Metodologie di Sintesi e Sviluppo Farmaceutico

Synthesis and Development Pharmaceutical Methodologies

Laurea Magistrale in Chimica a.a. 2018/2019

Drug Phases

How a drug do its job inside the body

- How a molecule act in a biological system
- How a molecule interact in its surrounding
- Columbian forces, HBA/HBD, Van de Waals forces

Marco L. Lolli University of Torino (UniTO)

FASI DELL'AZIONE DI UN FARMACO

Forma Farmaceutica

Compresse,
capsule,sciroppo, supposte,
preparazioni iniettabili, etc.

Fase Farmaceutica

Somministrazione
Liberazione dalla forma farmaceutica
Passaggio in soluzione

Fase Farmacocinetica

Assorbimento
Distribuzione
Metabolismo
Escrezione

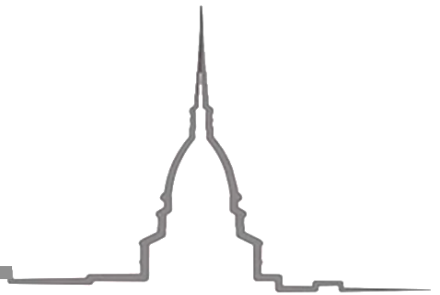
ADME

Fase Farmacodinamica

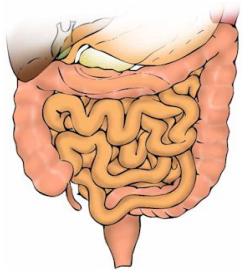
Interazione
Farmaco-sito d'azione

Biofase

Effetto



Fase **Farmacinetica**: *ciò che l'organismo fa al farmaco*



Assorbimento

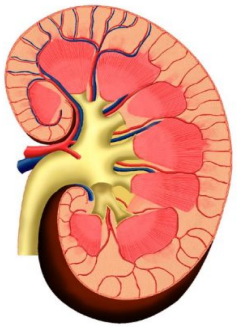
Distribuzione

F-P

F

metaboliti

sangue



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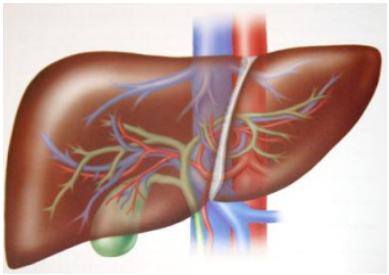


Metabolismo

Studia il movimento dei farmaci nell'organismo ed i processi che determinano la quantità di farmaco disponibile al sito di azione

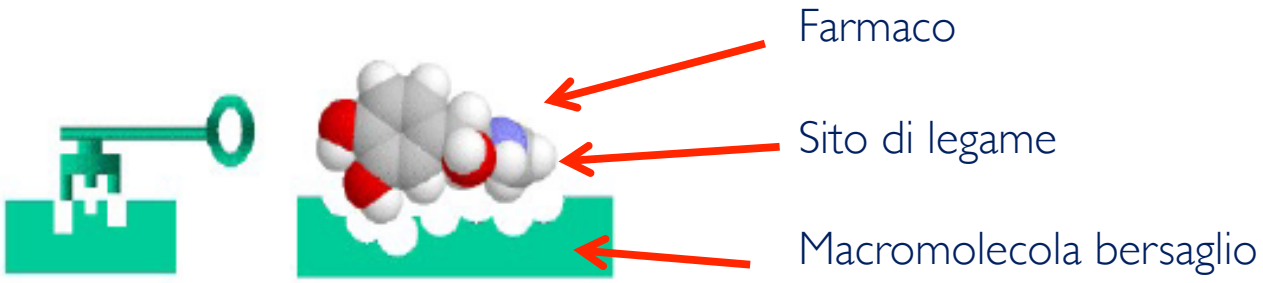
Fase **Farmacinetica**: *ciò che l'organismo fa al farmaco*

Da cosa dipende il tempo che intercorre dalla somministrazione all'effetto?

- *formulazione*
- *via di somministrazione (e.v.: l'assorbimento non è necessario)*
- *presenza di altri farmaci o alimenti*
- *proprietà chimico-fisiche del farmaco:*
  - *solubilità*
  - *grado di ionizzazione*
  - *lipofilia*
  - *dimensione molecolare*

Fase **Farmacodinamica**: *ciò che il Farmaco fa all'organismo*

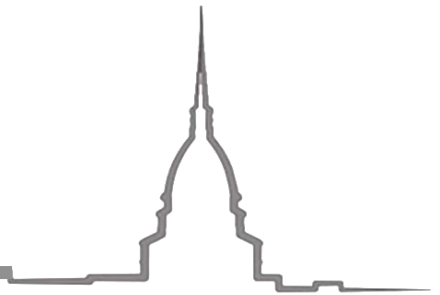
Studio del comportamento del farmaco nella BIOFASE ovvero di come interagisce con il suo bersaglio macromolecolare attraverso le interazioni di legame.



Fase **Farmacodinamica**: *ciò che il Farmaco fa all'organismo*

I farmaci possono essere:

- **Strutturalmente aspecifici:** non hanno un bersaglio molecolare ben definito
- **Strutturalmente specifici:** agiscono attraverso l'interazione con un recettore o un enzima



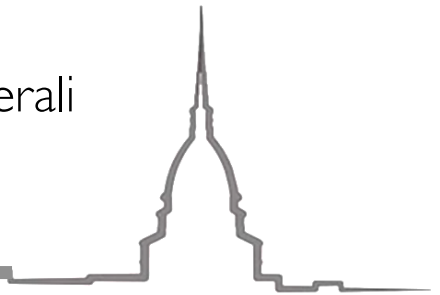
Farmaci strutturalmente aspecifici:

Esplicano la loro azione in funzione NON delle loro capacità di interazione con siti altamente elaborati sotto il profilo strutturale e conformazionale ma con generiche zone di interazione nella biofase, ad esempio le membrane.

Esempio:

- *diuretici osmotici*,
- *antiacidi* (es. $NaHCO_3$),
- *disinfettanti* (es. alcol, detergenti, agenti antiossidanti),
- *anestetici generali.*

La loro attività, insensibile a piccole modificazioni strutturali, dipende essenzialmente dalla loro concentrazione nella biofase e dalle loro caratteristiche chimico-fisiche (es. pK_a, potenziale redox, ecc.). Ad esempio l'azione degli anestetici generali è correlata al loro Log P.

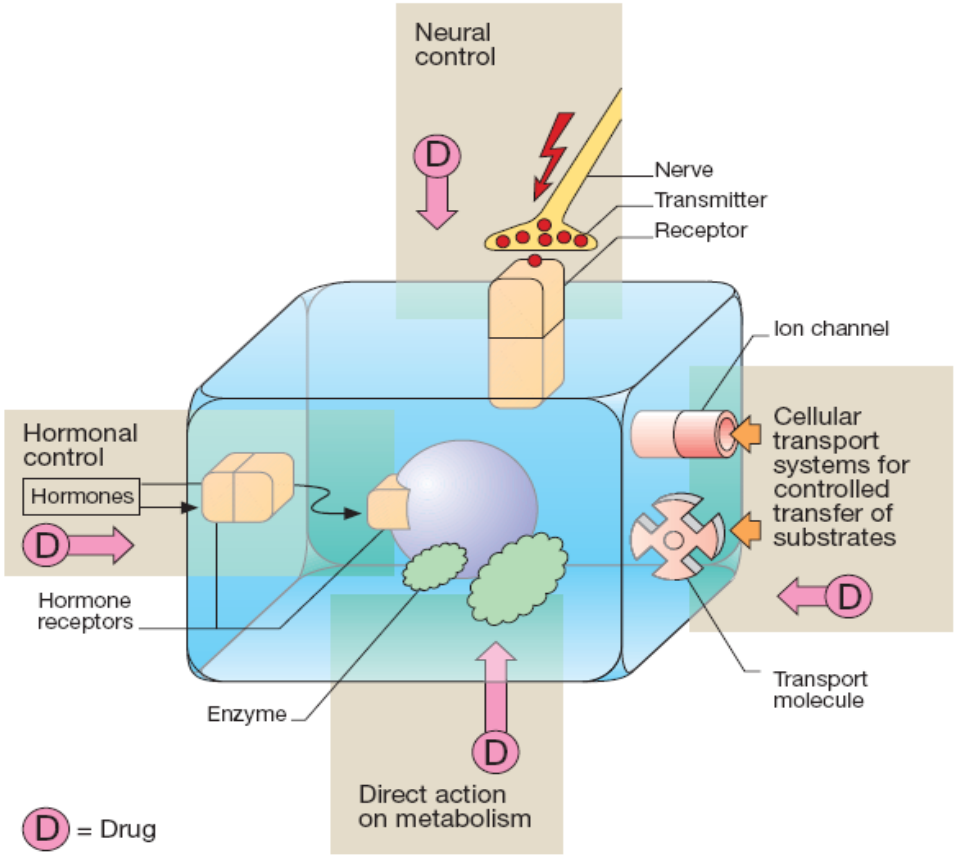


Farmaci strutturalmente specifici:

Caratterizzati da rigorosi requisiti strutturali, elevata potenza e da interazioni competitive.

Bersagli dei farmaci strutturalmente specifici:

Macromolecole quali proteine (enzimi, recettori, proteine di trasporto) ed acidi nucleici (DNA, RNA).



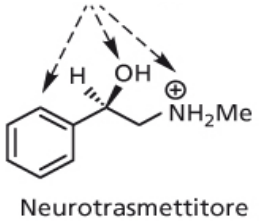
Drug targets:

- Recettori
- Enzimi

Gruppi funzionali e residui carboniosi presenti nel farmaco interagiscono con gruppi funzionali e residui carboniosi complementari presenti nel recettore.

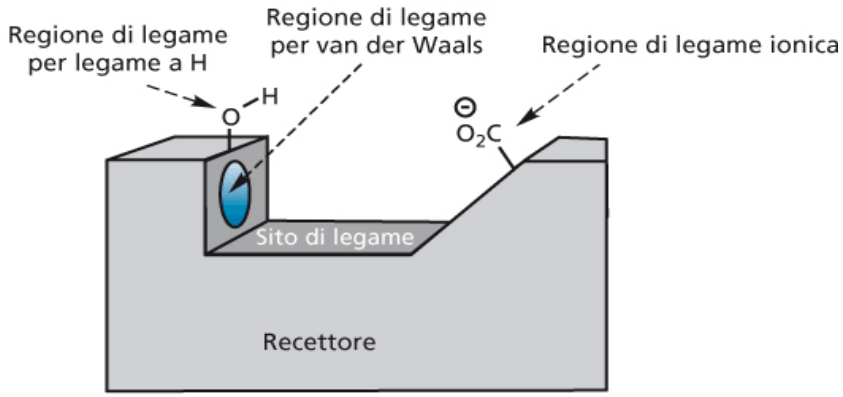
I primi si definiscono **gruppi di legame**, i secondi **siti di legame**.

