Catholic University of Louvain

Cellular and Molecular Pharmacology Unit

Vienna, Austria

Interactions of Ciprofloxacin with DPPC and DPPG: Fluorescence Anisotropy, ATR-FTIR and ^{31}P NMR Spectroscopies and Conformational Analysis

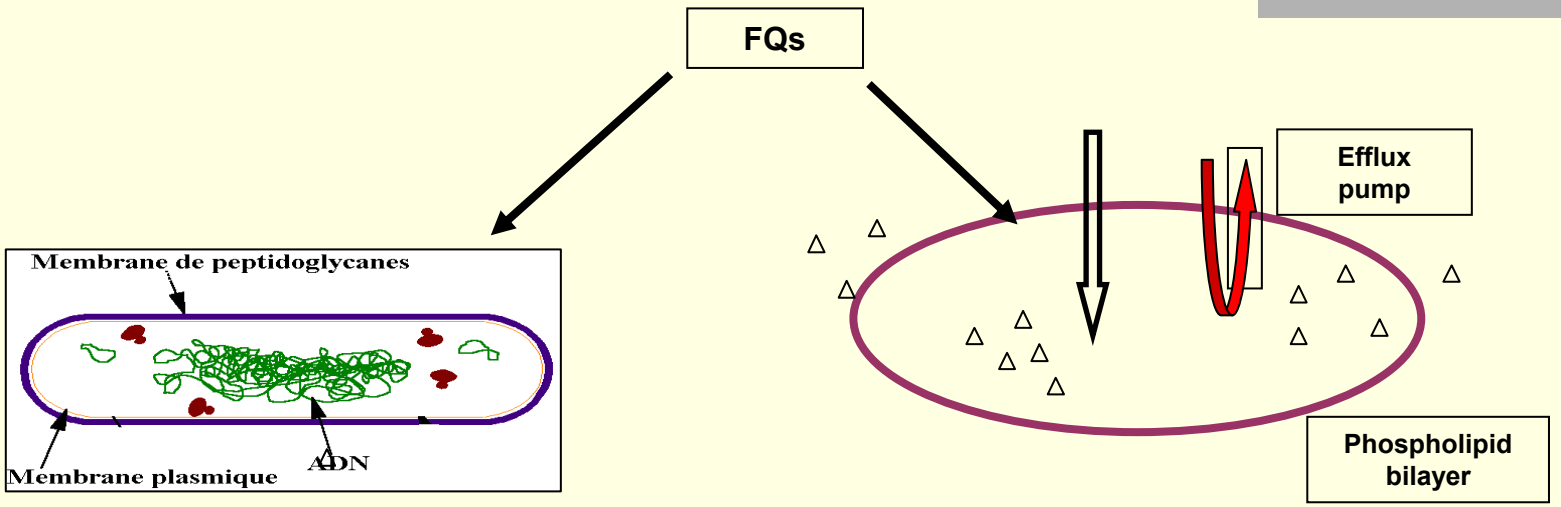
Hayet BENSIKADDOUR

Brussels, BELGIUM

May 13th, 2010

Introduction

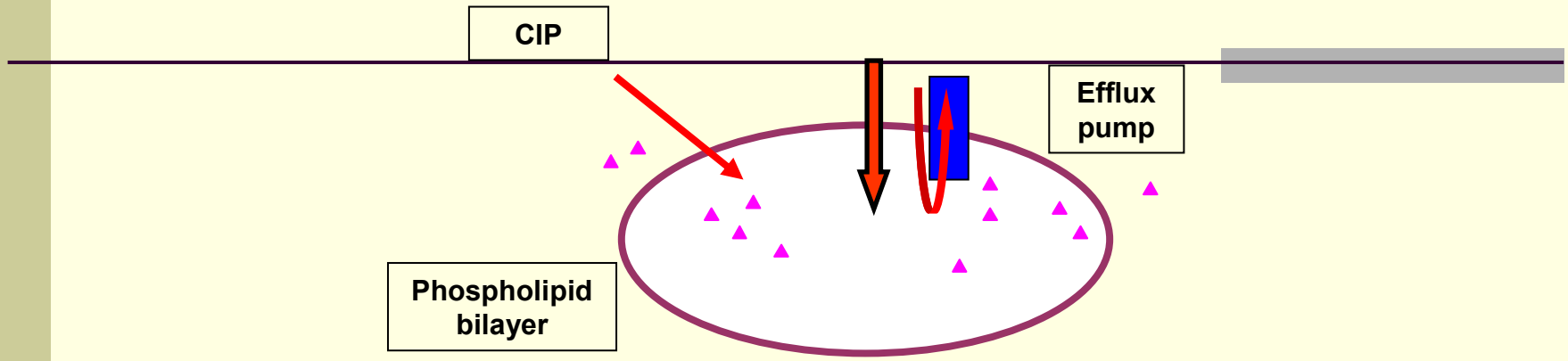
Fluoroquinolones (FQs)- lipids interaction



