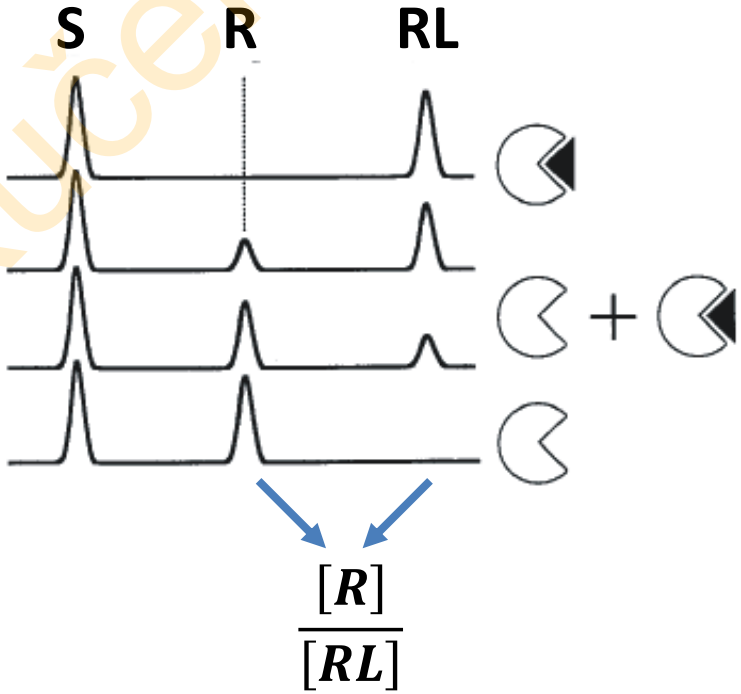
The electrophoretic mobility (μ) of a receptor protein (R) changes upon binding to the charged ligand (L) present in the electrophoresis buffer, due to changes in its charge-to-mass ratio. If the protein binds a charged ligand of relatively small mass, the change in mobility due to the change in mass is negligible relative to the change in mobility due to the change in charge.

Weak-to-moderate binding systems

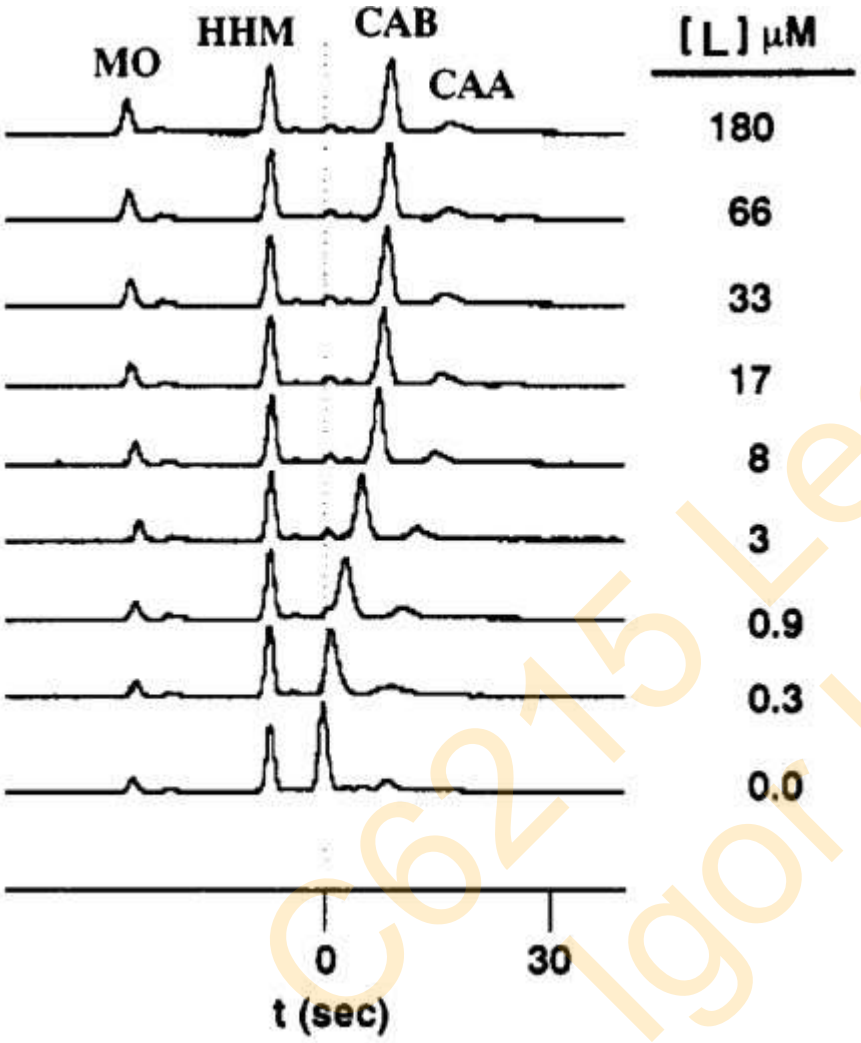


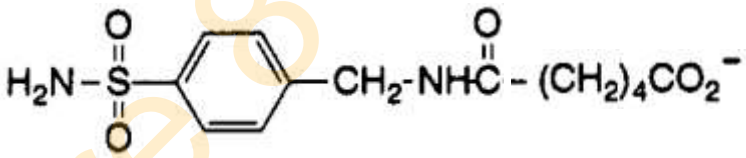
$$\frac{[RL]}{c_R} = \frac{\Delta\mu_{R+L}}{\Delta\mu_{RL}} = \frac{\Delta t}{\Delta t_{lim}}$$

Tight binding systems



Chu and Cheng, Cell. Mol. Life Sci. 1998, 54, 663



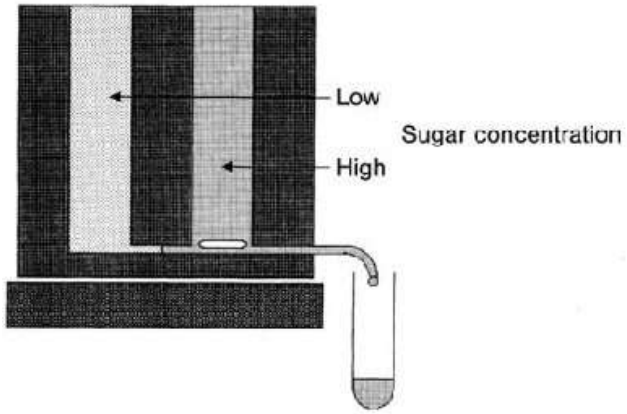


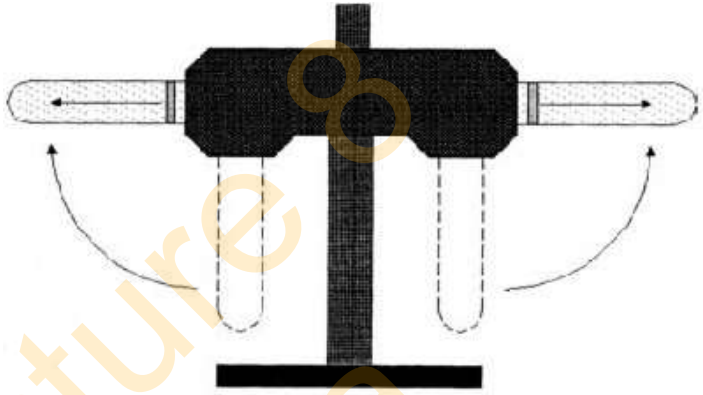
Affinity capillary electrophoresis of bovine carbonic anhydrase B (CAB) in 0.192 M glycine-0.025 M Tris buffer (pH 8.4) containing various concentrations of L. The total analysis time in each experiment was ~5.5 min at 30 kV using a 70-cm (inlet to detector), 50-μm open quartz capillary. Horse heart myoglobin (HHM) and mesityl oxide (MO) were used as internal standards.

