Membranes as meeting points for drugs, lipids and therapies

Salette Reis*, Marlene Lúcio, Cláudia Nunes, Marina Pinheiro and José L.F.C. Lima

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Next year

Degrees in FFUP

U.PORTO
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UNIVERSIDADE DO PORTO

- Mestrado Integrado em Ciências Farmacêuticas (300 ECTS)

- Mestrado em Análises Clínicas (120 ECTS)
- Mestrado em Controlo de Qualidade (120 ECTS)
- Mestrado em Tecnologia Farmacêutica (120 ECTS)
- Mestrado em Toxicologia Analítica Clínica e Forense (120 ECTS)

- Doutoramento em Ciências Farmacêuticas (240 ECTS)

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RESEARCH GROUPS AT REQUIMTE

Organic Chemistry

Chemical and Biochemical Engineering

Biochemistry and Biophysics

Analytical Chemistry

Food Chemistry

Physical and Inorganic Chemistry

Biological Chemistry

Our Research group

Salette Reis
Marlene Lúcio

Lines of research

- **1- Liposomes as cell membrane mimetic models systems for the study of drugs**
- Drugs for the treatment of tuberculosis

  Marina Pinheiro;

  Marta Oliveira;

  Mariana Arêde
- Nonsteroidal anti-inflammatory drugs

  Cláudia Pinho;
  Catarina Pinto Leite;
  Daniela Lopes
- Cardiovascular drugs

  Cláudia Carneiro
- Membrane toxicity studies

  Juliana Brittes

Lines of research

2 - Development of nanosystems for drugs delivery

- Silica nanotubes for the delivery of anti-inflammatory

  Cláudia Pinho

- Liposomes for the delivery of drugs for the treatment of tuberculosis

  Marina Pinheiro

- Lipid nanoparticles for the delivery of resveratrol

  Ana Rentão;
  Rafael Amaral

- Polymeric nanoparticles for drug delivery

  Catarina Alves
  Natacha Rosa

Lines of research

3 - Study of drug effect on enzymes activity at the lipid interface

- Studies with PLA2 and anti-inflammatory

  Ana Rute

4 – Enzymes/drugs binding studies

- Binding studies of drugs to plasma proteins

  Diogo Ribeiro;
  Ana Azevedo

Membranes as meeting points for drugs, lipids and therapies

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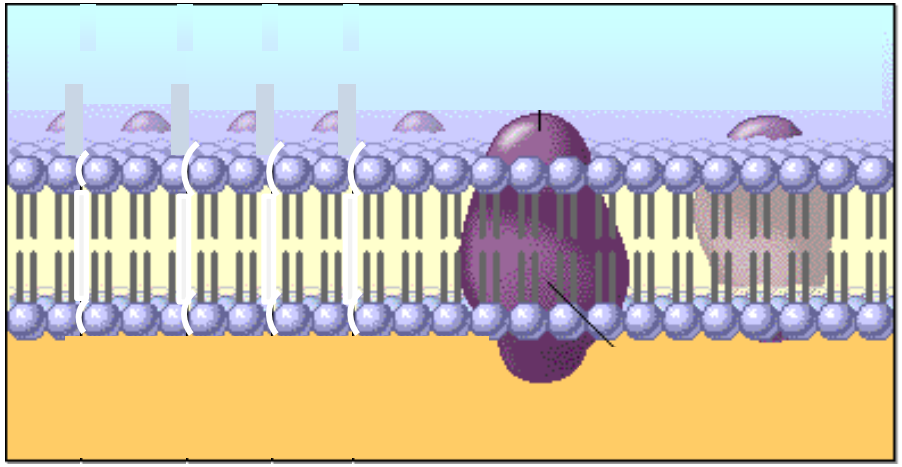
Why is it important to study how drugs interact with membranes ?



Drugs can act at the membrane level or must often pass across membranes before they can reach an intracellular target.

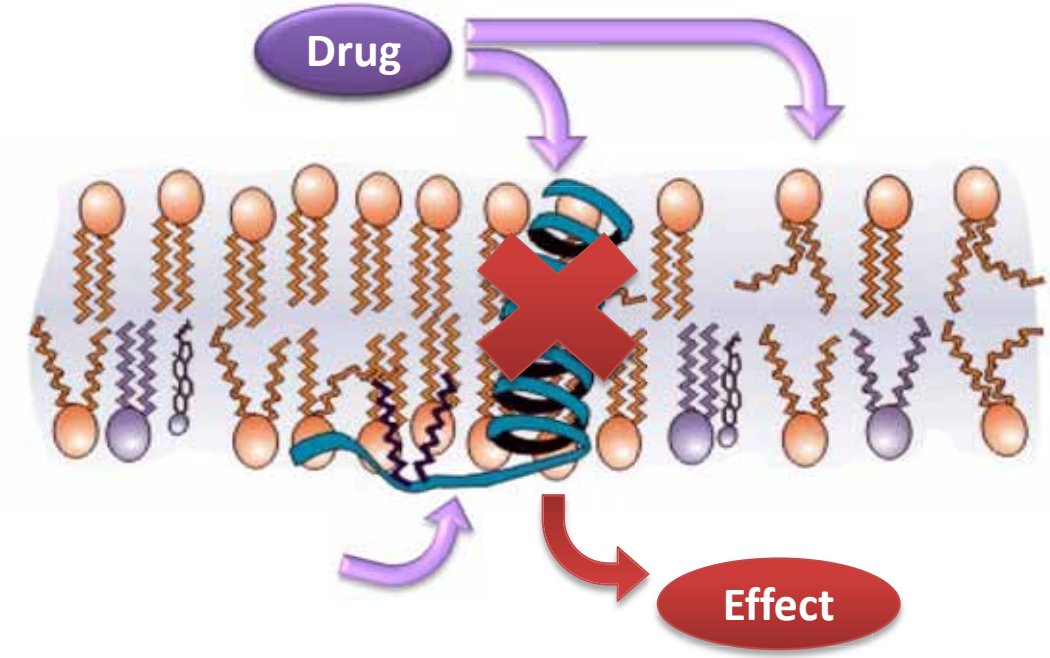
DRUG PENETRATION

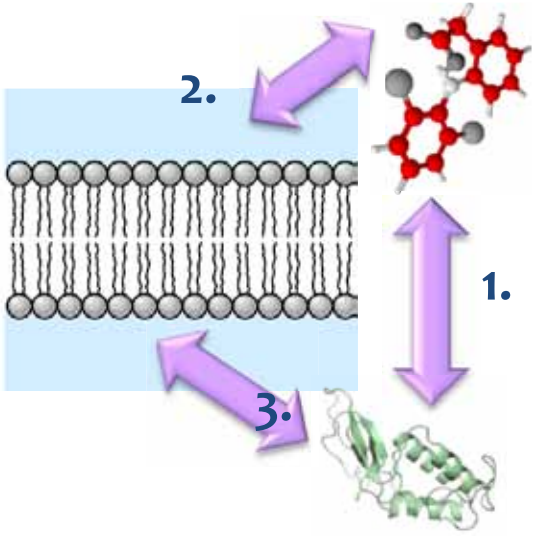
Why is it important to study how drugs interact with membranes ?

1. Drugs may interact with membrane proteins or receptors in the surface of the cell membrane producing their effect

2. Drugs may penetrate cell membrane to reach their intracellular target

3. Drugs may alter the cell membrane and change the activity of membrane enzymes/receptors

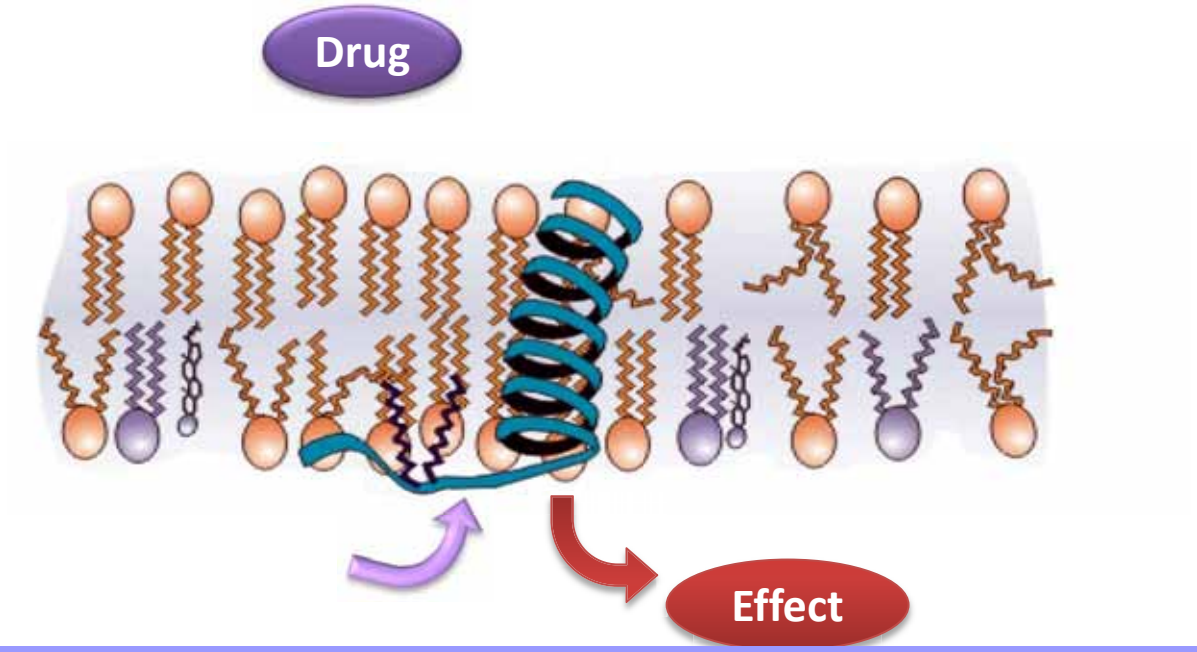




DRUG PENETRATION

Drug penetration into the lipid membrane can be evaluated by:

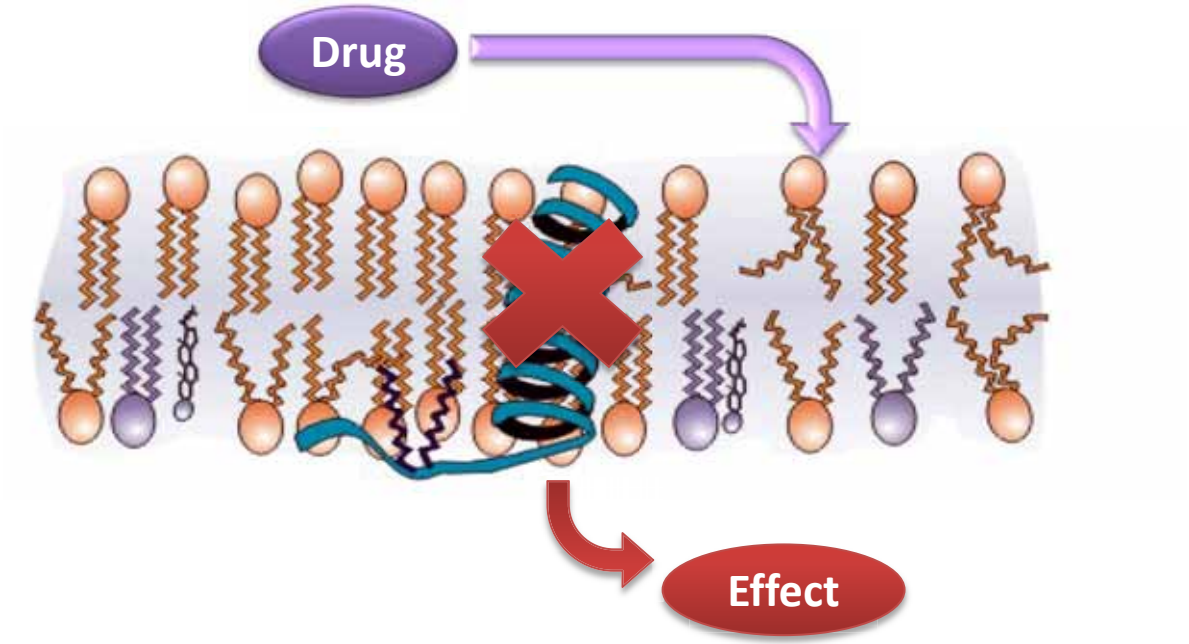
1. Determination of **membrane/water partition coefficient** (K_p);
2. Determination of **drug location** in the membrane



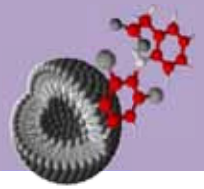
BIOPHYSICAL EFFECTS

The biophysical effects induced by the drugs in membranes can be evaluated by:

1. Membrane **microviscosity** and **phase transition** studies
2. Membrane **structure** and **order** studies



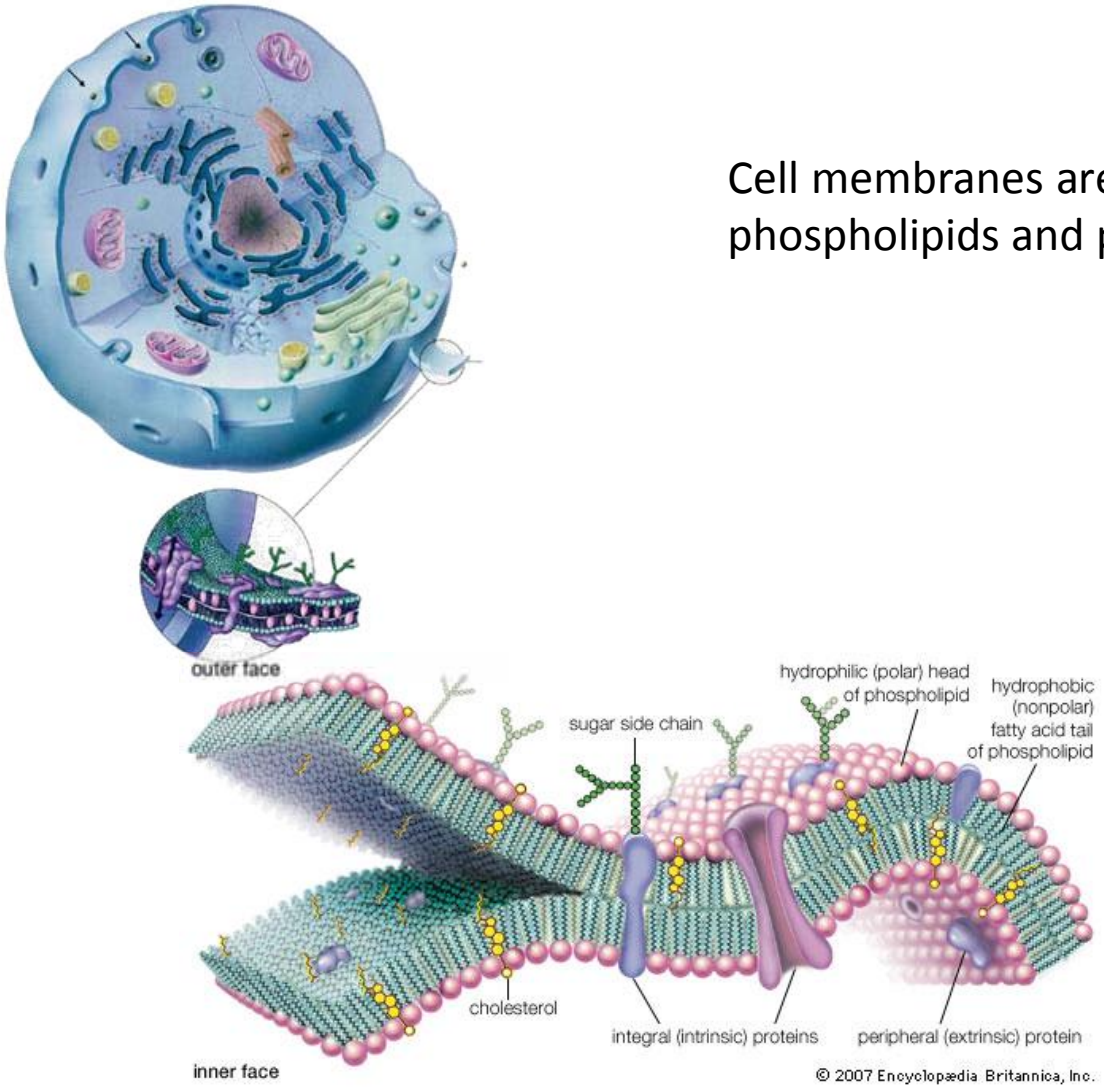
Aims of study



Drug-membrane interactions

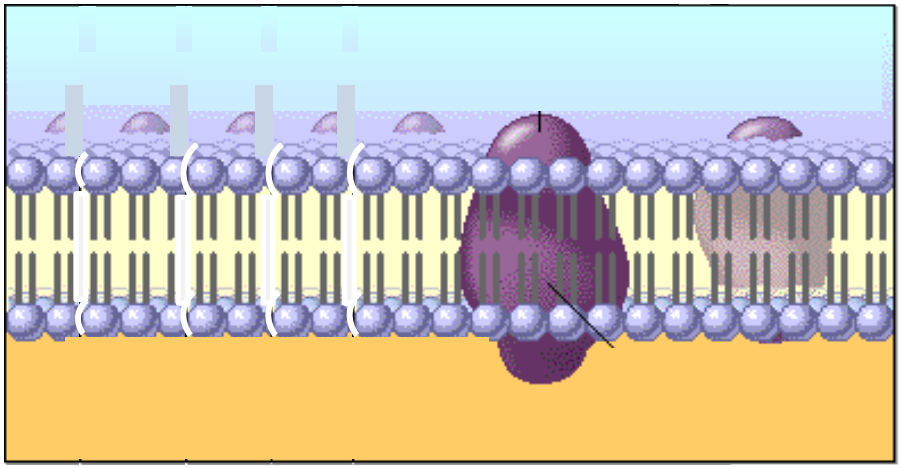
1. Understand drugs mode of action — Drug location
2. Predict drugs toxic effects
3. To reach better treatments — Partition coefficient
4. Understand membrane permeation — Membrane charge
5. Develop drug-delivery systems — Membrane biophysics

Why is it important to study how drugs interact with membranes ?



Cell membranes are lipid bilayers composed by phospholipids and proteins

Drug - membrane interactions



Drug membrane concentration

Drug membrane location

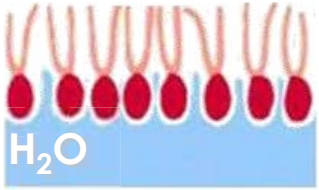
Surface charge

Modifications of membrane structure and membrane biophysical properties

Enzymatic inhibition

membrane model systems

Liposomes



Monolayers

The case of NSAIDs

Drug - membrane interactions

Drug membrane concentration

Drug membrane location

Surface charge

Modifications of membrane structure and membrane biophysical properties

Enzymatic inhibition

Toxicological effects

Non-steroidal Anti-inflammatory Drugs (NSAID) are the most widely used medicines for the treatment of fever, pain and inflammation.

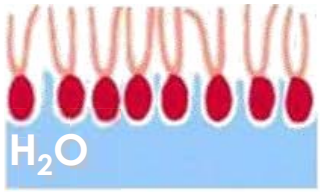
The case of NSAIDs

Drug - membrane interactions

- Drug membrane concentration
- Drug membrane location
- Surface charge
- Modifications of membrane structure and membrane biophysical properties
- Enzymatic inhibition

membrane model systems

Liposomes



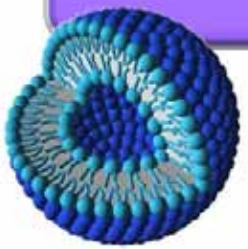
Monolayers

- Cell membrane
- Inflamated cell membrane
- mitochondrial membrane (i.m.m.)
- NSAID enzymatic activity
  - COX_1 and COX_2
  - PLA_2
- Gastric toxicity

Membranes as meeting points for the action and toxicity of therapeutics

The case of NSAIDs

membrane model systems



Liposomes

Monolayers

- Cell membrane
  - Inflamated cell membrane
- mitochondrial membrane
- NSAID enzymatic activity
  - COX_1 and COX_2
  - PLA_2
- Gastric toxicity

- EPC
  - DPPC
  - DMPC
  - DPPG
- DOPC /DOPE/TOCL
- DMPC/DPPE (7:3)
- DPPC (gel phase)

- pH
- temperature
- Ionic strength

The case of NSAIDs

Drug properties

Drug membrane concentration

membrane/aqueous phase partition

Drug membrane location

Surface charge

Med. Chem., 2 (5), 447-456 (2006)

What is the partition coefficient?

The **partition coefficient** of the drugs is a measure of their **lipophilicity** and thus their ability to interact with biomembranes

→ Pharmacological activity

→ Bioaccumulation and toxic effects

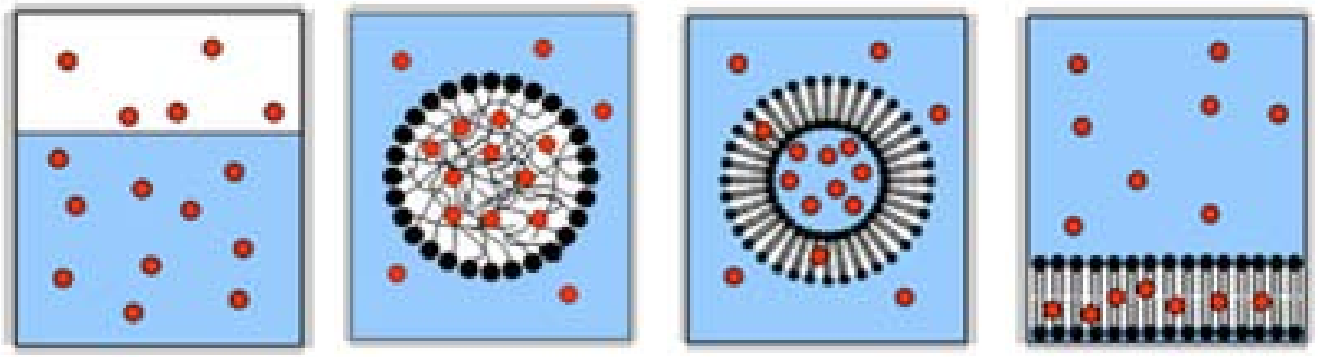
$$K_p = \frac{[Drug]_{lipid}}{[Drug]_{octanol}} \quad \text{or} \quad \frac{[Drug]_{lipid}}{[Drug]_{water}}$$

The partition coefficient of drugs is determined by their distribution in a biphasic system:

Drug

Aqueous Phase

Organic/lipidic Phase



Octanol/Water **Micelle/Water** **Liposome/Water** **Membrane/Water**

$K_{p\ membrane/water}$ versus $P_{octanol/water}$

Hydrophobic Drugs

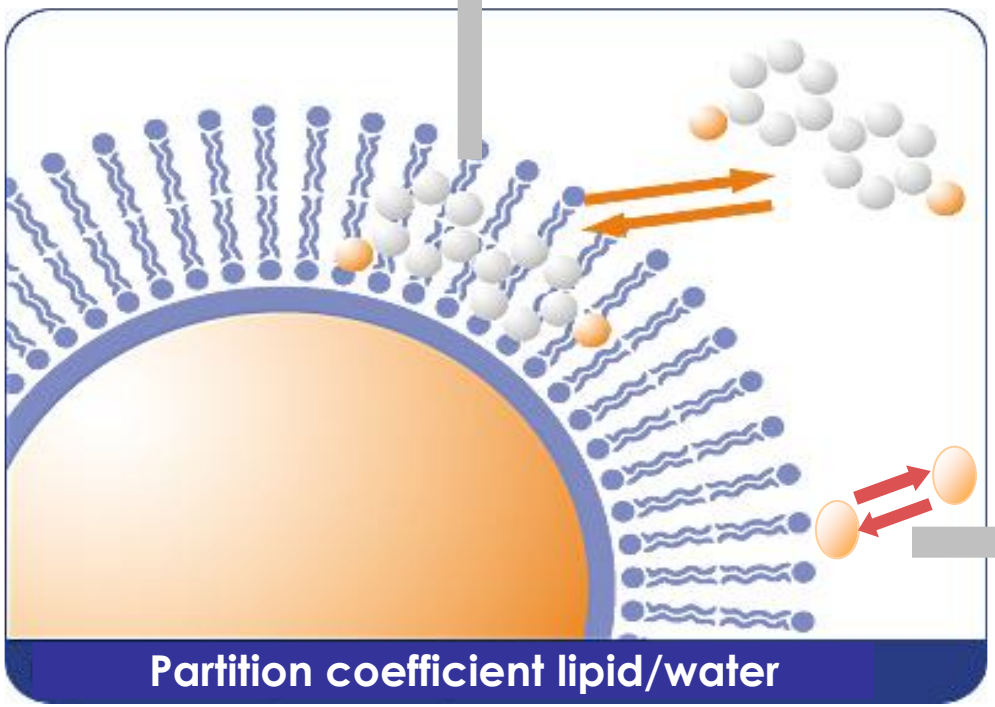
Hydrophobic interactions

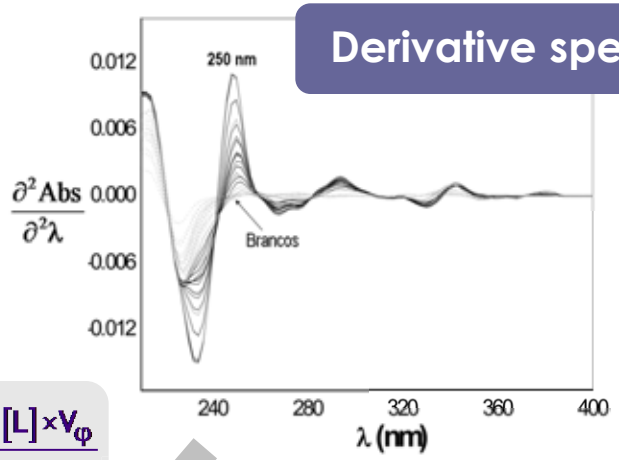
Partition coefficient octanol/water — P

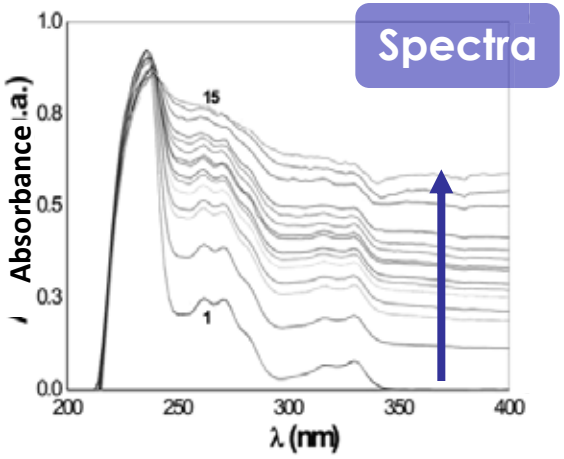
K_p — Partition coefficient lipid/water

Electrostatic interactions
Hydrogen bonds

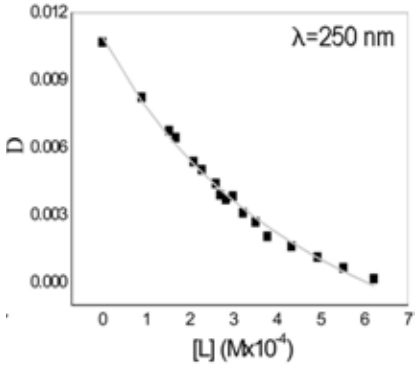
Hydrophilic drugs







$$Abs_f = Abs_a + \frac{(Abs_m - Abs_a) \times K_p \times [L] \times V_\varphi}{1 + K_p \times [L] \times V_\varphi}$$

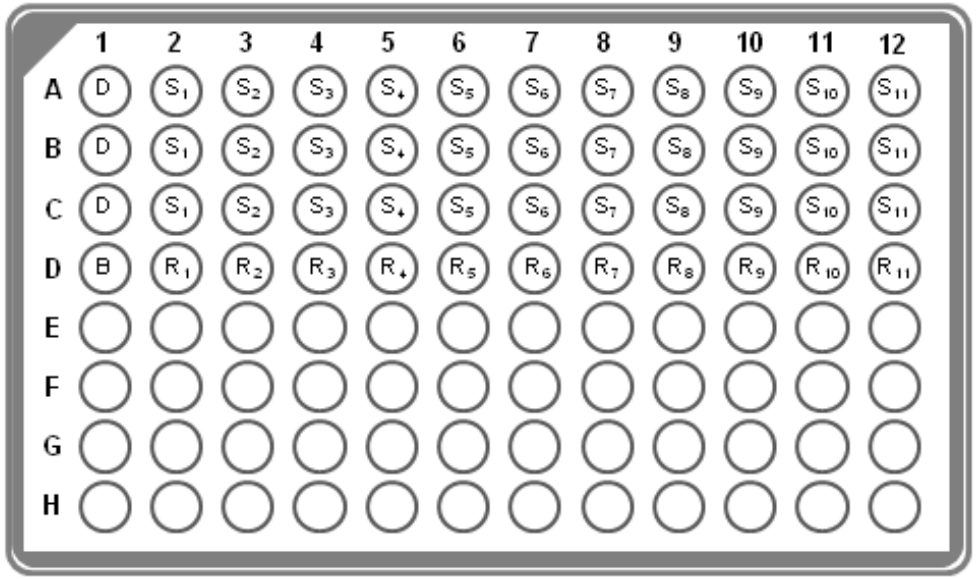


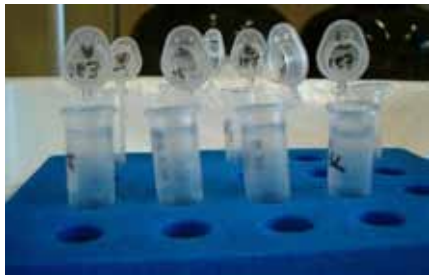
Derivative Spectroscopy method

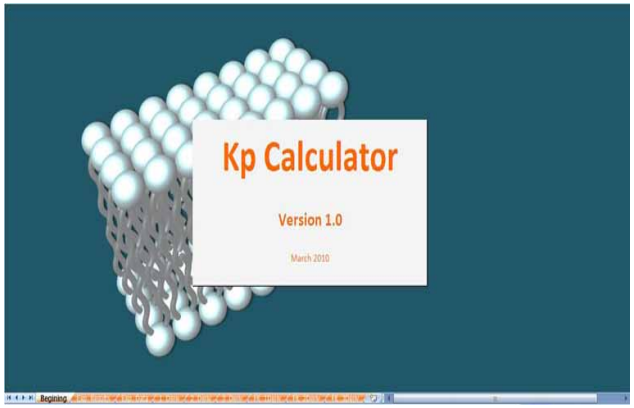
- Elimination of the light scattering interference
- Improved band resolution
- K_p determination without phase separation

