Catholic University of Louvain

Cellular and Molecular Pharmacology Unit

Interactions between Fluoroquinolones and lipids: Biophysical studies

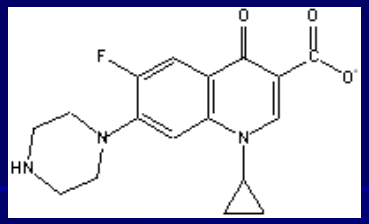
Hayet BENSIKADDOUR

Brussels, BELGIUM

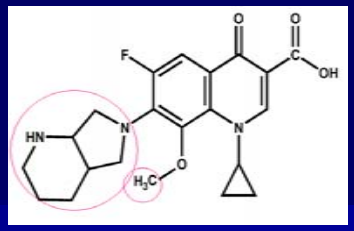
October 22th, 2008

Introduction
Interaction of FQs with eucaryotic cell

Ciprofloxacin (CIP)



Moxifloxacin (MXF)

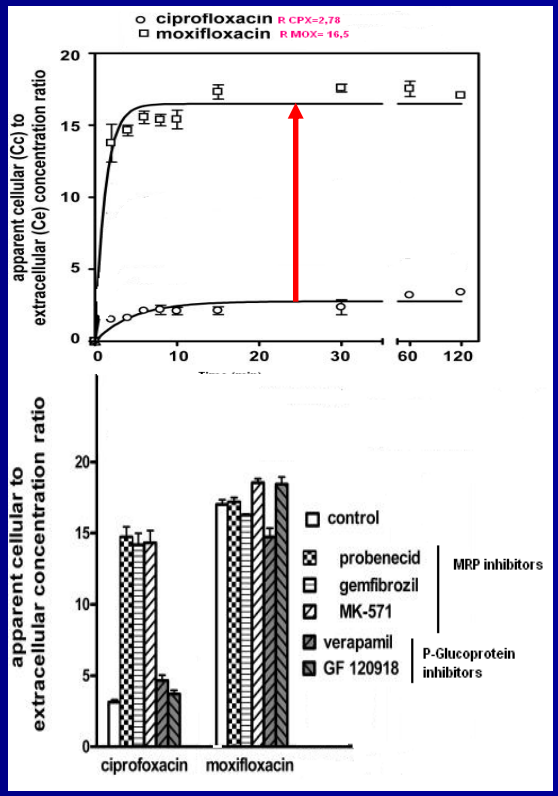


At cellular level :

- **Intracellular accumulation**

- **MRP and P Glycoprotein inhibitors had no effect on the accumulation of MXF**

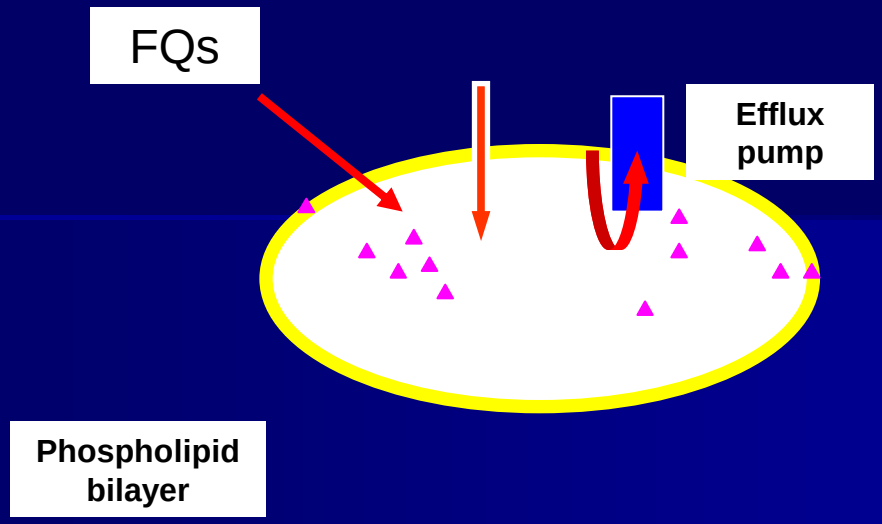
- **However MRP inhibitors had an effect on the accumulation of CIP**