➔ K_d = 2.1 μM

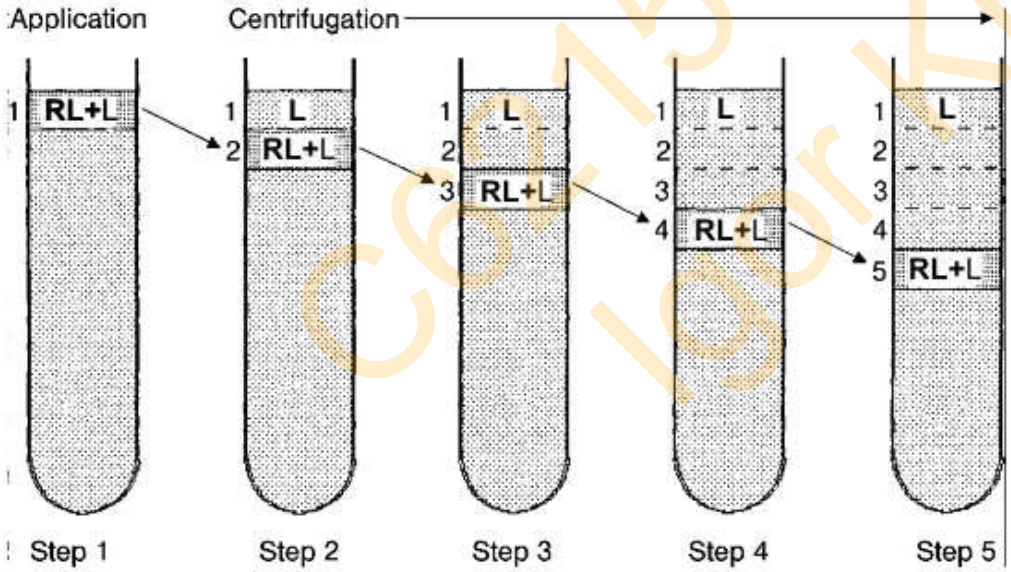
Chu et al., J. Med. Chem. 1992, 35, 2915

Sucrose gradient centrifugation after Draper and Hippel





R is applied in a large surplus (> 10-fold) over L, so that [R] ≈ c_R. When the R band migrates due to the centrifugation, it takes the bound ligand along, while free ligand is left at the starting position. Then the new distributions between free and bound ligand are repeatedly established.



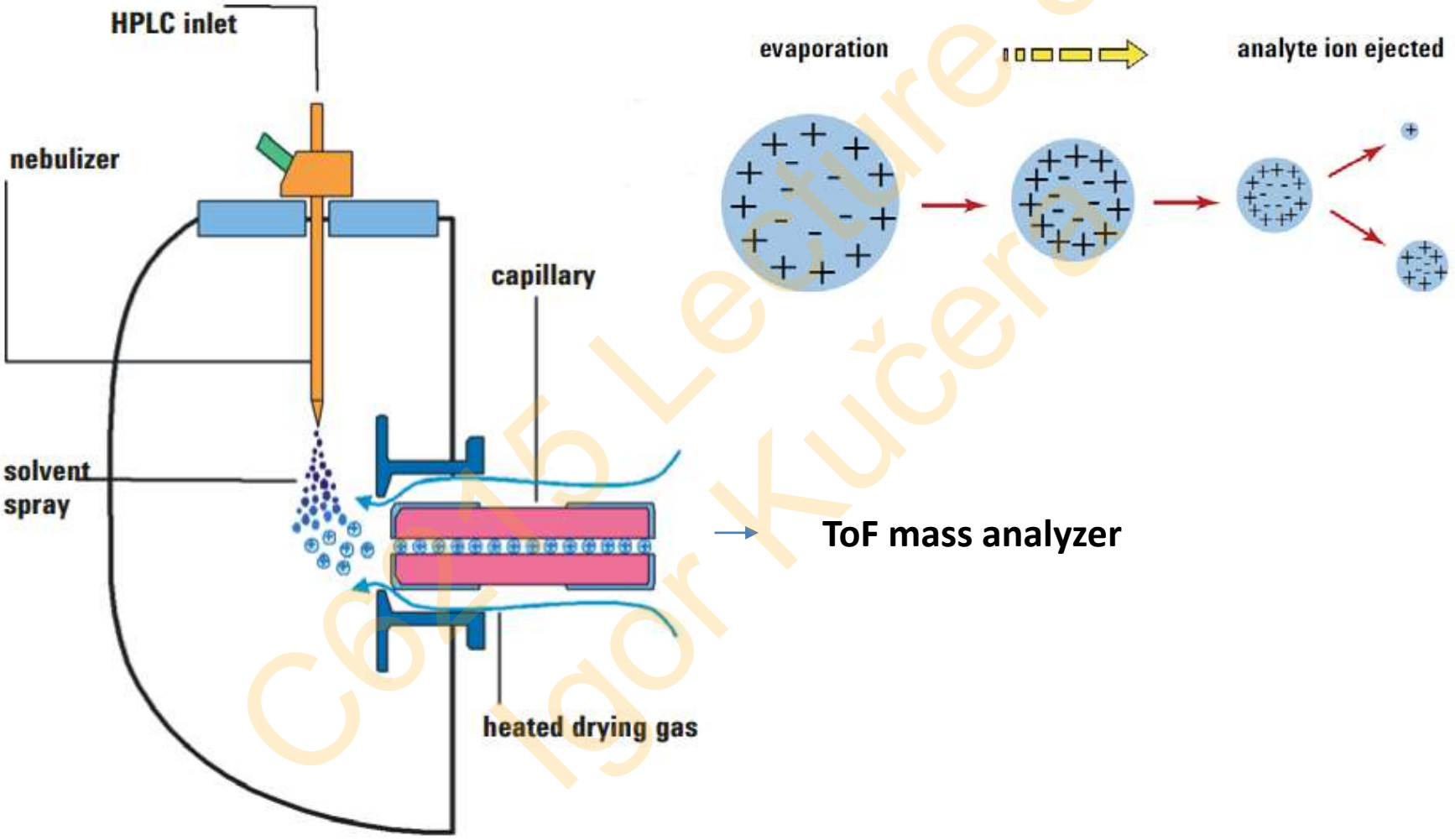
$$[RL]_1 = c_{L1}\frac{c_R}{c_R + K_d}$$

$$[RL]_2 = [RL]_1\frac{c_R}{c_R + K_d}$$

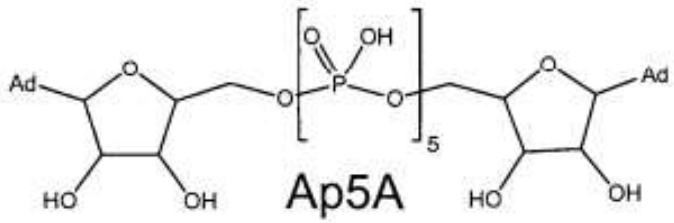
$$[RL]_i = c_{L1}\left(\frac{c_R}{c_R + K_d}\right)^i$$

Bisswanger, Enzyme Kinetics. Principles and Methods 2002

Electrospray ionization mass spectrometry



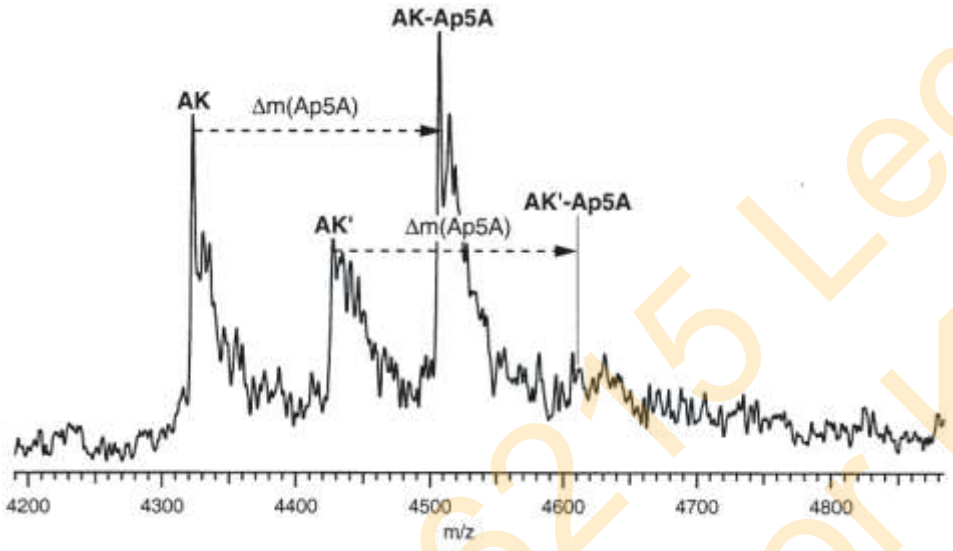
Concepts Guide, Agilent Technologies 2015



+

1) native chicken muscle adenylate kinase (AK)

2) chicken muscle adenylate kinase covalently modified with 8-N_3-ATP (AK´)



Spectrum of the reaction mixture (10 µM total protein) of AK and AK´ with 10 µM of Ap5A in 50 mM ($HNEt_3$)HCO3. Almost no binding to AK' is observed, suggesting that the inhibitor binds in the binding pocket of the protein.

