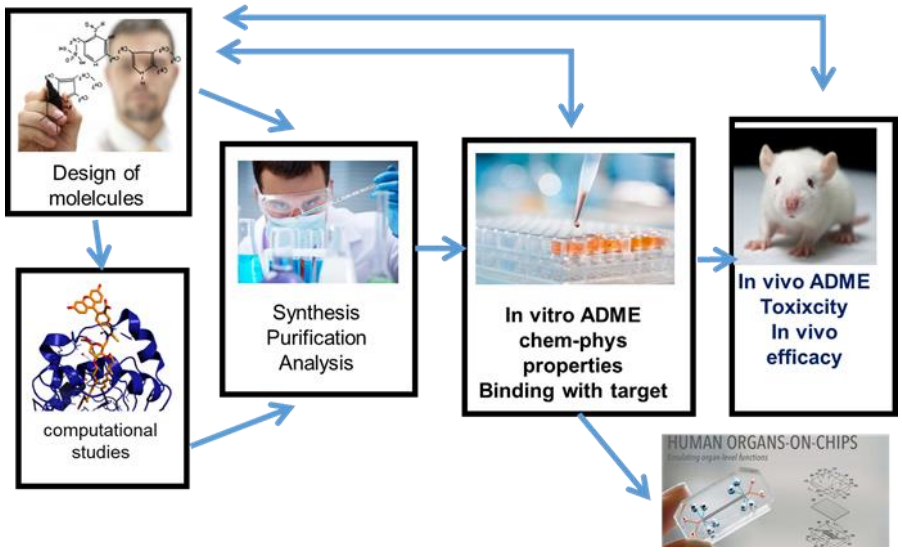
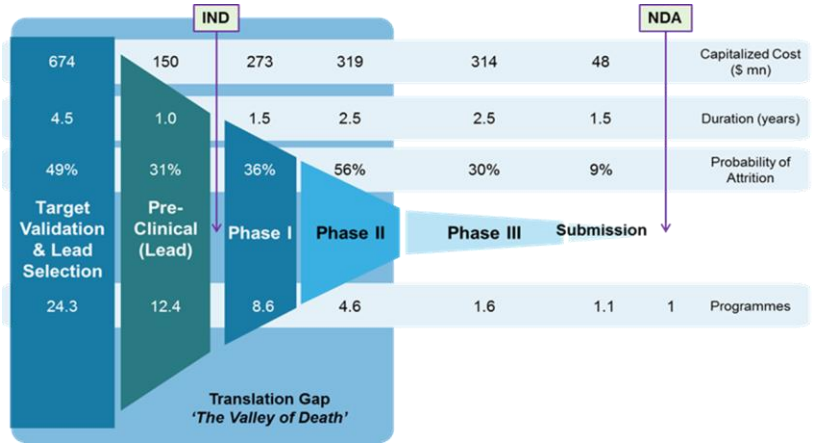
Medicinal Chemistry (6 cfu)
AA 2022-2023

Francesco Peri, Francesca Magli

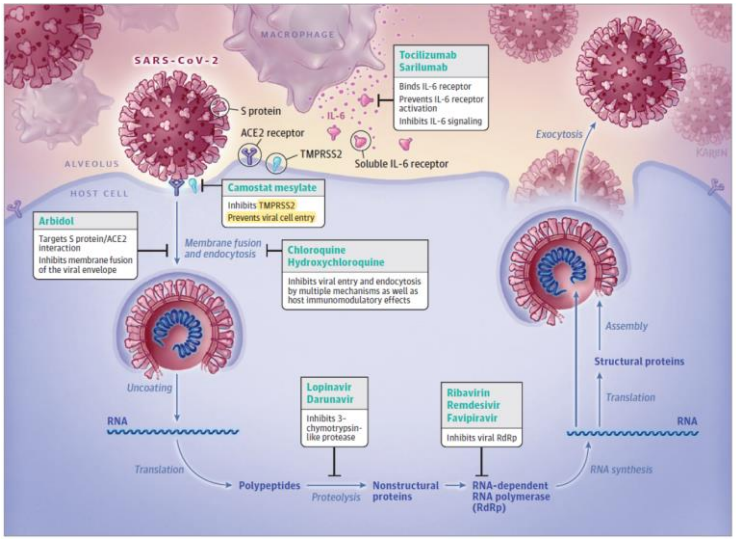


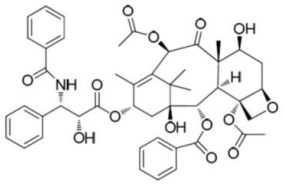


Medicinal Chemistry
AA 2022-2023
Prof. Francesco Peri
Prof. Francesca Magli

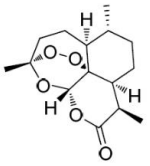
- 6 credits: 42 hours, 21 lessons
- **F. Peri: 35 hours, 17 lessons**
- **Ottobre: 6, 11, 13, 18, 20, 25, 27**
- **Novembre: 3, 8, 10, 15, 17, 22, 24**
- **F. Magli: 7 hours, 4 lessons**
- **13, 20, 22 Dicembre, 10 Gennaio 2023**
- **Esposizioni in classe: 29 Nov, 1, 6, 15 Dic**
- 1 cfu mondo del lavoro, Datwiler, case study + visita in azienda (Gennaio)
- 13 Gennaio, 11 Febbraio
- Oral Exam + personal work on a drug or a family of drugs + economic aspects of supply chain (production, logistic, distribution, marketing)
- Lessons in presence + streaming + recorded

«Storie di farmaci importanti» e relazioni personali su un farmaco o una classe di farmaci…con un occhio speciale agli antivirali in sperimentazione sul SARS-CoV2, ai farmaci da sostanze naturali ed ai farmaci biologici

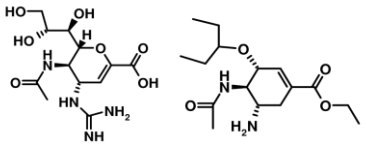




Taxol

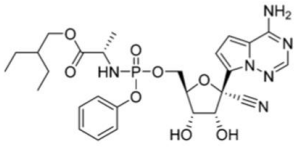


Artemisinin



Zanamavir

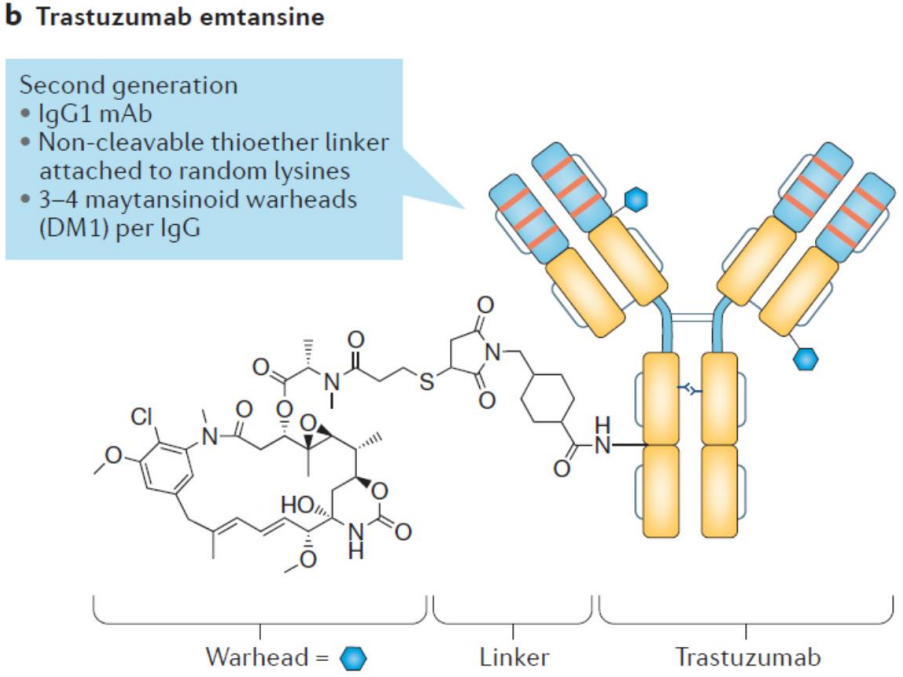
Oseltamivir



4a *Sp* and *Rp* isomers (~1:1)

Anti-influenzali

Remdesivir (approvato SARS-Cov»)



Programma da syllabus

Argomenti:

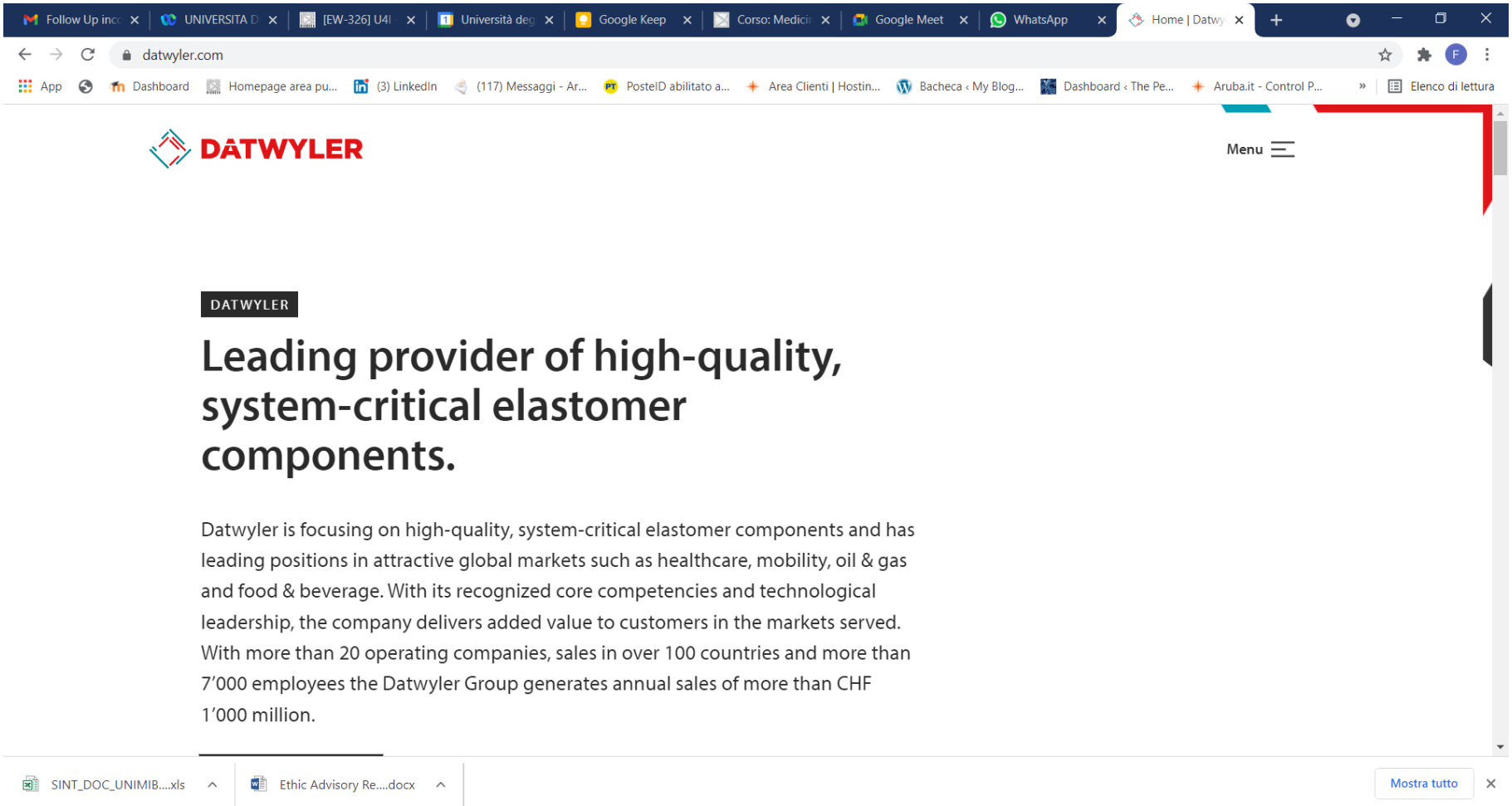
- 1 Rational Drug Design: progettazione di un farmaco, ligand- and structure-based drug design
- 2 Farmacocinetica e farmacodinamica
- 3 I trasportatori ed il rilascio: i nanocarriers e la terapia tumorale
- 4 Strategie per lo sviluppo di un farmaco, da hit a lead, analisi quantitativa della relazione struttura-attività
- 5 Farmaci da composti naturali, farmaci biologici, ADC (antibody-drug-conjugate)
- 6 Esempi di “storie di successo” di farmaci: antitumorali, antivirali, antibiotici, farmaci sul SNC, e tanto altro…

Supply chain del farmaco (Prof. F. Magli)

Al termine di questa attività formativa, lo studente dovrà dimostrare di essere in grado di:

- 1 Disegnare un ligando di un target farmacologico
- 2 Immaginare le fasi di sviluppo di un nuovo farmaco, inclusi gli aspetti economico-logisitico-gestionali
- 3 Immaginare come espandere una famiglia nota di farmaci congenerici (con la stessa azione e stesso target)

1 cfu mondo del lavoro



Contents

Contents

Pharmacokinetics (LADMET)

Liberation (prodrugs and drug delivery by nano-carriers)

Absorption, Distribution, Metabolism type I and II, Toxicity, covalent drugs

Some drug families:

Antibiotics

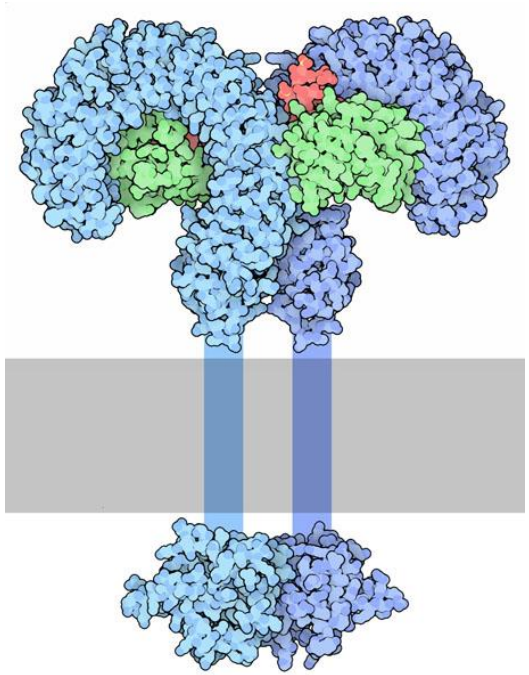
Antivirals

Antitumorals

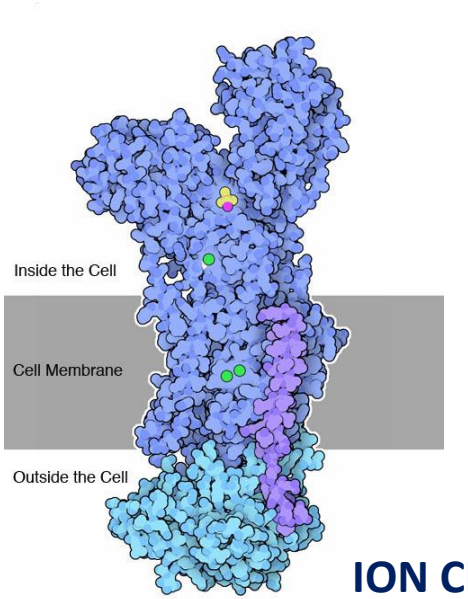
Some questions to start.....

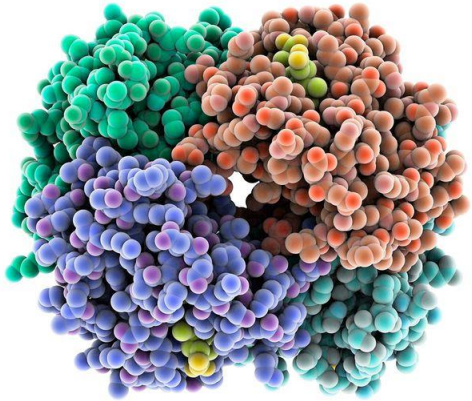
- PROTEINS, DNA, RNA (biopolymers) are the major targets of drugs:
- What is your knowledge on biopolymers?
- **In this course the focus will be on small organic molecules (drugs), whose pharmacological action is explained in terms of interaction with target proteins. Something on antibody-drug conjugates and biological drugs (proteins).**

Type of drug targets



RECEPTORS (50%)





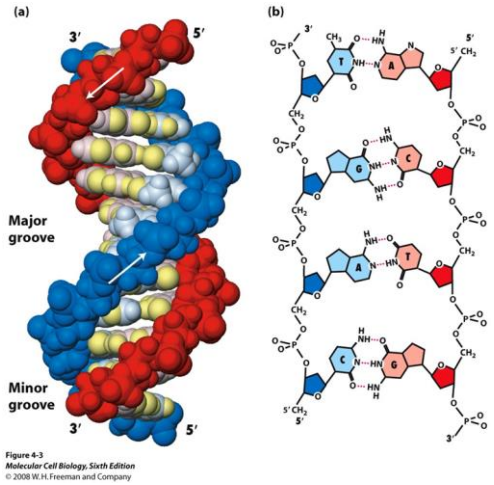
ENZYMES (30%)

ION CHANNELS (5%)

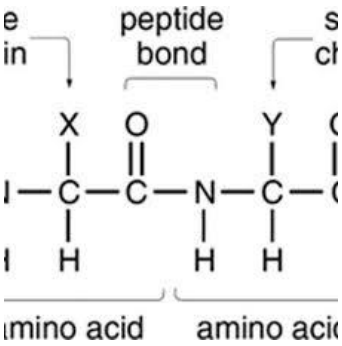
PROTEINS

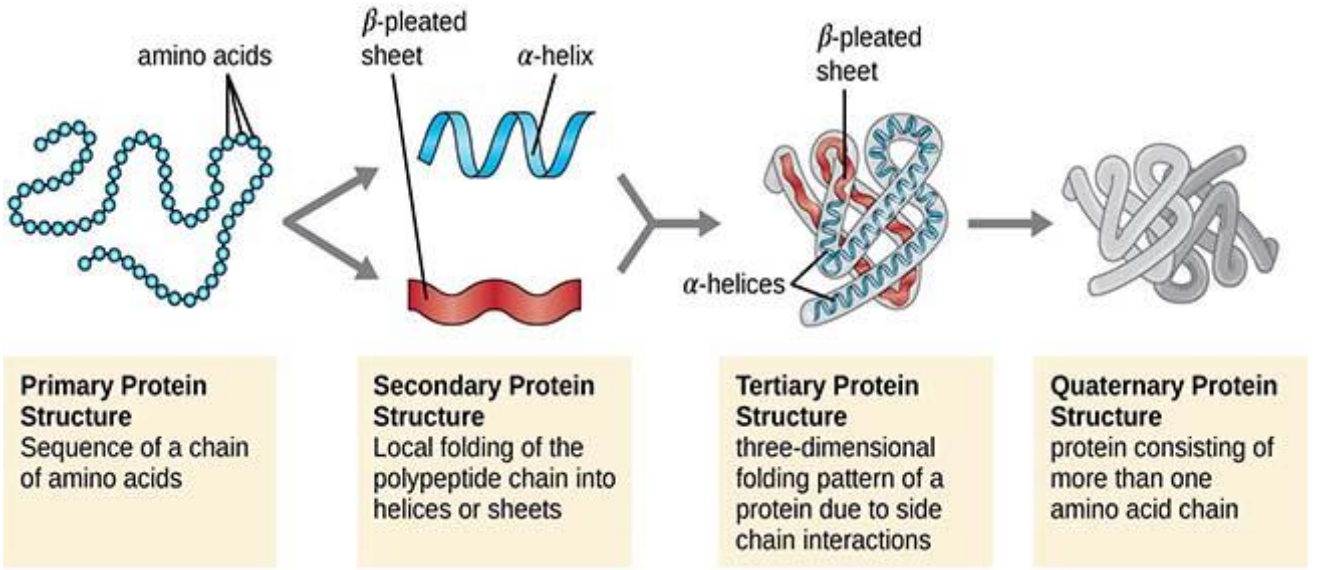
HORMONES/TRANSMITTERS 10%

NUCLEIC ACIDS (5%)