Anal. Bioanal. Chem. 377: 293-298 (2003)

K_p determination (MICROPLATE PROTOCOL)

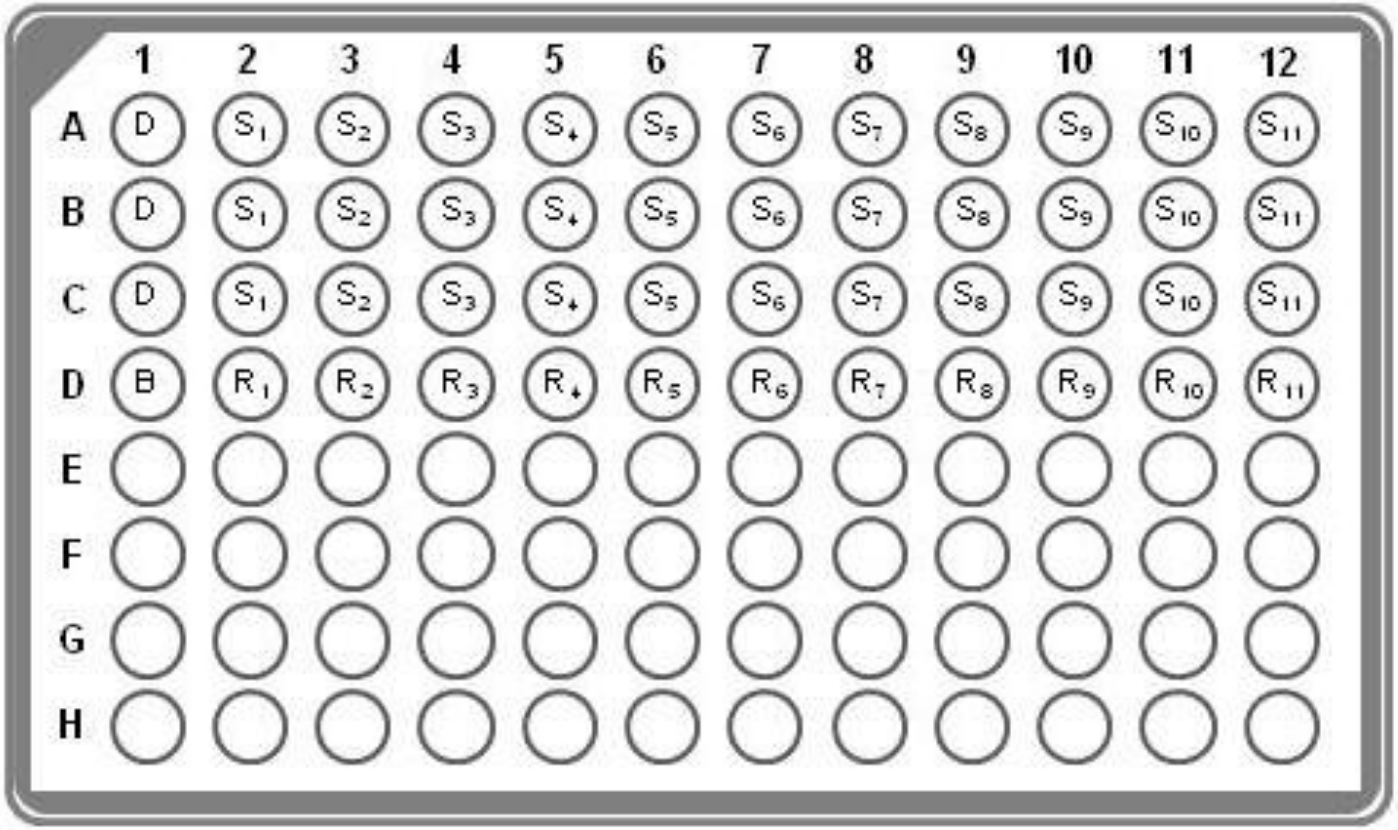




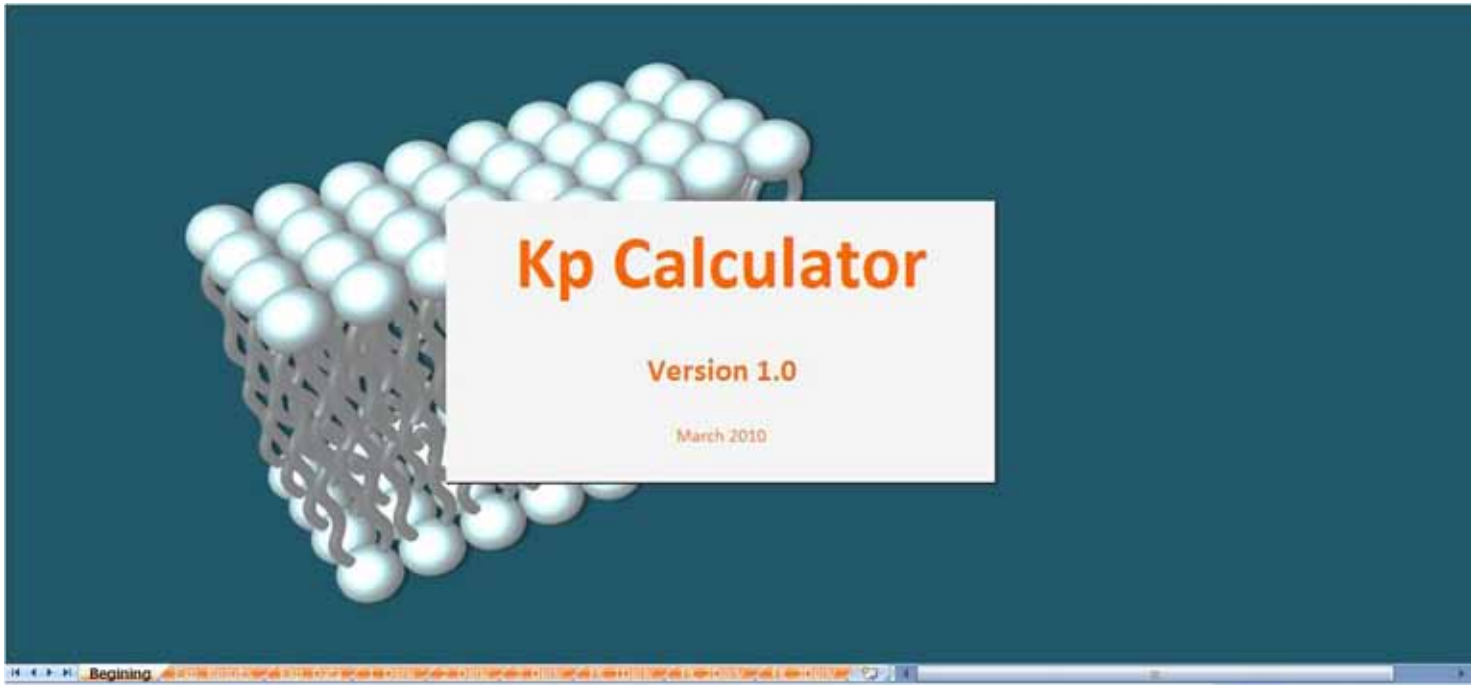


- Fast experimental protocol
- It is possible to measure Kp of several drugs at the same time
- Small amounts of samples
- Automatic data treatment

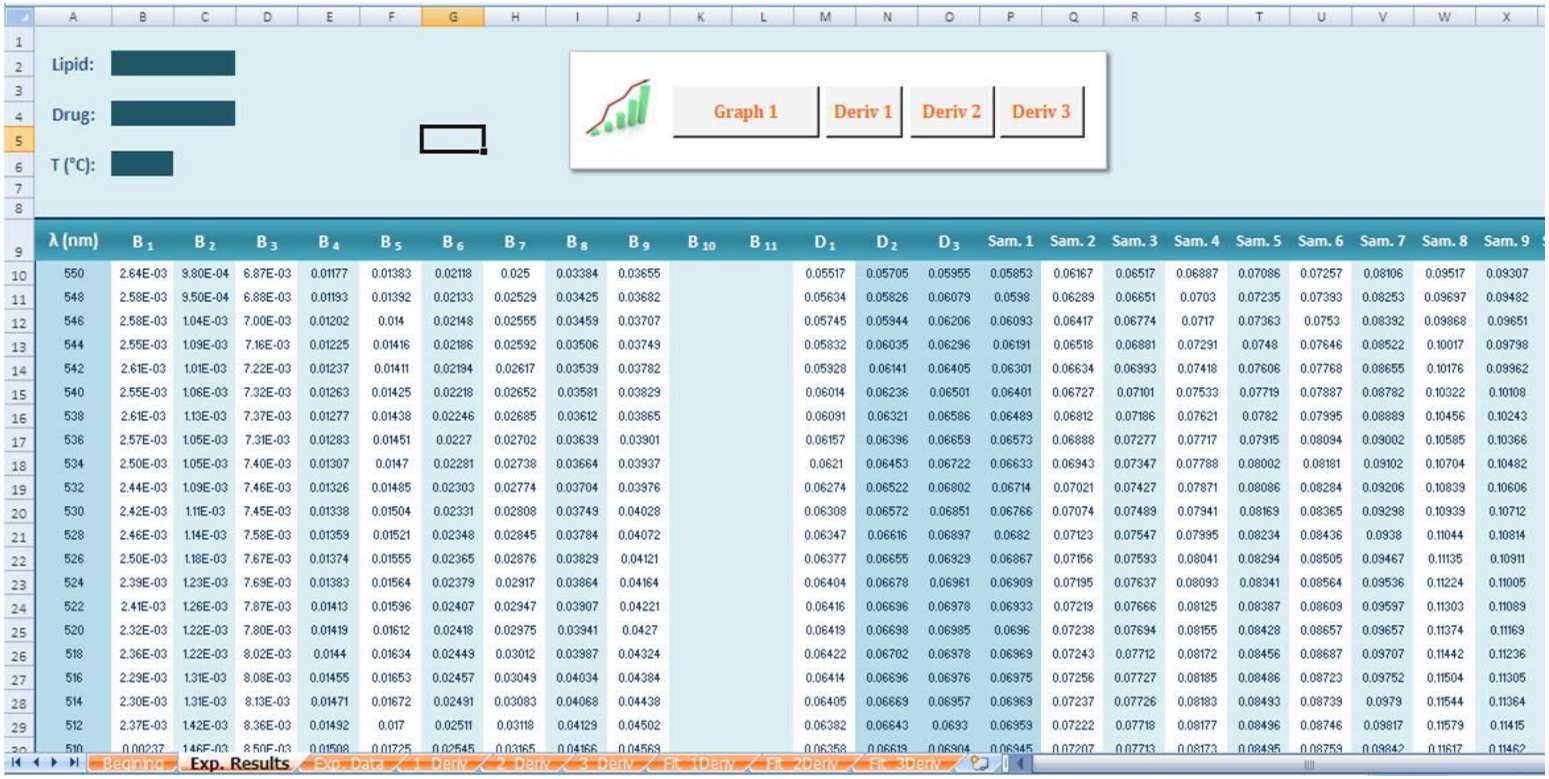
K_p determination (MICROPLATE PROTOCOL)



K_p determination (MICROPLATE PROTOCOL)

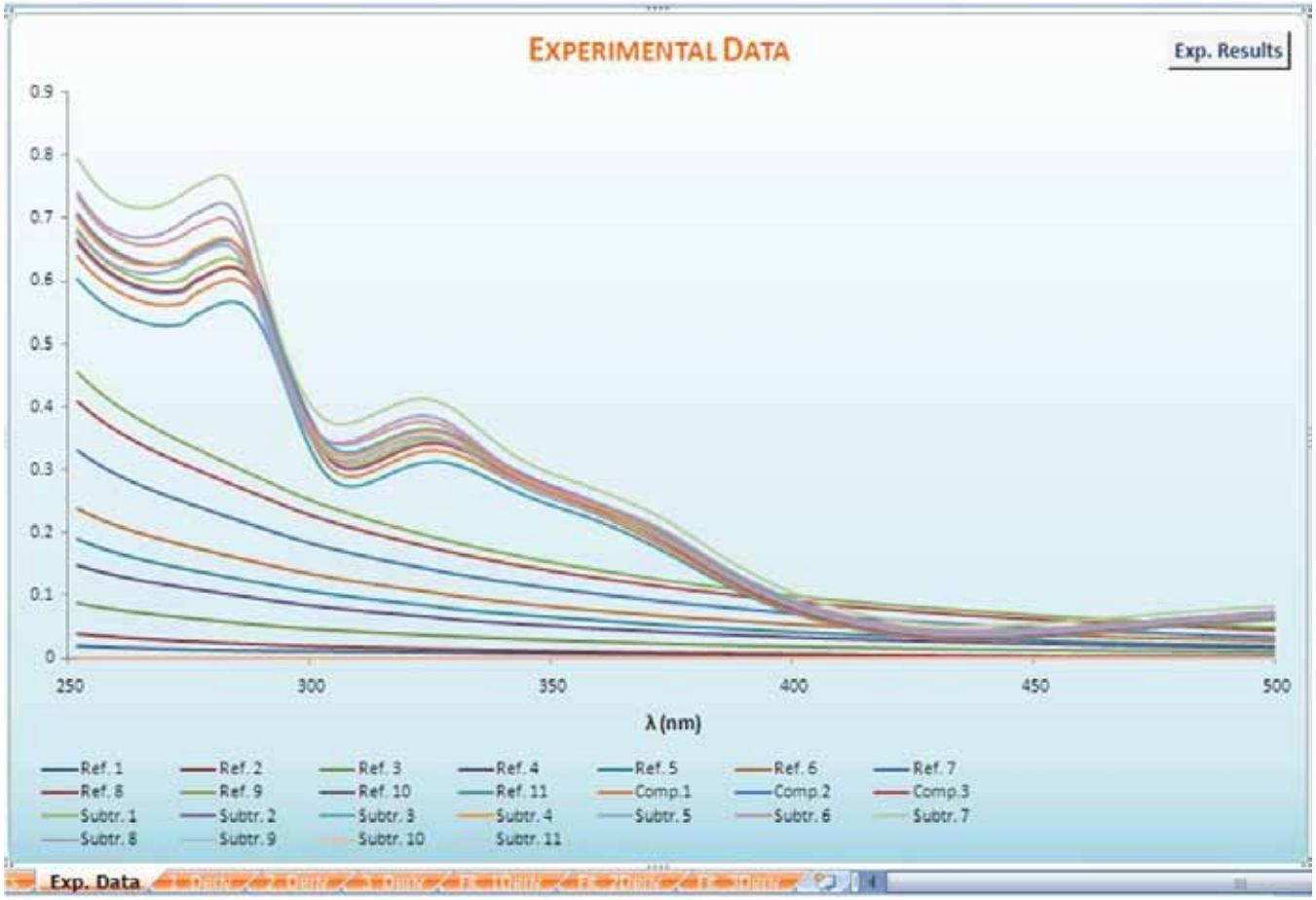


K_p determination (MICROPLATE PROTOCOL)