$$r = \frac{[RL]}{[R]} \Rightarrow K_d = \frac{c_L}{r} - \frac{c_R}{r+1}$$

Daniel et al., J. Am. Soc. Mass Spectrom. 2003, 14, 442

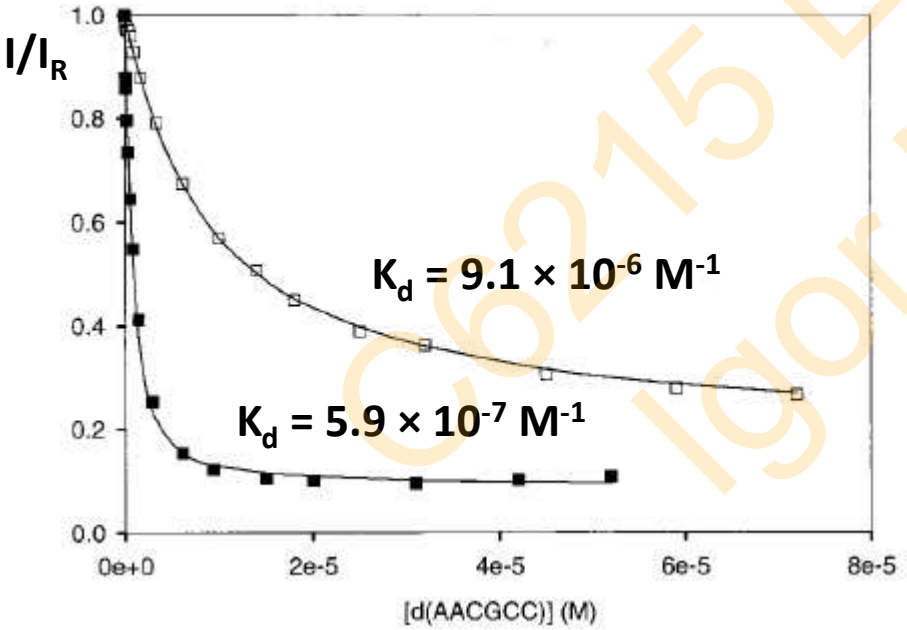
Fluorescence quenching titration

The fluorescence that is measured (I) is a sum of the fluorescence of the free and complexed receptor. Denoting the fluorescence intensities measured when the receptor is completely free and when is complexed as I_R and I_{RL} ($I_R > I_{RL}$), we get I as a function of [RL] as follows:

$$I = I_R \frac{[R]}{c_R} + I_{RL} \frac{[RL]}{c_R} = I_R \frac{c_R - [RL]}{c_R} + I_{RL} \frac{[RL]}{c_R} = I_R - \frac{I_R - I_{RL}}{c_R}[RL]$$

$$\frac{I}{I_R} = 1 - \left(1 - \frac{I_{RL}}{I_R}\right)\frac{[RL]}{c_R}$$

The functional dependence of [RL] on c_L is given by Eq. (5). When c_L changes from zero to infinity, [RL] increases from zero to c_R and I decreases from I_R to I_{RL}.



The viral nucleocapsid protein NCp7 contains two Trp residues that constitute sensitive intrinsic fluorescent probes. Binding of oligonucleotides to NCp7 is accompanied by fluorescence quenching.

FIGURE 2: Binding curves of (12−53)NCp7 with d(AACGCC). Peptide concentration was 0.8 μM in 50 mM Hepes, pH 7.5, in the absence (closed symbols) or in the presence of 100 mM NaCl (open symbols). The oligonucleotide concentration is expressed in strands.

Vuilleumier et al., Biochemistry 1999, 38, 16816

Titration with a fluorescent ligand

Suppose that the measured fluorescence intensity I depends linearly on the concentrations [L] and [RL] with the coefficients of proportionality ε_L and ε_{RL}. The intensity then can be expressed as

$$I = \varepsilon_L [L] + \varepsilon_{RL} [RL] = \varepsilon_L (c_L - [RL]) + \varepsilon_{RL} [RL] = \varepsilon_L c_L + (\varepsilon_{RL} - \varepsilon_L) [RL]$$

[RL] changes with c_L according to Eq. (5) from zero to c_R. After the receptor becomes saturated, adding more ligand cause only a linear increase in I with a slope of ε_L .

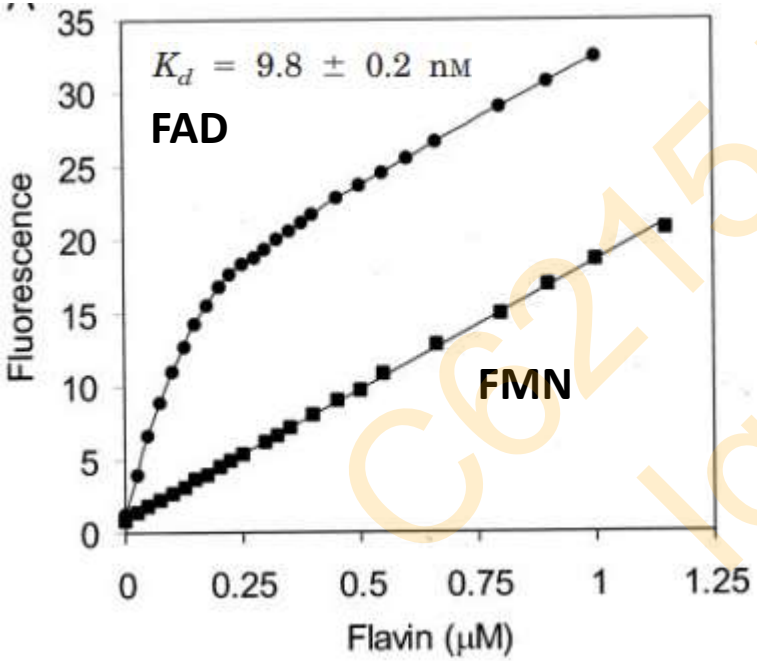


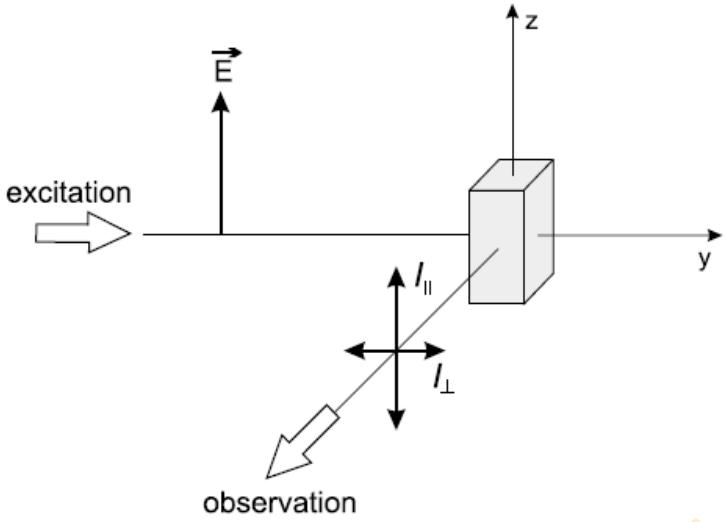
FIG. 5. **Binding of FAD and FMN to PheA2.** *A*, fluorescence changes upon titration of 170 nM PheA2 apoprotein with FAD (●) or FMN (■) in 50 mM potassium phosphate buffer, pH 7.0. The fluorescence emission was measured at 530 nm upon excitation at 450 nm. *B*,

The bound FAD fluoresces more than a free FAD molecule.

FMN either does not bind at all or does not change fluorescence upon binding. Measurement of fluorescence anisotropy might be informative here.

van den Heuvel et al., J. Biol. Chem. 2004, 279, 12860

Fluorescence anisotropy binding assays



$$r = \frac{I_{||} - I_{\perp}}{I} = \frac{I_{||} - I_{\perp}}{I_{||} + 2I_{\perp}}$$

The anisotropy r reflects the rotational diffusion of a fluorescent species.

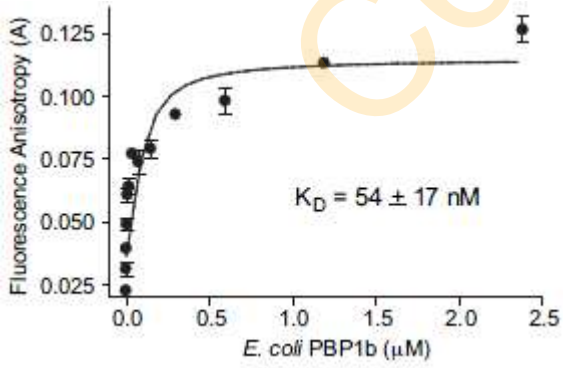
Additivity law of anisotropy:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} = \frac{\sum_i I_{\parallel}^i - \sum_i I_{\perp}^i}{I} = \sum_i \frac{I_{\parallel}^i - I_{\perp}^i}{I_i} \times \frac{I_i}{I}$$

In cases where the emission of a fluorescent ligand does not change upon binding to receptors, $I_L/I = [L]/c_L$, $I_{RL}/I = [RL]/c_L$ and

$$\mathbf{r = r_L \frac{[L]}{c_L} + r_{RL} \frac{[RL]}{c_L} = r_L \frac{c_L - [RL]}{c_L} + r_{RL} \frac{[RL]}{c_L} = r_L + \frac{r_{RL} - r_L}{c_L}[RL]}$$

