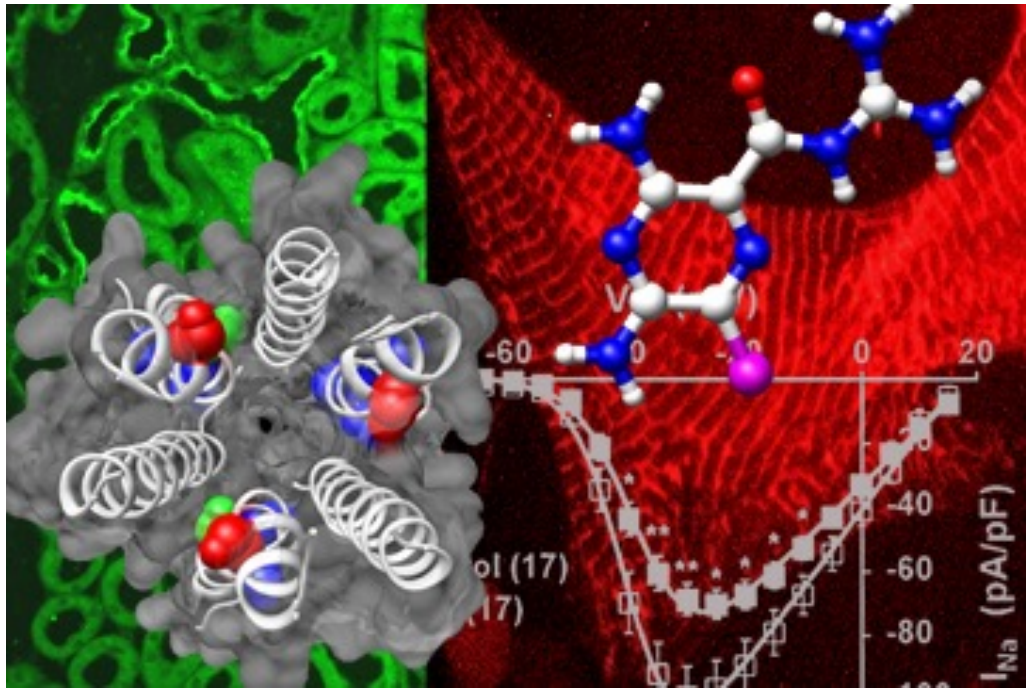
Pharmacology and Pharmacokinetics



Pharmacodynamics
Drug targets

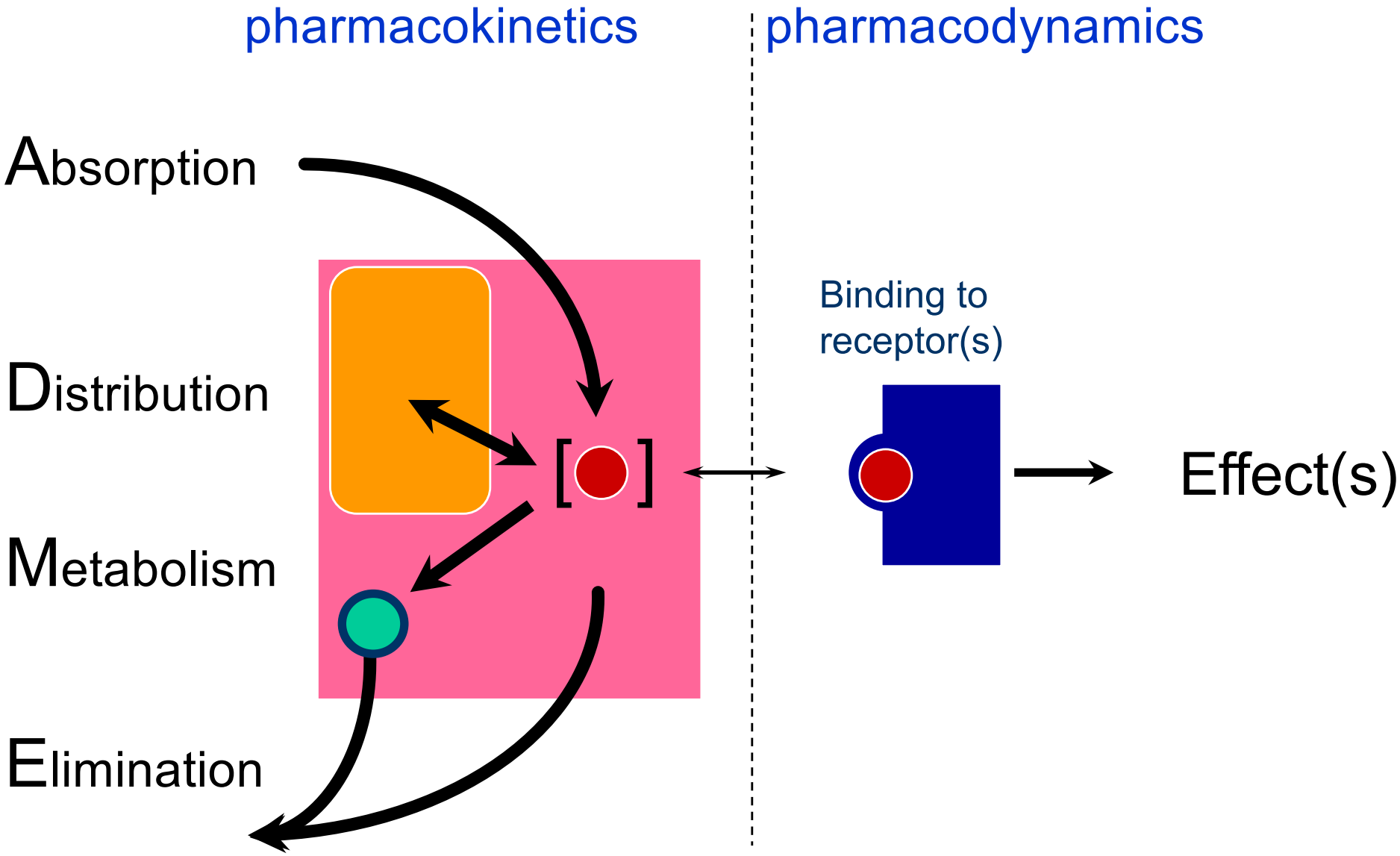
Stephan Kellenberger 021 692 5422 Stephan.Kellenberger@unil.ch
Department of biomedical Sciences, UNIL http://www.unil.ch/dsb

case

Pittsburgh, PA; November 1988: A 34-year-old man is dropped off by a private car at the ambulance entry of an emergency department. He is disheveled and unresponsive and is rushed by security guards into the department. He is quickly placed on a cardiac monitor, and intravenous access is established. His vital signs reveal a heart rate of 26 bpm and he is apneic. He has no palpable blood pressure but has a palpable slow pulse at his femoral artery. Fresh needle track marks, consistent with recent injections, are present in his left antecubital fossa. Physicians suspect he is a victim of the current epidemic of "superpotent" heroin, "China White," which is sweeping Allegheny County. Despite oral intubation, mechanical ventilation, advanced cardiac life support measures, and large intravenous doses of an **antidote**, the patient dies.
In 1988, the Pittsburgh, PA area experienced an epidemic of heroin abusers dying from accidental overdoses of a short-acting synthetic opioid agonist, 3-methyl fentanyl, known on the street as China White. These synthetic analogues of fentanyl were estimated to have 6000 times the **potency** of morphine. Pharmaceutical fentanyl has 75 to 100 times the potency of morphine.

→ what is the difference of the action of china white and morphine on the opioid receptors ?
→ what is the action of the antidote ?

General pharmacology:



Books about Pharmacology

In library

- **Rang & Dale's** *Pharmacology*
  - *J. M. Ritter, R.J. Flower, G. Henderson, Y.K. Loke, D. MacEwan and H. P. Rang, 9th edition , Churchill- Livingstone, 2020*
- **Golan :** *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy,*
  - *David E. Golan, Lippincott Williams & Wilkins, 4th edition, 2016*
- **Goodman & Gilman's :**
  The Pharmacological Basis of Therapeutics
  - *L.L. Brunton, R. Hilal-Dandan, B.C. Knollman, 13th edition, Mc Graw Hill, 2018*

Pharmacology and Pharmacokinetics (General Pharmacology)

Class of 13 x 2 hours

- 6 x 2 hours Pharmacokinetics (Dmitri Firsov)
- 7.5 x 2 hours Pharmacodynamics, drug targets (Stephan Kellenberger; March 31; April 7, 14, 28; May 5, 12, 19, 26)
- 1 hour: Drug development, clinical testing and commercialization (Stephan Kellenberger ; May 26)

Details to classes by Stephan Kellenberger

Pharmacodynamics: general principles and quantitative description of how drugs act : 6 hours including exercises *(March 31, April 7, 14)*

Targets for drug action: discussion of examples of various types of drug receptors: 8 hours *(April 28, May 5, 12, 19)*

Cellular aspects and some general principles of drug action : 1 hour *(May 26)*

Drug development : 1 hour *(May 26)*

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Exam and exam questions

- Written exam of 2h duration
- 6 questions per teacher (12 questions in total, ≈ 10 min per question)
- Type of exam questions shown towards the end of the class

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1. Pharmacodynamics

1.1. Binding interactions
1.2. Quantitative description of ligand-receptor binding
1.3. Relationship between ligand concentration, binding and effect

2. Drug targets

Target	*examples*
2.1. Receptors for physiological ligands	
Transmembrane receptors	
2.1.1. G-protein-coupled receptors	- *adrenergic receptors* - *opioid receptors*
2.1.2. ligand-gated ion channels	- *$GABA_A$ receptors (in pharmacodynamics part)*
2.1.3. kinase-linked receptors	- *insulin receptor*
Intracellular receptors	
2.1.4. nuclear receptors	- *pregnane X receptor*
2.2. Other targets/approaches	
2.2.1. enzymes	- *dihydrofolate reductase* - *tyrosine kinases* - *angiotensin-converting enzyme* - *COX inhibitors (in pain chapter)*
2.2.2. ion channels and transporters	- *voltage-gated Na channels*
2.2.3. protein therapeutics	- *GLP-1 receptor agonists* *-TNF-α monoclonal antibodies (e.g. infliximab)*
2.2.4. gene therapy	- *Nusinersen* - *Tisagenlecleucel /Axicabtagene ciloleucel*

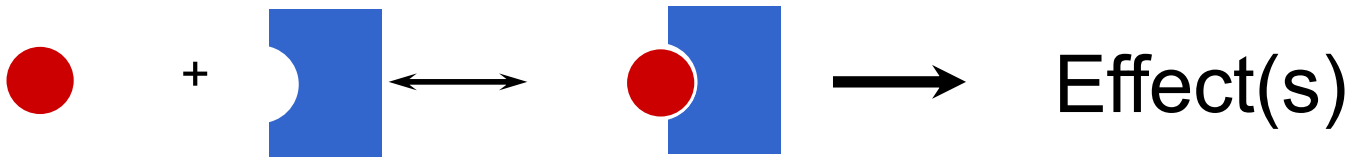
3. Some general aspects of pharmacology

a. Cancer and anti-infective therapy: selective targeting and development of resistance
b. Harmful effects of drugs

4. Drug development

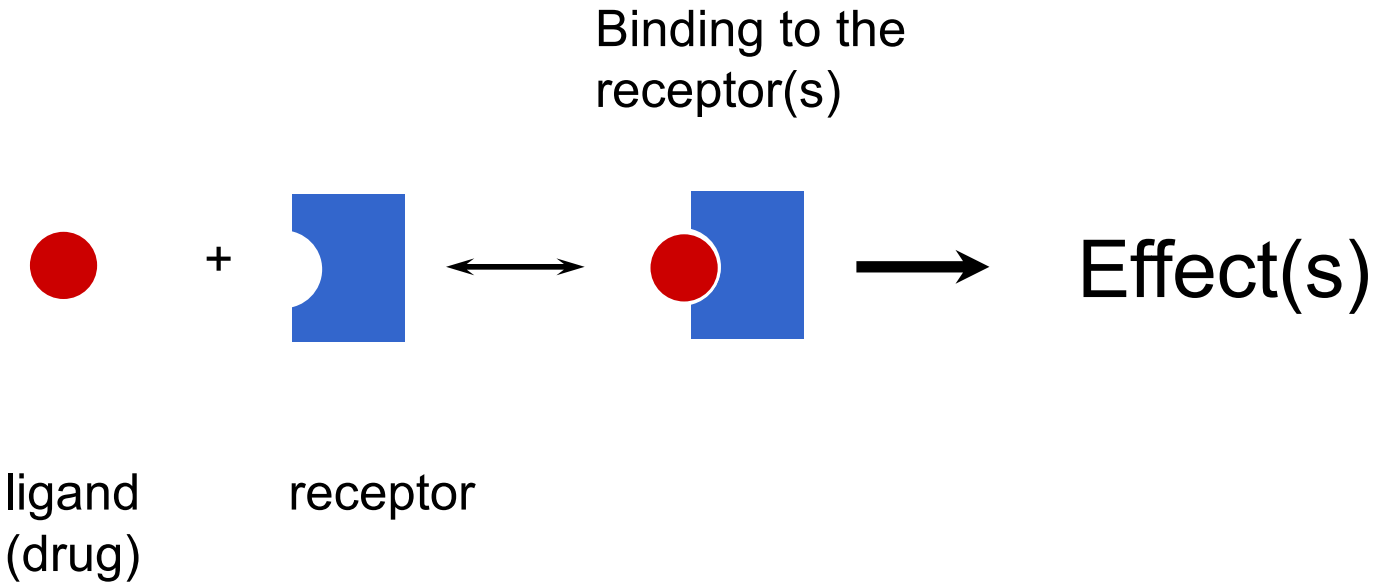
Contents of the part “pharmacodynamics”

- Part I: Introduction
- Part II: Binding of the ligand to the receptor
  - » interactions between ligand and receptor that govern binding
- Part III: Quantitative description of ligand-receptor binding
  - » binding at equilibrium
  - » association and dissociation kinetics
- Part IV: Relationship between ligand concentration, binding and effect
  - » concentration-effect relationship
  - » mechanisms/models of ligand→binding→effect coupling
  - » Agonists: definition, efficacy, potency
  - » Allosteric modulators
  - » Antagonists: types, quantitative description of their interaction with receptor and agonists
- Part V: Other aspects of receptor function and drug therapy



Pharmacodynamics

Part I Introduction, General aspects



Drugs act on specific receptors (drug targets)

Types of drug (and toxic substance) targets

Any functionally important constituent of a cell or an organism can be a drug target:

- Proteins (main topic of this course)
- Nucleic acids: e.g. mutagenic substances
- Lipids (mainly membrane lipids)

} Specific interaction

- \+ some drugs have non-specific toxic effects, e.g. strong acids or bases, or highly reactive substances.

