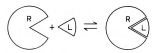
- Receptor - ligand interactions :
  - Equilibrium
  - Thermo & kinetics
  - Methods to determine
  - Efficacy

"Receptor" can also be "enzyme" or more general "protein"

1

Receptor - Ligand interaction

1-to-1 stoichiometry:



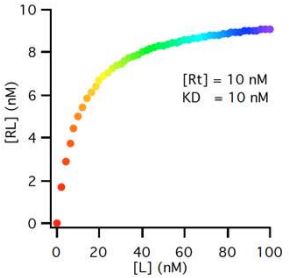
Association: $R + L \xrightarrow{k_{on}} RL$

Dissociation: $RL \xrightarrow{k_{off}} R + L$

At equilibrium: $k_{on} \cdot [R] \cdot [L] = k_{off} \cdot [RL]$

Dissociation constant: $K_D = \frac{k_{off}}{k_{on}} = \frac{[R] \cdot [L]}{[RL]}$

=> Langmuir isotherm: $[RL] = \frac{[R_T]}{1 + \frac{K_D}{[L]}}$



Langmuir: The absorption of gases on plane surfaces of glass, mica and platinum. JACS. 40, 1918, p.1361.

2

Receptor - Ligand interaction: How strong?

Gibbs free energy at equilibrium: $\Delta G^o = RT \ln(K_D)$

◇ 10-fold lower K_D decreases ΔG by -5.7 kJ/mol
(or ~1.4kcal/mol)

◇ Instead of K_D one often prefers: $pK_D = -\log(K_D)$

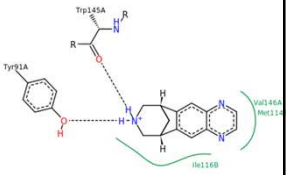
3

Receptor - Ligand interaction

Scaling interactions looking at the energy:

Covalent bonds	200 - 400	kJ/mole
Ion - dipole	50 - 200	
Anion-cation	6 at 3Å in water	
Dipole-dipole	5 - 50	
H-bond	4 - 13	
Ion - π	5 - 80	
π - π	0 - 50	
Van der Waals	2 - 4 per atom pair	
kT	2.48	

Varenicline bound to acetylcholine binding protein



4

Receptor - Ligand interaction: Who long?

Association: $$\frac{\delta[RL]}{\delta t} = k_{on} \cdot [R] \cdot [L] - k_{off} \cdot [RL]$$

Assuming $[L_T] >> [R_T]$: $$[RL]_t = [RL]_{eq} \cdot (1 - e^{-(k_{on} \cdot [L] + k_{off}) t})$$

Dissociation upon removal of ***L***: $$[RL]_t = [RL]_{eq} \cdot e^{-k_{off} \cdot t}$$

◇ The mean lifetime $\langle \tau \rangle$ of ***RL*** equals $1/k_{off}$

5

k_{off}, residence time and target potency

k_{off} vs k_{on} for 3 examples

streptavidin mutants vs biotin | HIV protease mutants vs saquinavir | Methyl transferase vs several inhibitors

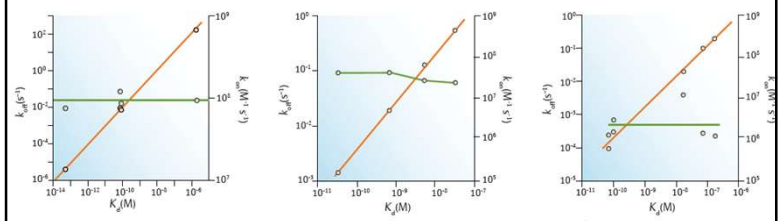
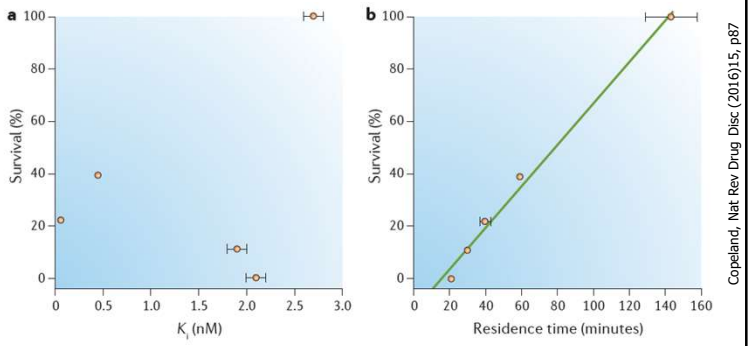