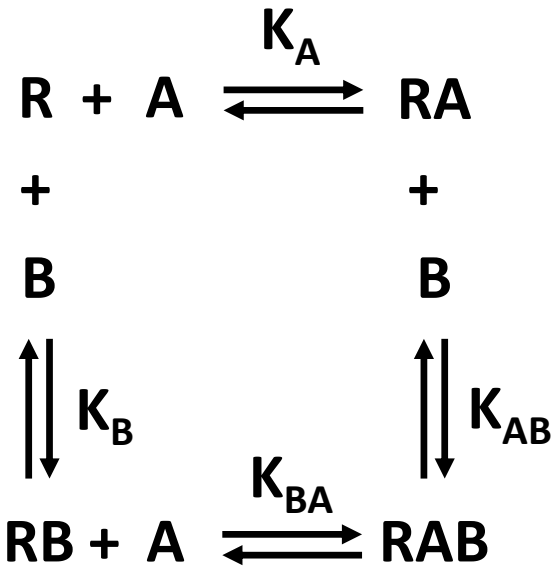
[RL] rises from zero to c_L and r from r_L to r_{RL} with increasing c_R



Interaction of fluorescein-labeled moenomycin with a penicillin-binding protein (PBP). The anisotropy of F-Moe increases during incubation with various concentrations of E. coli PBP1b, because of the formation of an F-Moe–PBP1b complex with a reduced rotational freedom.

Chang et al., Proc. Natl. Acad. Sci. 2008, 433

Binding of two different ligands



$$K_A = \frac{[R][A]}{[RA]} \qquad K_B = \frac{[R][B]}{[RB]}$$

$$K_{AB} = \frac{[RA][B]}{[RAB]} \qquad K_{BA} = \frac{[RB][A]}{[RAB]}$$

$$K_A K_{AB} = K_B K_{BA} \qquad \alpha = \frac{K_{BA}}{K_A} = \frac{K_{AB}}{K_B}$$

$\alpha < 1$ … positive cooperativity

$\alpha > 1$ … negative cooperativity

$$c_R = [R] + [RA] + [RB] + [RAB]$$

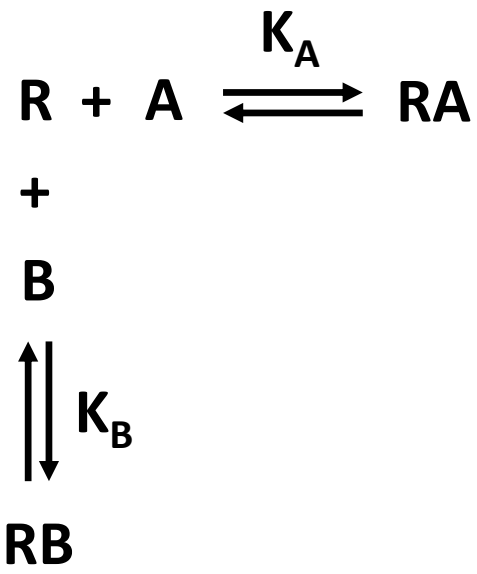
$$c_R = [R] + \frac{[R][A]}{K_A} + \frac{[R][B]}{K_B} + \frac{[R][A][B]}{\alpha K_A K_B}$$

$$[R] = \frac{c_R}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B} + \frac{[A][B]}{\alpha K_A K_B}}$$

$$[RA] = \frac{[A]}{K_A}[R] = \frac{c_R \frac{[A]}{K_A}}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B} + \frac{[A][B]}{\alpha K_A K_B}}$$

$$[RAB] = \frac{[A][B]}{\alpha K_A K_B}[R] = \frac{c_R \frac{[A][B]}{\alpha K_A K_B}}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B} + \frac{[A][B]}{\alpha K_A K_B}}$$

Competitive binding



Competitive binding can be viewed as an extreme case of negative cooperativity ($\alpha \rightarrow \infty$, $1/\alpha \rightarrow 0$).

Discarding the term containing α from the denominator of the expression for [RA] gives:

$$[\mathrm{RA}] = \frac{c_R \frac{[A]}{K_A}}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}} = c_R \frac{[A]}{[A] + K_A\left(1 + \frac{[B]}{K_B}\right)}$$

From this, it is apparent that the value of K_A, the dissociation constant of ligand A, is increased by a factor of $1 + [B]/K_B$, so that K_A is increased in direct proportion to the concentration of the competing ligand B.

Competitive binding (solution for total concentrations)

$$K_A = \frac{[R][A]}{[RA]} \qquad K_B = \frac{[R][B]}{[RB]}$$

$$c_A = [A] + [RA] \qquad c_B = [B] + [RB]$$

$$[RA] = \frac{[R]c_A}{K_A + [R]} \qquad [RB] = \frac{[R]c_B}{K_B + [R]}$$

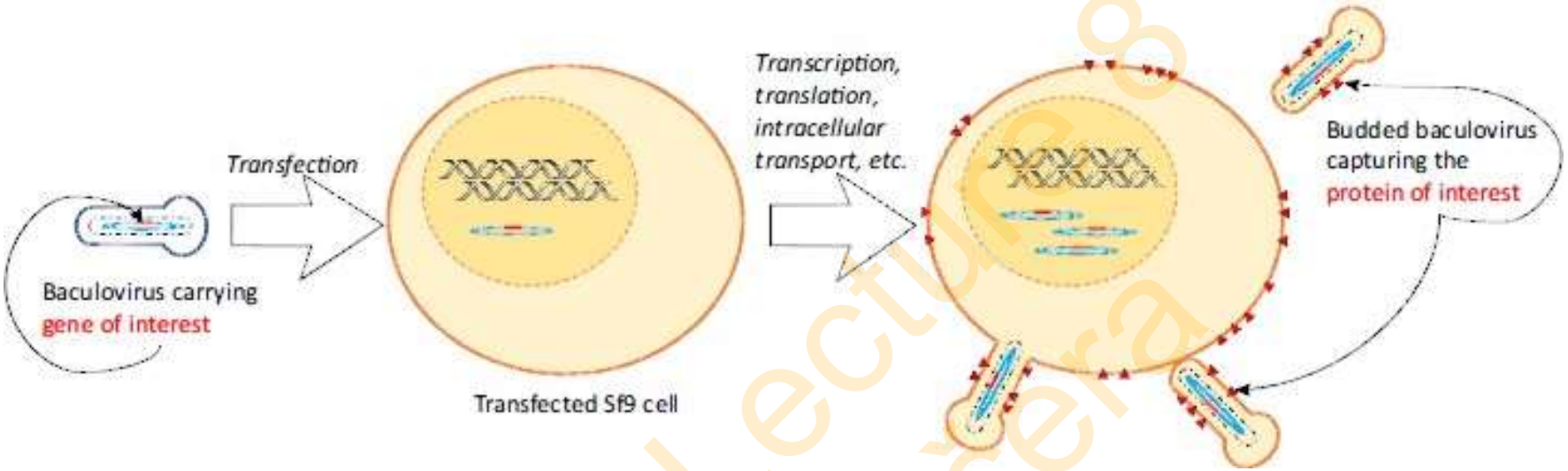
$$c_R = [R] + [RA] + [RB]$$

$$[R]^3 + (K_A + K_B + c_A + c_B - c_R)[R]^2$$
$$- \{K_B(c_A - c_R) + K_A(c_B - c_R) + K_A K_B\}[R] - K_A K_B c_R = 0$$

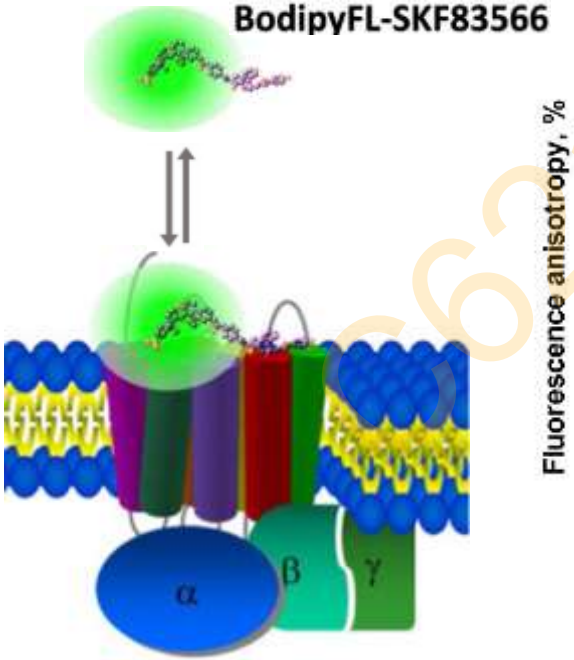
[R] obtained by solving this cubic equation is then used to calculate [RA] and [RB].