To kill intracelluar bacteria and to reach bacterial target, the FQS :

- **interact with cell membrane lipids bilayer : where they can be recognized by the membrane protein efflux pumps**
- **interact with bacterial membrane**

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



The mechanism of entrance of CIP involved:

- Diffusion process

- Recognition by efflux proteins in cell membranes and /or bacteria membrane

(Michot *et al.*,2004, Wang *et al.*, 2007, Périchon *et al.*,2007)

CIP interact with phospholipids

Role of lipids in this phenomena ?

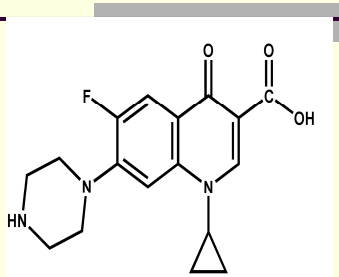
Aim of study

Characterization of the interactions between CIP with eucaryotic and procaryotic model lipids membrane (DPPC vs DPPG)

Materials

i) Antibiotics

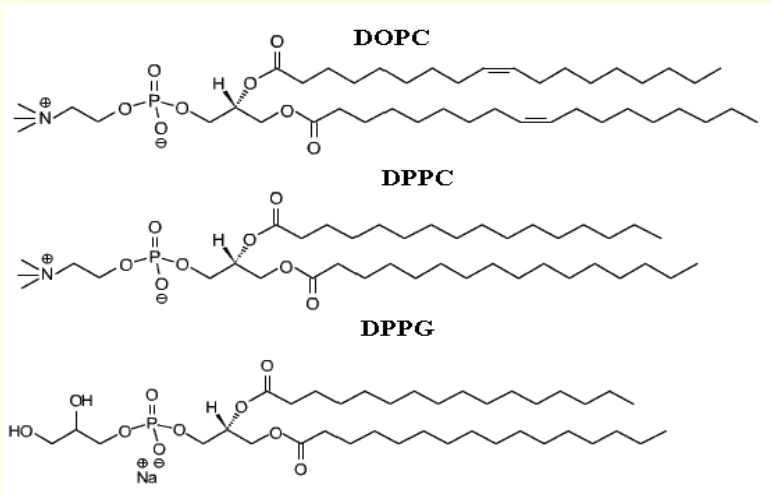
- **Ciprofloxacin**



ciprofloxacin

ii) Model membranes (Lipids)

- **Zwitterionic lipid (DPPC, DOPC)**
- **Negative charged lipid (DPPG)**
- **Liposomes: LUVs and MLV**



Methods

a) Binding parameters of Ciprofloxacin with lipids (DPPC, DOPC, DPPG)
Steady state anisotropy measurement

b) Effect of Ciprofloxacin on the mobility of phosphate heads of DPPC and DPPG
^{31}P Nuclear Magnetic Resonance (NMR)

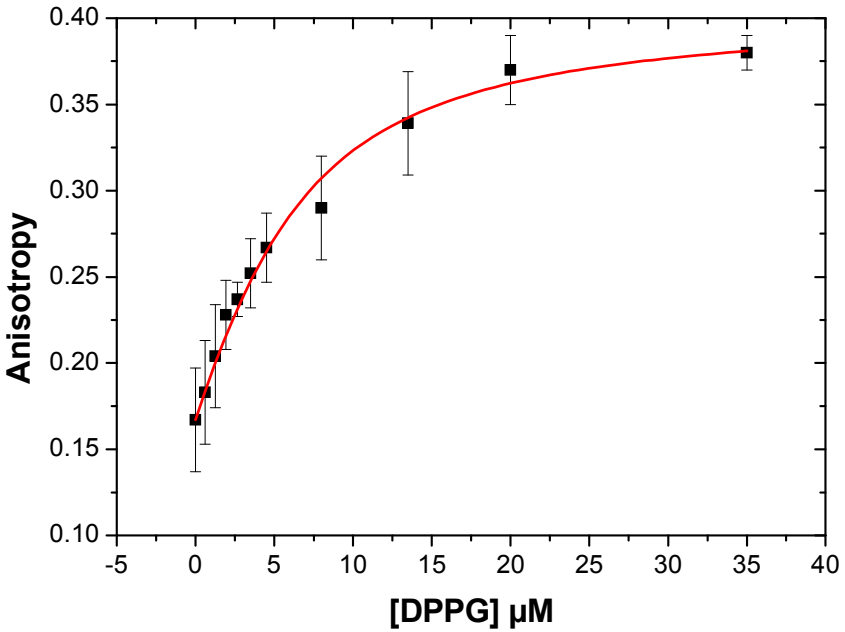
c) Ciprofloxacin effect on the melting temperature of DPPC and DPPG
Attenuated Total-Reflection- Fourier Transform Infrared Spectroscopy (ATR-FTIR