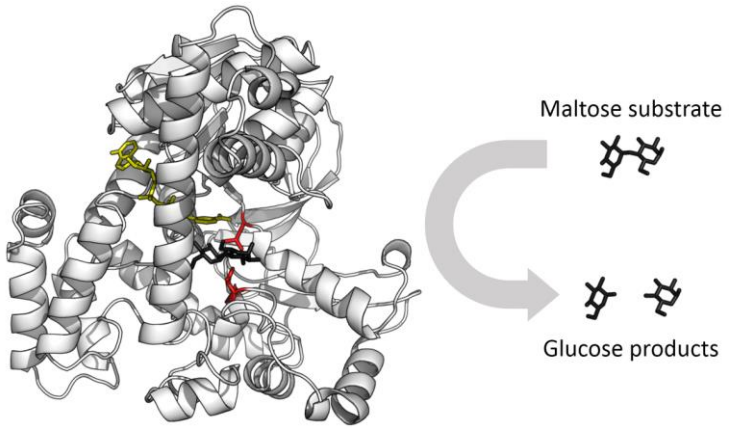


Structure of Proteins





ENZYMES



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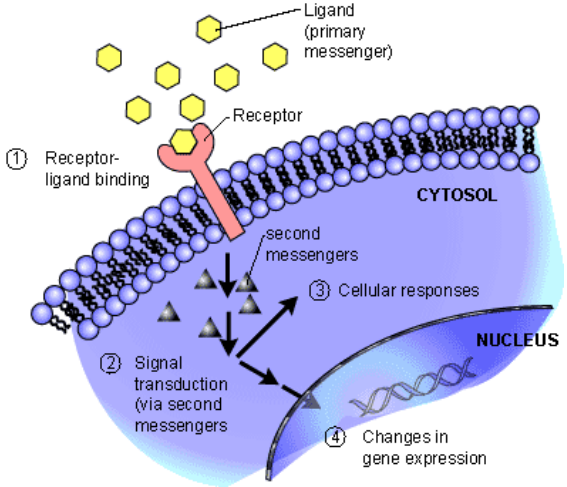
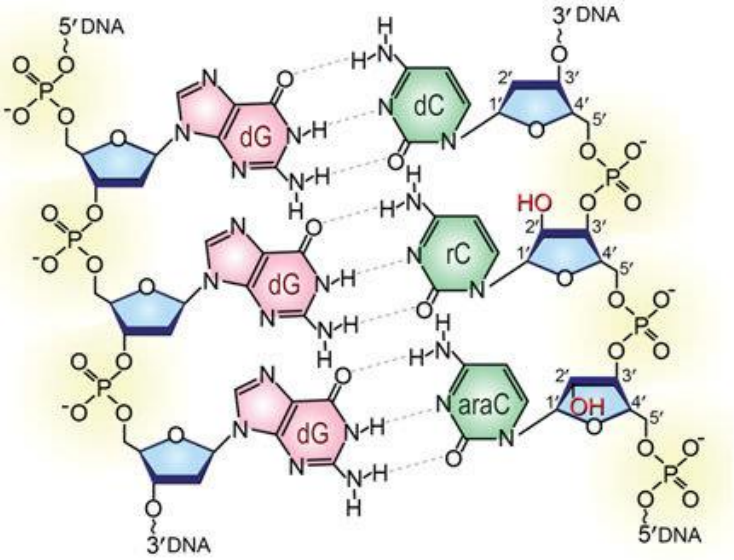
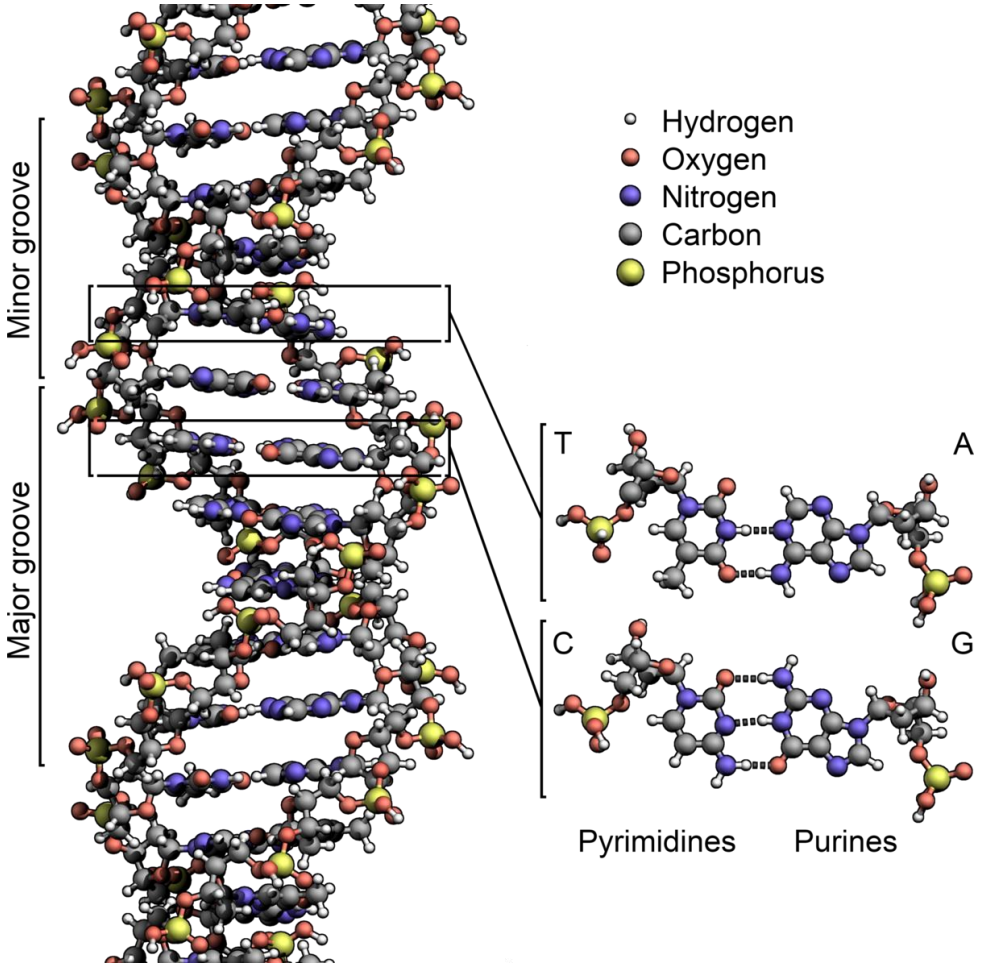
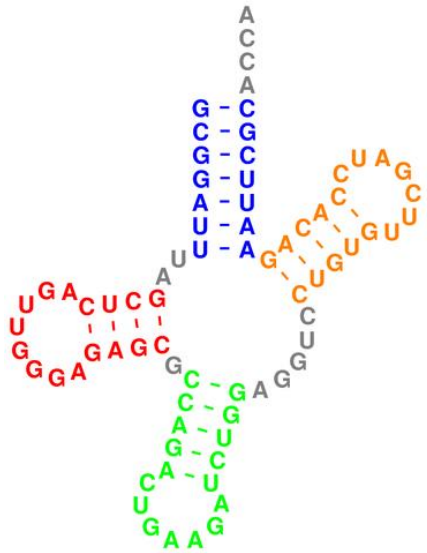


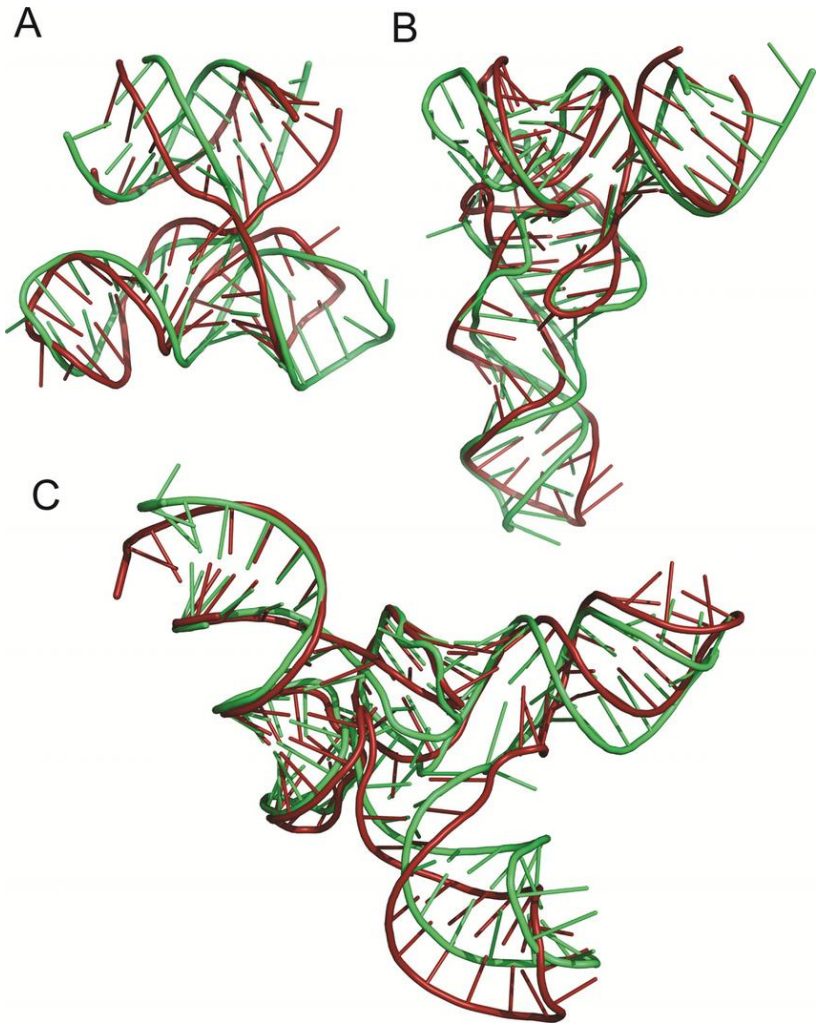
3D structure of DNA



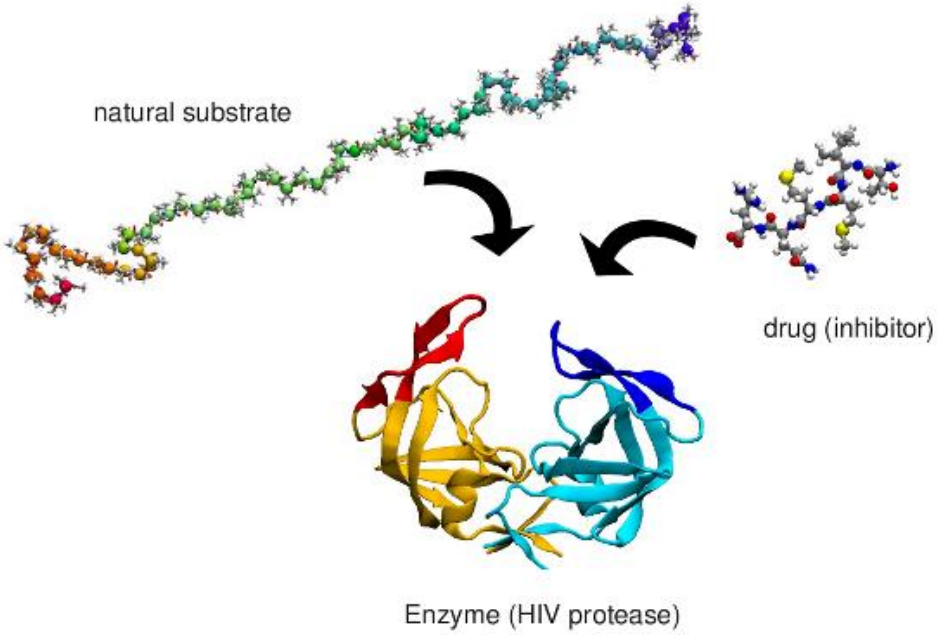


3D structures of RNA

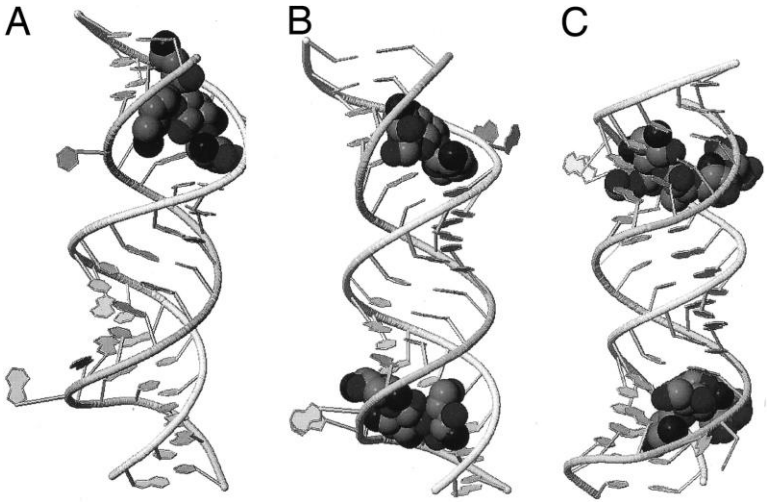




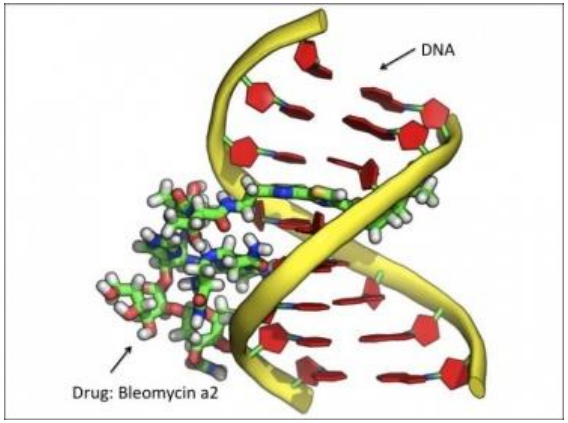
Interaction drug-target
small molecule/macromolecule (protein, DNA, RNA)



Drug/protein interaction

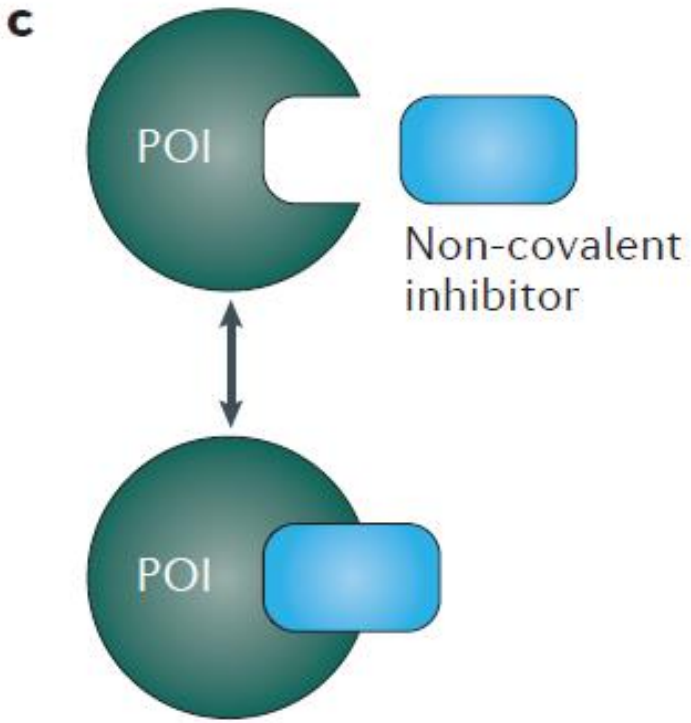


Drug/RNA complex

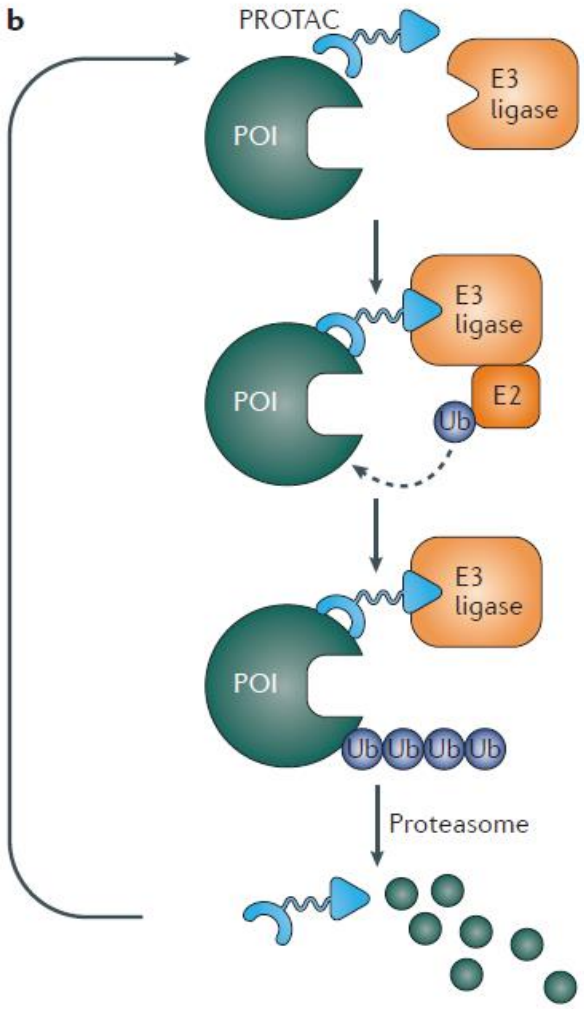


Drug/DNA complex

small-molecule therapeutic strategies occupation of a binding site on a protein or nucleic acid



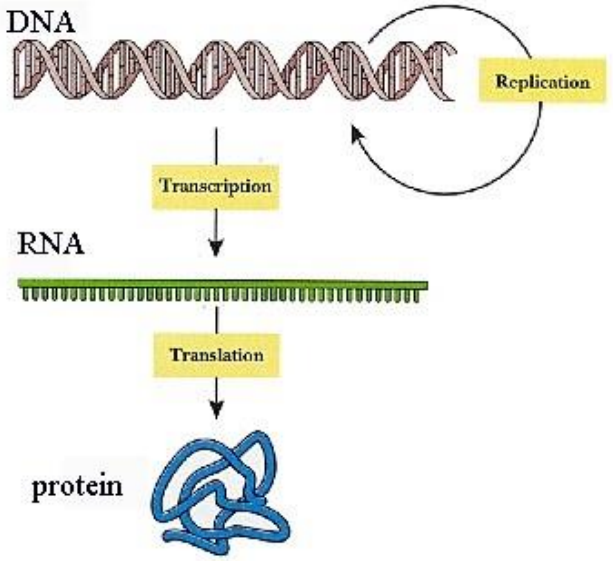
The PROTAC technology



- Based on the concept of event-driven pharmacology, proteolysis-targeting chimaera (PROTAC) and hydrophobic tagging (HyT) technologies utilize bifunctional molecules, whereby one end binds to the protein of interest while the other end hijacks cellular quality control mechanisms to induce the degradation of the target protein

Other methods to target proteins

- One method for modulating intracellular protein concentrations is through nucleic acid-based agents such as antisense oligonucleotides (ASOs) or agents that exploit RNA interference (RNAi), such as small interfering RNAs (siRNAs).