λ (nm)	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B 10	B 11	D 1	D 2	D 3	Sam. 1	Sam. 2	Sam. 3	Sam. 4	Sam. 5	Sam. 6	Sam. 7	Sam. 8	Sam. 9
550	2.64E-03	9.80E-04	6.87E-03	0.01177	0.01383	0.02118	0.025	0.03384	0.03655			0.05517	0.05705	0.05955	0.05853	0.06167	0.06517	0.06887	0.07086	0.07257	0.08106	0.09517	0.09307
548	2.58E-03	9.50E-04	6.88E-03	0.01193	0.01392	0.02133	0.02529	0.03425	0.03682			0.05634	0.05826	0.06079	0.0598	0.06289	0.06651	0.0703	0.07235	0.07393	0.08253	0.09697	0.09482
546	2.58E-03	1.04E-03	7.00E-03	0.01202	0.014	0.02148	0.02555	0.03459	0.03707			0.05745	0.05944	0.06206	0.06093	0.06417	0.06774	0.0717	0.07363	0.0753	0.08392	0.09868	0.09651
544	2.55E-03	1.09E-03	7.16E-03	0.01225	0.01416	0.02186	0.02592	0.03506	0.03749			0.05832	0.06035	0.06296	0.06191	0.06518	0.06881	0.07291	0.0748	0.07646	0.08522	0.10017	0.09798
542	2.61E-03	1.01E-03	7.22E-03	0.01237	0.01411	0.02194	0.02617	0.03539	0.03782			0.05928	0.06141	0.06405	0.06301	0.06634	0.06993	0.07418	0.07606	0.07768	0.08655	0.10176	0.09962
540	2.55E-03	1.06E-03	7.32E-03	0.01263	0.01425	0.02218	0.02652	0.03581	0.03829			0.06014	0.06236	0.06501	0.06401	0.06727	0.07101	0.07533	0.07719	0.07887	0.08782	0.10322	0.10108
538	2.61E-03	1.13E-03	7.37E-03	0.01277	0.01438	0.02246	0.02685	0.03612	0.03865			0.06091	0.06321	0.06586	0.06489	0.06812	0.07186	0.07621	0.0782	0.07995	0.08889	0.10456	0.10243
536	2.57E-03	1.05E-03	7.31E-03	0.01283	0.01451	0.0227	0.02702	0.03639	0.03901			0.06157	0.06396	0.06659	0.06573	0.06888	0.07277	0.07717	0.07915	0.08094	0.09002	0.10585	0.10366
534	2.50E-03	1.05E-03	7.40E-03	0.01307	0.0147	0.02281	0.02738	0.03664	0.03937			0.0621	0.06453	0.06722	0.06633	0.06943	0.07347	0.07788	0.08002	0.08181	0.09102	0.10704	0.10482
532	2.44E-03	1.09E-03	7.46E-03	0.01326	0.01485	0.02303	0.02774	0.03704	0.03976			0.06274	0.06522	0.06802	0.06714	0.07021	0.07427	0.07871	0.08086	0.08284	0.09206	0.10839	0.10606
530	2.42E-03	1.11E-03	7.45E-03	0.01338	0.01504	0.02331	0.02808	0.03749	0.04028			0.06308	0.06572	0.06851	0.06766	0.07074	0.07489	0.07941	0.08169	0.08365	0.09298	0.10939	0.10712
528	2.46E-03	1.14E-03	7.58E-03	0.01359	0.01521	0.02348	0.02845	0.03784	0.04072			0.06347	0.06616	0.06897	0.0682	0.07123	0.07547	0.07995	0.08234	0.08436	0.0938	0.11044	0.10814
526	2.50E-03	1.18E-03	7.67E-03	0.01374	0.01555	0.02365	0.02876	0.03829	0.04121			0.06377	0.06655	0.06929	0.06867	0.07156	0.07593	0.08041	0.08294	0.08505	0.09467	0.11135	0.10911
524	2.39E-03	1.23E-03	7.69E-03	0.01383	0.01564	0.02379	0.02917	0.03864	0.04164			0.06404	0.06678	0.06961	0.06909	0.07195	0.07637	0.08093	0.08341	0.08564	0.09536	0.11224	0.11005
522	2.41E-03	1.26E-03	7.87E-03	0.01413	0.01596	0.02407	0.02947	0.03907	0.04221			0.06416	0.06696	0.06978	0.06933	0.07219	0.07666	0.08125	0.08387	0.08609	0.09597	0.11303	0.11089
520	2.32E-03	1.22E-03	7.80E-03	0.01419	0.01612	0.02418	0.02975	0.03941	0.0427			0.06419	0.06698	0.06985	0.0696	0.07238	0.07694	0.08155	0.08428	0.08657	0.09657	0.11374	0.11169
518	2.36E-03	1.22E-03	8.02E-03	0.0144	0.01634	0.02449	0.03012	0.03987	0.04324			0.06422	0.06702	0.06978	0.06969	0.07243	0.07712	0.08172	0.08456	0.08687	0.09707	0.11442	0.11236
516	2.29E-03	1.31E-03	8.08E-03	0.01455	0.01653	0.02457	0.03049	0.04034	0.04384			0.06414	0.06696	0.06976	0.06975	0.07256	0.07727	0.08185	0.08486	0.08723	0.09752	0.11504	0.11305
514	2.30E-03	1.31E-03	8.13E-03	0.01471	0.01672	0.02491	0.03083	0.04068	0.04438			0.06405	0.06669	0.06957	0.06969	0.07237	0.07726	0.08183	0.08493	0.08739	0.0979	0.11544	0.11364
512	2.37E-03	1.42E-03	8.36E-03	0.01492	0.017	0.02511	0.03118	0.04129	0.04502			0.06382	0.06643	0.0693	0.06959	0.07222	0.07718	0.08177	0.08496	0.08746	0.09817	0.11579	0.11415
510	0.00237	1.46E-03	8.50E-03	0.01508	0.01725	0.02545	0.03165	0.04166	0.04569			0.06358	0.06619	0.06904	0.06945	0.07207	0.07713	0.08173	0.08495	0.08759	0.09842	0.11617	0.11462

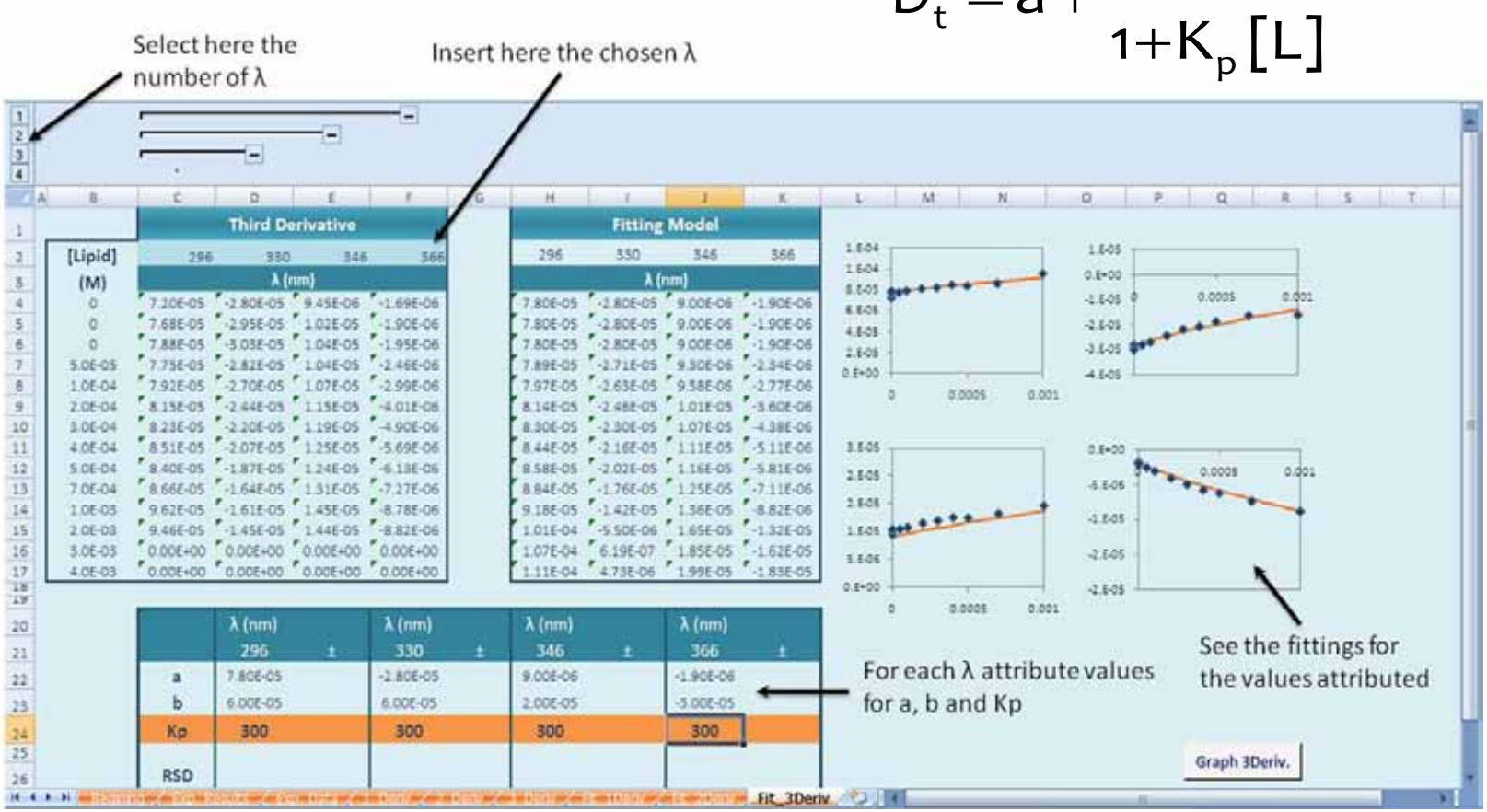
K_p determination (MICROPLATE PROTOCOL)



K_p determination (MICROPLATE PROTOCOL)

K_p determination (MICROPLATE PROTOCOL)

$$D_t = a + \frac{b\,K_p[L]}{1+K_p[L]}$$



The case of NSAIDs

Drug properties

Drug membrane concentration

membrane/aqueous phase partition

Drug membrane location

Surface charge

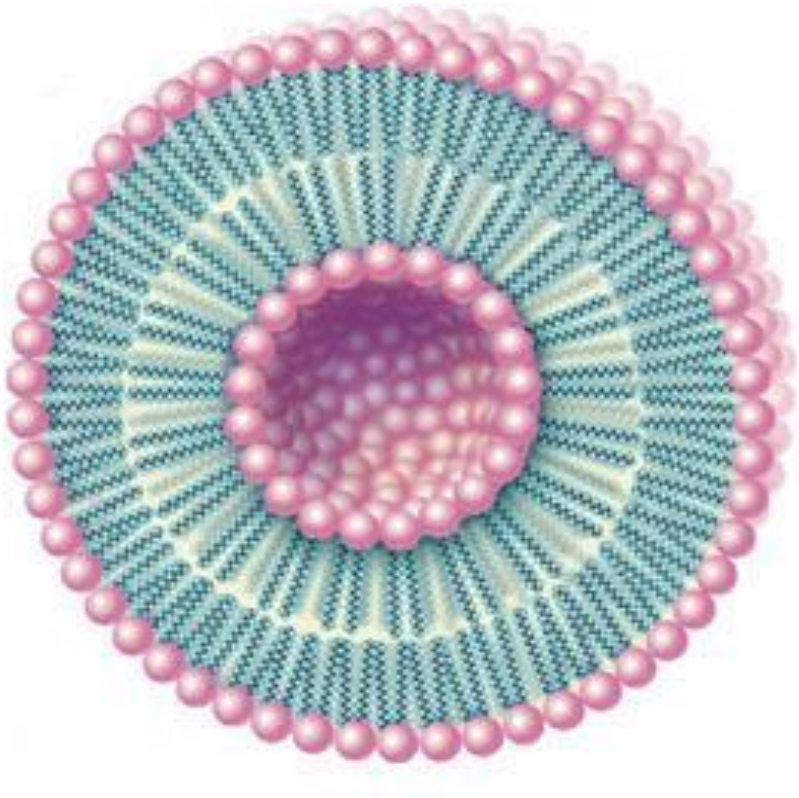
NSAID partition coefficient values (K_p) in LUV of EPC

NSAID	$K_{p\ LUV/water}$	$P_{octanol/water}$
Naproxen	2391 ± 473	2188
Nimesulide	360 ± 25	398
Tolmetin	220 ± 70	0,10
Meloxicam	685 ± 70	1,17
Lornoxicam	493 ± 81	63