Wang, FEBS Lett. 1995, 360, 111

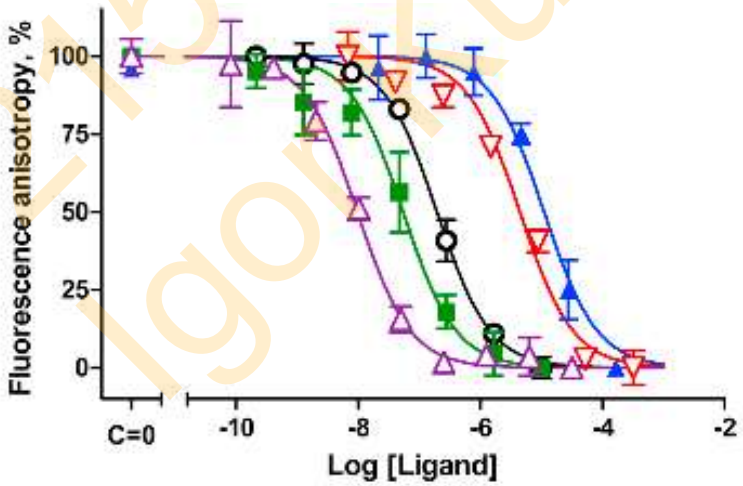
Fluorescence anisotropy-based competitive assays



Rinken et al., Trends Pharmacol. Sci. 2018, 39, 187



Dopamine D_1 receptor

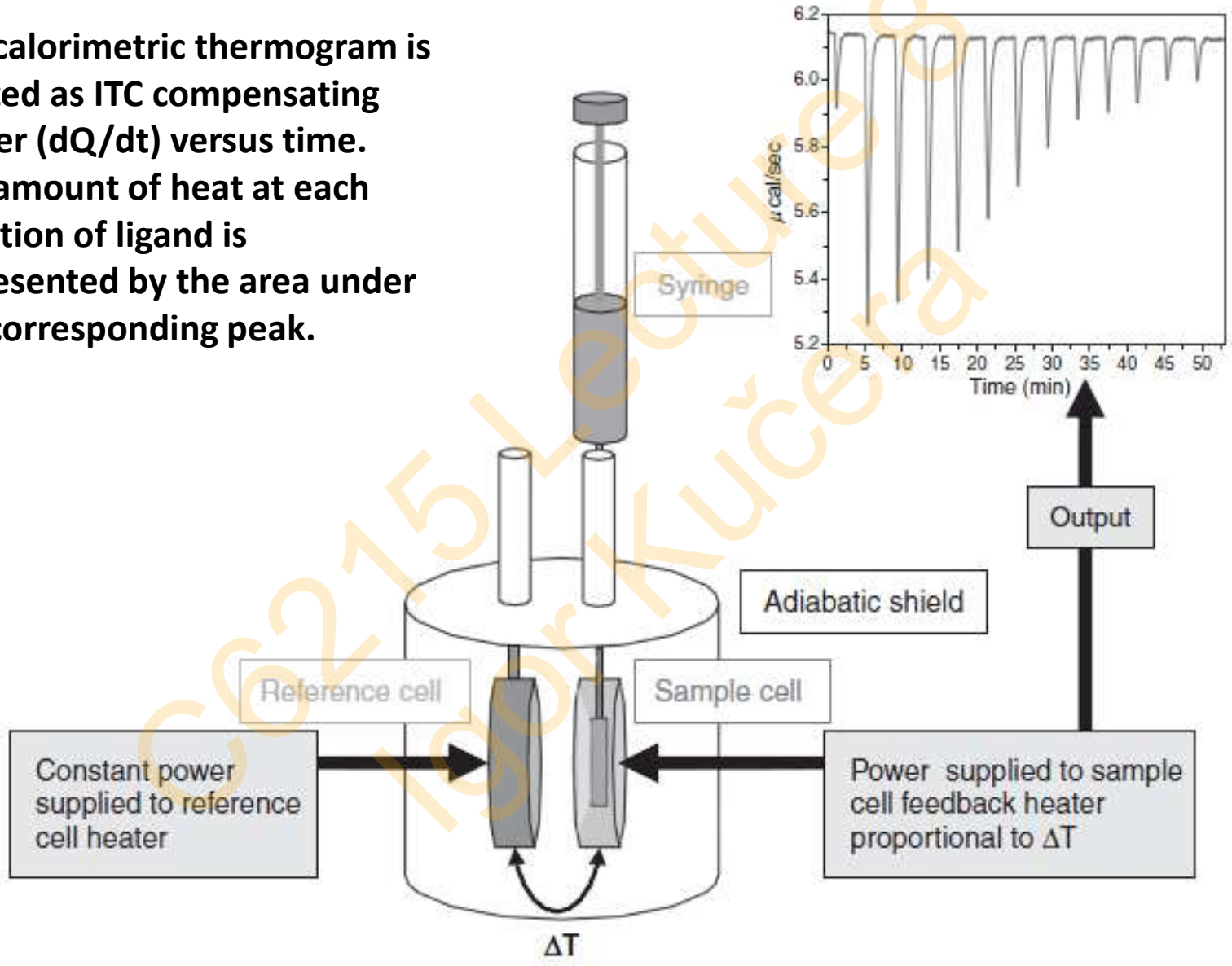


Agonists:	K_d (nM)
Dopamine	5300 ± 880
Apomorphine	1710 ± 150
A68930	38.5 ± 5.5
SKF81297	119 ± 21
SKF38393	1850 ± 370
NPA	1210 ± 110

Allikalt et al., Eur. J. Pharmacol. 2018, 839, 40

Isothermal titration calorimetry

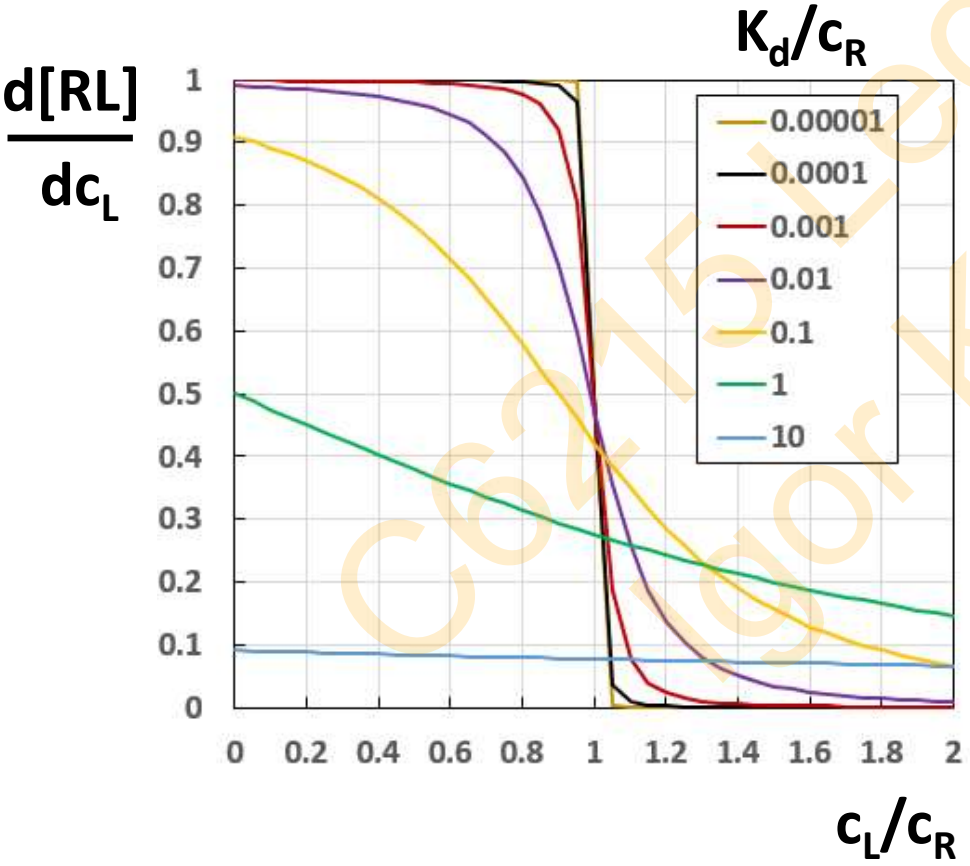
The calorimetric thermogram is plotted as ITC compensating power (dQ/dt) versus time. The amount of heat at each injection of ligand is represented by the area under the corresponding peak.