d) Assembly of CIP with phospholipids by molecular modeling
Hypermatrix procedure

Results

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

[CIP] = 5 µM



K_{app}= (8.6±0.5) 10^5 M^{-1}

a) Binding parameters of CIP with lipid vesicles

LUV liposomes Composition	$K_{app}(10^5\ M^{-1})$
DPPG	8.6±0.5
DPPC	2.5±0.1
DOPC:DPPG (1:1, M:M)	3.2±0.9
DOPC:DPPC (1:1, M:M)	1.1±0.2

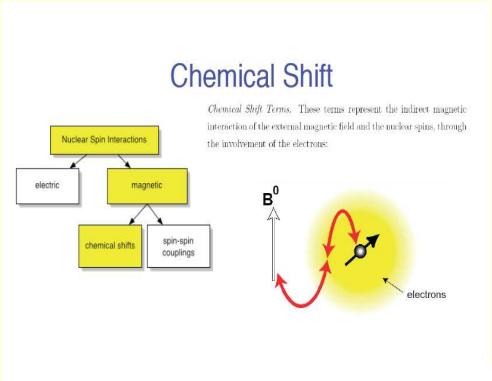
⇒ **CIP has more affinity for negative charge liposomes**

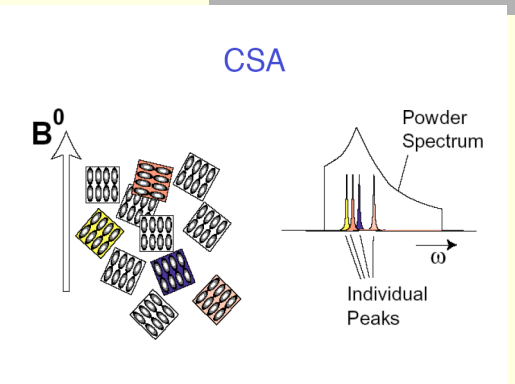
(DPPG Vs DPPC)

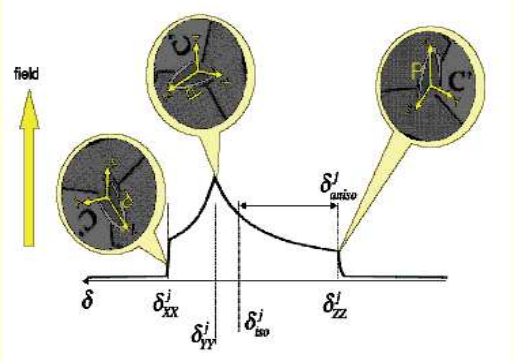
b) Mobility of phosphate heads of phospholipids

Nuclear Magnetic Resonance (^{31}P NMR)