Gruppi di legame:



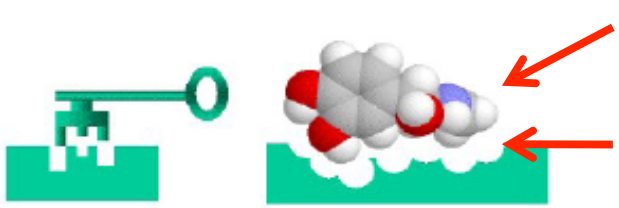
Neurotrasmettitore

Siti di legame:



Sito di legame (sito di *binding*): piccola e specifica zona della macromolecola in cui ha luogo l'interazione di legame tra il farmaco e la macromolecola stessa.

Tipicamente una insenatura o una fenditura nella superficie macromolecolare.
La complementarietà strutturale richiesta è analoga a quella richiesta tra una chiave ed una serratura.

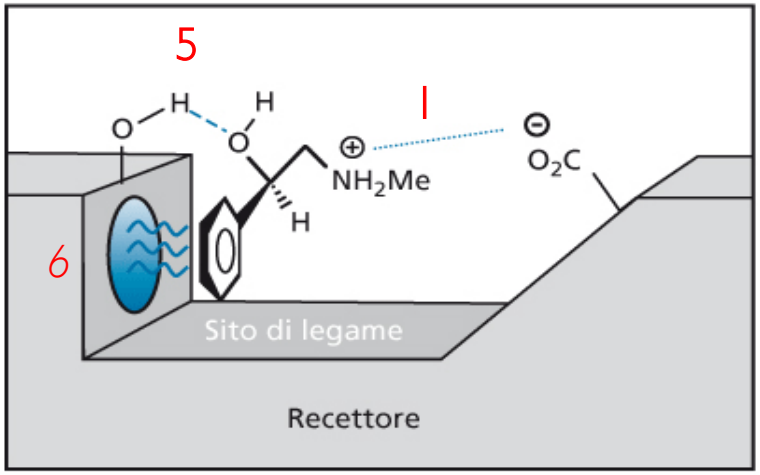


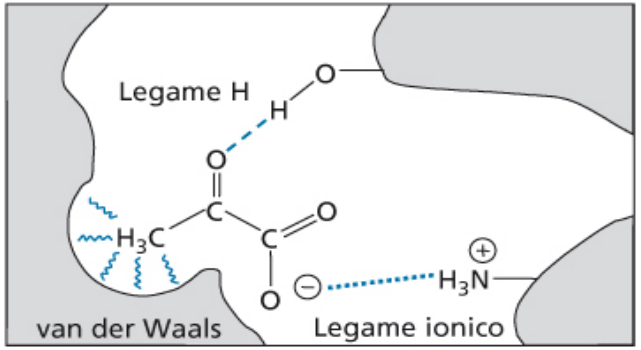
Interazioni coinvolte nel complesso Farmaco - Recettore

1. legame ionico
2. interazioni ione-dipolo
3. interazioni dipolo-dipolo
4. interazioni ione – dipolo indotto
5. legame idrogeno
6. forze di dispersione di van der Waals
7. interazioni idrofobiche
8. legami covalenti

Interazioni di natura elettrostatica

Possibili interazioni





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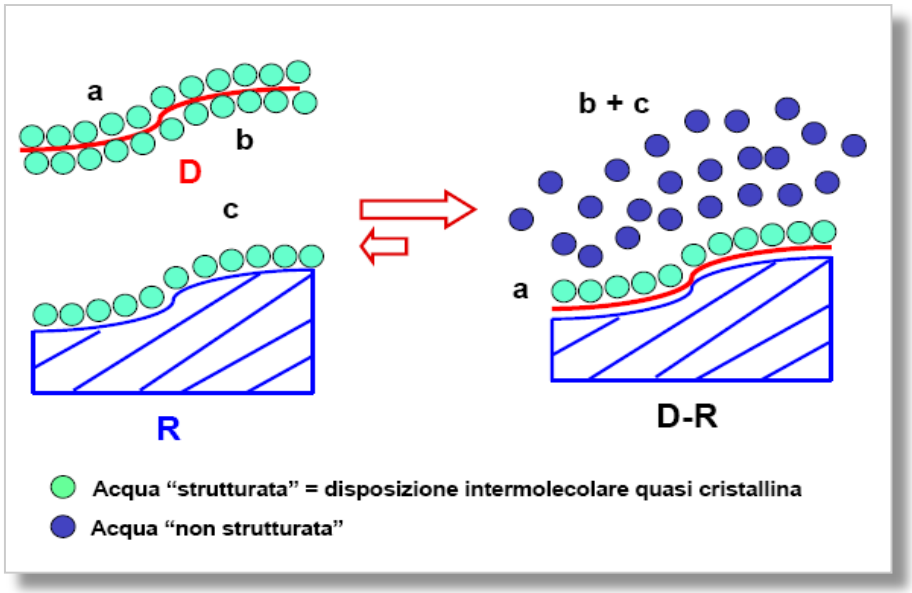
Stesse interazioni intermolecolari coinvolte in mp. bp e solubilità

L'acqua però non è in grado di solvatare regioni idrofobiche e non polari del ligando e del sito di legame. In questo caso le molecole d'acqua interagiscono tra loro più efficacemente del normale formando strati d'acqua molto ordinati. Ciò genera un'entropia negativa causata dall'aumento dell'ordine.

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



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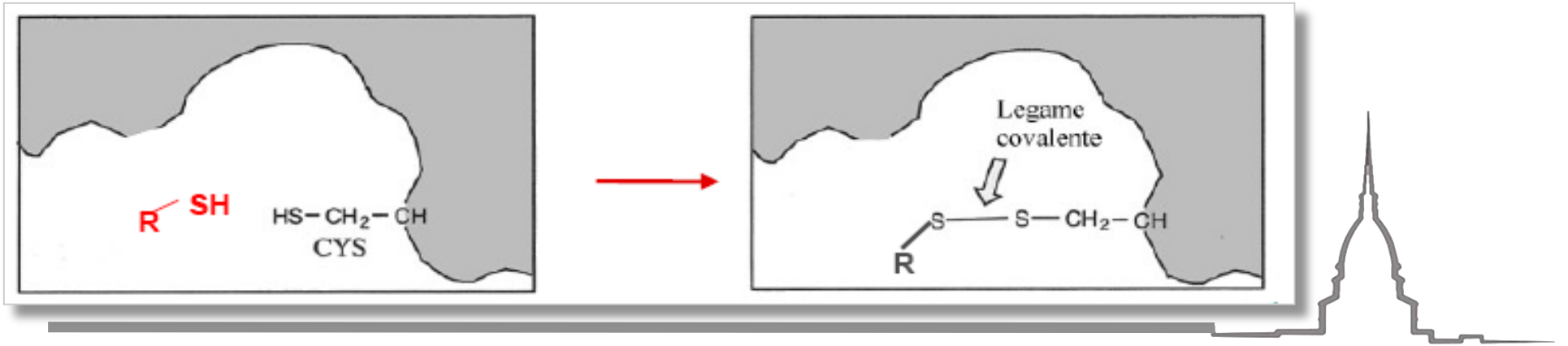
Stesse interazioni intermolecolari coinvolte in mp. bp e solubilità

Nel caso ci sia una reazione tra un gruppo funzionale del ligando e il recettore, allora c'è la possibilità che si formi un legame stabile, covalente, di una più alta energia (40-110 $kJmol^{-1}$). Si formerà pertanto un complesso irreversibile o difficilmente reversibile.

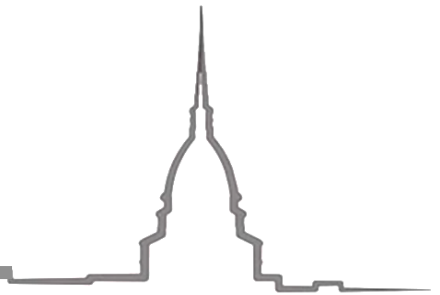


Druggability

Ligand side

….Understanding the difference between *biologically active* small molecules and *drugs* became a priority in the drug discovery process, and the importance of addressing pharmacokinetic properties early during lead optimization is a clear result…….

From:
Keller *at al.*
A practical view of 'druggability'. Current Opinion in Chemical Biology **2006**, 10:357–361



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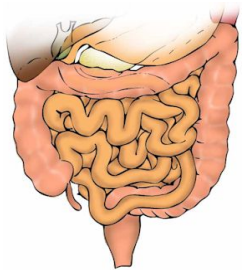

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metaboliti

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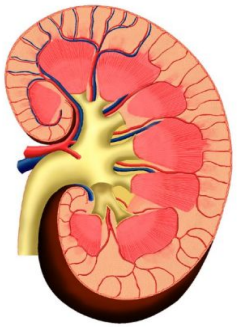

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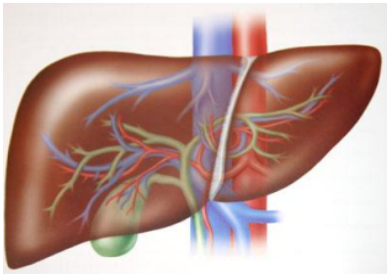

