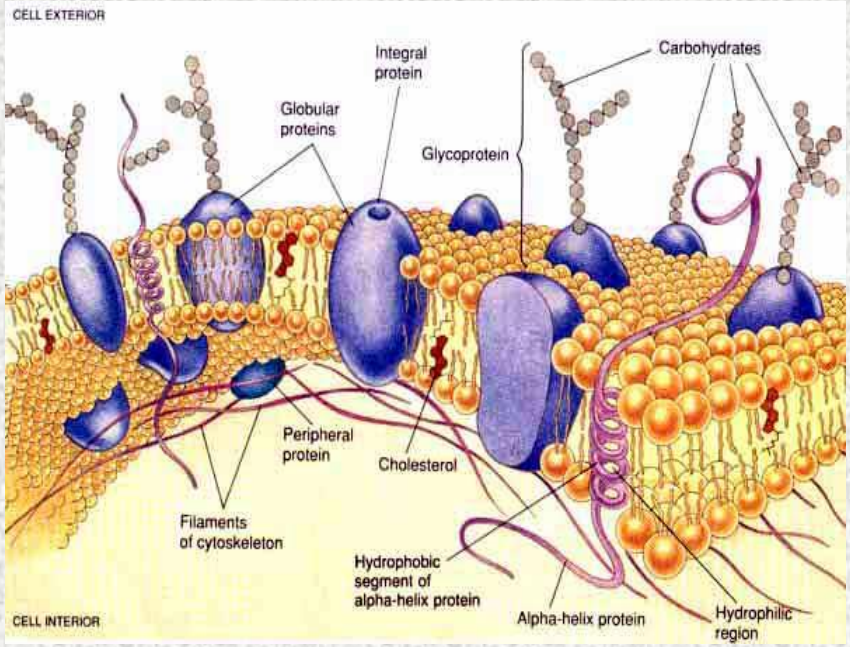
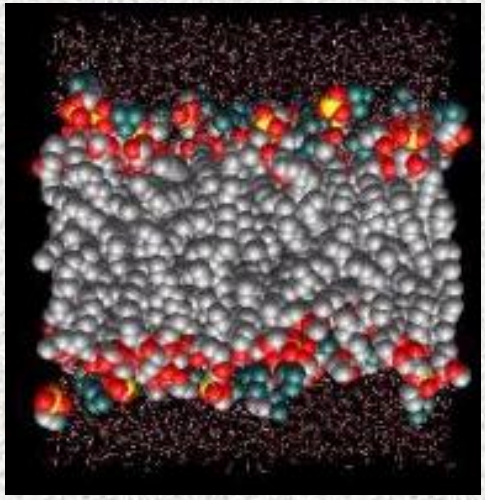
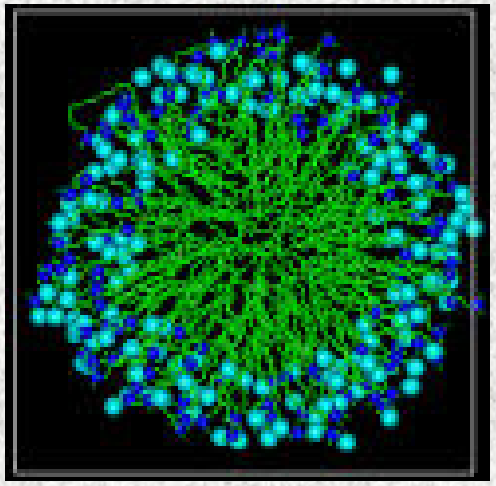
INSTITUTO DE QUÍMICA
USP

LBE
Laboratory of Structural Biology

THE MAGNETISM OF MEMBRANES
A PERSONAL ACCOUNT







Shirley Schreier

IFUSP setembro 2012

LABORATORY OF MOLECULAR BIOPHYSICS

LABORATORY OF STRUCTURAL BIOLOGY

FUNDAMENTAL RELATIONSHIP IN BIOLOGY

STRUCTURE ⇒ FUNCTION

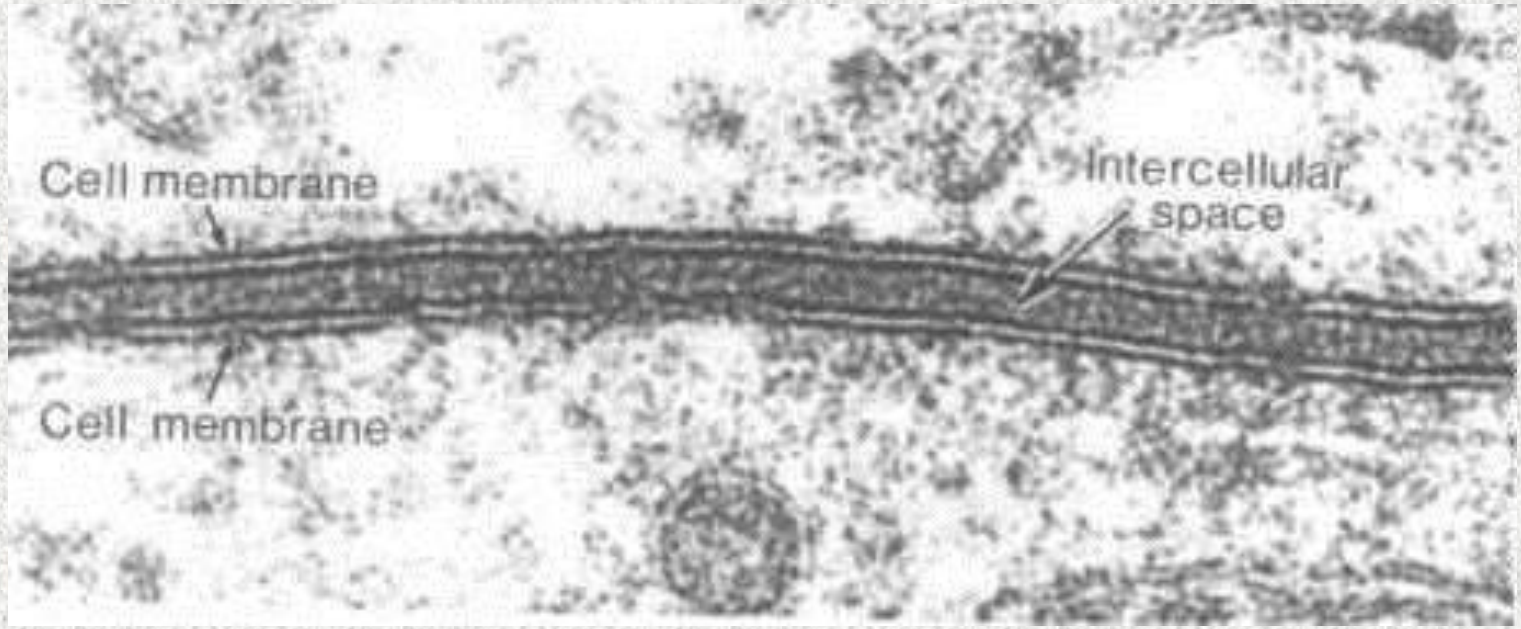
Spectroscopy:
Interaction between matter and electromagnetic radiation.

Spectroscopic Techniques :
Information about STRUCTURE and DYNAMICS.
Allow the study of conformational changes.

In particular, techniques that do not require transparency of the sample are convenient for the study of membranes.

The 70's - Decade of MEMBRANES

from STACTIC STRUTURES - electron microscopy, X rays



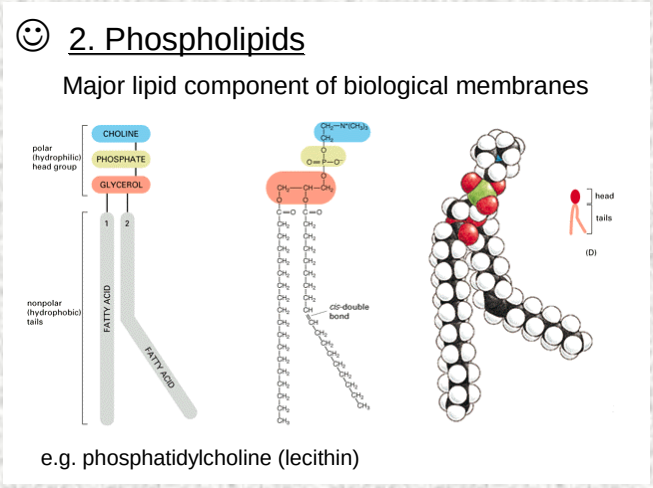
to DYNAMIC STRUCTURES

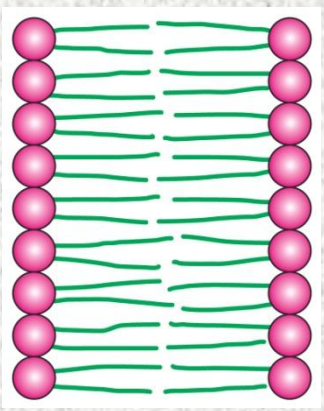
molecular organization and motion of lipids in bilayers

- **Rotation about the long molecular axis**
- **Intra-molecular motion (*trans-gauche* isomerization)**
- **Lateral diffusion**
- **Flip-flop**

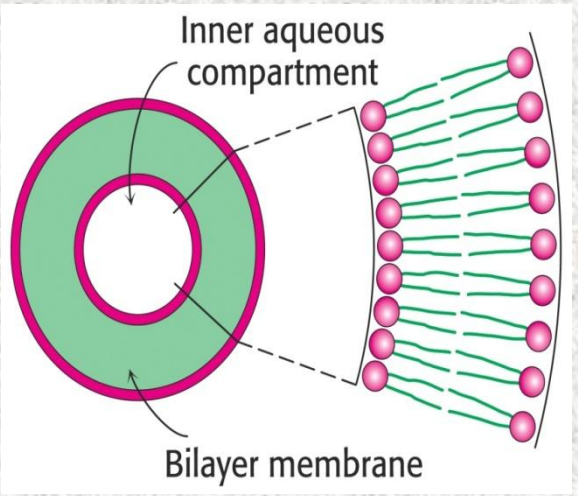
FLUID MOSAIC Model proposed in 1972, Singer & Nicolson, Science

•Phospholipids are amphipathic molecules





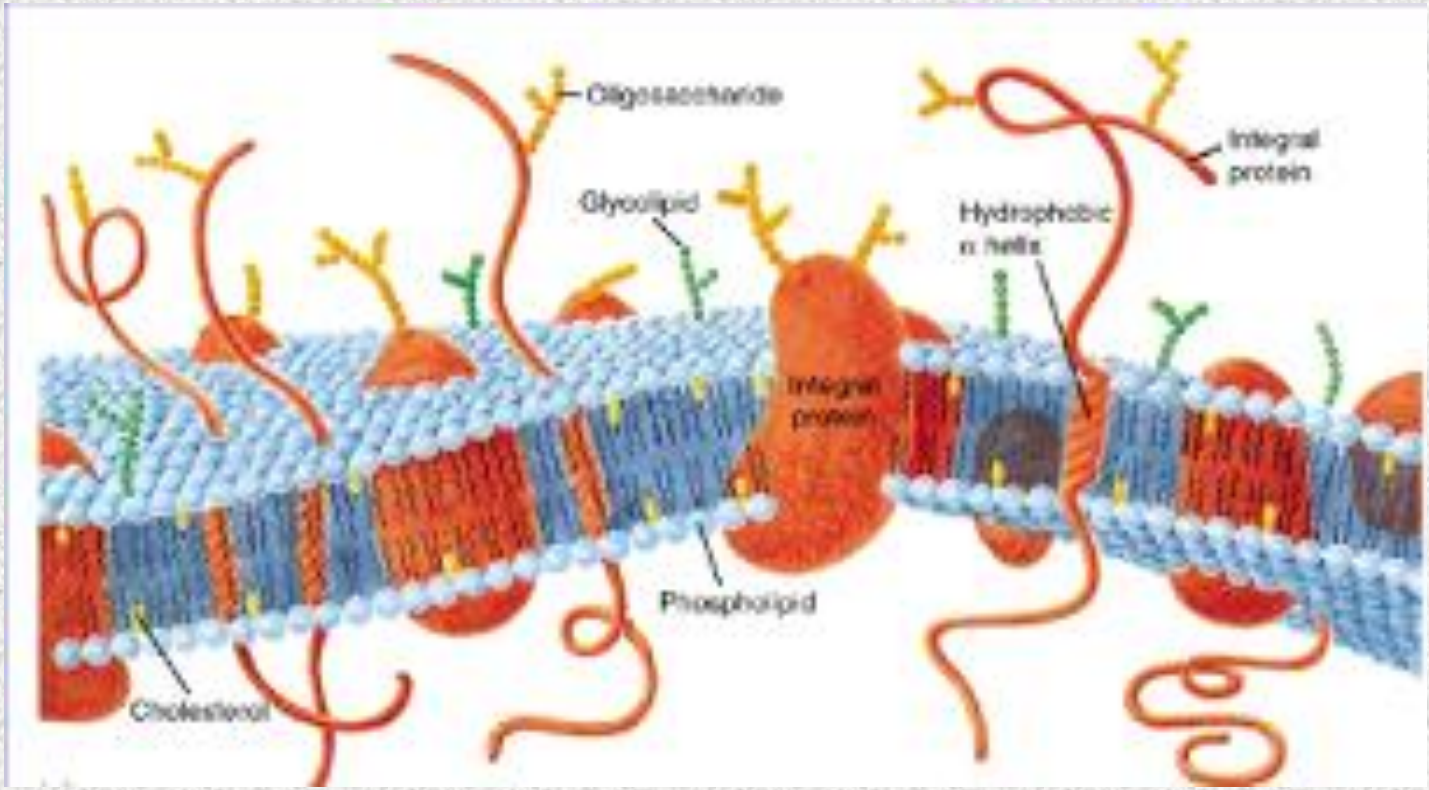
bilayers



lipid vesicles

Electron Paramagnetic Resonance,
Spin Label Method (H.M. McConnell, Stanford)

Lipídios, Lipossomos e Membranas Biológicas



A composição química das membranas biológicas

- PROTEINAS e LIPIDIOS: 40-60 %
- CARBOHIDRATOS baixa proporção

(+ Proteínas)

GLICOPROTEINAS

(+Lipídios)

GLICOLIPIDIOS

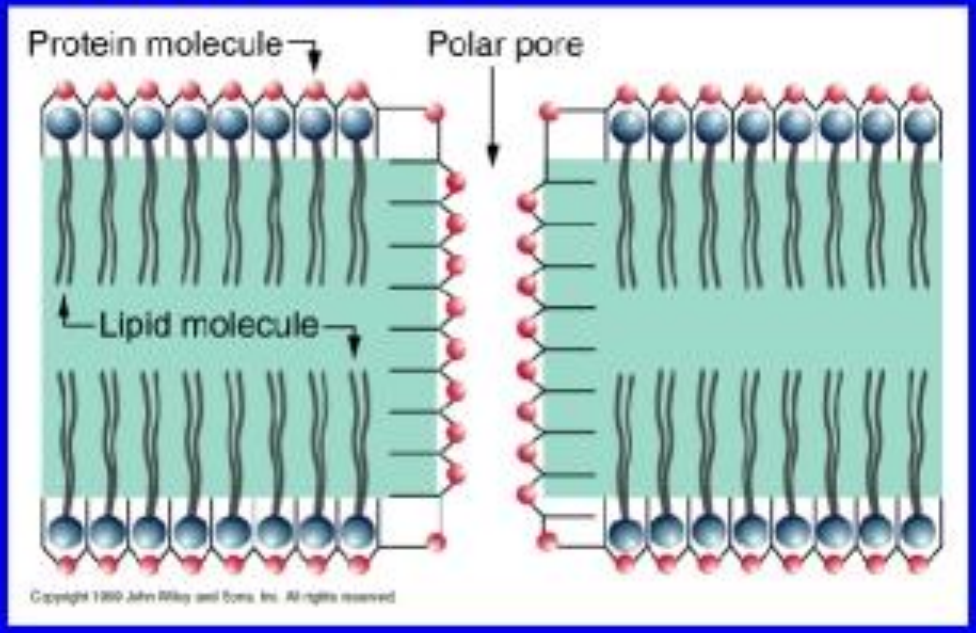
Associados com a membrana aparecem também:

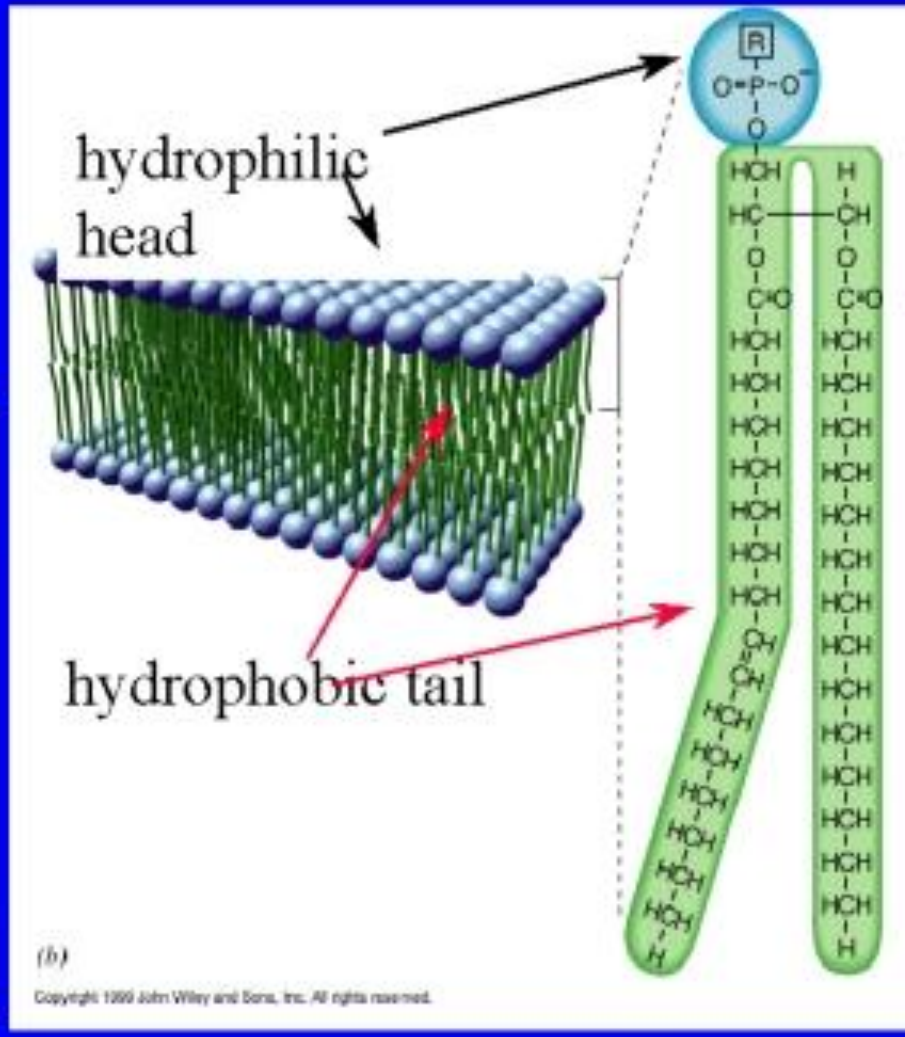
Íons minerais,
poliaminas e ácidos nucléicos.
água "associada"

EM of Membrane

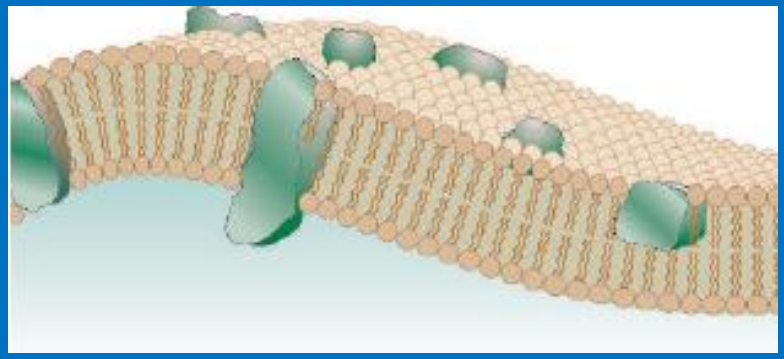
lipid bilayer

Davson-Danielli model of Bilayer with pore





Singer e Nicolson: *Modelo do Mosaico Fluido*



"A membrana formada por bicamada fosfolipídica fluida onde as proteínas difundem e ficam embebidas ou imersas "

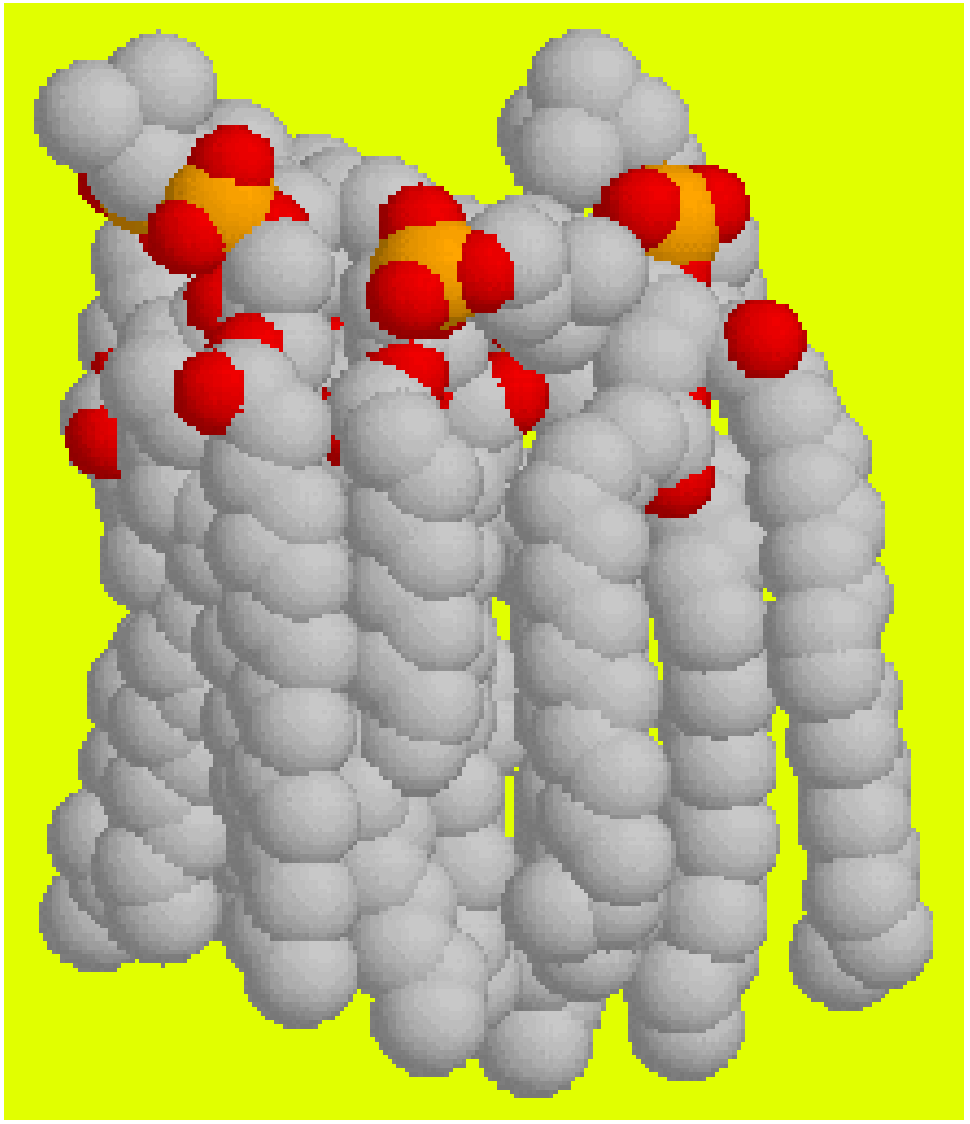
(Science, 1972:175, 720-731.)

Membrane Lipids

simple organization of amphipathic molecules

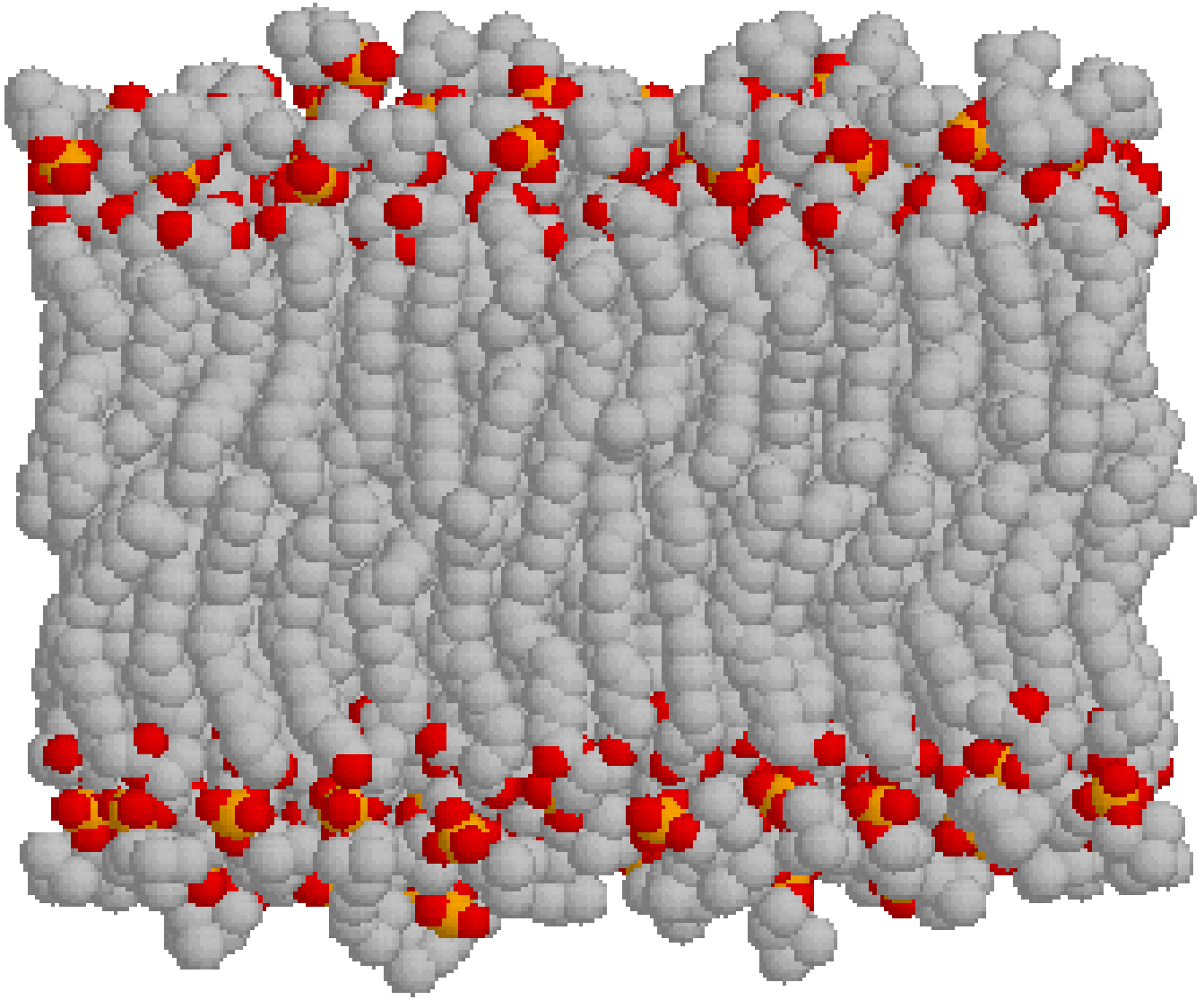
hydrophilic

hydrophobic



Membrane Lipids

cross-section of bilayer of glycerophospholipids

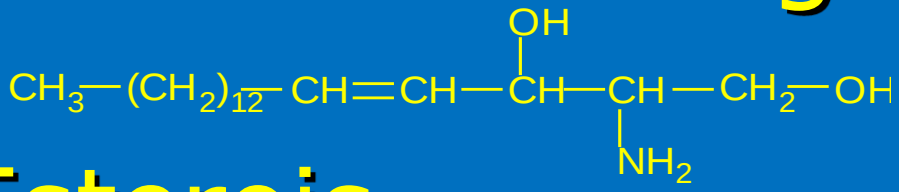


adapted from H. Heller *et al.* (1993) *J. Phys. Chem.* 97, 8343

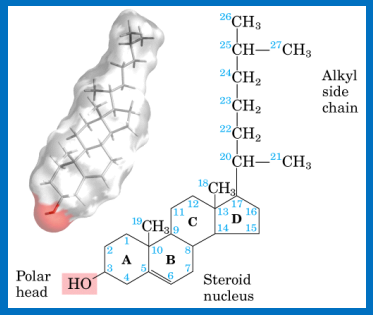
Lipídios que formam as Membranas Biologicas