Certain drugs take advantage of differences between the host organism (i.e. the human organism) and the invader organism (e.g. bacteria). These include antibacterial drugs, antifungal drugs, antiviral drugs and drugs used in cancer chemotherapy

Different uses of the term “receptor”

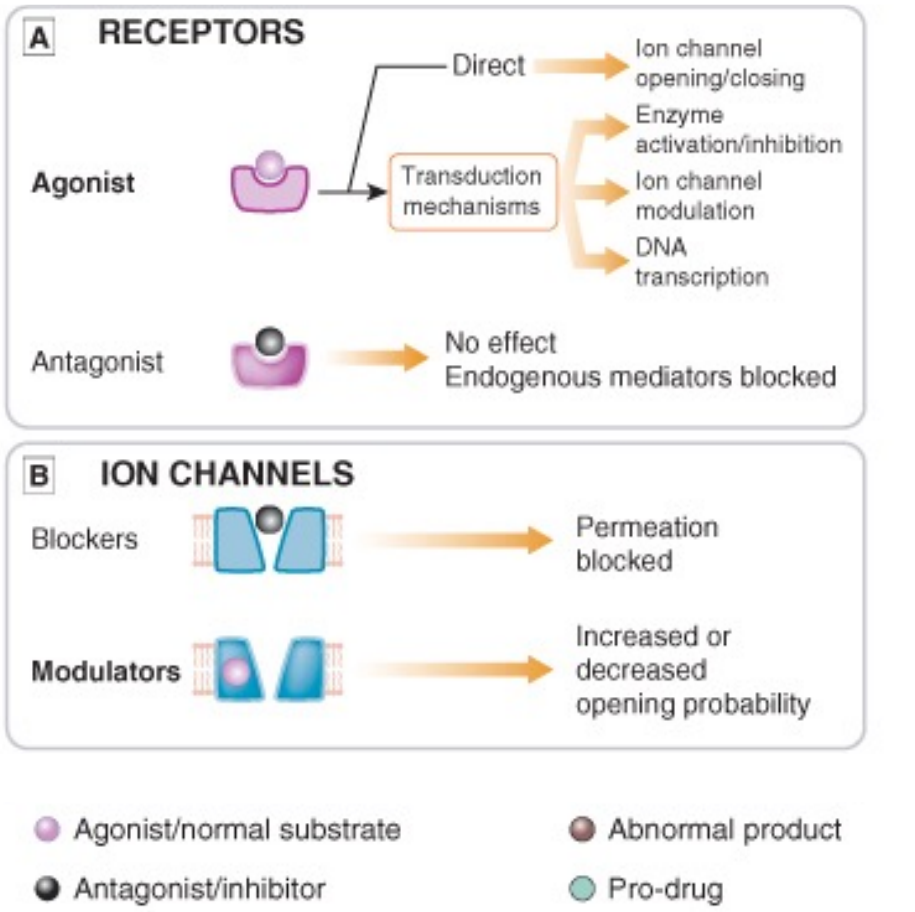
1) Receptors = protein molecules whose function is to recognize and respond to endogenous chemical signals

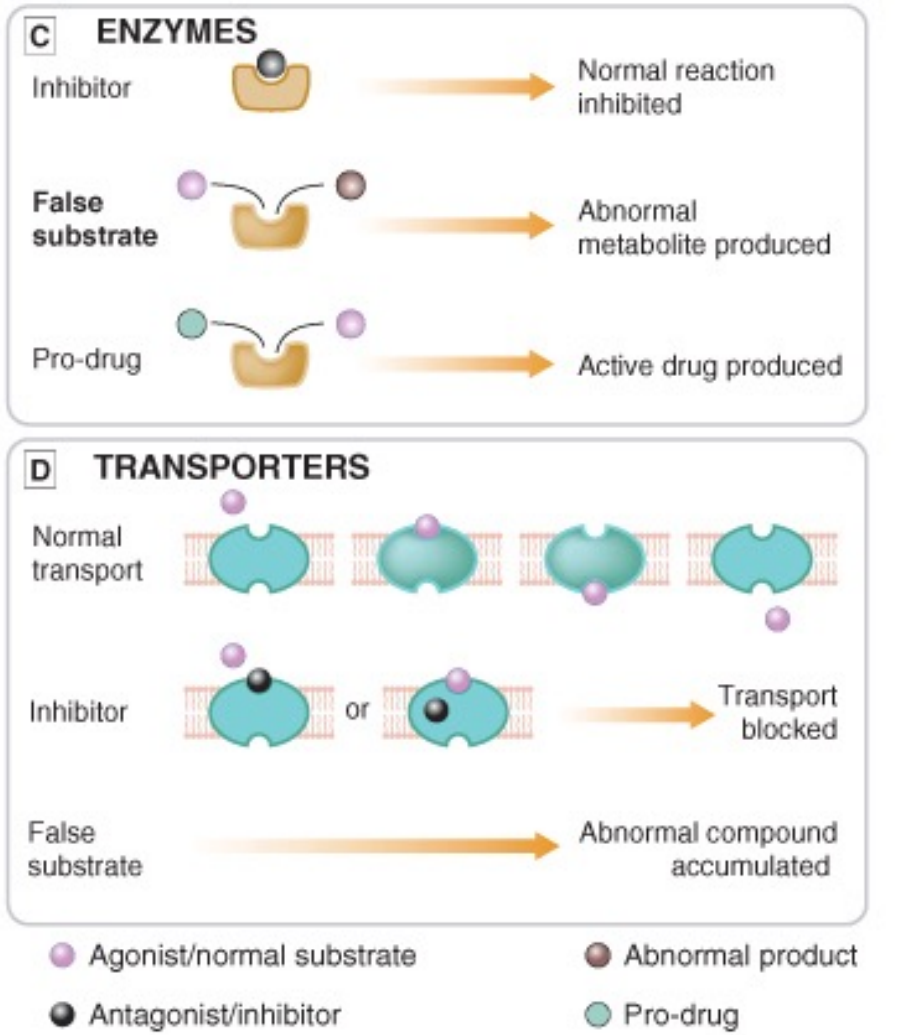
2) the term “drug receptor” or “receptor” in the context of ligands or drugs is also used for the drug target, even if the drug target may not be a receptor for physiological ligands.

3) receptor site = binding site of a drug or endogenous chemical on the target protein

Receptor sites on a protein for an exogenous ligand may be the same as that for endogenous ligands, or may be different

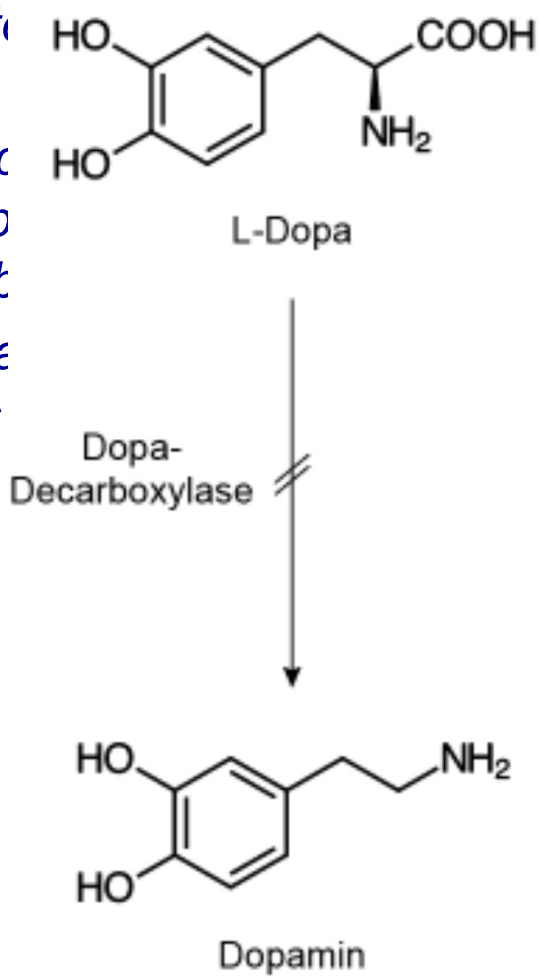
Classes of drug targets





Pro-drugs

- Some have no obvious benefits
- Examples where being a prodrug brings an advantage:
  - *Cyclophosphamide (cytotoxic drug, active only aft liver, oral administration possible)*
  - *Levodopa (Treatment of Parkinson, given with dec crosses blood-brain barrier and is activated to dop [less peripheral side effects, better crossing of bbb*
  - *Zidovudine (antiviral agent; phosphorylated to its a containing appropriate reverse transcriptase, thus the virus)*



monoclonal antibodies as drugs

- monoclonal antibodies are used to neutralize pathogens or other dangerous substances in the blood of the patient
- antibodies are different from conventional drugs in many aspects (they are big molecules, have a very strong and specific interaction with the target)
- Initially, monoclonal antibodies from mouse were used. Currently used mABs “humanized” to different degrees. This reduces the risk of an immune response, extends the plasma half-life and improves the ability of the antibody to activate human defense mechanisms
- name convention: name ending on –ximab = chimeric, -zumab = humanized antibody, -umab = human antibody

Type of mAb	Mouse part
chimeric	Variable regions
humanized	Hypervariable regions
human	-

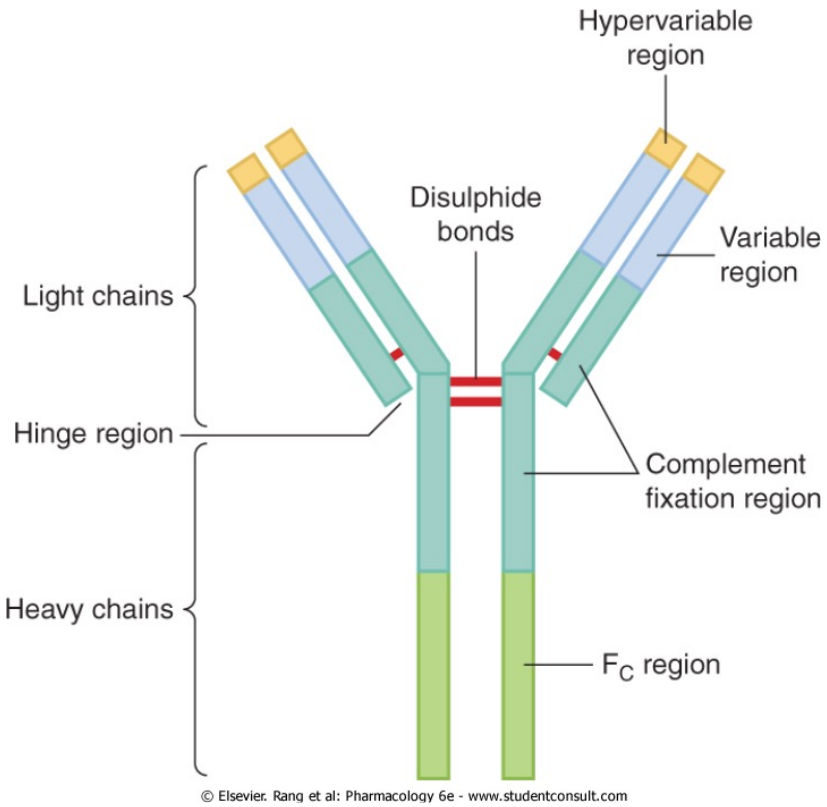
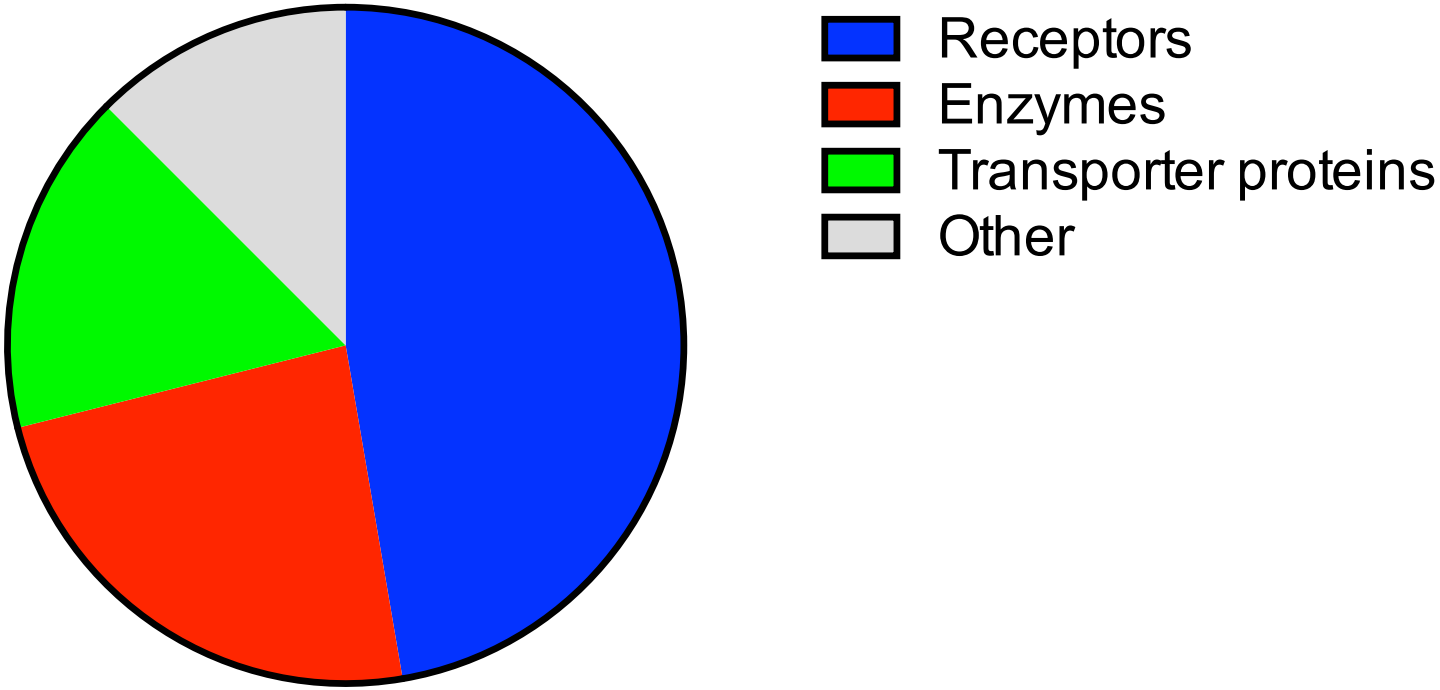