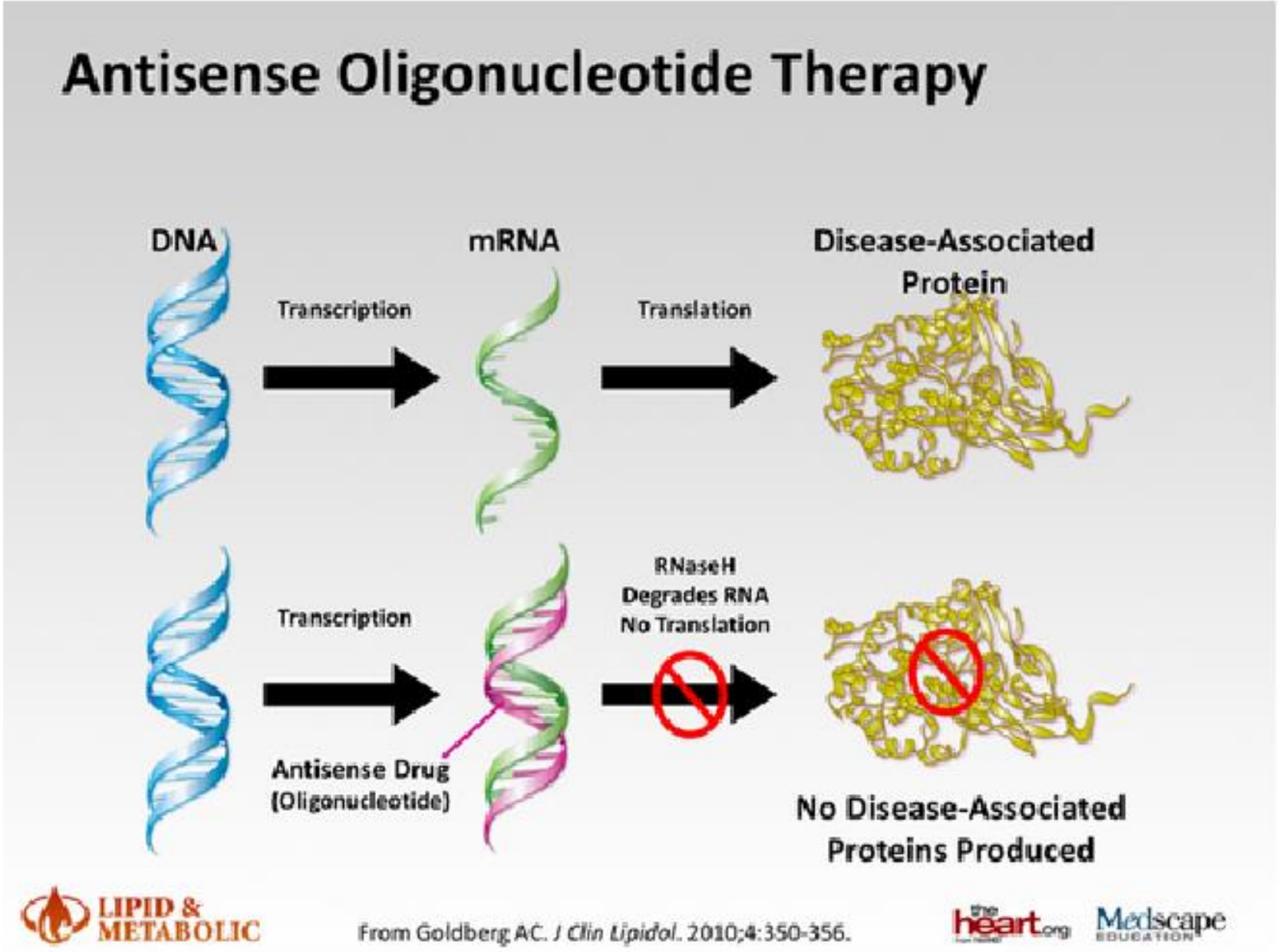
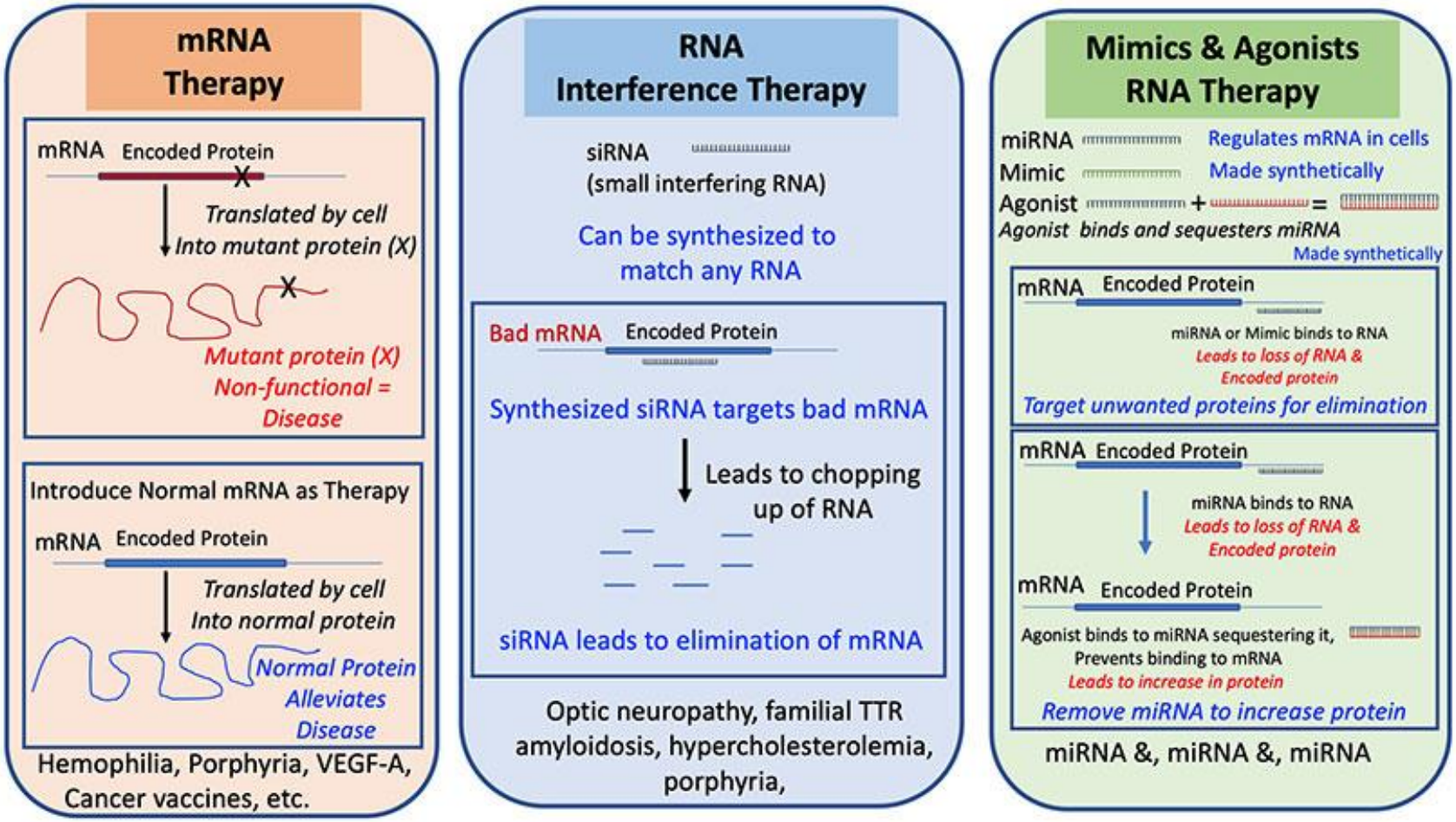


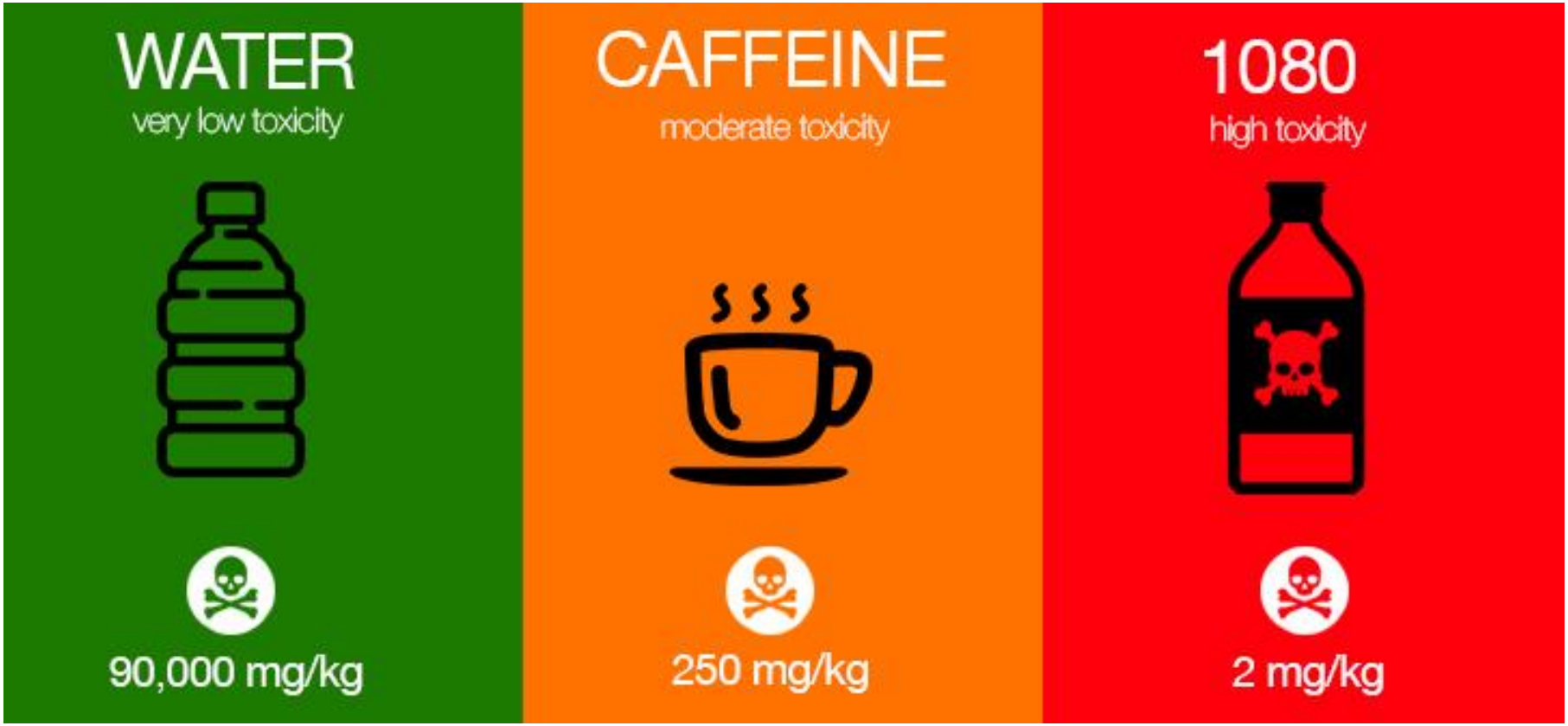
Figure 1: Cartoon representation of the antisense oligonucleotide therapy

RNA-based therapies



Off-target effects (side effects)

«The dose makes the poison»



"THE DOSE MAKES THE POISON"

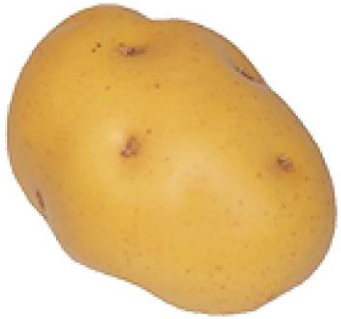
APPLE SEEDS

CONTAIN AMYGDALIN
~0.6g/kg of seeds

PEARS

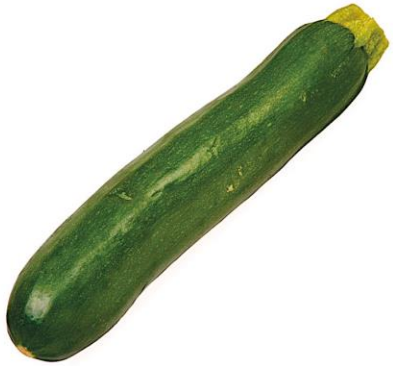
CONTAIN FORMALDEHYDE
~0.06g/kg

POTATOES



CONTAIN SOLANIN
~0.2g/kg
(higher in green potatoes)

COURGETTES



CONTAIN CUCURBITACIN E
Variable
(higher in bitter courgettes)

ALL OF THE FOOD ITEMS ABOVE CONTAIN NATURAL CHEMICALS THAT ARE TOXIC TO HUMANS. HOWEVER, THEY ARE USUALLY PRESENT IN VERY SMALL AMOUNTS, FAR BELOW THE HARMFUL DOSE.

JUST BECAUSE A CHEMICAL IS PRESENT, DOES NOT MEAN THAT IT IS HARMFUL IN THE *AMOUNT* PRESENT.

Definition of a drug

- A drug can be defined as a chemical that interacts with a biological system (human body) and produces a biological response.
- This definition also covers molecules contained in functional foods and poisons.
- Veterinary drugs and insecticides.
- Antibiotics, antiviral and antiparasitic drugs have to kill the microorganism....antitumoral drugs have to kill the tumor.... but these molecules have to be SAFE for the host organism...SELECTIVITY problem!!

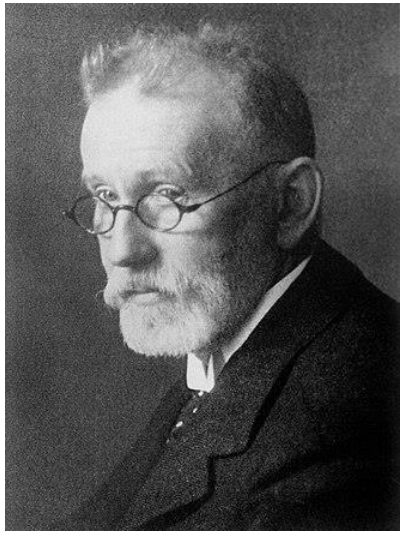
Definition of Medicinal Chemistry

- In 1974 IUPAC (International Union of Pure and Applied Chemistry) gave the following definition of medicinal chemistry:

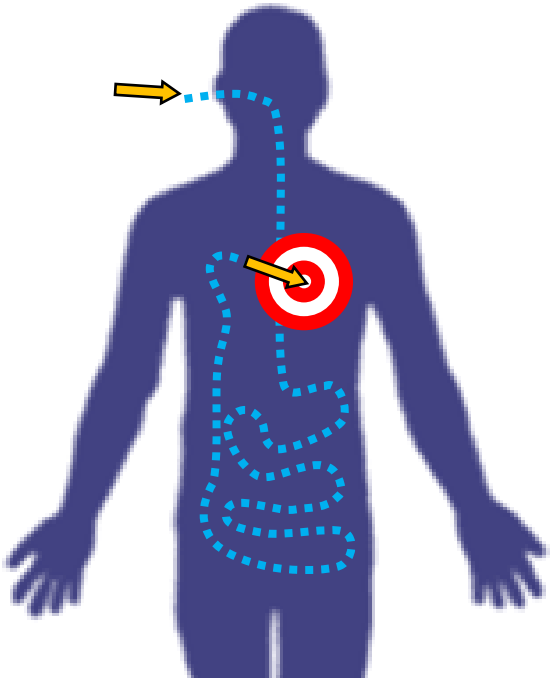
"Medicinal chemistry concerns the discovery, the development, the identification and the **interpretation of the mode of action** of biologically active compounds at the molecular level. Emphasis is put on drugs, but the interest of the medicinal chemist is not restricted to drugs but include bioactive compounds in general. Medicinal chemistry is also concerned with the study, identification and synthesis of the metabolic products of these drugs and related compounds"

Each drug has a MOLECULAR TARGET

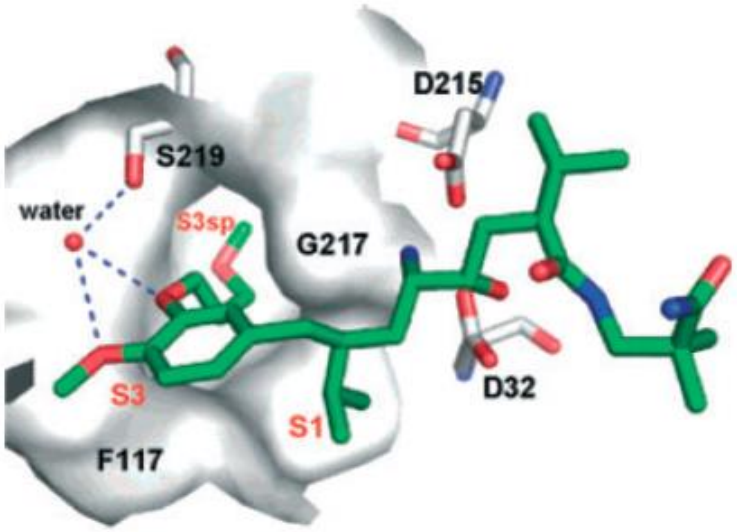
Paul *Ehrlich: "Corpora non agunt nisi fixata"*



- This is basically due to the fact that the pharmacological action of a drug is due to the interaction with a target (enzyme, receptor, ion channel).
- The side effects are due to the interaction with targets other than the principal one (off-target effects)



The supramolecular drug-target interaction: designing the complementarity

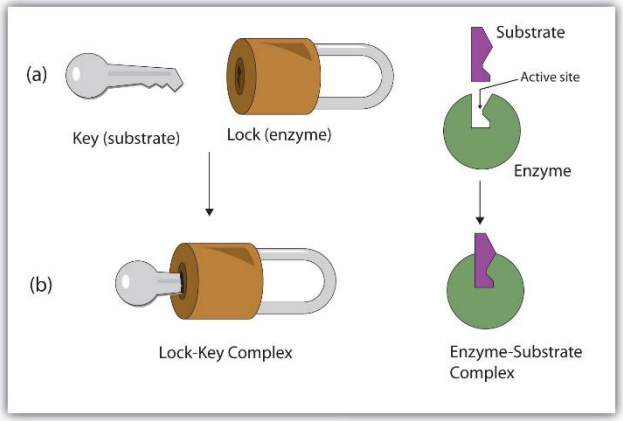


ALISKIREN-RENIN COMPLEX

Complementarity of shape and charge

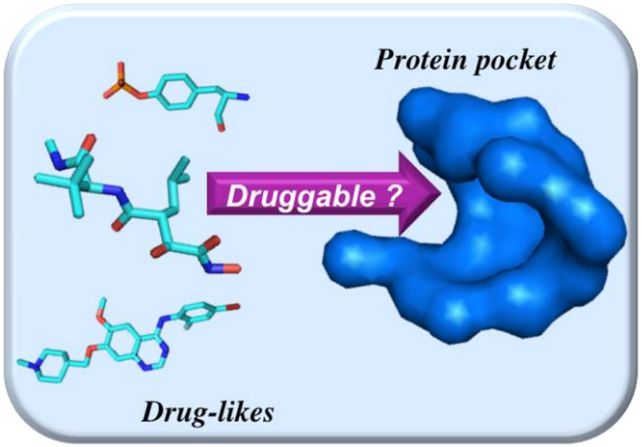
Formation of supramolecular interactions:

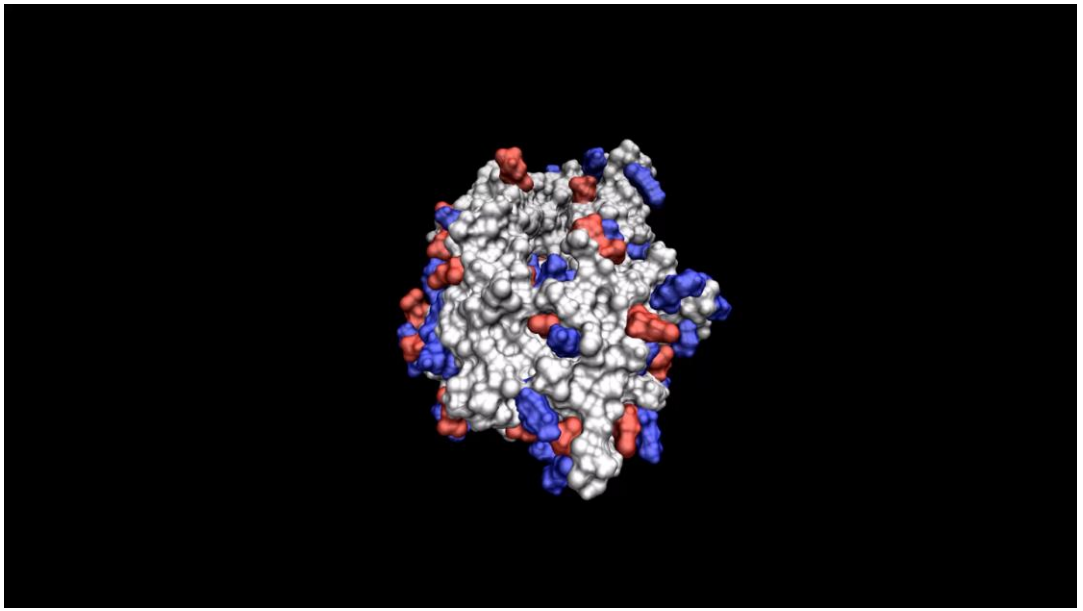
- polar interactions
- hydrophobic interactions
- aromatic-aromatic, aromatic-cation interactions
- Hydrogen bonds