Figure 2 | **Drug affinity (target potency) is often driven by drug–target residence time.** Correlation between the dissociation rate constant (k_{off}; orange circles) or association rate constant (k_{on}; green circles) with the equilibrium dissociation constant (K_d) for biotin binding to wild-type and mutant forms of streptavidin[16] (part **a**), saquinavir binding to wild-type and resistant mutants of HIV protease[17] (part **b**), and a series of aminonucleoside inhibitors binding to the protein methyltransferase DOT1L[18] (part **c**).

=> Affinity (=$1/K_d$) seems to be governed by k_{off}

6

k_{off}, residence time and efficacy



Copeland, Nat Rev Drug Disc (2016)15, p87

Figure 4 | ***In vivo* efficacy often depends on drug-target residence time.** Lu *et al.*[30] investigated the relationship between the residence time of a series of FabI enoyl-reductase inhibitors and *in vivo* activity. The plots presented here show the percent survival of mice 10 days after they were infected with the bacterium *Francisella tularensis* and then treated with the inhibitors. **a** | Correlation of percent survival with the inhibition constant (K_i). **b** | Correlation of percent survival with inhibitor residence time. Figure is adapted with permission from REF 5, Wiley.

7

- Methods to determine binding

8

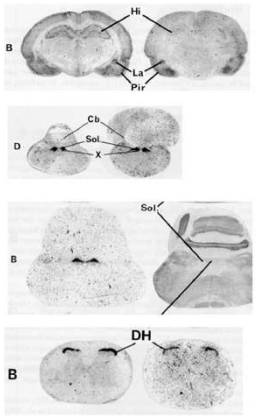
Localising receptors in tissues

Autoradiography of tissue slices incubated with radioactively labelled ligands

Hi: hipocampus
La: lateral layer
Pir : pyramidal layer

Cb : cerebellum
Sol : solaris nucleus tractus

DH : dorsal horn



See also:
www.proteinatlas.org

visible human project

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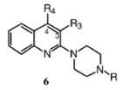
Quantifying receptor - ligand interaction

=> Understanding receptor ligand interaction, e.g. for medical chemistry

Table 2. 5-HT_3 Receptor Binding Affinities of Compounds **6a–n**: Effects of the Variation of the Substituents in Position 4 of the Quinoline Nucleus



6

compd	R	R_3	R_4	$K_i \pm$ SEM[a] (nM)
6a	CH_3	CH_3	COC_6H_5	0.84 ± 0.17
6b	CH_3	CH_3	$COOCH_2CH_3$	0.43 ± 0.02
6c	CH_3	CH_3	$CON(CH_2)_4$	1.6 ± 0.70
6d	CH_3	CH_3	$CON(CH_2CH_3)_2$	0.37 ± 0.09
6e	CH_3	CH_3	$CON(CH(CH_3)CH_3)_2$	2.2 ± 0.55
6f	CH_3	CH_3	$CON(CH_2CH_2CH_3)_2$	0.11 ± 0.004
6g	CH_3	CH_3	$CON(CH_2CH_2CH_2CH_3)_2$	0.78 ± 0.12
6h	CH_3	CH_3	$CON(CH_2CH_2CH_2CH_2CH_3)_2$	19 ± 2.4
6i	CH_3	CH_3	$CON(CH_2CH_2CH_2CH_2CH_2CH_3)_2$	188 ± 50
6j	CH_3	CH_3	$CON(CH_3)CH_2C_6H_5$	0.55 ± 0.15
6k	CH_3	CH_3	$CON(CH_3)CH_2C{\equiv}CH$	0.45 ± 0.12
6l	CH_3	CH_3	$CON(CH_3)p\text{-}Cl\text{-}C_6H_4$	13 ± 4.1
6m	CH_3	CH_2OH	CH_2OH	2.7 ± 0.52
6n	CH_3	$COOC_2H_5$	CH_3	0.17 ± 0.02