Figure 55-1 Production of engineered 'chimeric' and 'humanized' monoclonal antibodies. The Y-shaped antibody molecule consists of two main domains: the Fc (constant) domain and the Fab (antibody-binding) domain. At the tip of the Fab regions (on the arms of the 'Y') are the hypervariable regions that actually bind the antigen.. (After Walsh, 2004.)

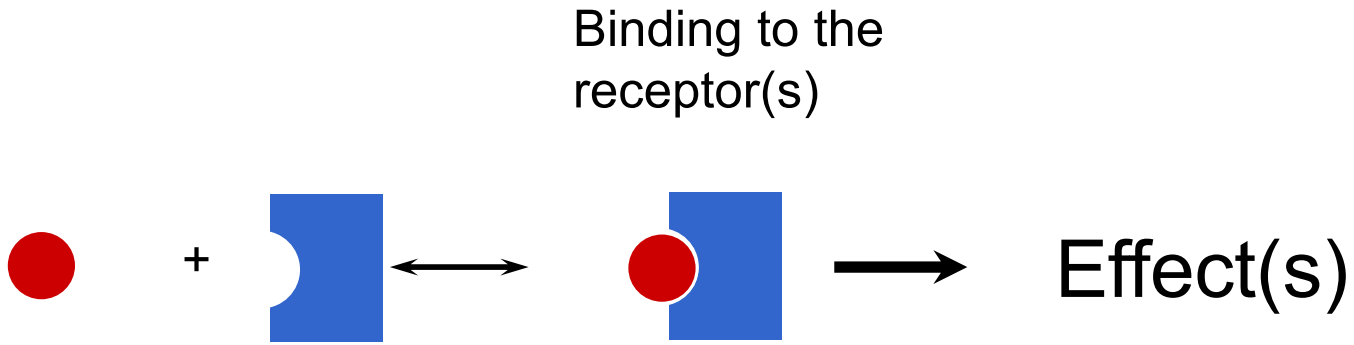
Classes of drug targets



Based on Rask-Andersen et al., Nat. Rev. Drug Disc. 10, pp 579, 2011

Concentration – effect relationship of drugs

(the principles of ligand binding described here and the quantitative description apply to drugs binding to their target or drug receptor, but also to any other endogenous or exogenous substance that binds to a receptor)



L + R ⟷ LR ⟶ Effect

Regarding receptors of endogenous ligands:

Different types of receptor – effector link

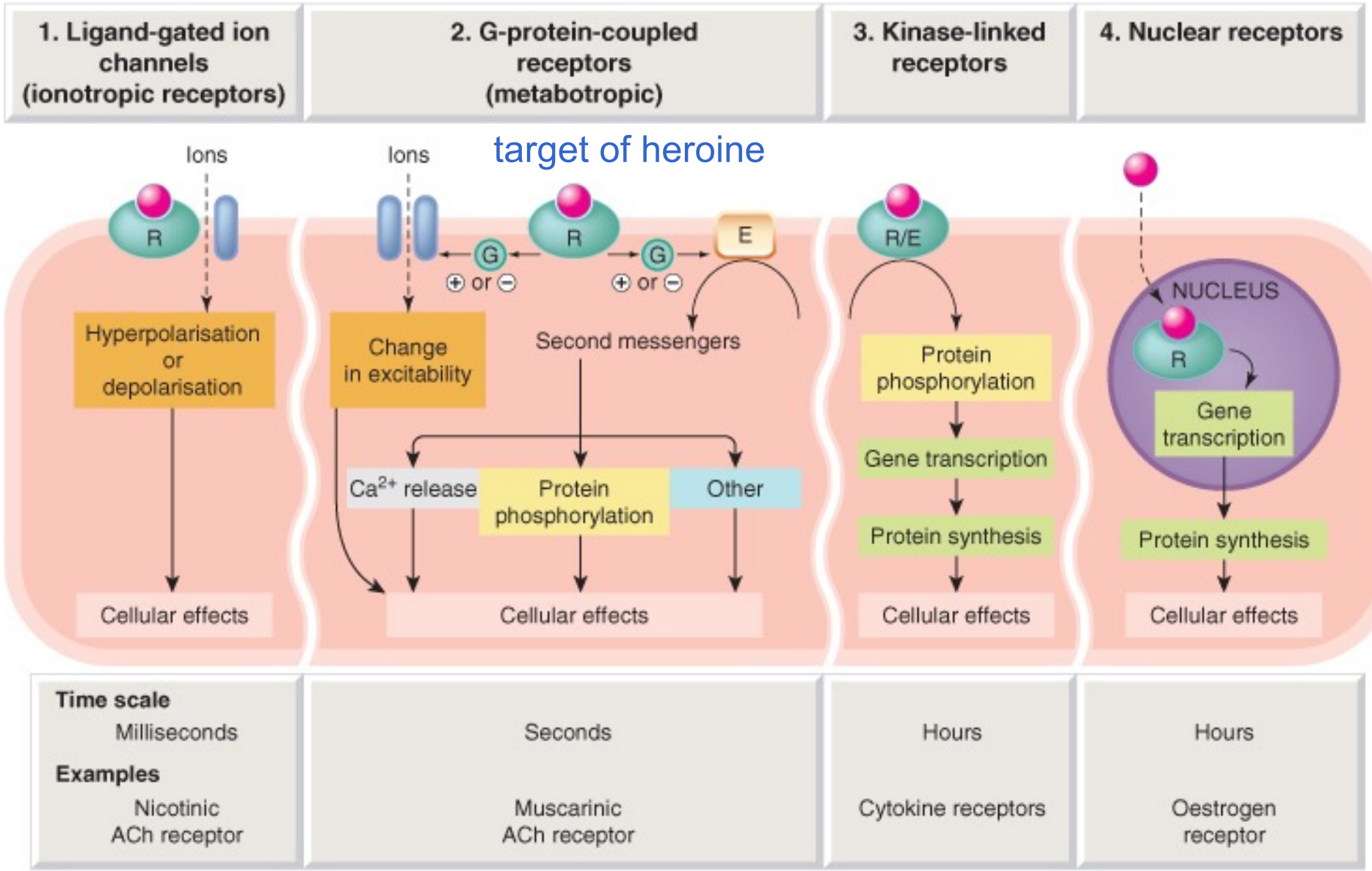
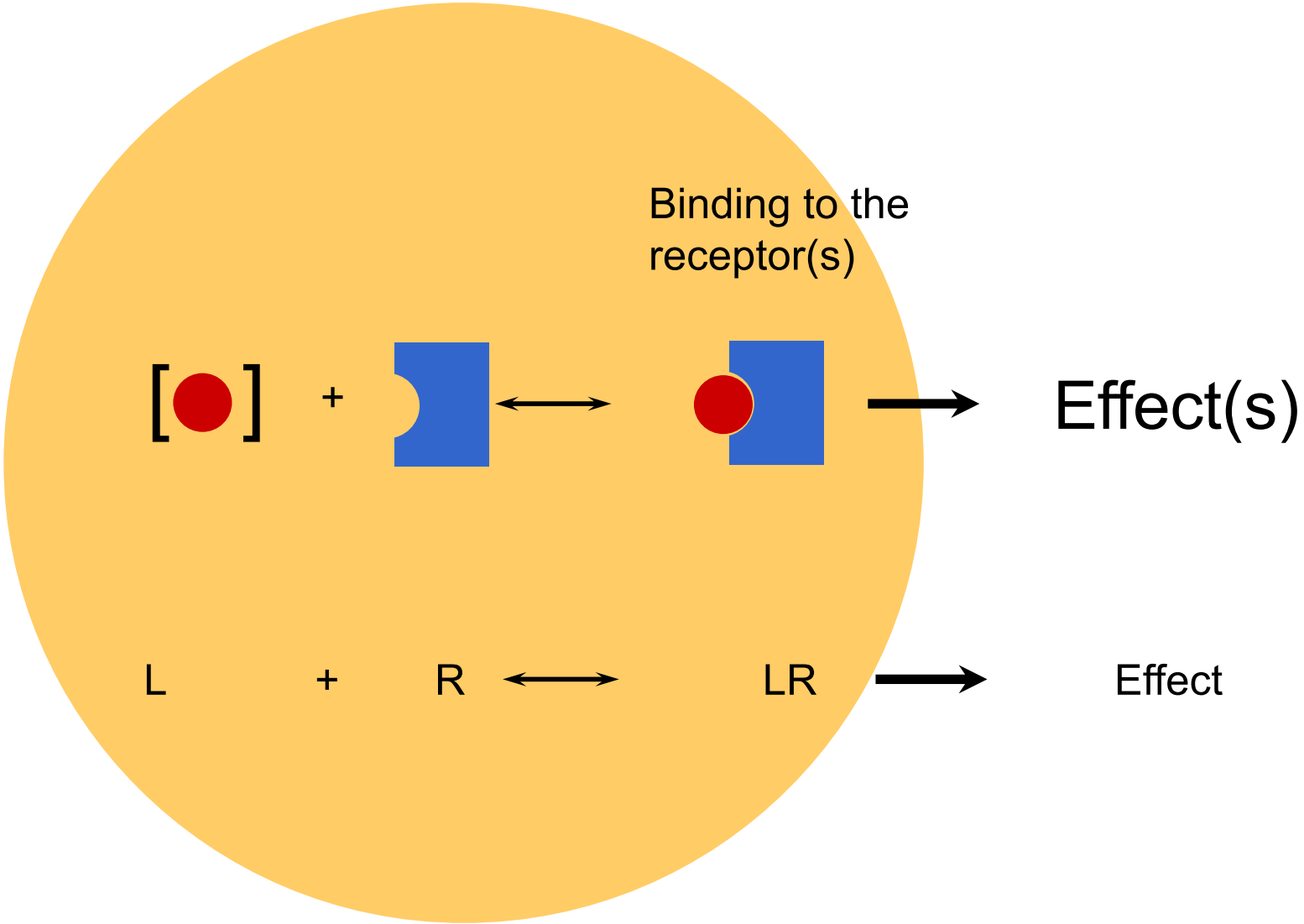


Figure 3.2 Types of receptor-effector linkage. (R, receptor; G, G-protein; E, enzyme; ACh, acetylcholine.)

Part II Binding of the ligand to the receptor

In this part we focus on the binding of the ligand to the receptor. The relationship between binding and response (effect) will be discussed in part IV.