Michot *et al.* (2004) Antimicrob.Agents.Chemother. 48: 2673-2682

Michot *et al.* (2005) Antimicrob.Agents.Chemother. 49: 2429-2437

Contribution of efflux pumps and lipids composition to resistance of bacteria to Fluoroquinolones (CIP, MXF)



The mechanism of entrance of CIP and MXF involved:

- **Diffusion process**

- **Recognition by efflux proteins in cell membranes (Michot *et al.*,2004) and /or bacteria membrane (Wang *et al.*, 2007; Périchon *et al.*,2007)**

FQs interact with phospholipids

Role of lipids in this phenomena ?

Aim of study

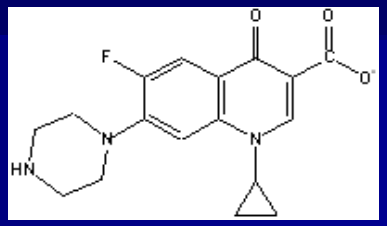
Investigation of interaction of FQs with model membranes, to elucidate the mechanism of their entry (CIP vs MXF) through the cytoplasmic membrane

Materials

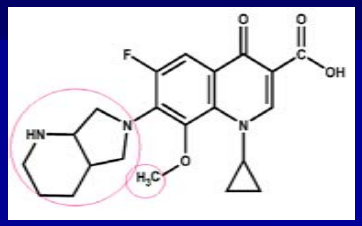
i) Antibiotics

- Ciprofloxacin
- Moxifloxacin

CIP



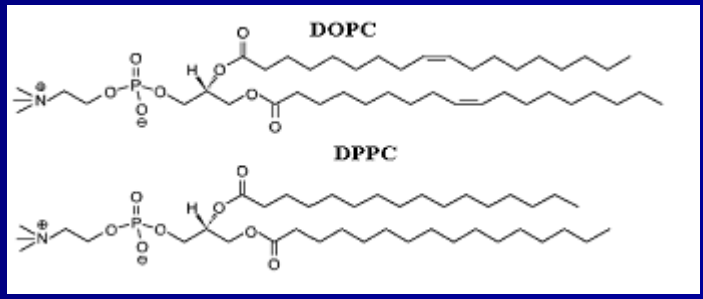
MXF



ii) Model membranes (Lipids)

- Monolayers
- Supported bilayers
- Liposomes SUVs

Zwiterionic lipids (Eucaryotic cells: DOPC, DPPC)



Methods

a) Effect of FQs on the membrane organisation:

Atomic Force Microscopy (AFM)

b) FQs stability within a lipid monolayer / compressibility

Langmuir

c) Effect of FQs on the conformation and orientation of phospholipids

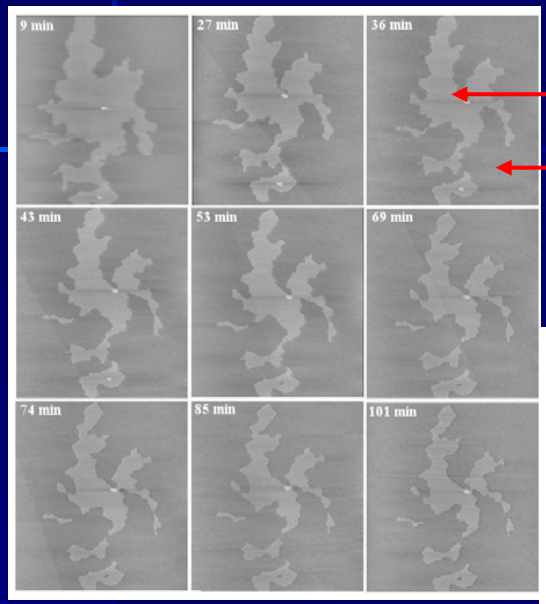
Attenuad Total-Reflection- Fourier Transform Infrared Spectroscopy (ATR-FTIR)

a) Effect of FQs on the membrane organisation:

CIP

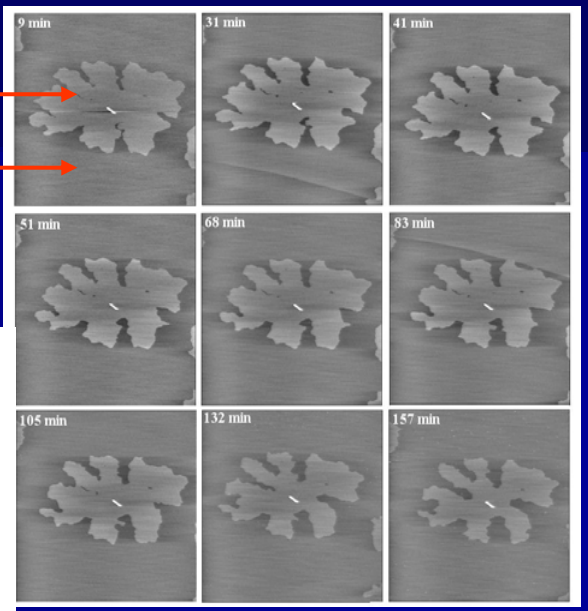
MXF

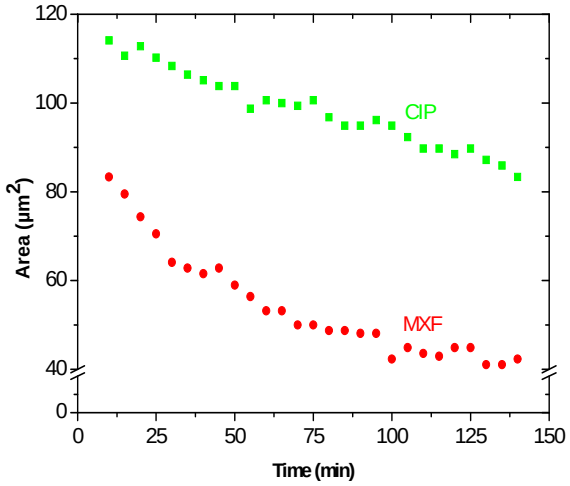
AFM Studies



DPPC

DOPC



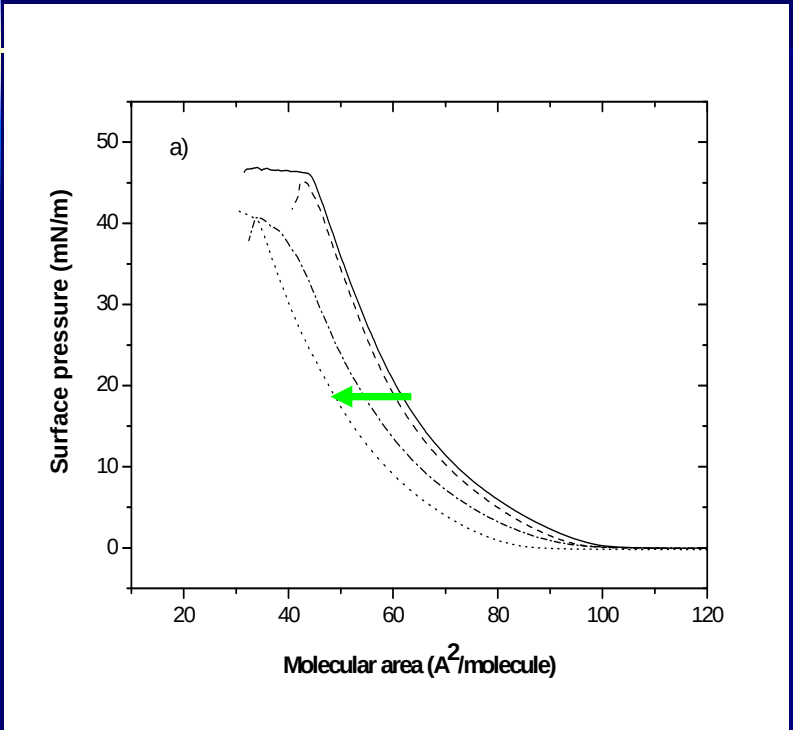


i) MXF induced in a more (57%) extent than CIP (27%), an erosion of the DPPC domains in the DOPC fluid phase