Emil Fischer's lock and key model

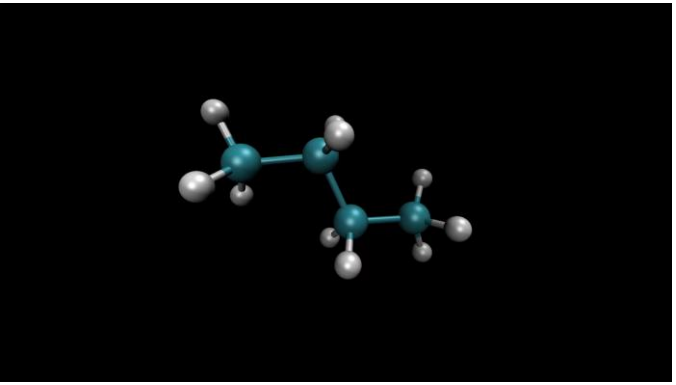
Looking for proteins druggable sites



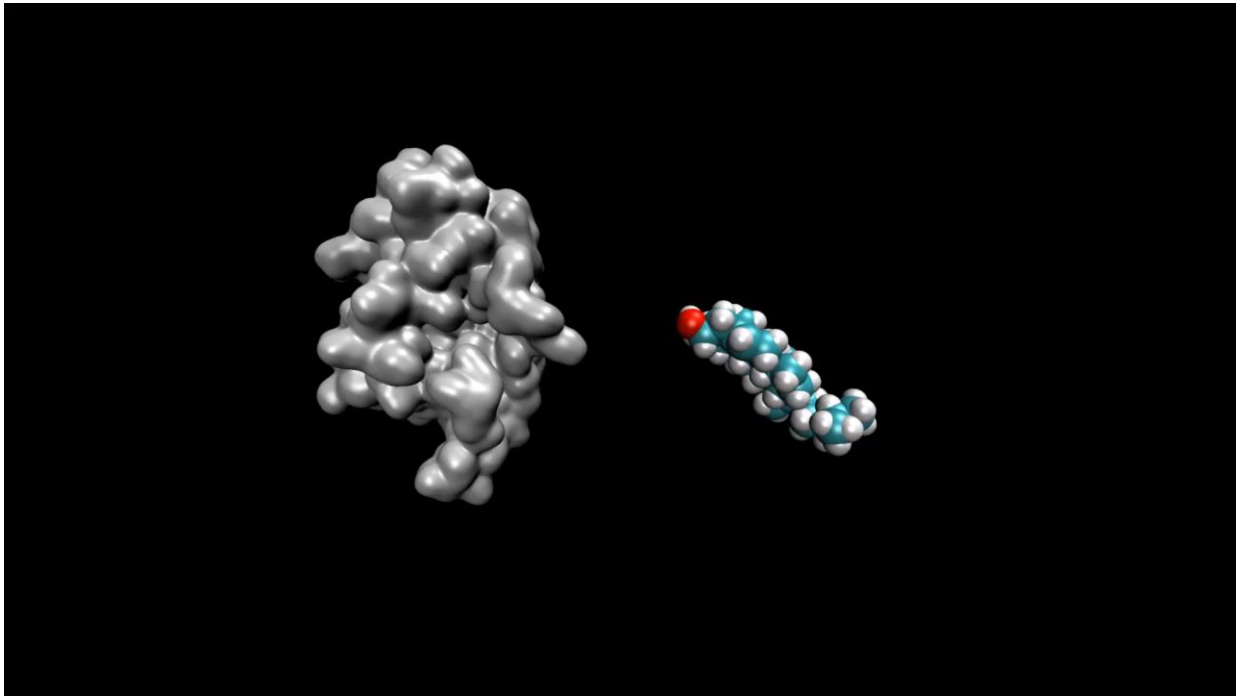


- **Computational models**
- **3D models (3D printers)**

Molecules move (rotation around bonds) and adapt each other

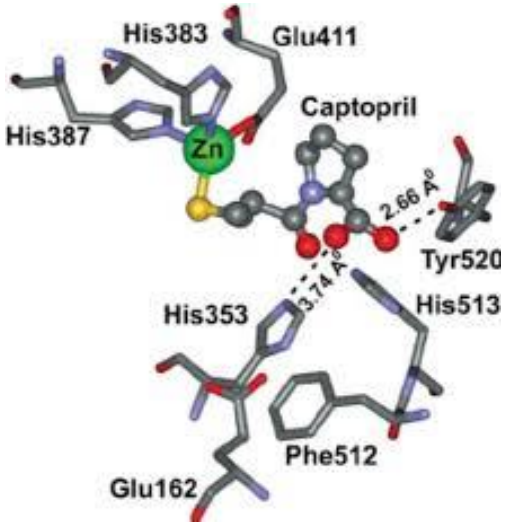


Butane's conformational motion: rotation around C-C bonds



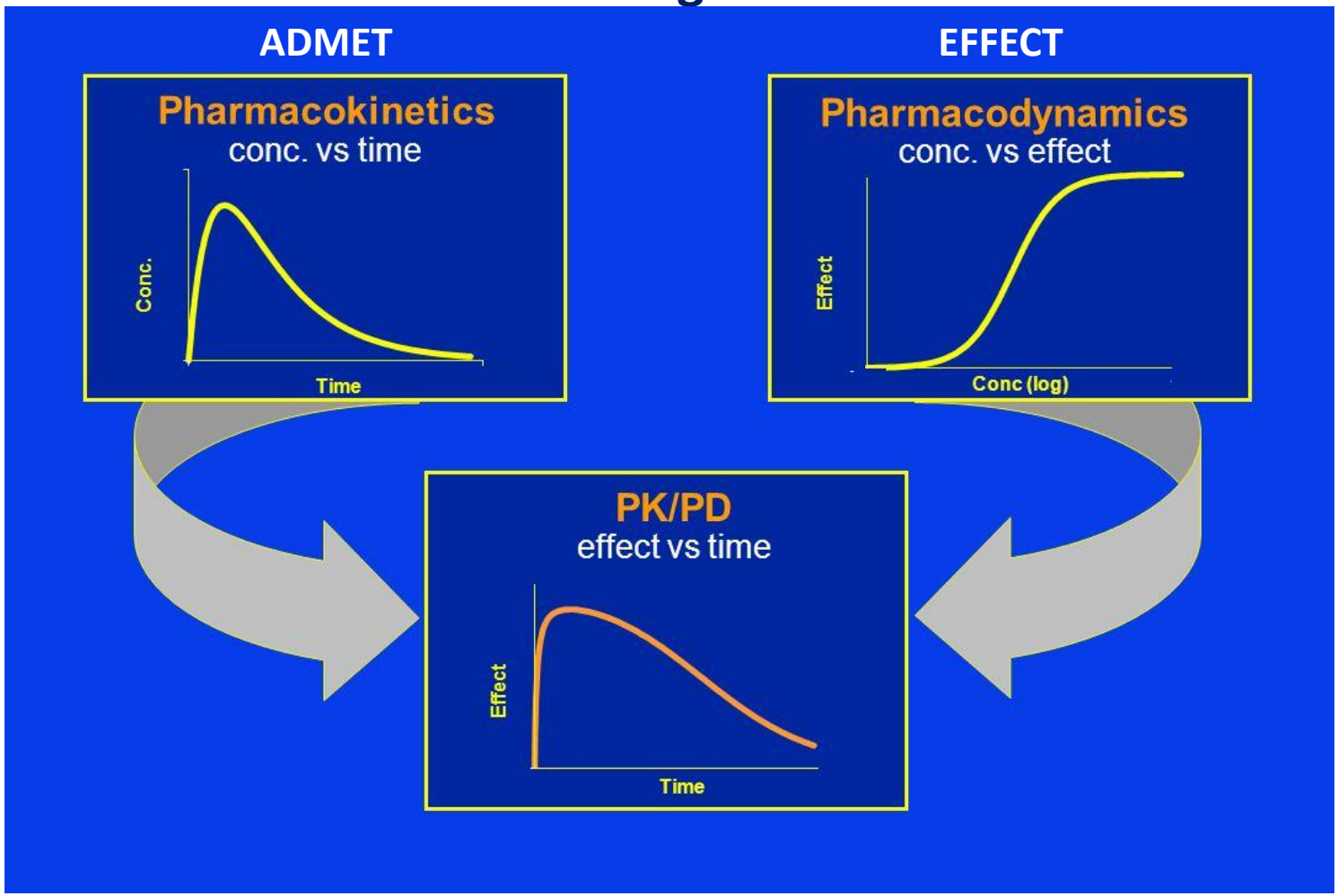
- Drug-target reciprocal adaptation (Daniel Koshland's induced fit model)

The intermolecular interactions that hold together the drug-target complex are NON-COVALENT: weak and additive

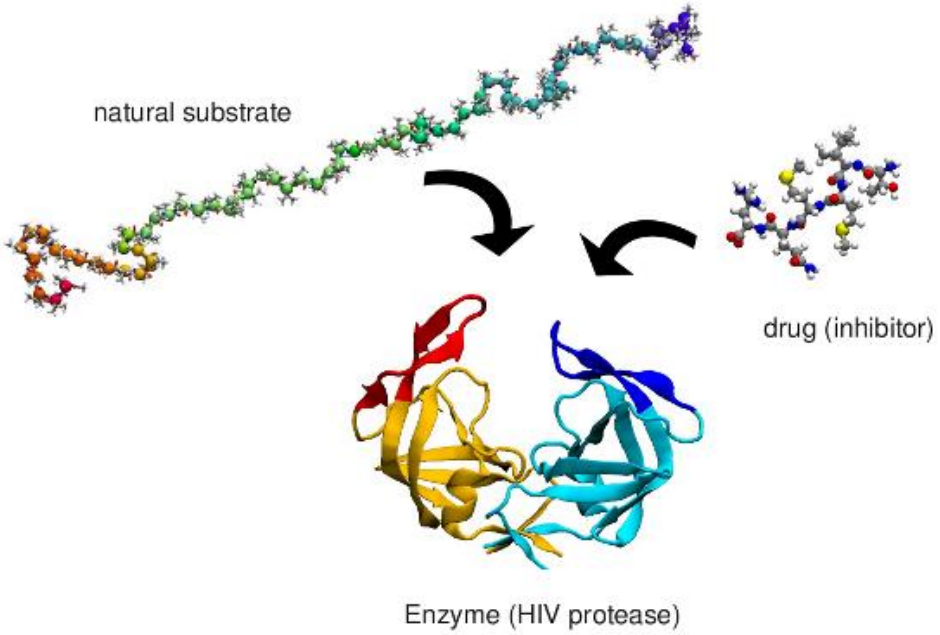


- Covalent bond = hundred kJ/mol
- NON COVALENT INTERACTIONS
- Ionic bond = 20 kJ/mol
- Hydrogen bond = 7-40 kJ/mol
- Van der Waals interactions = 1.9 kJ/mol
- IN SOME CASES THE NON-COVALENT INTERACYIONS EVOLVE TO GIVE COVALENT INTERACTIONS

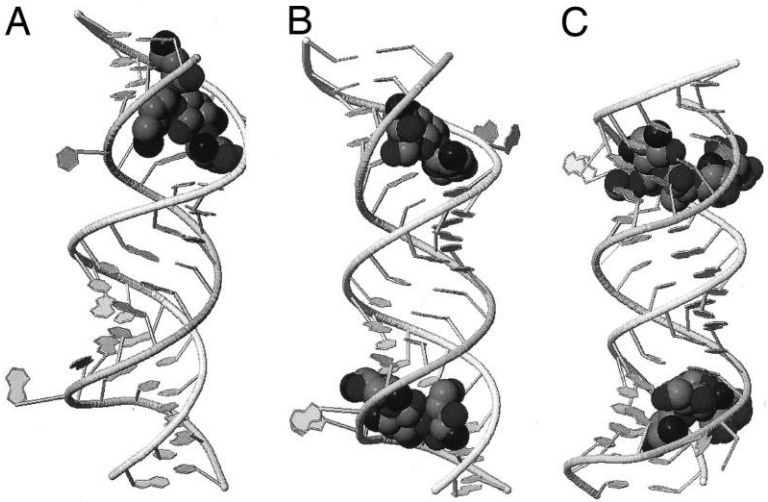
Pharmacodynamics and pharmacokinetics
This lesson is on pharmacodynamics
How to optimize drug effetc by optimizing interaction with target



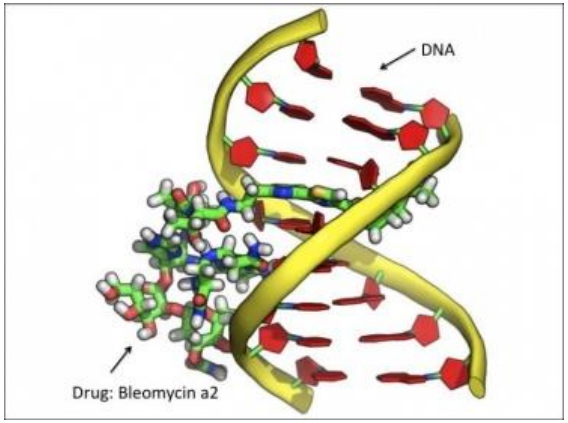
Interaction drug-target
small molecule/macromolecule (protein, DNA, RNA)



Drug/protein interaction



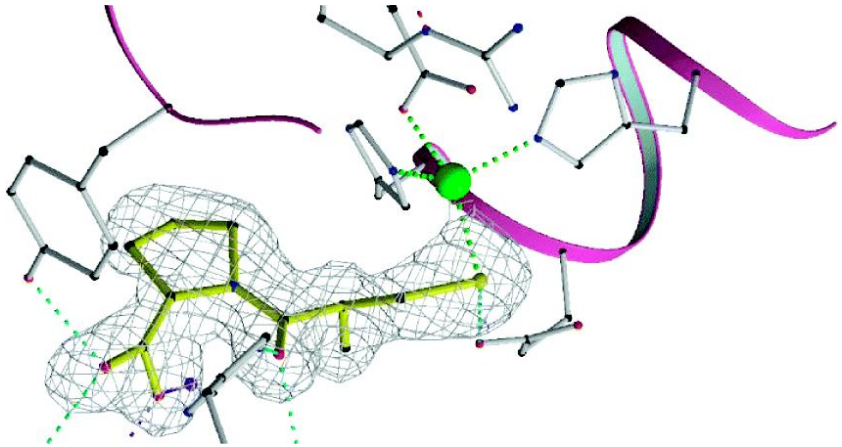
Drug/RNA complex



Drug/DNA complex

Drug interacting non-covalently with the target

- The anti-hypertensive captopril binds to the target enzyme ACE.



- Erhytromycin bound to its target (50S subunit of bacterial Ribosome)

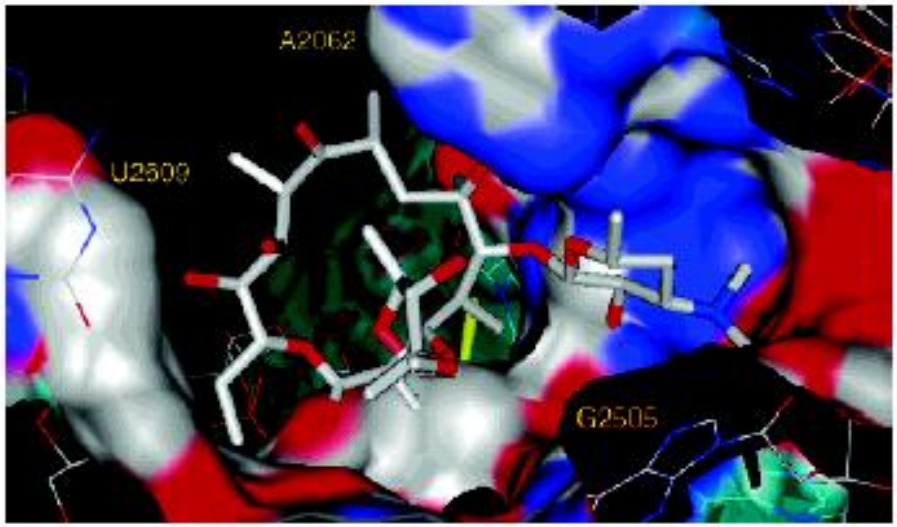
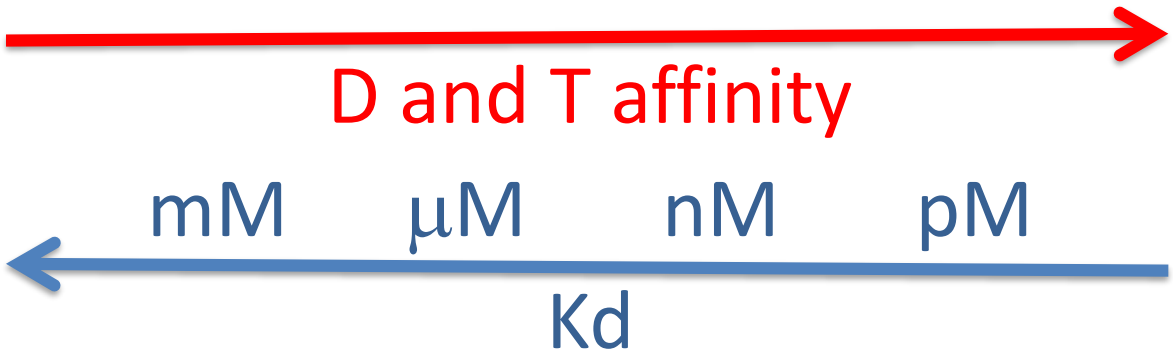


Figure 3. View of the erythromycin binding pocket on the 50S subunit of the *D. radiodurans* ribosome, showing the

Drug-Target interaction

$$D + T \underset{Kd}{\overset{Ka}{\rightleftharpoons}} DT$$

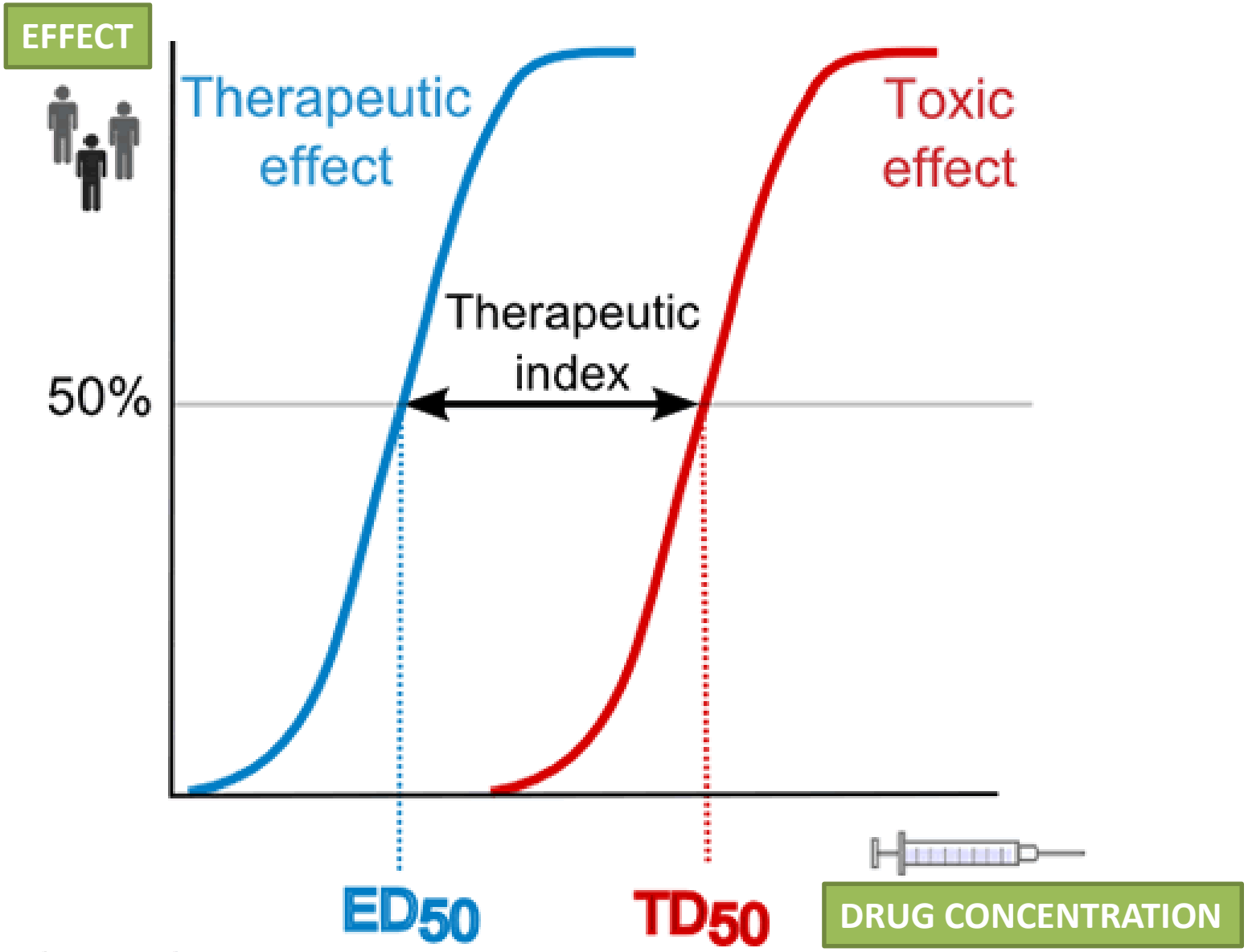
$$K_d = [D].[T]/[DT]$$



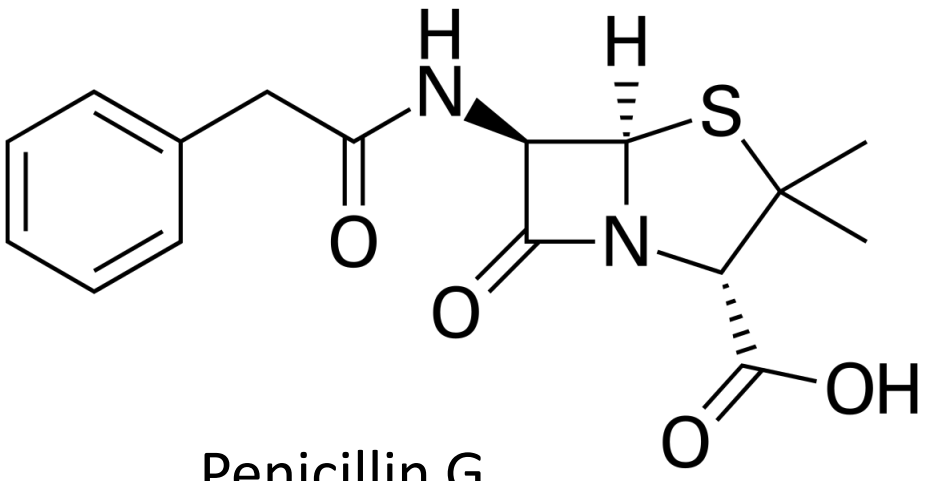
$$k_{off} = \frac{1}{\tau_R} \qquad K_d = \frac{k_{off}}{k_{on}}$$

The therapeutic index (TI)

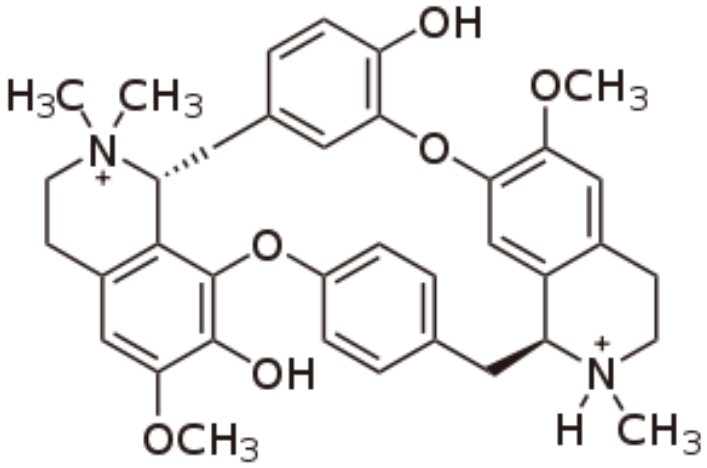
- Drugs are characterized by their *efficacy* to treat a given disease, by the *dose* necessary for therapeutic action and by the unwanted effects that they might cause at active doses (side *effects).*
- The *therapeutic index (TI) or Therapeutic Ratio (TR)* is a measure of **drug safety**: for a given drug, it is the ratio between the dose that gives toxic effects in 50% of the patients treated and the dose that cures 50% of them.