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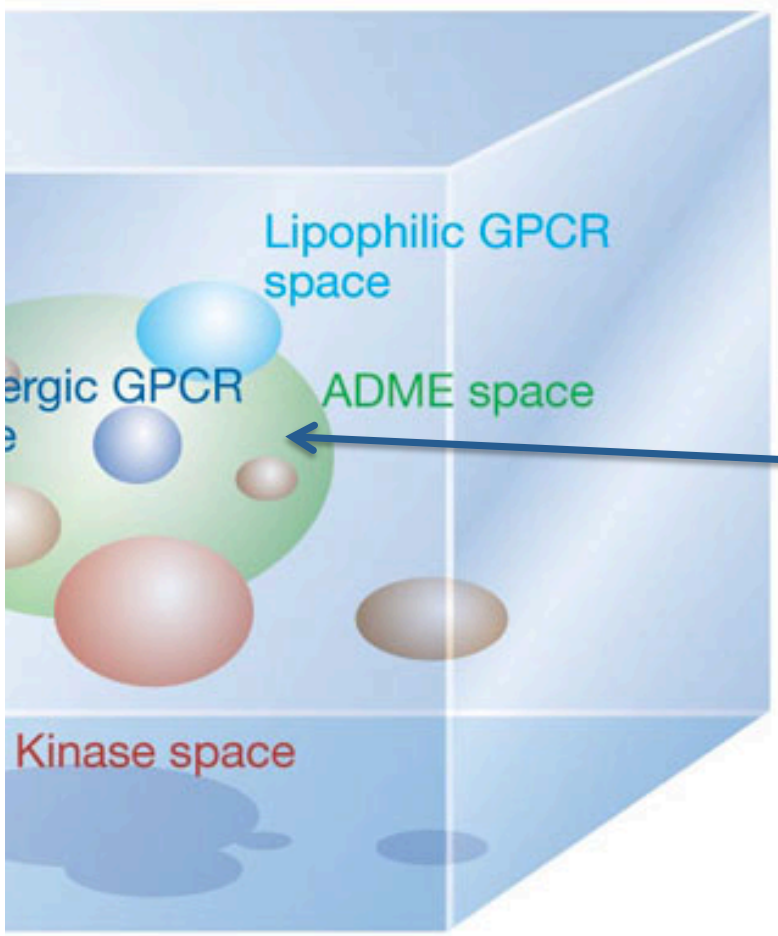
The Chemical space:

over 10^{60} conceivable compounds



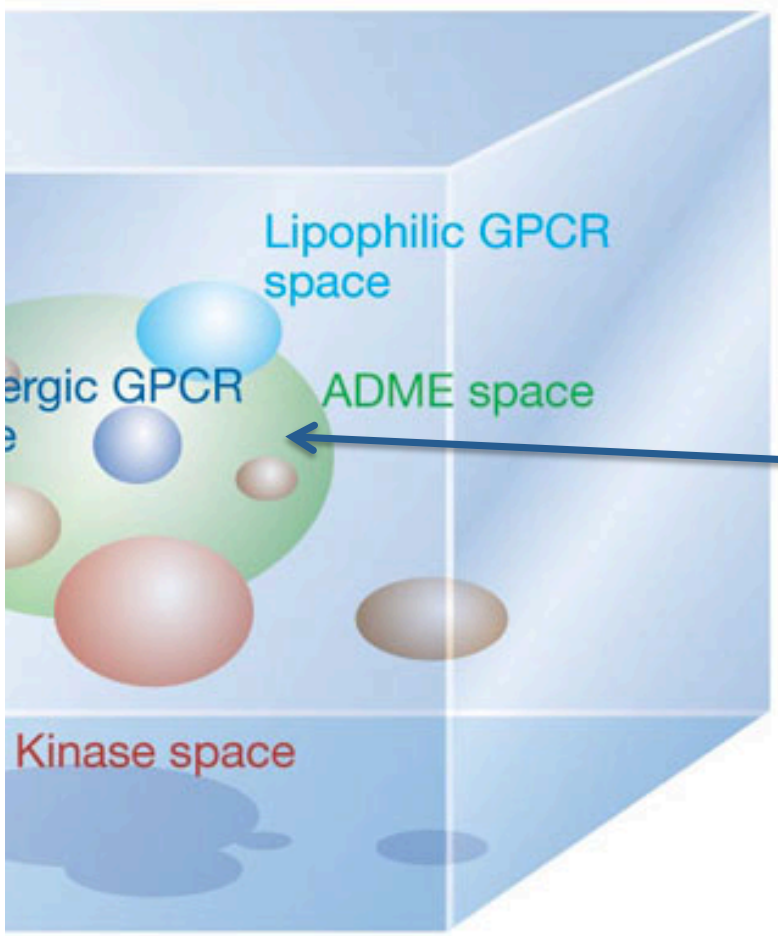
ADME- T Space

Adsorbtion
Distribution
Methabolism
Escretion

Here is where the drus are

The Chemical space:

over 10^{60} conceivable compounds



SOSA approach

Selective
Optimization
Side
Activities

Looking for inside the ADME space for new opportunities

Druggability

Ligand side

The Lipinski "rule of five"

In the original Lipinski paper was started hat poor absorption or permeation are more likely when:

1) In the structure are present more than 5 hydrogen-bond donors (HBD).
2) The MWT is over 500 Daltons
3) The LogP is over 5 (or MLogP is over 4.15).
4) There are more than 10 H-bond acceptors.
5) Substrates for biological transporters are exceptions to the rule.

Lipinski CA, Lombardo F, Dominy BW, Freeney PJ. *Experimental and computational approaches to estimate solubility and per- meability in drug discovery and development settings*. **1997**. *Adv Drug Deliv Rev 23*, 3 - 25.

Druggability

Target side

Journal of Medicinal Chemistry

Perspective

pubs.acs.org/jmc

New Frontiers in Druggability

Dima Kozakov,*,† David R. Hall,‡ Raeanne L. Napoleon,§ Christine Yueh,|| Adrian Whitty,*,§ and Sandor Vajda*,§,||

…….Many biologically compelling drug targets belong to other protein families that **lack such empirical proof of principle that they can be inhibited by small molecule drugs**. Consequently, approaches to assessing the druggability of potential drug targets, i.e., the likelihood of being able to identify a druglike small ligand that can modulate the activity of the target, have emerged as an important tool for target-based drug discovery.

J Med Chem. **2015**, 58(23), 9063 - 88.

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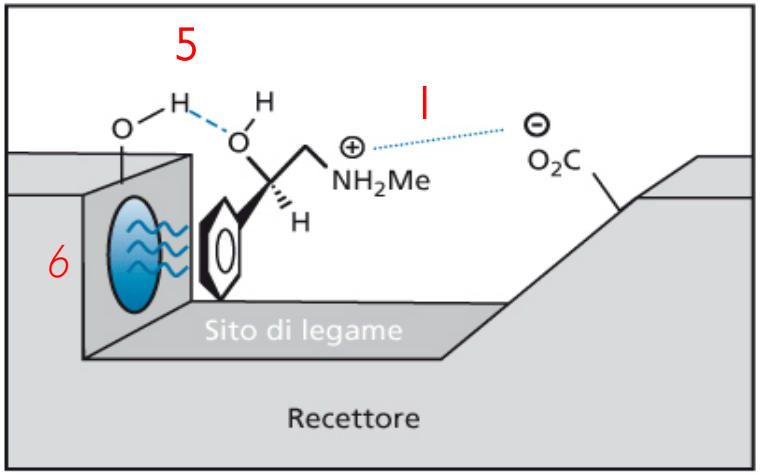
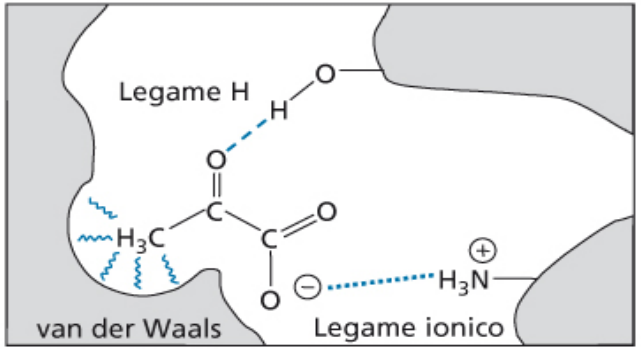
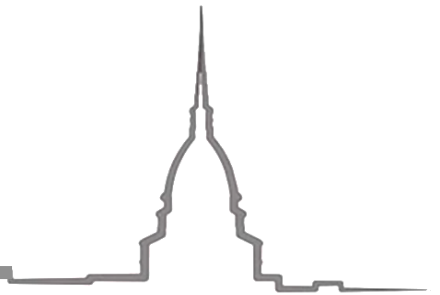




Intermolecular interaction forces

Weak forces that rule any biological related mechanism. Such forces rules also the involvement of a chemical structure with its surrounding, they influence both **pharmacokinetics** and **dynamic**

- Columbian forces
- Hydrogen bond
- Van der Waals



Electronegativity

"The tendency of an atom to attract electrons (or electron density) towards itself."

TABLE 1.3 The Electronegativities of Selected Elements[a]

IA	IIA	IB	IIB	IIIA	IVA	VA	VIA	VIIA
H 2.1								
Li 1.0	Be 1.5			B 2.0	C 2.5	N 3.0	O 3.5	F 4.0
Na 0.9	Mg 1.2			Al 1.5	Si 1.8	P 2.1	S 2.5	Cl 3.0
K 0.8	Ca 1.0							Br 2.8
								I 2.5

increasing electronegativity

increasing electronegativity

[a]Electronegativity values are relative, not absolute. As a result, there are several scales of electronegativities. The electronegativities listed here are from the scale devised by Linus Pauling.

(Di)polarity

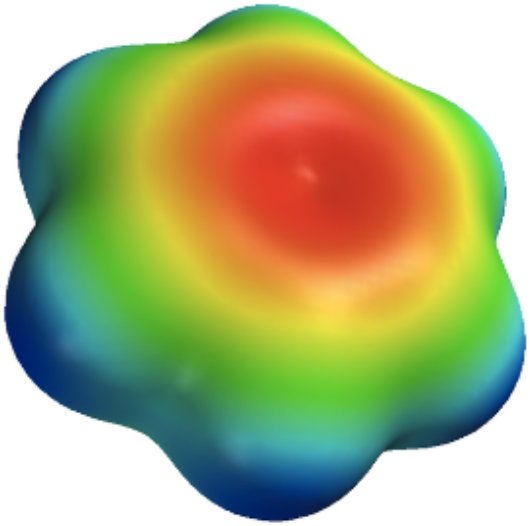
"A bond between two atoms with different electronegativity became polarized, a dipole with opposite charge. The value of the dipolar moment μ indicate such dipolarity

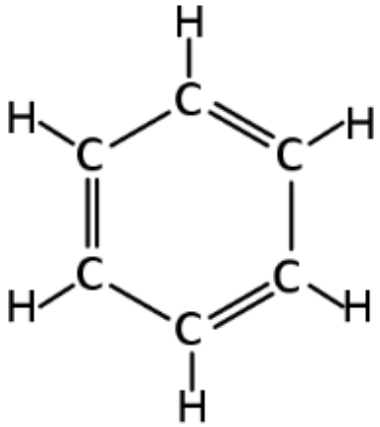
Table 1.4 The Dipole Moments of Some Commonly Encountered Bonds

Bond	Dipole moment (D)	Bond	Dipole moment (D)
H—C	0.4	C—C	0
H—N	1.3	C—N	0.2
H—O	1.5	C—O	0.7
H—F	1.7	C—F	1.6
H—Cl	1.1	C—Cl	1.5
H—Br	0.8	C—Br	1.4
H—I	0.4	C—I	1.2

Electrostatic potential maps

illustrate the charge distributions of molecules three dimensionally.