Freyer and Lewis, Methods in Cell Biology 2008, 84, 79

To find an expression for the heat per mole of ligand added, we first differentiate [RL], described by Eq. (5), with respect to c_L.

$$\frac{d[RL]}{dc_L} = \frac{1}{2}\left(1 + \frac{c_R - c_L - K_d}{\sqrt{(c_L + c_R + K_d)^2 - 4c_L c_R}}\right) = \frac{1}{2}\left(1 + \frac{1 - \frac{c_L}{c_R} - \frac{K_d}{c_R}}{\sqrt{\left(1 + \frac{c_L}{c_R} + \frac{K_d}{c_R}\right)^2 - 4\frac{c_L}{c_R}}}\right)$$

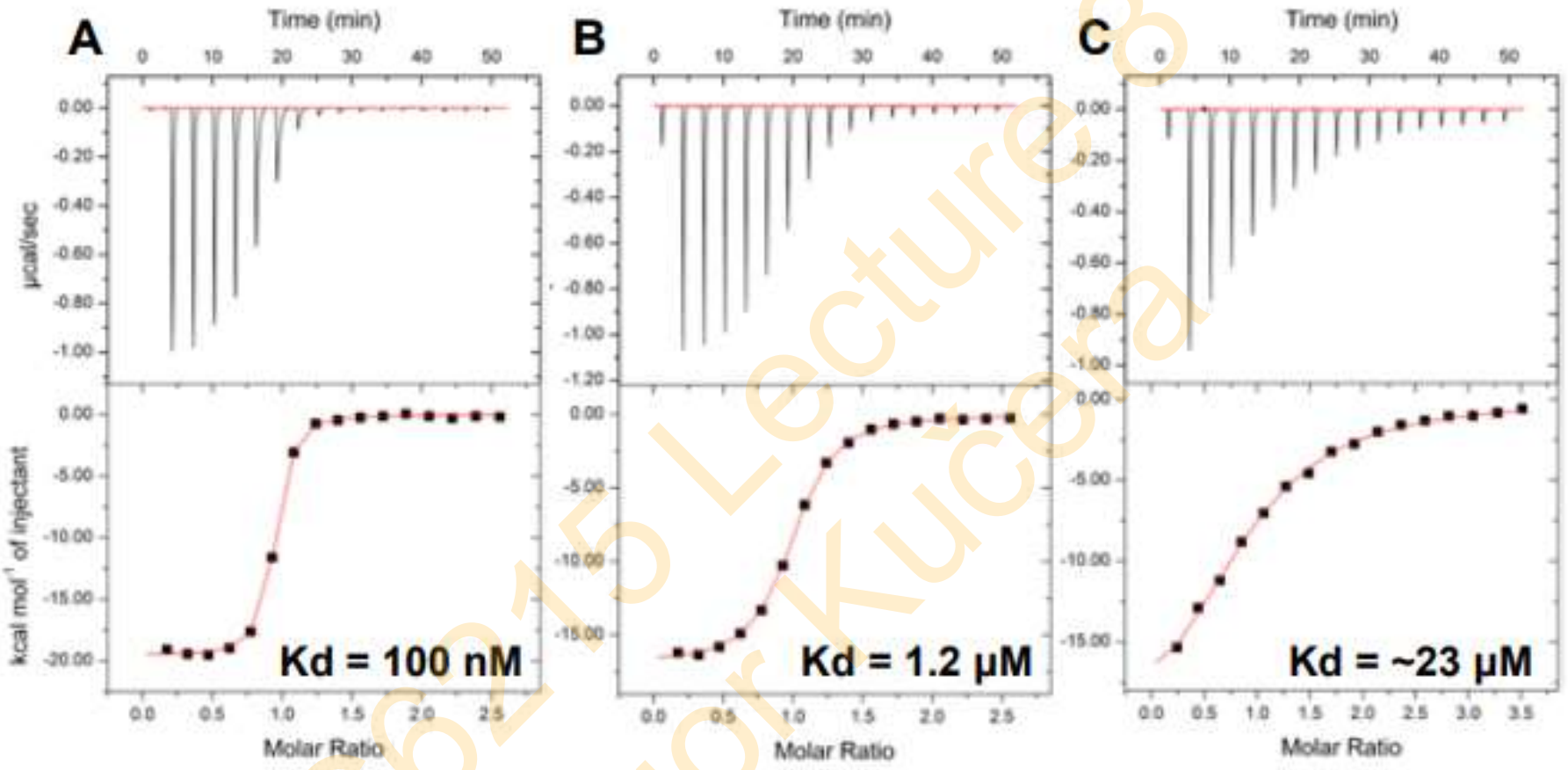


In the limit K_d/c_R approaching zero, this equation reduces to

$$\frac{d[RL]}{dc_L} = \frac{1}{2}\left(1 + \frac{1 - \frac{c_L}{c_R}}{\left|1 - \frac{c_L}{c_R}\right|}\right)$$

The change in [RL] can be related to the heat change as
$dQ = d[RL]\ \Delta H^0\ V$
where ΔH^0 is the molar enthalpy change of the ligand binding reaction and V the reaction volume.

Wiseman et al., Anal. Biochem. 1989, 179, 131



Titration of a protein with three different ligands. Each ligand binds to the protein with either A) a high affinity, B) a medium affinity or C) a low affinity. Top panels: experimental trace. The baseline is indicated in red. Bottom panels: the integrated heats upon injection (black squares) and the data fit (red line) after subtraction of the control data.

Harris and Scott, Biochemist 2019, 41, 4

Pseudo-first order transient kinetics

$$R + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RL$$

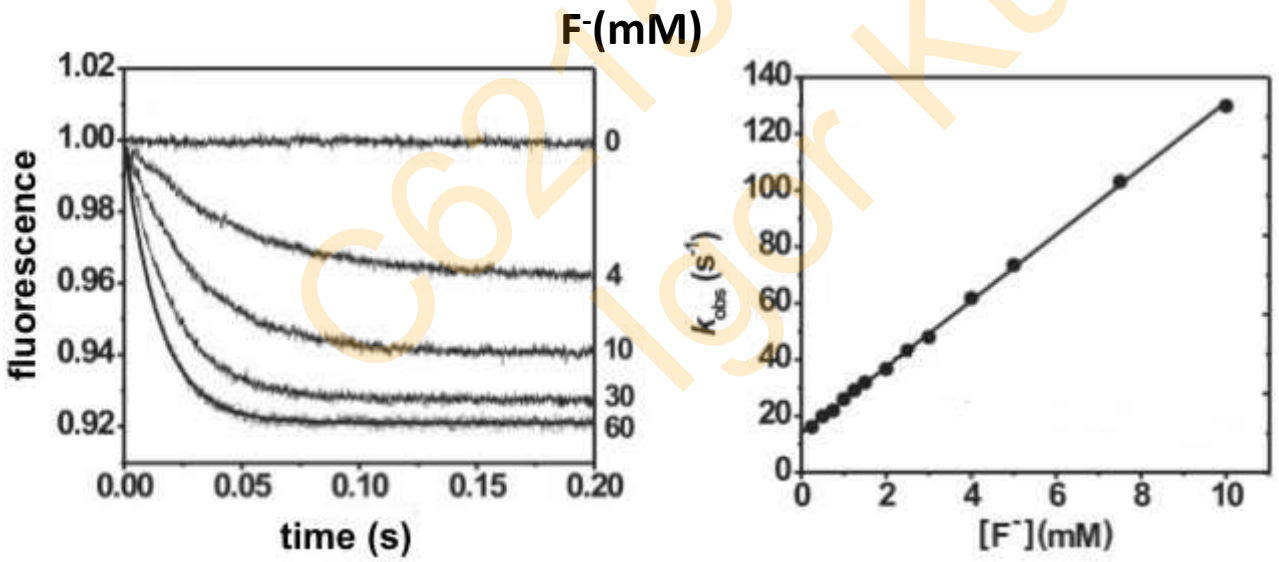
$$c_R = [R] + [RL] \quad [L] \approx c_L >> c_R$$

$$\frac{d[R]}{dt} = -k_1 c_L [R] + k_{-1}(c_R - [R])$$

$$\frac{d[R]}{dt} = k_{-1} c_R - \underbrace{(k_1 c_L + k_{-1})}_{k_{obs}} [R]$$

$$[R] = [R]_\infty + ([R]_0 - [R]_\infty) e^{-k_{obs} t}$$

$$[RL] = ([R]_0 - [R]_\infty)\left(1 - e^{-k_{obs} t}\right)$$



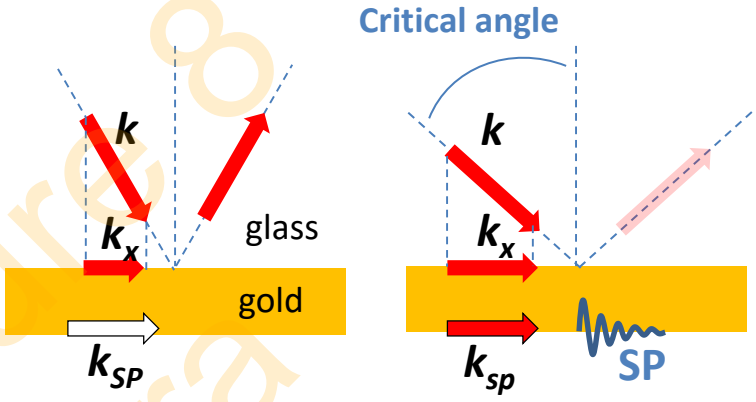
Fluoride binding to 2.5 µM tyrosinase
λ_{ex} = 285 nm
λ_{em} = 320 nm