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Quantifying and localising receptor - ligand interactions

Receptor

Ligand

Assay format

11

Heterogeneous methods : Separation needed

How to determine ***RL*** without affecting the equilibrium?

=> be fast, with separation times < $1/k_{off}$

Compare 2 standard separation methods:

- Rapid filtration separation in ~ 1 sec
- Gel filtration ~10-300 sec

=> Only applicable in case high-affinity or very slow ligands

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Quantifying receptor - ligand interaction

Rapid filtration:
- incubation mixture is sucked through a filter by vacuum
- receptor proteins are retained by the filter

Manual filtering

- radioactivity on filter is quantified by scintillation counting

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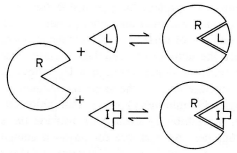
Ligand-binding assays with competition

Reversible inhibitor ***I*** competing for the **same** binding site:



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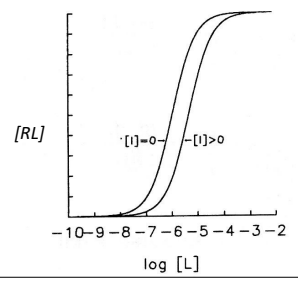
Receptor - Ligand interaction: Competition

Reversible inhibitor ***I*** competing for the same binding site:
- Binding isotherm of ***L*** shifts to higher concentrations
  => apparent higher K_D
- Maximal binding is unaffected

Semi-logarithmic plot



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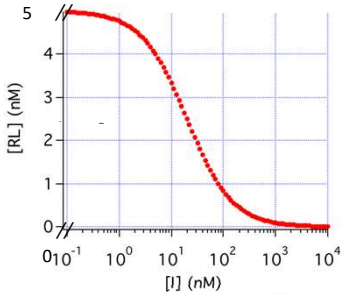
Receptor - Ligand interaction : Competition

Increasing concentrations of ***[I]*** will compete out ***L*** for binding to receptor

e.g. $[R_T]=[K_D]=[K_I]=[L]$= 10 nM

IC_{50}: ***[I]*** of half-maximal inhibition of binding of ***L*** to ***R***



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Quantifying receptor - ligand interaction

=> Understanding receptor ligand interaction, e.g. for medical chemistry

3568 *Journal of Medicinal Chemistry, 2005, Vol. 48, No. 10* *Cappelli et a*

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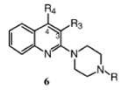

6

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Methods to quantify receptor - ligand interactions

	Label-free	Labelled
Homogeneous	ITC, NMR *CETSA*	radioactive fluorescence
Heterogeneous	GC, LC, MS	radioactive fluorescence

Criteria for evaluation:
- Through-put
- Reproducibility
- Cost
- Working range
- Ease of handling
- Environment
- ..

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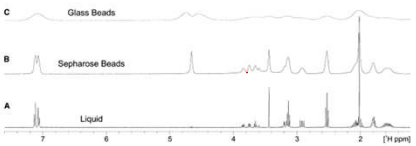
NMR for drug screening

TINS : **T**arget **I**mmobilized **N**MR **S**creening

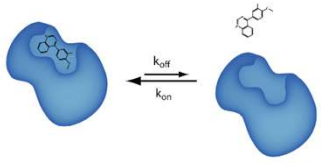
Small molecules in solution
A) in liquid
B) with sepharose
C) with glass beads



What change upon binding ?