Usually but not necessarily always, red indicates the lowest electrostatic potential energy, and blue indicates the highest electrostatic potential energy. Intermediary colors represent intermediary electrostatic potentials.

red < **orange** < **yellow** < **green** < **blue**

most negative electrostatic potential

most positive electrostatic potential

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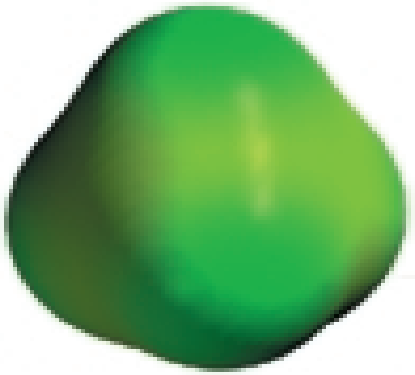
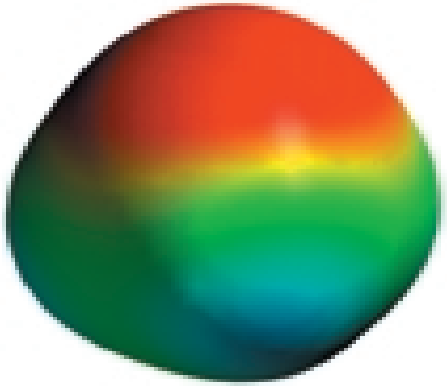
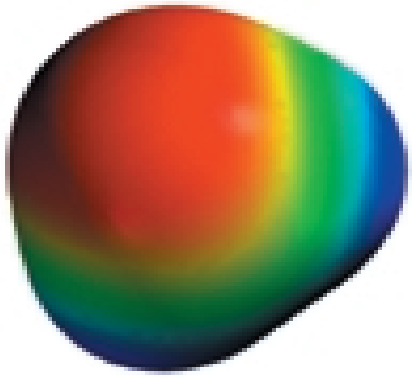


electrostatic potential map for methane



electrostatic potential map for ammonia



electrostatic potential map for water

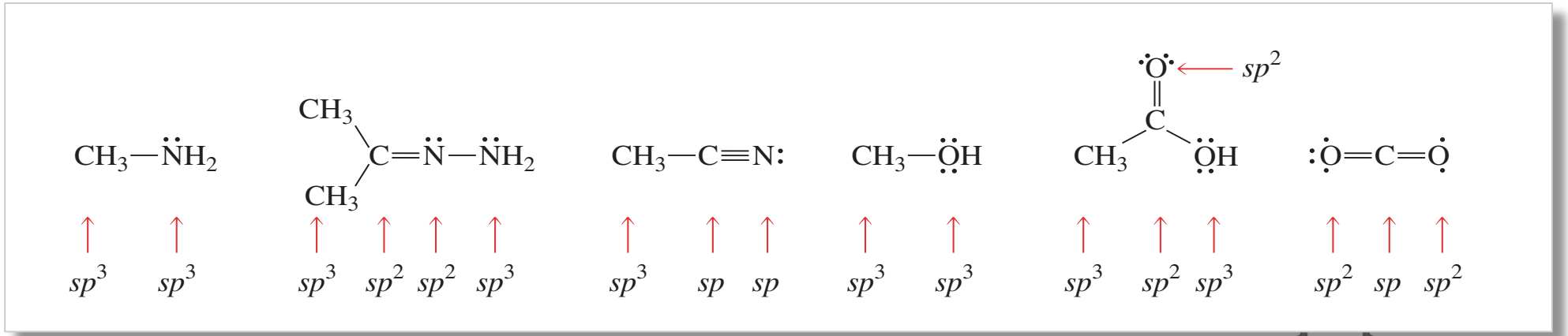
red < **orange** < **yellow** < **green** < **blue**

most negative electrostatic potential

most positive electrostatic potential

Molecular orbital

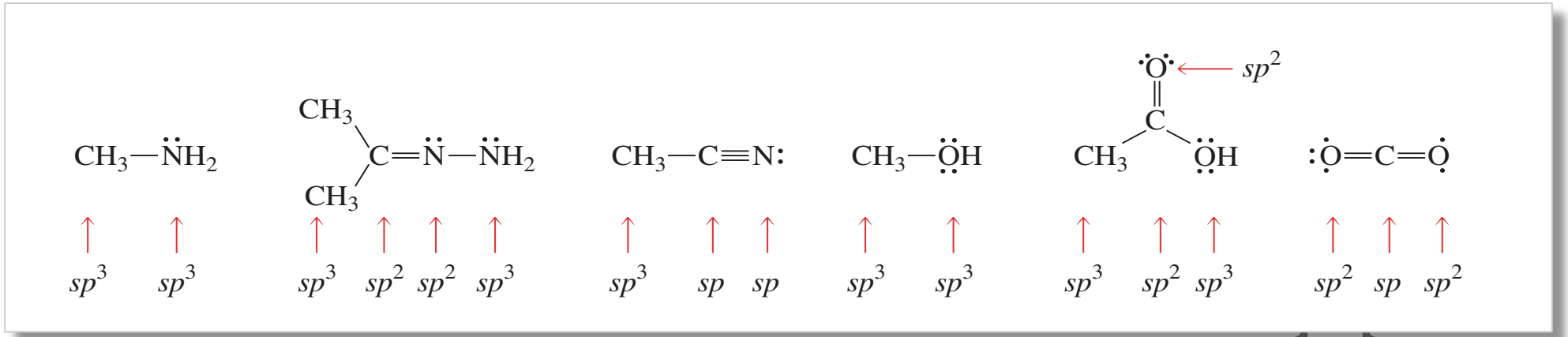
"A molecular orbital (MO) is a mathematical function describing the wave-like behavior of an electron in a molecule. This function can be used to calculate the probability of finding an electron in any specific region."



Inductive effect

"The inductive effect is an experimentally observable effect of the transmission of charge through a chain of atoms in a molecule, resulting in a permanent dipole in a bond"

- Symbols: + I (donation)
  – I (attraction)"

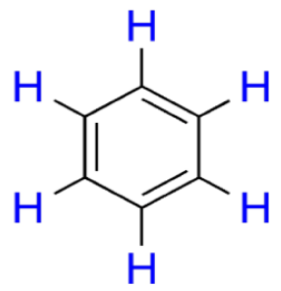


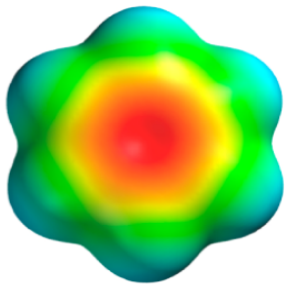
Electrostatic potential maps

illustrate the charge distributions of molecules three dimensionally.

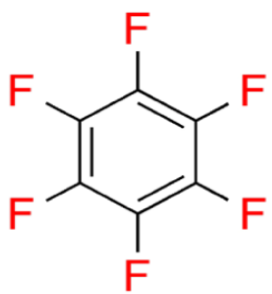
A

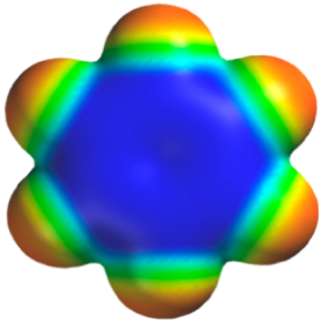
Benzene





Perfluorobenzene





C

Aromatic Ring	LogP	Surface Area ($Å^2$)	Polarizability ($Å^3$)	Quadrupole Moment ($C\ m^{-2}$)
Benzene	2.03	115.07	49.37	-29.9×10^{-40}
Perfluorobenzene	2.98	150.26	51.28	$+31.7 \times 10^{-40}$

Pace & Gao, Acc.Chem.res., 2013, 907-915

Modulation of the inductive effects on the benzene ring

Fluorination is well tolerated without introducing much steric pertubation (C-F 1.34 Å, C-H 1.09 Å)

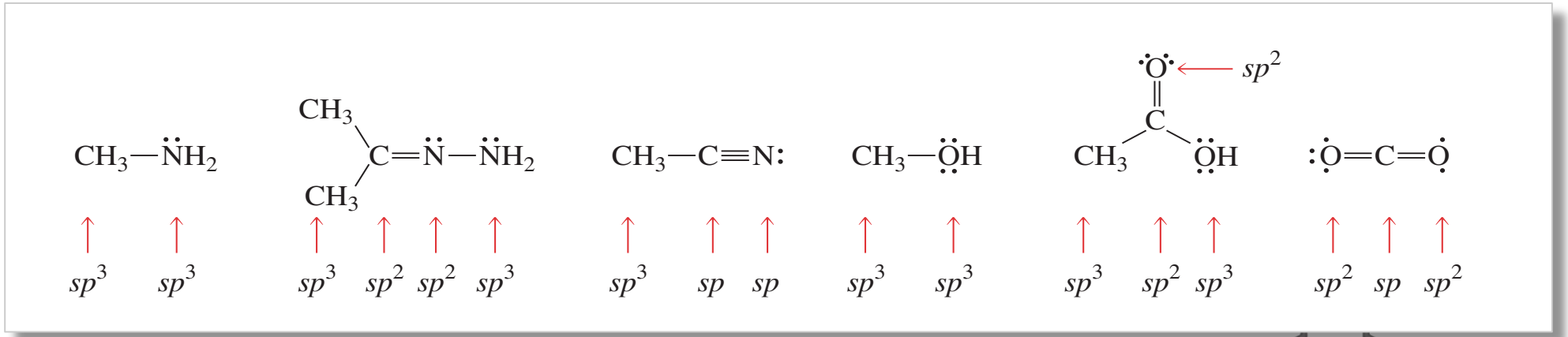
Usually increases the hydrophobicity of the amino acid and thus favors protein folding and stability

Nonexistent in biology, so fluorinated residues can be tracked in complex biological systems with zero background (NMR spectroscopy)

Mesomeric effect

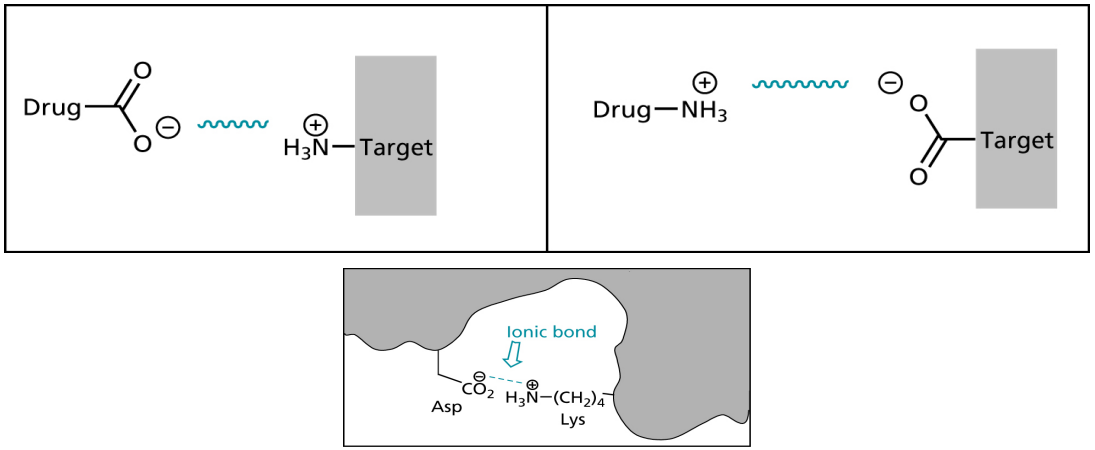
"The capacity of an atom (a functional group) to stabilize a charge o a radical by the generation of a resonance hybrid"