Models of ligand – receptor interaction

- lock and key model (1894, Emil Fischer)

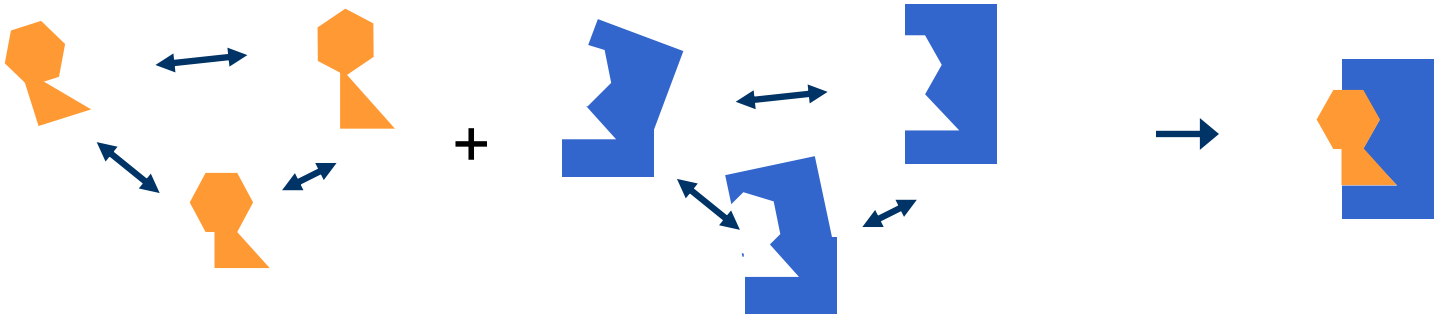
  The shapes of the ligand and the receptor are exactly complementary

- induced fit (Koshland)

  Optimization, in which both the receptor and the ligand adapt their structure in order to bind to each other

- Conformation selection

  Several conformations of the receptor and the ligand exist, and binding occurs preferentially between those conformations that are complementary



Example of conformational change induced by ligand binding

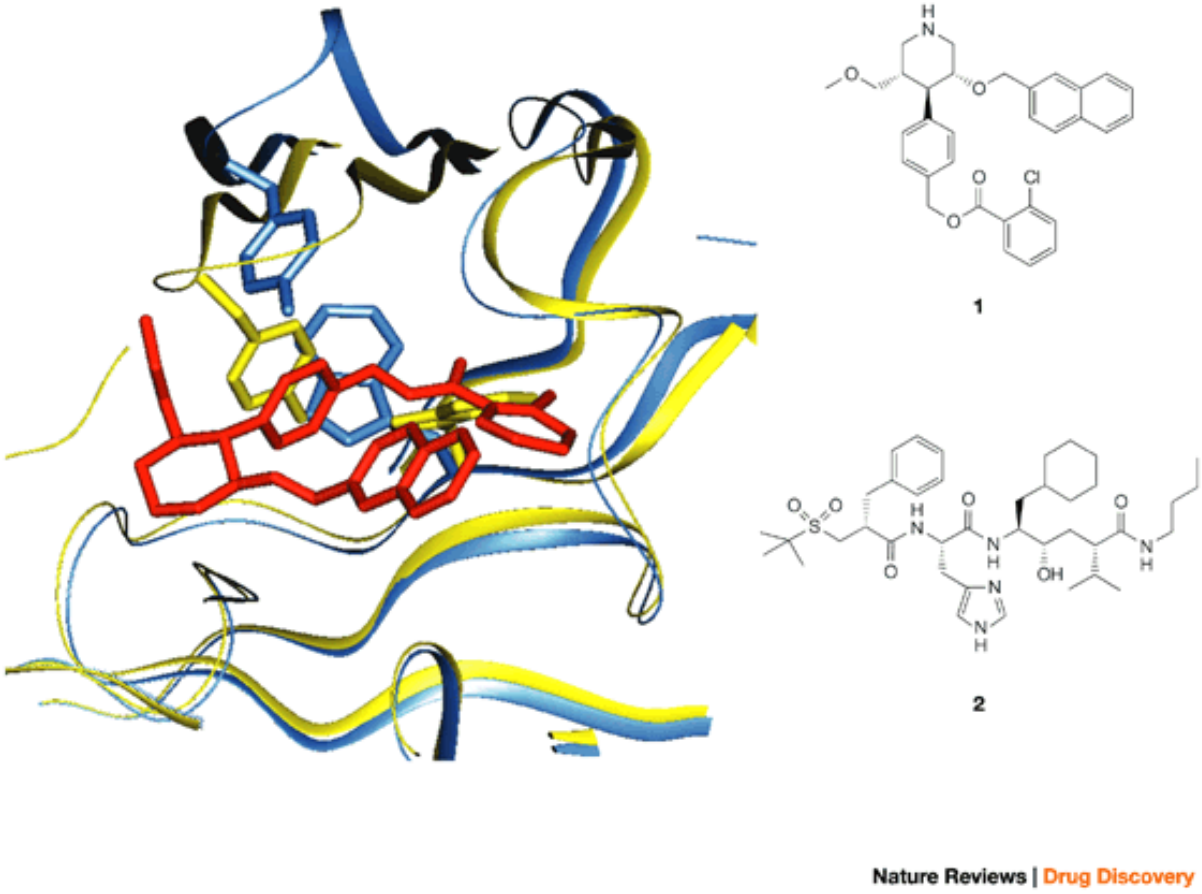


Figure 2 | **Novel conformation of renin observed on binding a non-peptidomimetic ligand.** The complex between renin (blue) and compound (1) in red overlaid with renin (yellow) bound to a transition-state analogue inhibitor (2). For clarity, (2) has been hidden and only those residues within a 10 Å sphere around the active site are displayed. The side chains for residues Trp39 and Tyr75 from each complex are displayed as thick lines in blue for the complex with (1) and yellow for the complex with (2). The differences in the positions are particularly striking, but many other changes in the conformation of the protein are also required to accommodate (1). The changes can be likened to a local refolding of the protein. (Protein Data Bank code 1RNE for the renin–(2) complex; the coordinates for the complex with compound (1) were kindly provided by Dr H.-P. Märki, F. Hoffmann-La Roche Ltd). (*Nature Reviews Drug Discovery* **2**, 527-541 (2003))

forces involved in the binding of the ligand to the receptor

All types of intermolecular forces can be involved in the binding between a drug molecule and its receptor, for example:

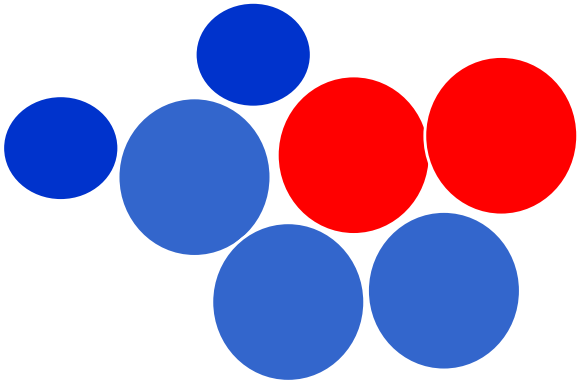
	interaction energy
• van der Waals forces	0.05-1 kJ/mol (per atom pair)
• Ionic interactions	20-500 kJ/mol
• Hydrogen bonding	5-25 kJ/mol
• Covalent bonding	150-500 kJ/mol

All these forces act only on very short distances

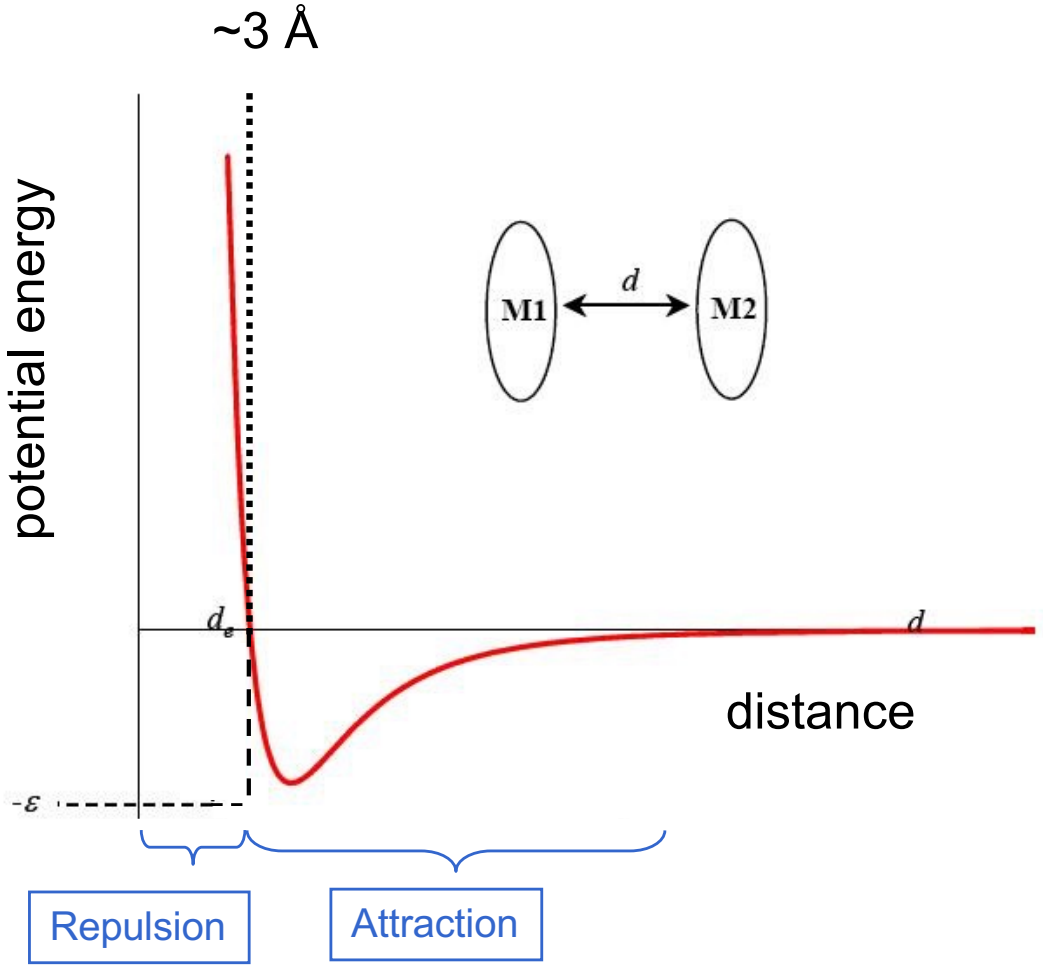
forces involved in the binding of the ligand to the receptor (2)

van der Waals forces

- Direct atom – atom contact, which depends essentially on the geometry of the molecules
- Very short distance of action
- Important for ligand dissociation
- Most important type of interaction for lipophilic molecules, e.g. side chains of lipophilic amino acids



distance dependence of van der Waals forces (2a)



At distances of ~3-8 Å these interactions are attractive (*due to transient dipole formation= London dispersion forces)* and the interaction energy is proportional to $1/d^6$; at distances < 3 Å they are repulsive.

forces involved in the binding of the ligand to the receptor (3)

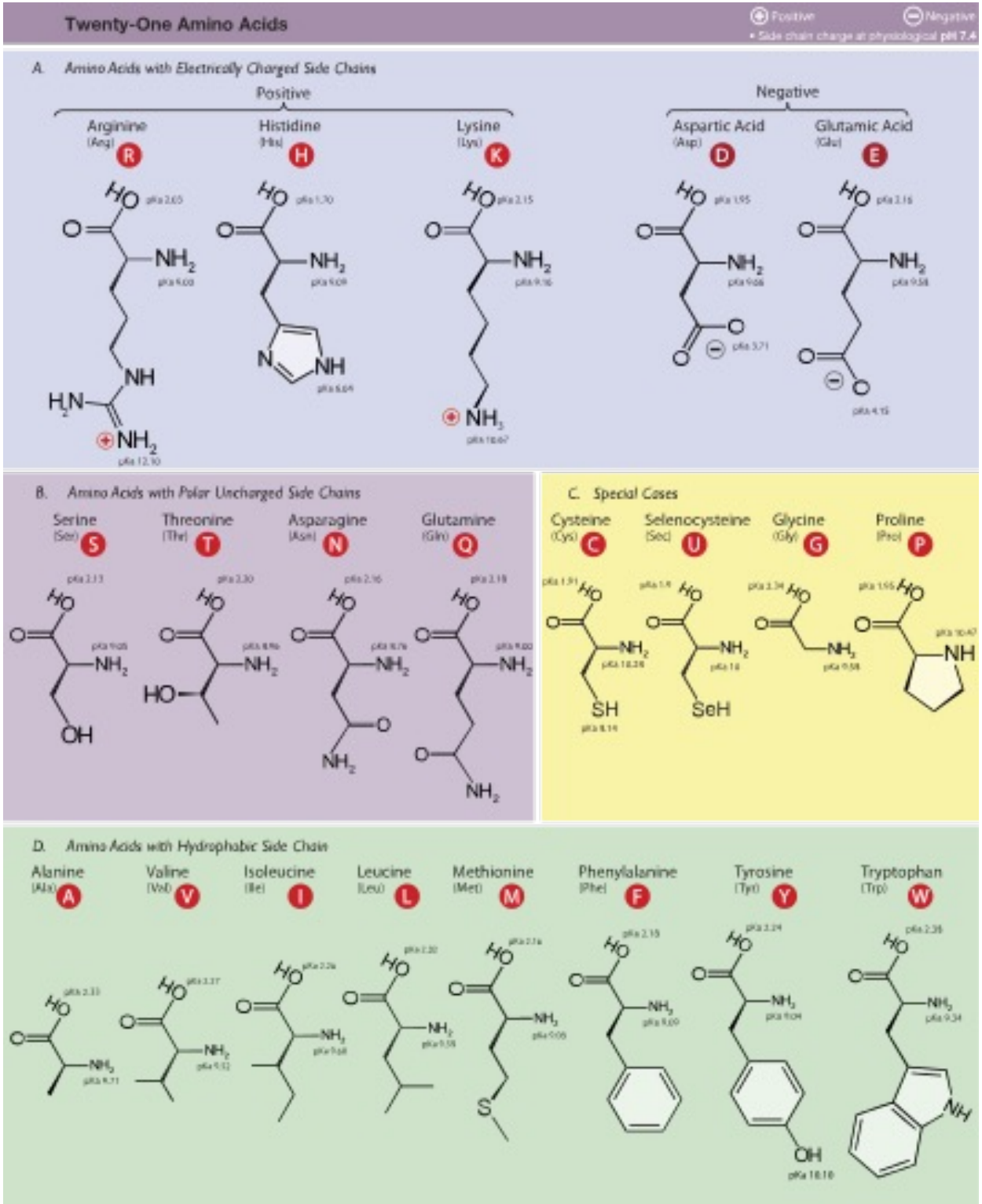
Ionic interactions

Occur between atoms of opposite charges

- act on longer distances and are stronger than van der Waals and hydrogen bonds *(the interaction energy is proportional to 1/distance between the charges)*
- They can direct the ligand – receptor encounter by orienting the ligand relative to the binding site, thereby influencing the association