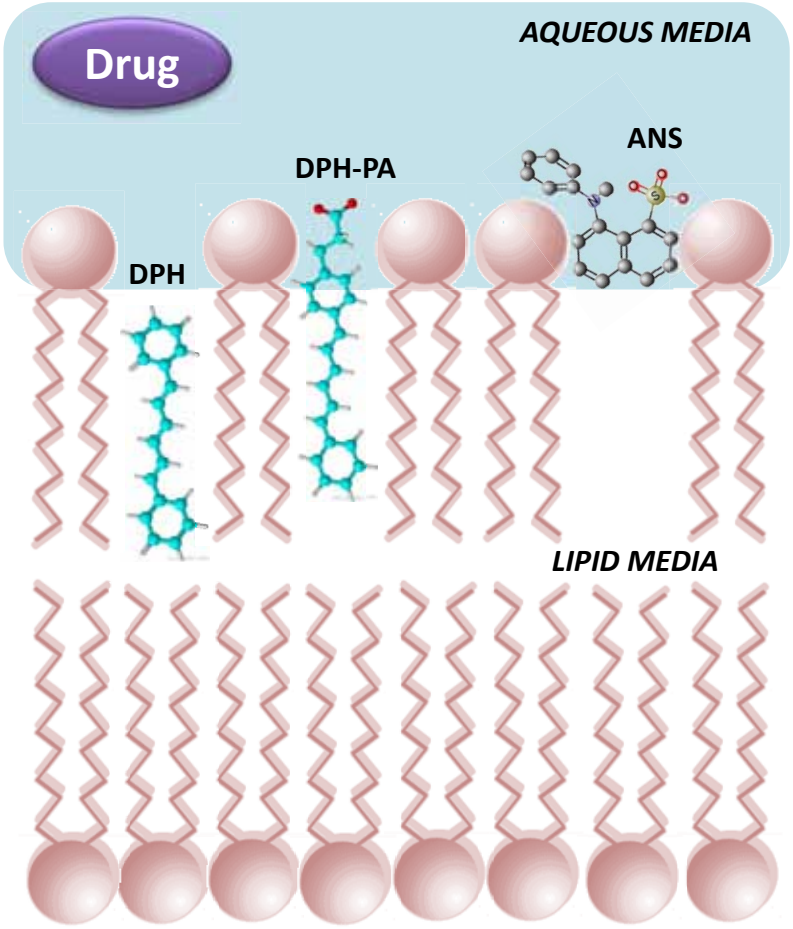
Hydrophobic drug/membrane interactions

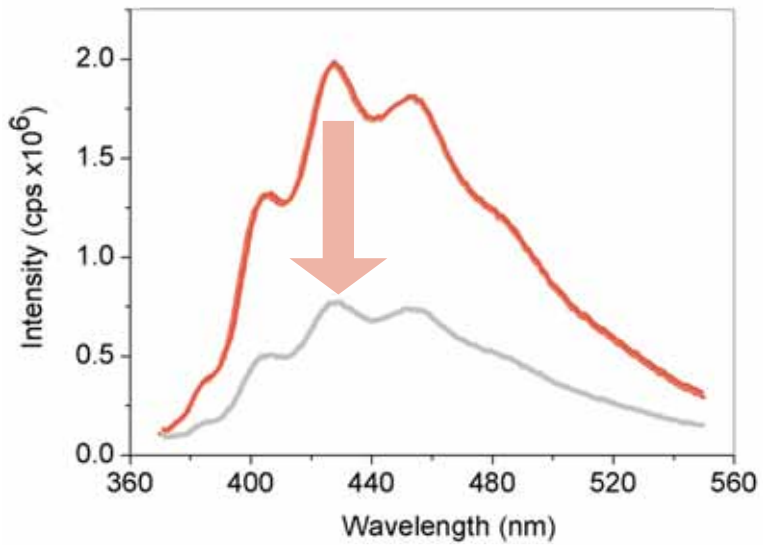
Hydrophobic and electrostatic drug/membrane interactions

Why is important to predict location of drugs in the membrane?



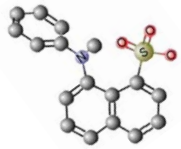
How can we predict the location of drugs in the membrane?






DPH-PA


DPH


ANS

DPH-PA: 1-(4-(6-phenyl)-1,3,5-hexatrienylpropionic acid

DPH : 1,6-Diphenyl-1,3,5-hexatriene

ANS : 1-anilinonaphthalene-8-sulfonic acid

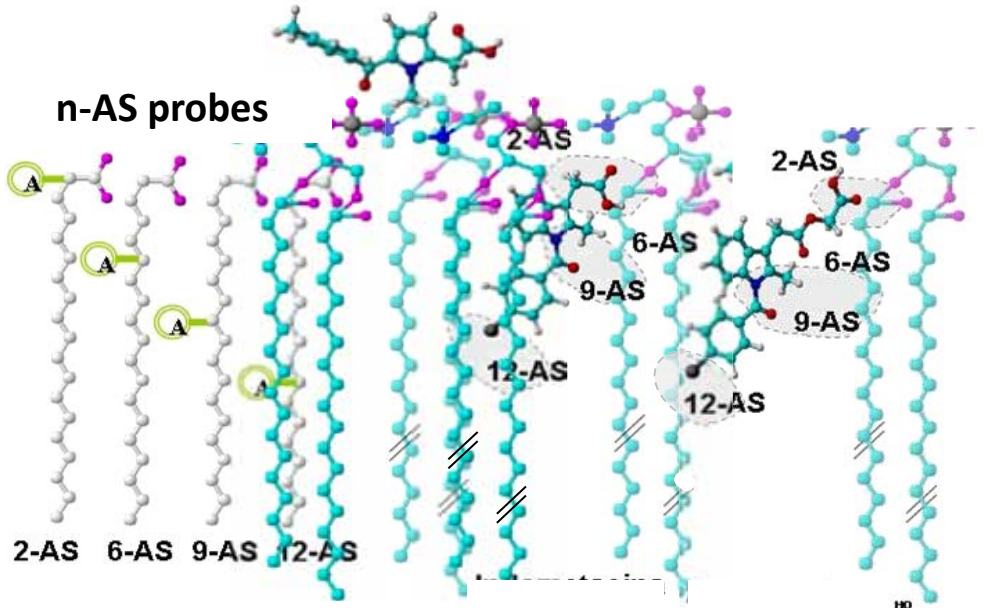
The case of NSAIDs

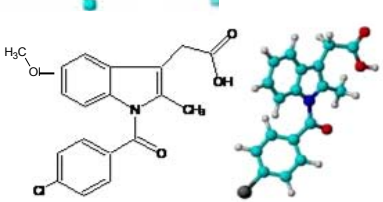
Drug properties

Drug membrane concentration

Drug membrane location

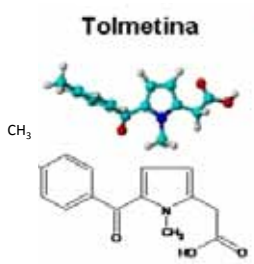
Surface charge





INDOMETHACIN

ACEMETACIN



TOLMETIN

Anal Bioanal Chem (2003) 377 : 293–298
PCCP 6 (2004) 1493–1698

Chemical Physics Letters 471 (2009) 300–309

The case of NSAIDs

Drug properties

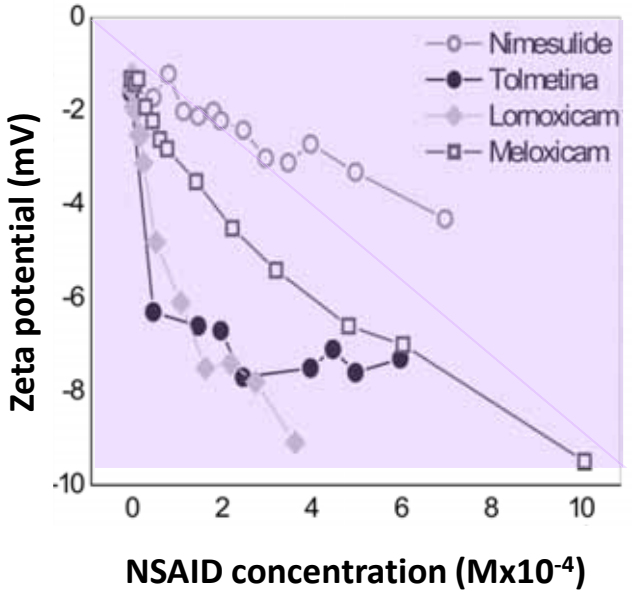
Drug membrane concentration

Drug membrane location

Surface charge

The polar heads of the phospholipids can affect the function of a membrane since this is the part of the lipids that is present on the membrane surface, and is responsible for the interaction with the surrounding.

The electrostatics of the lipid membranes affects membrane-protein interactions, domain formation and other membrane functions.



The case of NSAIDs

Modifications of membrane structure and membrane biophysical properties

- **Dynamic properties**
- Membrane phase transitions
- Membrane structure

Why study membrane dynamic properties?

Modifications membrane dynamic properties (diffusion, permeability, fluidity bilayer, order, packing) may modify the functionality of receptors in vivo presumably due to changes in the movement or orientation of proteins which float within matrix of the lipid bilayer.

The case of NSAIDs

Modifications of membrane structure and membrane biophysical properties

- **Dynamic properties**
- Membrane phase transitions
- Membrane structure

Fluidity corresponds to all the lipid dynamic aspects such as:

Microviscosity and **order**.

X-rays

- **Differential scanning calorimetry (DSC)**
- **Langmuir isotherms**
- **Fluorescence anisotropy**

BIOPHYSICAL EFFECTS:
Microviscosity and phase transition

The case of NSAIDs

Modifications of membrane structure and membrane biophysical properties

Dynamic properties

Membrane phase transitions

Membrane structure

Tp – pre-transition temperature

Effect in T_p

Drug interacts with polar head groups

Tm – Main phase transition temperature

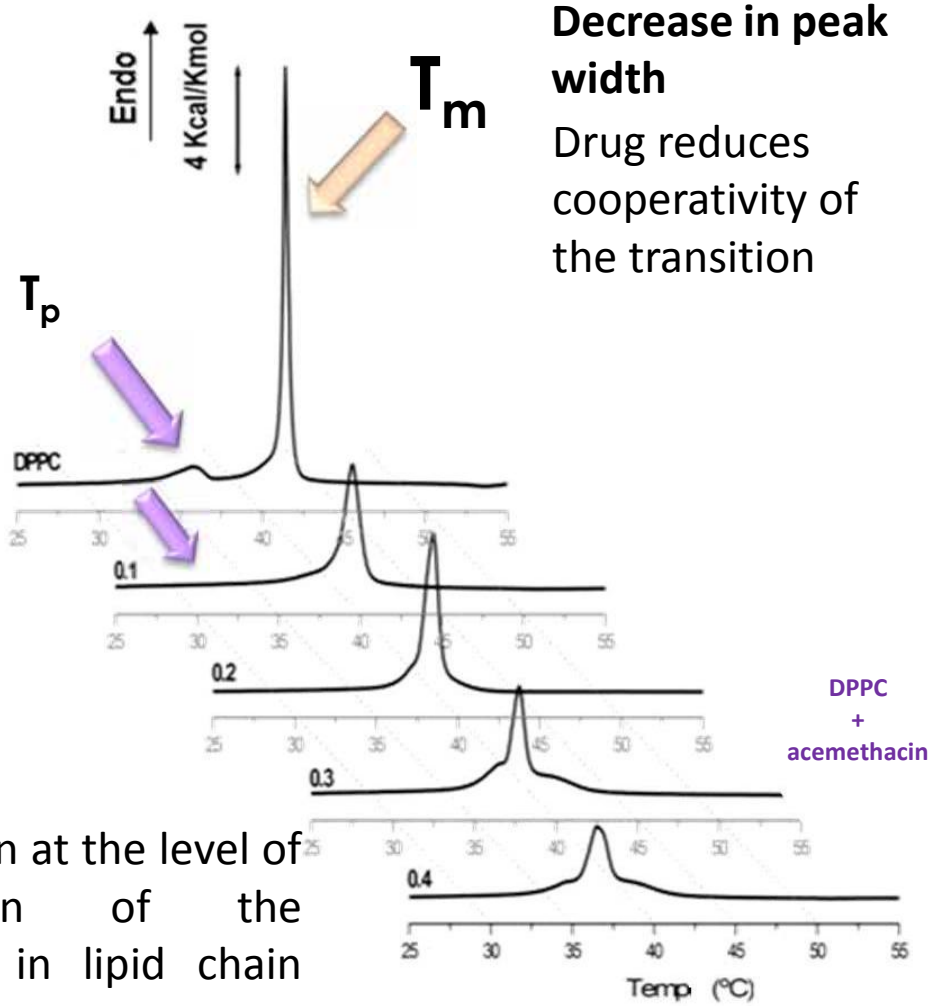
Effect in T_m

↓ T_m indicates that drug induces destabilization

Effect in ΔH

↓ ΔH indicates insertion at the level of hydrophobic region of the membrane, changes in lipid chain packing.

DSC results interpretation



Decrease in peak width

Drug reduces cooperativity of the transition

BIOPHYSICAL EFFECTS:
Microviscosity and phase transition

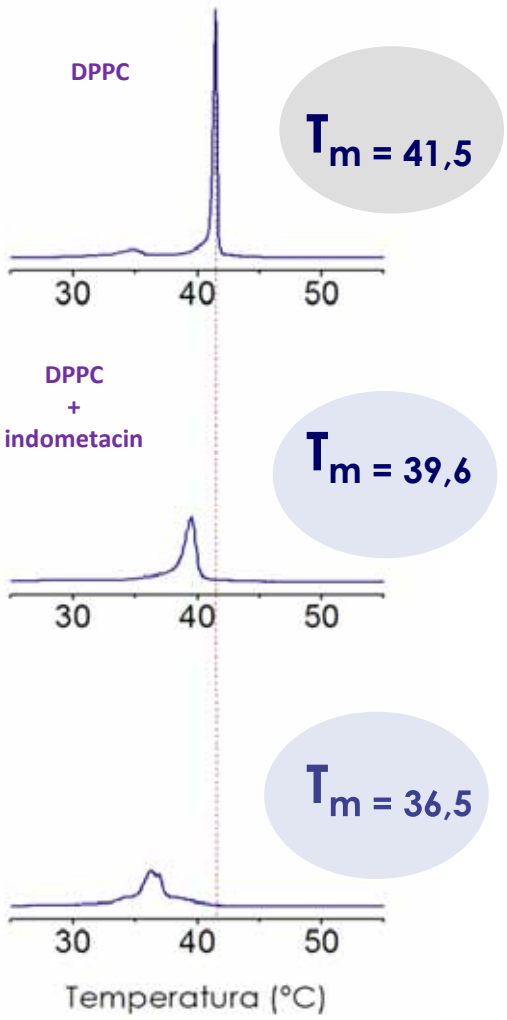
The case of NSAIDs

Modifications of membrane structure and membrane biophysical properties

Dynamic properties

Membrane phase transitions

Membrane structure



Effects of NSAIDs:

- Decrease of the **main phase transition temperature** (T_m) .

