Louvain Drug Research Institute

Cellular and Molecular Pharmacology Unit

UCL Université catholique de Louvain

Interactions between Fluoroquinolones and lipids: Biophysical studies

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May 25th, 2011

Promoter Prof. Marie-Paule Mingeot-Leclercq

Plan of the presentation

I- Introduction

- ✓ Pharmacology of fluoroquinolones
- ✓ Mechanism of resistance of fluoroquinolones: efflux pumps phenomenom

II- Aims of the Thesis

III- Materials and Methods

IV- Results

- ✓ Investigation of the interaction of two fluoroquinolones (CIP and MXF) with model lipid membranes

Bensikaddour et al., 2008: *Biophysical Journal* 94: 3035-3046

- ✓ Investigation of the interaction of CIP with eukaryotic and prokaryotic model lipids membranes (DPPC vs DPPG)

Bensikaddour et al., 2008: *Biochimica et Biophysica Acta* 1778: 2535-2543

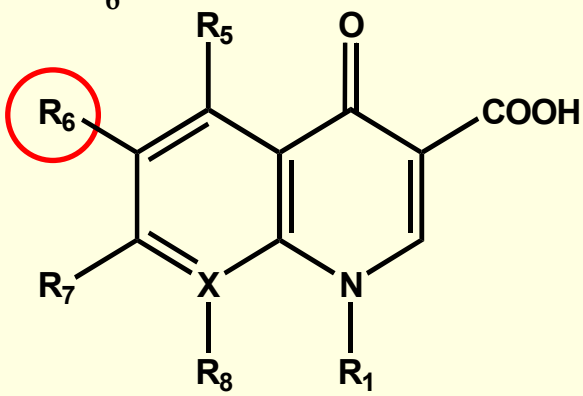
V- General Conclusion

VI- Perspectives

I- Introduction

Pharmacology of Fluoroquinolones

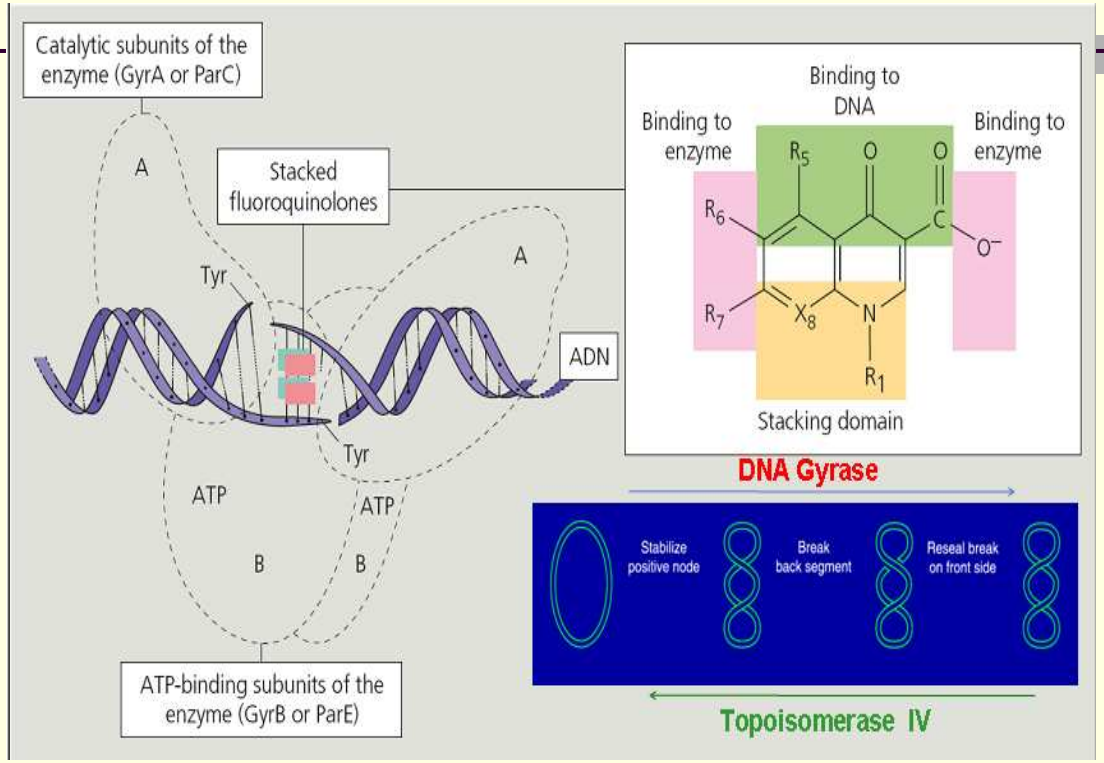
- ✓ **Synthetic origin compounds: Quinoline ring and Fluor atom at R_6**
- ✓ **Bactericide activity**
- ✓ **Based on chemical structure and antibacterial activity: 3 generations**



- **1st generation (Quinolones)** ⇒ Treatment of urinary tract infections (1960-1970)
- **2nd generation** ⇒ Systematic use in the (1980-1990)
- **3rd generation** ⇒ Treatment of respiratory tract infections (from1990-xxxx)

I- Introduction

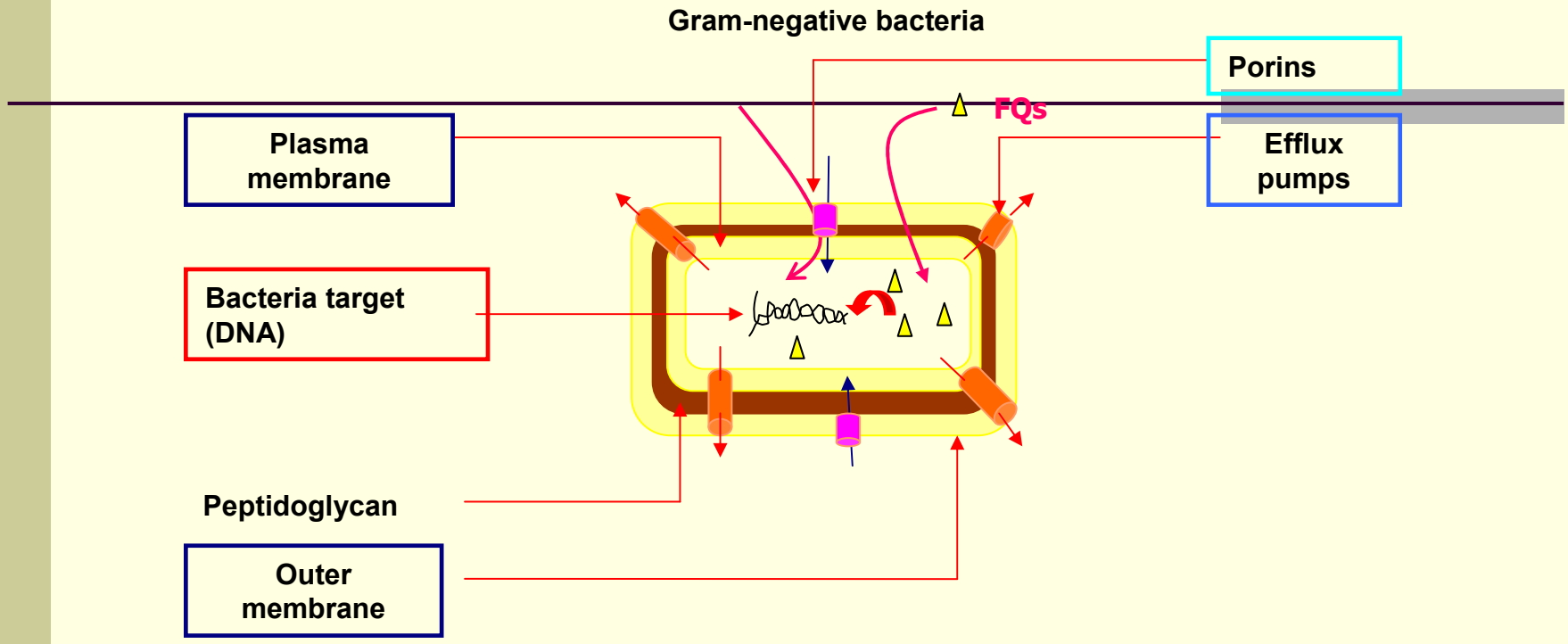
Pharmacology of fluoroquinolones



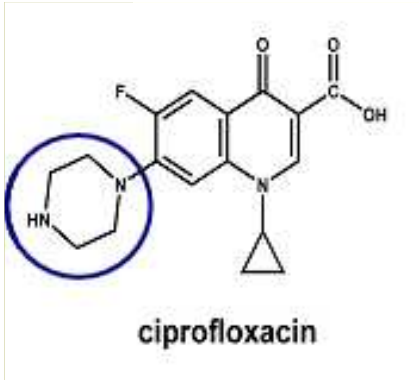
✓ **Bacterial targets are DNA gyrase and Topoisomerase IV.**

I- Introduction

Mechanisms of resistance

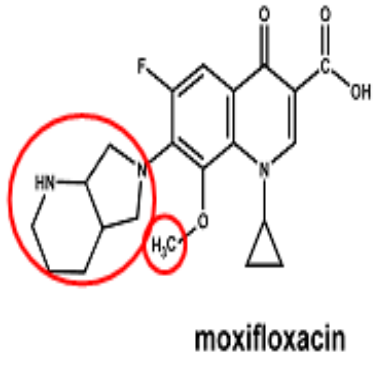


Resistance due to target enzymes mutation: - mutations in topoisomerase IV (ParC or ParE) - mutations in DNA gyrase (GyrA or GyrB)
- resistance due to a protective mechanism (ex. plasmid qnr)
- Resistance due to altered access of drug to target enzymes: ✓ Difficulty to diffuse through the membrane ✓ Efflux system (efflux pumps in bacteria ex: AcrAB-TolC of *E.coli*)



I- Introduction

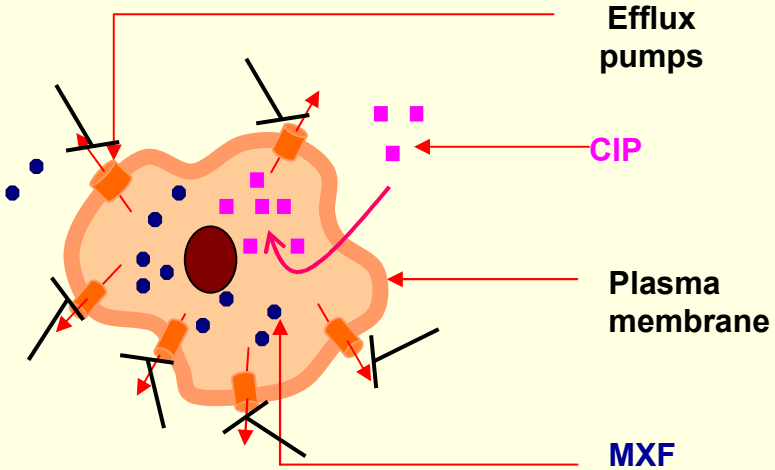
Previous studies on accumulation of CIP and MXF



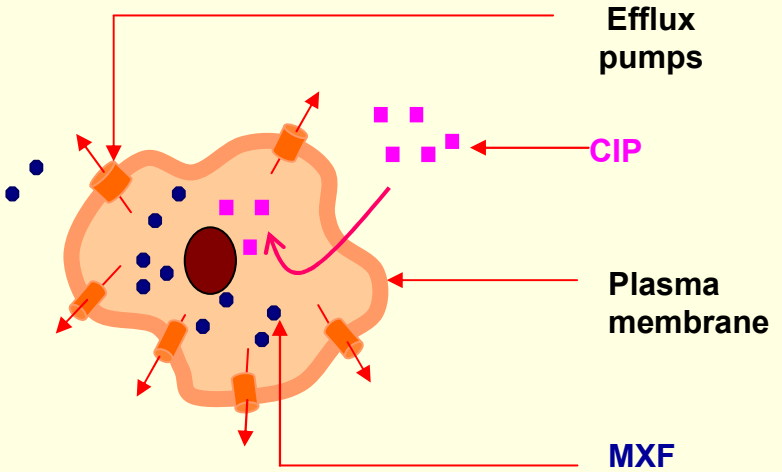
Eukaryotic cells

J774 macrophages cells

+ MRP inhibitors



- MRP inhibitors



✓ **MRP inhibitors had no effect on the accumulation of MXF**

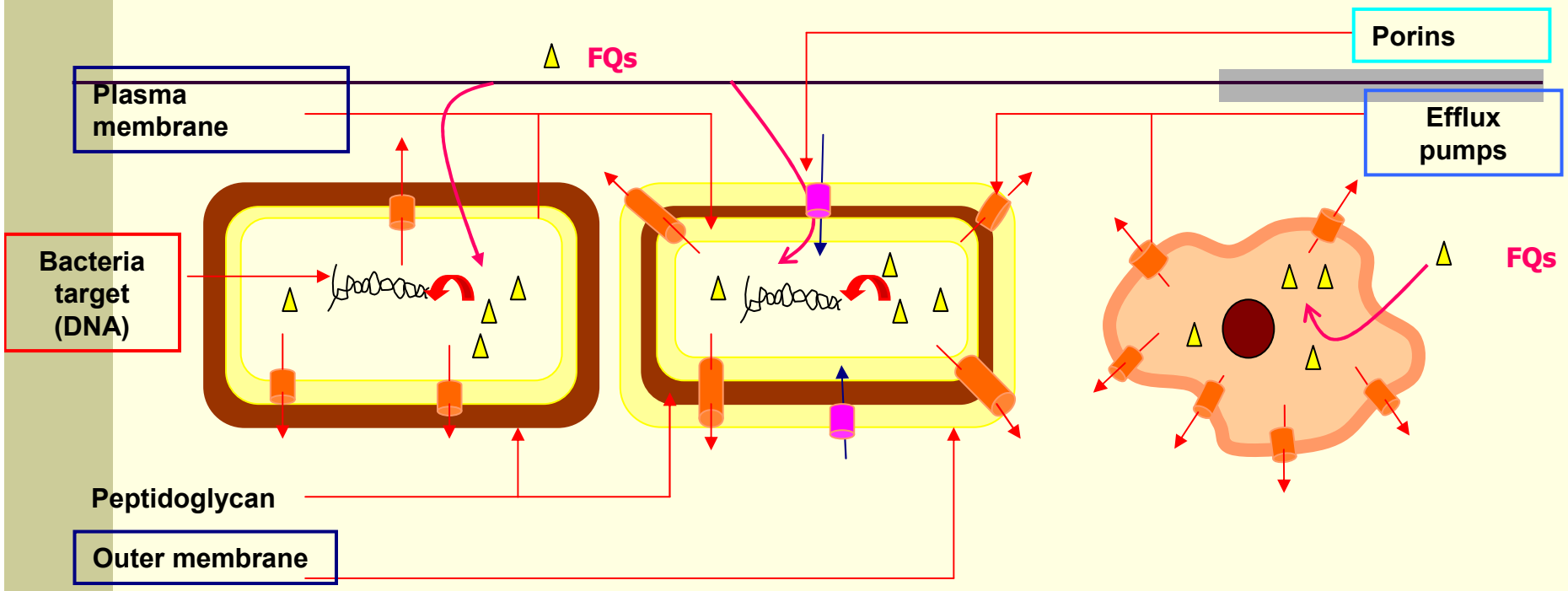
✓ **MRP inhibitors increase the accumulation of CIP**

✓ **Intracellular accumulation**

✓ **Accumulation of MXF >>>CIP**

Michot *et al.* (2004, 2005), Marquez *et al.*,(2009).