Within the receptor protein

- side chains of charged amino acids (R, K, E, D, H)
- Partial charge of the carbonyl (=O) of peptide bonds
- π electrons of aromatic amino acids
- Polarization induced by α helices



forces involved in the binding of the ligand to the receptor (4)

Hydrogen bonds R – C=O···H – NH – X

- Of the same nature as hydrogen bonds within a protein
- Distance between acceptor and donor (N, O): 2.7 -3.1 Å
- it is a dipole-dipole type interaction, depending on $1/d^6$

Covalent bonds

for example R – S=O . . . H – S – X R – S=S – X

- Generally practically irreversible

Generally, binding occurs in two phases:

- First, rapid phase: rough orientation of the ligand within its receptor site
- Second, slow phase : binding by the short-range interactions described above

Illustration of interactions of the ligand-receptor complex

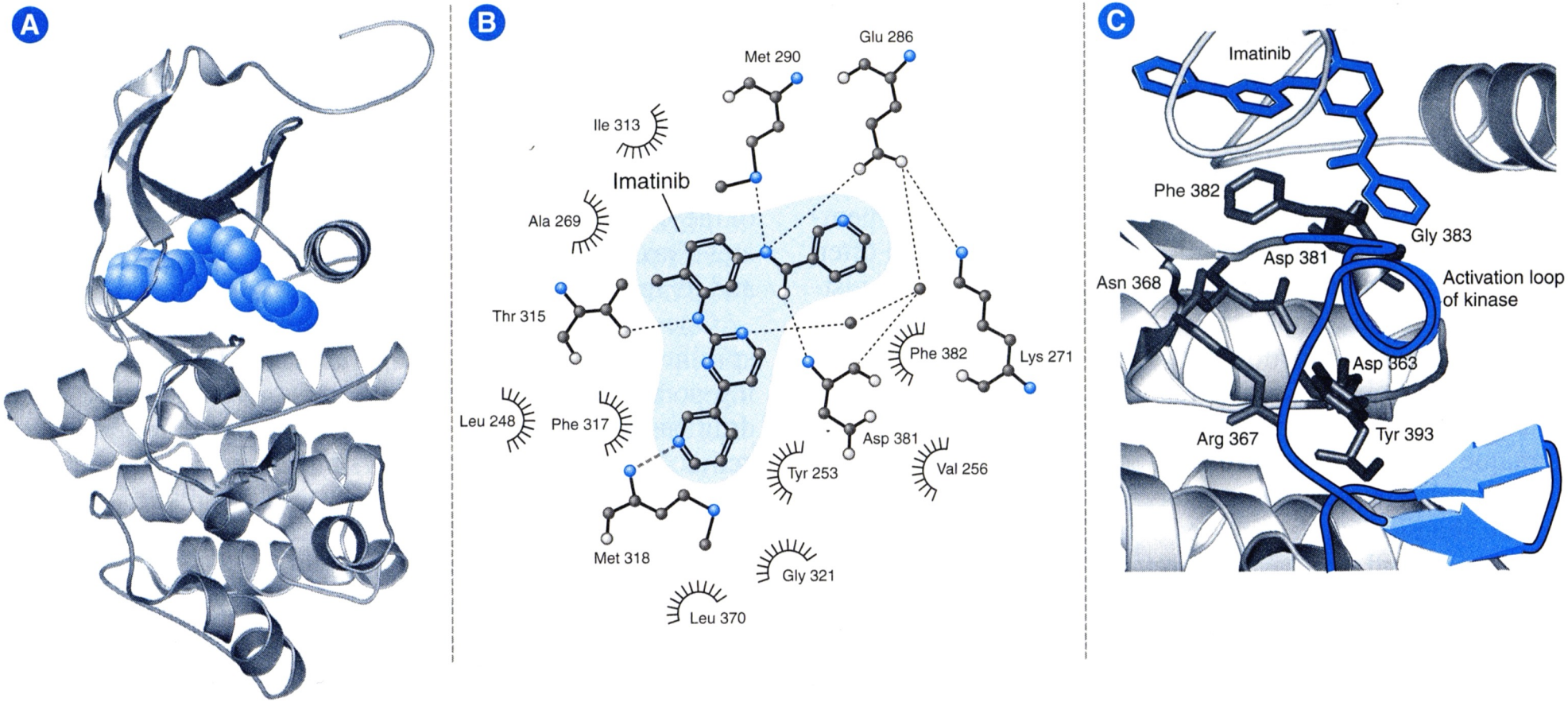


Figure 1-2. Structural Basis of Specific Enzyme Inhibition: Imatinib Interaction with the BCR-Abl Kinase. **A.** The kinase portion of the BCR-Abl tyrosine kinase is shown in a ribbon format (gray). Imatinib, a specific inhibitor of the BCR-Abl tyrosine kinase, is shown as a space-filling model (blue). **B.** Detailed diagram of the intermolecular interactions between imatinib and amino acid residues in the BCR-Abl protein. Hydrogen bonds are indicated by dashed lines, while van der Waals interactions (indicated by halos around the amino acid name and its position in the protein sequence) are shown for nine amino acids with hydrophobic side chains. **C.** The interaction of imatinib (blue) with the BCR-Abl protein (gray) inhibits phosphorylation of a critical activation loop (shown here in a blue-highlighted ribbon format), thus preventing catalytic activity.

Part III: Quantitative Description of ligand – receptor binding

aim :

- Determination of the ***affinity*** (**dissociation constant Kd**):
  - *Which concentration is required to occupy the sites and have an effect?*

- Determination of the ***kinetics*** of binding
  - *How much time is needed to occupy the receptors?*
  - *How long do the receptors stay occupied?*

Quantitative description of ligand binding: basic scheme (1)

Situation: imagine an experiment with a tissue, e.g. a cardiac muscle, containing a total number of receptors N_{tot} for a specific ligand, e.g. adrenalin. If the tissue is exposed to adrenalin at a concentration x_A until an equilibrium is reached, a certain number of receptors, N_A, will be occupied and the number of vacant receptors will be reduced to N_{tot}-N_A. Normally the number of ligand molecules is much higher than the number of receptors. For this reason, binding will not appreciably decrease the concentration x_A. The magnitude of the response produced by adrenalin is related to the number of receptors occupied, so it is useful to consider what quantitative relationship is predicted between N_A et x_A. The reaction can be represented by:

$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} LR$$

L		R		LR
ligand (drug)		*free receptor*	k_{off}	*ligand-receptor complex*
x_A		$(N_{tot} - N_A)$		(N_A)

The Law of Mass Action (which states that the rate of a chemical reaction is proportional to the product of the concentrations of reactants) can be applied to this reaction.

Rate of forward reaction = $k_{on} \cdot x_A \cdot (N_{tot} - N_A)$ (1)

Rate of backward reaction = $k_{off} \cdot N_A$ (2)

Quantitative description of ligand binding: equilibrium binding

At equilibrium, the two rates are equal: :

$$k_{on} \cdot x_A \cdot (N_{tot} - N_A) = k_{off} \cdot N_A \qquad (3)$$

The number of occupied receptors, N_A is:

$$N_A = \frac{N_{tot} \cdot x_A}{x_A + k_{off} / k_{on}} \qquad (4)$$

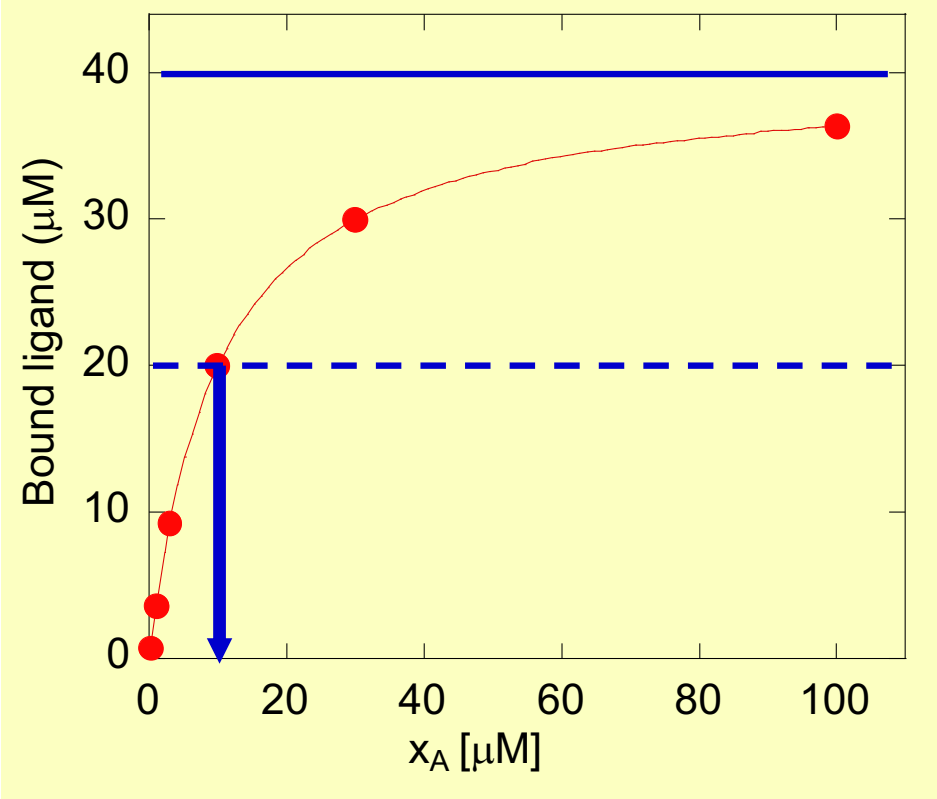
Based on the definition of the **dissociation constant $K_d = k_{off} / k_{on}$** , (5)
the equation can be written as:

$$N_A = \frac{N_{tot} \cdot x_A/K_d}{x_A/K_d + 1} = \frac{N_{tot}}{1 + K_d/x_A} \qquad (6)$$ (Hill-Langmuir equation)

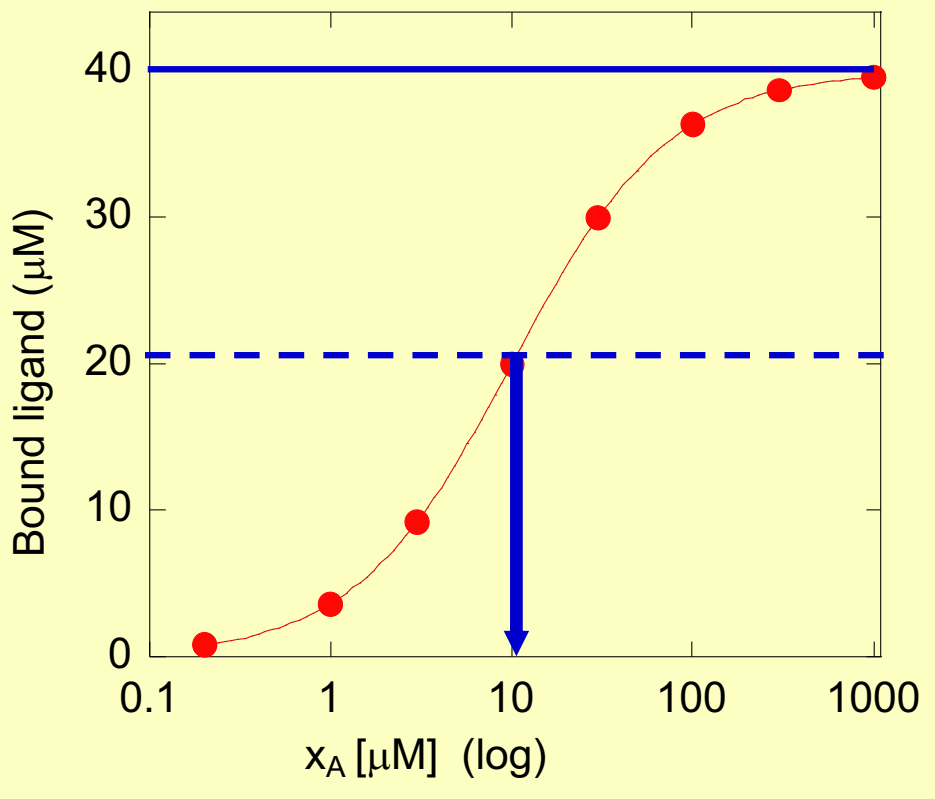
The **dissociation constant K_d** is a characteristic of the drug and of the receptor; it has the dimensions of concentration and is numerically equal to the concentration of drug required to occupy 50% of the sites at equilibrium. The K_d is thus a measure of the affinity between ligand and receptor. The higher the affinity of the drug for the receptors, the lower will be the value of K_d.