Chemical shift anisotropy $\Delta\sigma$





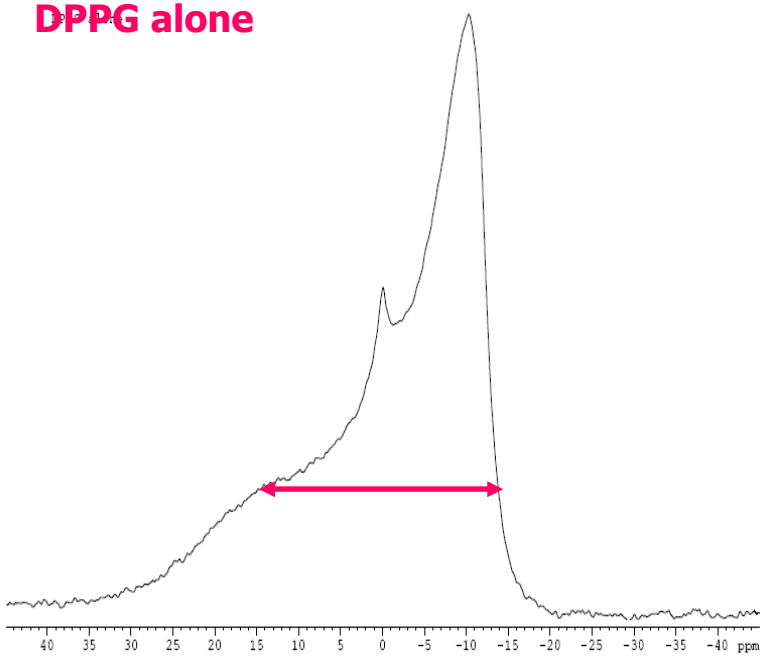


$\Delta\sigma = \sigma_{//} - \sigma_{\perp}$
$\sigma_{//}$: The low field shoulder
$\sigma_{\perp}$: The high field peak

b) CIP effect on the membrane mobility

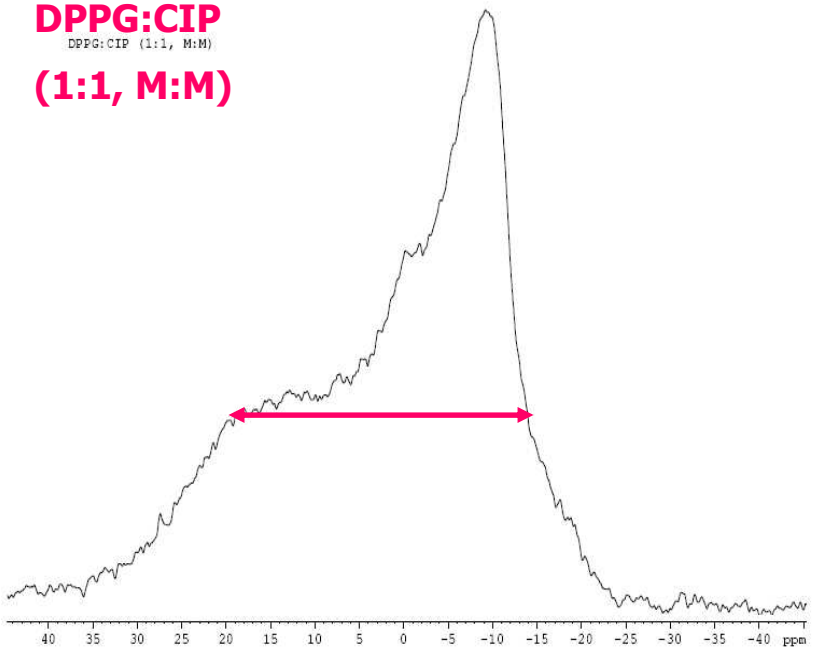
i) DPPG

DPPG alone



DPPG:CIP

(1:1, M:M)