- Decrease of **cooperativity** of the transition temperature (narrower phase transition peaks)

Increase of membrane fluidity

The case of NSAIDs

Modifications of membrane structure and membrane biophysical properties

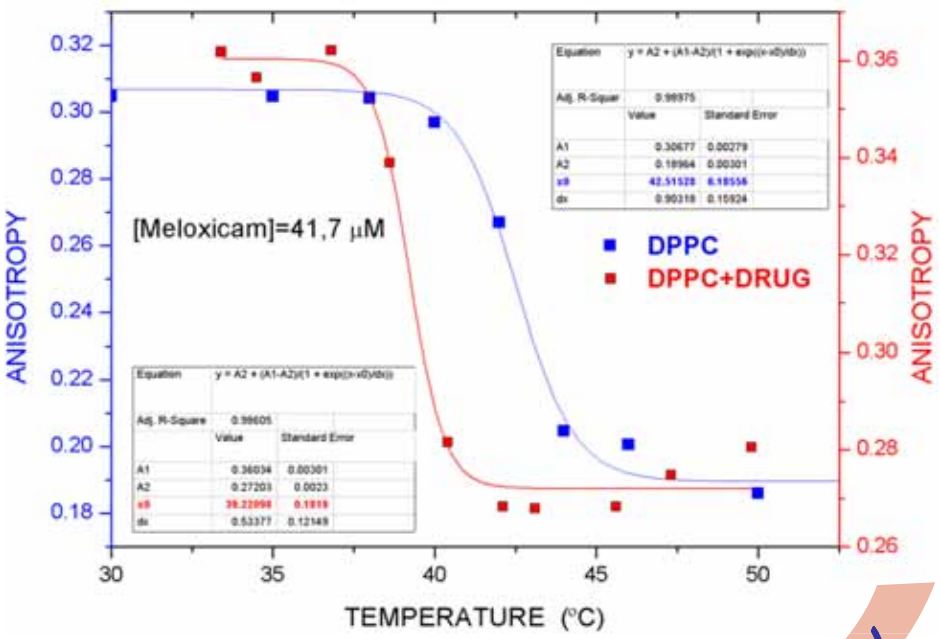
Dynamic properties

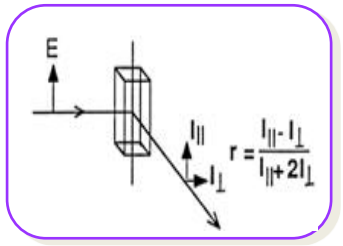
Membrane phase transitions

Membrane structure

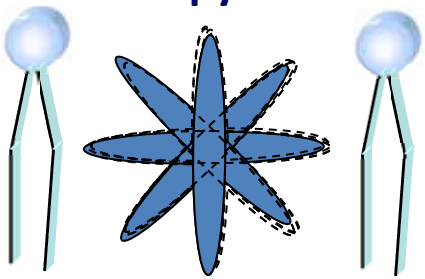
Steady-state anisotropy

Anisotropy (r) measurements indicate the rotation capacity of a fluorescent probe during the excited lifetime.





Anisotropy values



Probe rotation

influences

Membrane microviscosity

study

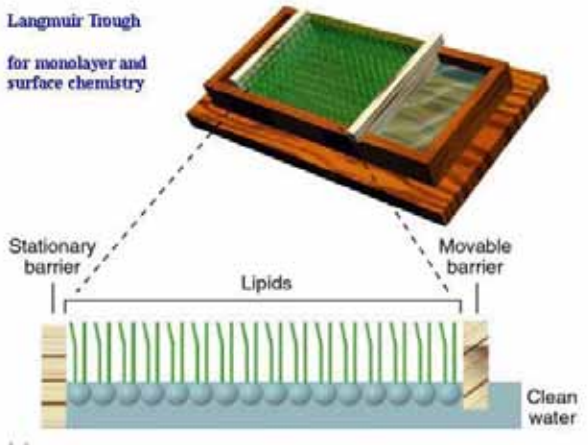
The case of NSAIDs

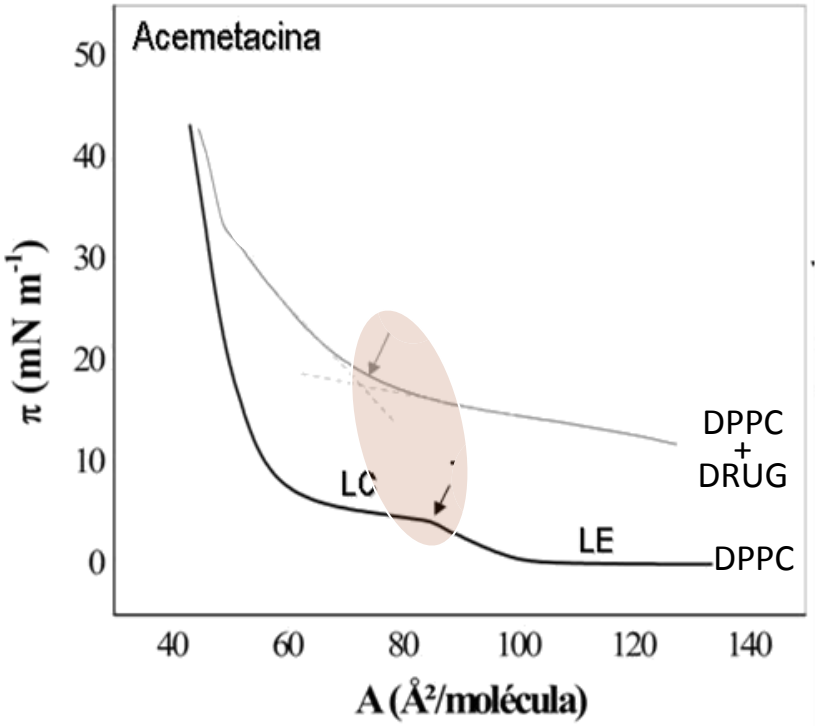
Modifications of membrane structure and membrane biophysical properties

Dynamic properties

Membrane phase transitions

Membrane structure





Drug has an effect in membrane phase transition

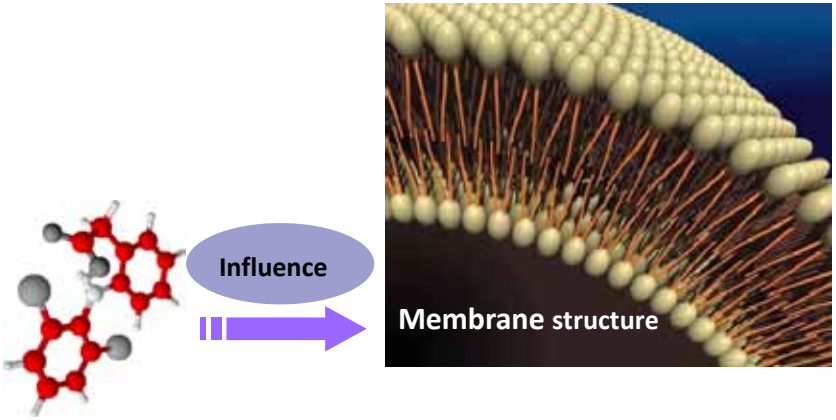
Increase of membrane fluidity

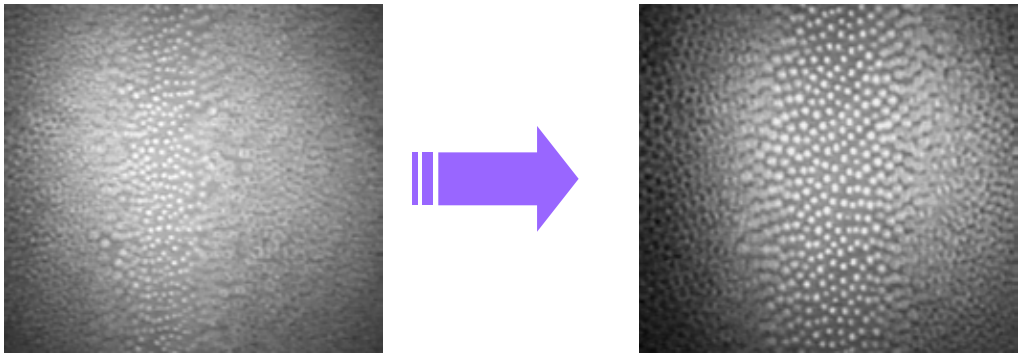
Langmuir, 24, 4132-4139 (2008)

The case of NSAIDs

Modifications of membrane structure and membrane biophysical properties

- Dynamic properties
- Membrane phase transitions
- **Membrane structure**





DMPC + DPPE (7:3)

(BAM – Brewster angle microscopy)

The case of NSAIDs

Modifications of membrane structure and membrane biophysical properties

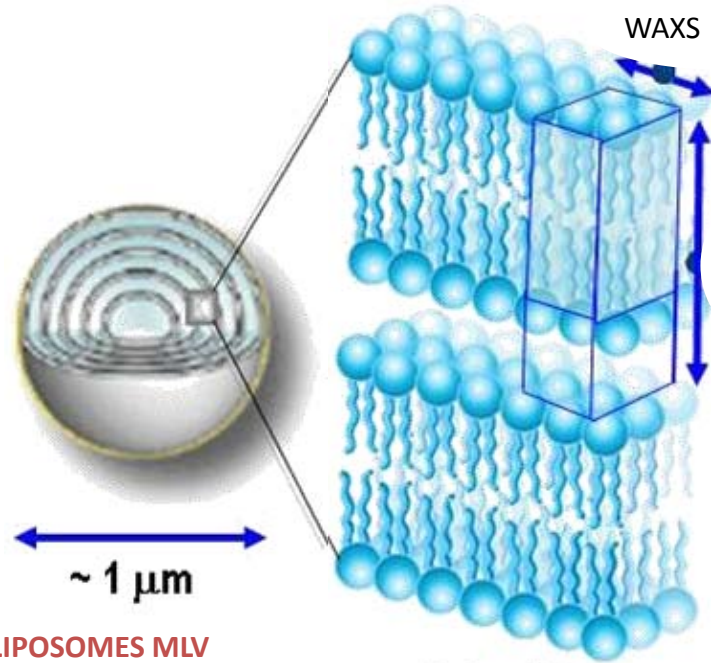
- Dynamic properties
- Membrane phase transitions
- **Membrane structure**

X-RAY SCATTERING

WIDE ANGLE (WAXS)

SMALL ANGLE (SAXS)

WAXS MEASURES LIPID PACKING



SAXS MEASURES BILAYER THICKNESS

LIPOSOMES MLV

X-rays resolution

~ 4 - 1000 Å

The case of NSAIDs

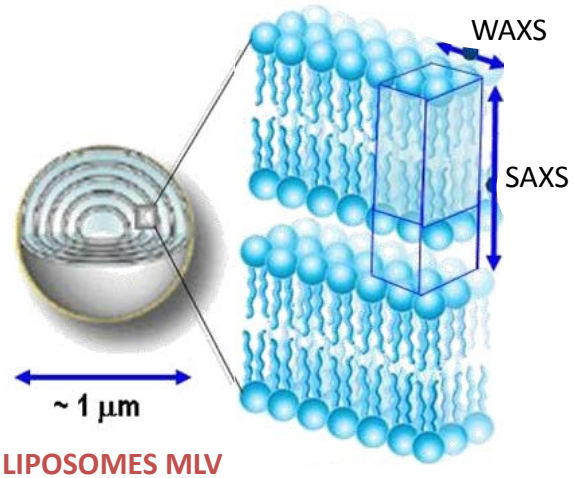
Modifications of membrane structure and membrane biophysical properties

- Dynamic properties
- Membrane phase transitions
- **Membrane structure**

X-RAY SCATTERING

WIDE ANGLE (WAXS)

SMALL ANGLE (SAXS)



LIPOSOMES MLV

X-rays resolution

~ 4 - 1000 Å

SAXS

(Gel phase)	20 °C d (Å)
DPPC	63,7 ± 0,5
DPPC + Nimesulide	63,7 ± 0,5
DPPC + Acemetacin	74,6 ± 0,5
DPPC + Indomethacin	69,7 ± 0,5
	77,0 ± 0,5

No visible perturbation of the gel phase

d doesn´t change

d increases

Increases the water layer thickness

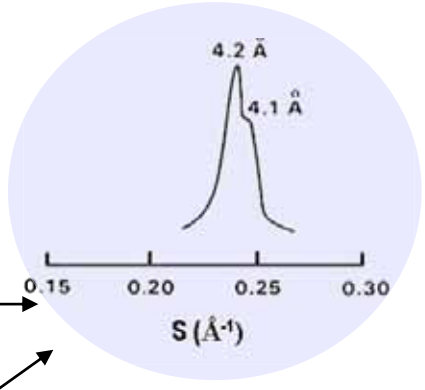
Langmuir, 24, 4132-4139 (2008)

BIOPHYSICAL EFFECTS:
Structure and order

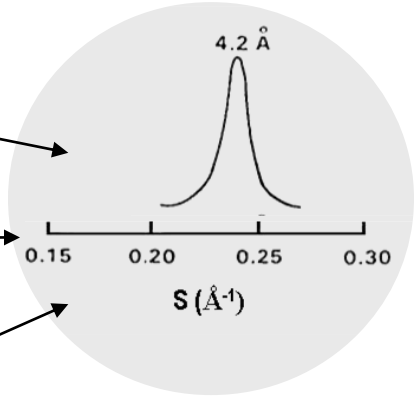
WAXS

(Gel phase)	c_{NSAID} (mol%)	20 °C d (Å)	20 °C ξ
DPPC	0	4,25	178 ± 10
		4,16	113 ± 10
DPPC + Nimesulide	10	4,25	168 ± 10
		4,15	102 ± 10
	40	4,21	83 ± 10
DPPC + Acemetacin	10	4,19	85 ± 10
	40	4,20	79 ± 10
DPPC + Indomethacin	10	4,20	72 ± 10
	40	4,20	94 ± 10

Orthorrombic packing



Hexagonal packing



Drugs penetrate into the bilayer changing the headgroup conformation altering the lipid packing