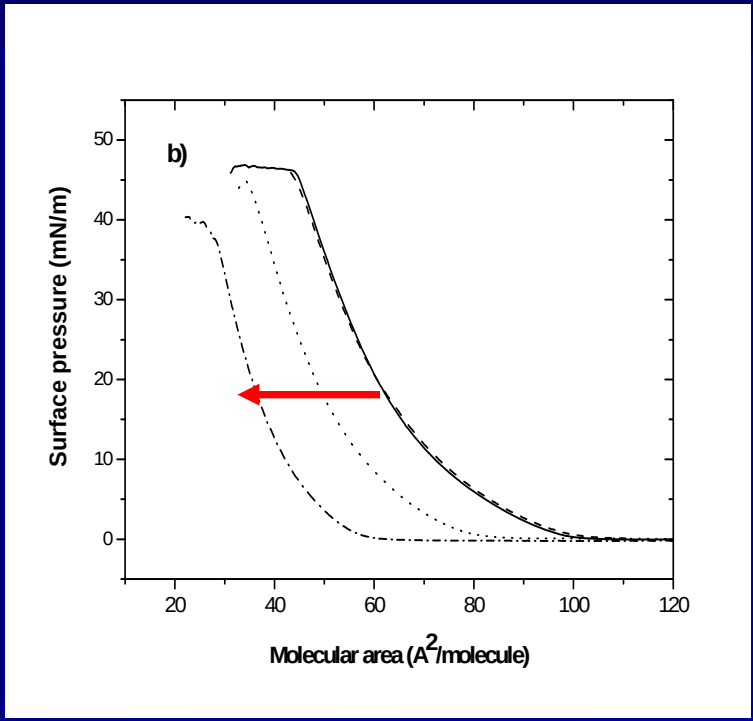
b) FQs stability within a lipid monolayer / compressibility