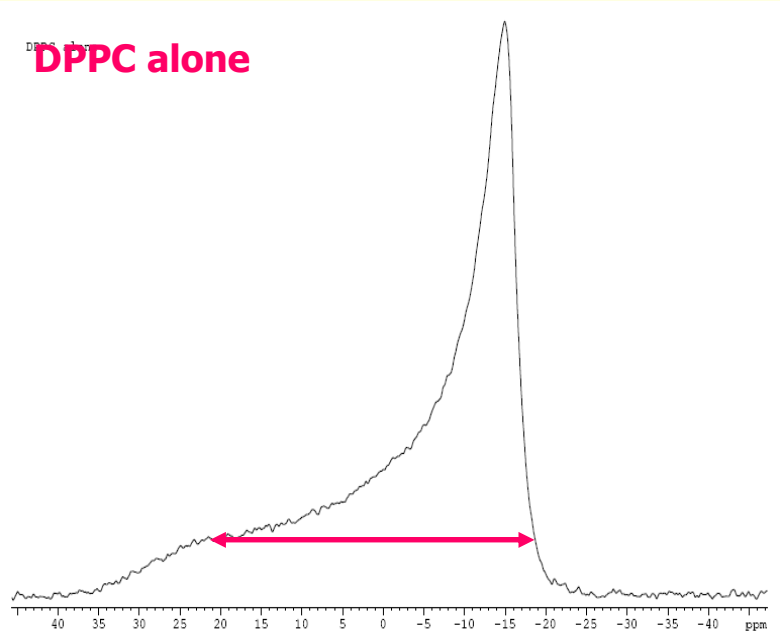


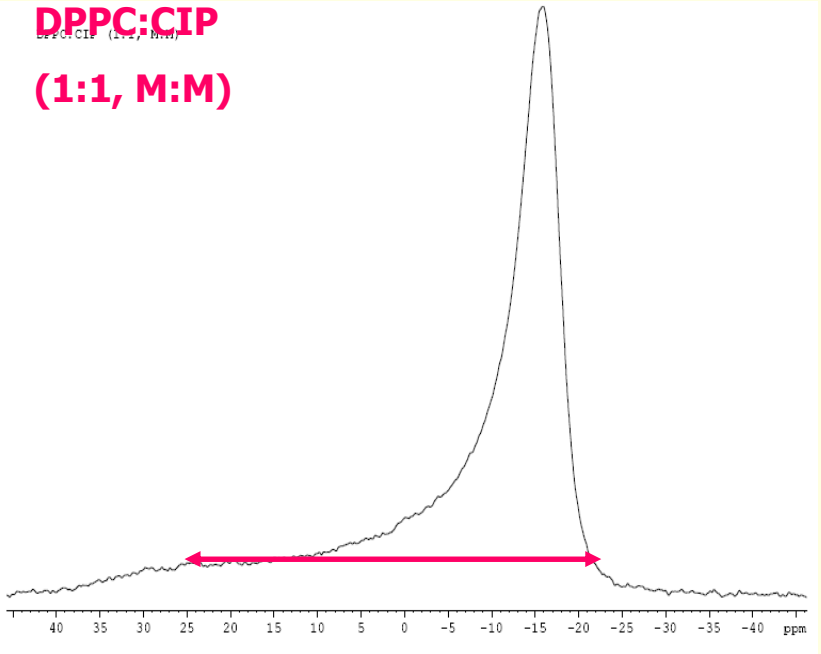
$\Delta\sigma$=25.5±0.5 ppm

$\Delta\sigma$=34.0±1.0 ppm

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

b) CIP effect on the membrane mobility
ii) DPPC