- Derivados do Glicerol

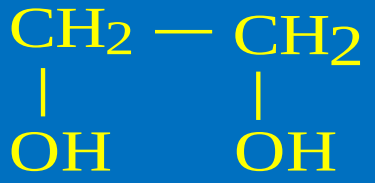
- Derivados da Esfingosina



- Os Esterois

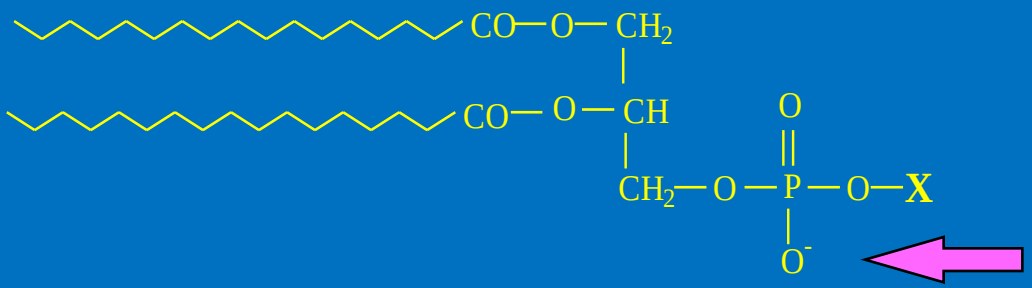


- Os Fosfolipídios Dióis



Fosfoglicerídeos

a) 1,2 DIACIL FOSFO GLICERÍDEOS



X = — H

ácido fosfatídico

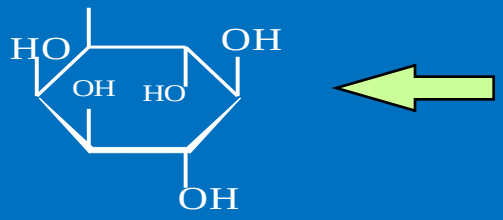
fosfatidilcolina

fosfatidiletanolamina

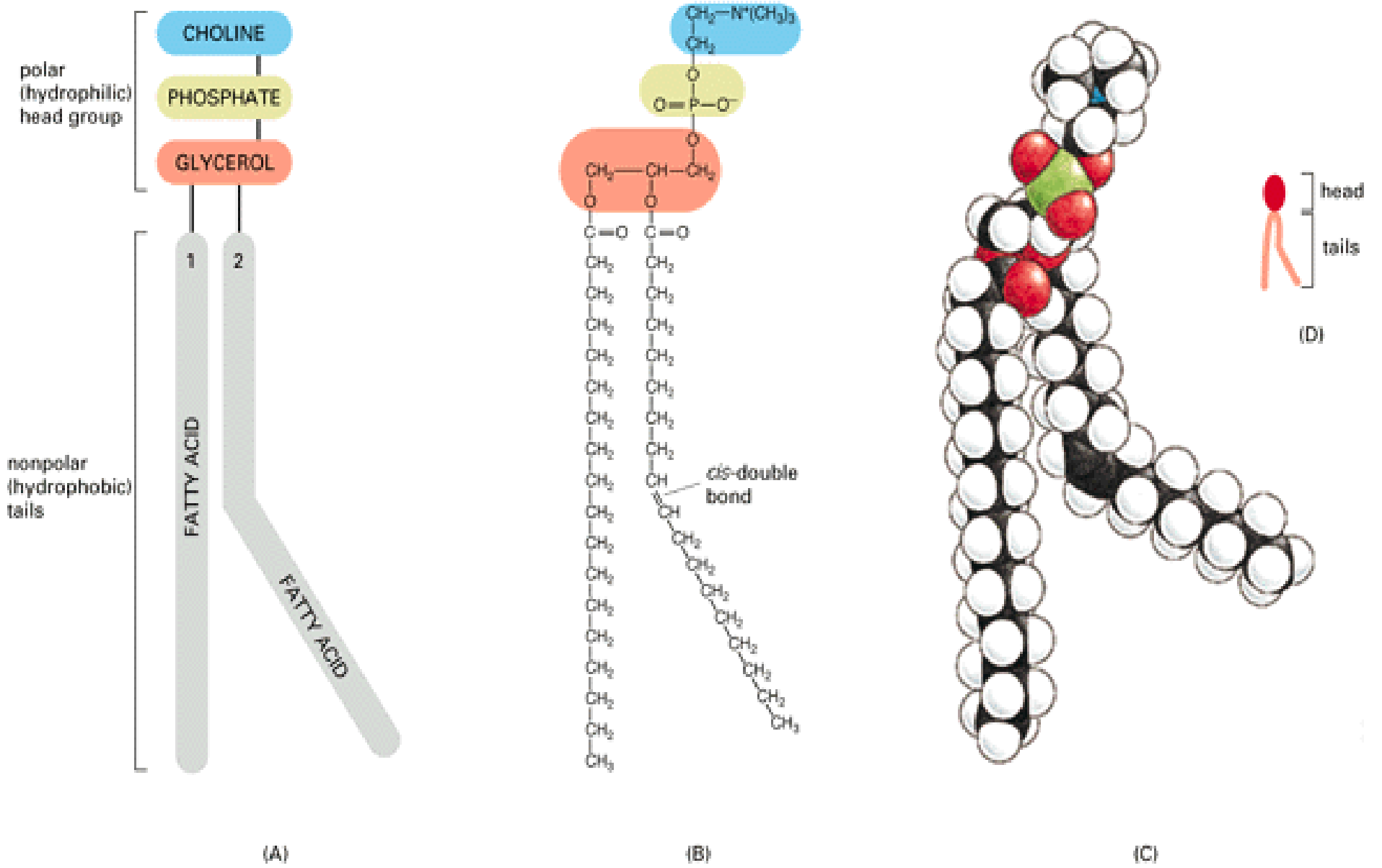
fosfatidilserina

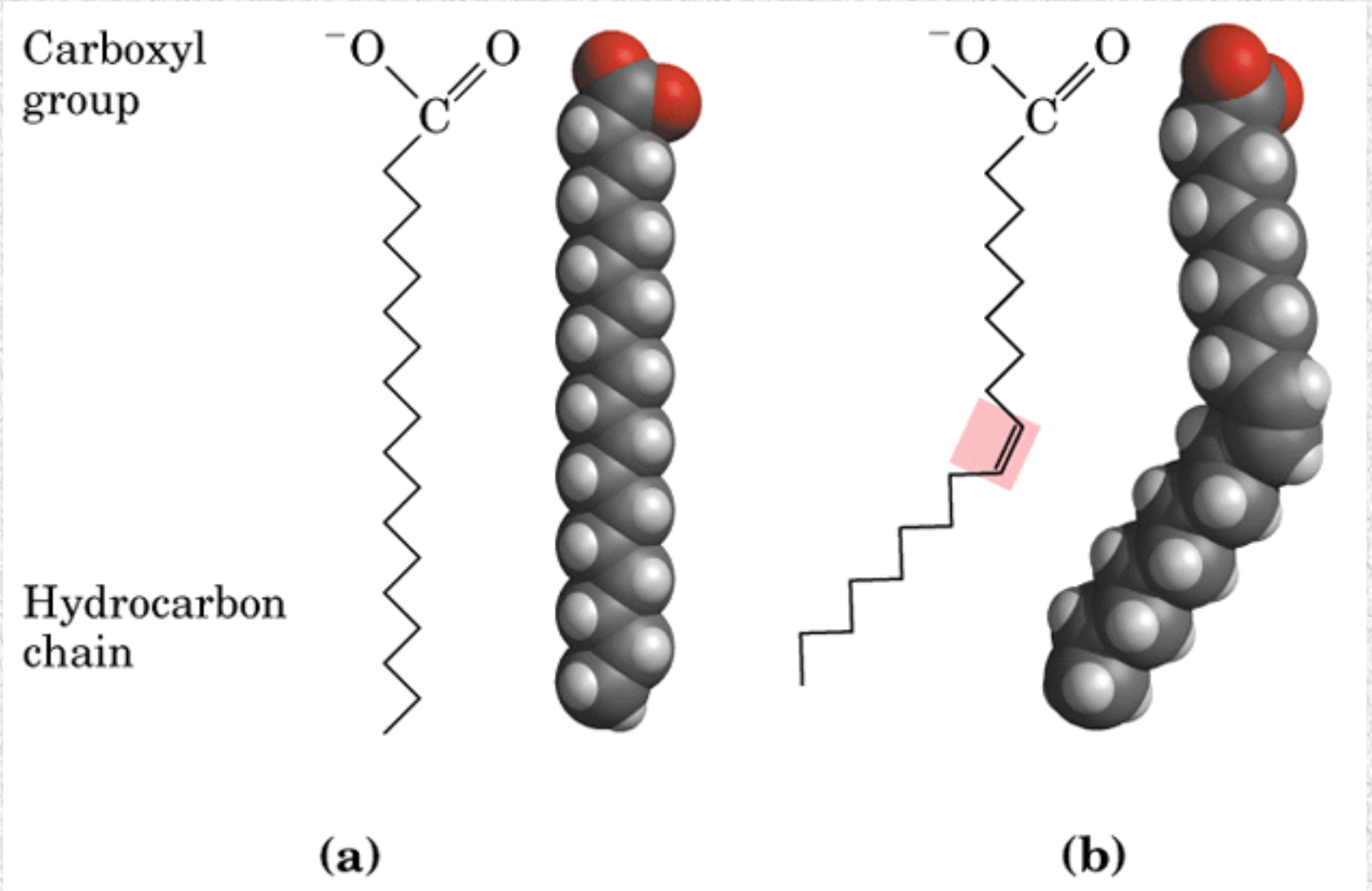
fosfatidilglicerol

fosfatidilinositol



TODAS SÃO ESTRUTURAS ANFIPATICAS


CHOLINE
PHOSPHATE
GLYCEROL
polar (hydrophilic) head group
1
2
FATTY ACID
FATTY ACID
nonpolar (hydrophobic) tails
cis-double bond
head
tails
(A)
(B)
(C)
(D)


Carboxyl group
Hydrocarbon chain
(a)
(b)

- Os ácidos graxos saturados de número par mais abundantes na natureza são:

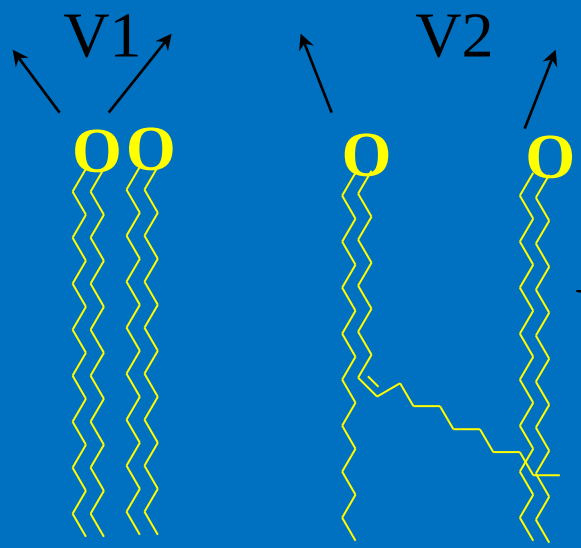
C14:0, ácido mirístico
C16:0, ácido palmítico
C18:0, ácido esteárico

- Ácidos graxos de número impar existem apenas nos gangliosídios e nos cerebrosídeos: e*sfingoglicolipídios*

- Entre os insaturados, encontram-se:

C16:1 (9c): palmitoleico
C18:1 (9c): oleico
C18:2 (9, 12): linoleico
C 18:3 (6, 9, 12) γ ou (9,12, 15) α linolénico
C20:4 (5, 8, 11, 14) araquidónico.

Presença de um AG insaturado nos PL



V2 > V1

Diminuição da interação entre moléculas vizinhas
Enfraquecimento das interações de *Van der Waals*

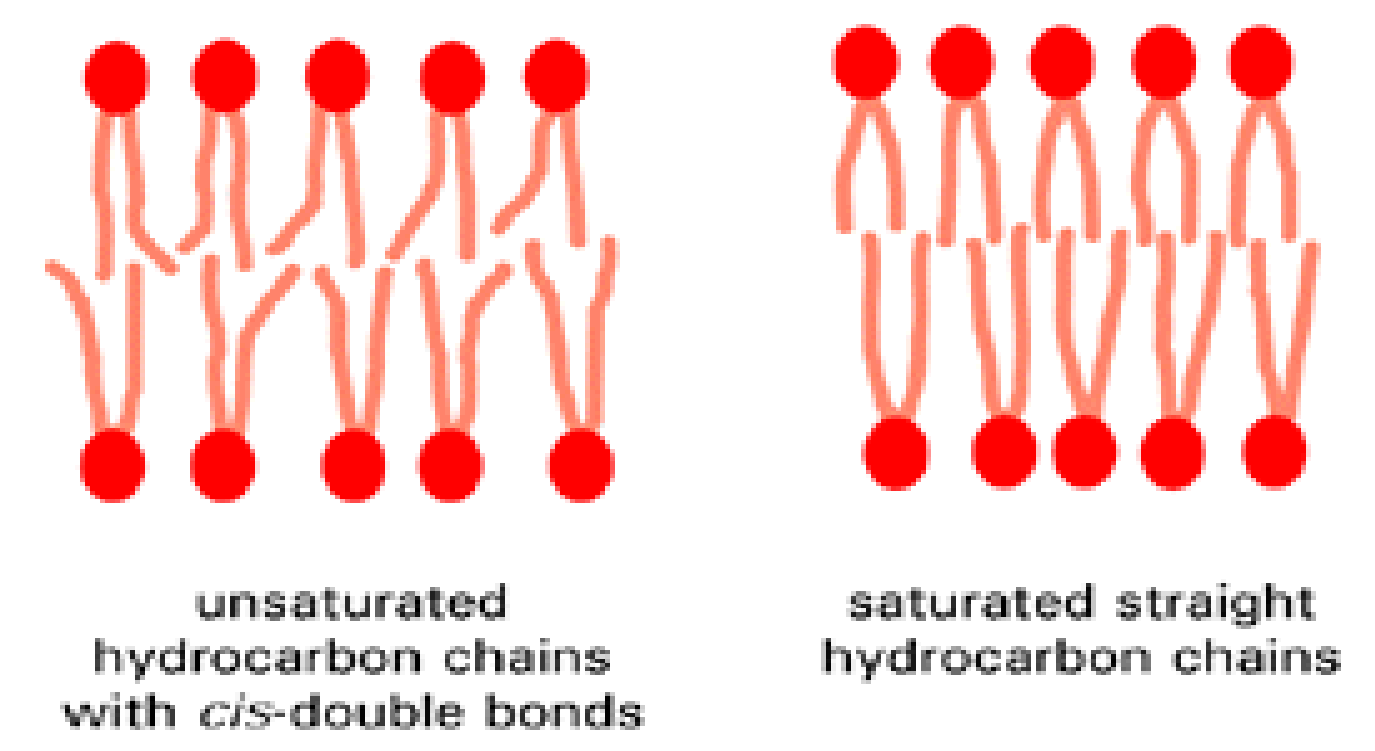
Aumento da fluidez

DSPC Tm = 60 ^{0}C
DOPC Tm = -22 ^{0}C

Aumento da fluidez sem "sacrificar" a hidrofobicidade.

Influence of *cis*-double bonds in hydrocarbon chains.

The double bonds make it more difficult to pack the chains together and therefore make the lipid bilayer more difficult to freeze.