Curves of equilibrium binding

$$N_A = \frac{N_{tot}}{1 + K_d/x_A}$$



Linear scale



Logarithmic scale

For Kd = 10 μM and N_{tot} corresponding to 40 μM.

N_{tot} = total number of receptors, also called « N_{max} » or « B_{max} »

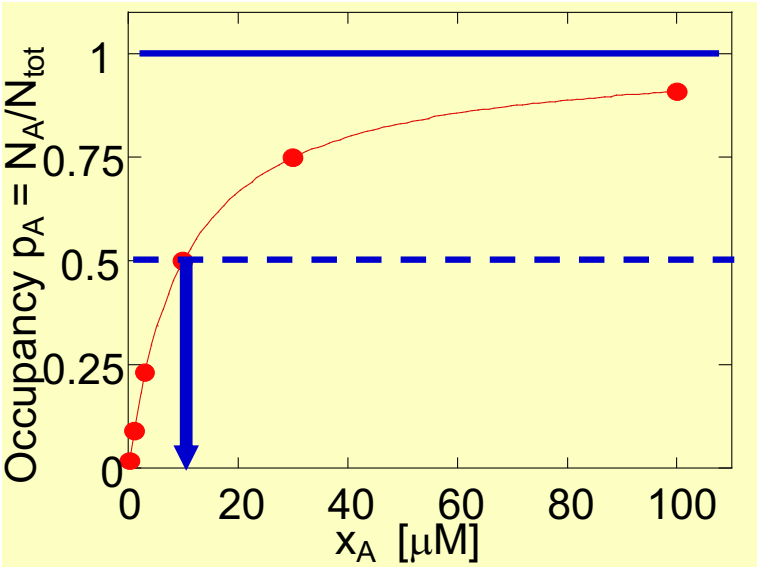
Receptor occupancy

The proportion of receptors occupied, or **occupancy** (*p*A), is equal to the number of receptors occupied by the ligand (N_A), divided by the total number of receptors (N_{tot}):

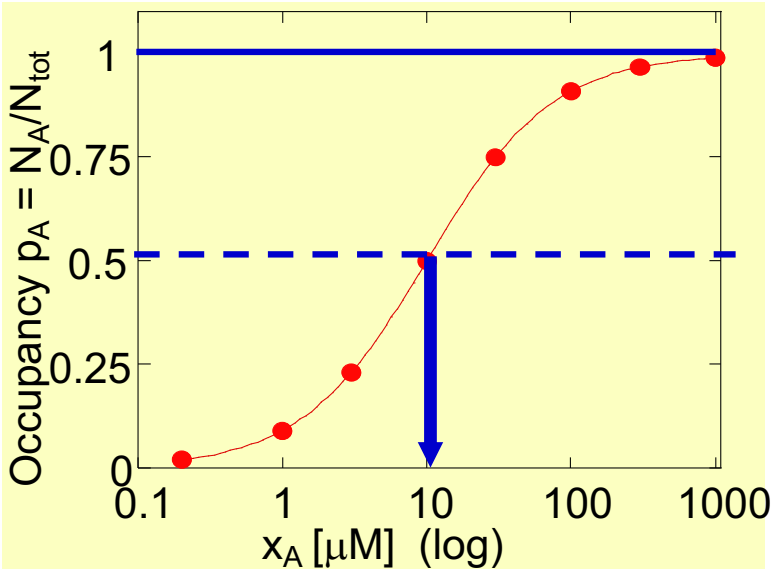
$$pA = N_A/N_{tot} \qquad (7)$$

The Hill-Langmuir equation (6), in the form normalized to N_{tot}, is:

$$pA = \frac{1}{1 + K_d/x_A} \qquad (8)$$



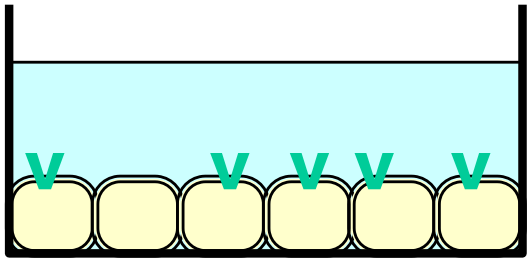
Linear scale



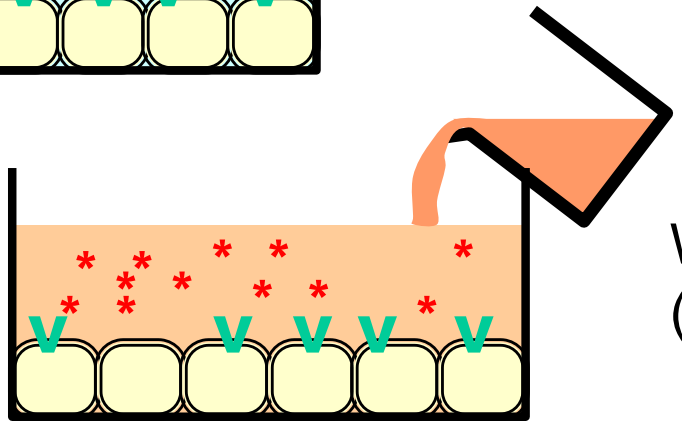
Logarithmic scale

Example with Kd = 10 μM

Measuring equilibrium binding

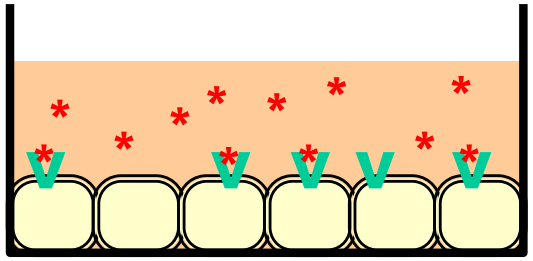


t = 0



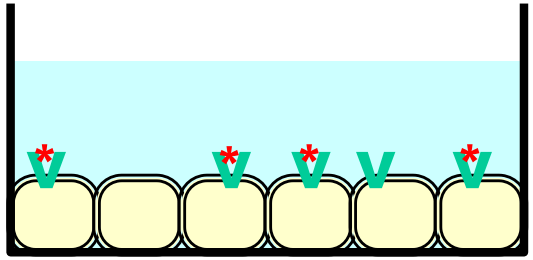
With different concentrations of (radio-labeled) ligand

Incubation time long enough to reach an equilibrium



elimination of the “hot” solution

for example by rapid rinsing



Elimination of the unbound “hot” (= labeled) ligand in a binding experiment

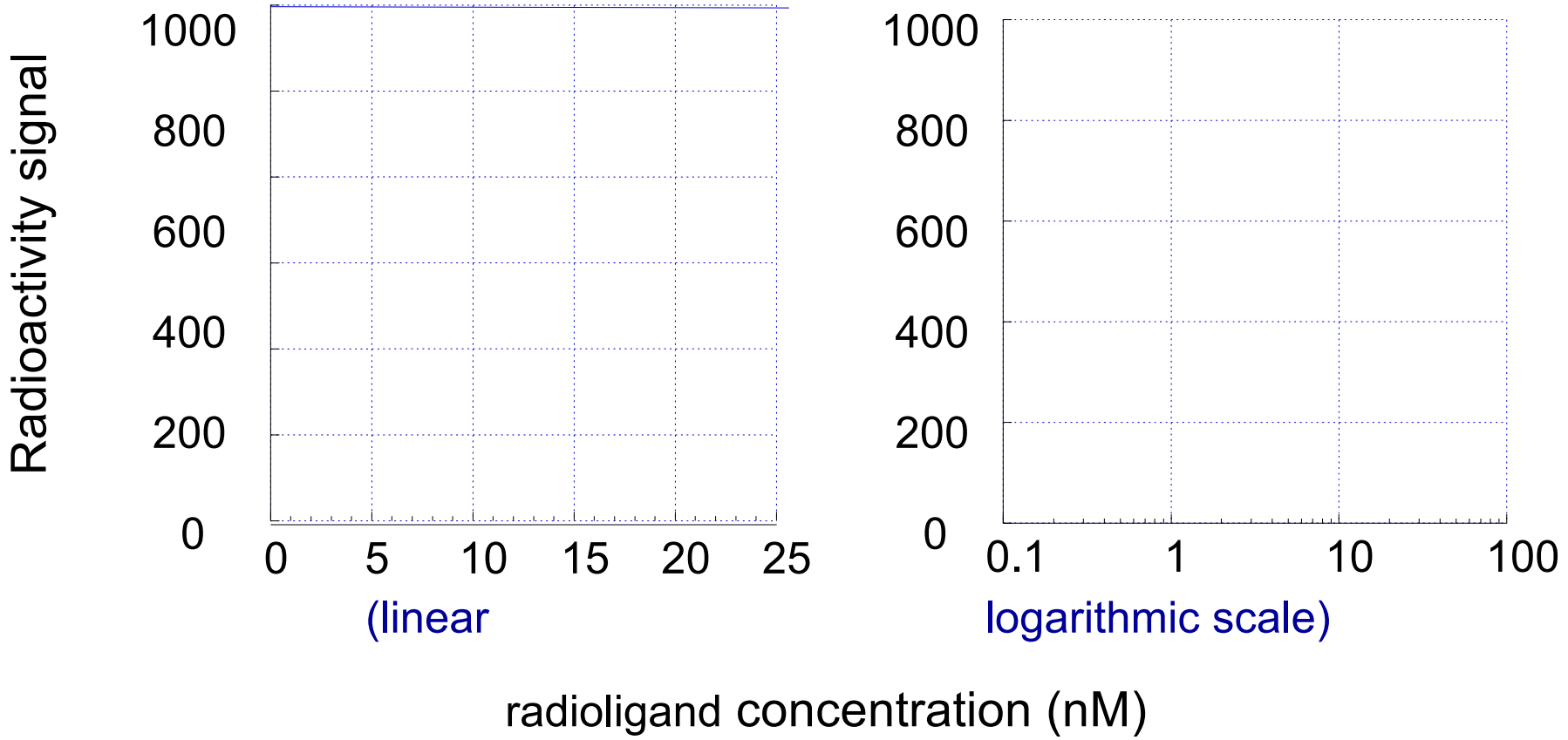
- By rinsing if the receptor is attached to a fixed support (e.g. cells in a petri dish)
- By filtration, if the receptors are attached to a particle (cell, vesicles, etc.)
- By fixing the ligand on an acceptor, for example activated charcoal

The elimination of the unbound hot ligand needs to be much faster than the dissociation of the ligand-receptor complex.

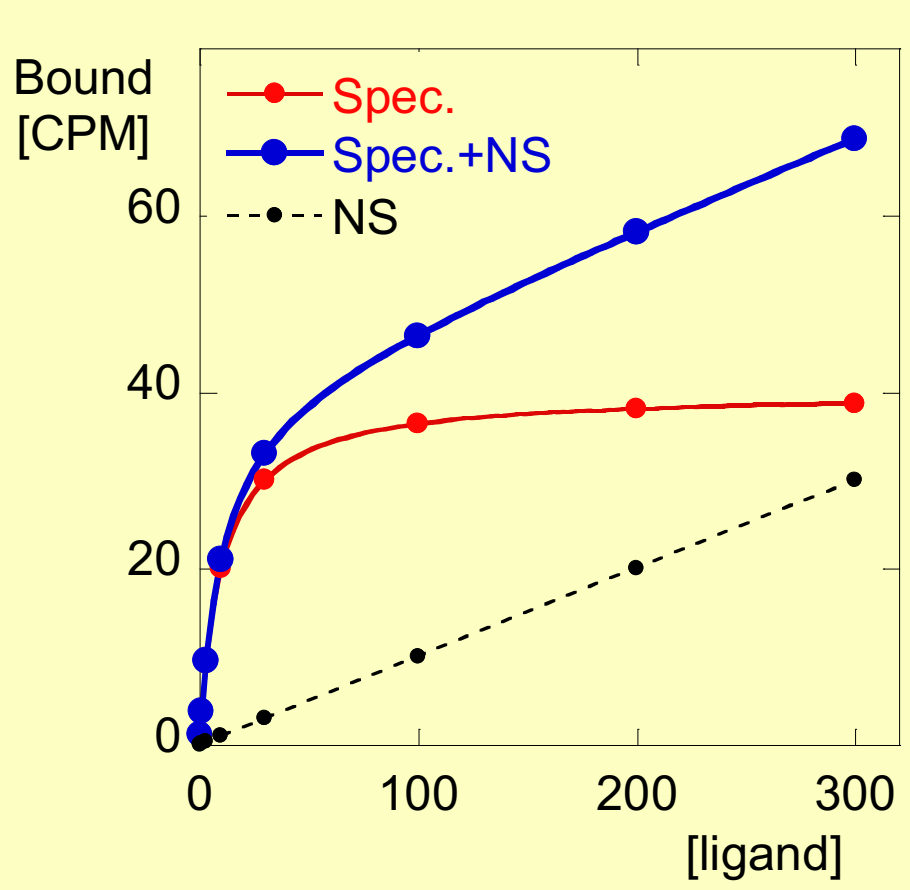
Analysis of the binding measurement

Radioligand: [3H]Ro-15-1788, ligand of GABAA receptors, binding to brain tissue

concentration radioligand (nM)	0.1	0.3	1	2	4	6	8	12	16	20
radioactivity bound (e.g. in cpm)	36.2	104	277	426	573	677	739	824	897	964