EFFECT
Therapeutic effect
Toxic effect
Therapeutic index
50%
ED50
TD50
DRUG CONCENTRATION
PharmacologyCorner.com

- Higher is TI safer is the drug
- High TI: **penicillin.**
- Drug with lower TI are more dangerous
- Low IT: **tubocurarin** is a mortal poison that is used at very low doses to relax musculature and prepare patients to surgery; **cardiac glycosides**



Penicillin G



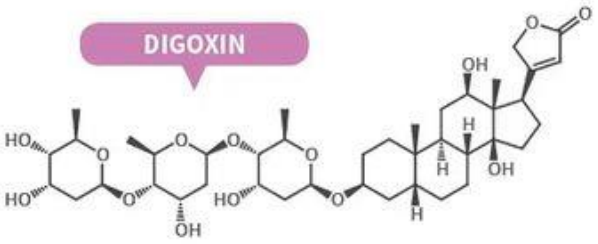
tubocurarin

THE CHEMISTRY OF FOXGLOVES

The vibrance of foxgloves belies their poisonous nature; but the same compounds that make them poisonous can also be used in medicine.

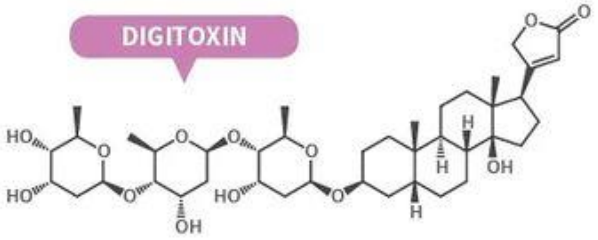
POISONOUS FOXGLOVES

DIGOXIN



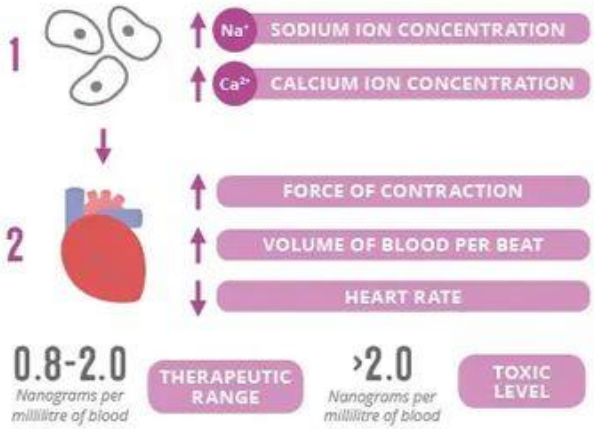
All parts of the foxglove contain compounds called cardiac glycosides, including the structurally similar digoxin and digitoxin. Ingestion of these compounds can cause nausea, vomiting, diarrhoea, and an irregular heart beat. They disable cell sodium-potassium ion pumps, leading to increased cell sodium and calcium ion concentration. This slows the heart rate, which can lead to a heart attack and death.

DIGITOXIN



FOXGLOVES IN MEDICINE

Though poisonous in large amounts, in small doses digoxin can be used to manage some heart conditions, including abnormal heart rhythms and heart failure. It increases the force of the heart's contraction and consequently the volume of blood pumped with each beat, and also causes the heartbeat to slow.



0.8-2.0 *Nanograms per millilitre of blood* — THERAPEUTIC RANGE — **>2.0** *Nanograms per millilitre of blood* — TOXIC LEVEL

The therapeutic levels of digoxin don't differ greatly from those at which toxic effects are seen, and as such dosages must be carefully monitored.

- **Biological activity**:

- IC_{50}: inhibitory concentration at which there is 50% of inhibition

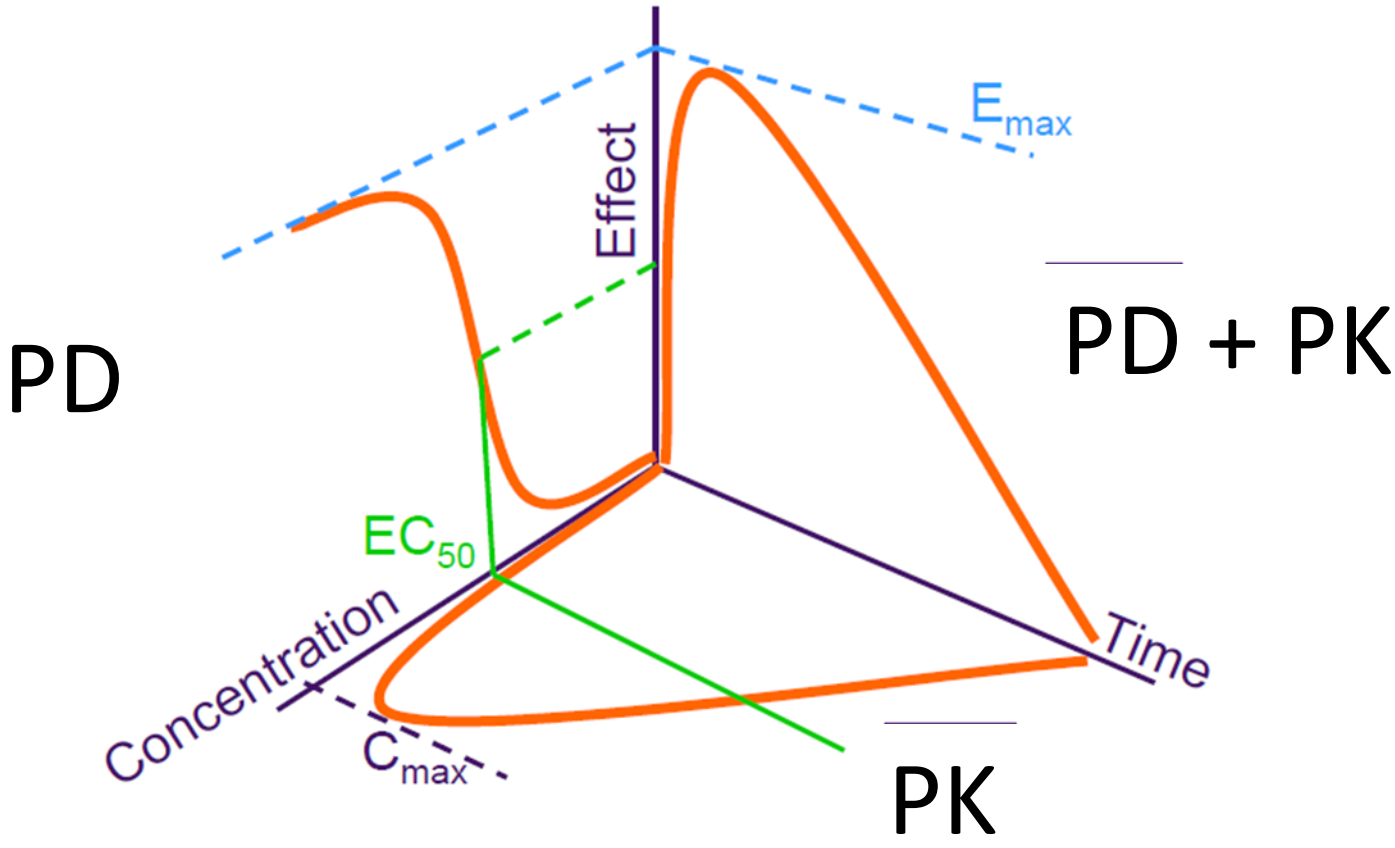
- $AC_{50}(ED_{50})$: drug concentration required for 50% of activity

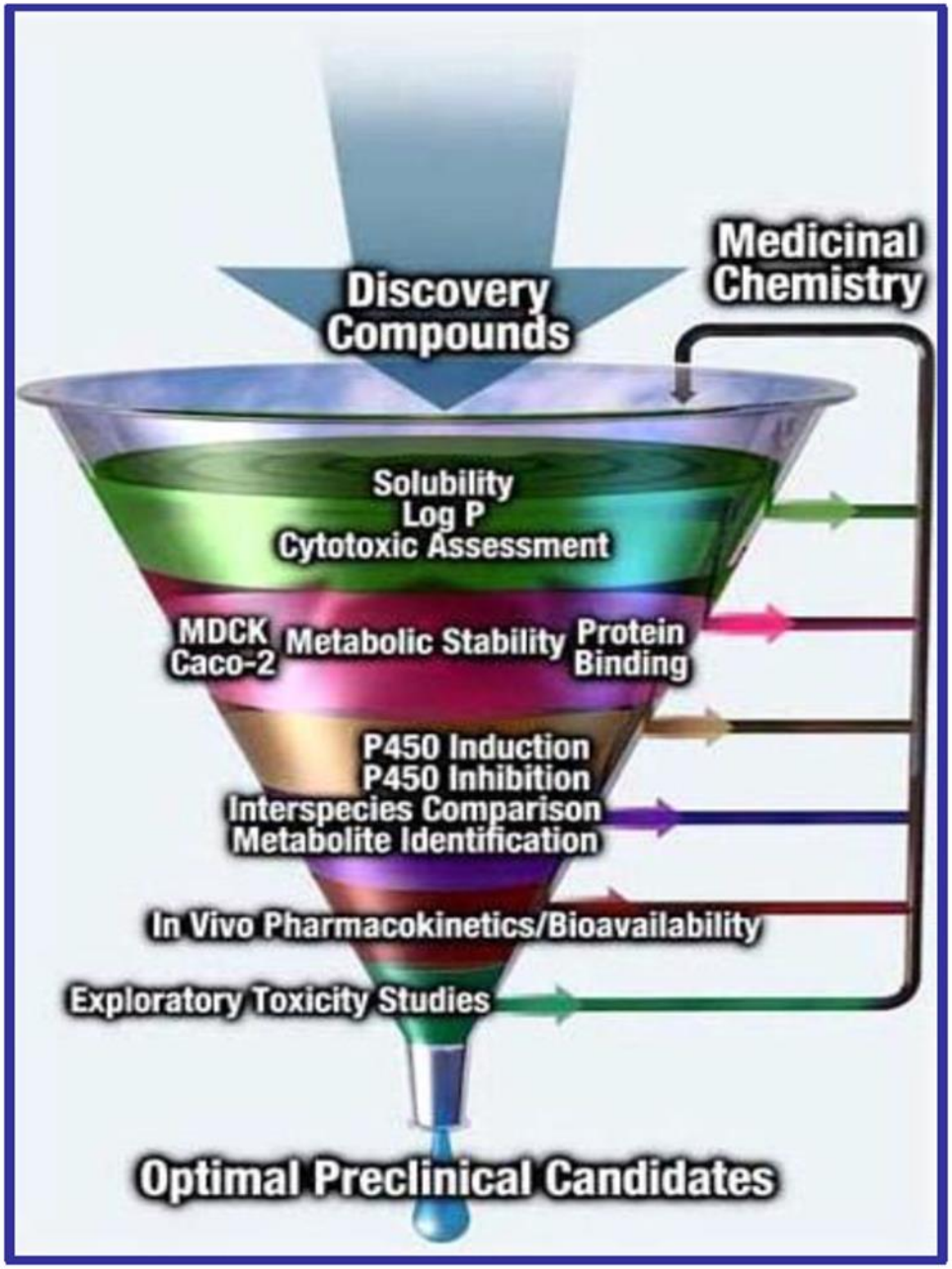
- These quantities decrease increasing the potency of a drug (lower concentration is required to obtain the same activity or effect)

- $1/IC_{50}$ or $1/AC_{50}$ or $\log 1/IC_{50}$ are directly proportional to the biological activity

PK/PD Inter-relationship

(Dose)-Time-Concentration-Effect



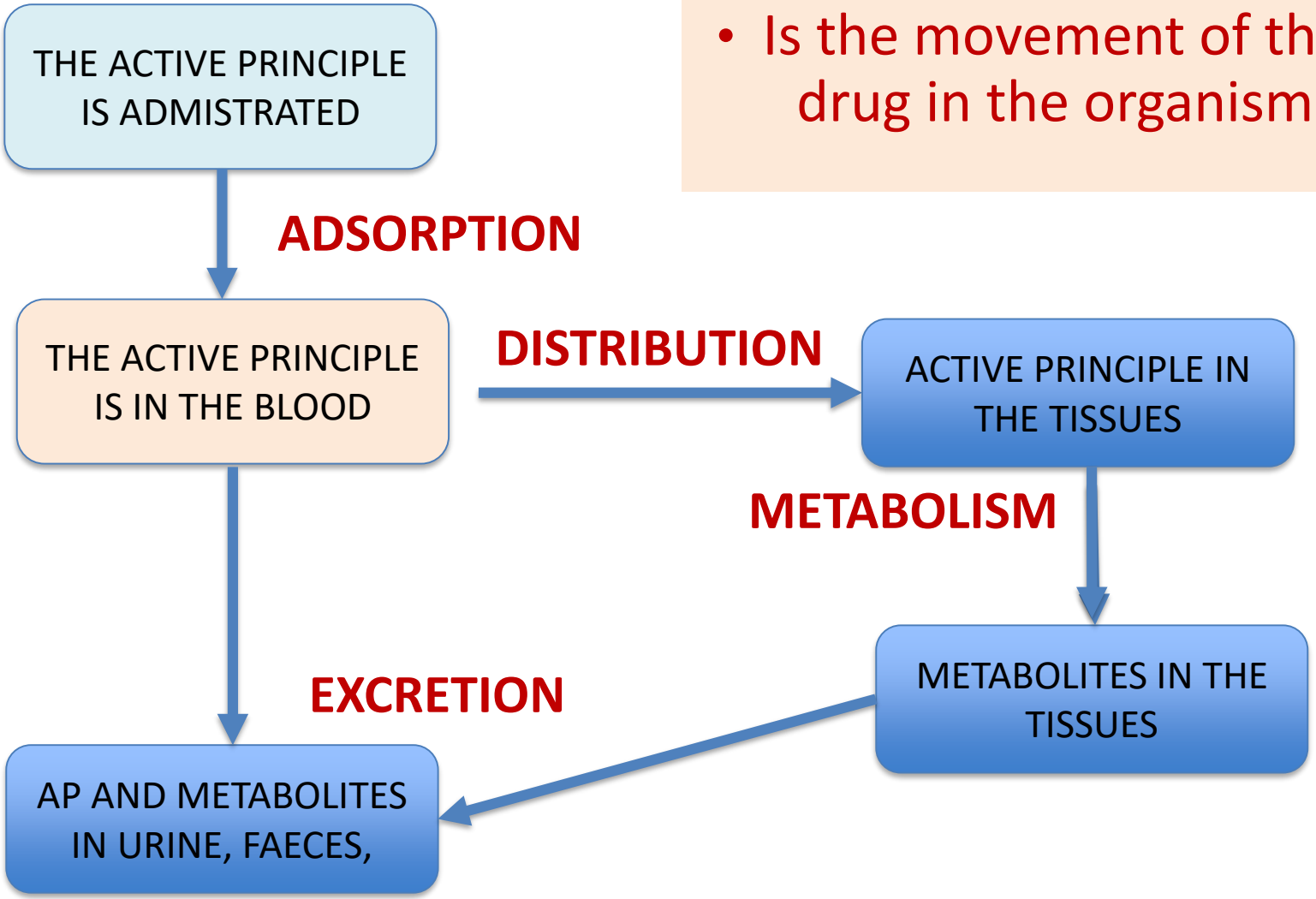

Medicinal Chemistry
Discovery Compounds
Solubility
Log P
Cytotoxic Assessment
MDCK
Caco-2
Metabolic Stability
Protein Binding
P450 Induction
P450 Inhibition
Interspecies Comparison
Metabolite Identification
In Vivo Pharmacokinetics/Bioavailability
Exploratory Toxicity Studies
Optimal Preclinical Candidates

Pharmacokinetics: Liberation, Absorption, Distribution, Metabolism, Escretion, Toxicity (LADMET)

- The administration route of the ACTIVE PRINCIPLE:
- **enteral** (from the alimentary tract)
- **parenteral** (direct injection into the blood stream)
- **other non-parenteral routes** (intramuscular or subcutaneous injection, transdermal systems, sprays, etc.)

Pharmacokinetic

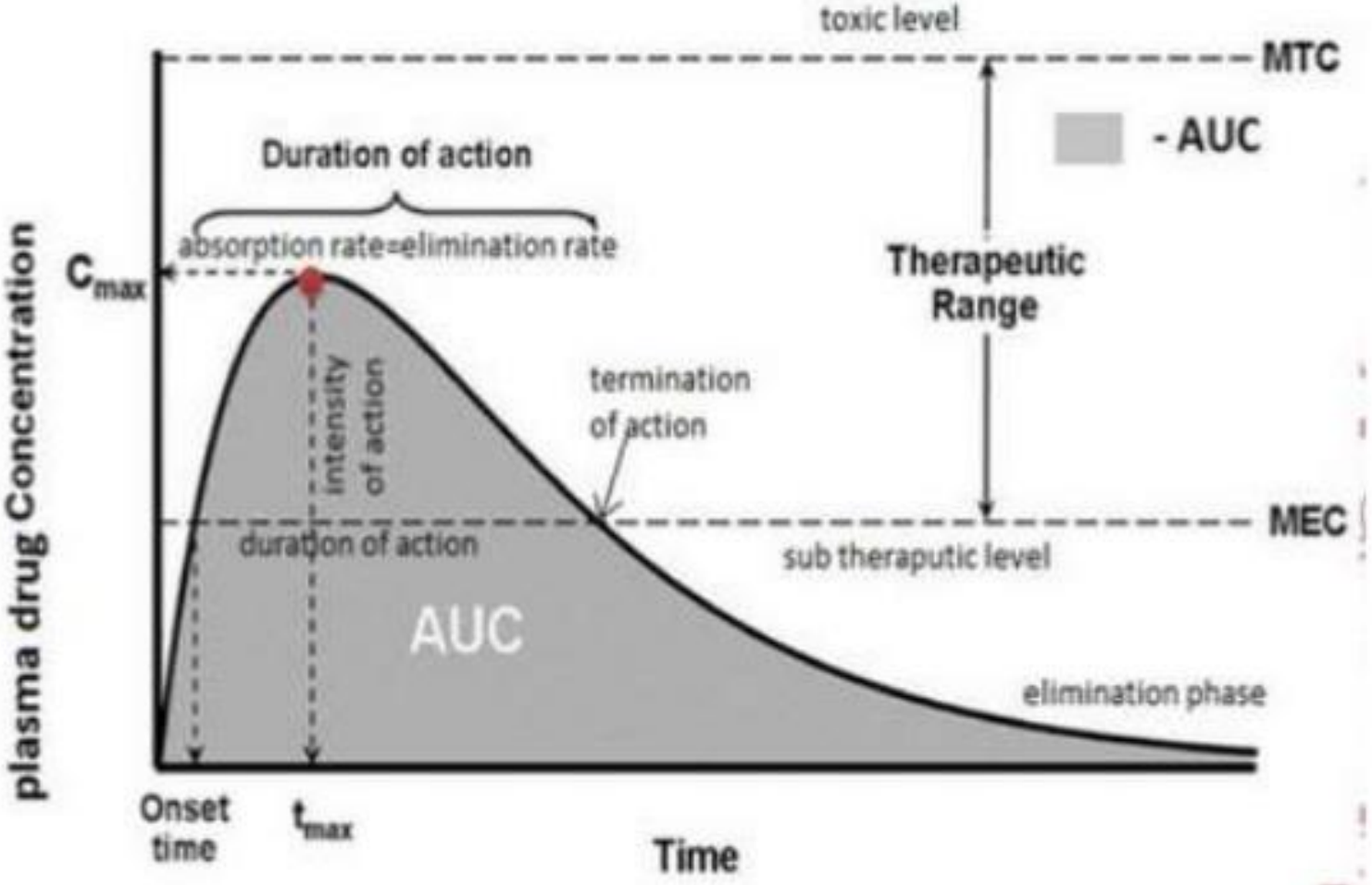
- Is the movement of the drug in the organism



LADMET

- **Adsorption and Distribution (in the blood and tissues!!)** of a drug determine its bioavailability.
- **Bioavailability**: fraction of drug that reaches the general circulation in a given time
- A and D: capacity of the drug has to cross several cell membranes, determined by these factors:
- MW
- Log P
- pKa
- Capacity to form H-bonds