I-Introduction



To reach intracellular bacteria target, CIP and MXF interact with membrane lipids bilayer:

- ✓ where they can be recognized by the efflux pumps proteins in eukaryotic cells
- ✓ and /or the porins and efflux pumps proteins in prokaryotic cells

Investigation of interaction of FQs with model membranes at molecular level

II- Aims of Thesis

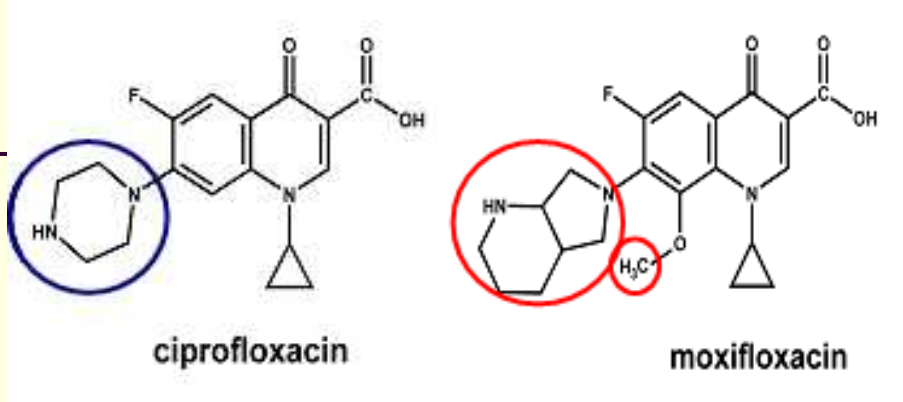
Characterize the effect of two fluoroquinolones, CIP and MXF, on the physicochemical properties of the major phospholipids of both the eukaryotic and prokaryotic membranes:

✓ *Investigation of the interaction of two fluoroquinolones (CIP and MXF) with model lipid membranes.*

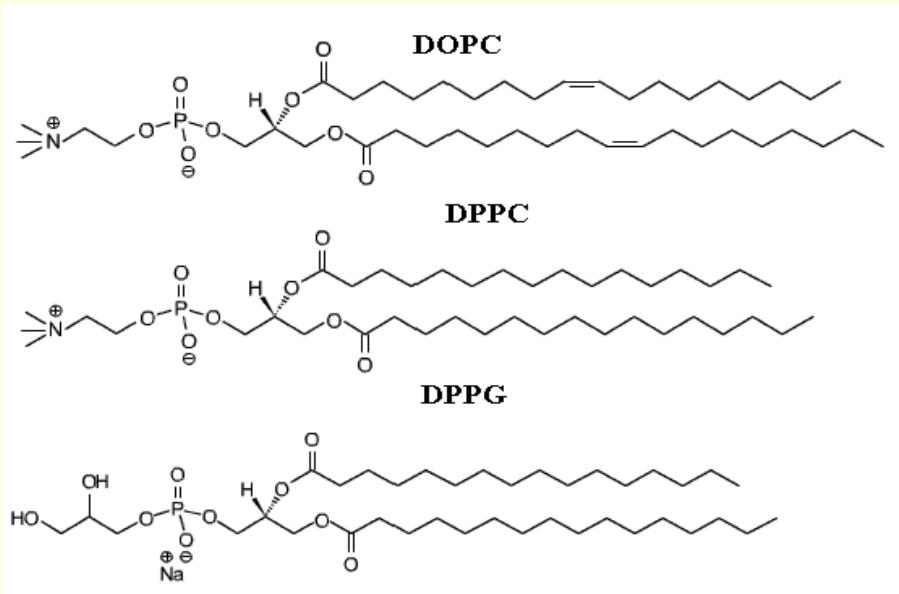
✓ *Investigation of the interaction of CIP with eukaryotic and prokaryotic model lipids membranes (DPPC vs DPPG).*

III- Materials and methods

- ✓ Antibiotics: CIP and MXF

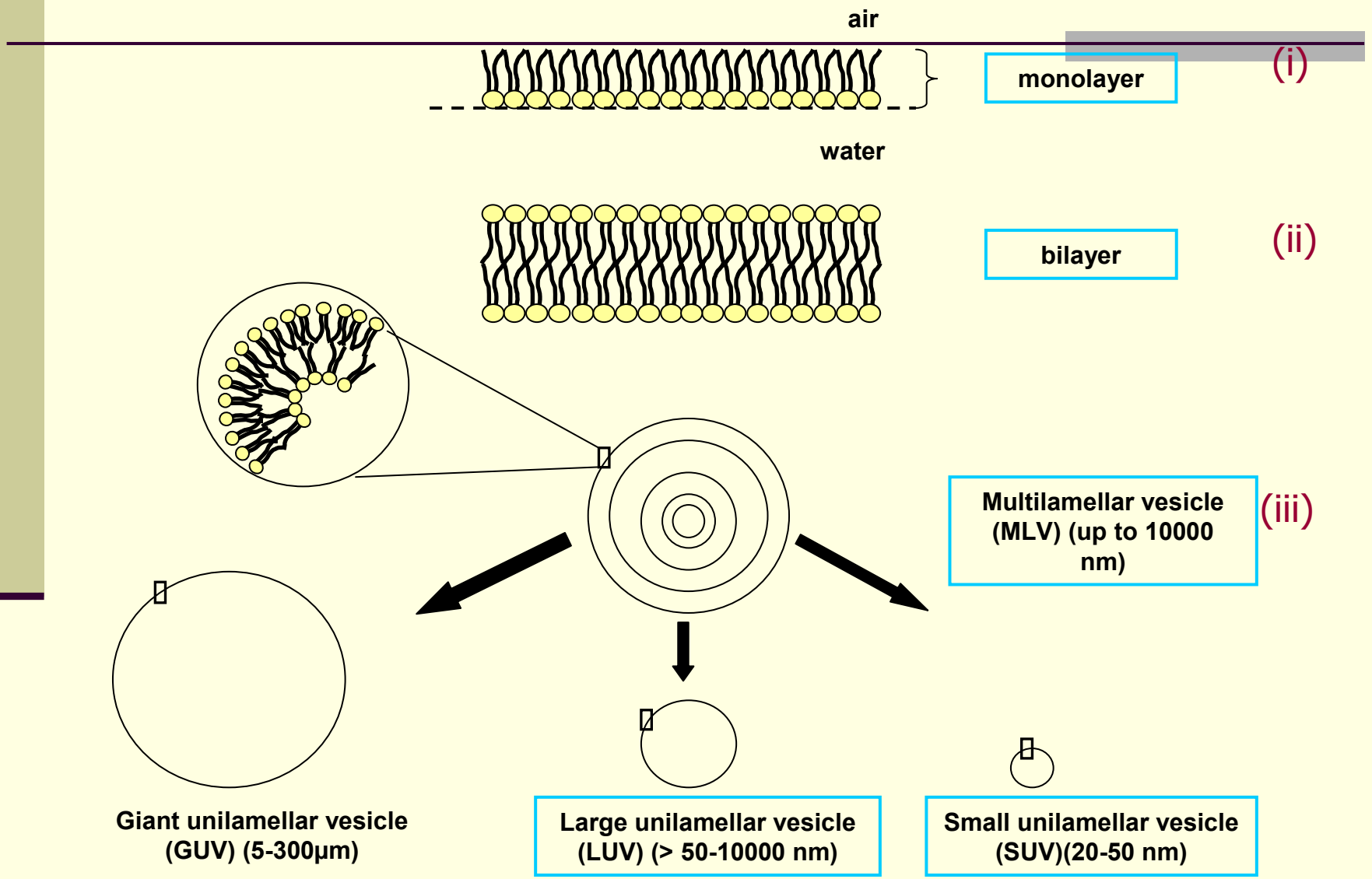


- ✓ Zwitterionic phospholipids (Eukaryotic cells: DOPC, DPPC)
- ✓ Anionic phospholipids (Prokaryotic cells: DPPG)



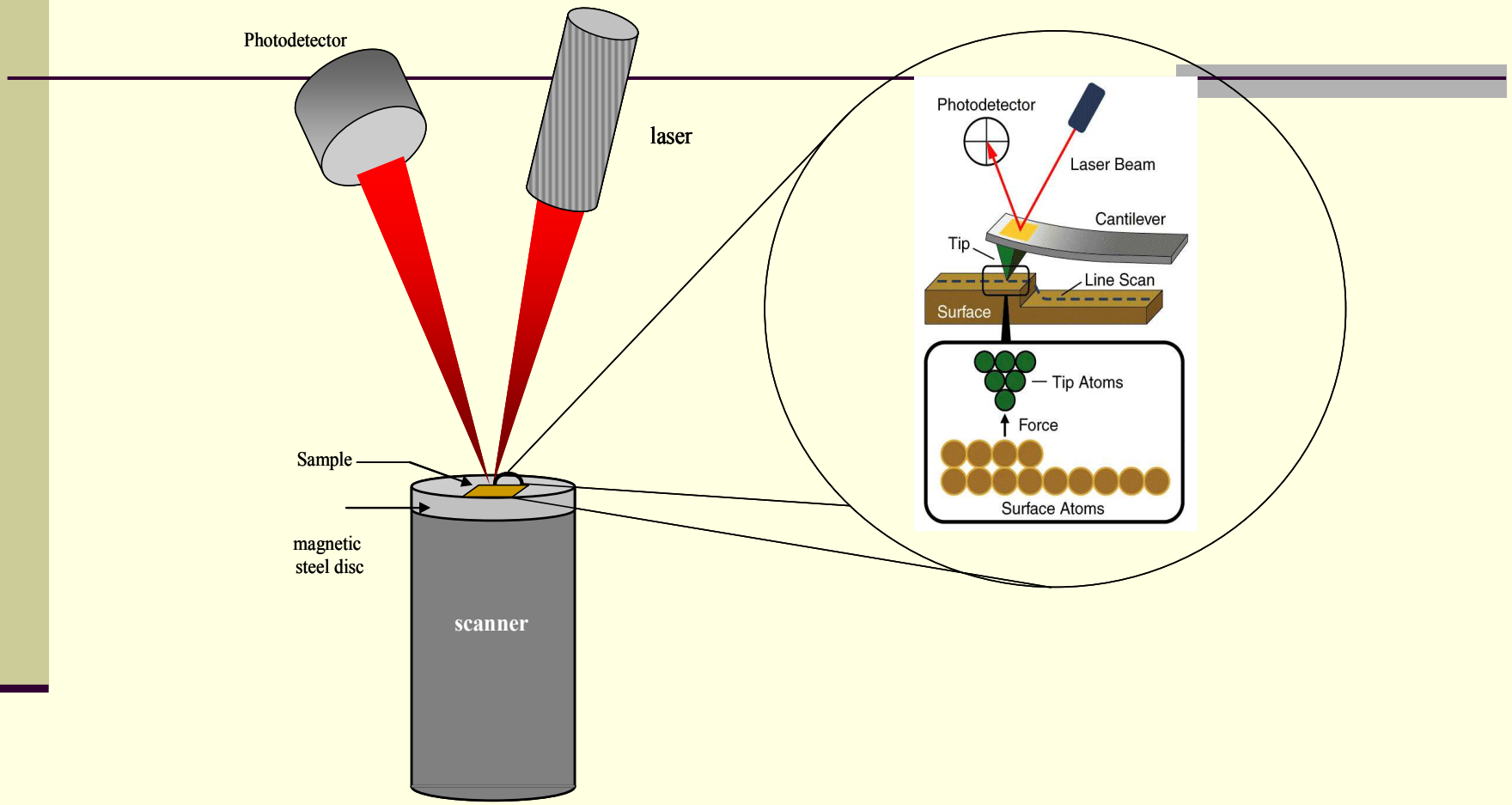
III- Materials and methods

Models of lipids membranes



III- Materials and methods

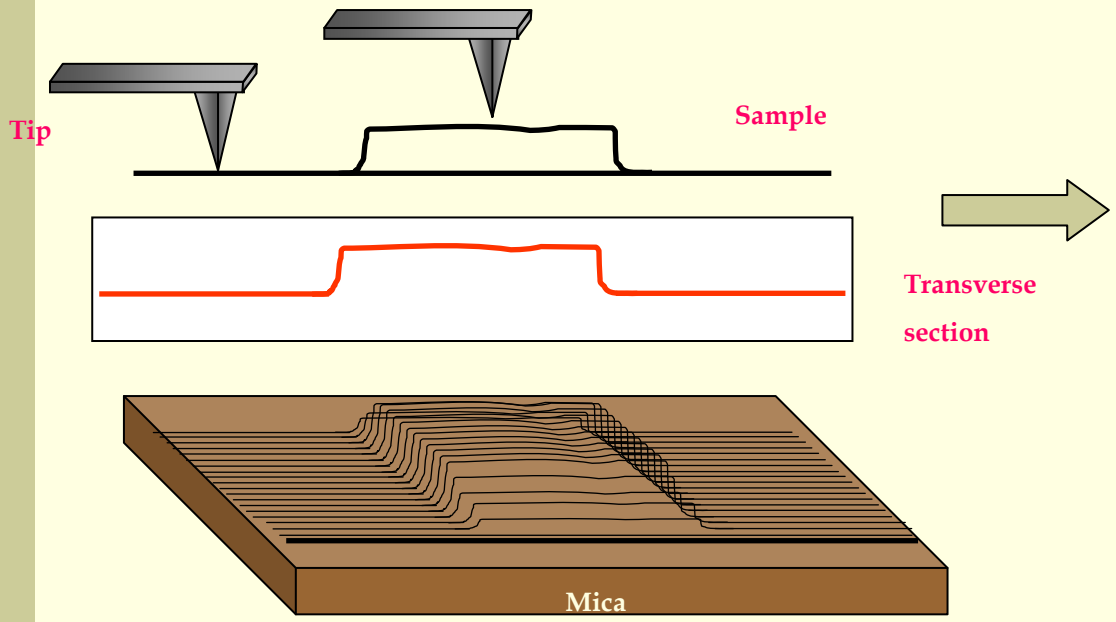
i) Atomic Force Microscopy (AFM)