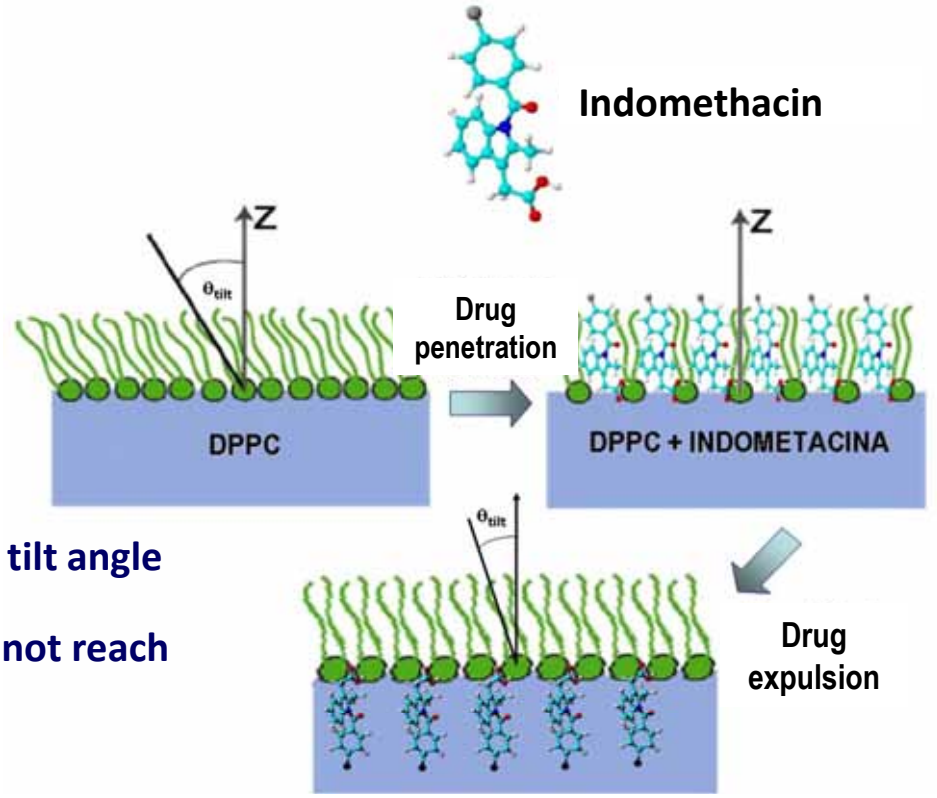
Langmuir, 24, 4132-4139 (2008)
Soft Matter, 7, 3002-3010 (2011)

The case of NSAIDs

Modifications of membrane structure and membrane biophysical properties

- Dynamic properties
- Membrane phase transitions
- **Membrane structure**

GRAZING INCIDENT X-RAY DIFRACTION (GIXD)



Drug penetrates into the monolayer changing the tilt angle

When the drug is squeezed out, the tilt angle does not reach the normal value

Even in the aqueous phase drug is still interacting with the membrane

The case of NSAIDs

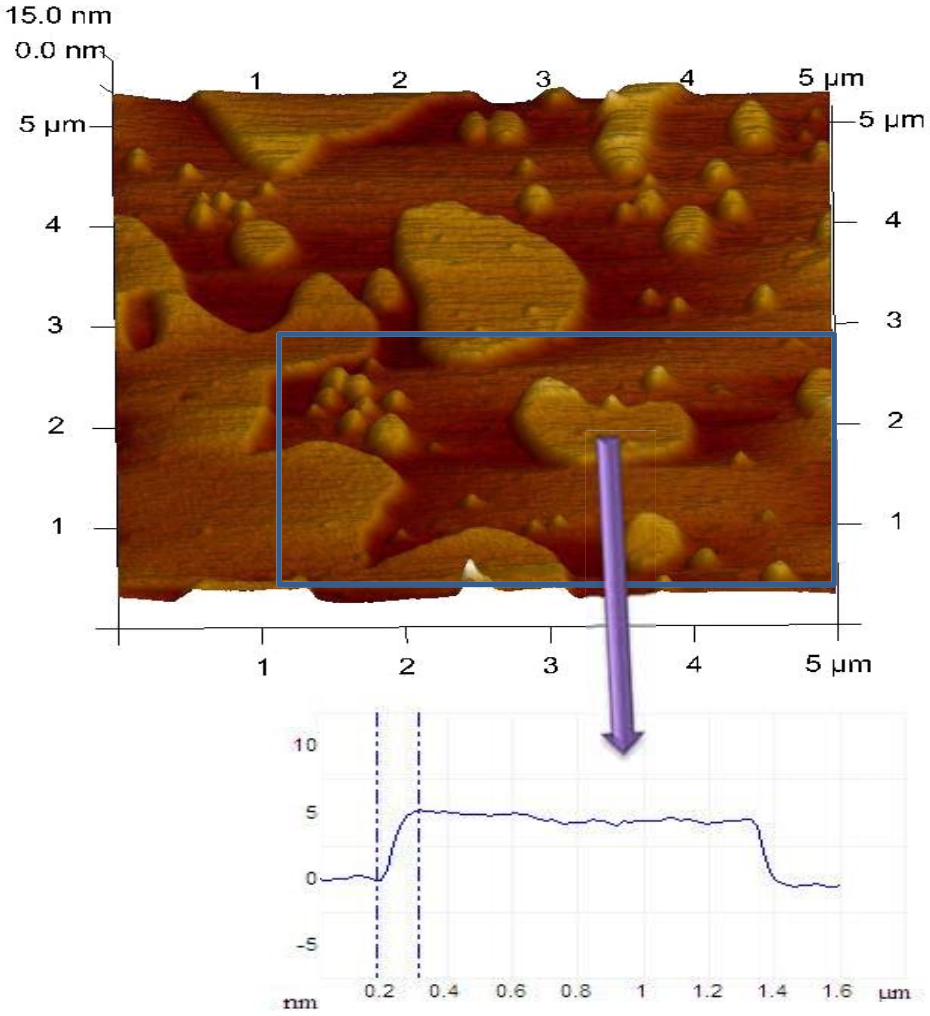
Modifications of membrane structure and membrane biophysical properties

- Dynamic properties
- Membrane phase transitions
- **Membrane structure**

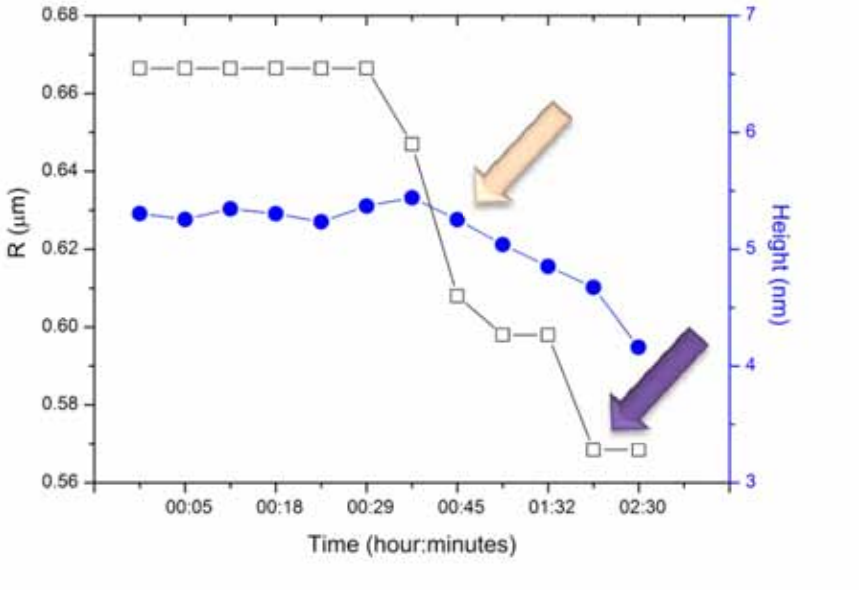
Supported lipid bilayer (SLB) of DPPC pH 7.4 (gel phase)

Before addition of drug

AFM example of results interpretation



BIOPHYSICAL EFFECTS:
Structure and order - AFM

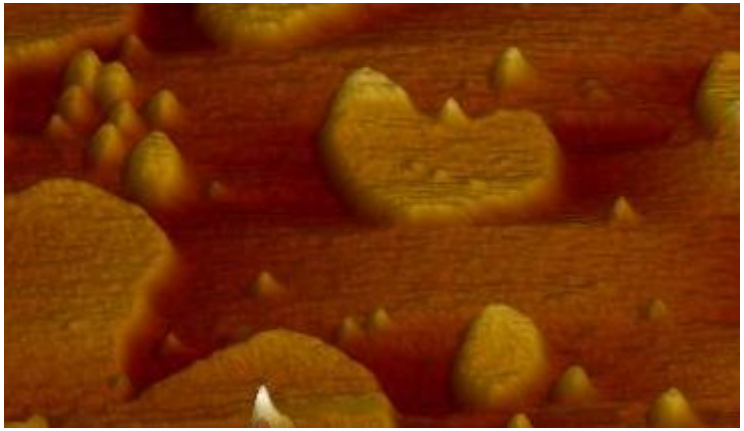


Lipid domain diameter decreases

Lipid bilayer thickness decreases

These effects can be correlated with local gastric toxicity

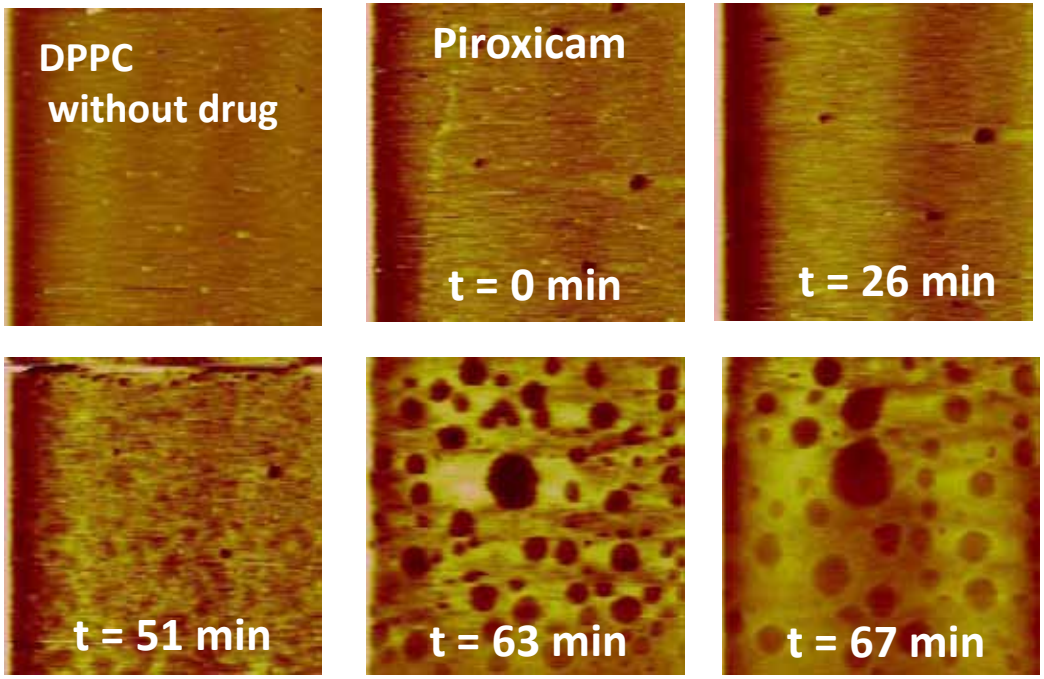
AFM example of results interpretation



Supported lipid bilayer (SLB) of DPPC pH 7.4

After addition of drug (Tolmetin)

AFM example of results interpretation

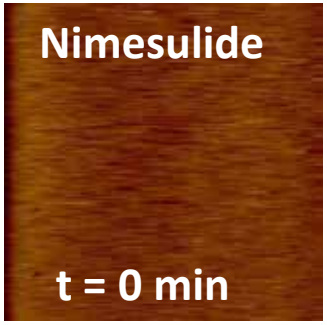


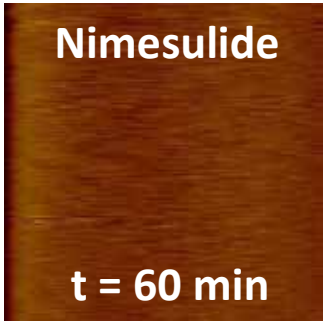
Piroxicam causes holes in the lipid bilayer

The number and the diameter of the holes in the lipid bilayer increase with time

These effects can be correlated with local gastric toxicity

AFM example of results interpretation





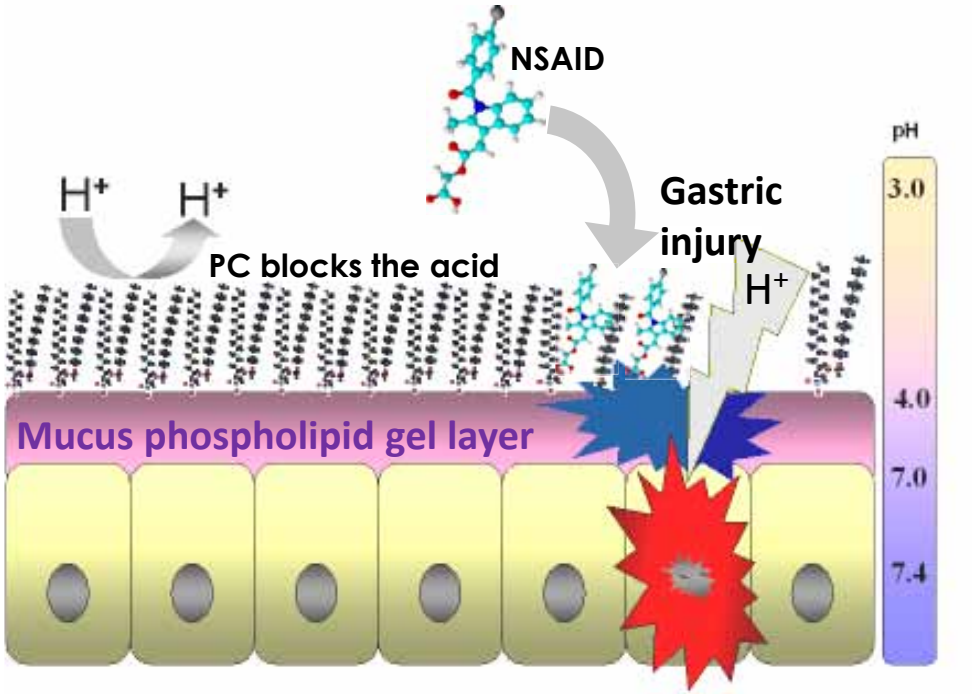
Nimesulide has no visible effect on the lipid bilayers

These effects can be correlated with local gastric toxicity

This drug has the less gastric effects

Gastric toxicity is the most common problem with the use of NSAIDs

Stomach is protected with a layer of DPPC in the gel phase



Drug effects presented in lipid bilayers of DPPC can be correlated with gastric toxicity

Effects of NSAIDs:

Modifications of membrane structure and membrane biophysical properties

- **Increase the water layer** between the bilayers

- **Change the tilt angle** between the phospholipid molecules

-**Change the headgroup orientation** of the bilayer phospholipids.