- Symbol: +M (donating)
  – M (attracting)"

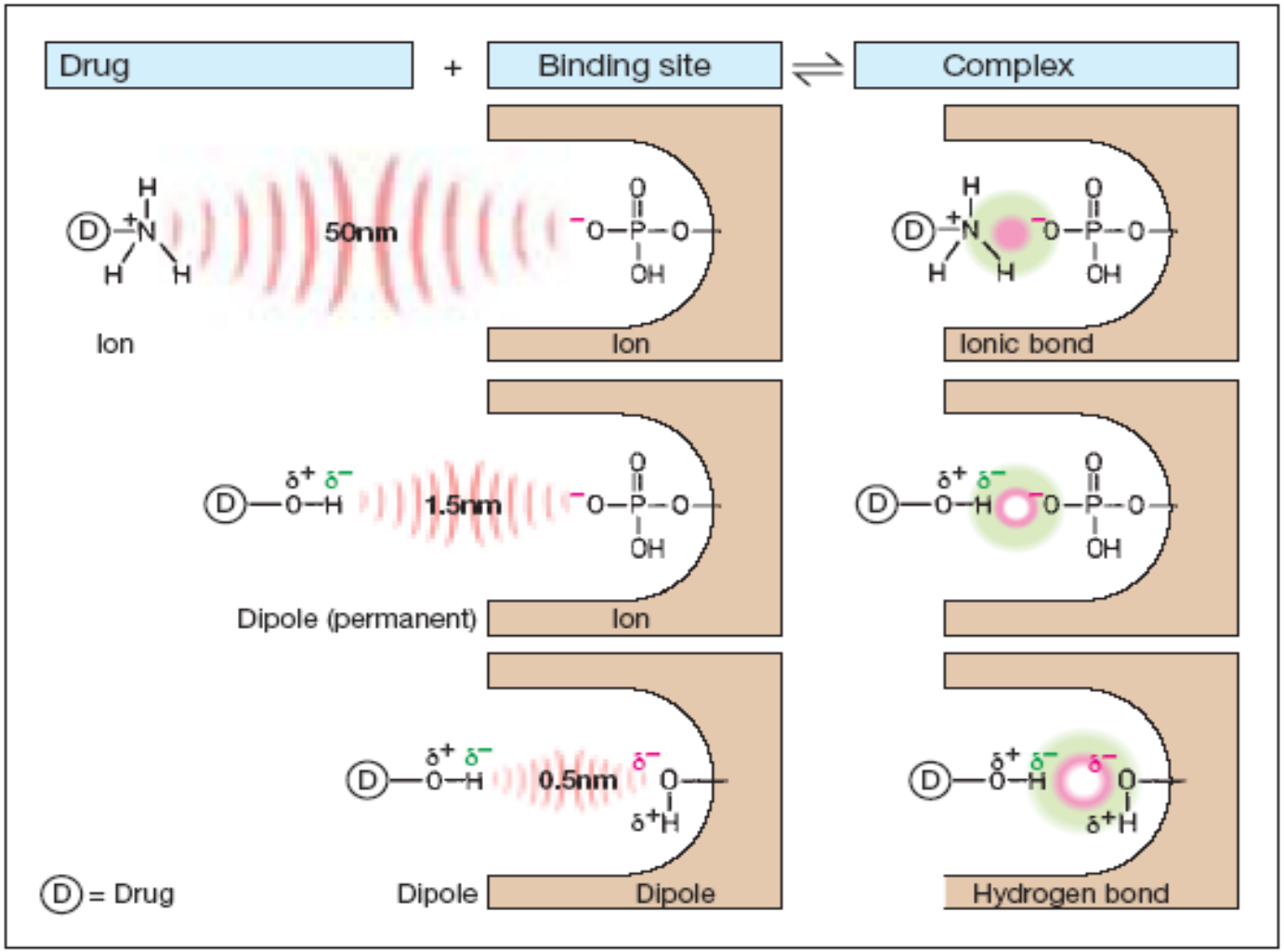


Ionic bond (Columbian forces)

- Strongest of the intermolecular bonds
- Ionic bonds are the most important initial interactions as a drug enters the binding site
- Takes place between groups of opposite charge
- The strength of the ionic interaction is inversely proportional to the distance between the two charged groups (drops off less rapidly with distance than with other forms of intermolecular interactions)
- Stronger interactions occur in hydrophobic environments



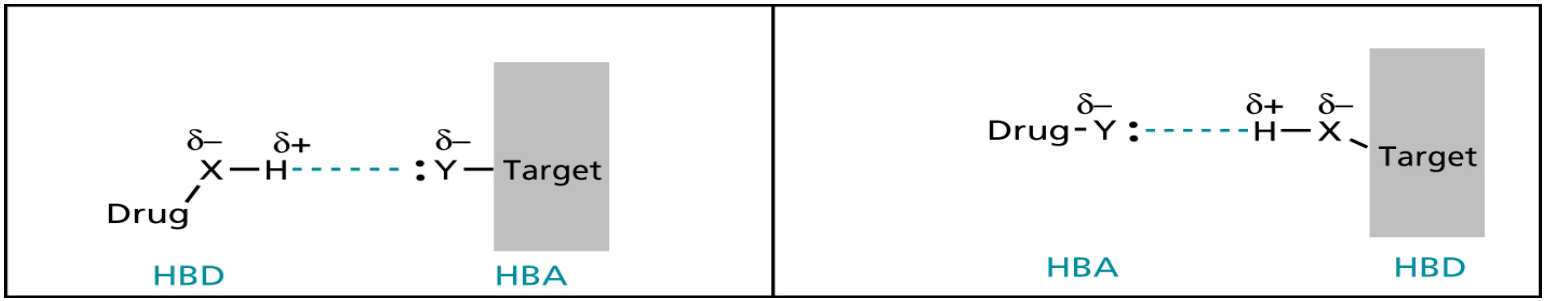
Ionic bond (Columbian forces)

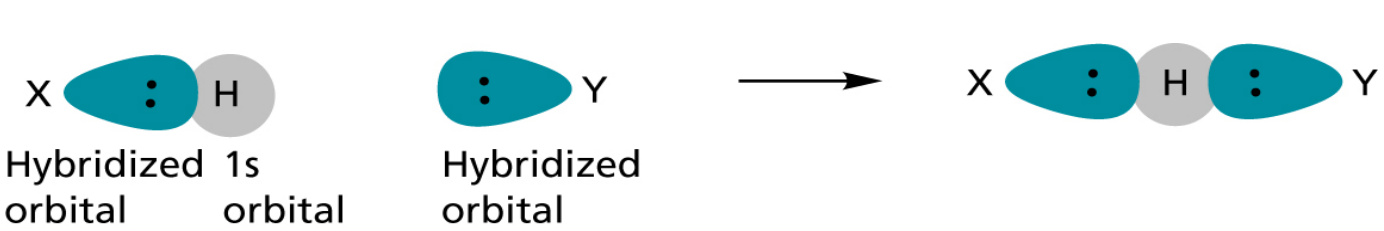


A. Electrostatic attraction

Hydrogen Bond (HB)

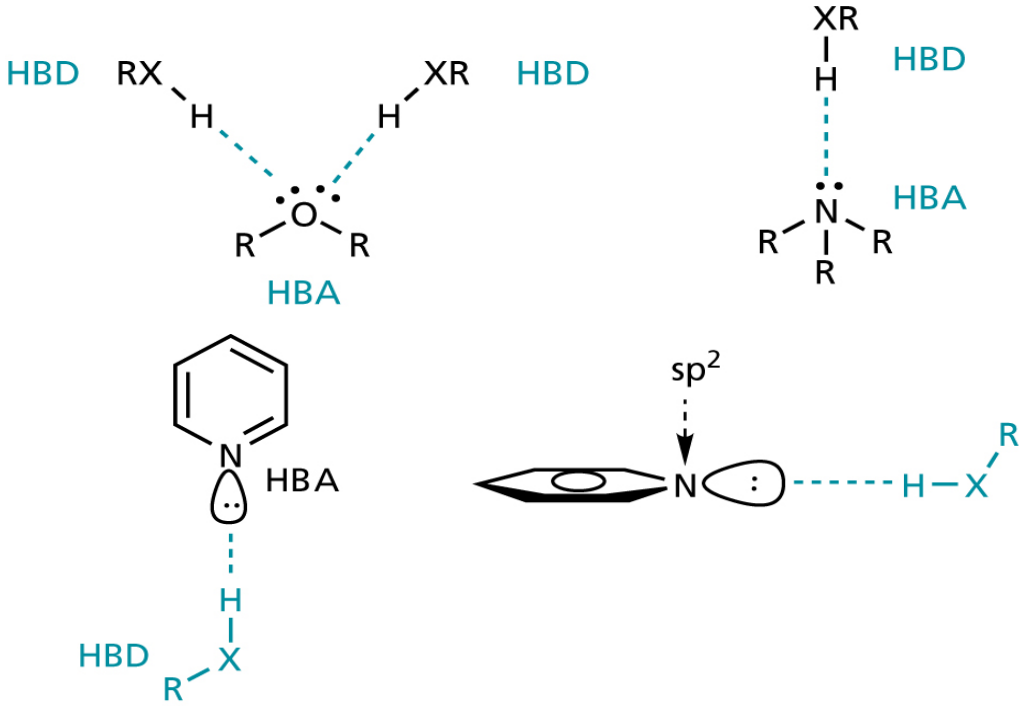
- Vary in strength, ten times less then a covalent bond.
- Weaker than electrostatic interactions but stronger than van der Waals interactions
- A hydrogen bond takes place between an electron deficient hydrogen and an electron rich heteroatom (Nitrogen or Oxygen)
- The electron deficient hydrogen is usually attached to a heteroatom (O or N)
- The electron deficient hydrogen is called a hydrogen bond donor (HBD)
- The electron rich heteroatom is called a hydrogen bond acceptor (HBA)