Linear fit:
k_1 = 11.7 $mM^{-1}s^{-1}$
k_{-1} = 13.9 s^{-1}

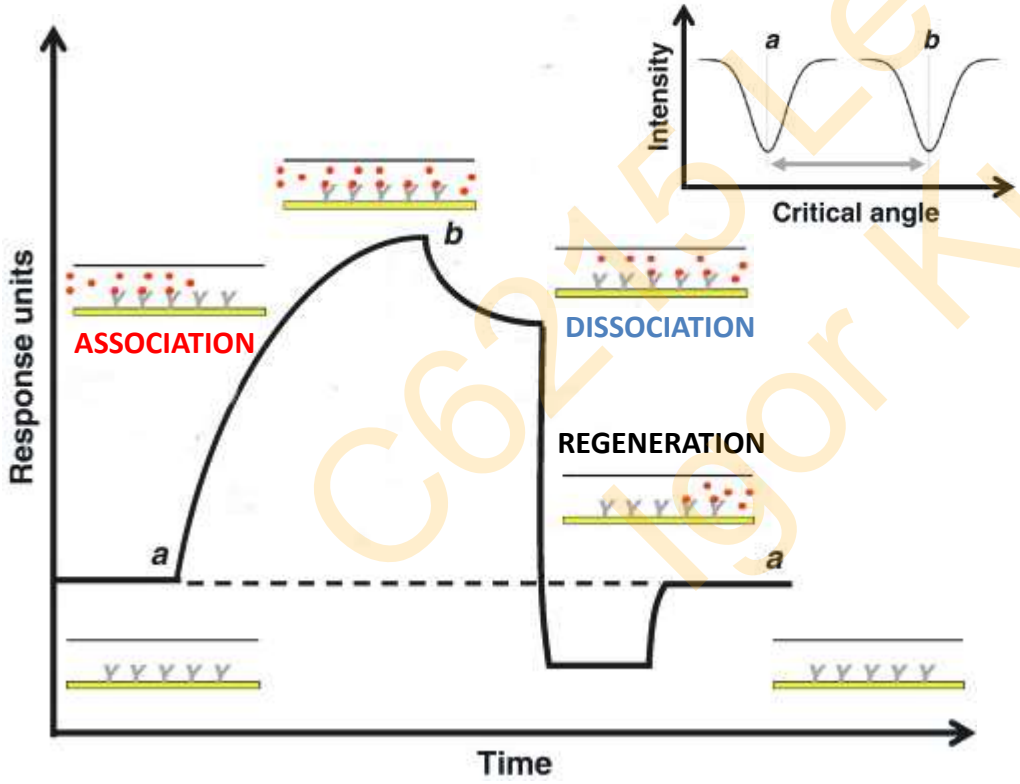
Tepper et al., J. Biol. Chem. 2004, 279, 13425

Surface plasmon resonance (SPR)

The measurement of the SPR is done using monochromatic and polarized light. Although incident light is totally reflected at the interface glass-gold, the electromagnetic field component does penetrate a short (tens of nanometers) distance into the metal creating an exponentially attenuated 'evanescent wave', which can excite surface plasmons. Due to resonance energy transfer between the evanescent wave and surface plasmons (SP), the intensity of the reflected light is reduced at a specific incident angle. The resonance angle at which the intensity minimum occurs is a function of the refractive index of the solution close to the gold layer.



k = wave vectors



Patching, Biochim. Biophys. Acta 2014, 1838, 43

Response is

$$\sim\left(1 - e^{-(k_1 c_L + k_{-1})\cdot t}\right)$$

for ASSOCIATION and

$$\sim e^{-k_{-1}\cdot t}$$

for DISSOCIATION.

Enzyme reaction (equilibrium approximation)

$$\text{enzyme substrate complex} \xrightarrow{k_{cat}} \text{enzyme + product}$$

k_{cat} (s^{-1}) = catalytic rate constant, molecular activity, „turnover number“

By applying previous considerations to a case with R = E (enzyme) and L = S (substrate), the rate of enzyme reaction $v = k_{cat}[ES]$ can be expressed in three ways as

$$v = k_{cat} c_E \frac{[S]}{[S] + K_d}$$

Requires [S] to be known, which is rarely the case in *in vivo* conditions.

$$v = k_{cat} \frac{(c_S + c_E + K_d) - \sqrt{(c_S + c_E + K_d)^2 - 4 c_S c_E}}{2}$$

$$v = k_{cat} c_E \frac{c_S}{c_S + K_d}$$

Holds only if $c_E << K_d$; then [ES] is small and $c_S \approx [S]$.

In all three cases, v tends to $k_{cat} c_E$ (= limiting or “maximum“ rate) as substrate concentration tends to infinity.

In-vivo concentrations of substrates and enzymes

When working with isolated enzymes, the substrate concentrations are usually held at least 1000 times higher than that of the enzyme (i.e., $c_S >> c_E$). Under *in vivo* conditions, however, the actual c_S/c_E ratio may be significantly lower. For various enzymes of glycolysis in mammalian skeletal muscle it ranges from 0.057 (for glyceraldehyde 3-phosphate dehydrogenase) to 62 (for phosphofructokinase).

Enzyme	Active site (μM)	Metabolite (substrate)	Concentration (μM)
Phosphofructokinase	24.1	Fructose 6-phosphate	1500
Aldolase	809.3	Fructose 1,6-bisphosphate	80
Triosephosphate isomerase	223.8	Dihydroxyacetone phosphate	160
Glyceraldehyde 3-phosphate dehydrogenase	1398.6	Glyceraldehyde 3-phosphate	80
Phosphoglycerate kinase	133.6	1,3-Bisphospho-glycerate	50
Pyruvate kinase	172.9	Phosphoenolpyruvate	65
Lactate dehydrogenase	296.0	Pyruvate	380

Punekar, Enzymes: Catalysis, Kinetics and Mechanisms 2018

Competition of two substrates for a single enzyme

When substrates A and B react with a single enzyme E without formation of any ternary complex containing A and B, then in mixtures of A and B each substrate acts as a competitive inhibitor of the other:

$$\begin{array}{l} \\ + \ A \ \underset{}{\overset{K_A}{\rightleftharpoons}} \ EA \ \xrightarrow{k_{cat}^A} \\ E \\ + \ B \ \underset{}{\overset{K_B}{\rightleftharpoons}} \ EB \ \xrightarrow{k_{cat}^B} \end{array}$$

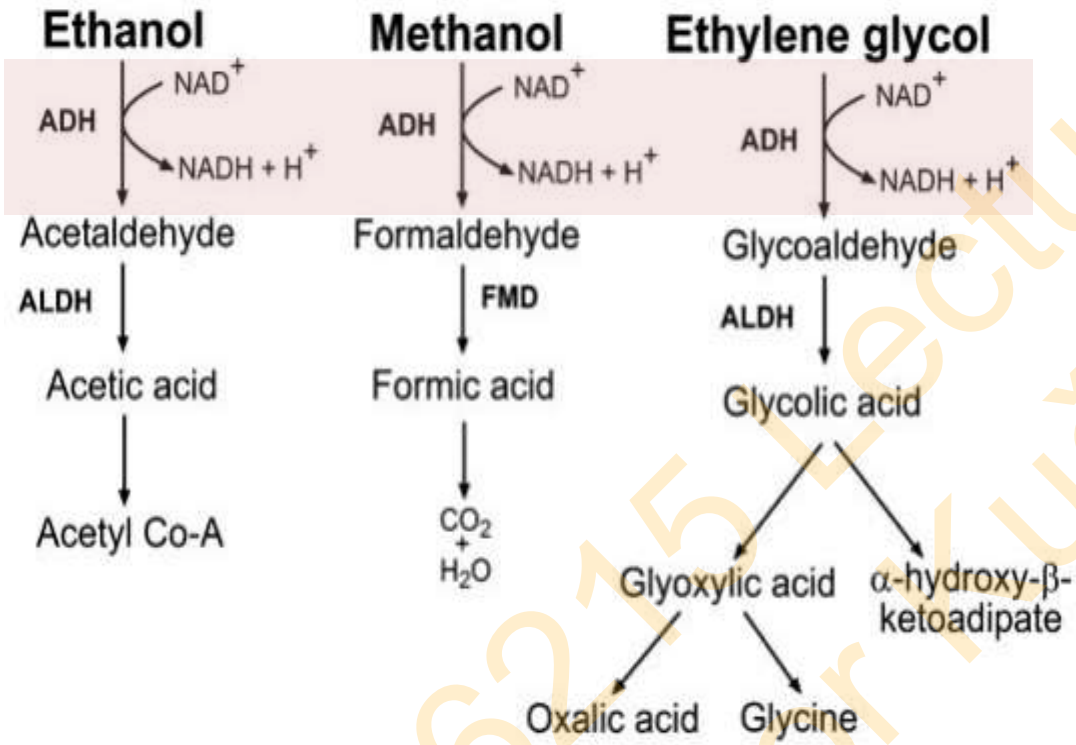
$$v_A = k_{cat}^A[EA] = k_{cat}^A c_E \frac{\frac{[A]}{K_A}}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}} = k_{cat}^A c_E \frac{[A]}{[A] + K_A\left(1 + \frac{[B]}{K_B}\right)}$$

$$v_B = k_{cat}^B[EB] = k_{cat}^B c_E \frac{\frac{[B]}{K_B}}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}} = k_{cat}^B c_E \frac{[B]}{[B] + K_B\left(1 + \frac{[A]}{K_A}\right)}$$

$$\frac{v_A}{v_B} = \frac{\frac{k_{cat}^A}{K_A}[A]}{\frac{k_{cat}^B}{K_B}[B]}$$

=> The relative rate of turnover of two competing substrates is defined by their relative concentrations and k_{cat}/K values. The k_{cat}/K ratio is called specificity constant (or kinetic efficiency) and is used as a measure of the substrate preference of an enzyme .