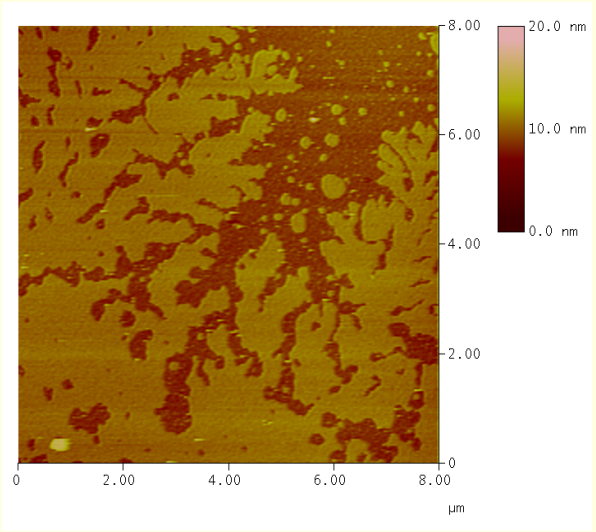


✓ Measure the forces resulting from different interactions between a tip, which is attached to a cantilever and the atoms of the sample surface.

i) Atomic Force Microscopy

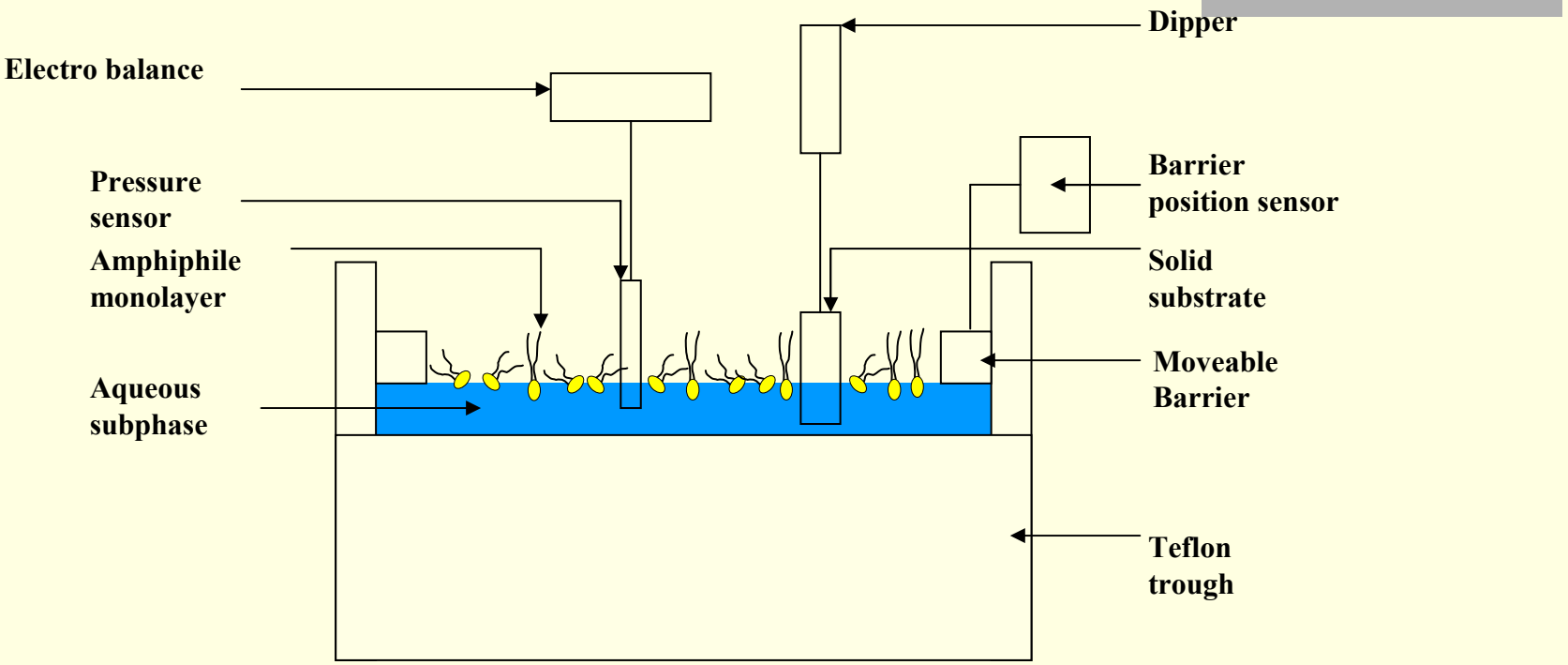
How it's work?





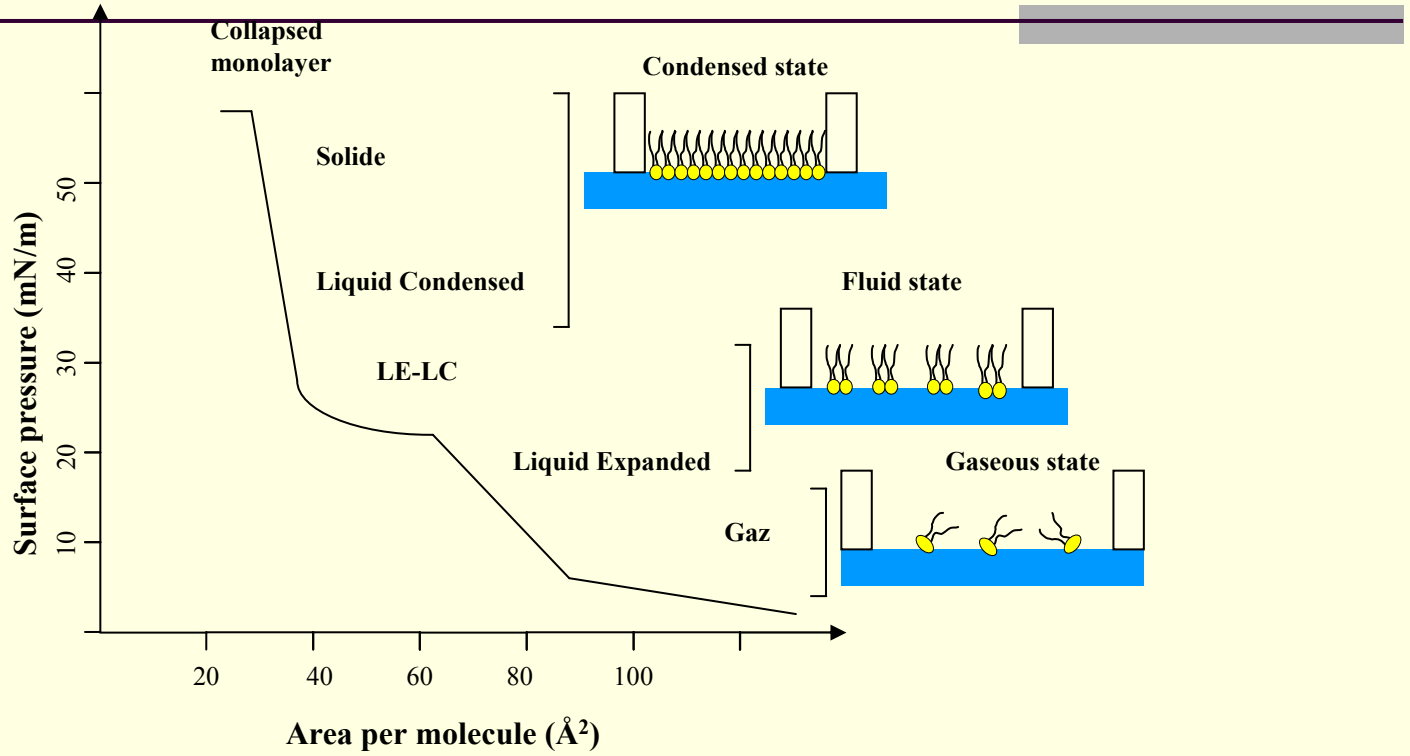
✓ *Surface topography*
✓ *Lipid domain structure*

ii) Langmuir-Blodgett technology (LB)



✓ Measure the surface pressure/area (π-A) of monomolecular layer upon compression

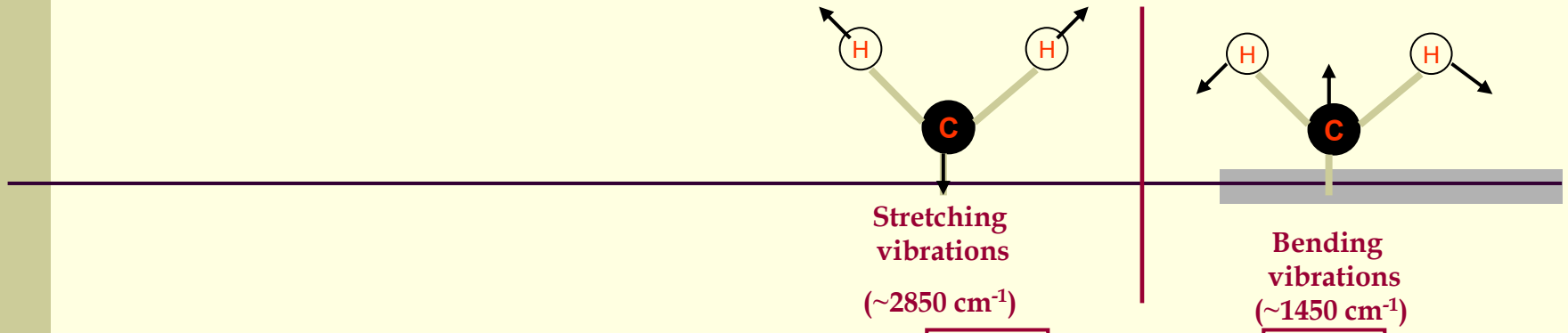
ii) Pressure/area (π-A) isotherm curve for a phospholipid monolayer



Compression of lipids (↓ molecular area) ⇒ ↑ lateral pressure

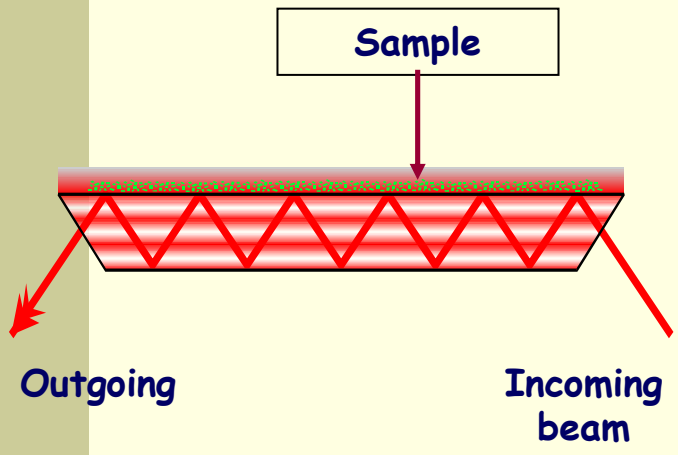
- ✓ ***Lipid molecular arrangement***
- ✓ ***Lipid state transition***
- ✓ ***Lipid packing***

iii) Fourier Transform Infrared Spectroscopy (FTIR)

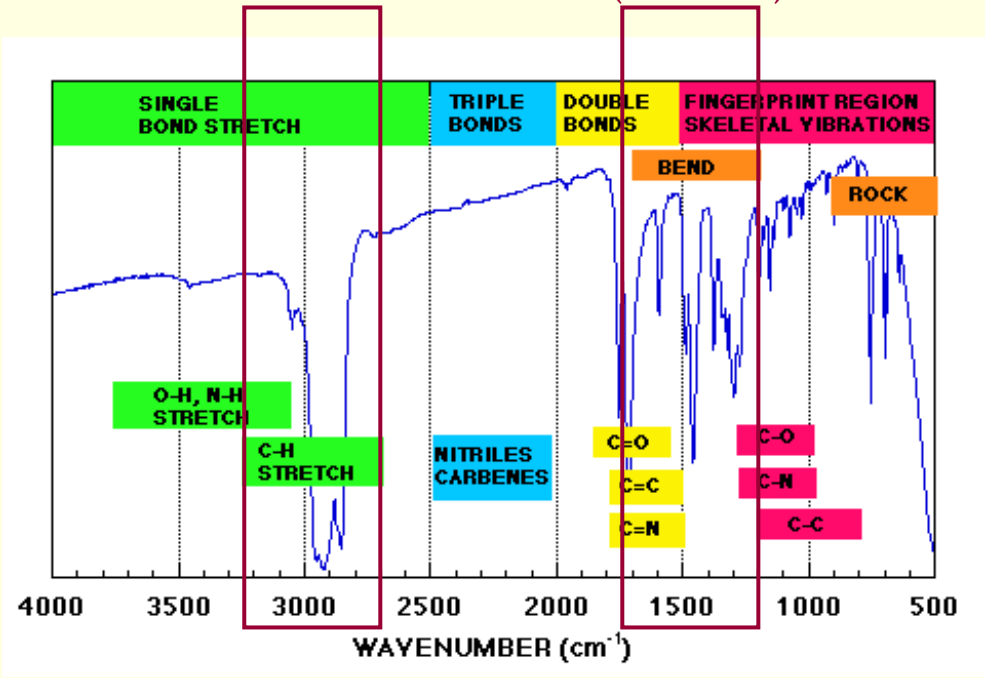


Stretching vibrations (~2850 cm^{-1})

Bending vibrations (~1450 cm^{-1})