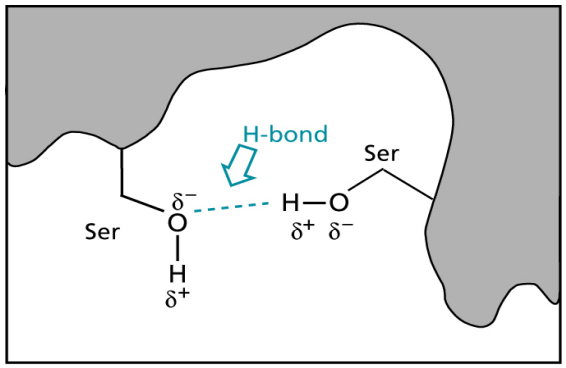


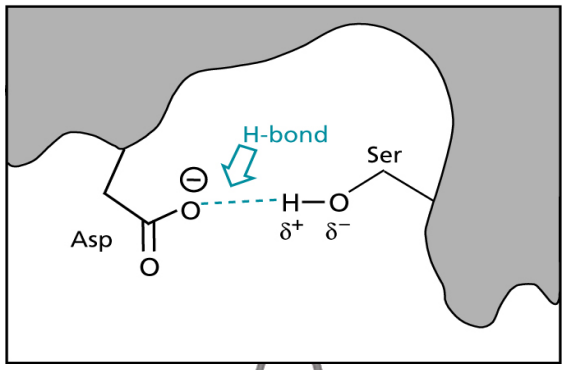


Hydrogen Bond (HB)

- The interaction involves orbitals and is directional
- Optimum orientation is where the X-H bond points directly to the lone pair on Y such that the angle between X, H and Y is 180°

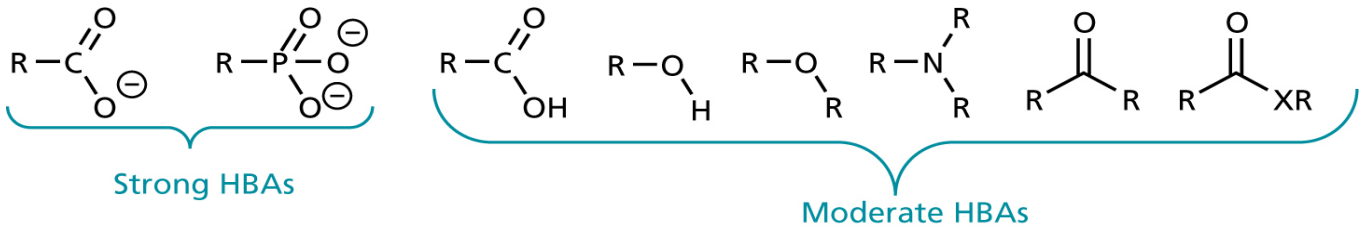


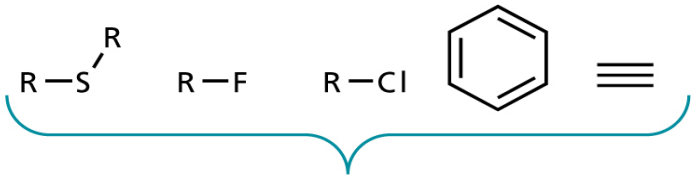




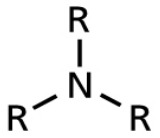
Hydrogen Bond (HB)

- Examples of strong hydrogen bond acceptors (HBA)
  - carboxylate ion, phosphate ion, tertiary amine
- Examples of moderate hydrogen bond acceptors
  - carboxylic acid, amide oxygen, ketone, ester, ether, alcohol
- Examples of poor hydrogen bond acceptors
  - sulfur, fluorine, chlorine, aromatic ring, amide nitrogen, aromatic amine
- Example of good hydrogen bond donors (HBD)
  - Quaternary ammonium ion

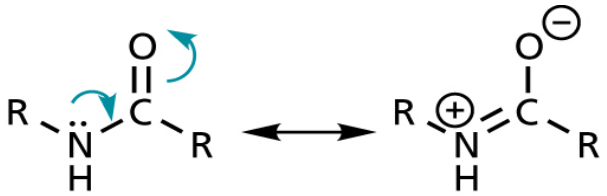




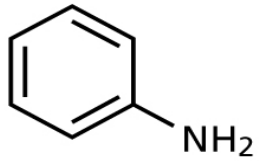
Hydrogen Bond (HB)



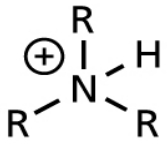
Tertiary amine—good HBA



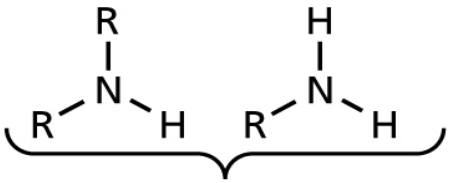
Amide—N acts as poor HBA



Aniline—N acts as poor HBA



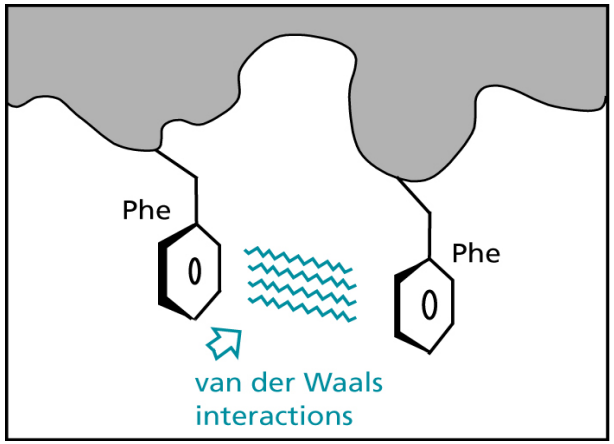
Quaternary ammonium ion
(stronger HBD)

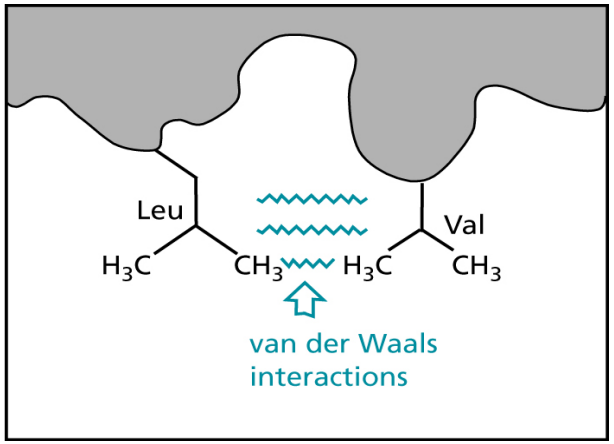


Secondary and primary
amines

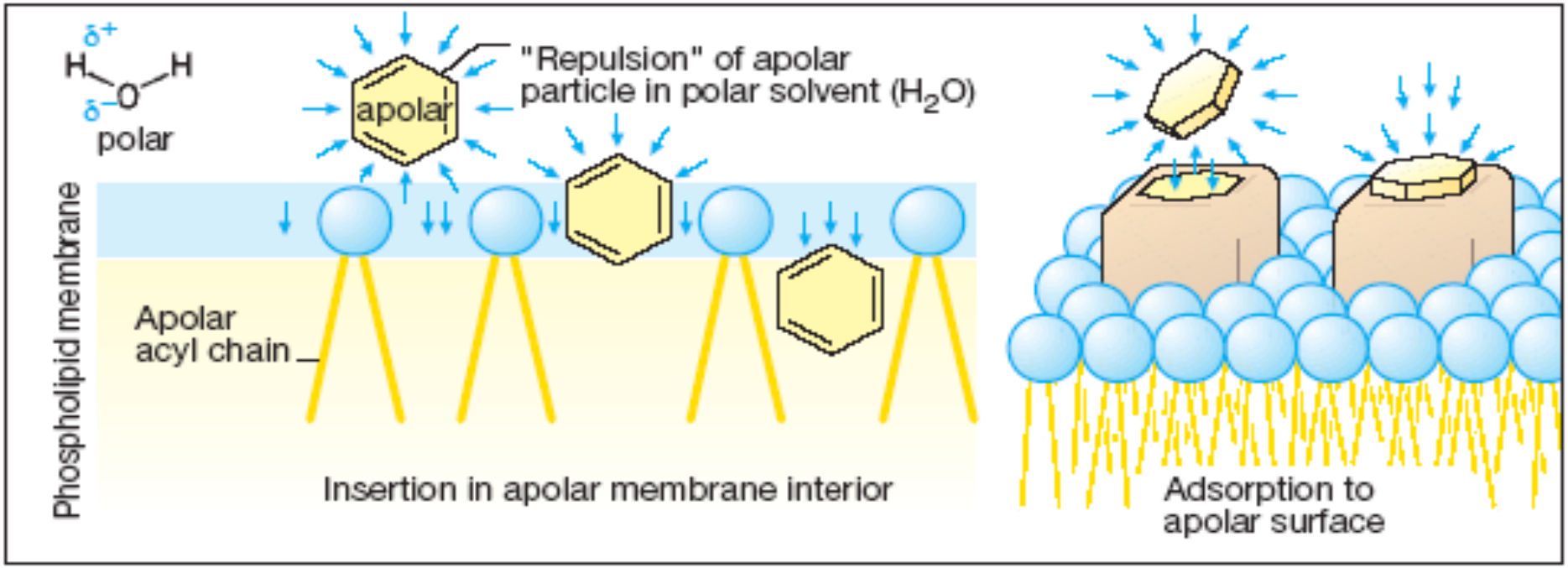
Van der Waals interactions

- Very weak interactions
- Occur between hydrophobic regions of the drug and the target
- Due to transient areas of high and low electron densities leading to temporary dipoles
- Interactions drop off rapidly with distance
- Drug must be close to the binding region for interactions to occur
- The overall contribution of van der Waals interactions can be crucial to binding