Esterois

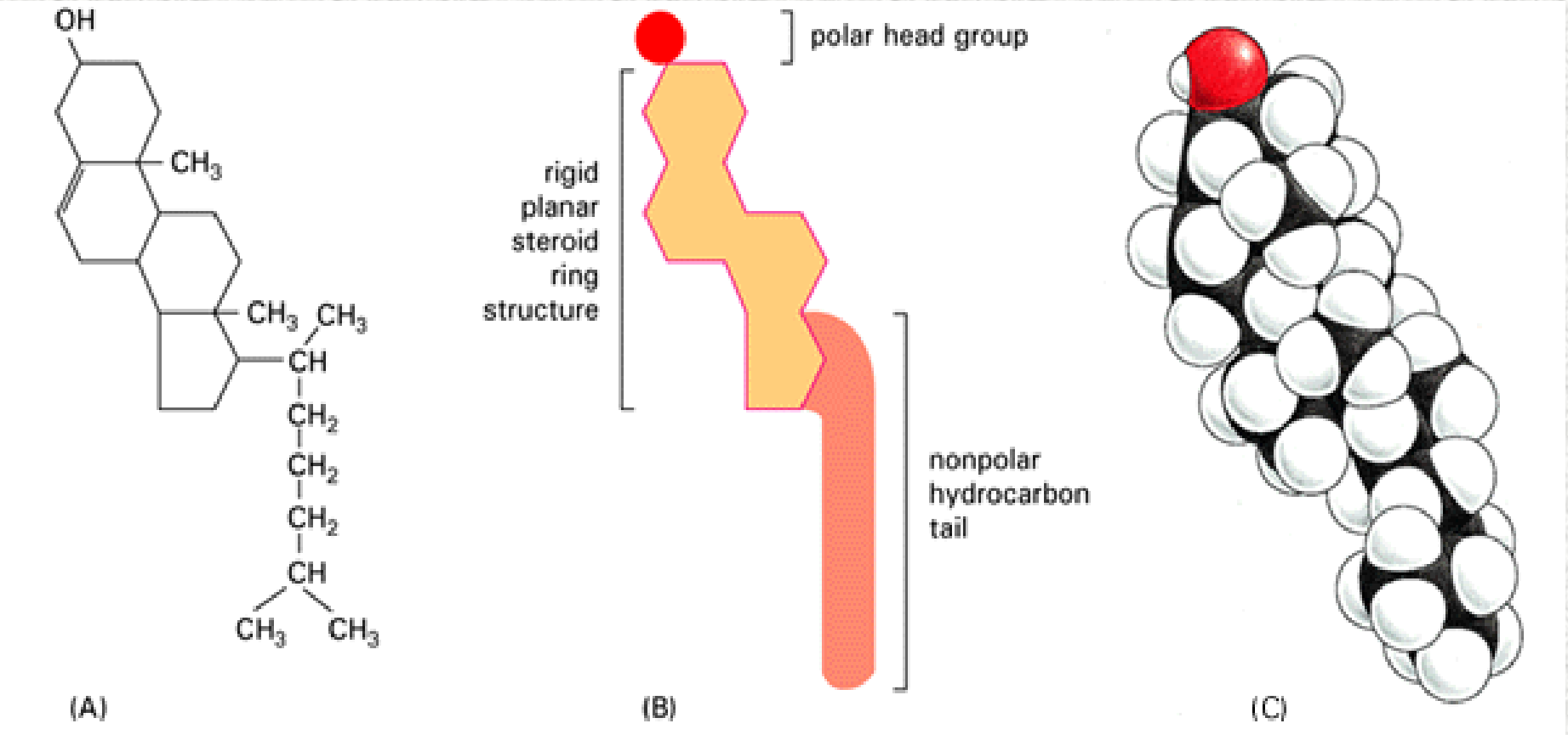
Moléculas mais compactas e apolares que os lipídios descritos anteriormente

Derivados do *ciclopentanoperidrofenantreno*, um hidrocarboneto tetracíclico saturado.

Presentes no mundo animal, vegetal, microbiano.

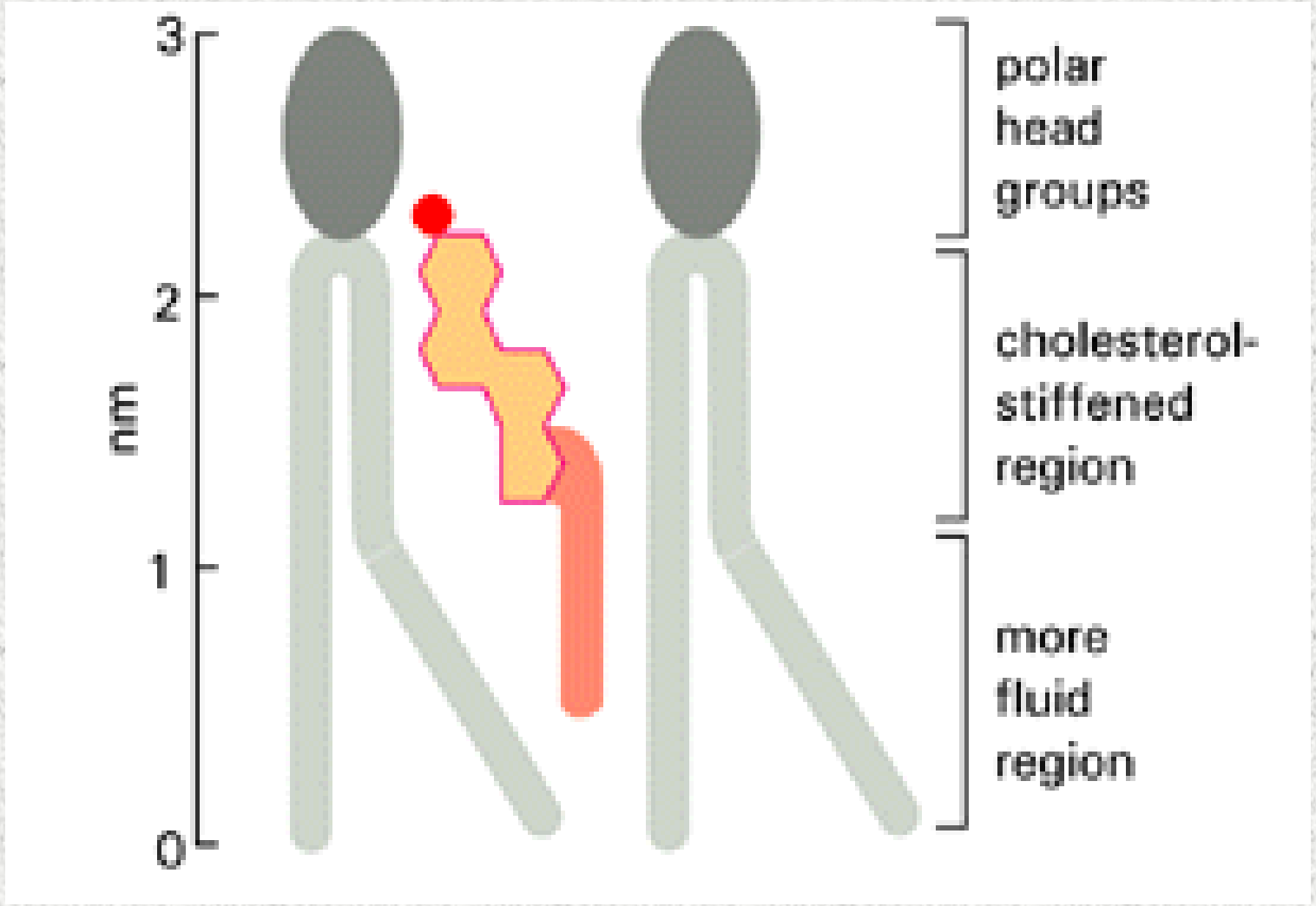
ESTEROIS TIPICOS:

Animais: Colesterol
Plantas: Fitosteroles: Estigmasterol e sitosterol
Leveduras e fungos: Micoesterois: Ergosterol
Procariotos contêm muito pouco ou nenhum colesterol



The structure of cholesterol

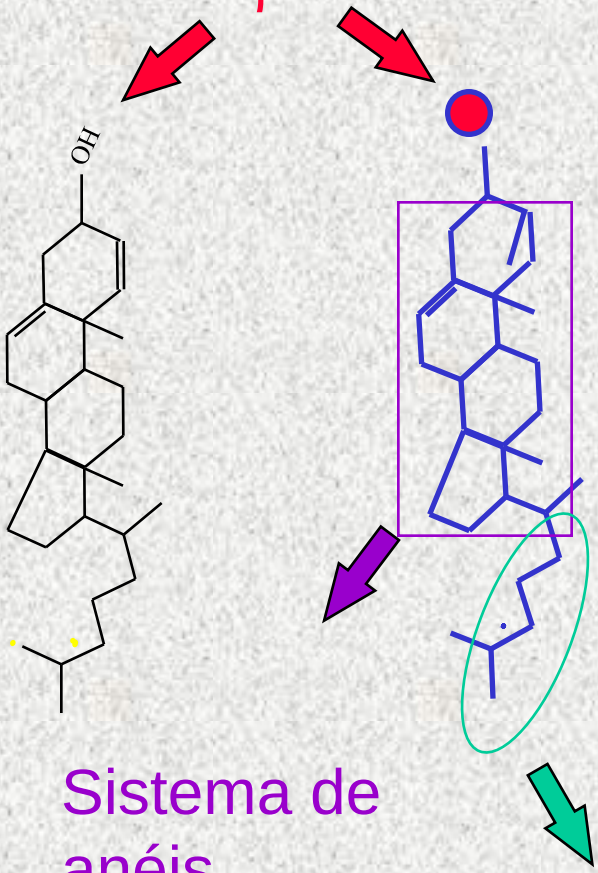
Cholesterol is represented by a formula in (A), by a schematic drawing in (B), and as a space-filling model in (C).



Cholesterol in a lipid bilayer. Schematic drawing of a cholesterol molecule interacting with two phospholipid molecules in one leaflet of a lipid bilayer.