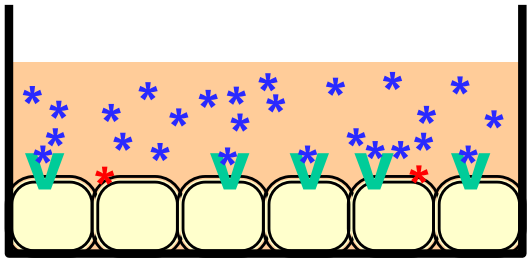
Theory and reality….. : specific and non-specific sites



non-specific sites: very low affinity, very high N

Measuring non-specific binding

- Repeat the binding measurement in the presence of an excess (e.g. a 100-fold excess) of “cold” (= non-radiolabeled) ligand. The cold ligand occupies 99% of the specific receptor sites, but binds only to a small fraction of the non-specific sites, due to their very high number and low affinity. Under this condition, the binding of the hot ligand represents essentially the non-specific sites
- The binding data are then fit with an equation that comprises a parameter for non-specific binding:

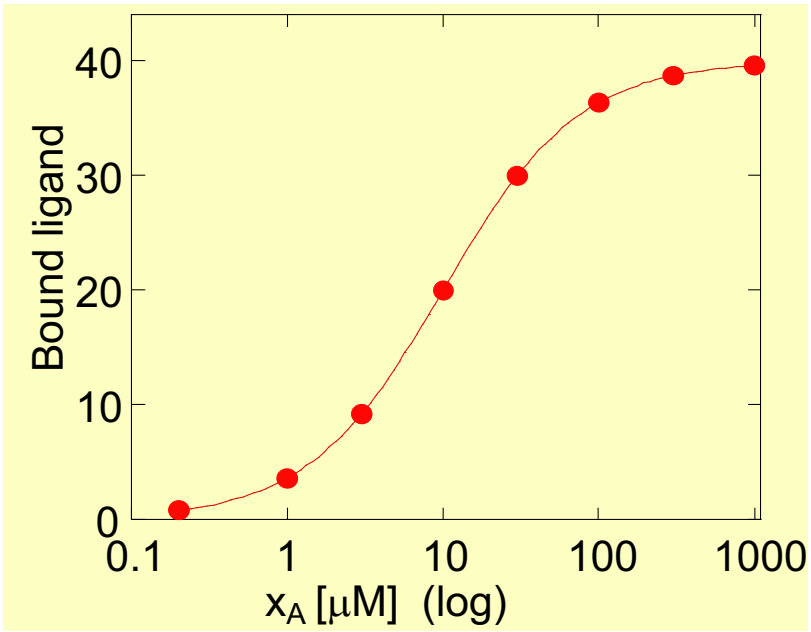


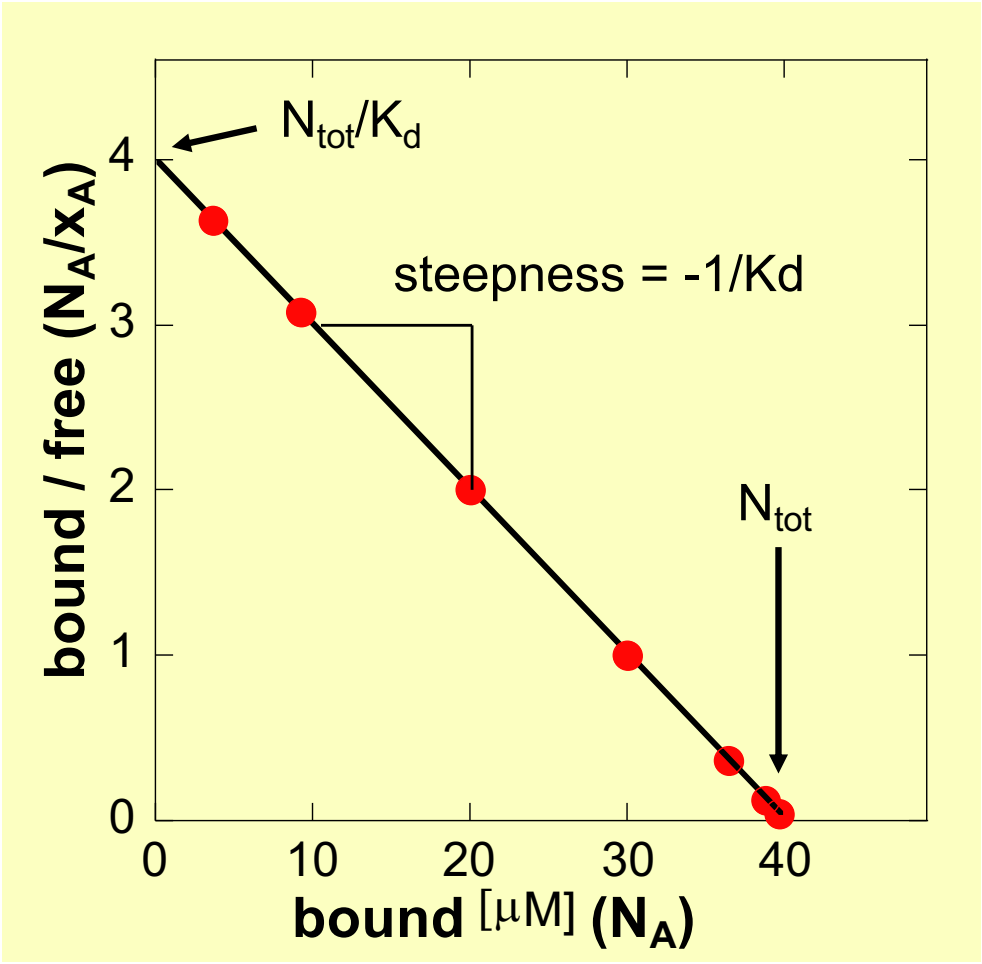
$$N_A = \frac{Ntot}{1 + Kd / x_A} + k_{ns} \cdot x_A \qquad (9)$$

Analysis of the binding measurement

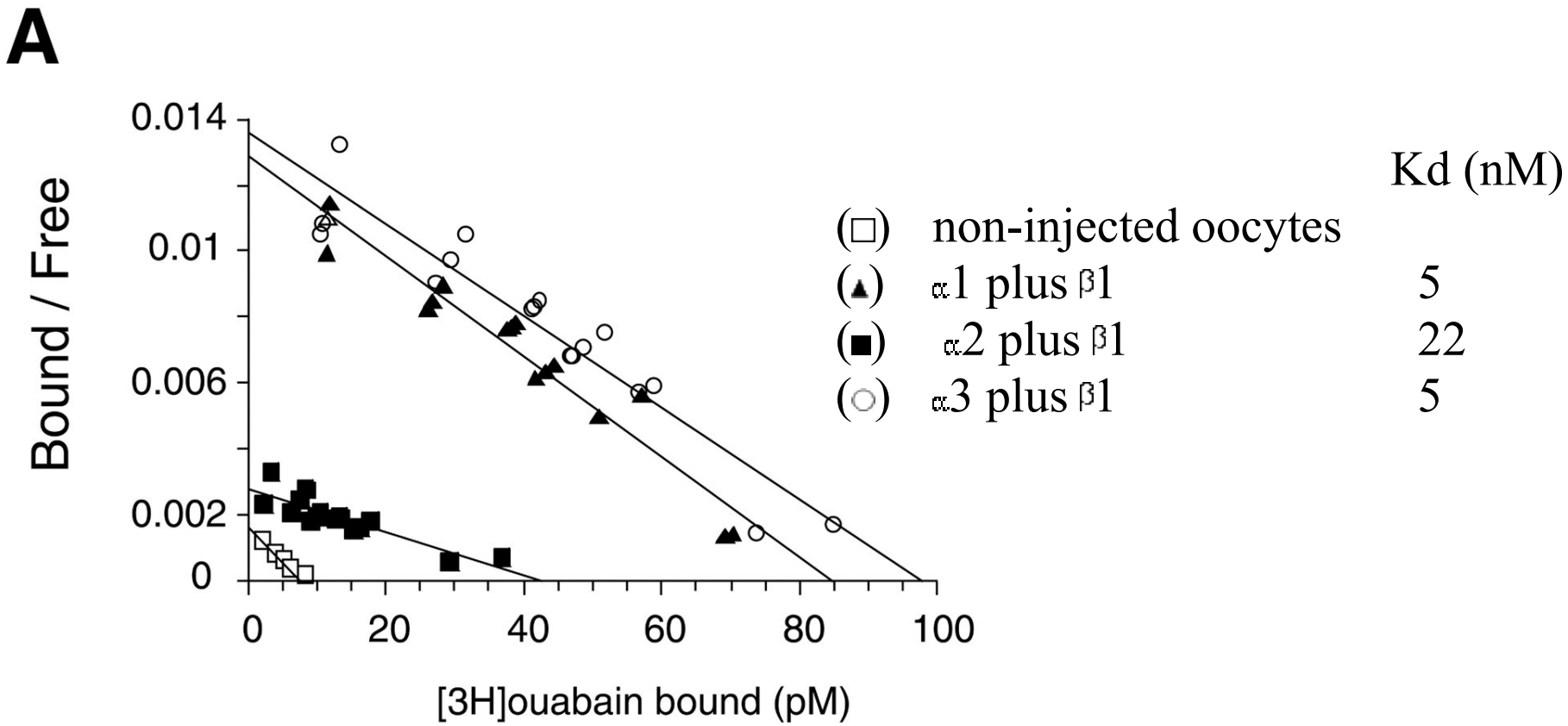
concentration radioligand (nM)	0.1	0.3	1	2	4	6	8	12	16	20
radioactivity bound (p.e. en cpm	36.2	104	277	426	573	677	739	824	897	964
non-specific binding	1.2	4	12	24	48	72	96	144	192	240
specific binding										

Binding: Scatchard plot



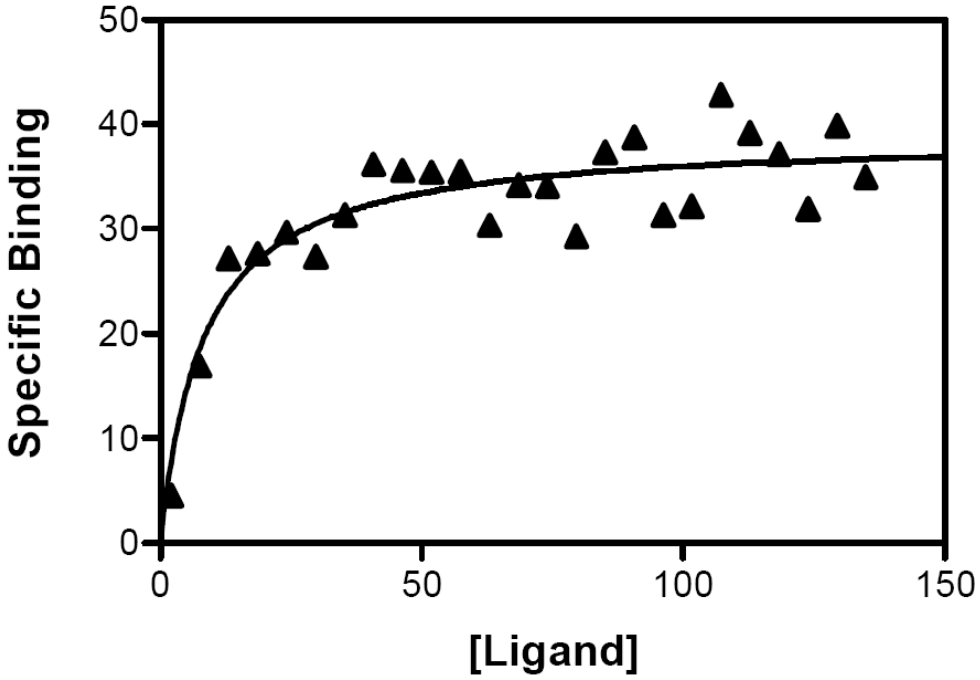


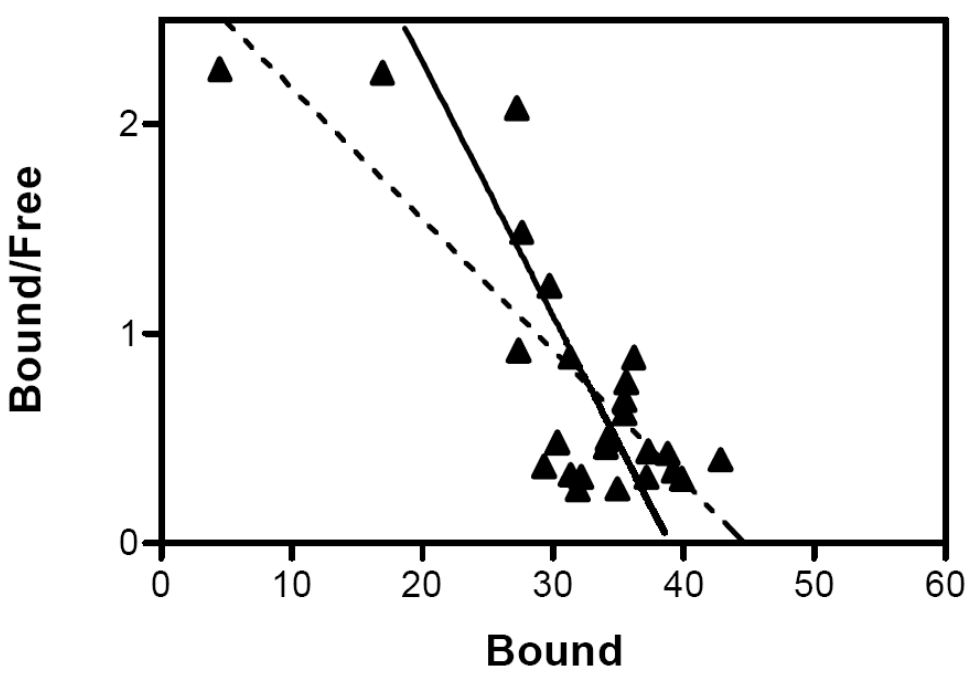
Example of a scatchard plot



Ouabain affinity of human Na,K-ATPase isozymes. Oocytes were not injected (*ni*) or injected with different α and β cRNAs as indicated. Three days later, microsomes were prepared as described under "Experimental Procedures." Ouabain binding measurements were carried out in Na-ATP conditions (see "Experimental Procedures") in the presence of 10^{-9} to 5×10^{-8} M [^{3}H]ouabain for 5 h either in the absence or presence of 5 mM K^+. *A*, representative Scatchard plots of ouabain binding data obtained with microsomes from non-injected oocytes (□) or from oocytes injected with α1 plus β1 (▲), α2 plus β1 (■), and α3 plus β1 (○) cRNAs in the absence of K^+. Data from equilibrium ouabain binding measurements obtained with microsomes from noninjected oocytes were subtracted from those obtained with microsomes from injected oocytes One out of four similar experiments is shown. (Crambert et al., JBC 275, pp1976)