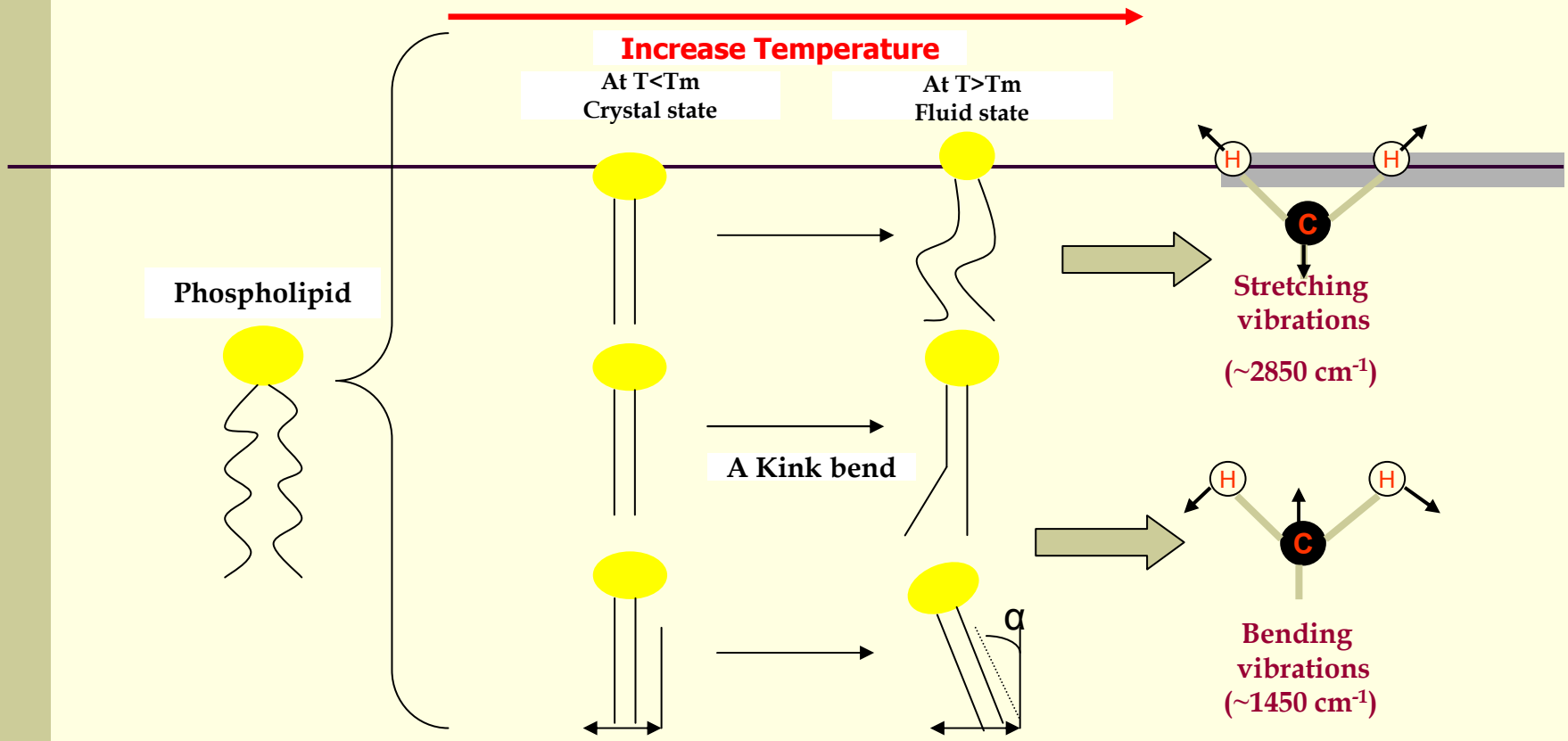


Germanium Plate



- ✓ **IR beam is focused into the ATR crystal witch is covered with lipid layer**
- ✓ **The light travels inside the plate by internal reflections from one surface of the plate to the other**
- ✓ **Absorption of the energy by the sample provides ATR-FTIR spectra and information about the structure of the system.**

iii) Fourier Transform Infrared Spectroscopy (FTIR)



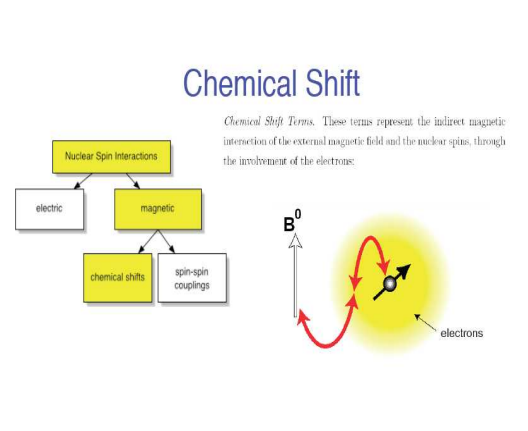
✓ ***Non polarized spectra for :***

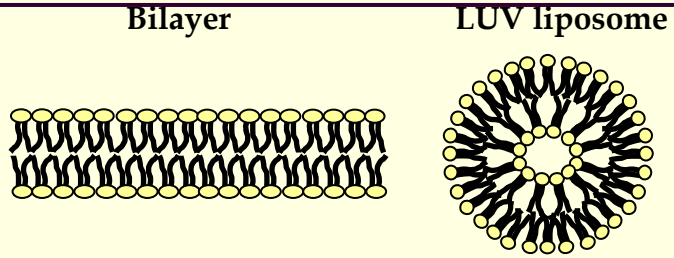
- *state behavior* (CH_2 *asymmetric and symmetric stretching bands 2800-3000* cm^{-1})
- *conformation determination of lipids:* (CH_2 *wagging bands (1400* cm^{-1}))

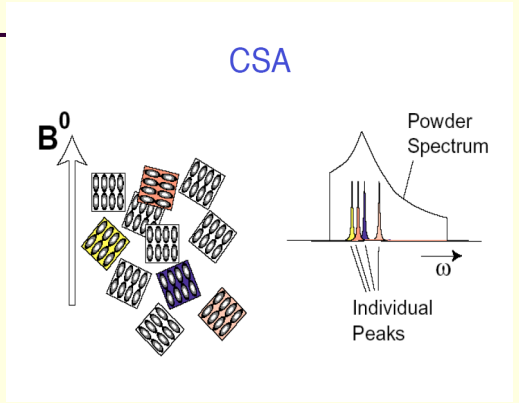
✓ ***Polarized spectra for determination of*** *lipids orientation: Dichroism measurements of the* CH_2 *wagging band (1400* cm^{-1}))

iv) Nuclear Magnetic Resonance spectroscopy (NMR)

Chemical shift anisotropy $\Delta\sigma$



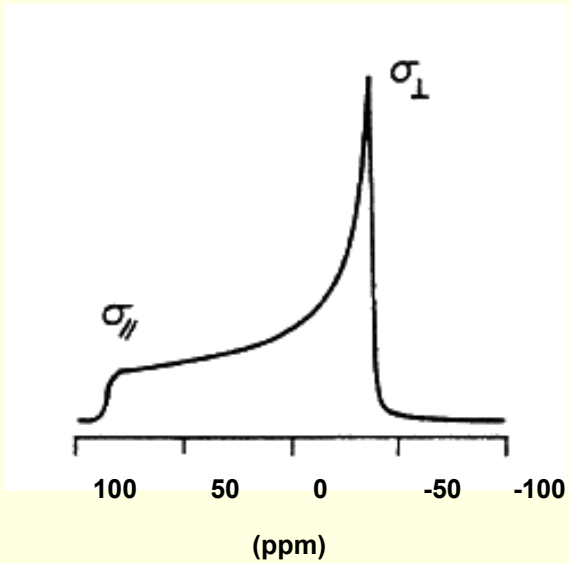




$\Delta\sigma = \sigma_{//} - \sigma_{\perp}$

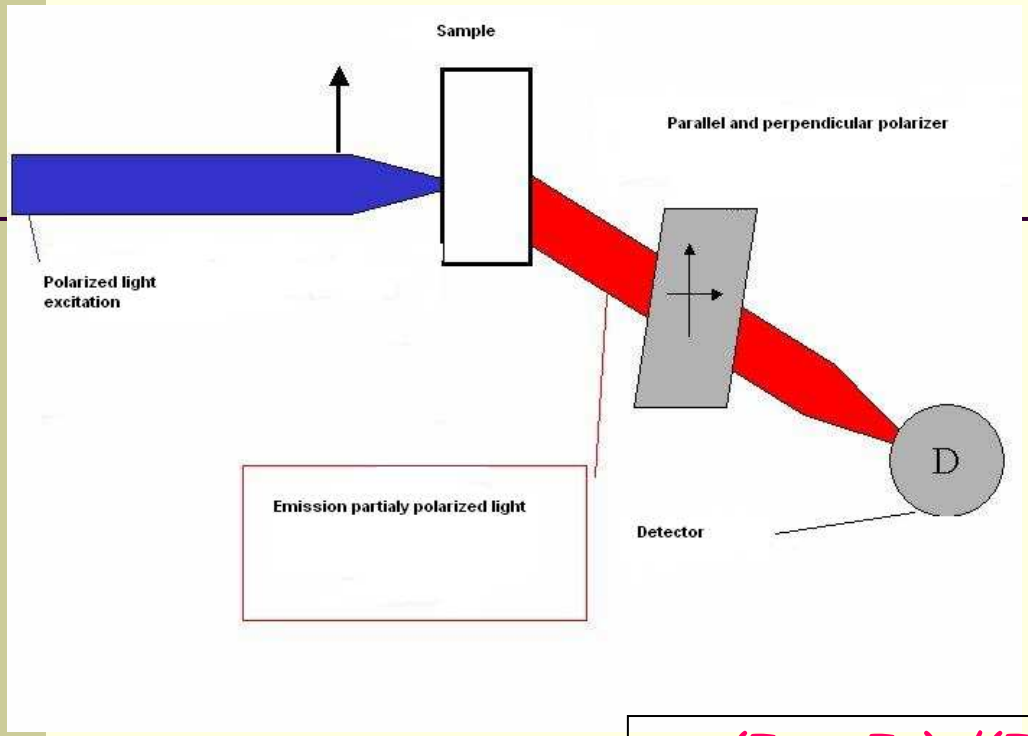
$\sigma_{//}$: The low field shoulder

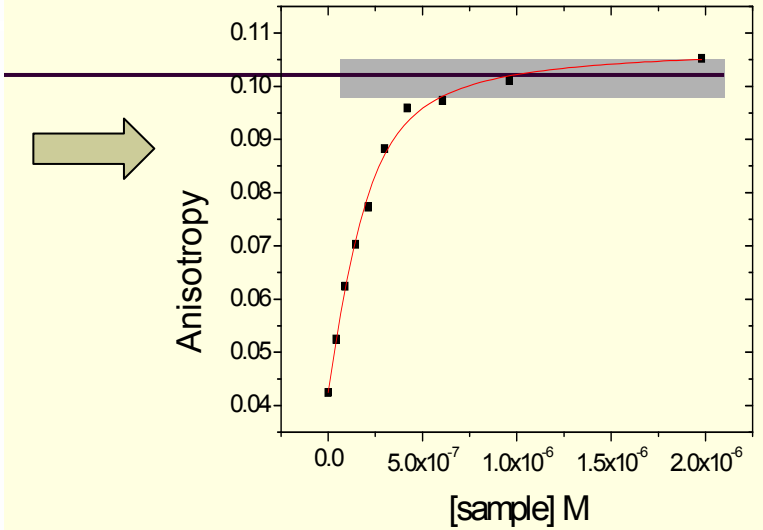
$\sigma_{\perp}$: The high field peak



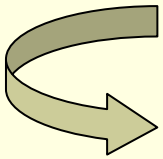
- ✓ *Mobility of phosphate heads of phospholipids*
- ✓ *Drug effect on the orientation of phospholipids head groups (^{31}P NMR).*

v) Steady state anisotropy fluorescence





$$r = (I_{//} - I_{\perp}) / (I_{//} + 2\, I_{\perp})$$



L_{free} => $r = r_0$

$L_{bound} + R$ => $r \uparrow$

- ✓ ***Binding parameters of drugs to lipids (stoichiometry, affinity)***

i) Investigation of the interaction of two fluoroquinolones (CIP and MXF) with model lipid membranes

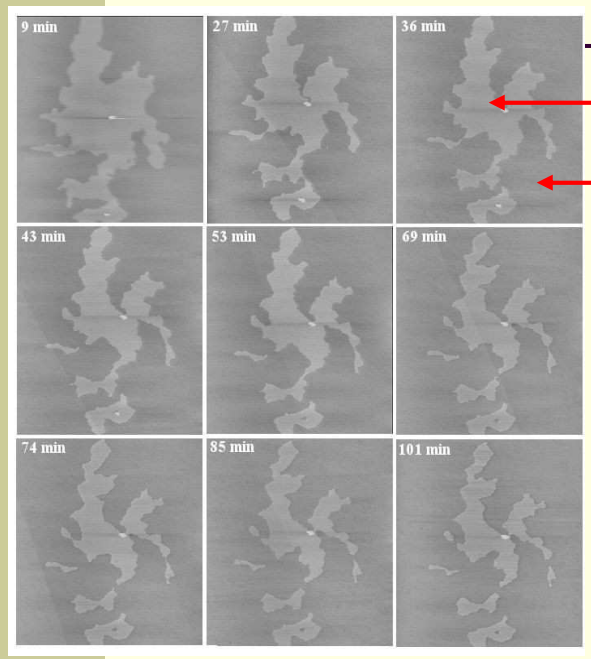
Chapter I

a) Effect of FQs on the membrane organization

AFM Studies

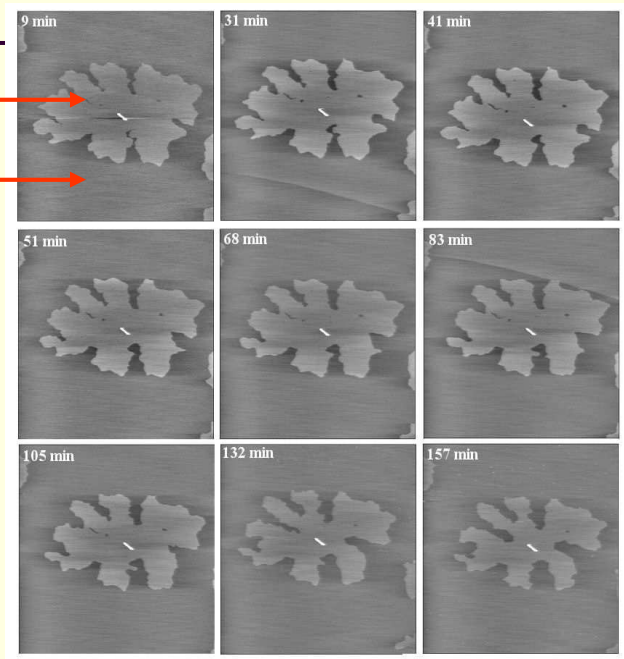
(Collaboration with Pr Y. Defrene (UCL)