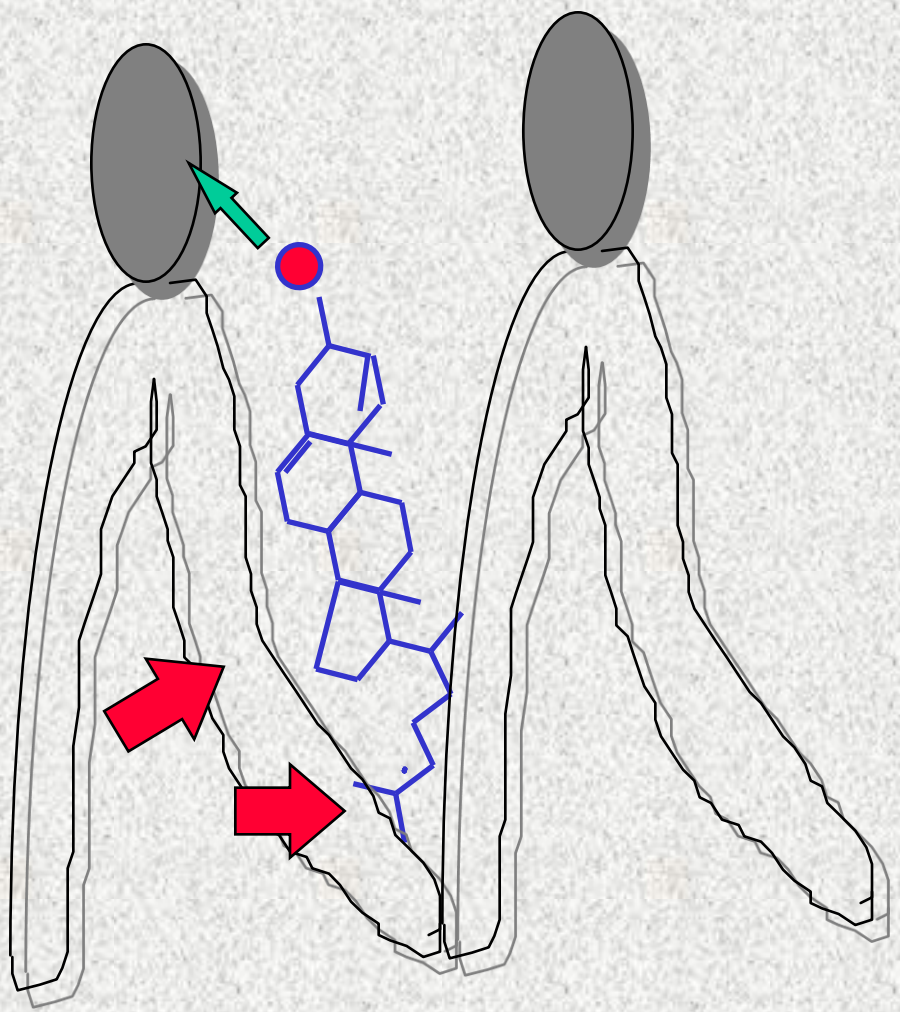
Cabeça polar

β



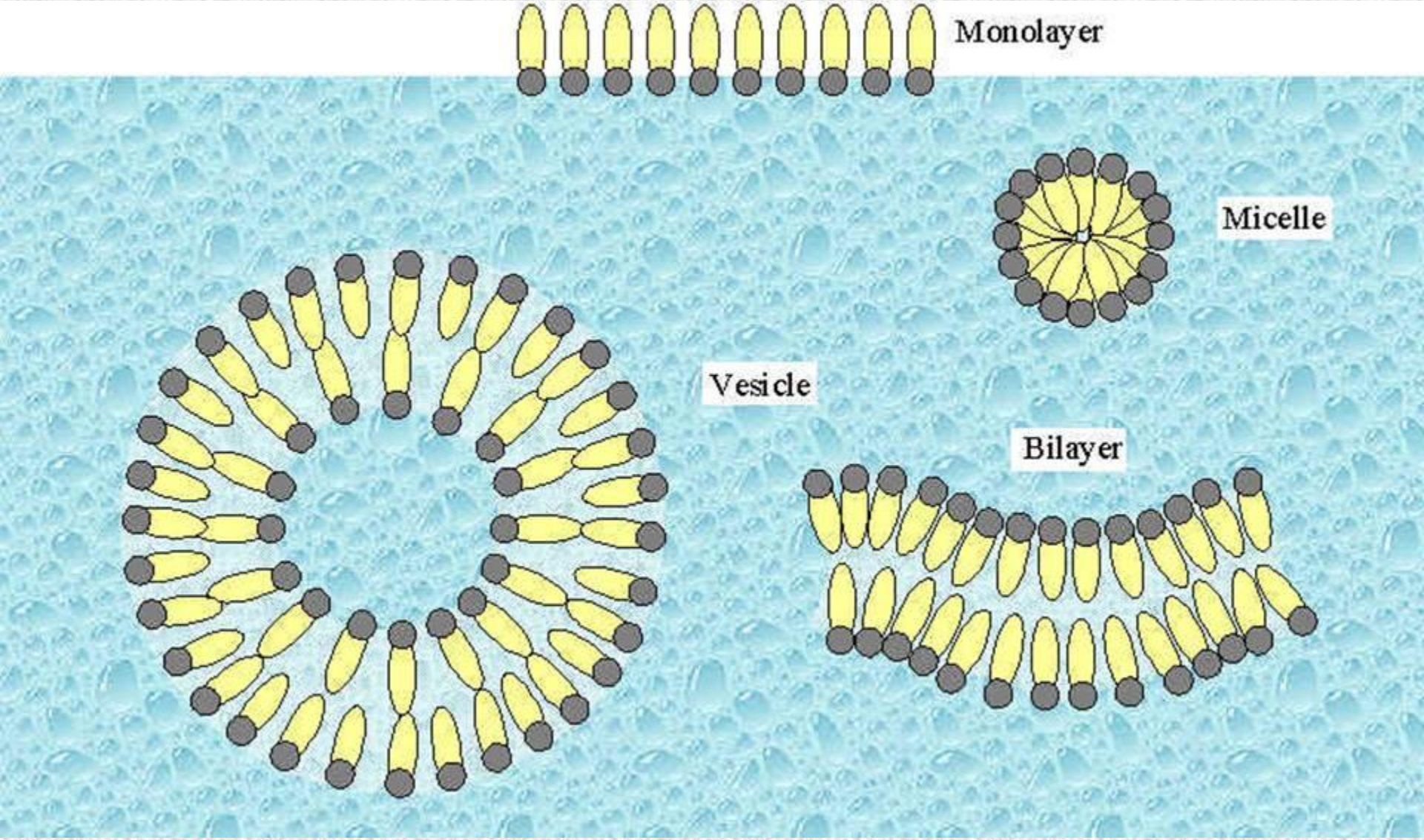
Sistema de anéis

Cauda não polar= livre rotação

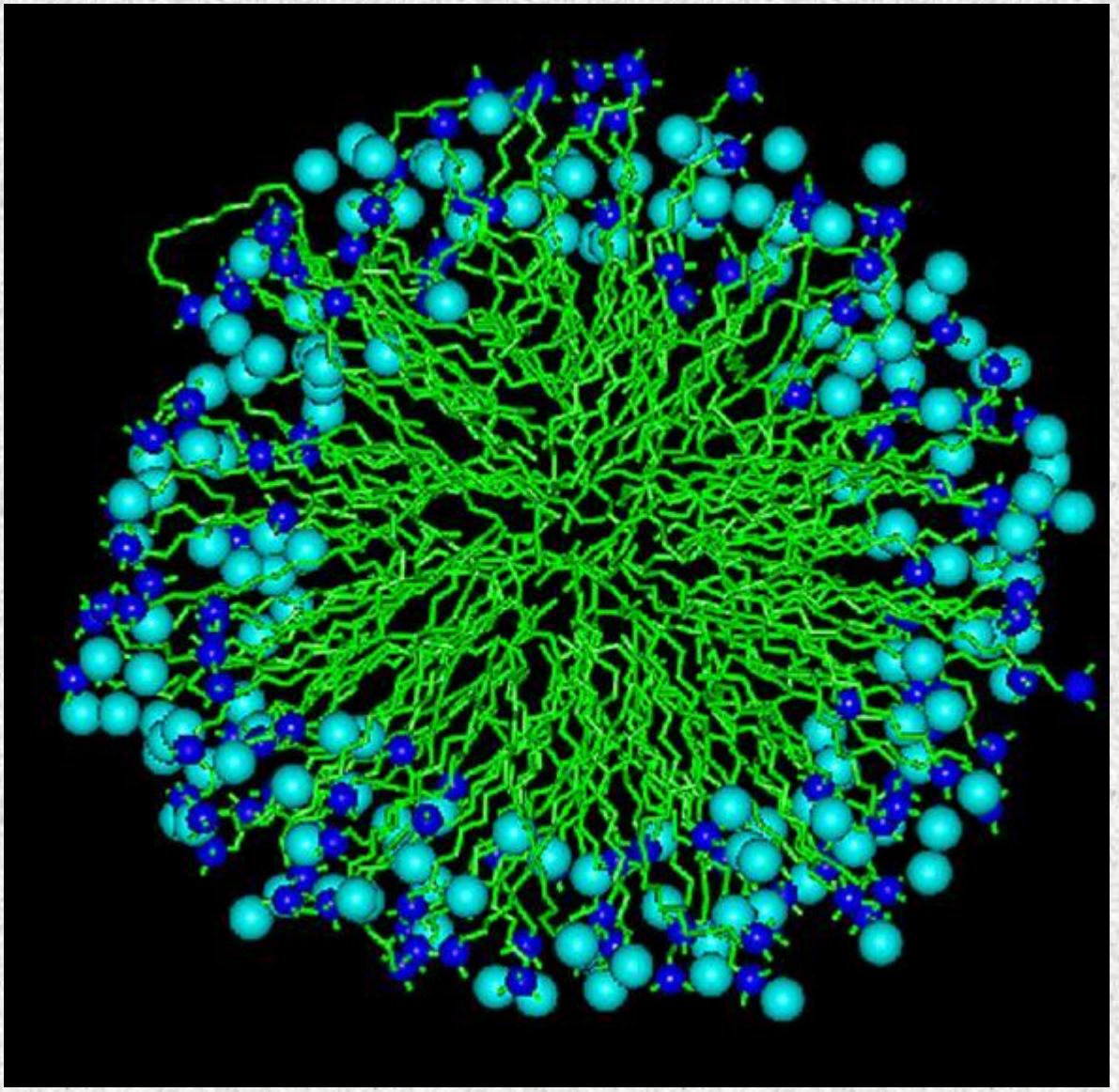


Localização do colesterol na membrana

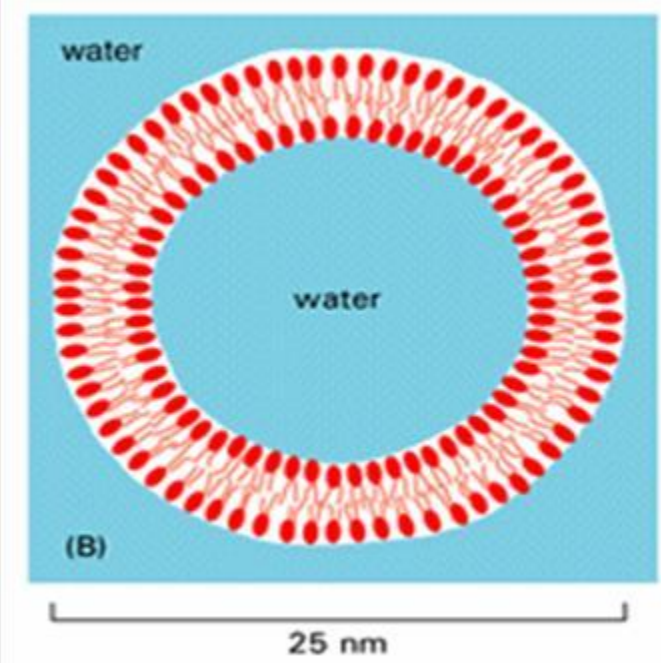
Algunos agregados de los lipidos

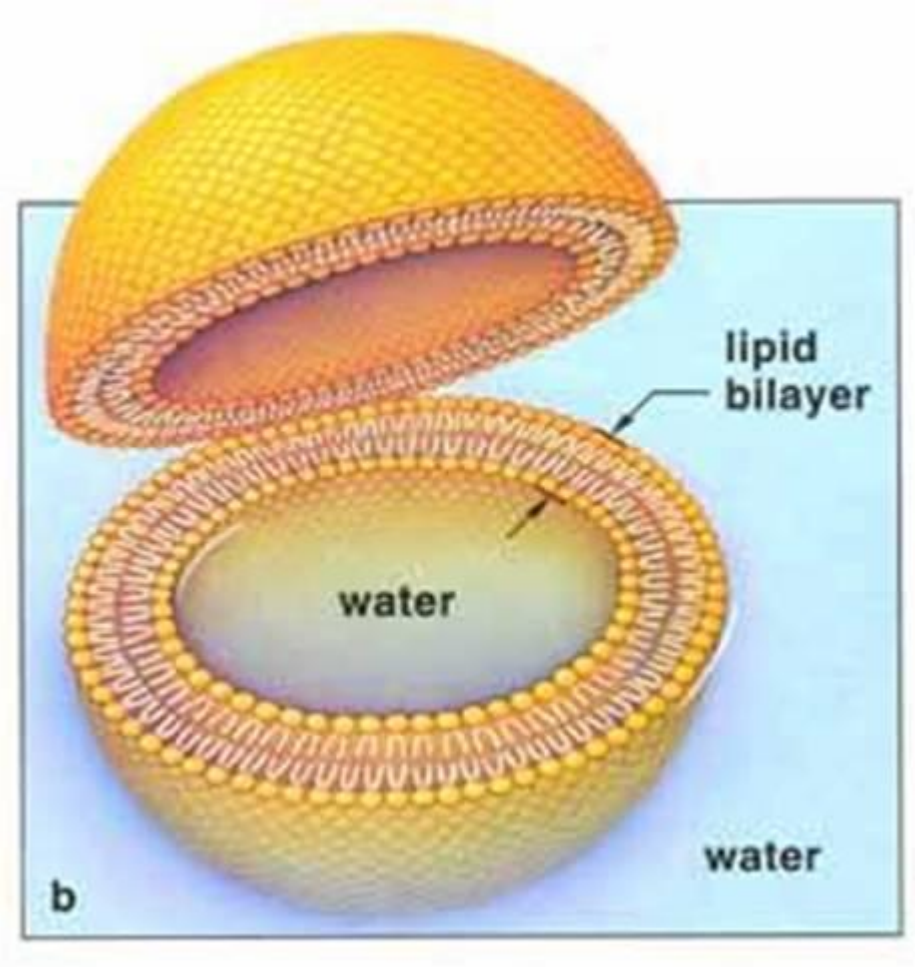


Micela clásica

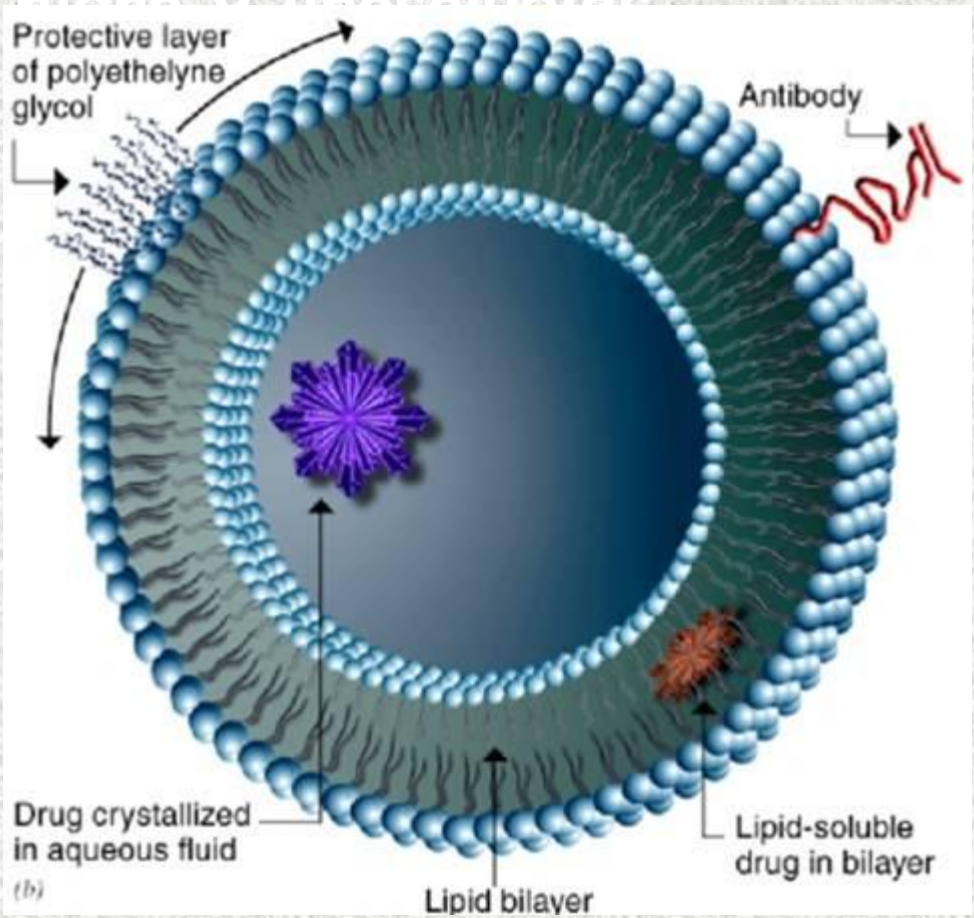


Liposomas (arreglo laminar)

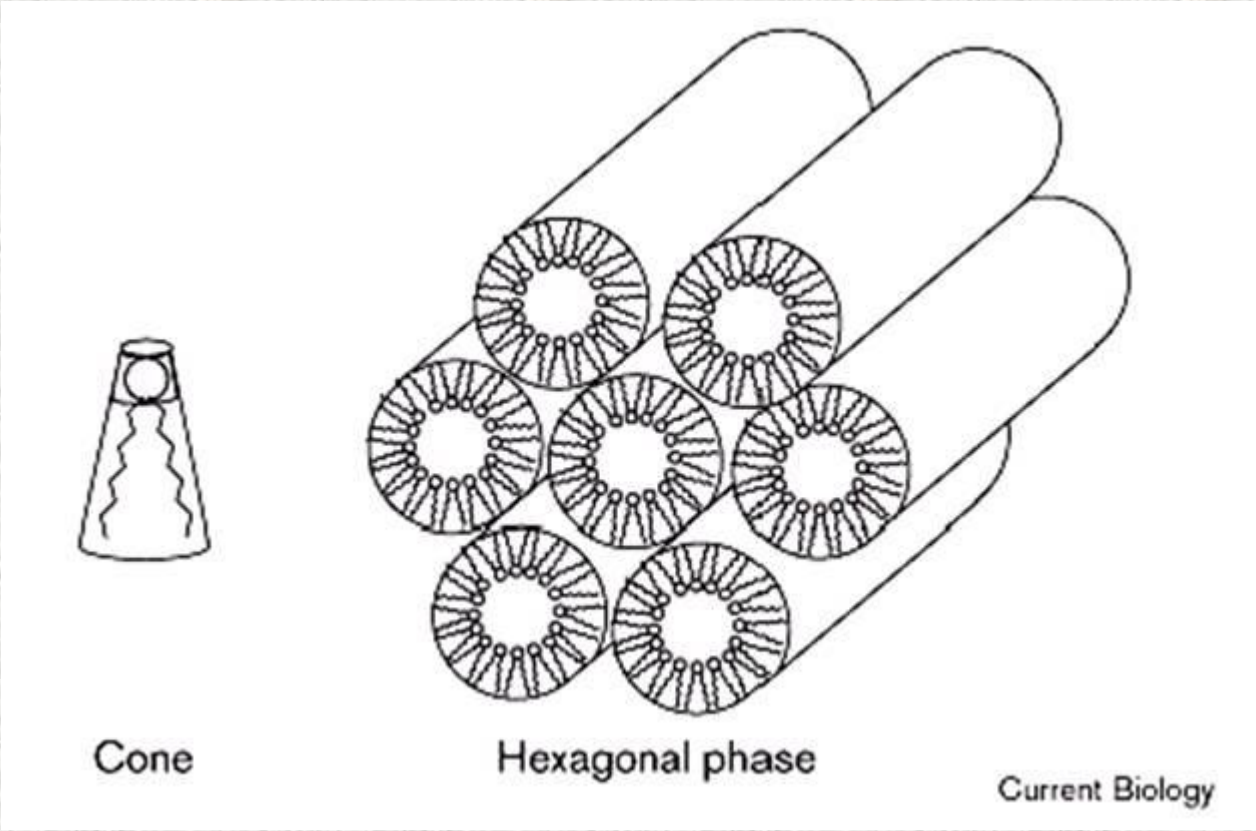




Los liposomas se utilizan como sistemas modelo en los estudios de membrana. Además, son utilizados como vehículos para transportar sustancias hidro y liposolubles.