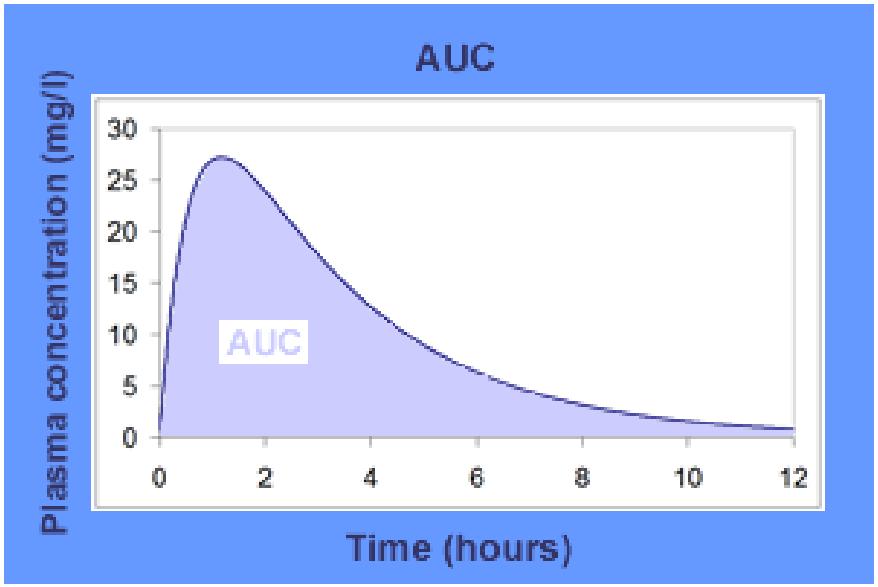
Basic PK considerations



Plasma Drug Concentration Vs Time Graph

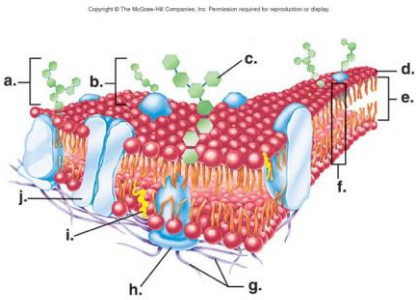
Absorption and Bioavailability: the AUC

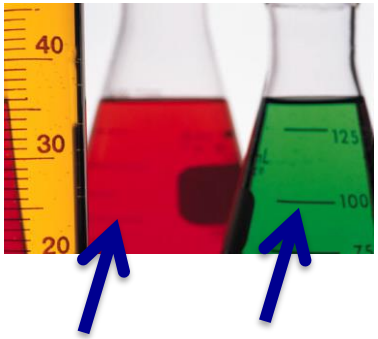
The area under the plasma **drug** concentration-time curve (**AUC**) reflects the actual body exposure to **drug** after administration of a dose of the **drug** and is expressed in mg*h/L. This area under the curve is dependant on the rate of elimination of the **drug** from the body and the dose administered



Human organism

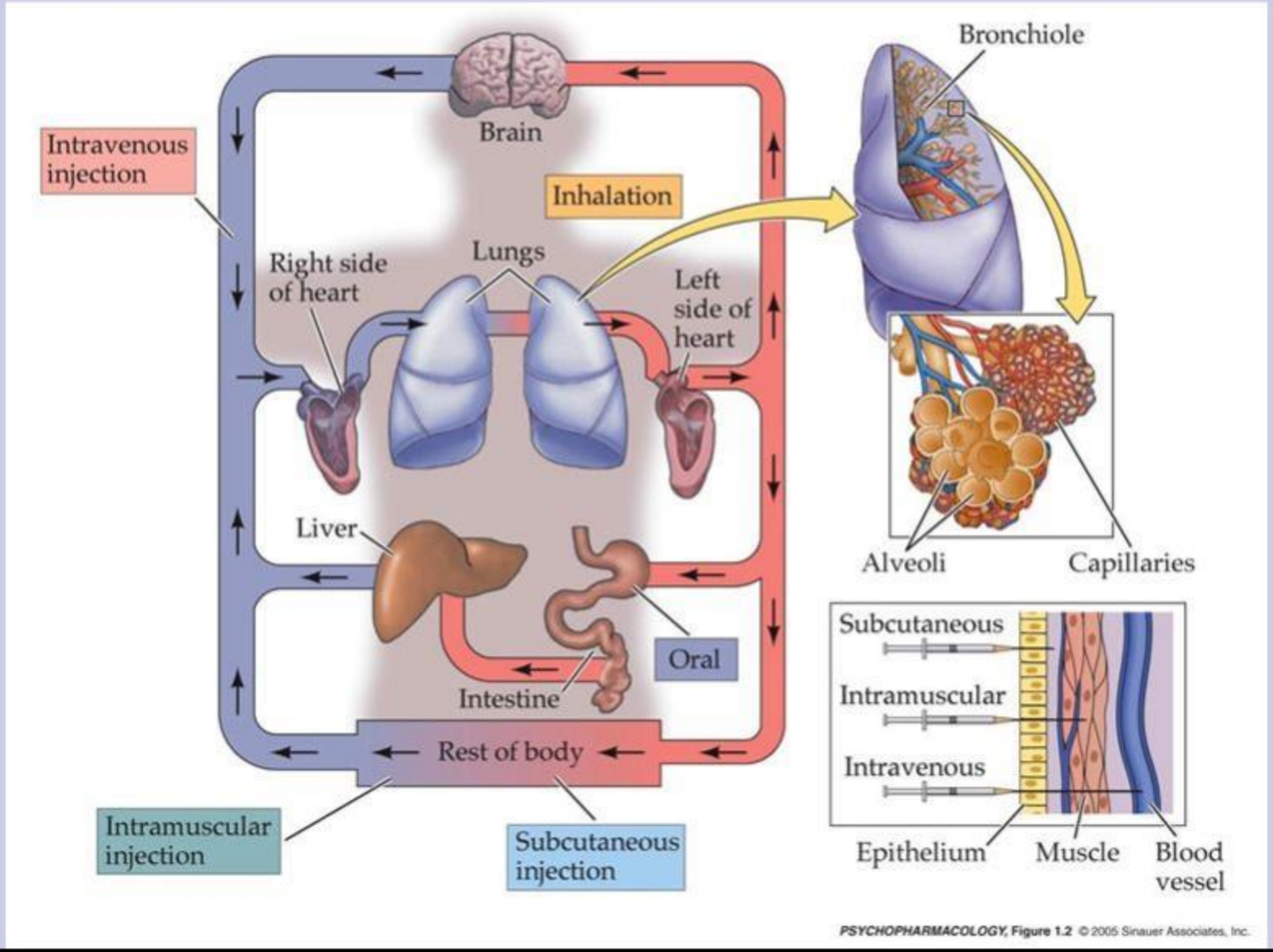
- Hydrophylic media: blood, other body fluids
- Hydrophobic environment: cell membranes, tissues barriers, blood-brain barrier (BBB), CNS

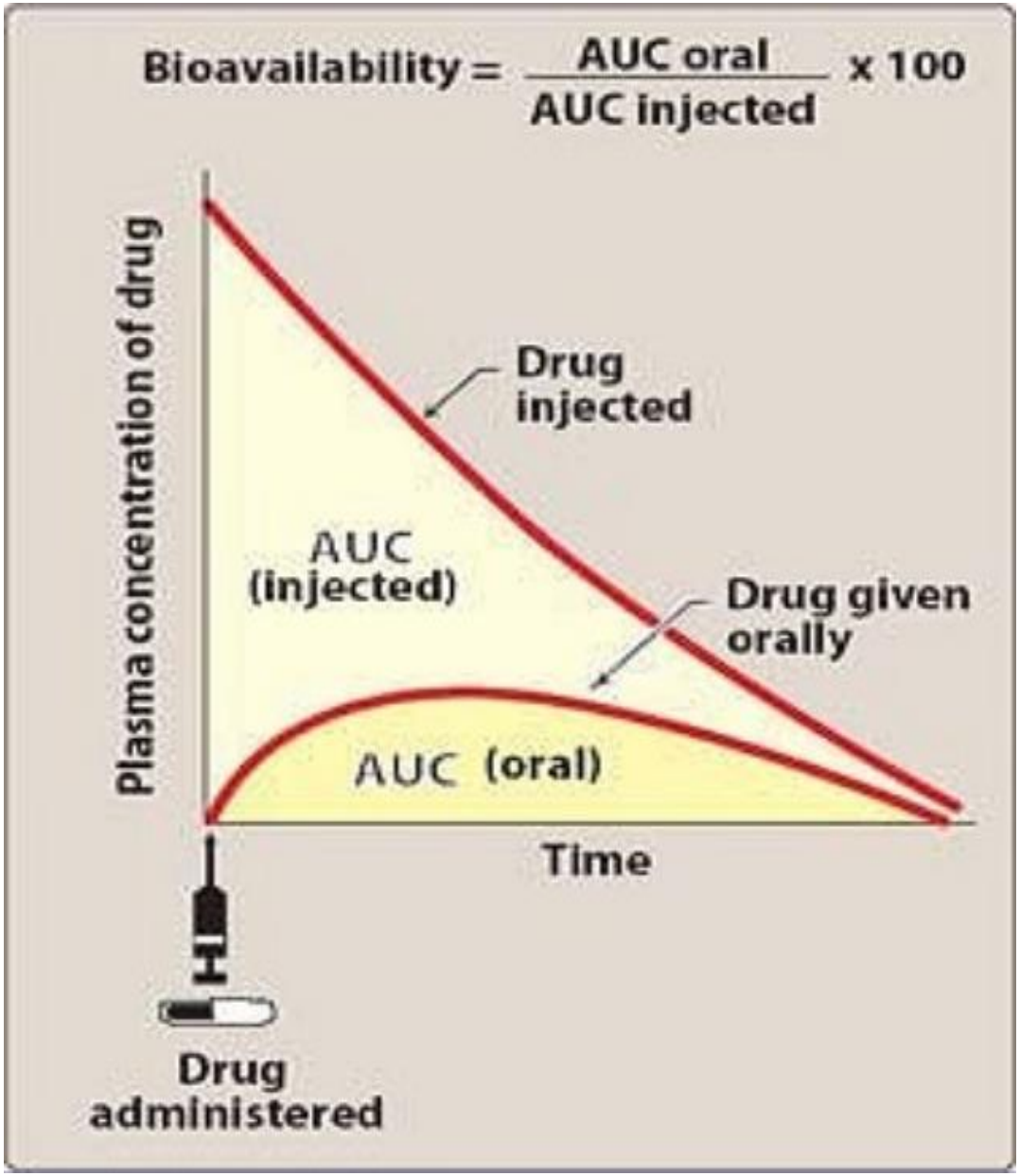




Octanol/water

Routes of Drug Administration





BIOAVAILABILITY

- **Bioavailability is the fraction (in %) of the administered drug dose that reaches chemically unaltered the bloodstream**

The Lipinski's "rule of five" RO5 for drugs

A great contribution drug discovery was given by the work of C. Lipinski. By examining a set of orally active commercial drugs, in 1997, he observed that molecules that are characterized by at least two of the following properties **will present good absorption for development as drugs**:

a) molecular weight (MW) <500 Da

b) Log *P* <5

c) No more than 5 H–bond donor groups (OH, NH)

d) the sum of N and O atoms (H bond acceptors) no more than 10

Lipinsky's rule of five

1. H-bond donors ≤ 5 (expressed as the sum of OHs and NHs)

2. Molecular weight ≤ 500 DA

Good *in vivo* drug absorption and permeation

3. log P ≤ 5

4. H-bond acceptors ≤ 10 (expressed as the sum of Ns and Os)

Updated RO5

Good bioavailability if:

- Partition coefficient log *P* in −0.4 to +5.6 range
- Molecular weight from 180 to 500
- Number of atoms from 20 to 70 (includes H-bond donors [e.g.;OH's and NH's] and H-bond acceptors [e.g.; N's and O's])
- Polar surface area no greater than 140 $Å^2$

Also the 500 molecular weight cutoff has been questioned.

Polar surface area and the number of rotatable bonds has been found to better discriminate between compounds that are orally active and those that are not for a large data set of compounds in the rat.

In particular, compounds which meet only the two criteria of:

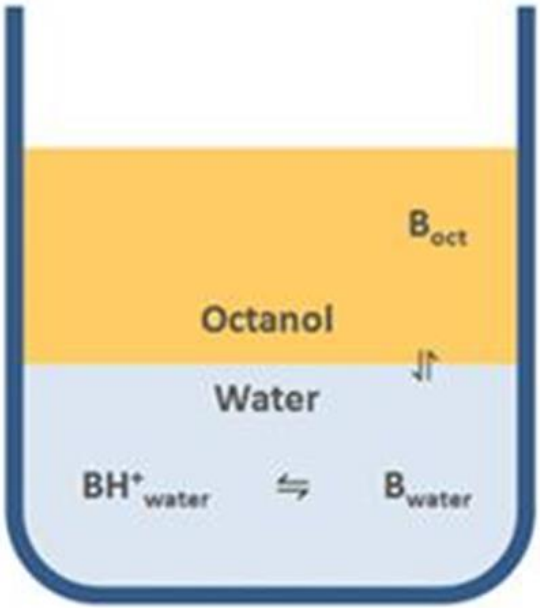
- 10 or fewer rotatable bonds and
- polar surface area equal to or less than 140 $Å^2$
- are predicted to have good oral bioavailability

The partition coefficient log P

- Partition coefficient, P is the ratio of concentrations of a compound (drug, D) in the two phases of a mixture of immiscible solvents at equilibrium. Normally one of the solvents chosen is water, the second octanol. Octanol mimics the properties of lipid bilayers of cellular membranes.

Log P = log ($[D]_{octanol}/[D]_{water}$)

$$pK_a = \log \frac{[BH^+_{water}]}{[H^+][B_{water}]}$$

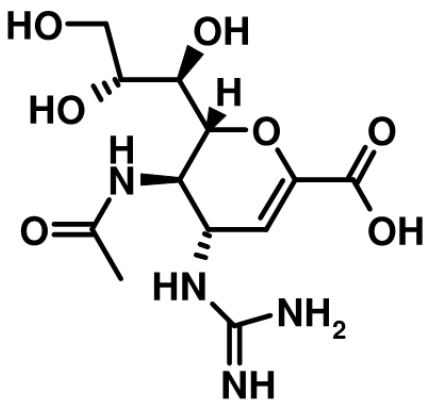


$$\log P = \log \frac{[B_{oct}]}{[B_{water}]}$$

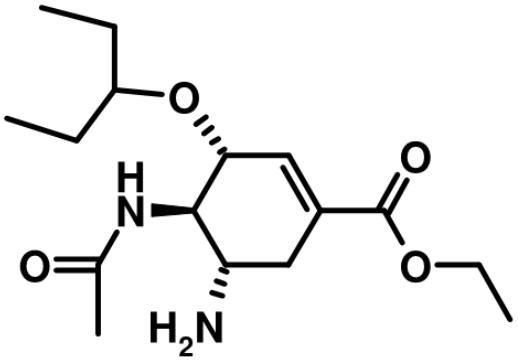
$$P = \text{Partition Coefficient} = \frac{\text{Concentration of neutral species dissolved in partition solvent}}{\text{Concentration of neutral species dissolved in water}}$$

Drugs

- Hydrophobic, apolar, logP > 0
- Hydrophilic, polar, logP < 0
- Intermediate polarity, logP about 0



Zanamavir



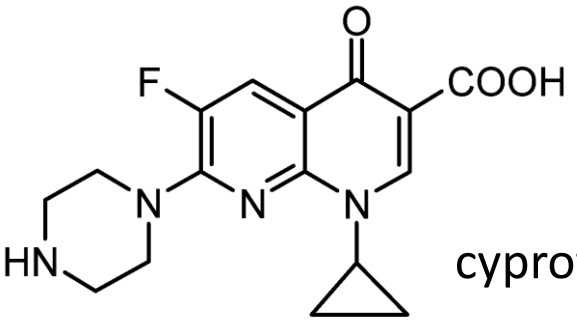
Oseltamivir

Log P = -3

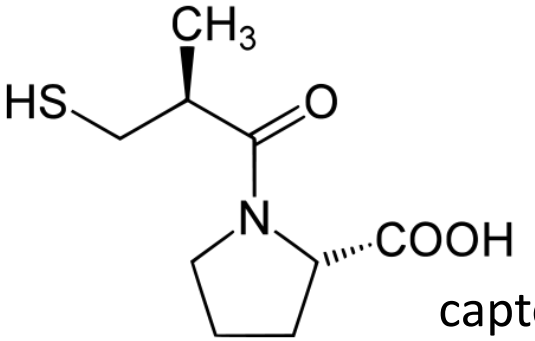
Log P = 1.16

- The rules of Lipinsky have been derived from a large set of pharmacologically active compounds presents in the market in '80-'90.....a lot of aromatic (hydrophobic) compounds!
- Several valuable medicines do not meet all the ‘rules’ of Lipinski. Here are just a few examples: Aliskiren (anti-hypertensive); Cyclosporin (immunosuppressant)
- All natural product-based drugs [Lovenox , Oxycontin , Taxotere , Tacrolimus , Pulmicort and Nasonex (quinone steroids); erythromycin-based antibiotics, b-lactam- based antibiotics etc.]