Langmuir studies

CIP

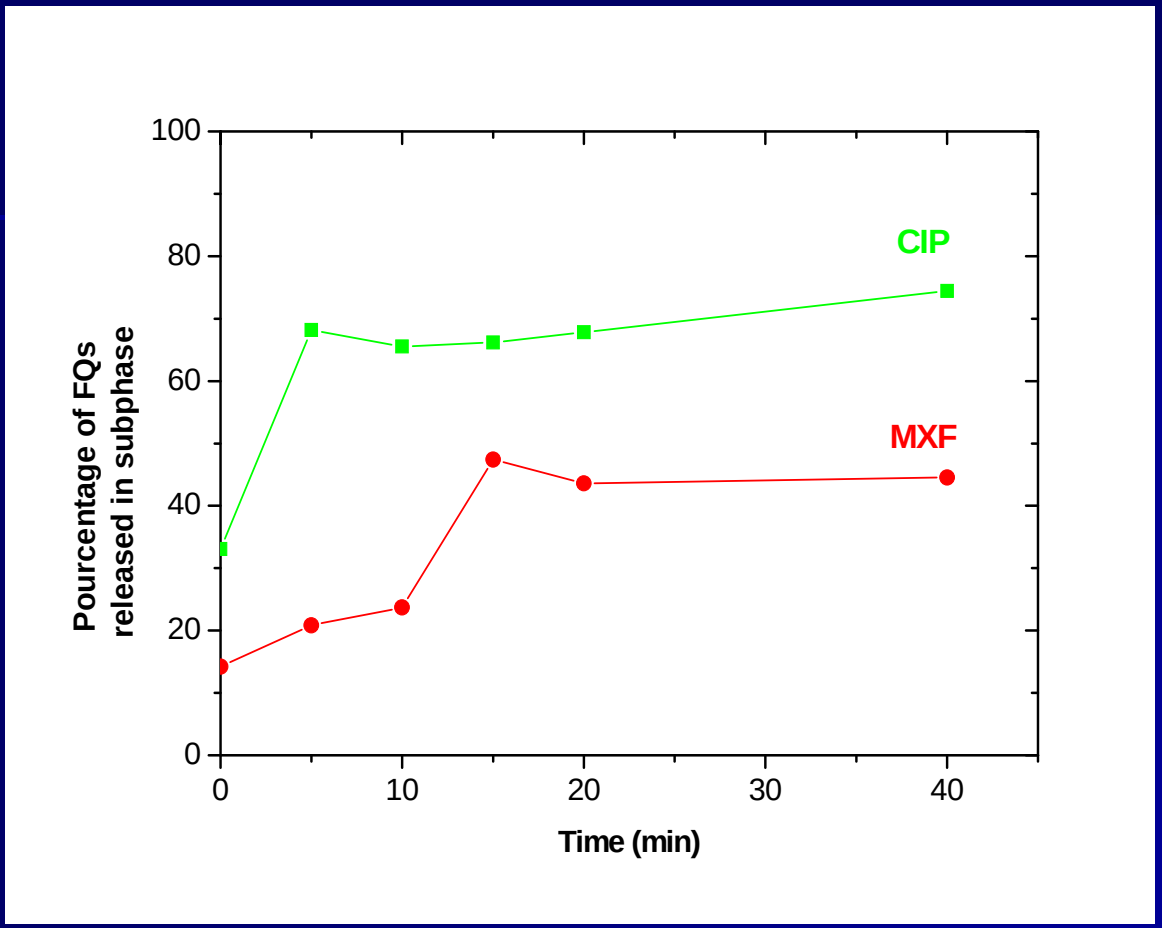


MXF



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

Phase transfer studies



iii) MXF has a lower propensity (as compared to CIP) to be released from lipid to aqueous phase