- **Increase membrane fluidity**

- **Formation of holes in gel bilayer**

More toxic drugs have higher effects

The case of NSAIDs

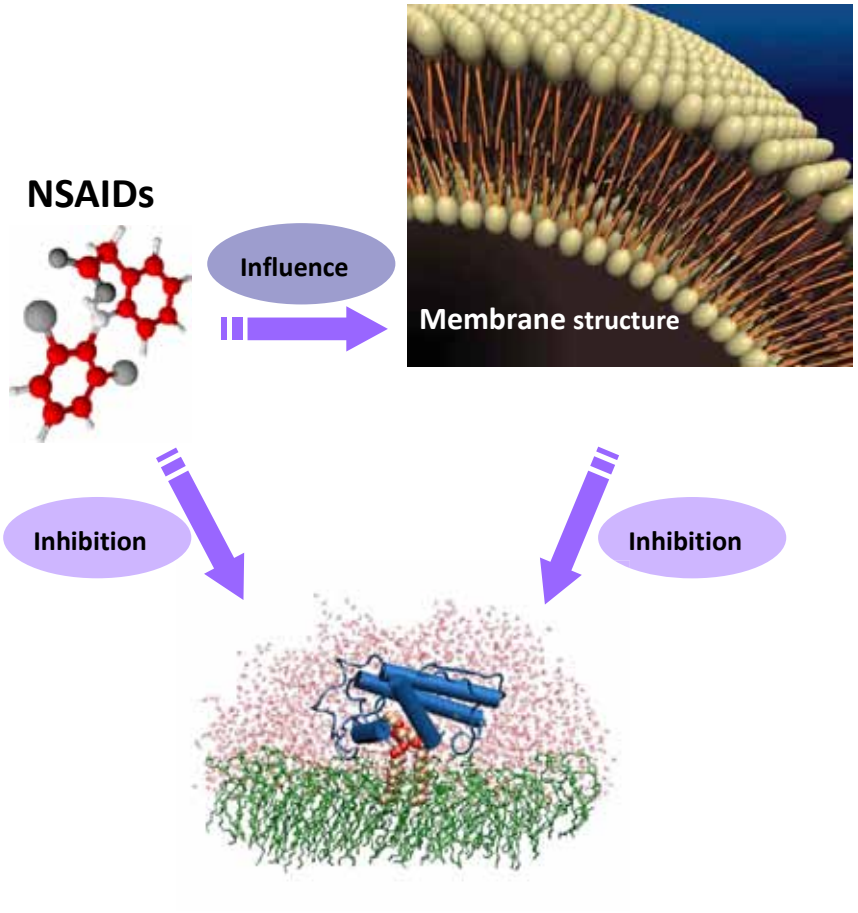
Modifications of membrane structure and membrane biophysical properties

Drugs can interact directly with enzymes

or

Drugs can interact with lipid bilayers changing membrane interface

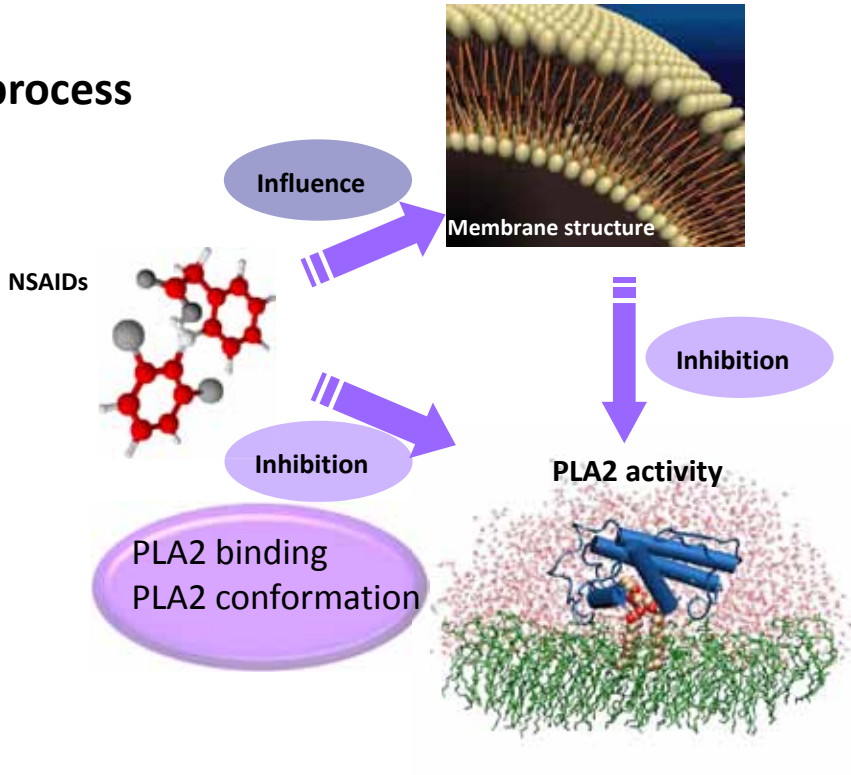
This can lead to enzymatic inhibition



This two mechanisms were studied for PLA2

PLA2 is an enzyme involved in the inflammatory process

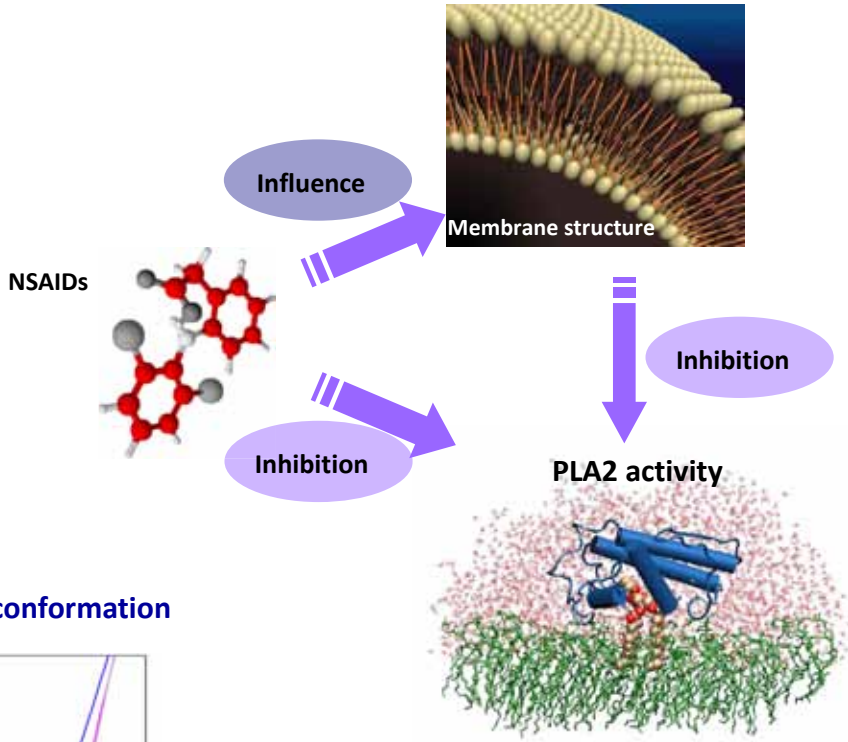
PLA2 is water soluble but acts at the membrane level hydrolising membrane phospholipids



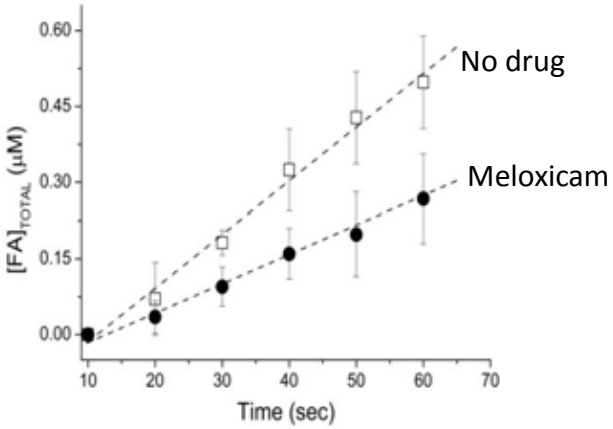
Biophysical Chemistry 152, 109–11 (2010)

How the PLA2 inhibition mechanism was studied?

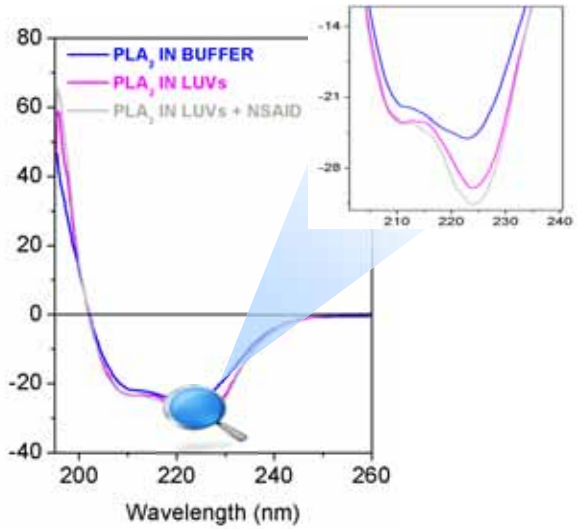
- Enzyme activity and Inhibition studies
- Enzyme conformation studies
- Drug-enzyme binding studies
- Effect of drugs in membrane interface



Fluorescence studies with ADIFAB probe



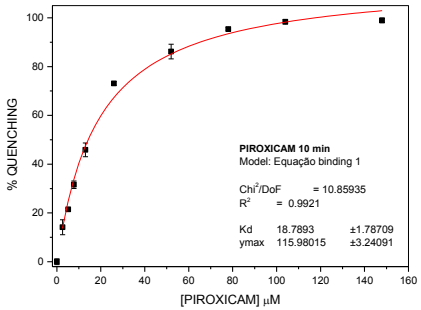
Circular dicroism (CD) – enzyme conformation



Fluorescence quenching of PLA2 intrinsic fluorescence

$$y = \frac{y_{máx}}{1 + \frac{Kd}{[L]}}$$

Kd=18.87



Biophysical Chemistry 152, 109–11 (2010)

Chemistry and Physics of lipids (in press) (2011)

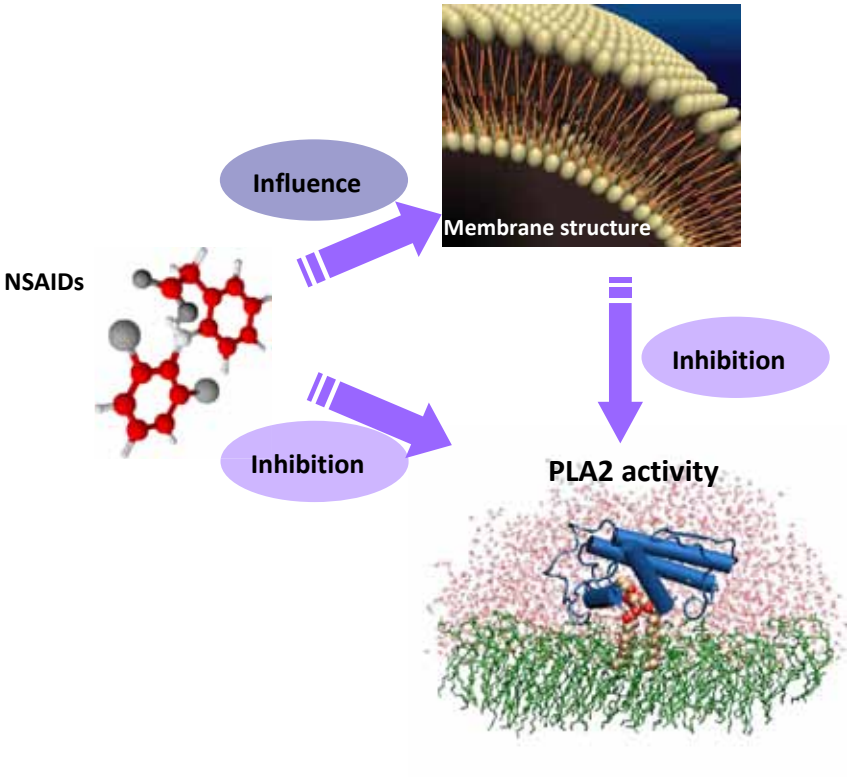
How the PLA2 inhibition mechanism was studied?

Enzyme activity and Inhibition studies

Enzyme conformation studies

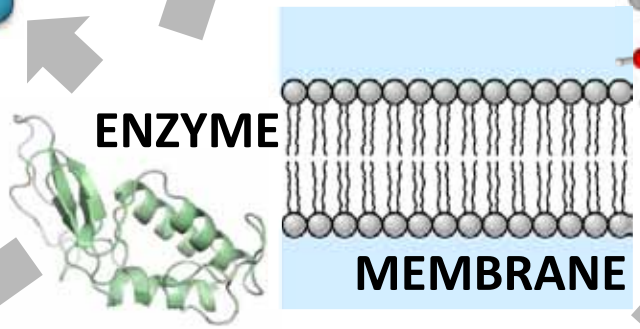
Drug-enzyme binding studies

Effect of drugs in membrane interface



These studies prove that the effects of drugs on membrane biophysical properties contributes to their mechanism of action

Biophysical Chemistry 152, 109–11 (2010)
Chemistry and Physics of lipids (in press) (2011)



ENZYME

MEMBRANE

DRUG

CONFORMATION

CD, FLUORESCENCE

MEMBRANE LOCATION

FLUORESCENCE QUENCHING

INHIBITION

FLUORESCENCE
LANGMUIR ISO-THERMS

MEMBRANE / WATER PARTITION

DERIVATIVE SPECTROSCOPY

BINDING

FLUORESCENCE

STRUCTURE

AFM, BAM , SAXS
WAXS

PHASE TRANSITIONS

DSC , FLUORESCENCE ANISOTROPY
LANGMUIR ISOTHERMS

Study the role of membrane on the mechanisms of action and toxicity of therapeutics

- **CONFORMATION** — CD, FLUORESCENCE
- **MEMBRANE LOCATION** — FLUORESCENCE QUENCHING
- **INHIBITION** — FLUORESCENCE LANGMUIR ISO-THERMS
- **MEMBRANE / WATER PARTITION** — DERIVATIVE SPECTROSCOPY
- **BINDING** — FLUORESCENCE
- **STRUCTURE** — AFM, BAM , SAXS WAXS
- **PHASE TRANSITIONS** — DSC , FLUORESCENCE ANISOTROPY LANGMUIR ISOTHERMS

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Daniela Lopes
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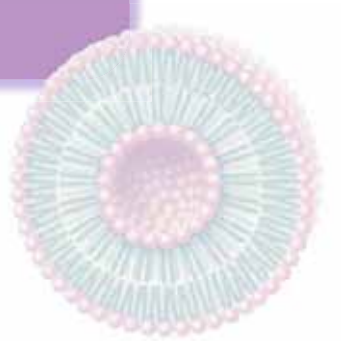
Rafael Amaral
Ana Rentão
Catarina Alves
Natacha Rosa

José L. F. C. Lima

OBRIGADA



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DESY

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