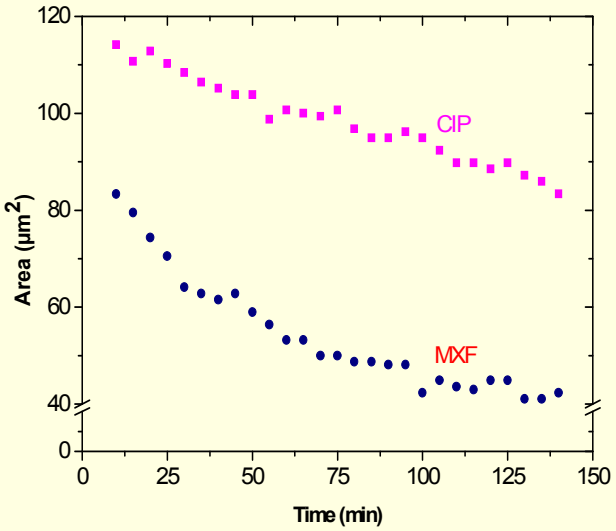
CIP **MXF**



DPPC

DOPC





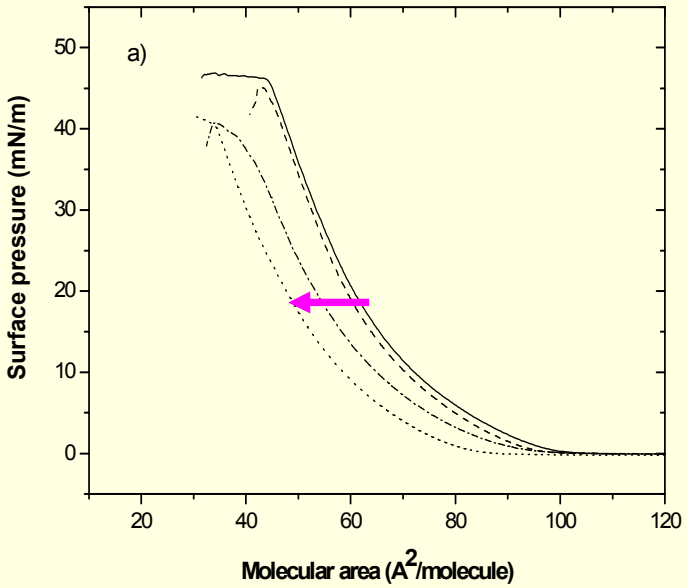
i) MXF induced in a more (57%) extent than CIP (27%), an erosion of the DPPC domains in the DOPC fluid phase

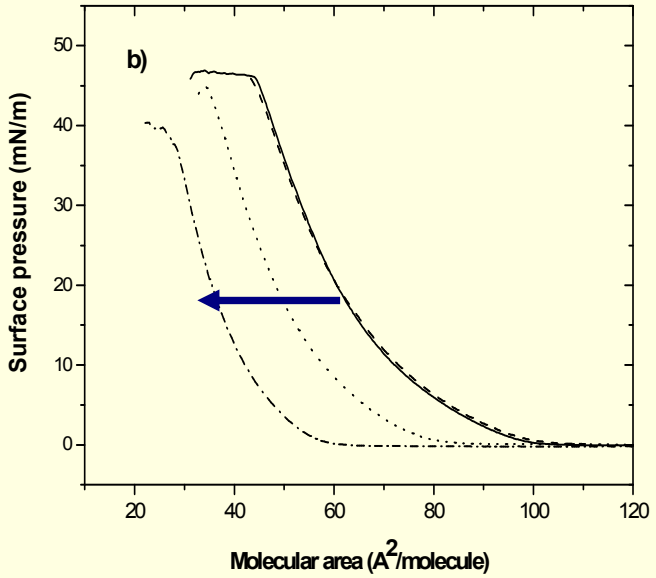
b) FQs stability within a lipid monolayer / compressibility

Langmuir studies

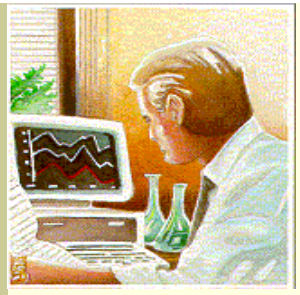
(Collaboration with Dr M. Deleu (FSAG, Gembloux))

CIP **MXF**





ii) MXF induced a higher shift of the surface pressure-area isotherms of DOPC:DPPC:FQs monolayer towards the lower area per molecule as compared to CIP.



c) ATR-FTIR Data analysis

(Collaboration with Pr E.Goormaghtigh (SFMB, ULB)

✓ Conformation analysis

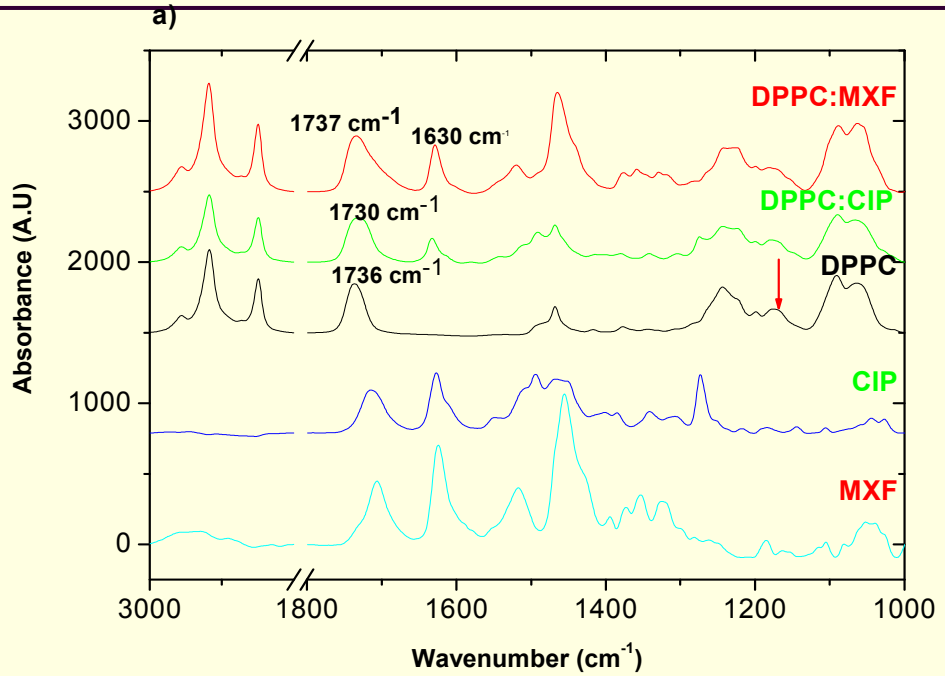
⇒ Non polarized spectra of DPPC and DPPC:FQS with molar ratio of 1:1

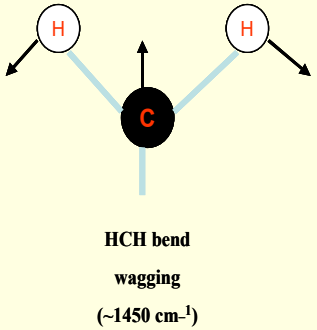
✓ Orientation analysis

⇒ Dichroic spectra ($A_{//}$- $A_{\perp}$) of DPPC and DPPC: FQs with molar ratio of 1:1

C i) Conformation analysis

Non polarized spectra of drug, DPPC and DPPC:FQS with molar ratio of 1:1





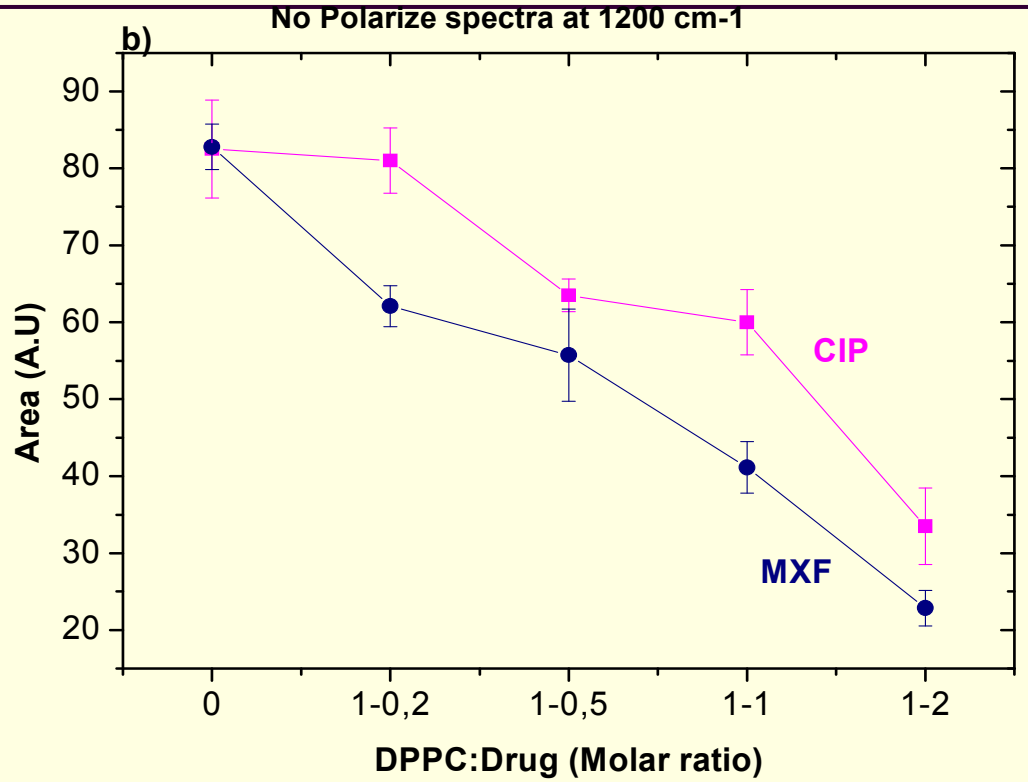
✓The drug spectrum appeared in the DPPC: drug mixture spectrum, notably at 1630 cm^{-1}.

✓In DPPC: drug spectra, the DPPC ν(C=O) band at 1736 cm^{-1} was modified in terms of frequency and shape

⇒ a modification of the interfacial lipid carbonyl groups

C i) Conformation analysis

Analysis of the lipid C-H wagging ($\nu_w(CH_2)$)⇒ Information on the proportion of the chains in the all-trans conformation



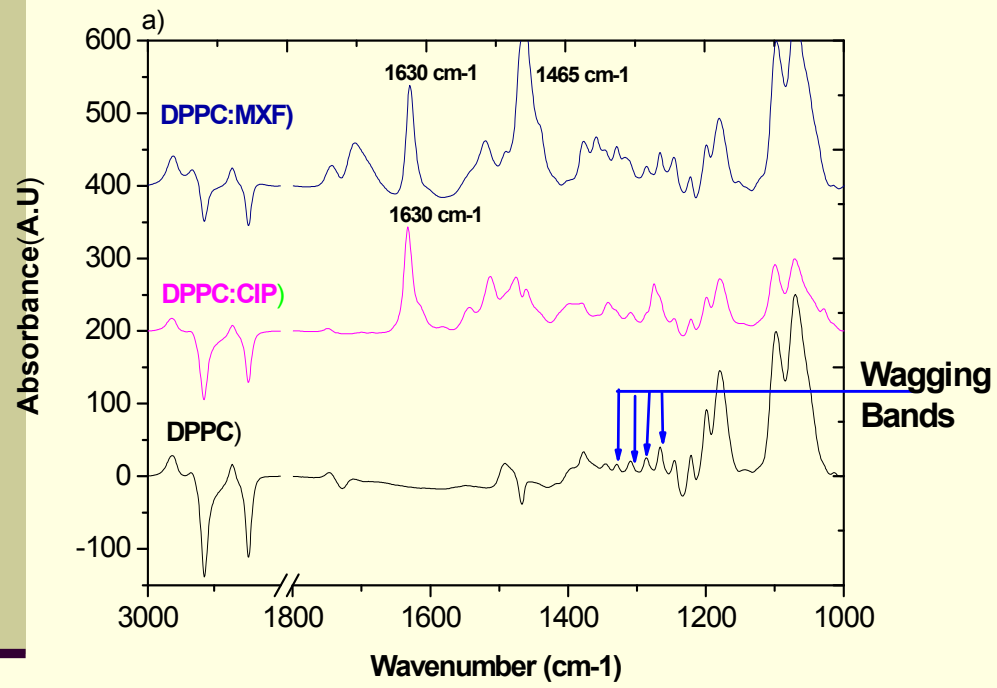
✓ **Area evolution of DPPC peak at 1206-1193 cm^{-1} as function of increasing amounts of FQs : ↓ 60% for CIP, ↓ 72% for MXF.**

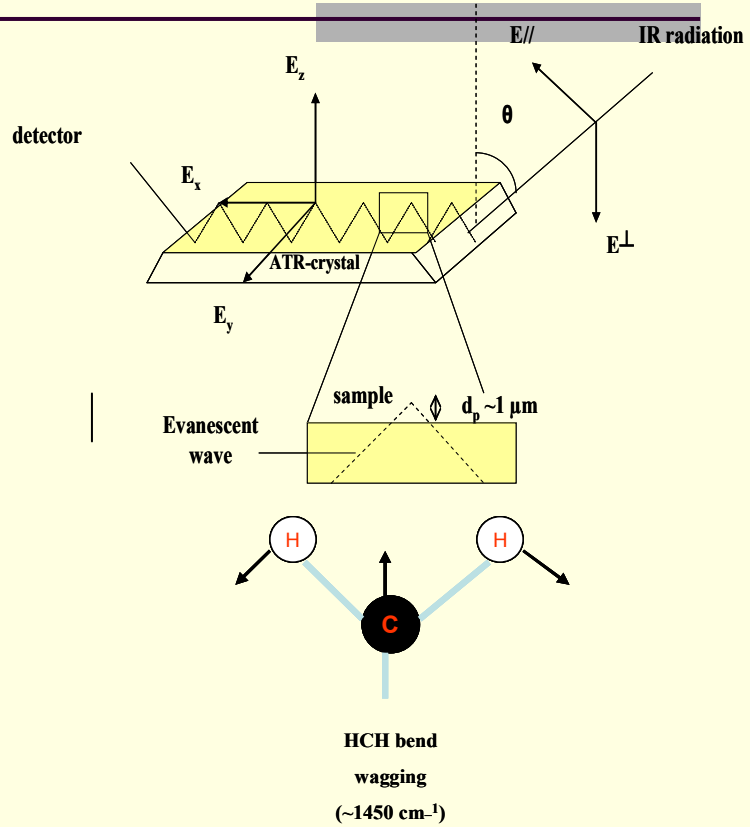
⇒ The all-trans configuration of the alkyl chain of DPPC decreased more in the presence of MXF.