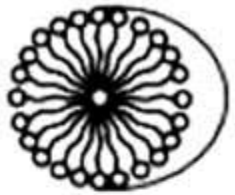
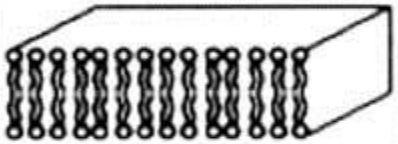
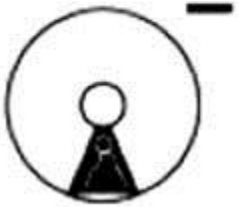


Fase hexagonal (H_{II})



Lee (2000) Membrane lipids: It's only a phase. *Curr. Biol.* 10: R377-R380

Hipótesis geométrica del Polimorfismo lipídico

Lipid Species	Molecular Shape	Lipid Phase
Lysophospholipids Detergents	Type-I  Inverted Cone / Wedge	 Micellar
Phosphatidylcholine Sphingomyelin Phosphatidylserine Phosphatidylinositol Phosphatidylglycerol Phosphatidic acid (PA) Cardiolipin (CI) Digalactosyldiglyceride	Bilayer-preferring  Cylinder	 Bilayer
Phosphatidylethanolamine CI + Ca^{2+} PA + low pH + Ca^{2+}/ Mg^{2+} Monogalactosyldiglyceride Unsaturated fatty acids Ceramide Diacylglycerol Cholesterol	Type-II  Cone	 Hexagonal-II

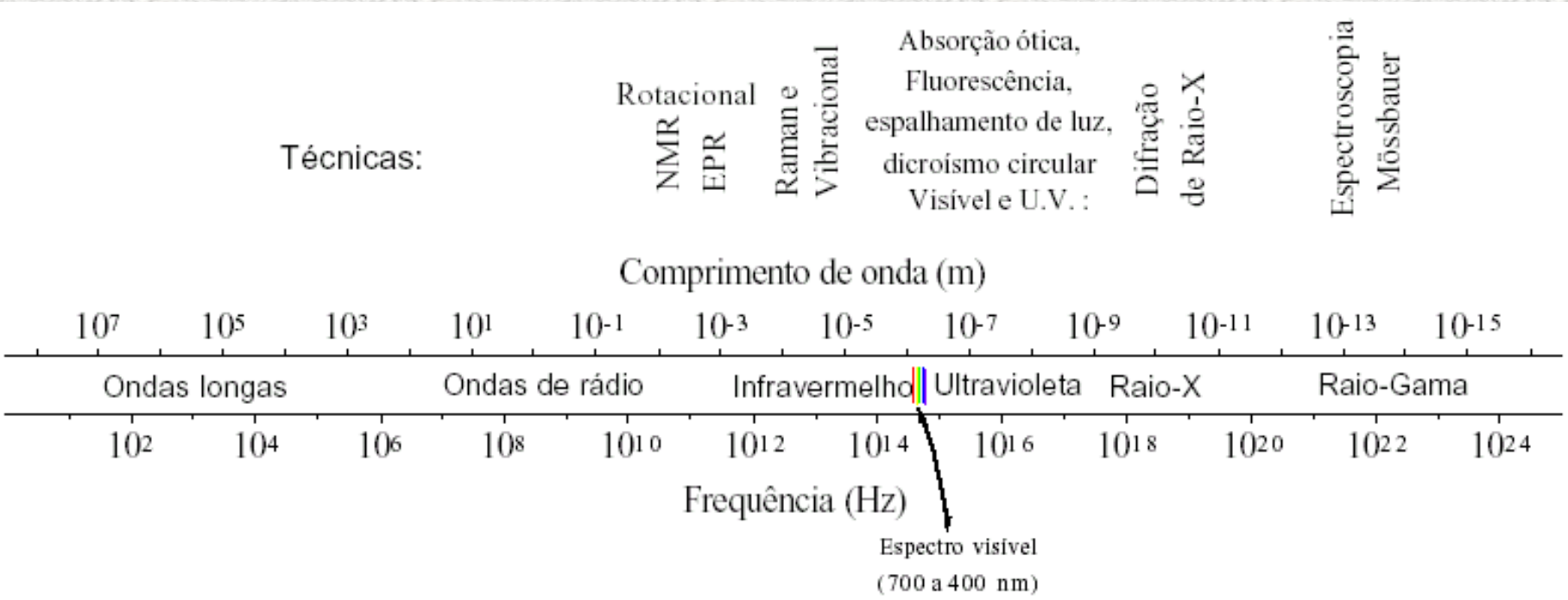
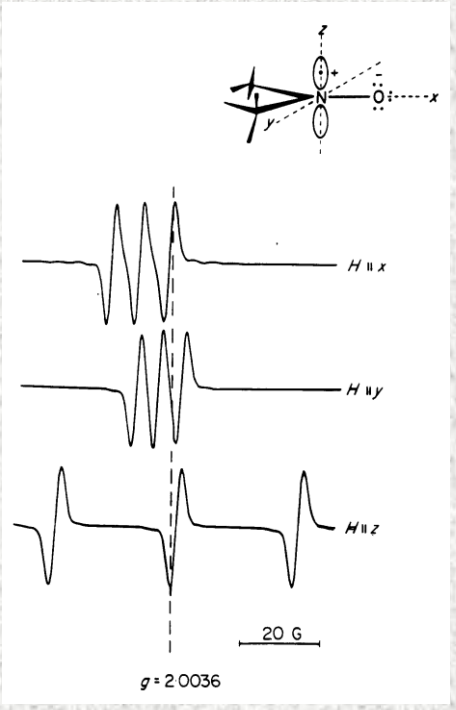


Figura 1. O espectro eletomagnético e freqüências típicas utilizadas pelas técnicas espectroscópicas mais utilizadas para estudas sistemas biológicos.

Electron Paramagnetic Resonance

- **Interaction between magnetic field and electron spin magnetic moment**
- **Spectra are sensitive to orientation and motion**
- **Microwave radiation**
- **Frequency corresponds to that of molecular motion**



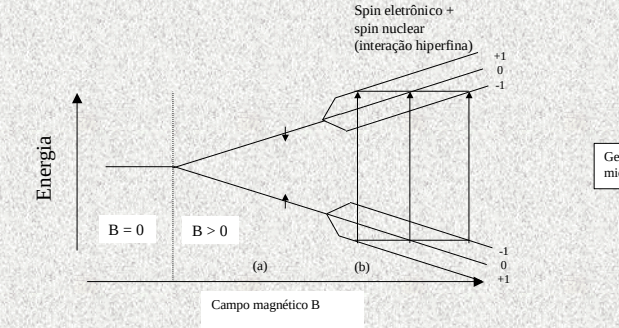


Figura 1 - Níveis de energia do elétron desemparelhado do grupo nitróxido (N-O) num campo magnético: (a) sem interação e (b) com interação com o spin do N (estrutura hiperfina).

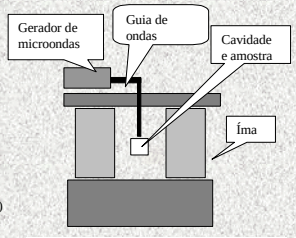
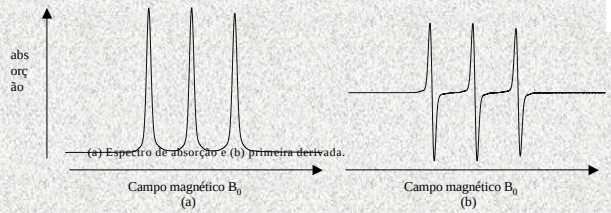
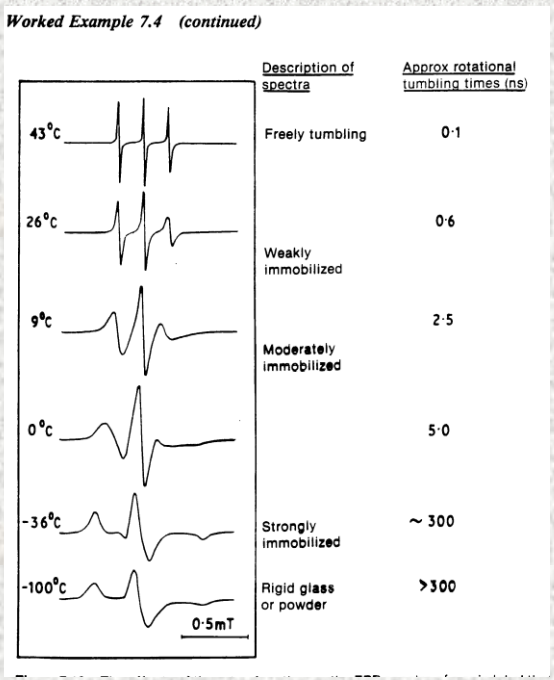


Figura 10. Diagrama de um espectrômetro de EPR. As microondas (MW) percorrem a ponte e atingem a cavidade onde está a amostra.

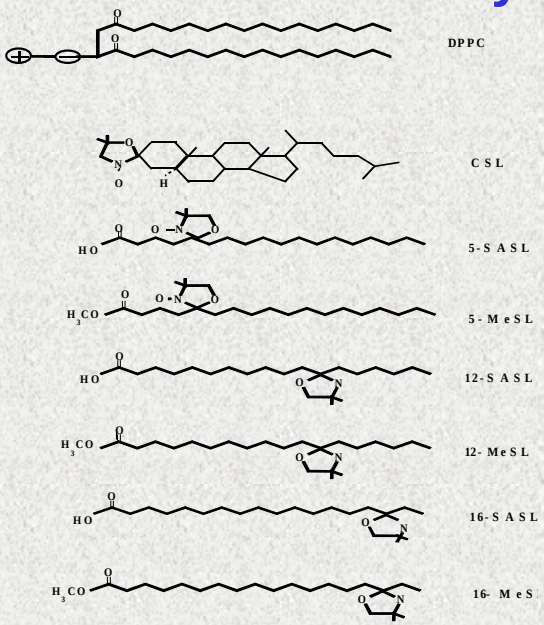


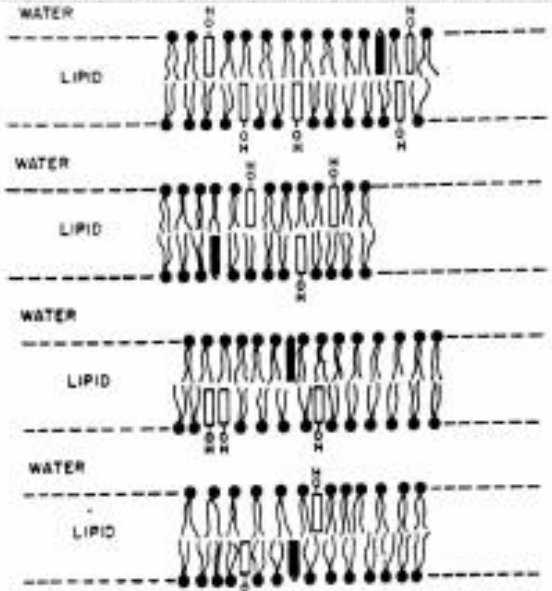
(a) Espectro de absorção e (b) primeira derivada.