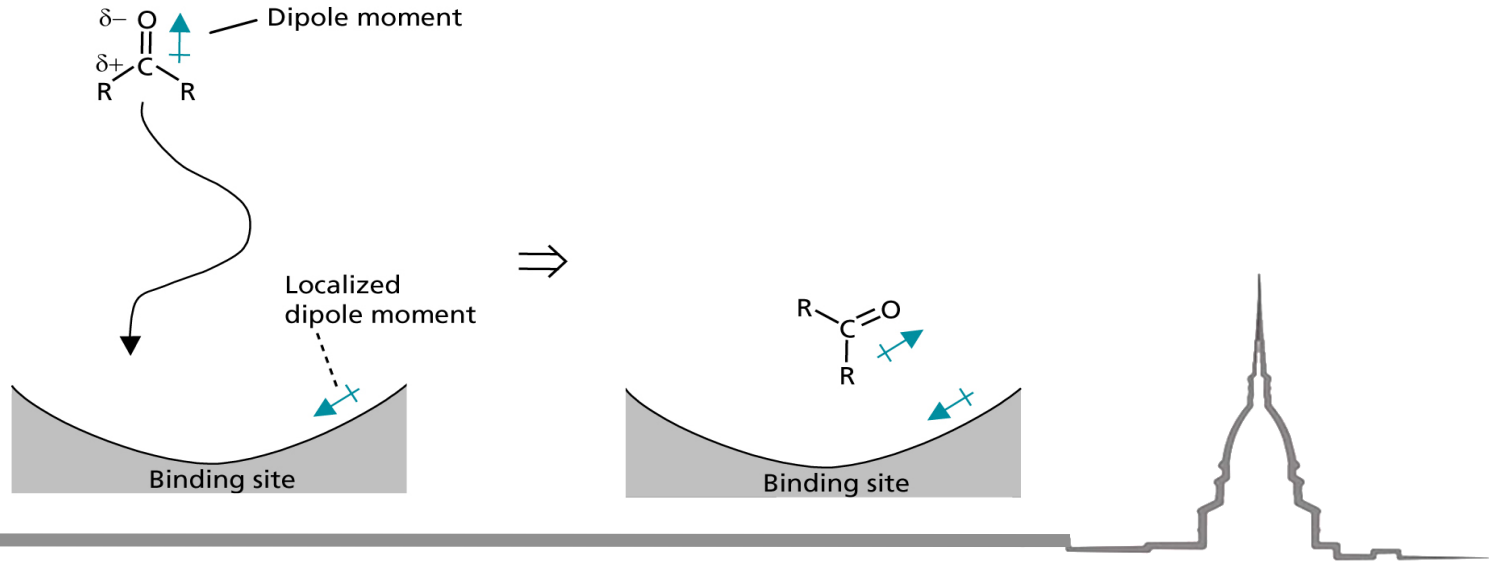
Van der Waals interactions



C. Hydrophobic interaction

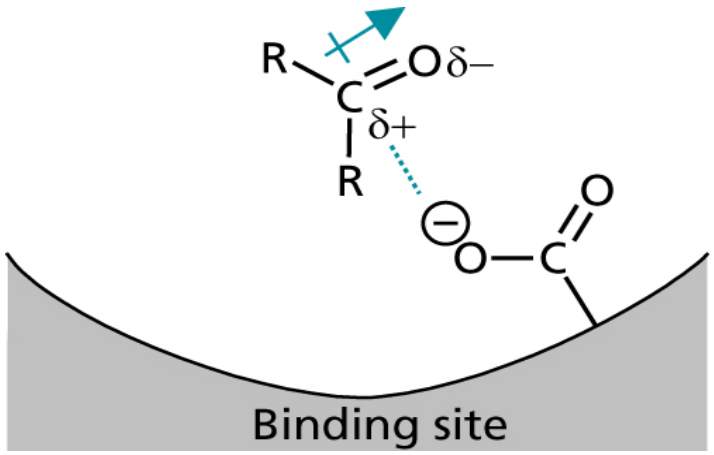
Dipole-Dipole interactions

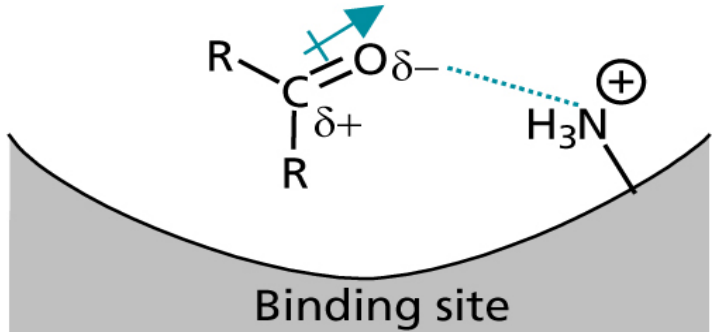
- Can occur if the drug and the binding site have dipole moments
- Dipoles align with each other as the drug enters the binding site
- Dipole alignment orientates the molecule in the binding site
- Orientation is beneficial if other binding groups are positioned correctly with respect to the corresponding binding regions
- Orientation is detrimental if the binding groups are not positioned correctly with respect to corresponding binding regions
- The strength of the interaction decreases with distance more quickly than with electrostatic interactions, but less quickly than with van der Waals interactions



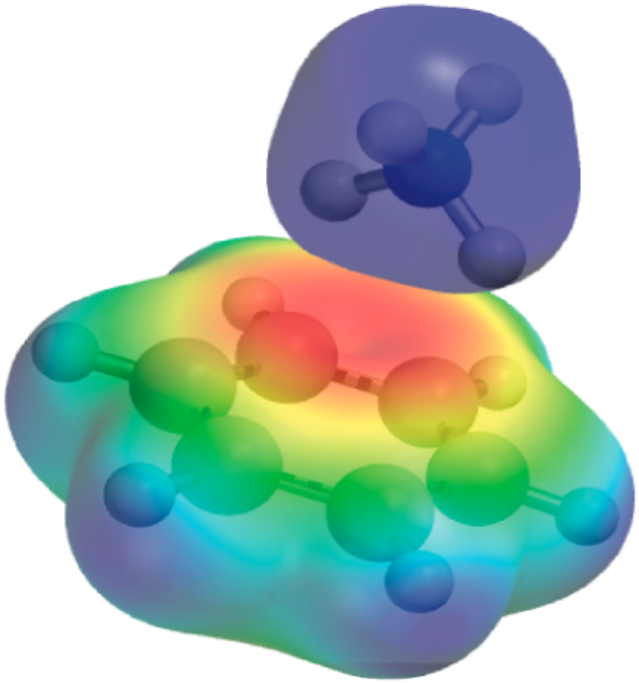
Dipole-ion interactions

- Occur where the charge on one molecule interacts with the dipole moment of another
- Stronger than a dipole-dipole interaction
- Strength of interaction falls off less rapidly with distance than for a dipole-dipole interaction



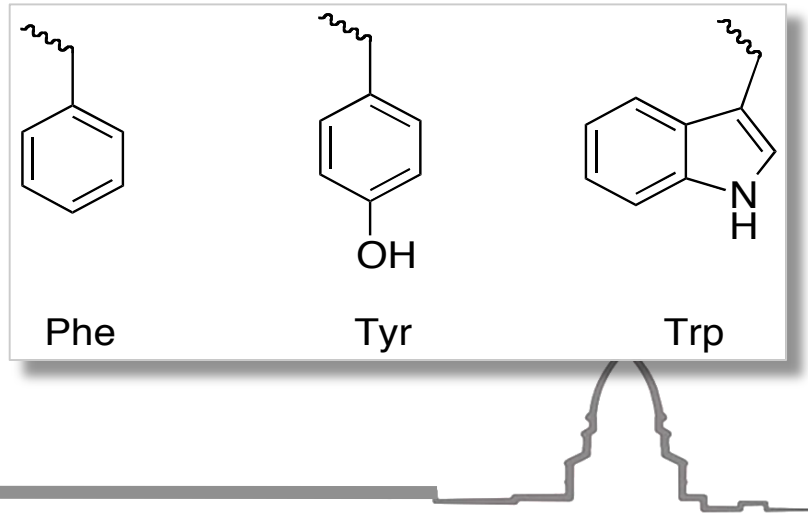


Cation- π interaction



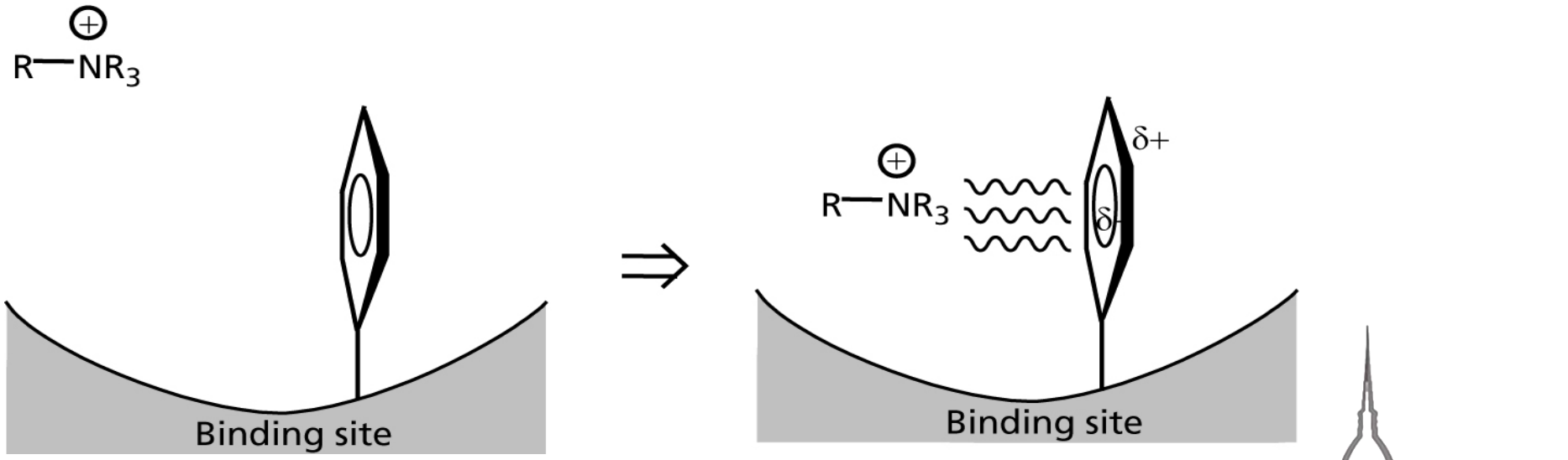
Cation-π interaction is a strong noncovalent binding interaction that contributes to protein secondary structure and to diverse ligand-receptor interaction.