c) Effect of FQs on the conformation and orientation of phospholipids

ATR-FTIR studies

i) Conformation analysis

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

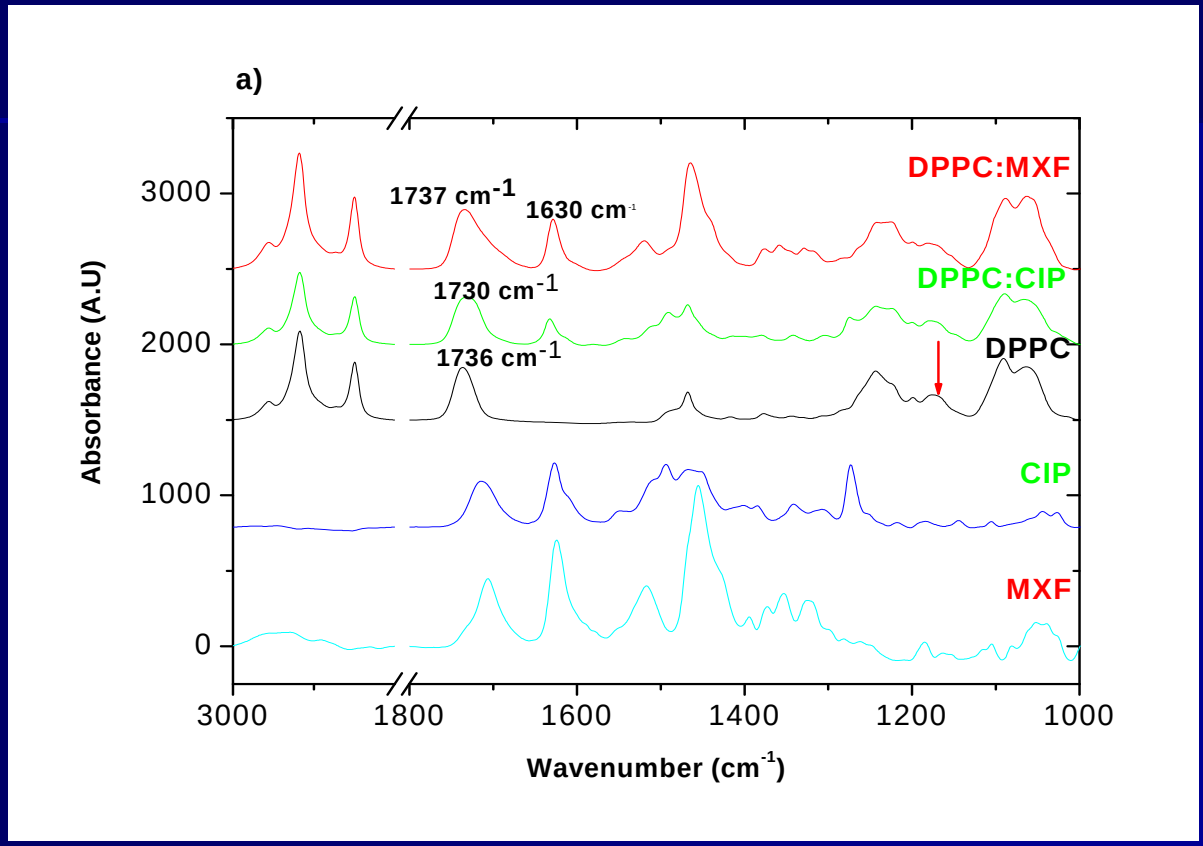
ii) Orientation analysis

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

Goormaghtigh E *et al* ., 1999: Biochim.Biophys.Acta. 1422:105-185

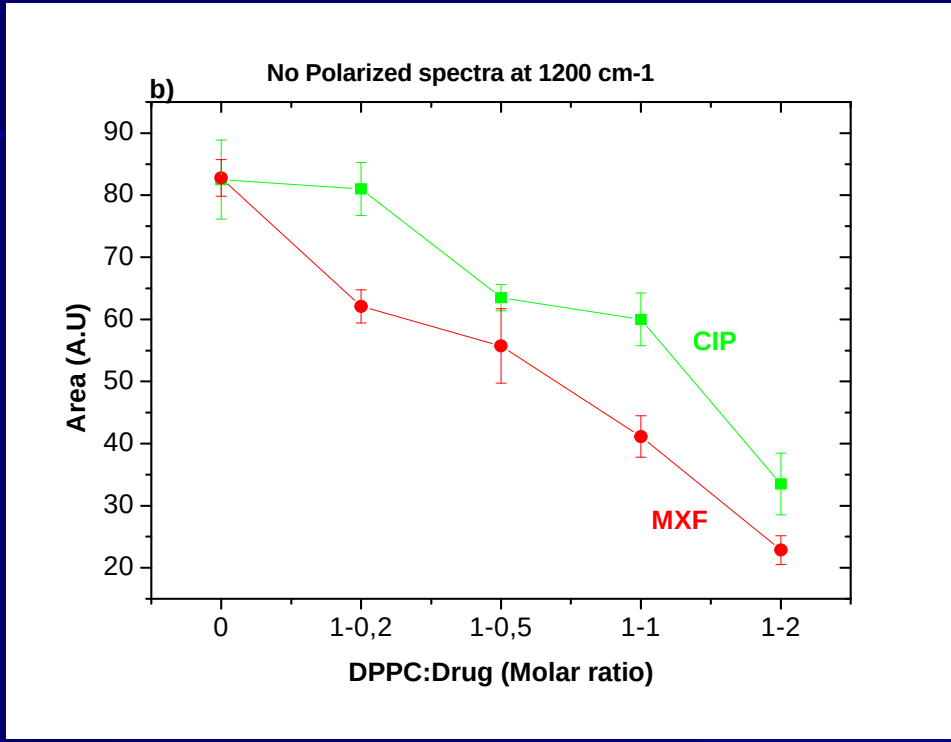
i) Conformation analysis