Drugs following the Lipinski rule

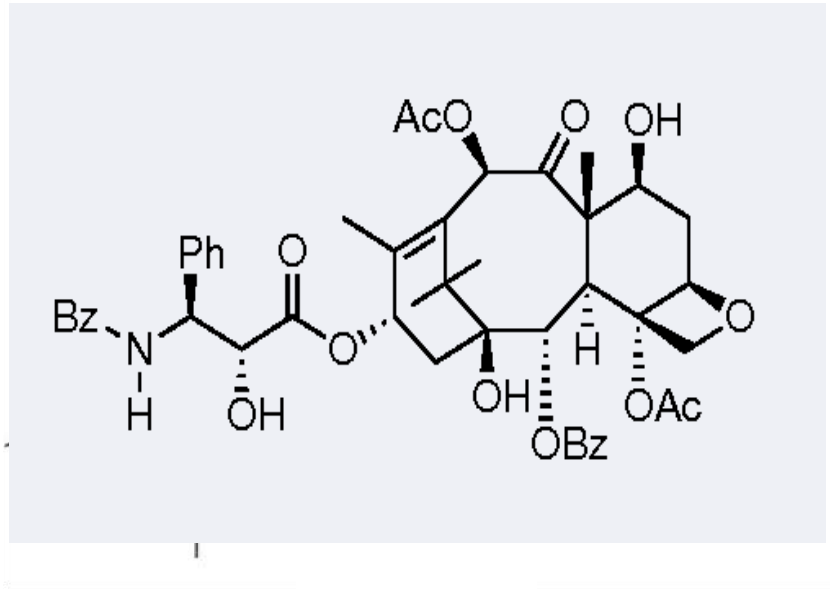


cyprofloxacin

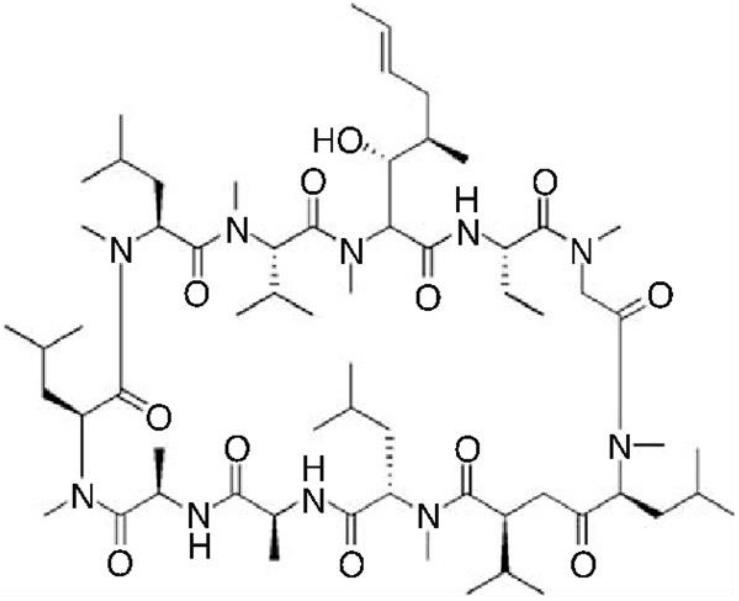


captopryl

Drugs that do not follow the Lipinsky rule



Taxol

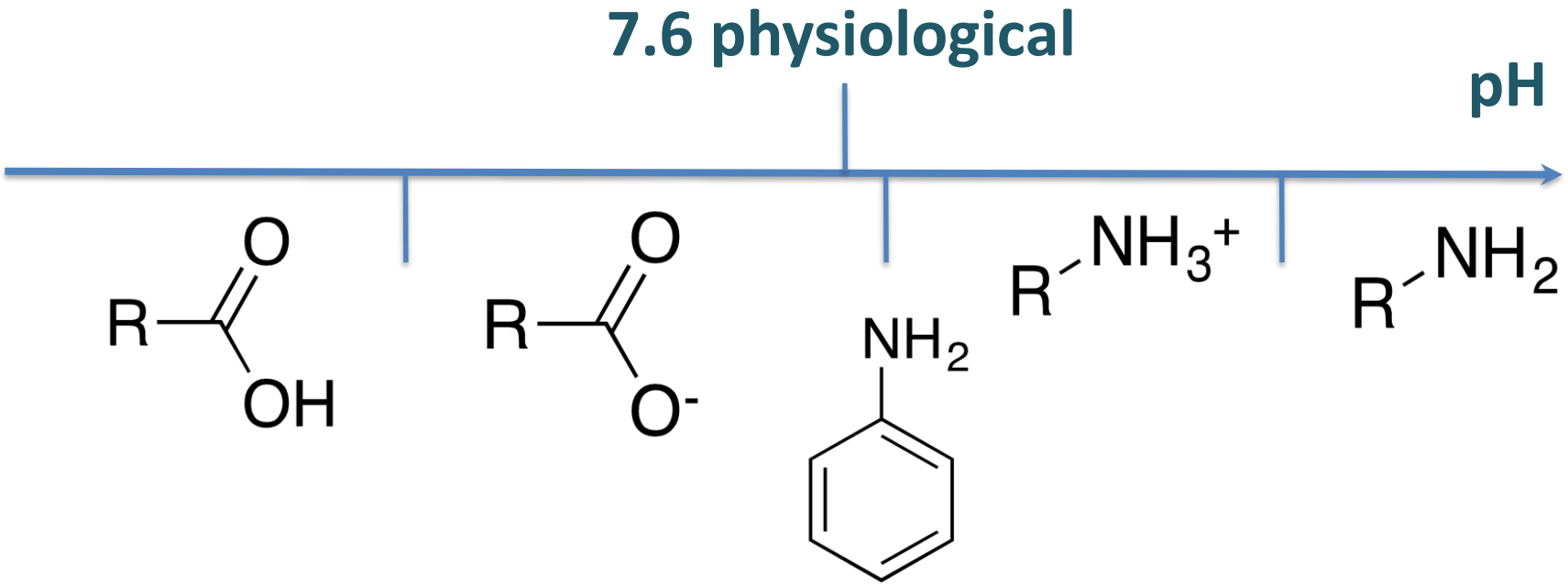


cyclosporin

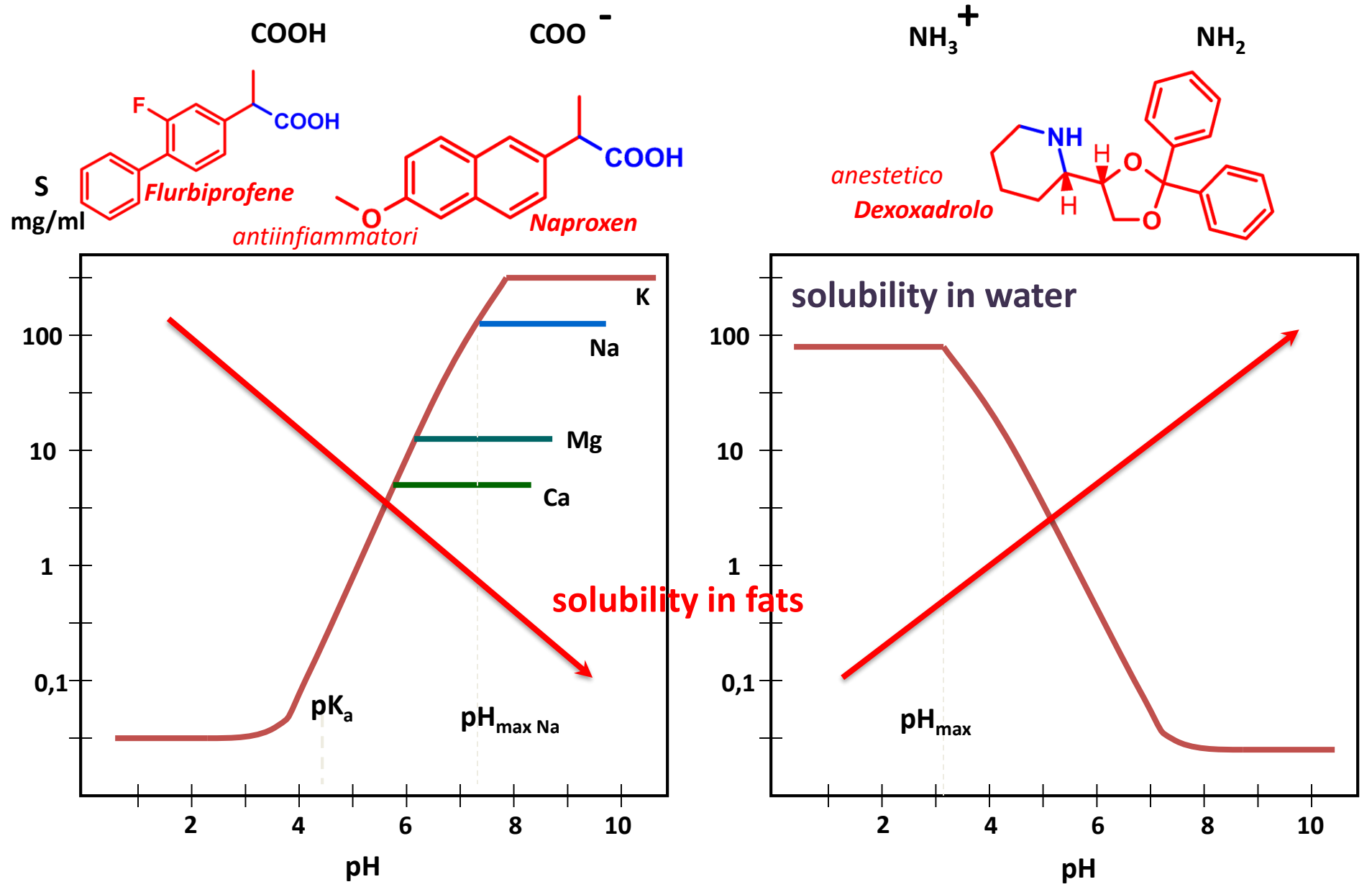
The pKa: the the charged/neutral balance

- Drugs containing amines and carboxylic acids are charged (respectively + and –) and have problems to cross cellular membranes.
- Aromatic amines are particularly suitable groups because at neutral pH are mainly protonated (pKa between 7 and 9) but also present a percentage of neutral form that easily crosses membranes and enter into tissues.
- Acidity and basicity are modulated by the insertion in the molecule of electron withdrawing (EWG) and electro donating (EDG) groups.

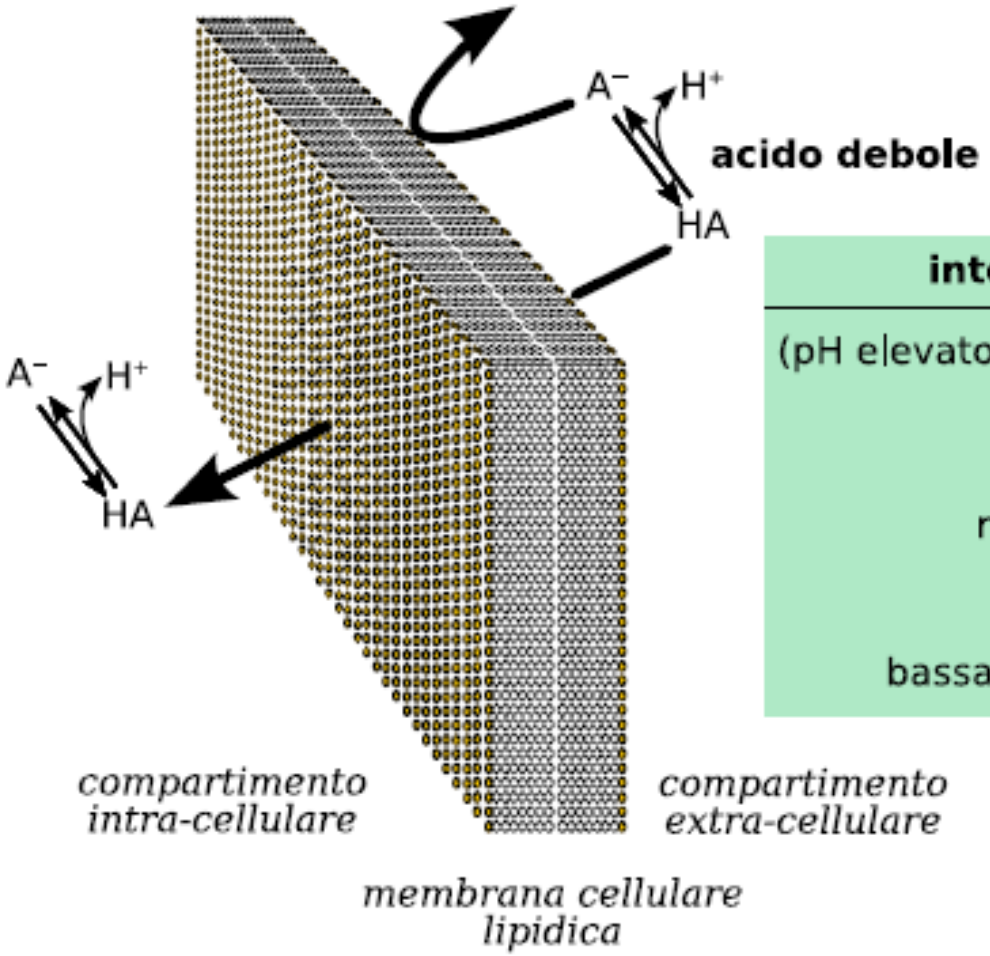
protonation state of organic molecules in dependence of pH



Water solubility and pH :

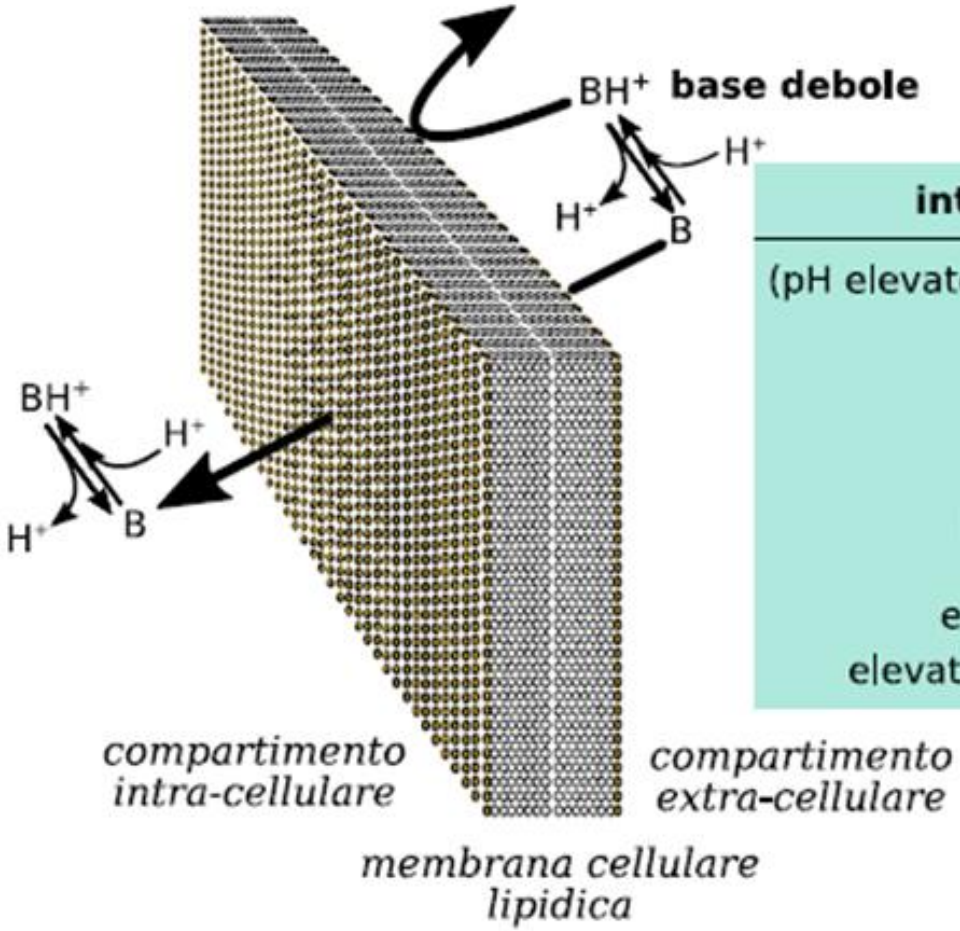


Absorption of carboxylic acids



intestino	stomaco
(pH elevato = bassa $[H^+]$)	(pH basso = elevata $[H^+]$)
$R–COO^-$	$\rightleftharpoons$ R–COOH
non protonato	protonato
carico	non carico
basso $P_{olio/acqua}$	elevato $P_{olio/acqua}$
bassa permeabilità	elevata permeabilità

Absorption of amines

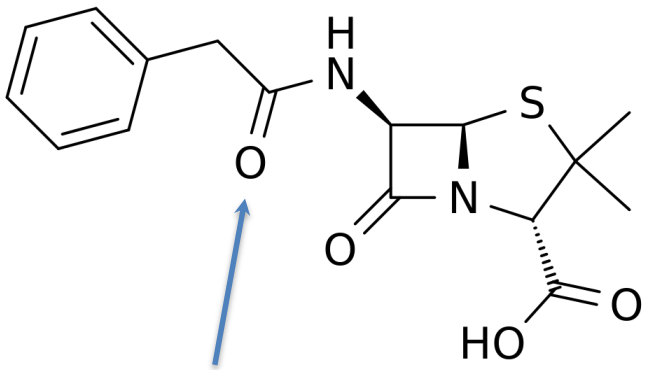


intestino	stomaco
(pH elevato = bassa $[H^+]$)	(pH basso = elevata $[H^+]$)
	
non protonato	protonato
non carico	carico
elevato $P_{olio/acqua}$	basso $P_{olio/acqua}$
elevata permeabilità	bassa permeabilità

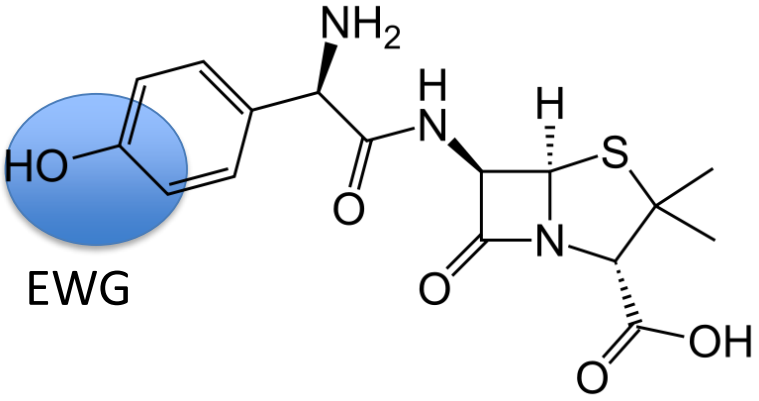
pH stability

- Oral stability: resistance to the stomach acidic pH
- The oral stability can be improved by design

penicillin G
NOT ORALLY ACTIVE
(injectable i.v.)

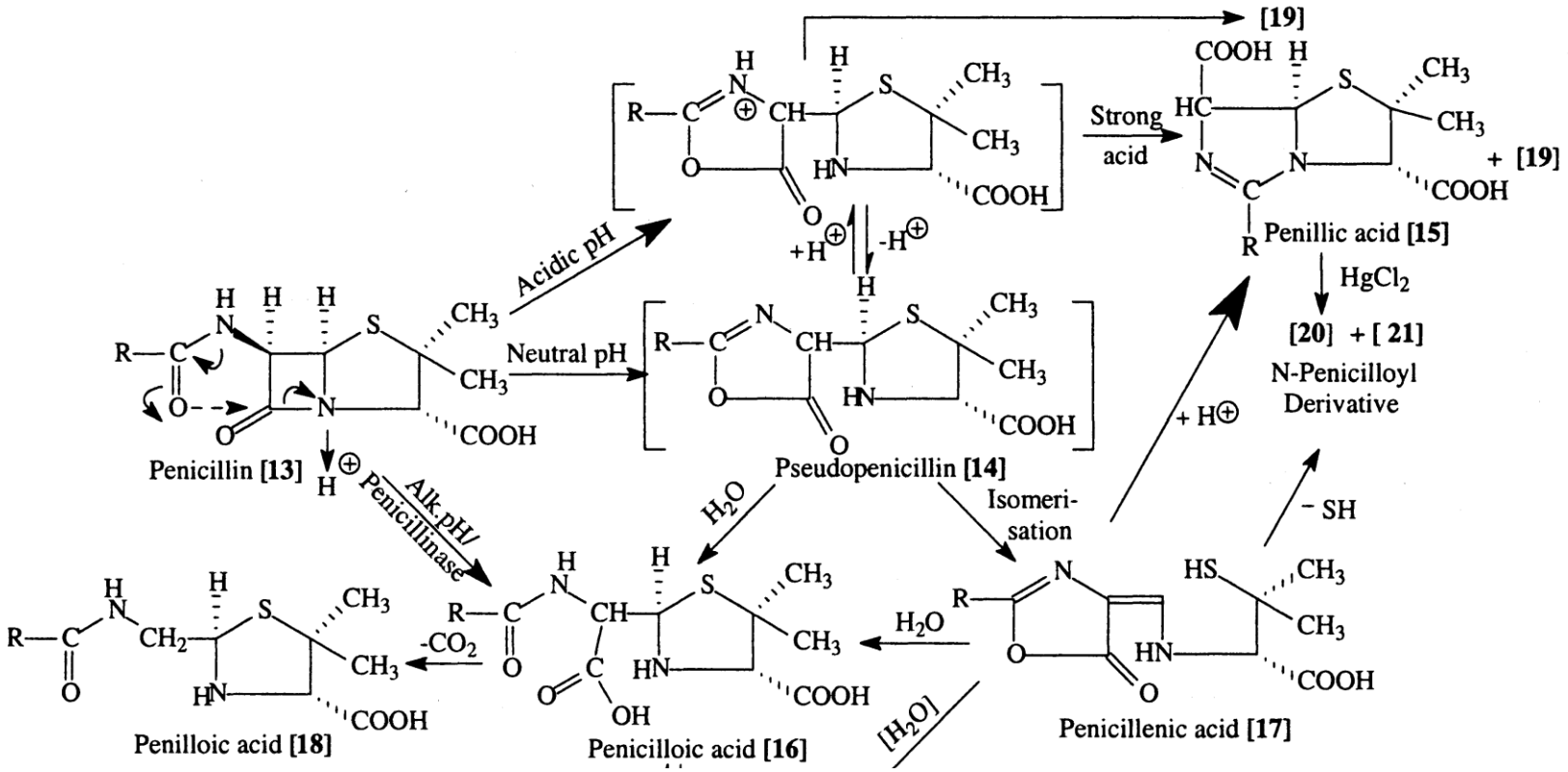


Instability

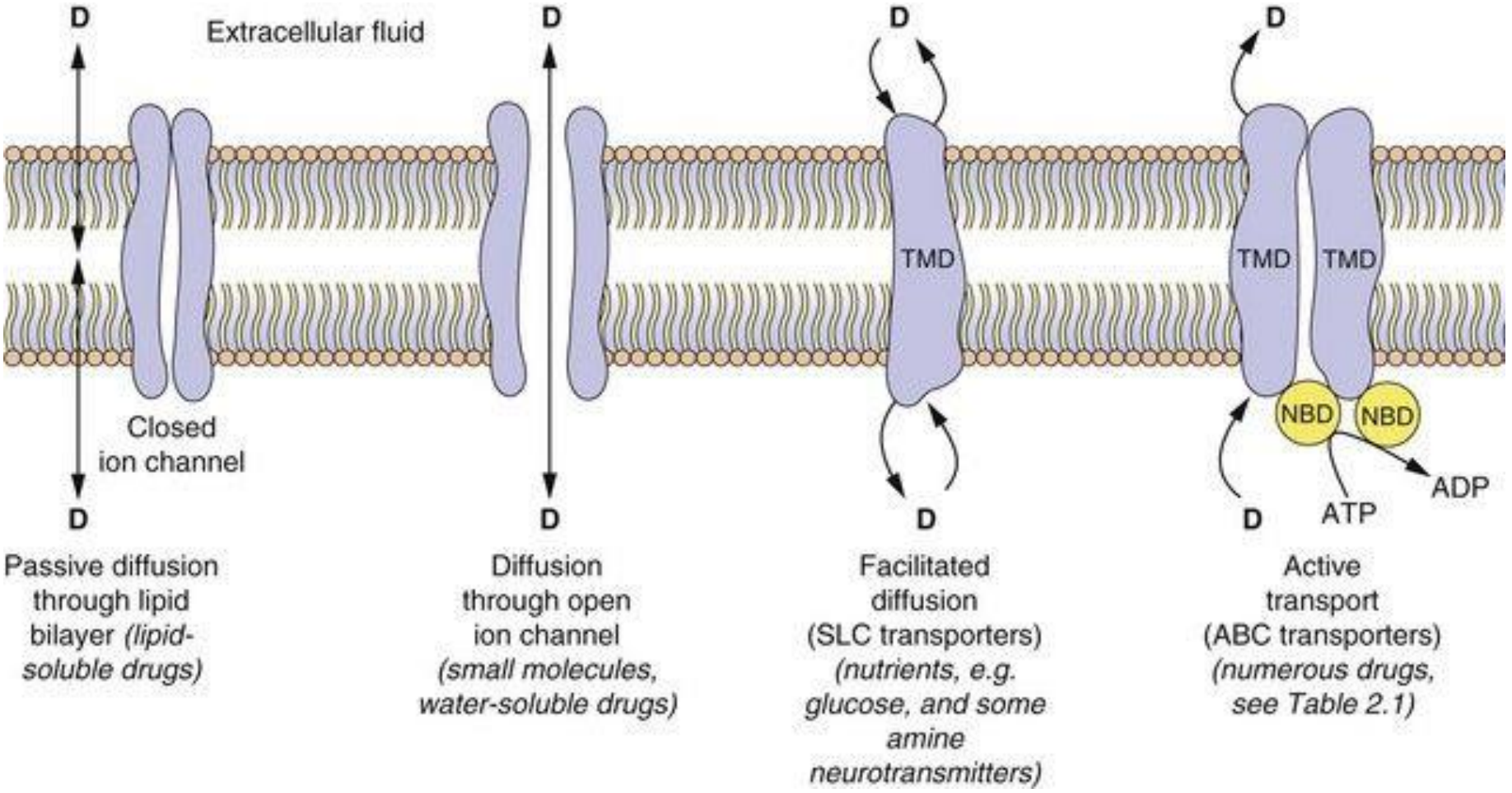


amoxicillin (ORALLY ACTIVE)