Ligand properties

bound		free
protein	chemical environment	solvent
ω_{bound}	chemical shift	ω_{free}
slow	rotational tumbling	fast
fast strong positive	transverse relaxation NOE cross-peaks	slow weak negative
slow	translational diffusion	fast



Protein properties

bound		free
ligand	chemical environment	solvent/protein
ω_{bound}	chemical shift	ω_{free}

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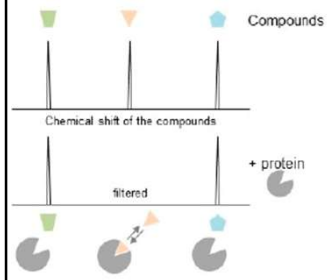
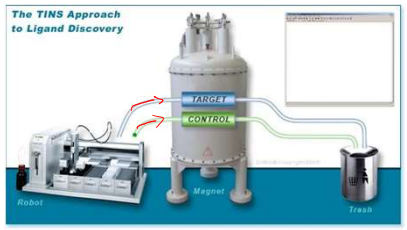
NMR for drug screening

How to screen efficiently ?
Mix & measure



Industrial platform
www.zobio.com



Up to 100 compounds per run

Sugiki, Molecules 2018, 23,148

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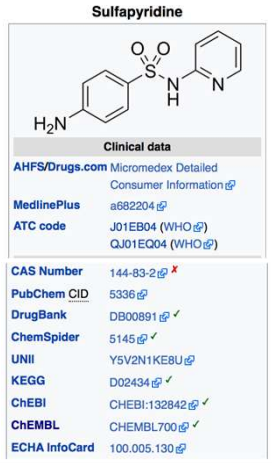
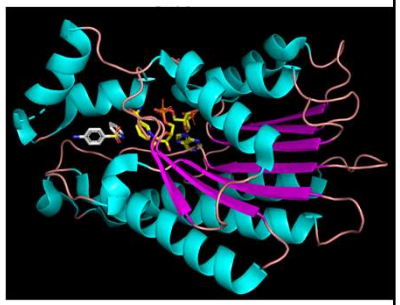
Ligand - Protein interactions :: Information data bases



Protein structure-based :

PDB at http://www.rcsb.org
e.g. sulfapyridine bound to
sepiapterin reductase
4HWK.pdb



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- Efficacy

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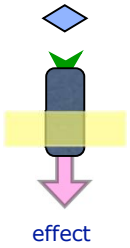
Ligand effect on receptor activity & cellular response



Ligand nomenclature :

- Activation of effect =>Agonist
- No effect =>Antagonist
- Inhibition of effect =>Inverse agonist

Manner of ligand action :

- Reversible or irreversible
- Competitive or noncompetitive

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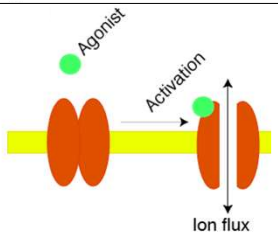
Receptor activation : The most simple case

Agonist-binding induced activation

e.g. ligand-gated ion channel

ligand binding → channel opening



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Receptor activation

Ligand binding is followed by a structural change that leads to opening of an internal channel

Del Castillo-Katz equation (1957): $L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} RL \underset{\alpha}{\overset{\beta}{\rightleftarrows}} R^{Open}L$

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Receptor activation

Only binding

$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} RL$$

$$[RL] = \frac{[R_T]}{1 + K_D / [L]}$$

50 % of R_T as RL when

$$[L] = K_D$$

Binding & activation

$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} RL \underset{\alpha}{\overset{\beta}{\rightleftarrows}} R^{Open}L$$

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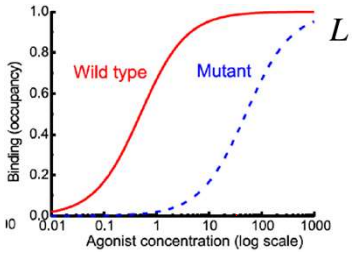
Receptor activation - Effect of mutations

The ligand-concentration dependency of binding and activation can be affected by mutations, below a hypothetical example:



$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} RL \underset{\alpha}{\overset{\beta}{\rightleftarrows}} R^{Open}L$$

Q: What is affected by the mutation?