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}**

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

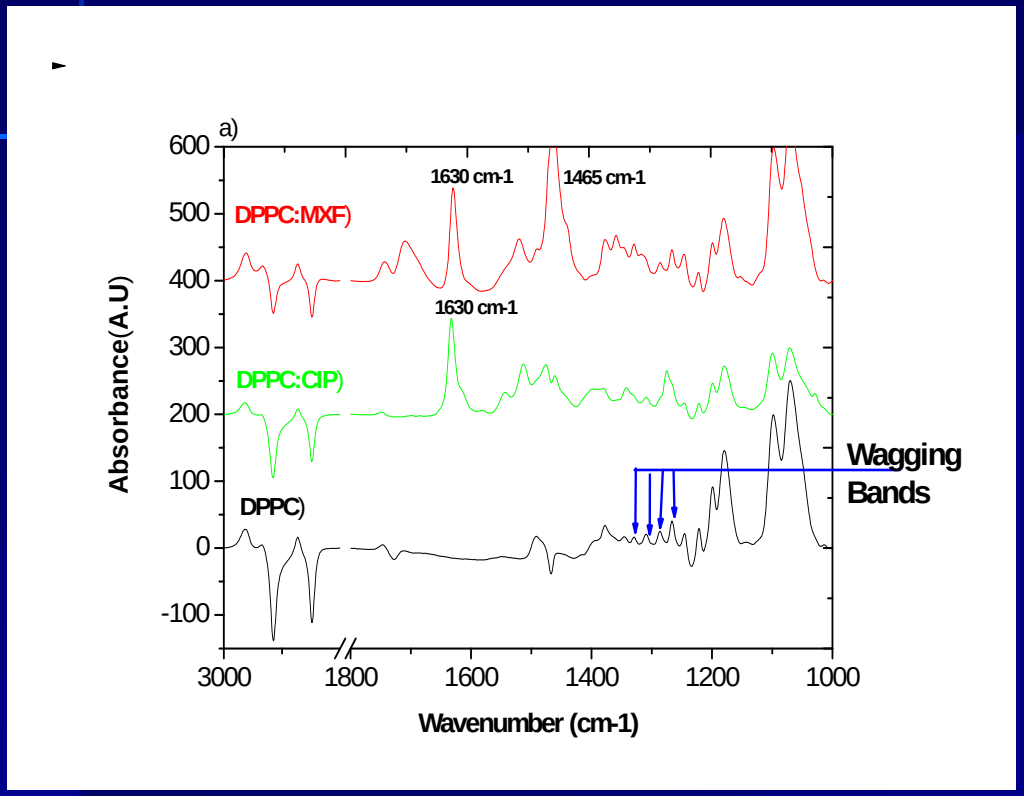


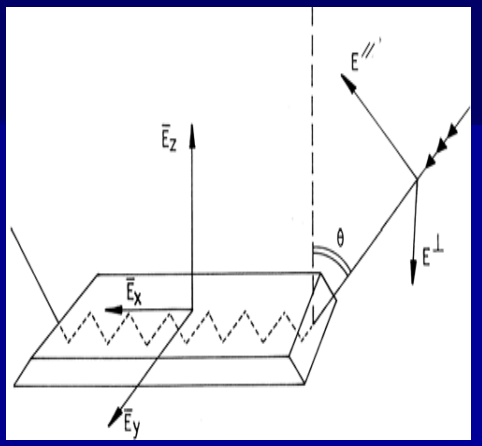
- Area evolution of DPPC peak at 1206-1193 cm^{-1} as function of increasing amounts of FQs : ↓ 60 % for CIP, ↓ 72 % for MXF

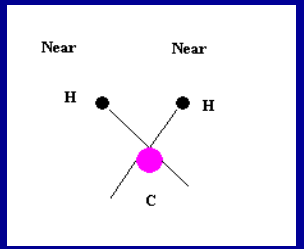
⇒ The all-trans configuration of the alkyl chain of DPPC decreased more in the presence of MXF.