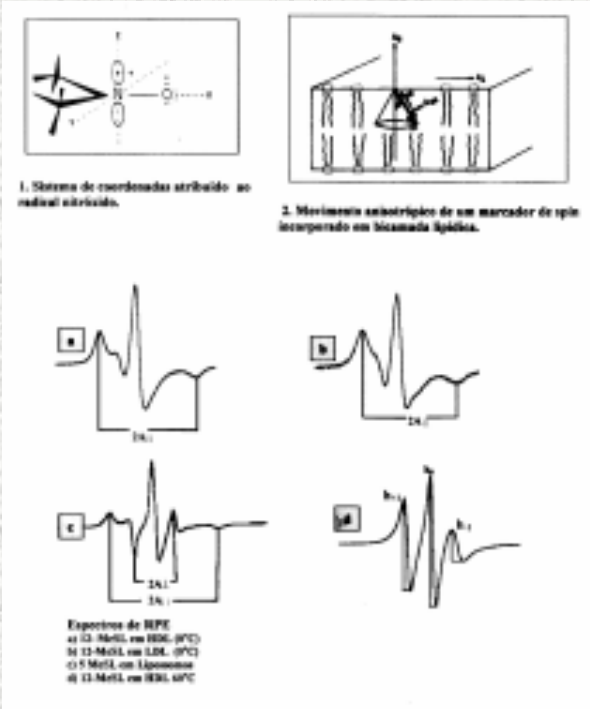


Lipid spin labels

amphipathic molecules that can be intercalated in the bilayer



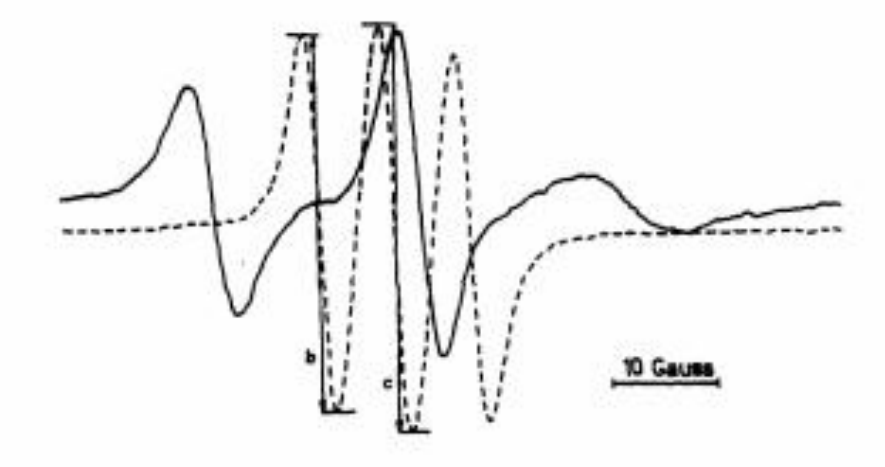




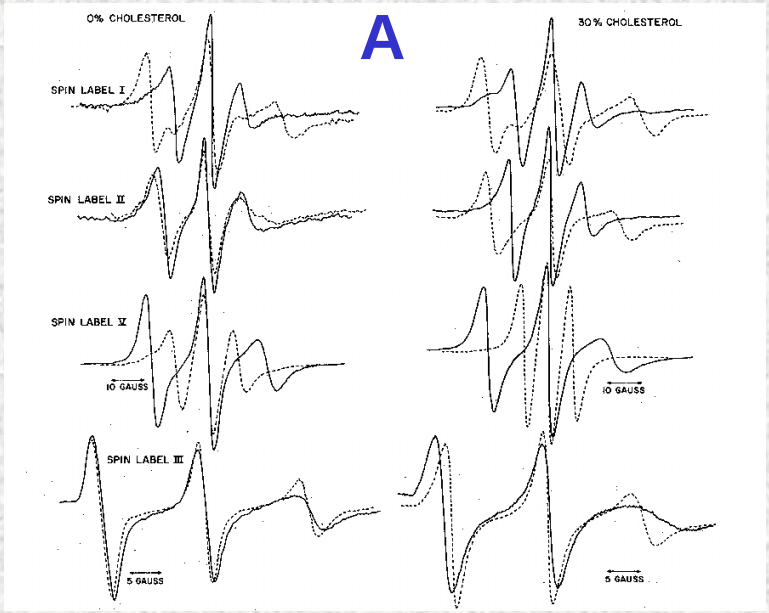
Magnetic field

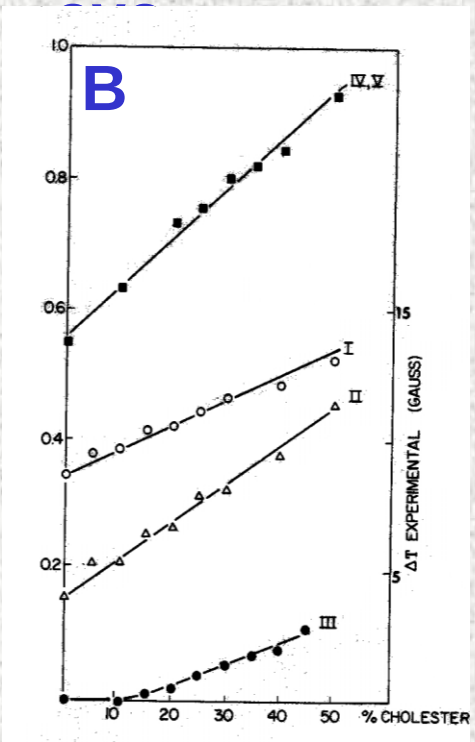
—— // to bilayer surface

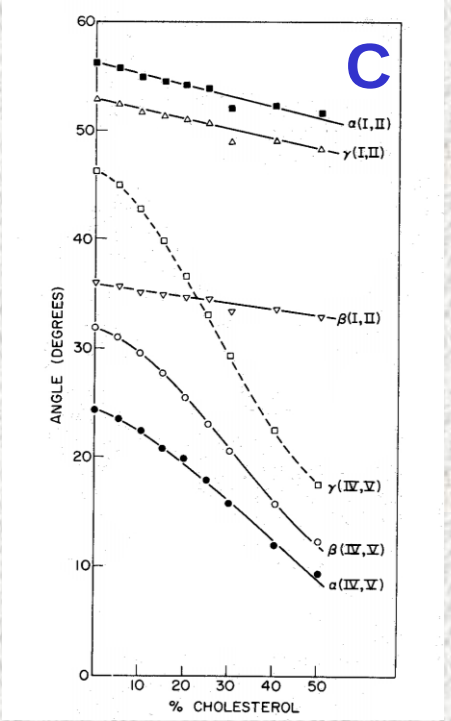
----- ⊥ to bilayer surface



Effect of cholesterol on egg phosphatidyl choline (PC) bilayers







A- EPR spectra of planar bilayers

Magnetic field —— // and ----- ⊥ to the bilayer surface

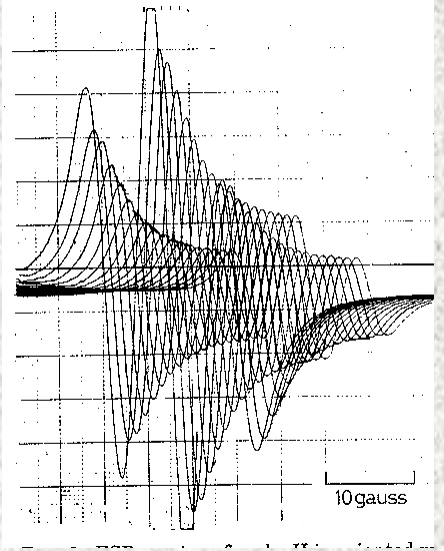
B- Theorder parameter, S, increases with increasing cholesterol

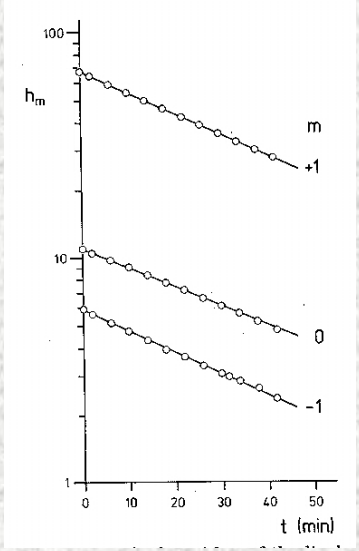
C- The angle that measures the amplitude of motion decreases

Calculations showed that the population of *trans* rotamers increases, i.e., the lipid acyl chains become more extended

Schreier-Muccillo et al, Chem. Phys. Lipids 10: 11-27 (1973)

Study of bilayer permeability by means of the kinetics of reduction of the N-O group by ascorbate





- The deeper the location of the N-O group, the slower the kinetics.
- Cholesterol increases the molecular packing, therefore the kinetics are slower.
- The activation energy increases.
- The reaction rate as a function of polar headgroup charge increases in the order: negative < zero < positive

TABLE I

HALF-TIMES ($t_{1/2}$) AT 19°C FOR THE REACTION BETWEEN ASCORBATE (10^{-2} M, pH 6.2–6.8) AND SPIN PROBES IN MULTIBILAYERS OF VARIOUS COMPOSITIONS

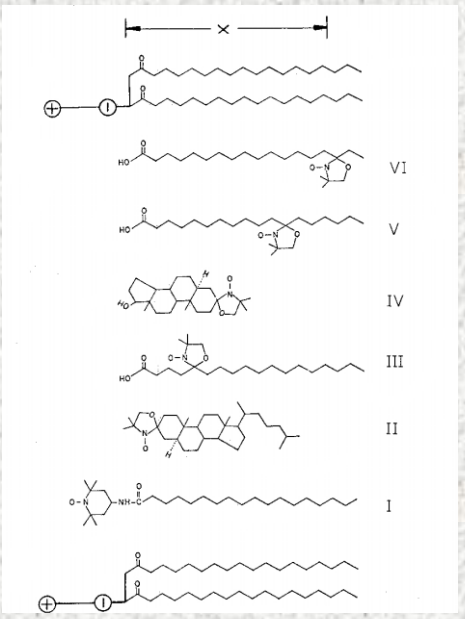
Spin probe	$t_{1/2}$ (min)			
	PC	PC (65%) + chol (35%)	PSer (30%) + PC (35%) + chol (35%)	HDTMA (15%) + PC (30%) + chol (35%)
II	—	3.2	12	5.9[a]
III	9.5	11.5	51	—
IV	16	29	85	16
V	30	60	136	—
VI	32	—	166	34

[a] [Ascorbate] = 10^{-3} M

TABLE II

ACTIVATION ENERGIES (E_a) FOR THE REACTION BETWEEN ASCORBATE (10^{-2} M, pH 6.2–6.8) AND SPIN PROBES IN MULTIBILAYERS OF VARIOUS COMPOSITIONS

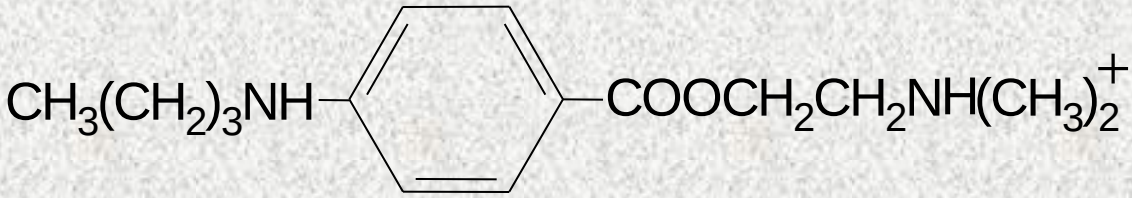
Spin probe	E_a (kcal/mol)			
	PC	PC (65%) + chol (35%)	PSer (30%) + PC (35%) + chol (35%)	HDTMA (15%) + PC (50%) + chol (35%)
II	—	14	15	15
III	6	13	—	—
IV	6	16	14	16
V	6	15	—	—



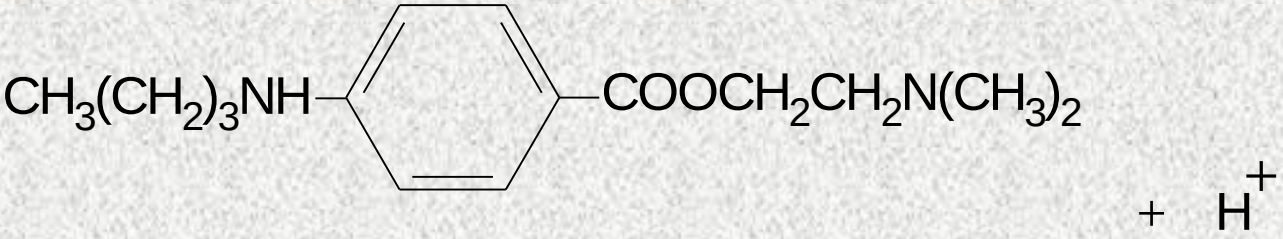
Schreier-Muccillo et al, Arch. Biochem. Biophys. 1976

MEMBRANE-LOCAL ANESTHETIC INTERACTION

The local anesthetic tetracaine (TTC)

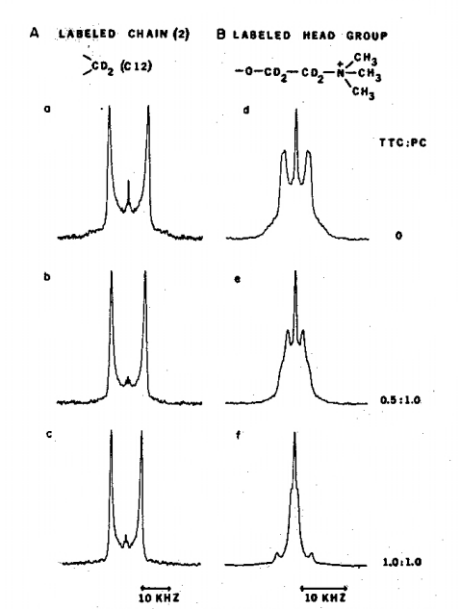


Low pH



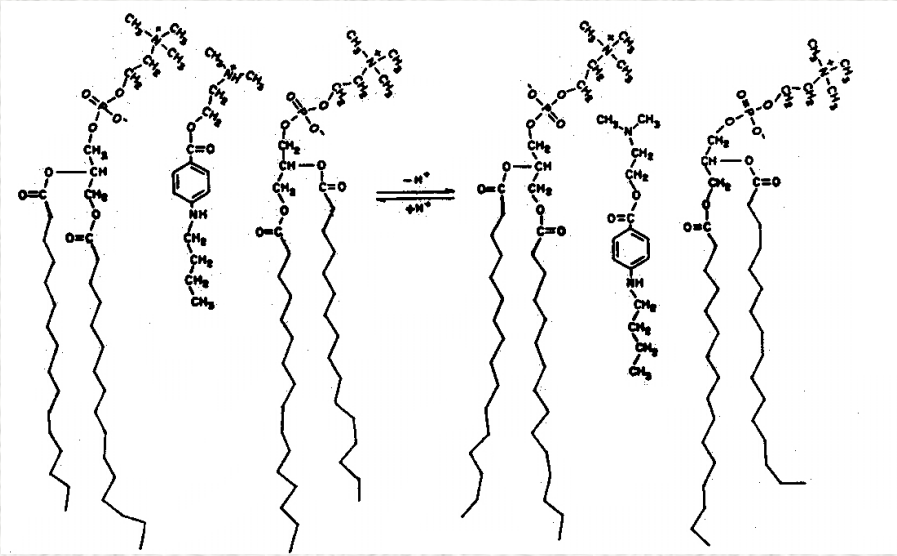
High pH

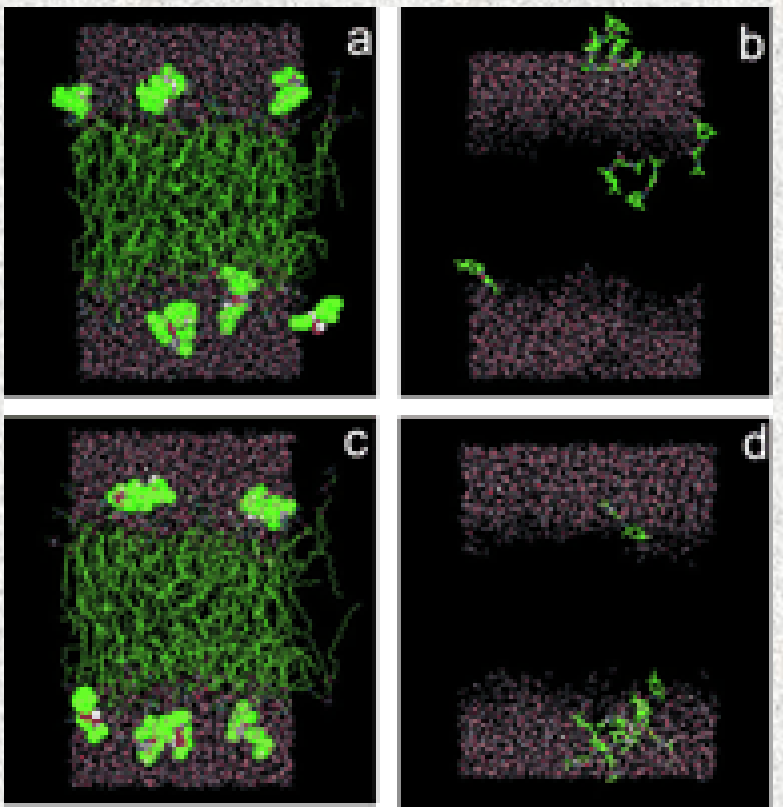
DEUTERIUM NUCLEAR MAGNETIC RESONANCE
MEMBRANE - LOCAL ANESTHETIC INTERACTION



- Work at a pH where only one form is present, either the charged form or the neutral form.
- Know the partition coefficient to evaluate how much of the total amount added is actually bound.

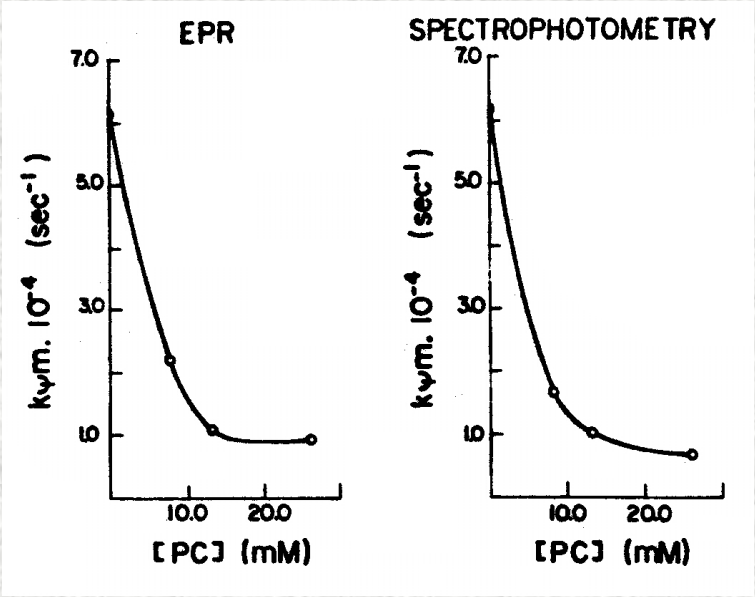
DETERMINATION OF ANESTHETIC LOCATION IN THE MEMBRANE

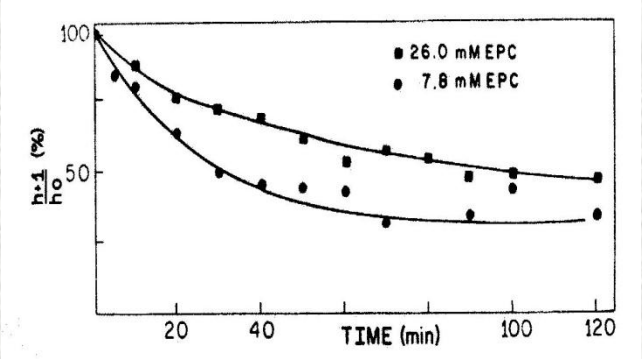




Boulanger et al, Biochemistry 1981

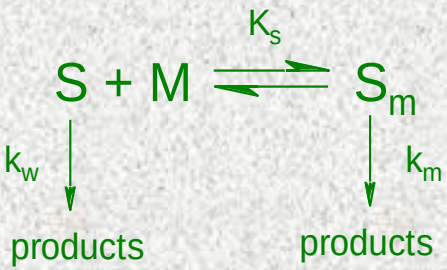
EFFECT OF MEMBRANES ON THE KINETICS OF REACTIONS THAT OCCUR IN THE AQUEOUS PHASE





The kinetics of spectral changes of a probe in the membrane allow monitoring the kinetics of hydrolysis of a solute that undergoes partitioning between water and membrane.