The amino acid side chains that can contribute to cation-π interactions in protein structure:

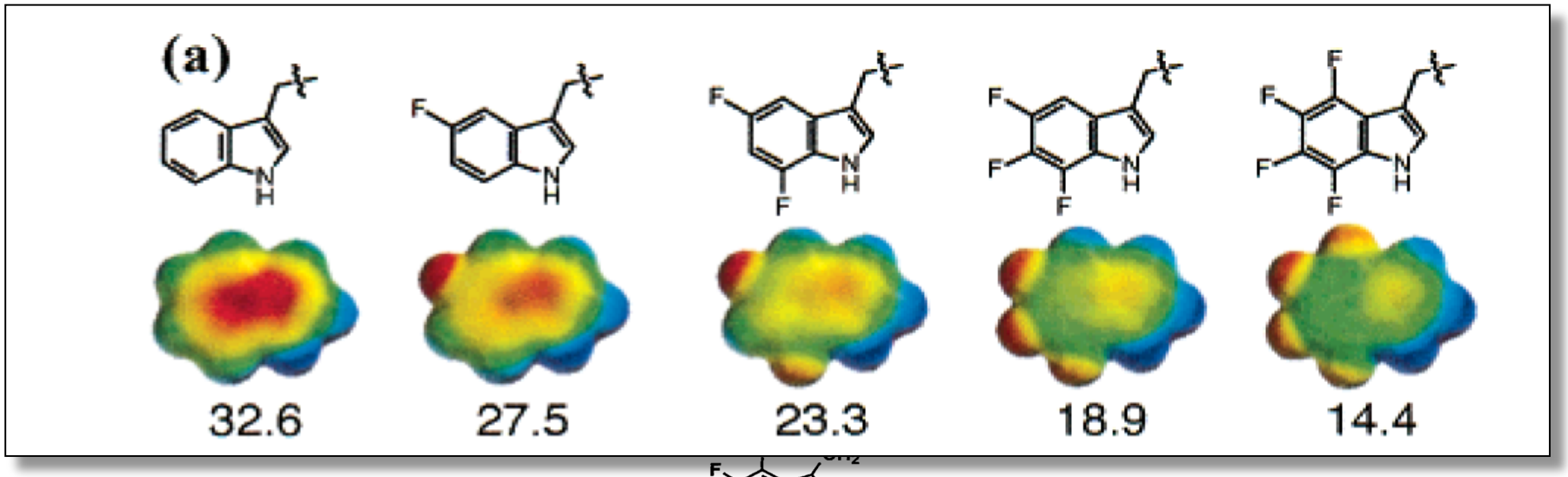


Dipole-ion interactions

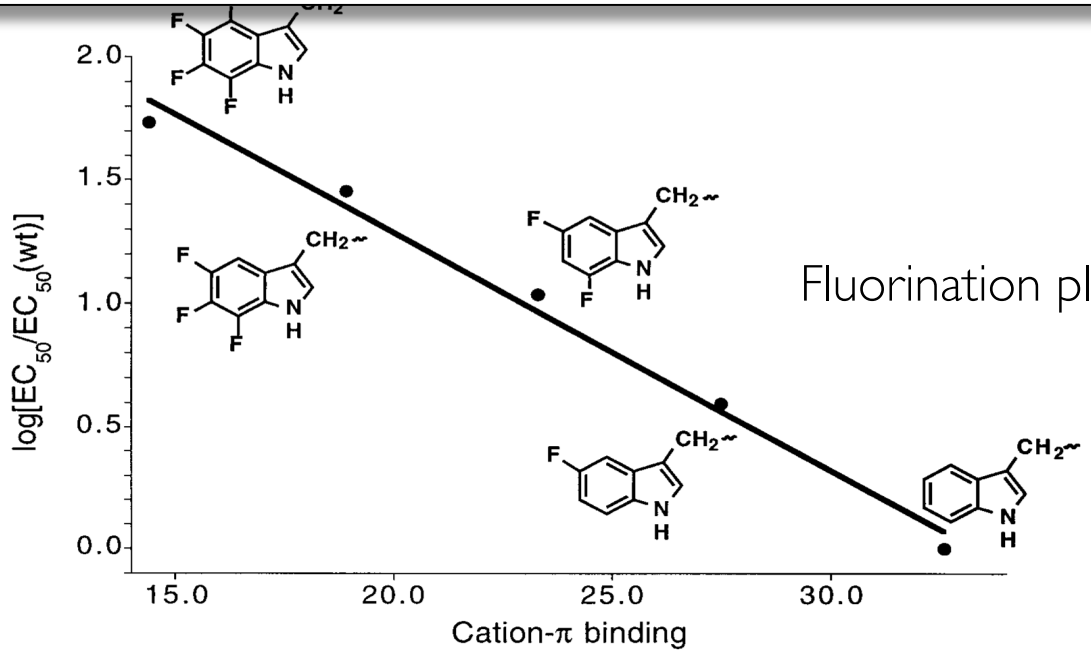
- Occur where the charge on one molecule induces a dipole on another
- Occurs between a quaternary ammonium ion and an aromatic ring



Probing/identifying the cation-π interactions



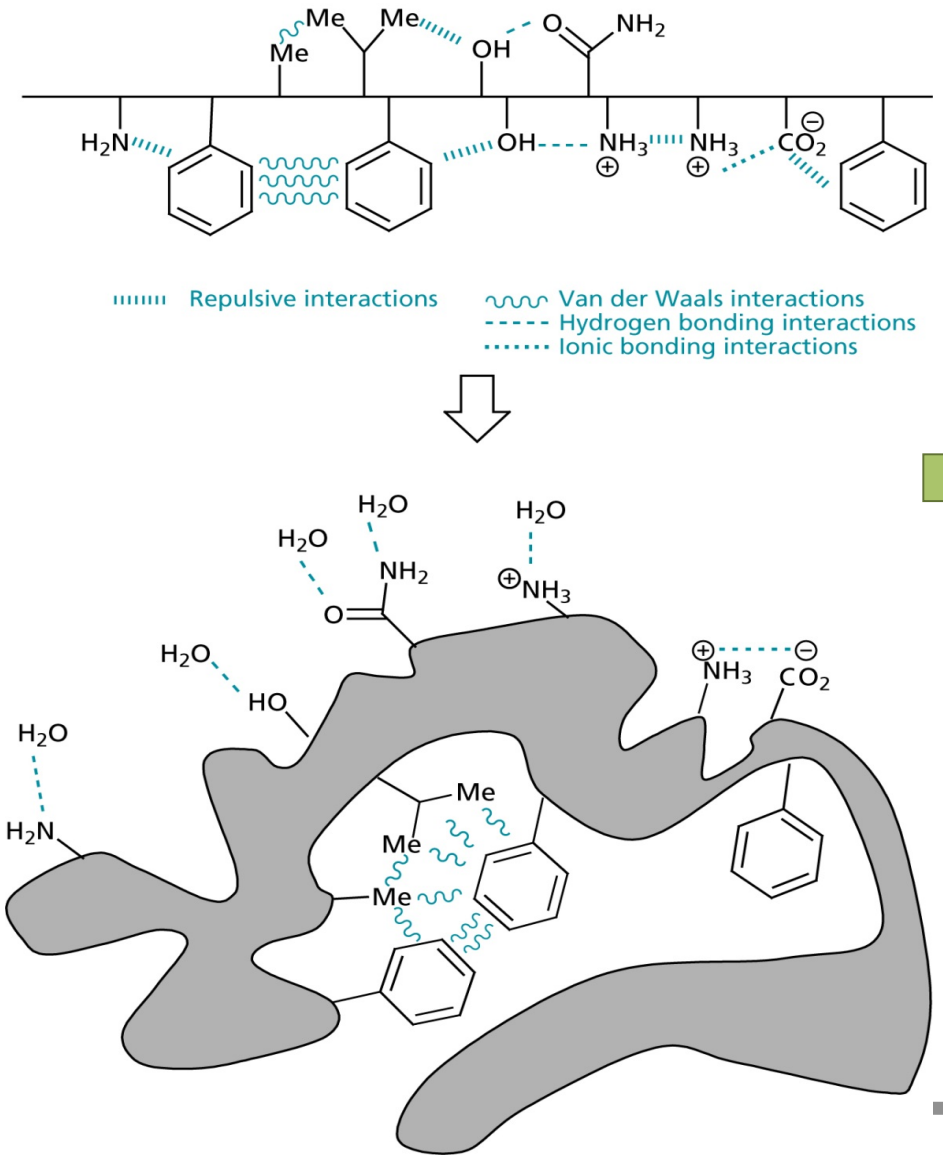
Gas-phase cation-π binding energy (kcal/mol) of Trp and fluorinated analogues to Na^+

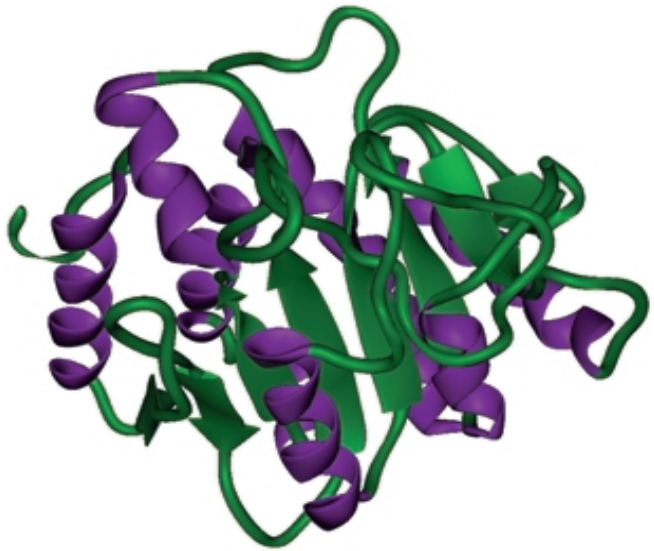


Fluorination plot

Esempio:

Tertiary protein structure





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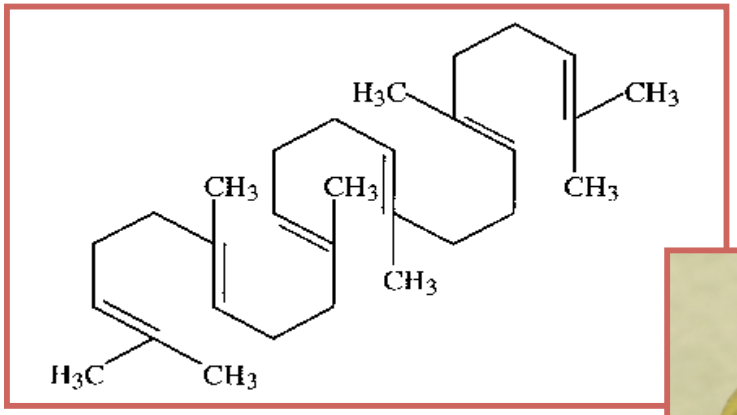
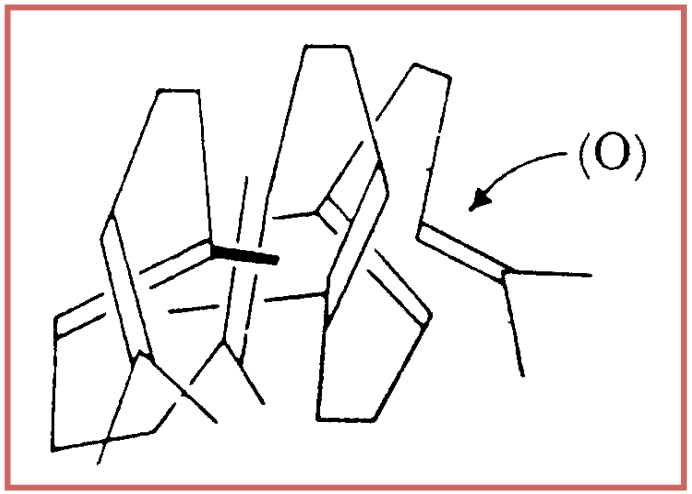
University of Torino (UniTO)

Esempio:

Squalene *quasi-ball* configuration



SQUALENE



Squalene is a very very old molecule, old as our earth, that during the time has acquired the property to adopt a configuration called "quasi ball configuration" both in water solution or into enzyme
(called in this case a prechair-preboat-prechair-preboat all trans conformation)