C ii) Orientation analysis

Dichroic spectra ($A_{//} - A_{\perp}$) of DPPC and DPPC: FQs

with molar ratio of 1:1



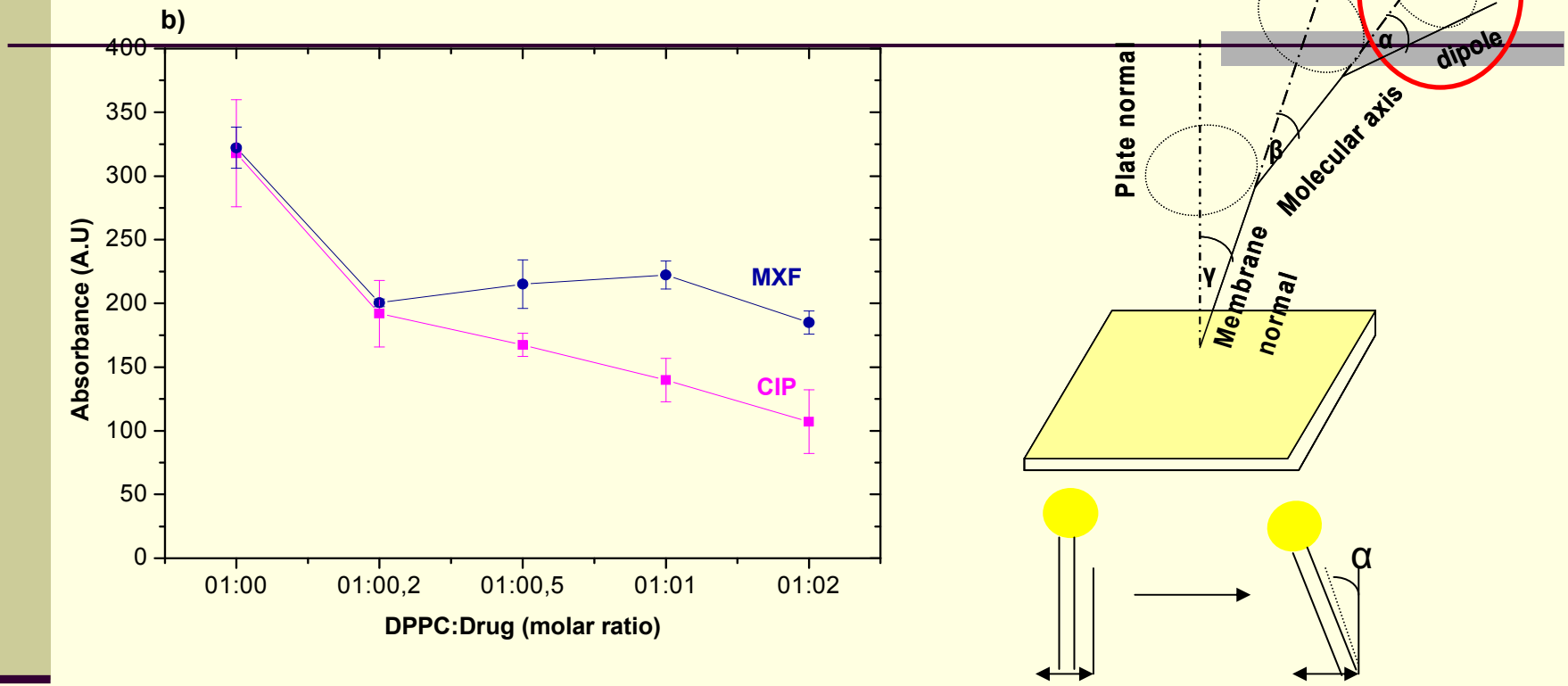


✓ **The dichroic spectra of DPPC:FQs mixture displayed strong dichroism for bands assigned to the drug (at 1630 and 1465 cm^{-1})**

⇒ **a well-organized, well-defined orientation of the drug in the DPPC bilayer.**

C ii) Orientation analysis

Analysis of the lipid C-H wagging ($\nu_w(CH_2)$) band ⇒ Estimation of the orientation of the lipid acyl chain



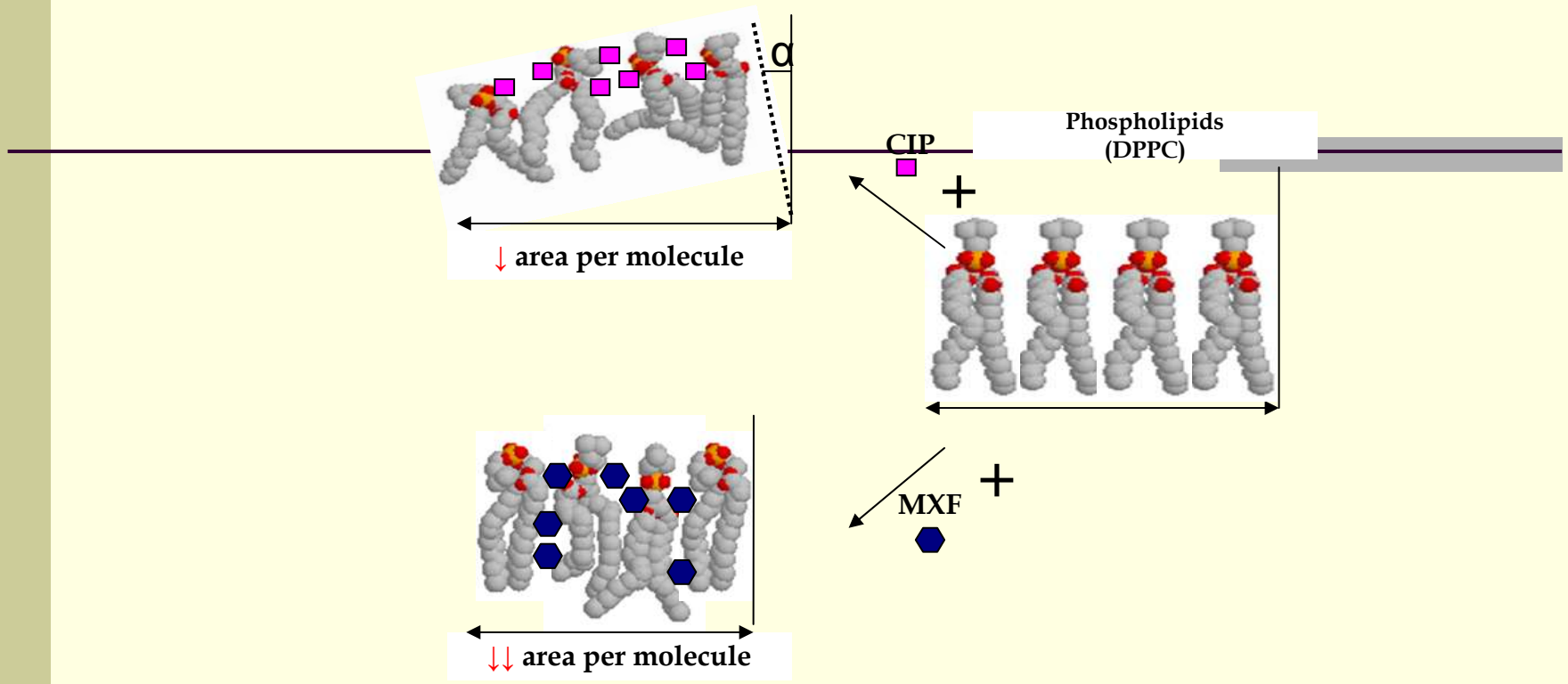
✓ **Area evolution of the wagging band $\nu_w(CH_2)$ of DPPC as a function of lipid:Drug molar ratio, indicated :**

- ↓ 60% of the area for CIP, ↓ 30% for MXF.

- The tilt between the acyl chain of DPPC and a normal at the germanium surface was (27°) in the presence of CIP and remained unchanged (20°) in the presence of MXF

⇒ MXF induced less disorder than CIP

Summary 1



- ✓ **MXF induced in a more extent than CIP an erosion of the DPPC domains in the DOPC fluid phase.**
- ✓ **MXF induced a higher shift of the surface pressure-area isotherms of DOPC: DPPC: FQs monolayer towards the lower area per molecule as compared to CIP.**
- ✓ **MXF had a higher tendency to decrease the number of all-trans conformation, as compared to CIP.**
- ✓ **CIP induced a disorder and modifies the orientation of the acyl chain.**

Bensikaddour et al., 2008: *Biophysical Journal* **94**: 3035-3046

ii) Investigation of the interaction of CIP with eukaryotic and prokaryotic model lipids membranes (DPPC vs DPPG)

Chapter II

Phospholipids composition (%) of bacteria membrane and humain cells membrane

Lipid	Eukaryotic cells		Prokaryotic cells		
	Human alveolar macrophages	Human Erythrocyte membrane	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Staphylococcus pneumoniae*
PE	20.6	6.2	60	-	-
PC	**31**	**24.2**	-	-	-
PS	20.7	2.6	Traces		
PG+CL			**21+11**	**58+42**	**50+50**
PI+PA				-	-
SM	6.6	18.9	-	-	-
Chol	7..9	48.1			
Others	13.2				

- ✓ **The bacterial membranes are composed largely of anionic lipids (PG).**
- ✓ **The eukaryotic cell membranes are rich with zwitterionic lipids.**

Wolkers et al., (2002), Epand *et al*. (2007), Lohner and Prenner, (1999).

1) Binding of CIP to Lipids

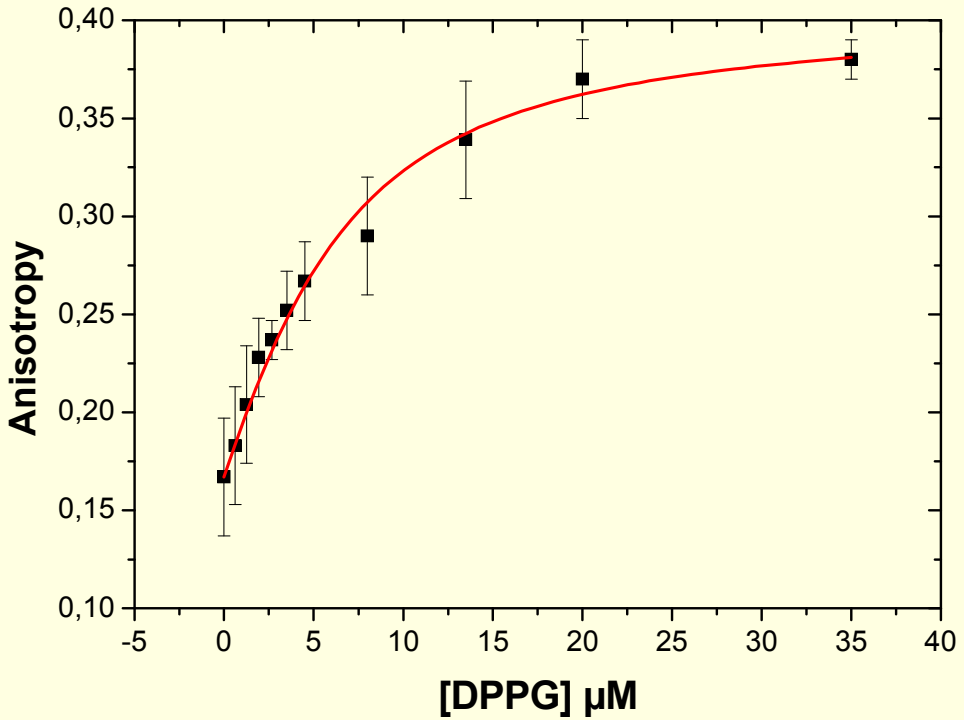
a) Size of LUVs in the presence of CIP by quasi-elastic light scattering

Lipid-Drug Molar ratio	Average size (nm) LUV	
	DPPC	DPPG
1:0	101±1	124±1
1:0.2	100±1	155±2
1:0.5	98±1	195±2
1:1	105±1	256±5

⇒ CIP increased the size of DPPG liposomes

a) Binding of CIP to Lipids by steady state anisotropy titration

[CIP] = 5 µM



$$r = (I_{//} - I_{\perp}) / (I_{//} + 2\, I_{\perp})$$

K_{app}= (8.6±0.5) 10^5 M^{-1}

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a) Binding parameters of CIP with Lipids vesicles

LUV liposomes Composition	Zeta potential (mV)	$K_{app}(10^5\ M^{-1})$
DPPG	-(66 ± 4) (83 ± 12 %)	8.6±0.5
DPPC	-(5 ± 1) (96 ± 5 %)	2.5±0.1
DOPC:DPPG (1:1, M:M)	-(34 ± 4) (100%)	3.2±0.9
DOPC:DPPC (1:1, M:M)	-(6 ± 1) (100%)	1.1±0.2