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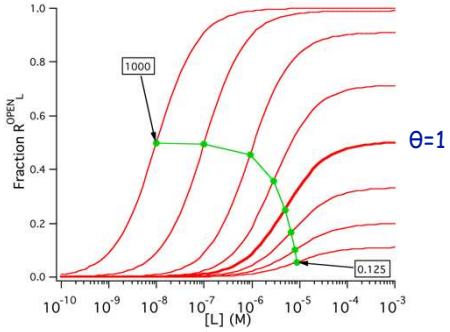
Ligand-gated ion channels : The power of θ

E.g. K_D = 10^{-5} M & θ ranges from 0.125 to 1000



θ => affects both potency (EC_{50}) & efficacy

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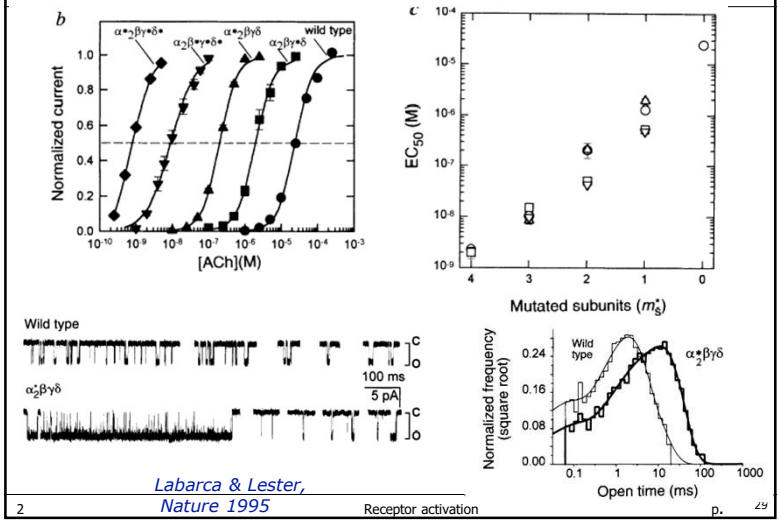
Receptor activation - Effect of mutations



Labarca & Lester, Nature 1995

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Relating ligand binding and cellular effect : *Dis*proportionality

Binding isotherms with altered slopes

Single receptors have multiple binding sites for the same ligand.

Ligand binding might or not affect affinity for binding to remaining sites

$R + nL \leftrightarrow RL_n$

In a linear mechanism: $K_D^{global} = \frac{[R]\cdot[L]^n}{[RL_n]} = \prod_{i=1}^{n} \frac{[RL_{i-1}]\cdot[L]}{[RL_i]} = \prod_{i=1}^{n} K_{Di}$

Hill equation: $K_{Di} = K_{Dj}$ => $[RL_n] = \frac{[R_T]}{1+\left(K_D/[L]\right)^n}$ Hille, 1910

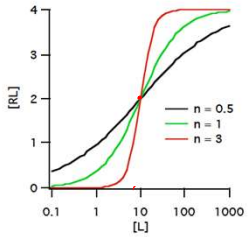
n : Hill coefficient

30

Relating ligand binding and cellular effect : *Dis*proportionality

Binding isotherms with altered slopes

Hill equation $[RL_n] = \frac{[R_T]}{1+\left(K_D/[L]\right)^n}$



):403-9.

s in the analysis

Tuk B[1], van Oostenbruggen MF.

Positive cooperativity → Small [L] variations around EC50 have strong effect

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Further reading:

Stryer

Colquhoun 1998 : Receptor - ligand interactions

Colquhoun 2006 : Historical perspective

Copeland 2016 : Drug-target residence time

Next week:

Molecular interactions

https://www.proteinatlas.org

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