ii) Orientation analysis

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





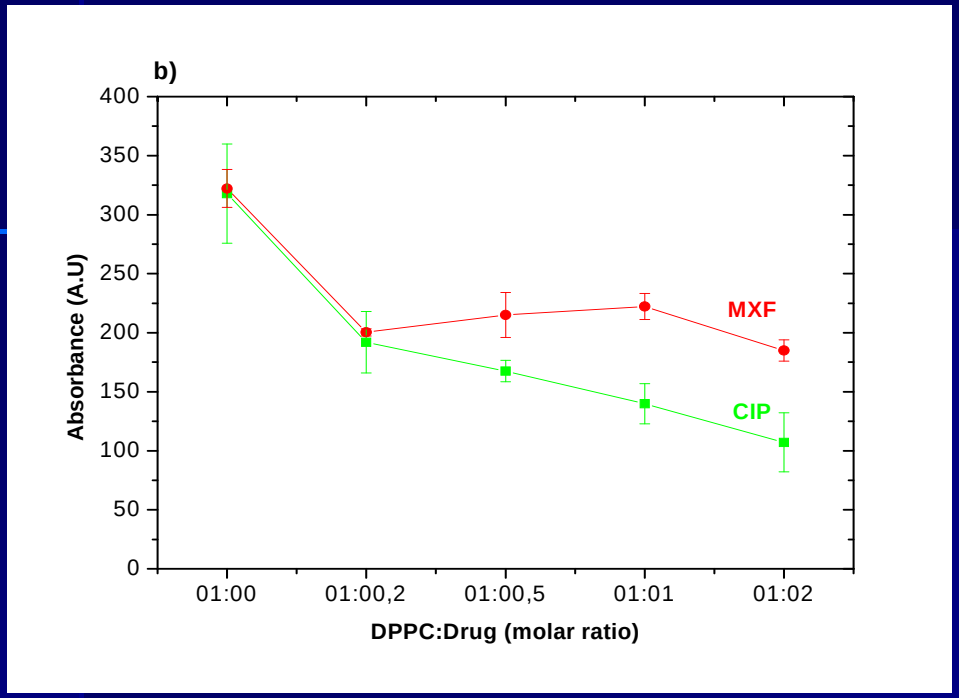


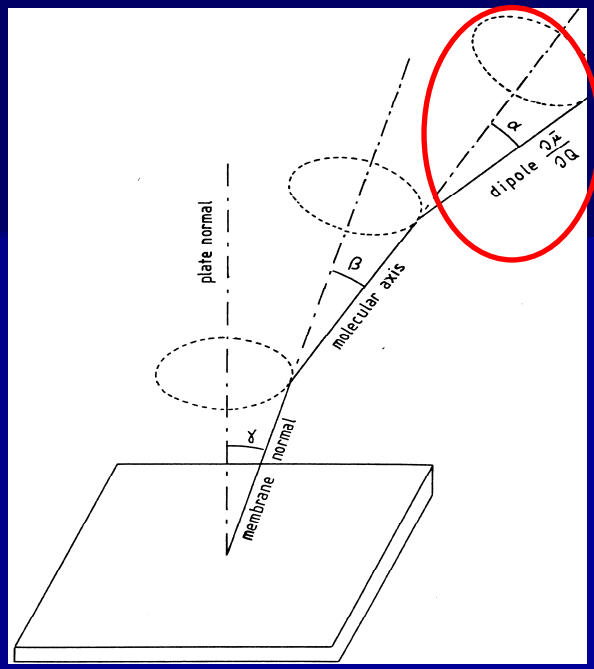
Out of plane wagging

- 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

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 angle 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 induces less disorder than CIP

Conclusion

- MXF induced, to a greater extent than CIP, an erosion of DPPC domains in the DOPC fluid phase
- MXF induced a decrease of the molecular area of DOPC/DPPC/ FQ monolayer
- MXF had a higher tendency to decrease the number of all-trans conformations (as compared to CIP), probably creating a pocket in which MXF can be located.
- CIP induced a disorder and modified the orientation of the acyl chain in relation to its higher ability to be released in an aqueous phase after monolayer compression

Acknowledgements

- Marie-Paule Mingeot-Leclercq, Nathalie Fa, Ingrid Burton (FACM, UCL)
- Yves F. Dufrène (CIFA) and André Schank (CHIM, UCL, Louvain la neuve, Belgium)
- Erik Goormaghtigh (SFMB ,ULB, Brussels, Belgium)
- Laurence Lins, Robert Brasseur and Magali Deleu (FSAGx , Gembloux, Belgium)
- FACM Team…

Thanks for your attention