Problems with scatchard plot



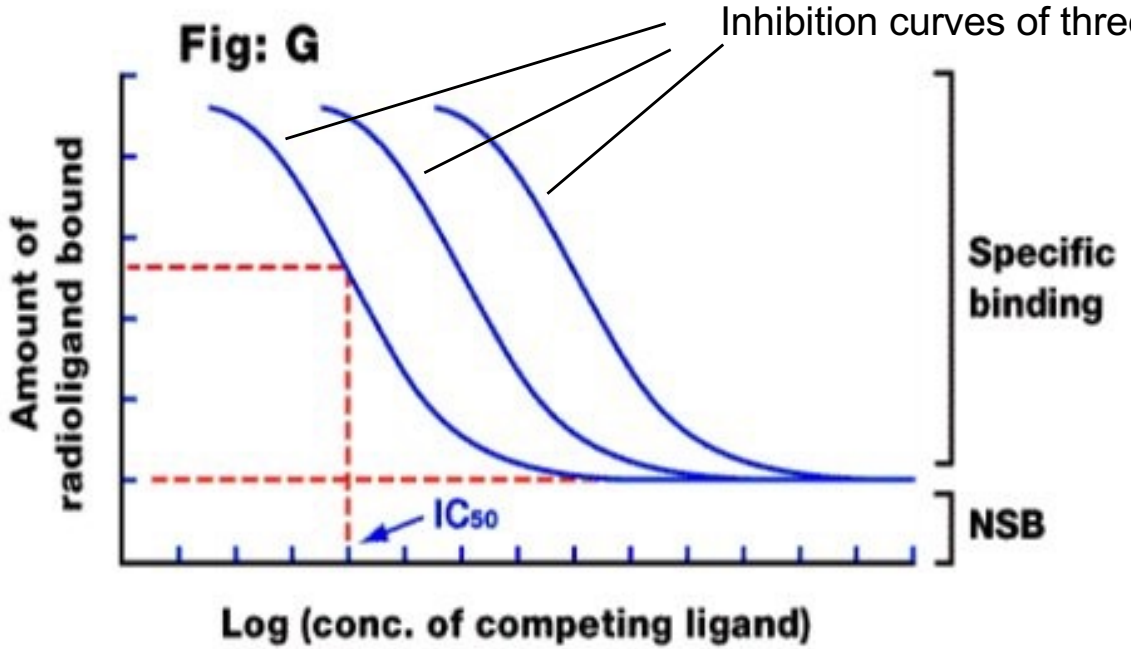


While Scatchard plots are useful for visualizing data, they are not the most accurate way to analyze data.

The problem is that the linear transformation distorts the experimental error. Linear regression assumes that the scatter of points around the line follows a Gaussian distribution and that the standard deviation is the same at every value of X. These assumptions are not true with the transformed data. A second problem is that the Scatchard transformation alters the relationship between X and Y. The value of X (bound) is used to calculate Y (bound/free), and this violates the assumptions of linear regression.

Competition binding assay

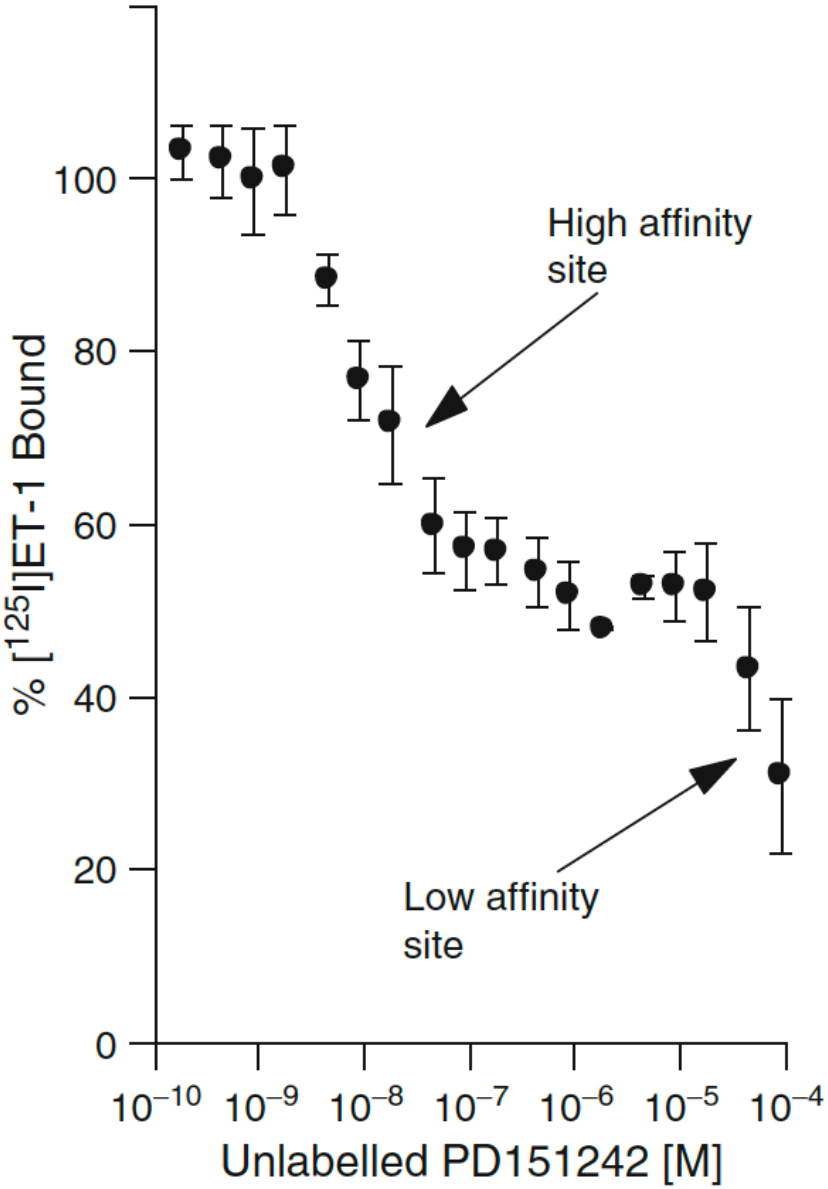
Receptor binding techniques: competition (inhibition or displacement) assays



In this type of assay, a single concentration of radioligand is used in every assay tube. The ligand is used at a low concentration, usually at or below its KD value. The level of specific binding of the radioligand is then determined in the presence of a range of concentrations of other competing non-radioactive compounds, in order to measure the potency with which they compete for the binding of the radioligand.

(Figure from the Glaxo Wellcome Pharmacology guide)

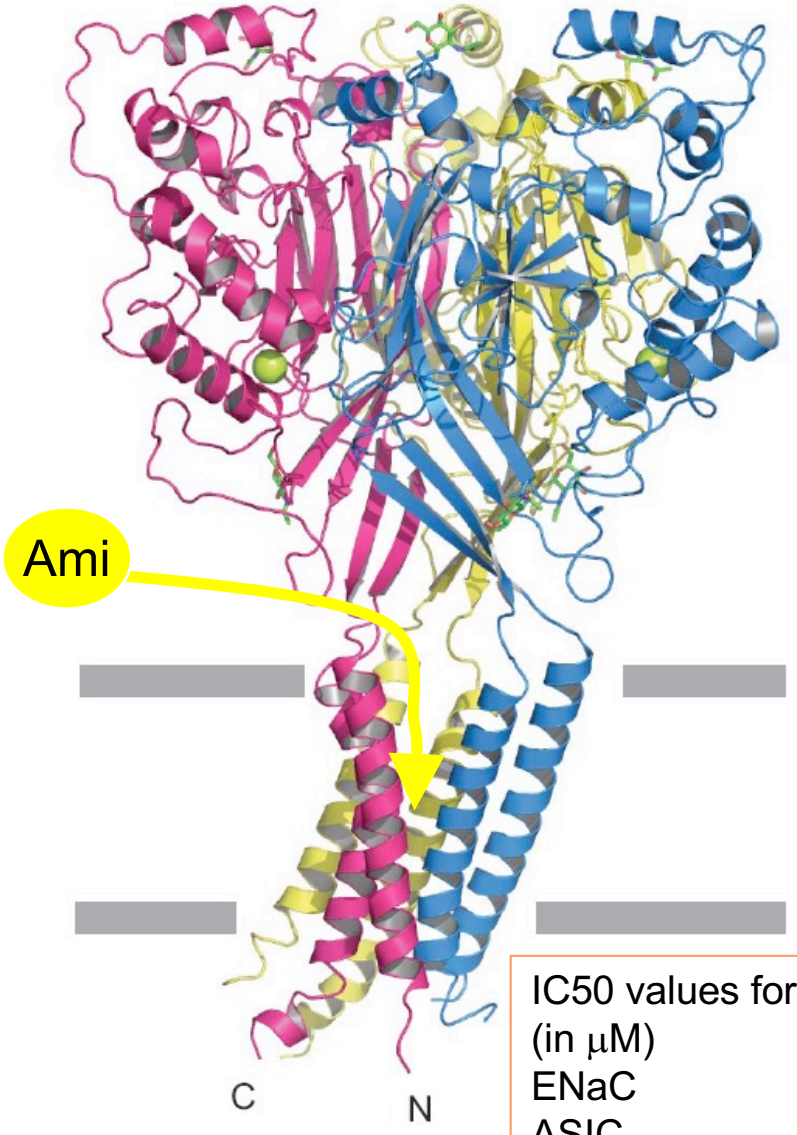
Example competition binding

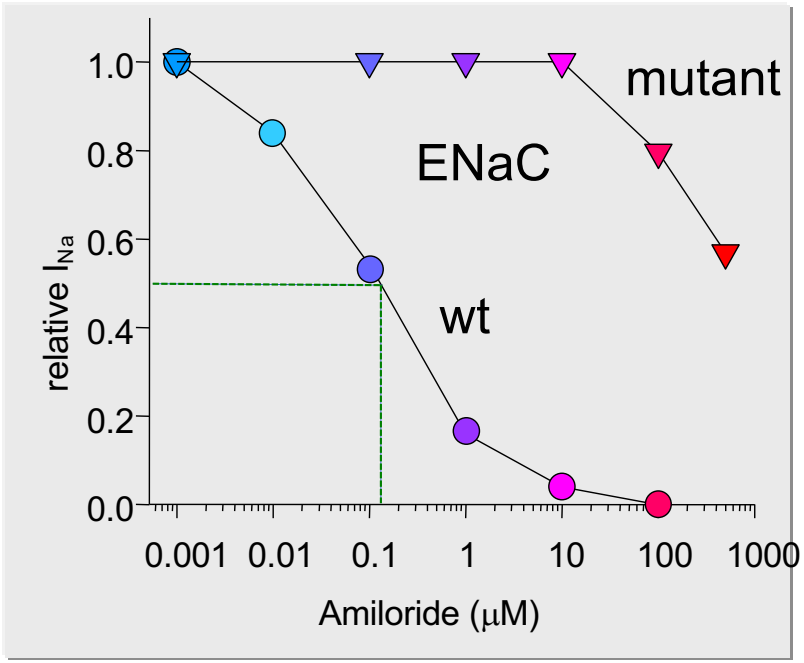


Tissue: Human heart ventricle
Radioactive ligand: [125 I]-Endothelin-1 (which binds to both Endothelin-A and –B receptor subtypes with the same affinity)
Unlabeled ligand: PD151242
In the left ventricle of the human heart the two receptor types are present in a ratio of about 60% ET_A : 40% ET_B

Fig. 4. Competition binding curve for the inhibition of a fixed concentration of [^{125}I]-ET-1 (0.1 nM) binding to ET receptors by increasing concentrations of unlabeled PD151242 in sections of human ventricle. Over the concentration range tested, PD151242 competed in a biphasic manner and a two-site fit was preferred to a one-site or three-site model using LIGAND. The high-affinity site corresponded to the endothelin ET_A receptor ($K_D = 7.2 + 2.8$ nM), the low-affinity site to the ET_B receptor, $K_D = 104 + 23$ µM. Each value represents the mean ± s.e.m. of three individuals.

Example: Selectivity of a ligand is relative





IC50 values for amiloride inhibition (in μM)	
ENaC	0.1
ASIC	10-200
Na+/H+ antiporter	80
Cav, T-type	30