Management of toxic alcohol ingestions



Ethanol has 10 to 20 times greater affinity for alcohol dehydrogenase (ADH) than the other alcohols. At a serum concentration of 1 g ethanol/L, the conversion of other alcohols by ADH is completely inhibited.

Kraut, Kurtz, Clin. J. Am. Soc. Nephrol. 2008, 3, 208

General principles in the treatment of alcohol intoxications

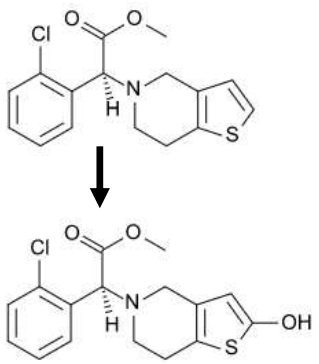
Gastric lavage, induced emesis, or use of activated charcoal to remove alcohol from gastrointestinal tract needs to be initiated within 30 to 60 min after ingestion of alcohol.

Administration of ethanol or fomepizole to delay or prevent generation of toxic metabolites needs to be initiated while sufficient alcohol remains unmetabolized.

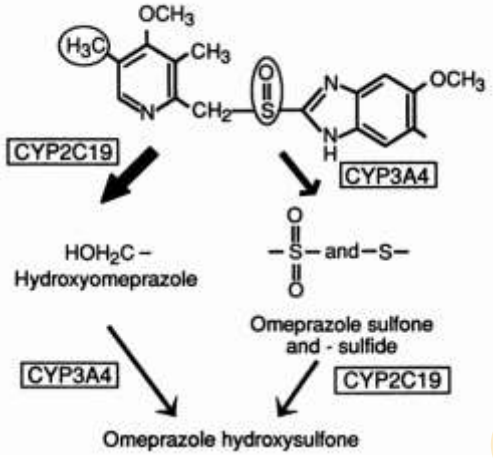
Dialysis helpful in removing unmetabolized alcohol and possibly toxic metabolites and delivering base to patient to ameliorate metabolic acidosis.

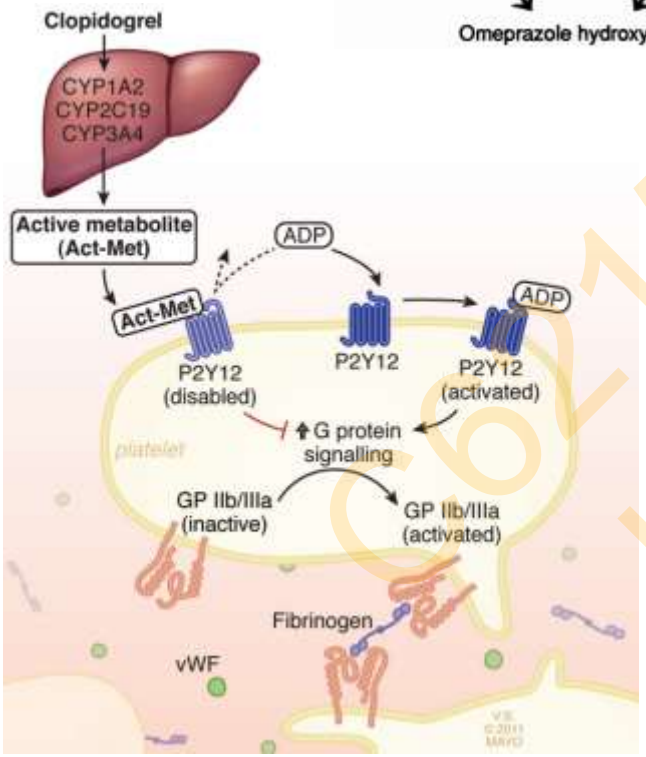
An example of drug interactions

CLOPIDOGREL



OMEPRAZOLE





Clopidogrel is a prodrug of the thienopyridine family, which has little, if any, platelet inhibitory effect in its original state. Its active form is produced by metabolism of the prodrug through the cytochrome P450 system of the liver, most particularly by the enzyme CYP2C19.

Proton pump inhibitors (PPIs) irreversibly block the gastric proton pump responsible for the acidification of the stomach. Elimination of these drugs occurs almost entirely by cytochrome P450 metabolism to inactive or less active metabolites.

PPIs are often coadministered to patients receiving clopidogrel to reduce the risk of gastrointestinal bleeding.

=> There is reduced inhibition of platelet aggregation by clopidogrel when PPIs are coadministered.

Meyer, Yale J. Biol. Med. 1996, 69,203

Comin, Kallmes, Am. J. Neuroradiol. 2011, 32, 2002

An enzyme reaction at low and high concentrations of substrate

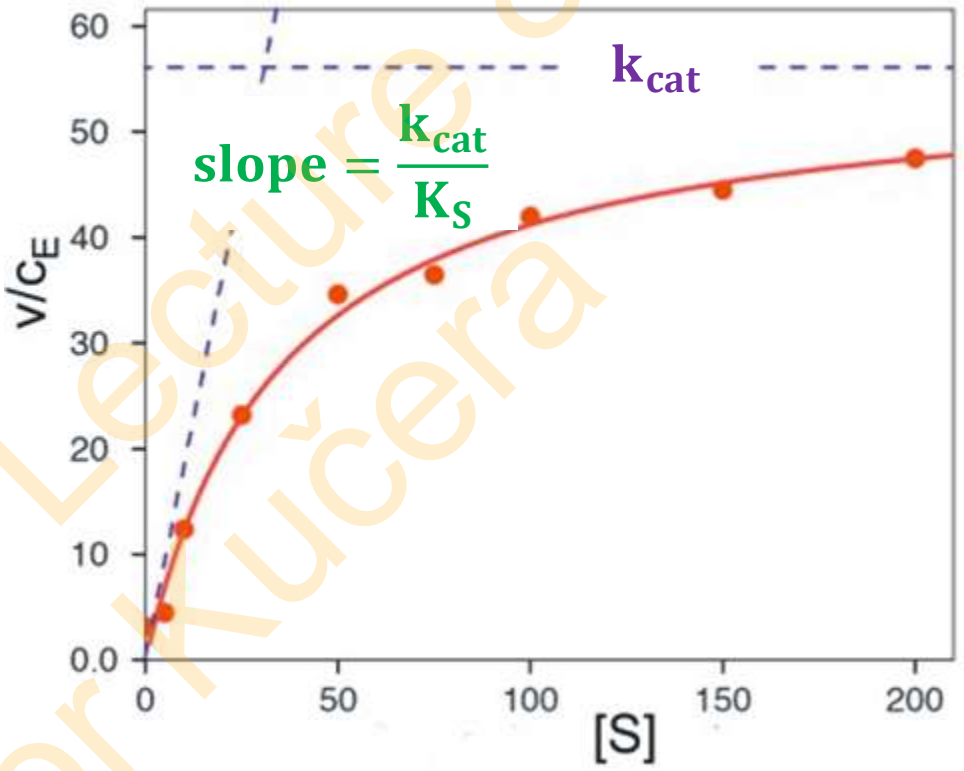
$$v = \frac{v_{lim}[S]}{[S] + K_S}$$

[S] << K_S :

$$v \approx \frac{v_{lim}}{K_S}[S] = \frac{k_{cat}c_E}{K_S}[S]$$

[S] >> K_S :

$$v \approx v_{lim} = k_{cat}c_E$$



=> An enzyme reaction can be appropriately characterized by the parameters specificity constant (k_{cat}/K_S) and catalytic rate constant (k_{cat}).

Johnson, Beilstein J. Org. Chem. 2019, 15, 16