Instability of penicillin to acidic conditions (stomach)

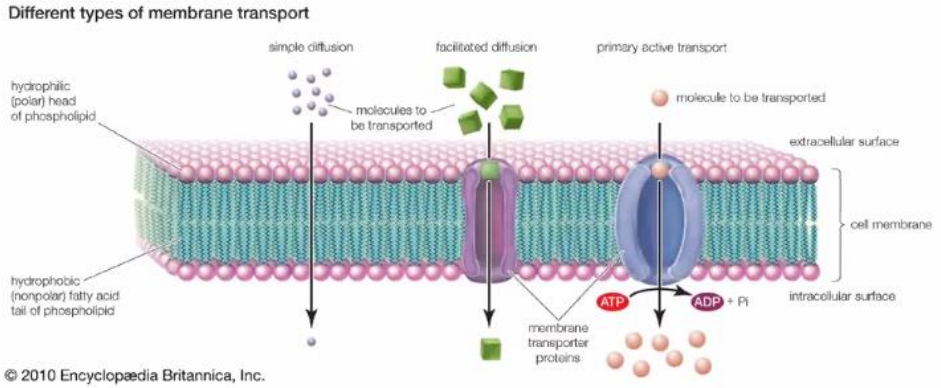


DRUGS ADSORPTION THROUGH MEMBRANES, TISSUE BARRIERS

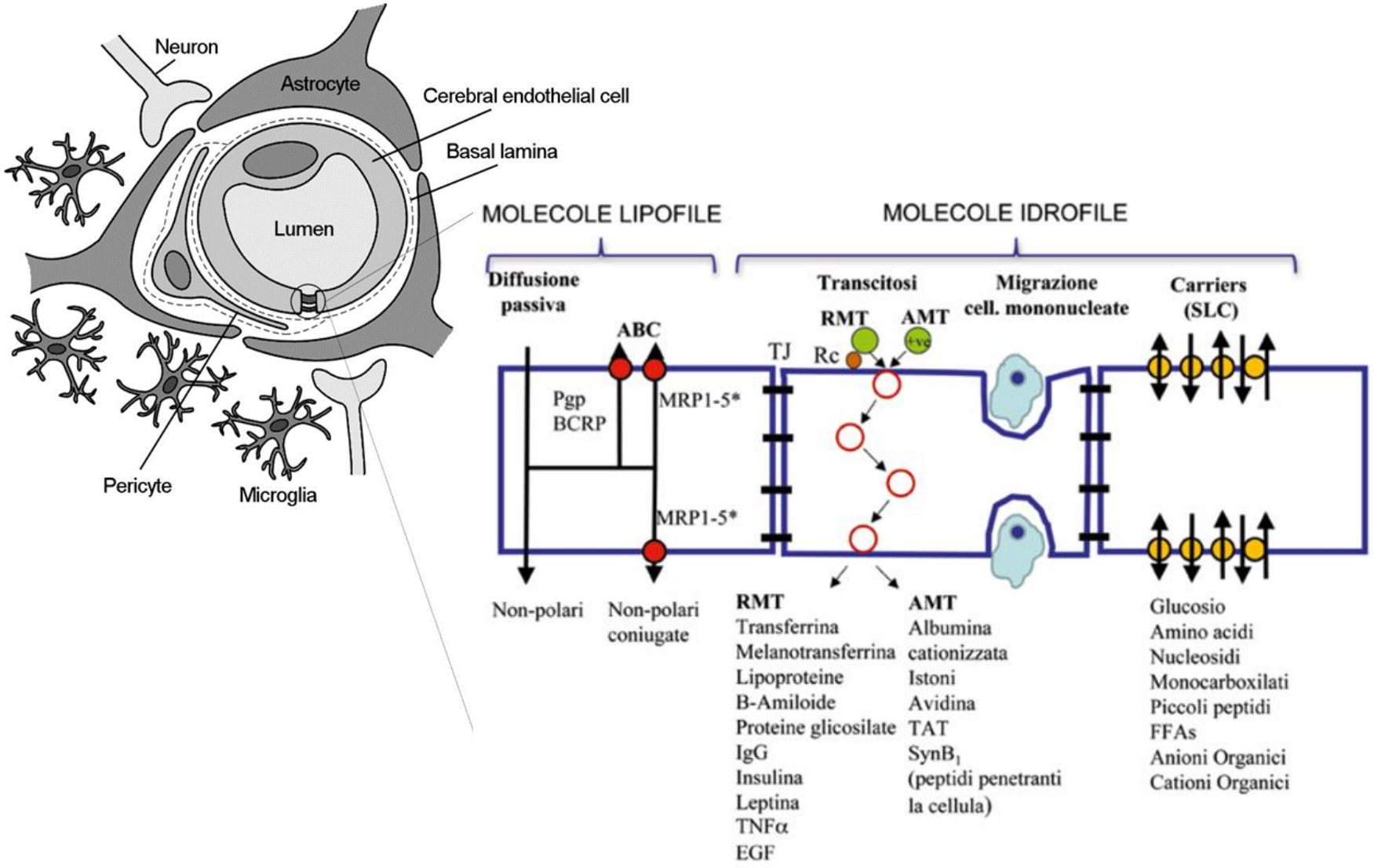


Transporters are Everywhere!

- All biological membranes contain transporters
- Still many of them and their function are unknown
- **It doesn't mean they aren't there!**
  - 10% of human genome encodes
- Solute Carriers (SLCs)
  - Facilitated diffusion
- ATP-Binding Cassette (ABCs)
  - Against concentration gradient

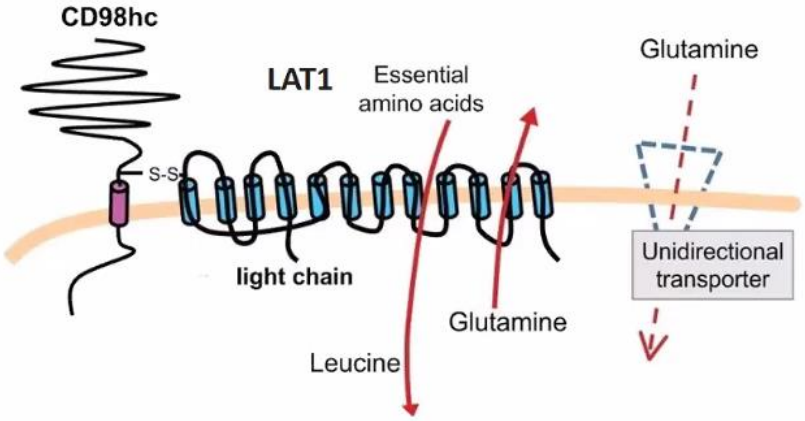


Distribution: the blood-brain barrier (BBB) penetration



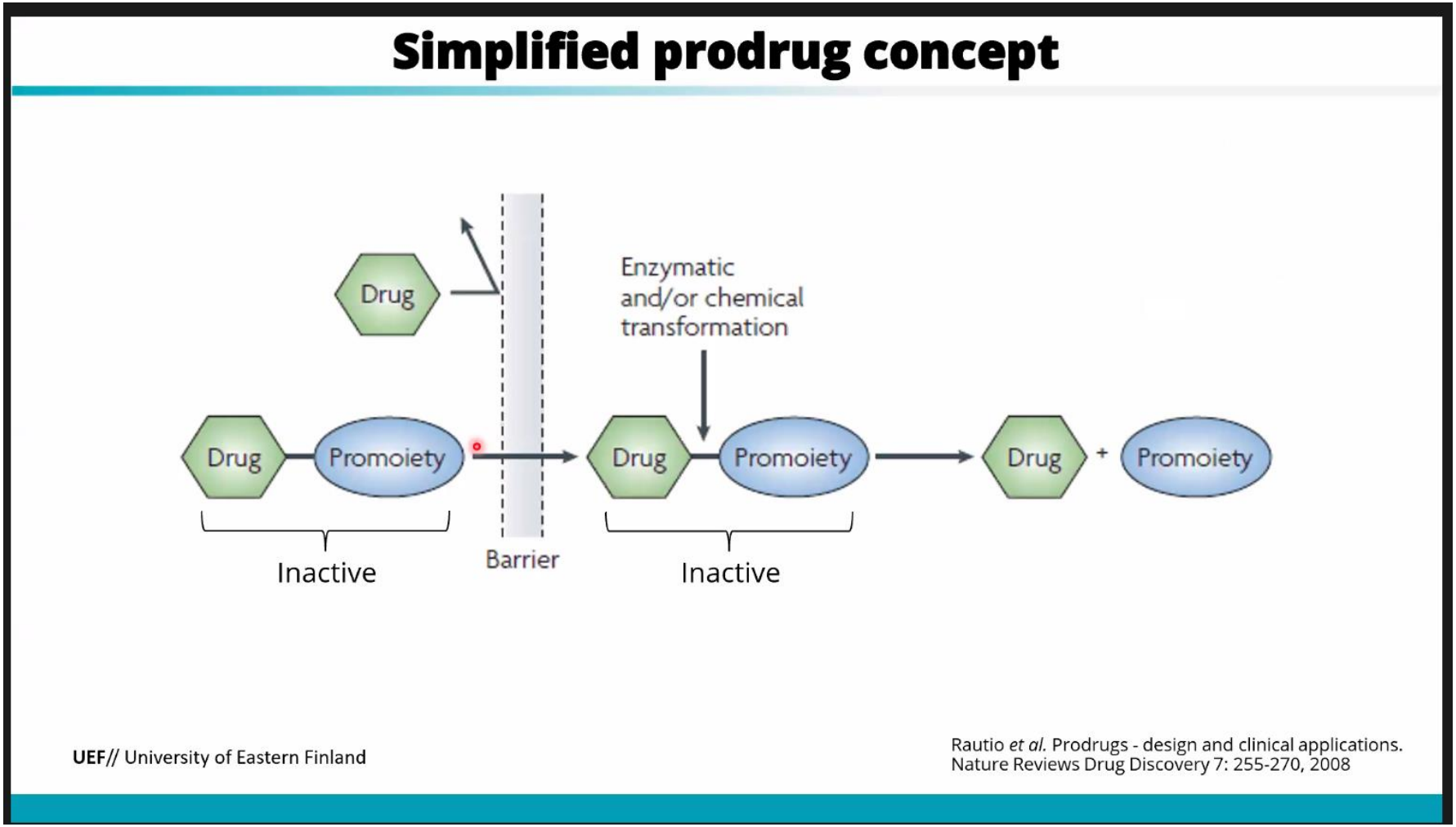
L-Type Amino Acid Transporter 1 (LAT1)

- Na^+- and pH- independent heterodimeric transmembrane protein (SLC)
- Carries bulky & neutral AAs into the cells (L-Leu, L-Phe, L-Trp, L-Tyr…)
- Highly expressed
  - **Blood-Brain Barrier (BBB)**
    - Luminal and abluminal sides
  - Several types of cancer cells
- Carries also drugs
  - **Brain/tumor drug disposition!**



Cantor et al, J. Cell Sci. 2012, 125, 1373-1382

Prodrugs helps in adsorption

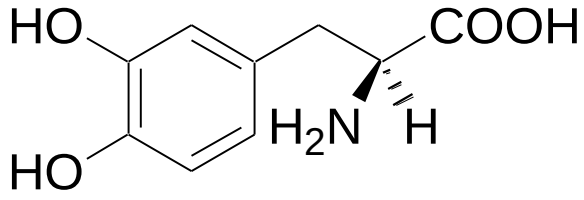


Prodrugs

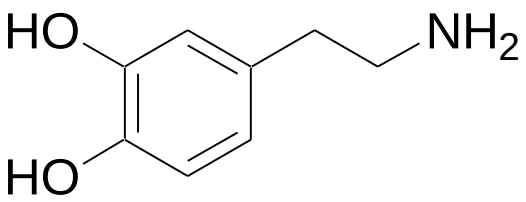
- During 2006–2008, many prodrugs entered the market. Most are esters or amides that are hydrolyzed in vivo.
- Prodrugs are inative compounds that are activated into the corresponding drug in the organism by a chemical or enzymatic trasformation.
- Prodrug strategies to overcome some problems linked to: 1) drug stability in acids, 2) membrane permeability, 3) bad flavour, 4) short half-life
- The other products generated in the activation process should be not toxic for the organism
- Approximately 10% of all marketed drugs worldwide can be considered prodrugs.

The "Troian horse" approach

- The drug is masqued into a prodrug that mimics the substrate of a TRANSPORT PROTEIN
- The LEVODOPA is the prodrug of DOPAMINE and mimics the natural amino acid Tyrosine that is carried into the CNS by BBB transporters
- Dopamine is used to cure Parkinson that is caused by a deficit in this neurotransmitter.
- After dopamine enters into the CNS, a decarboxylase enzyme transforms it into dopamine.

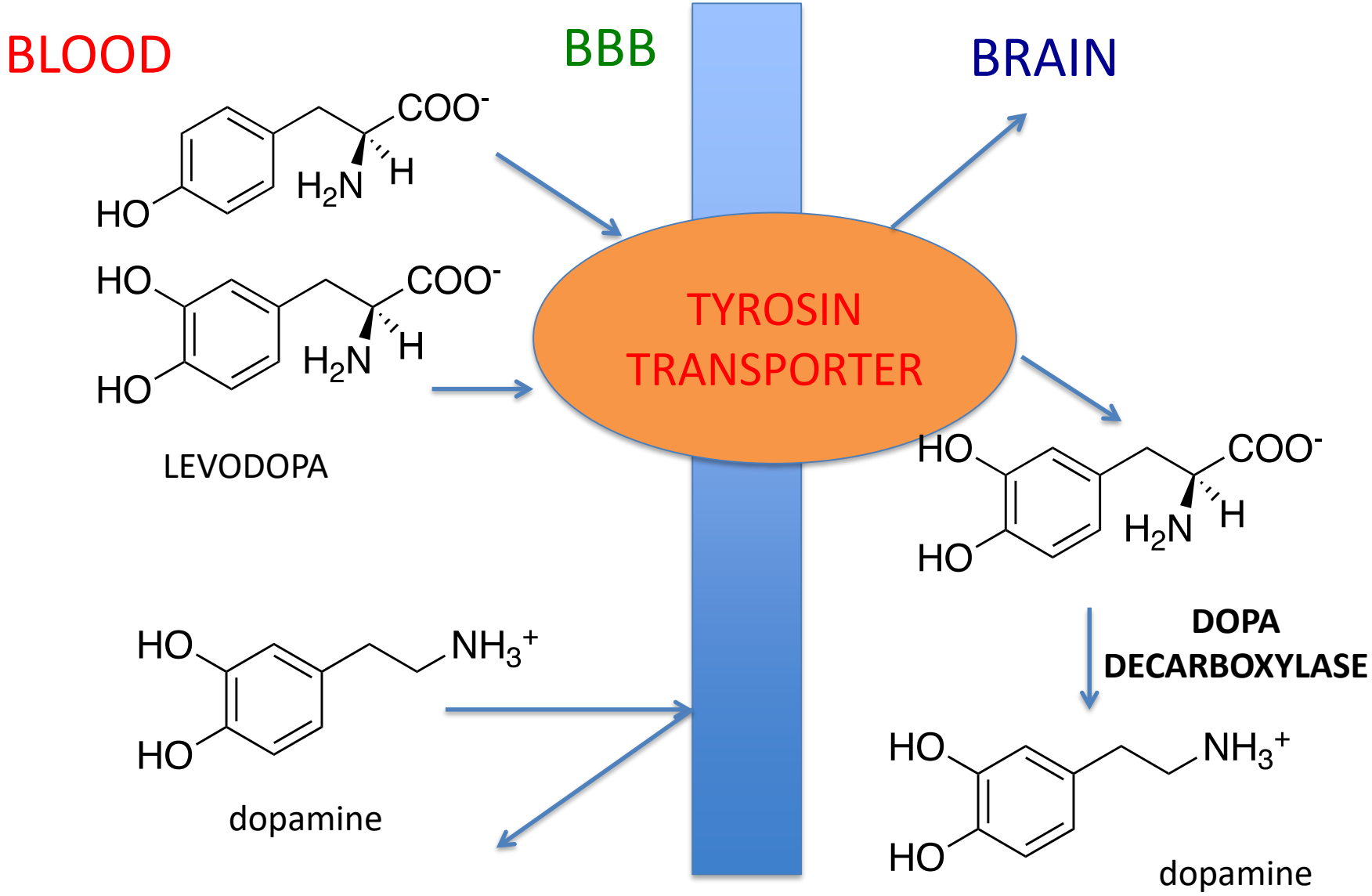


LEVODOPA

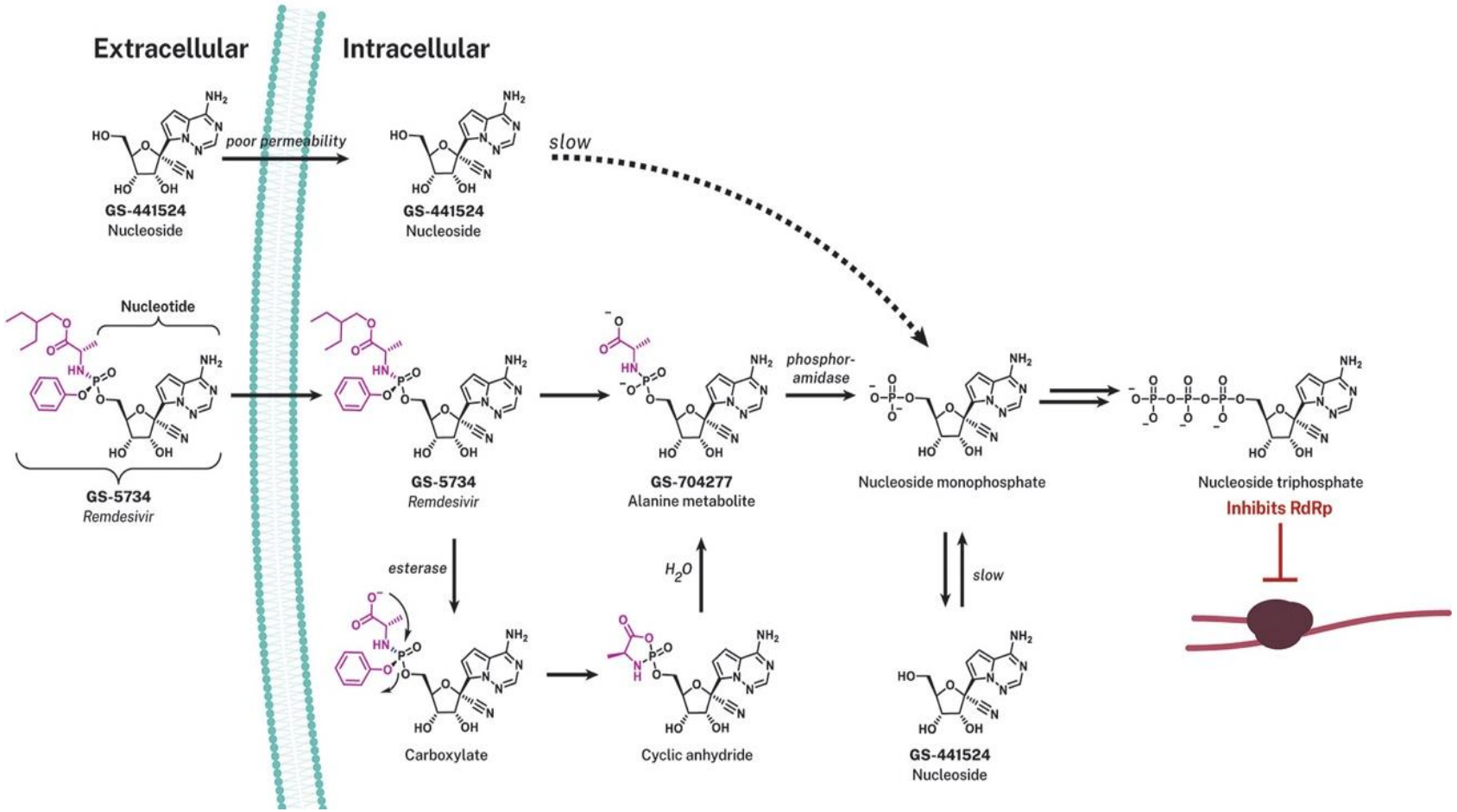


DOPAMINA

levodopa is transported into the CNS and then decarboxylated to dopamine



The ProTide remdesivir



Eastman R.T., Roth J.S., Brimacombe K.R. *et al. ACS Cent. Sci.* 2020, 6, 672−683 DOI: 10.1021/acscentsci.0c00489