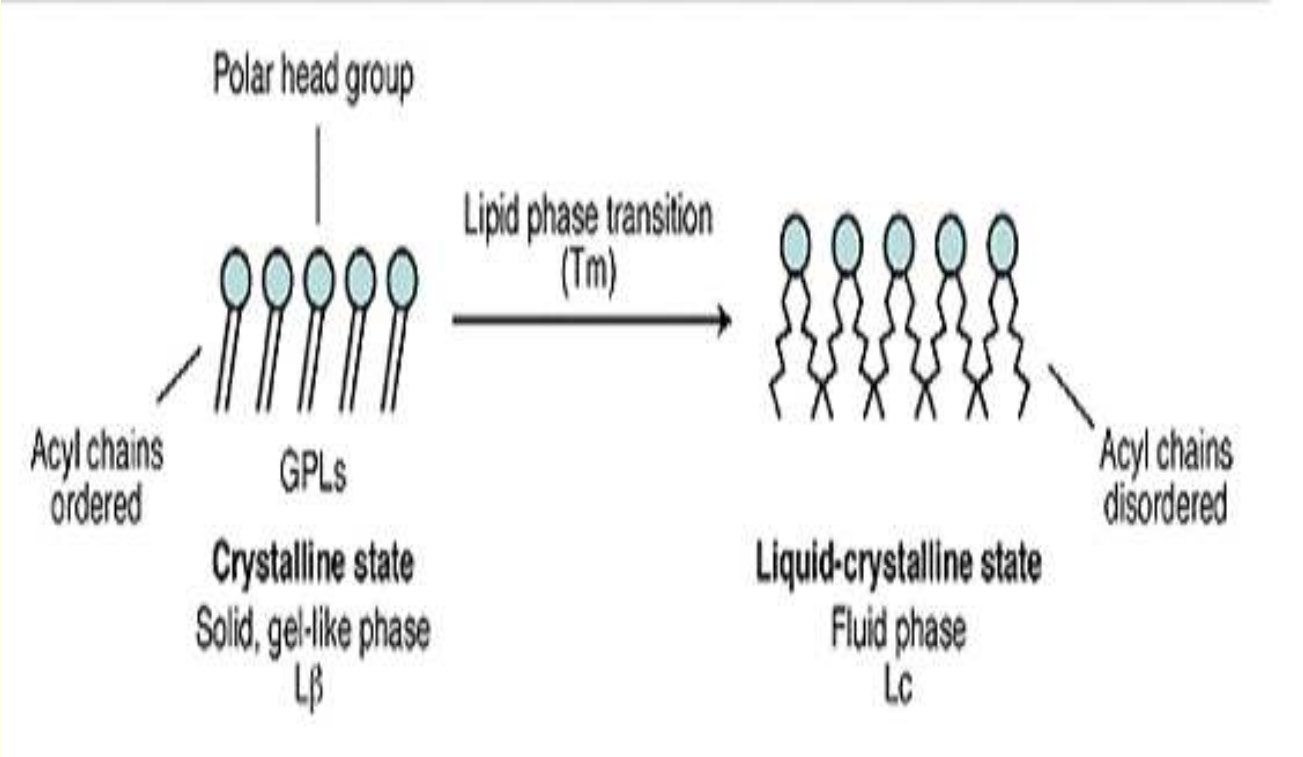


$\Delta\sigma$=41.5±0.5 ppm

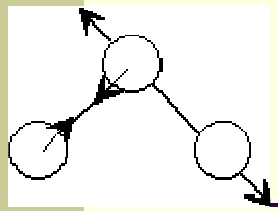
$\Delta\sigma$=46.6±0.1 ppm

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

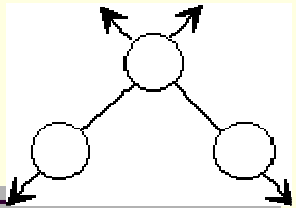
c) CIP effect on the melting temperature of lipid