⇒ **CIP has more affinity for negative charged liposomes (DPPG Vs DPPC)**

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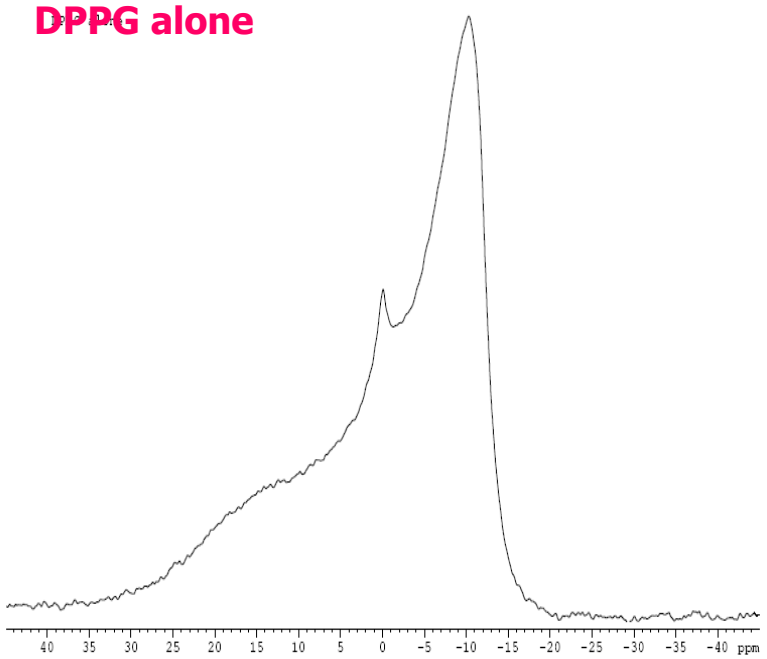
2) CIP effect on the membrane mobility

(Collaboration with Pr K. Snoussi (CHIM, UCL))

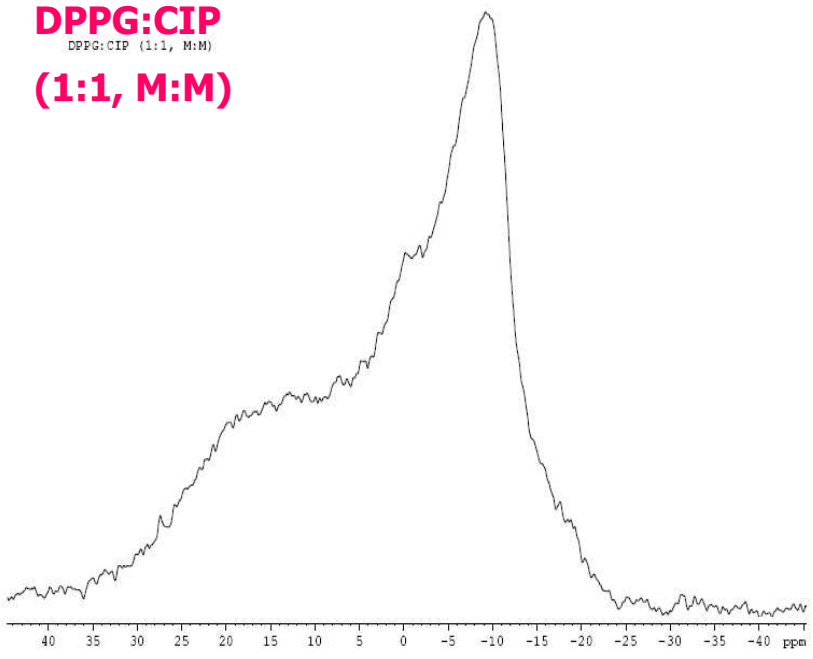
i) DPPG

DPPG alone



DPPG:CIP

(1:1, M:M)



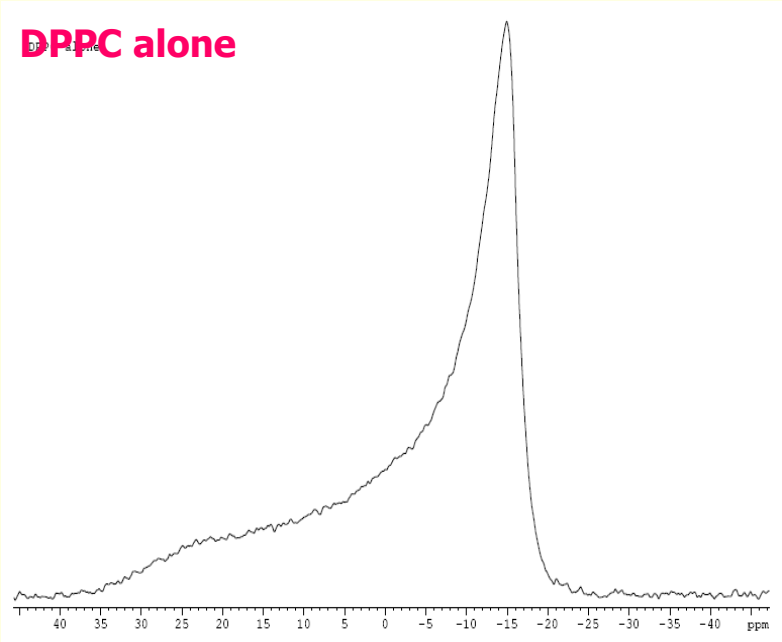
$\Delta\sigma$=25.5±0.5 ppm

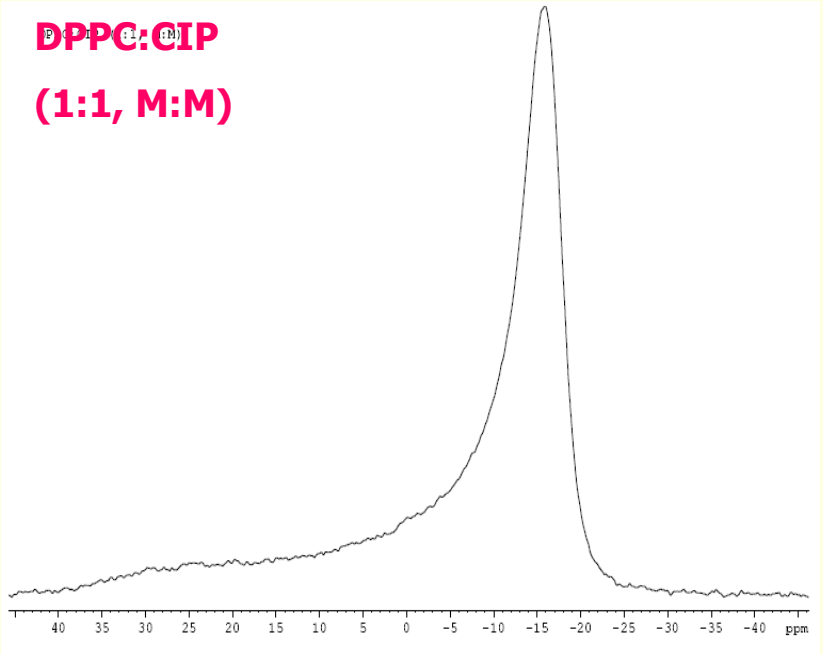
$\Delta\sigma$=34.0±1.0 ppm

$\Rightarrow$ The difference of $\Delta\sigma$ of DPPG:CIP and DPPG is 9 ppm

2) CIP effect on the membrane mobility

ii) DPPC





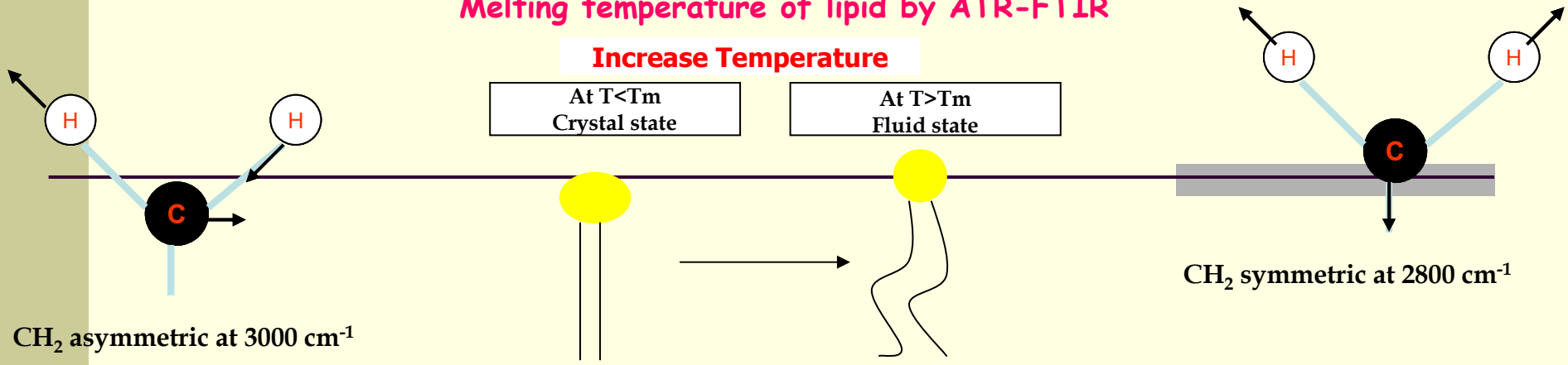
$\Delta\sigma$=41.5±0.5 ppm

$\Delta\sigma$=46.6±0.1 ppm

⇒The difference of $\Delta\sigma$ of DPPC:CIP and DPPC is 5 ppm

3) CIP effect on the thermotropic behavior of lipids

Melting temperature of lipid by ATR-FTIR

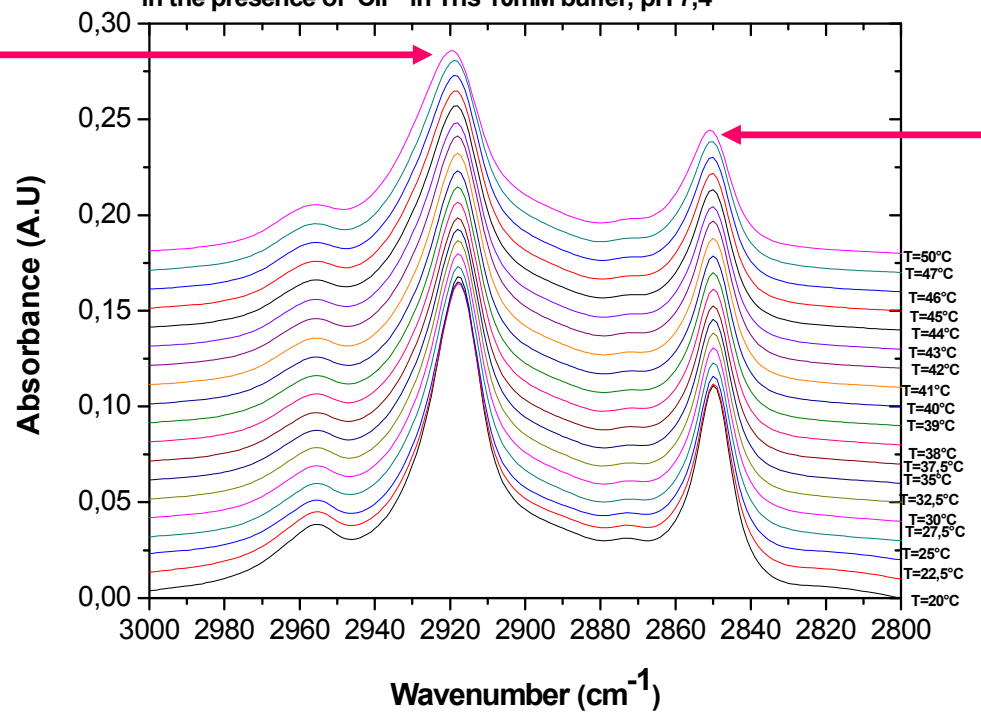


CH_2 asymmetric at 3000 cm^{-1}

CH_2 symmetric at 2800 cm^{-1}

CH_2 Stretching vibrations

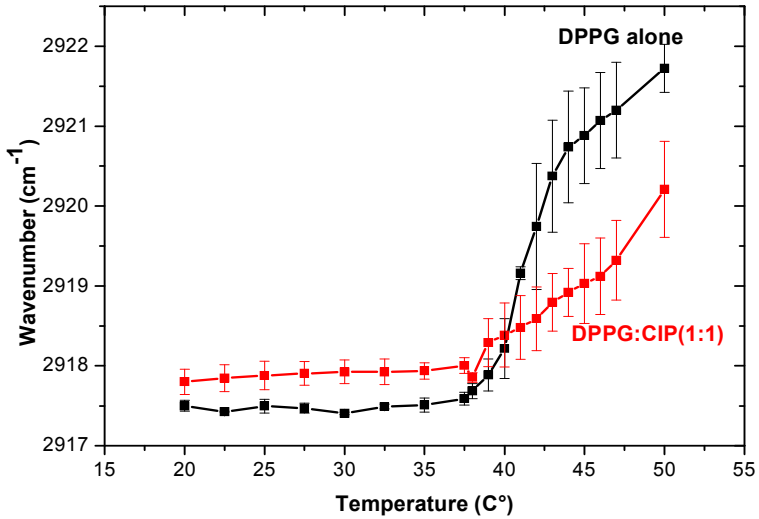
Evolution of stretching vibration bands of CH_2 of DPPG as function of temperature in the presence of CIP in Tris 10mM buffer, pH 7,4



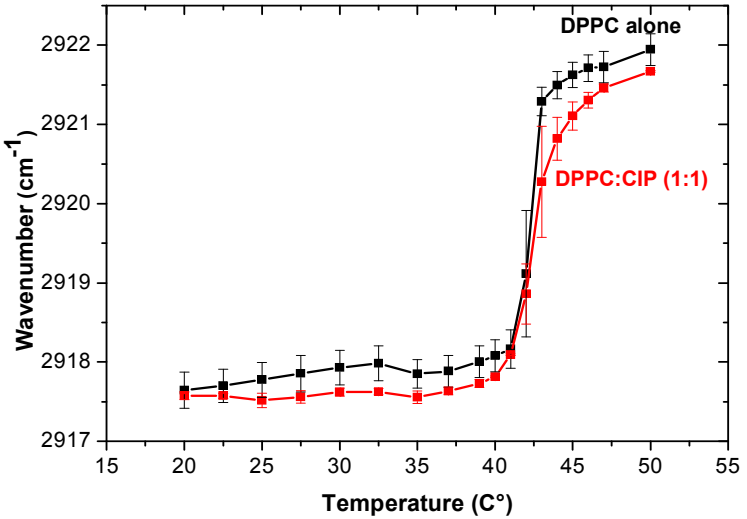
3) CIP effect on thermotropic curve of DPPG and DPPC

CH_2 asymmetric stretching band

Melting temperature of DPPG and DPPG:CIP (1:1)



Melting temperature of DPPC and DPPC:CIP (1:1)



Tm(DPPG)= 40 C°, Tm(DPPC)=42 C°

- ✓ **CIP did not affect dramatically the DPPC melting curve**
- ✓ **CIP : ↓ order of acyl chain of DPPG < Tm**

  ↑order of acyl chain of DPPG >Tm

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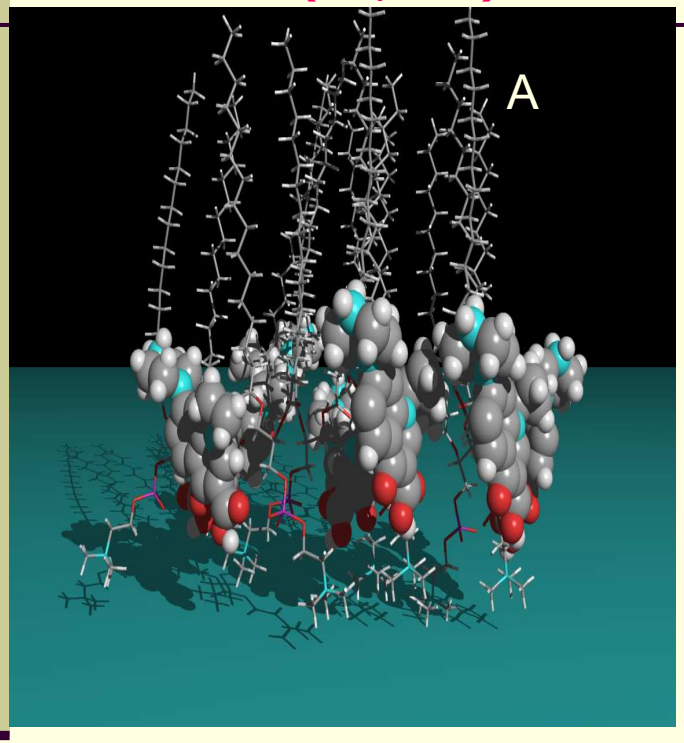
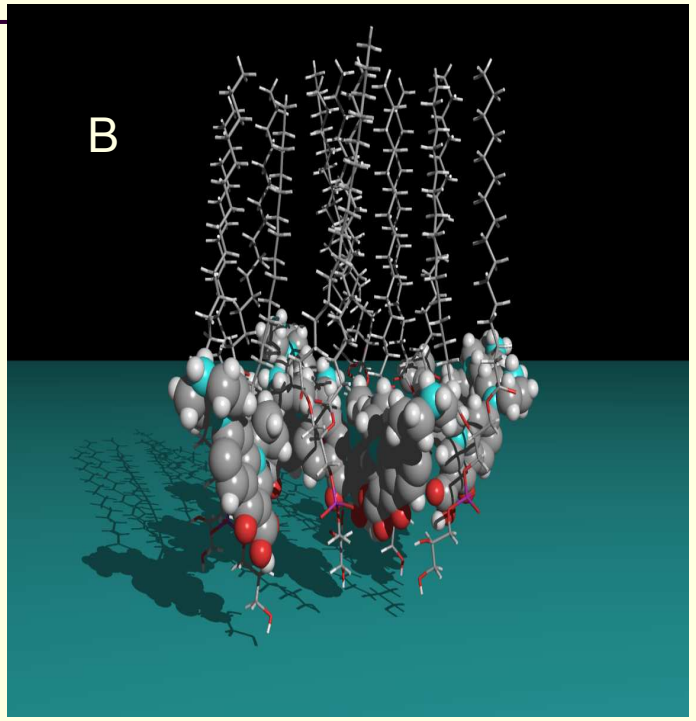
4) Assembly of CIP with phospholipids by molecular modeling

(Collaboration with Dr L. Lins (CBMN, Gembloux)

DPPC:CIP (1:1, M:M)



DPPG:CIP (1:1, M:M)

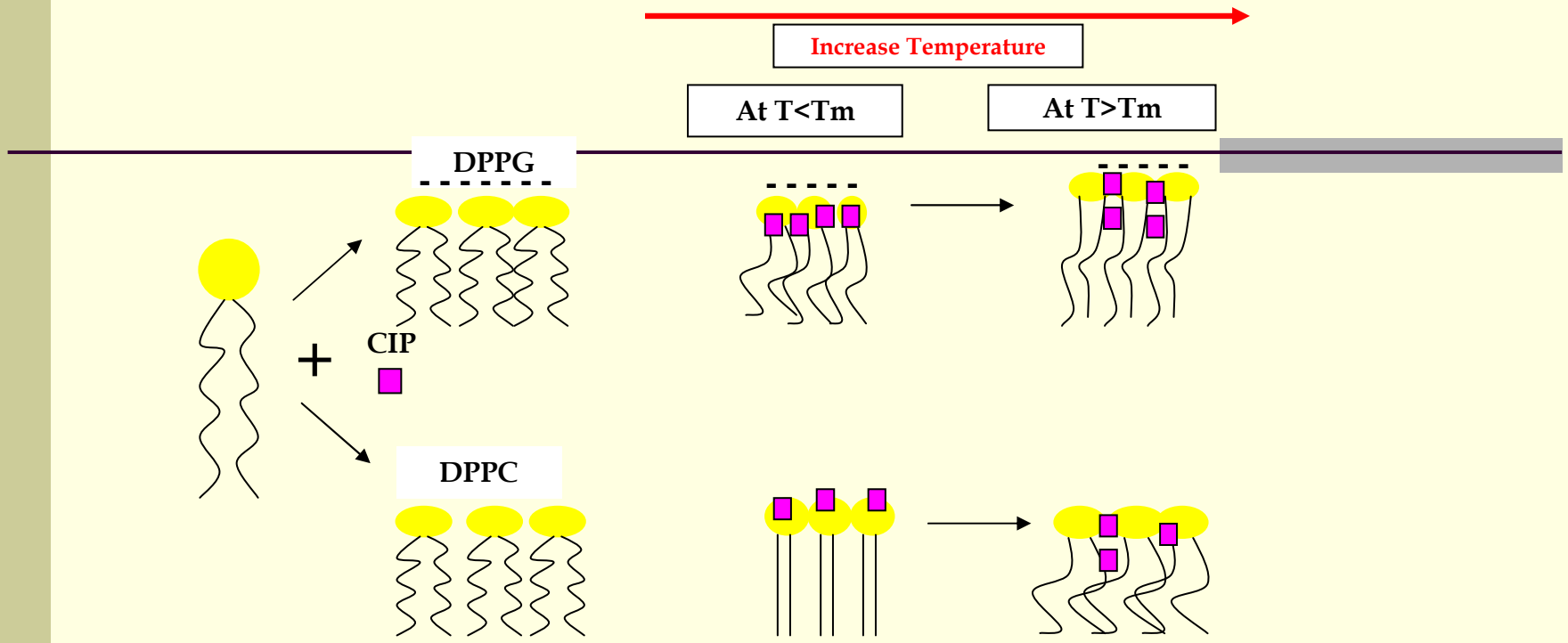


E = - 44.4 Kcal/mol

E = - 53.4 Kcal/mol

=> The interaction of DPPG:CIP is more stable than DPPC:CIP

Summary 2

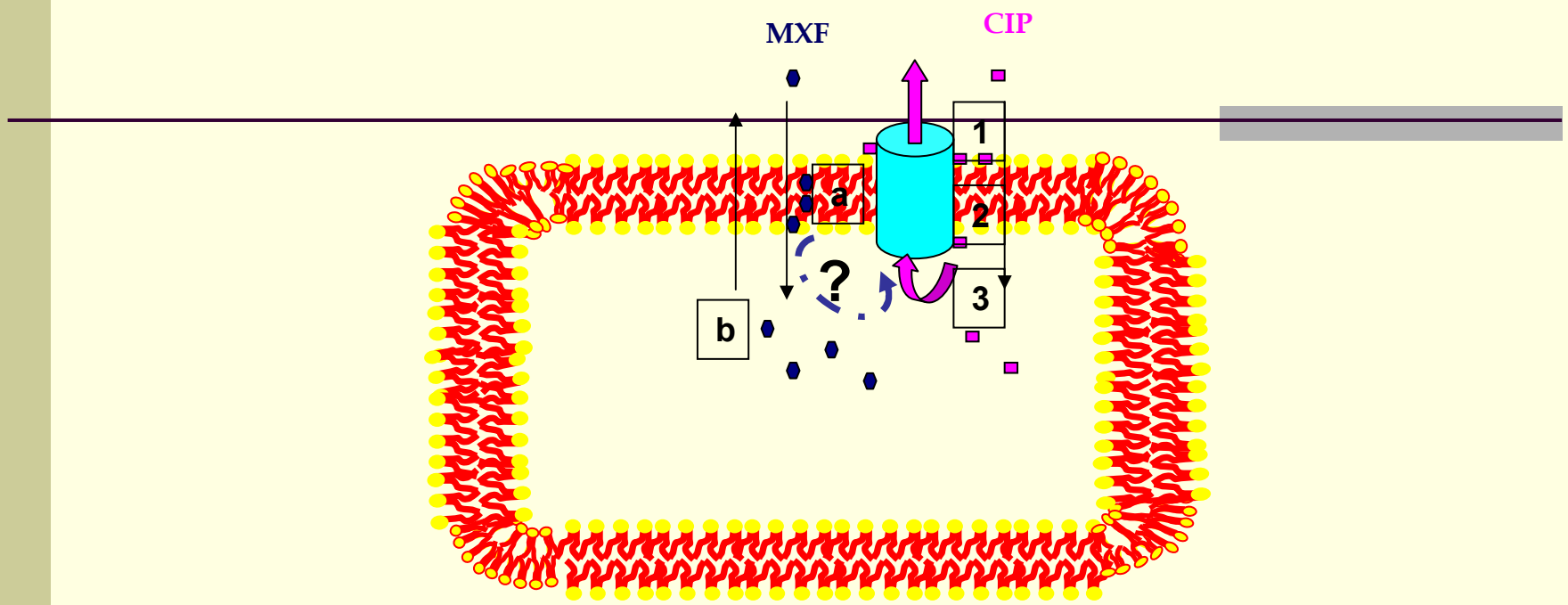


The results showed that ciprofloxacin:

- ✓ **had more affinity to the negatively charged lipid**
- ✓ **reduced more the mobility of DPPG than DPPC**
- ✓ **had no dramatically effect on the melting curve of DPPC**
- ✓ **decreased the order of acyl chain of DPPG at pre-transition temperature and increased the order at post-transition temperature**

Bensikaddour et al., 2008: *Biochimica et Biophysica Acta* **1778**: 2535-2543

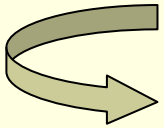
V- General Conclusion



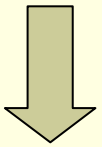
- ✓ **CIP and MXF had an effect on physicochemical properties of lipids**
- ✓ **MXF seems primarily to have a packing effect**
- ✓ **CIP modifies the orientation of acyl-chain and has more affinity to the anionic lipid**
- ✓ **Different effects that we observed between CIP and MXF on lipids probably reflect a difference in their localization into the lipid bilayer**

VI- Perspectives

- ✓ **Relationship between the membrane composition and the permeability of these compounds (CIP and MXF) by the Parallel Artificial Membrane Permeability Assay**
- ✓ **Molecular modeling of MRP4 protein in the lipid bilayer in the absence and the presence of either CIP or MXF**
- ✓ **Are there correlation between lipid composition and efflux pumps protein expression?**



Determination of the lipids composition in J774 cells wild type and those overexpressed MRP4 protein (resistant to CIP)

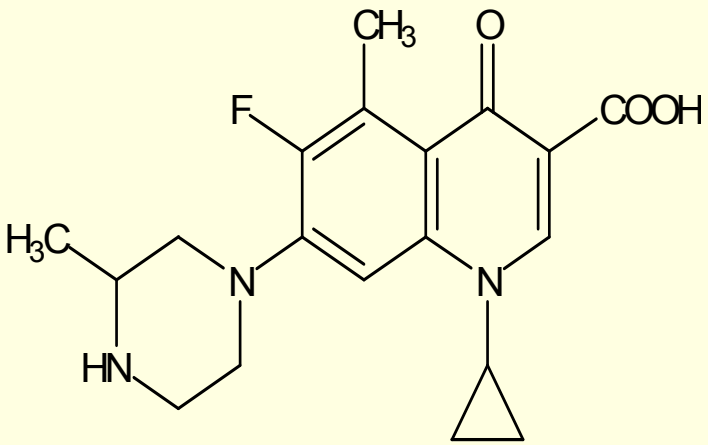


An alteration in the sphingolipids composition was observed: an increased level of GluCer, and a decreased content in Cer and in SM

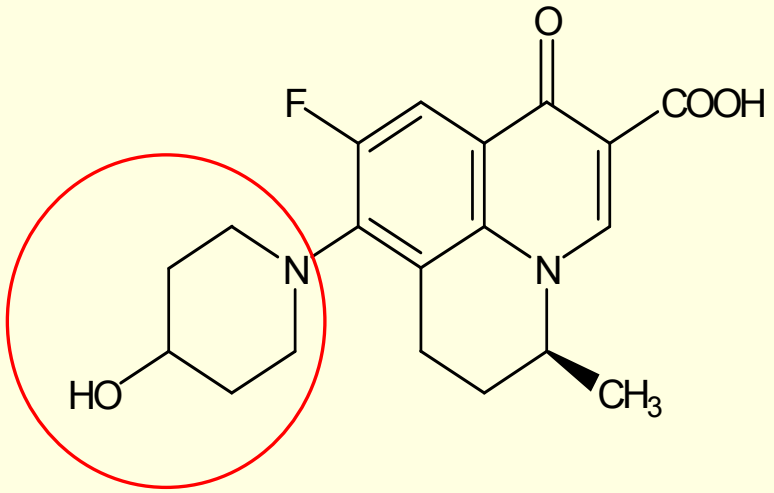
(Bensikaddour et al., Unpublished data)

VI- Perspectives

- ✓ **Application to others fluoroquinolones (role of lipophilicity, the basic function (piperazine))**



Grepafloxacin (Log P=2.48)



nadifloxacin

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- ✓ Dr L. Lins (CBMN, Gembloux)
- ✓ Pr M. Fillet (ULg, Liège)
- ✓ FACM'S members
- ✓ My Family

Thanks' for your attention !