c) Melting temperature of lipid by ATR-FTIR

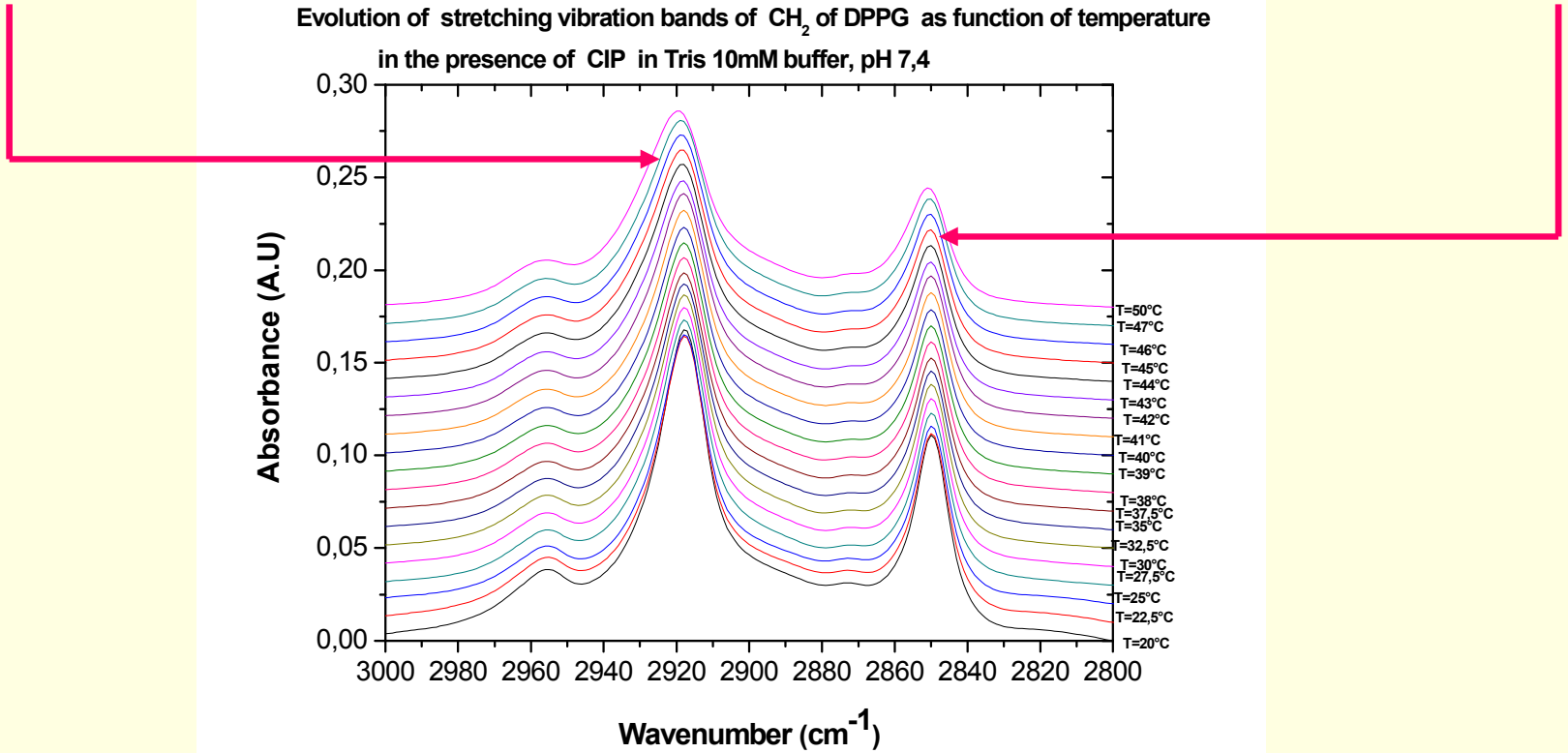


CH_2 Stretching vibrations



CH_2 Asymmetric at 3000 cm^{-1}

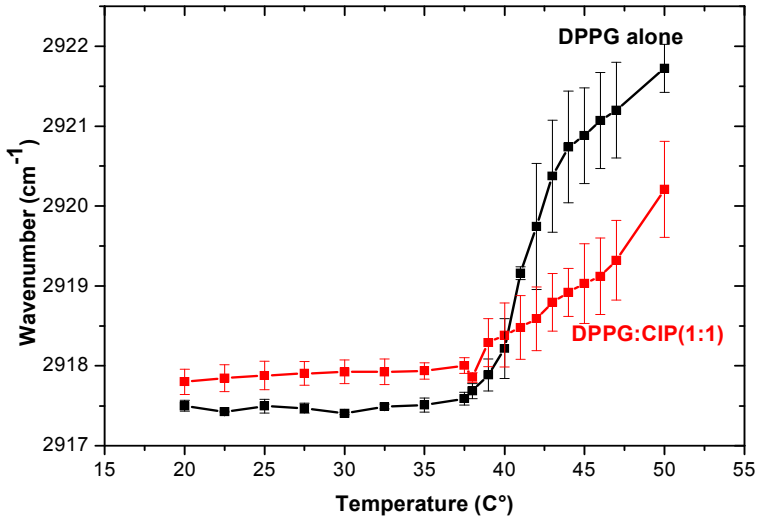
CH_2 symmetric at 2800 cm^{-1}



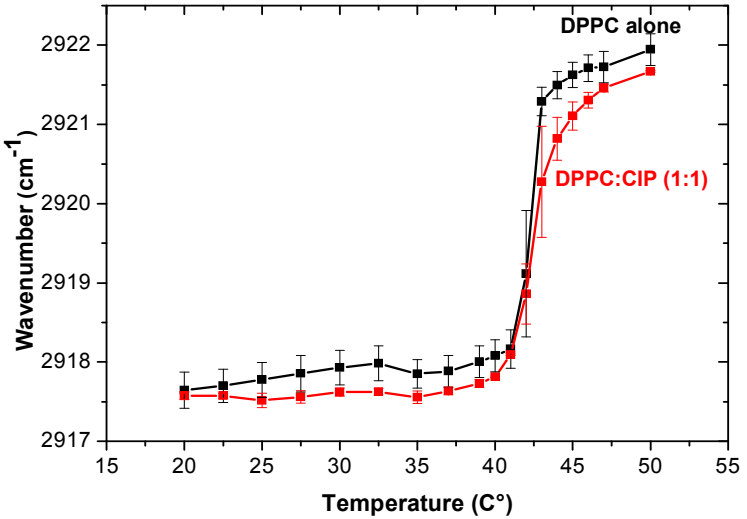
c) CIP effect on the melting temperature of DPPG and DPPC

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°

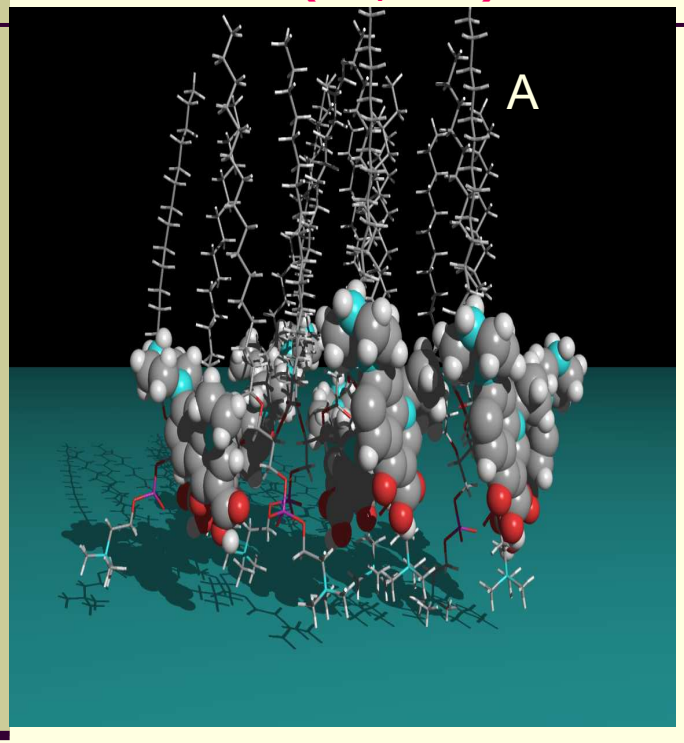
- CIP did not affect dramatically the DPPC melting curve

- CIP : ↓ order of acyl chain of DPPG < Tm

↑order of acyl chain of DPPG >Tm

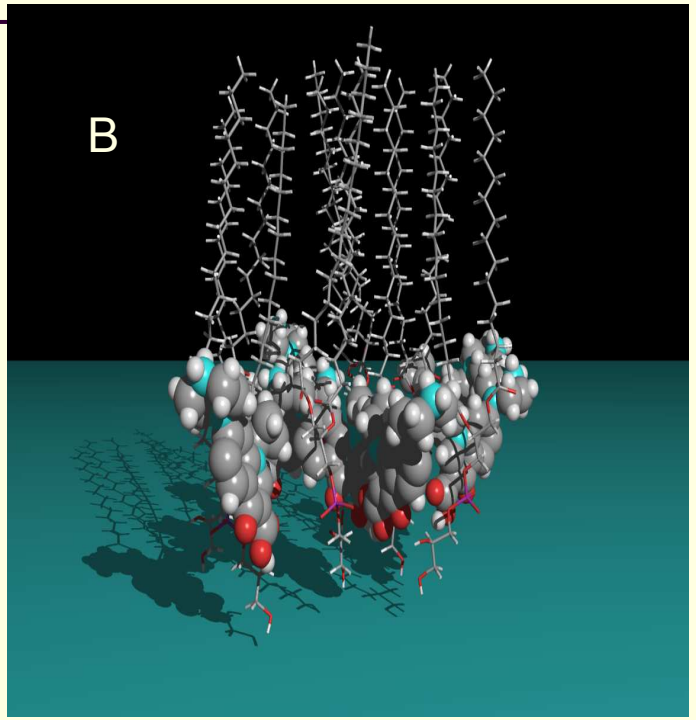
d) Assembly of CIP with phospholipids by molecular modeling using the Hypermatrix procedure

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



E = - 44.4 Kcal/mol
Area = 66 **Å²**

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



E = - 53.4 Kcal/mol
Area = 76 **Å²**

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

Conclusion

- The binding constants K_{app} were in the order of $10^5 M^{-1}$ and the affinity appeared dependent on the negative charge of liposomes: DPPG > DOPC: DPPG (1:1) > DPPC > DOPC: DPPC (1:1)

- The major effect of CIP on DPPG as compared to DPPC suggested a role of the electrostatic interactions between CIP and lipids and the importance of the nature of the polar heads of phospholipids

Bensikaddour H. *et al*., (2008): *Biochim Biophys Acta* **1778:**2535-2543

Acknowledgement

- Prs: M-P. Mingeot, P-M Tulkens, F. Van Bambeke (FACM, UCL, Brussels, Belgium)
- Pr Goormaghtigh Erik (SFMB, ULB, Brussels, Belgium)
- Pr Karim Snoussi (CHIM,UCL, Louvain-la-Neuve, Belgium)
- Dr Laurence Lins (CBMN, Gembloux, Belgium)
- FEBS for Young Travel Award

Thanks for your attention !