Note: hydrolysis occurs in the aqueous phase

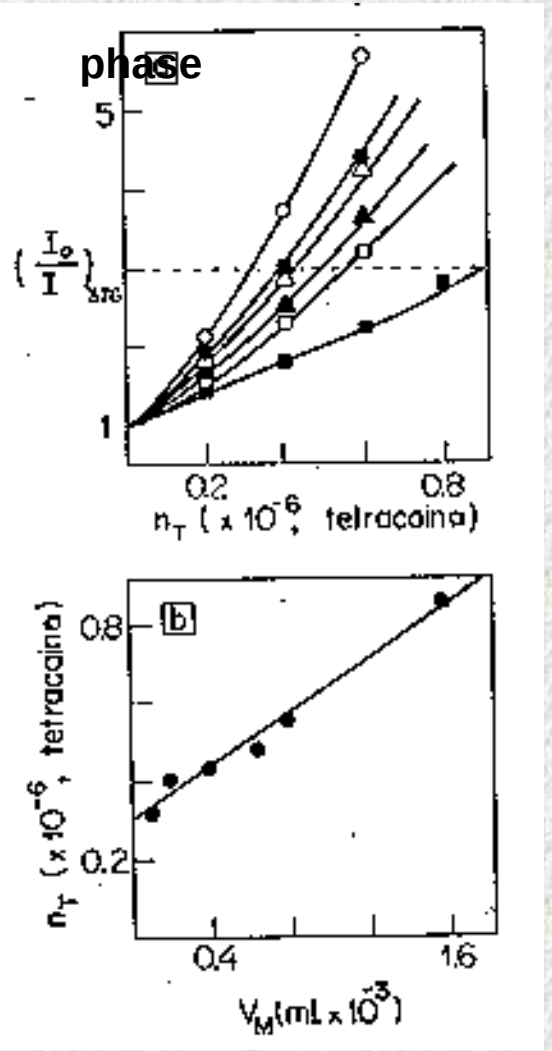


$$k_\psi = \frac{k_m [M] K_s + k_w}{1 + [M] K_s}$$

Bianconi et al, Biochem. Biophys. Res. Commun. 1988

METHODS FOR THE DETERMINATION OF MEMBRANE-WATER PARTITION COEFFICIENT – P

Based on the effect of solute on membrane structure



P= [solute] in the membrane / [solute] in the aqueous phase

1-vary solute concentration at fixed membrane concentrations

A line parallel to the abcissa

indicates that the effect is the same

i.e., same solute:lipid ratio

IN THE MEMBRANE

Lissi et al BBA, 1021 (1990)

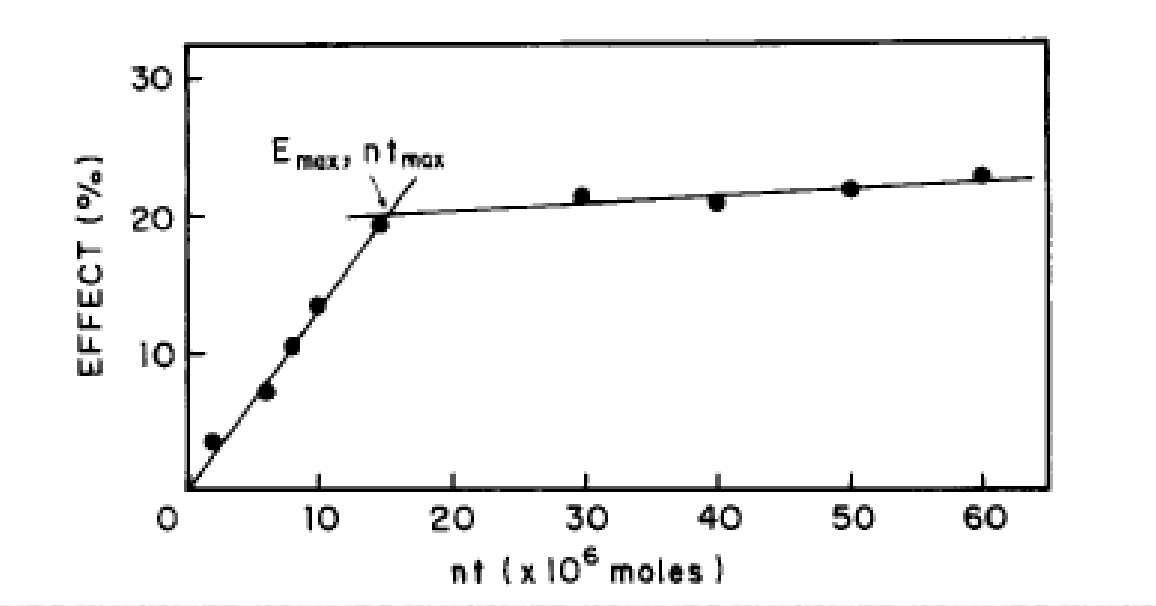
2. Based on solute effect on membrane properties and knowledge of solute's aqueous solubility (Sw)

One curve is obtained for one fixed membrane concentration.

The breakpoint corresponds to WATER SATURATION.

No more solute partitions into the membrane

$SOLUTE_{solid} \leftrightarrow SOLUTE_{in\ solution} \leftrightarrow Solute_{in\ the\ membrane}$



de Paula and Schreier, BBA 1240 (1995)

Peptide-Membrane Interaction

Questions we want to answer

1- does the peptide bind?

2- does it undergo conformational changes?

3- what are the intra-molecular interactions involved in the conformational change ?
e.g. hydrogen bonding - modulated by local polarity

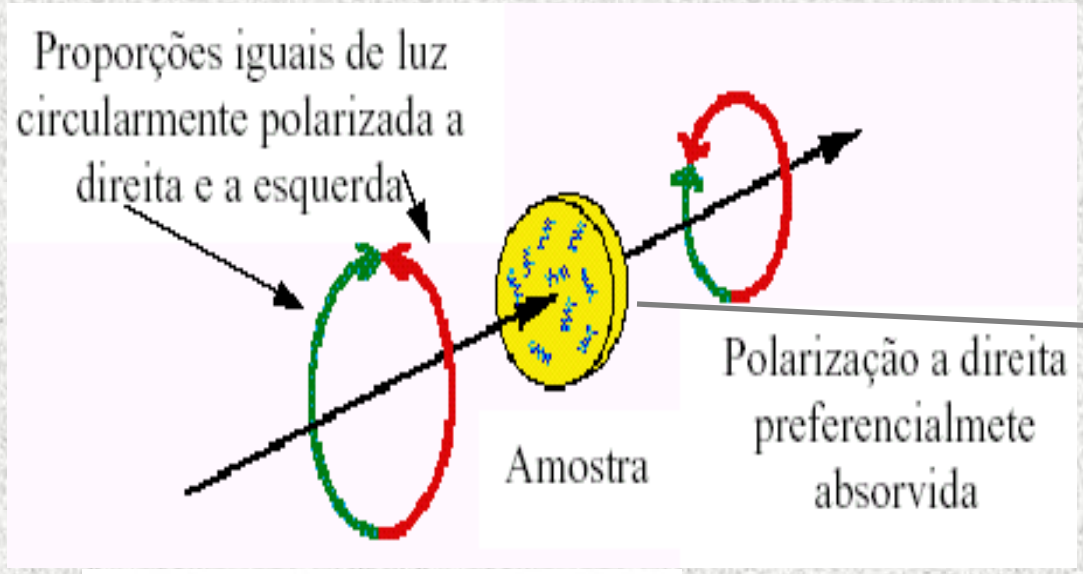
4- what are the effects on peptide physico-chemical properties?
e.g. pK changes - modulated by both local polarity and peptide conformation

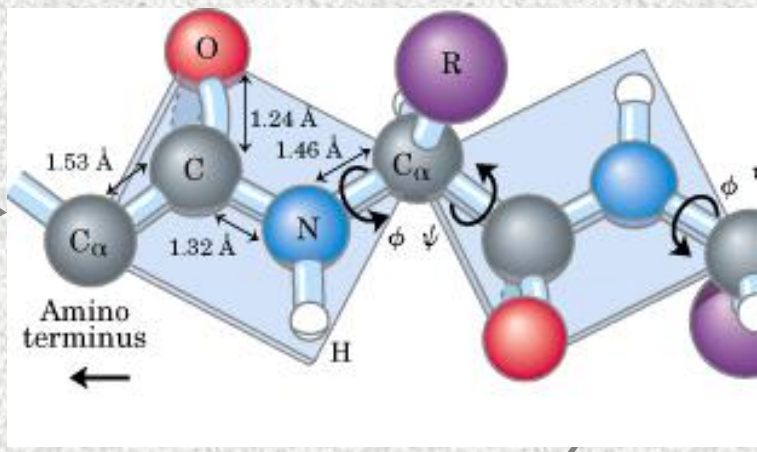
5- what are the interactions modulating peptide-membrane interaction?
e.g. electrostatic, hydrophobic, van der Waals, hydrogen bonding

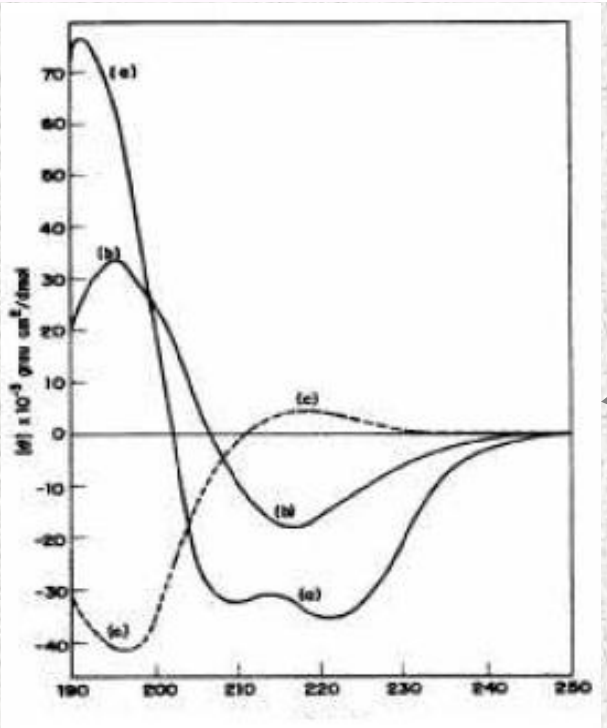
6- peptide location (membrane depth of penetration)?
on the surface, transmembrane

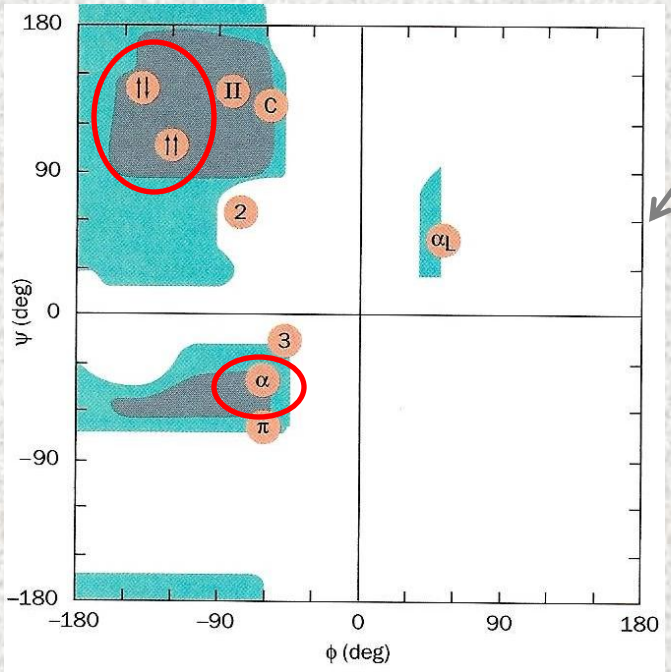
7- what is the effect of peptide binding on lipid molecular organization?

Circular Dichroism of Proteins









Fluorescence

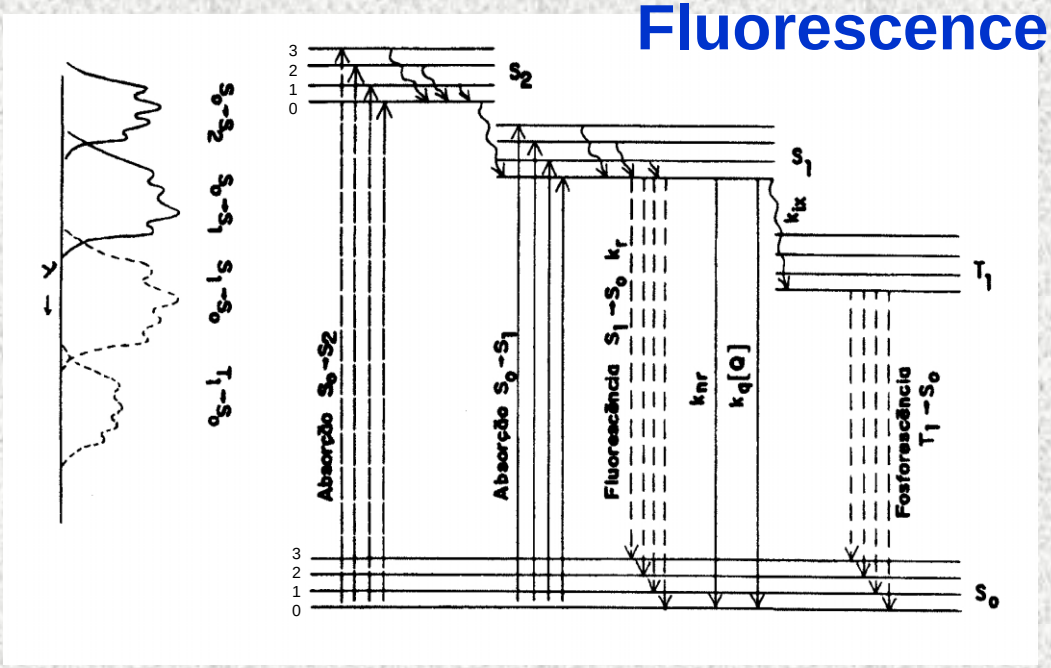
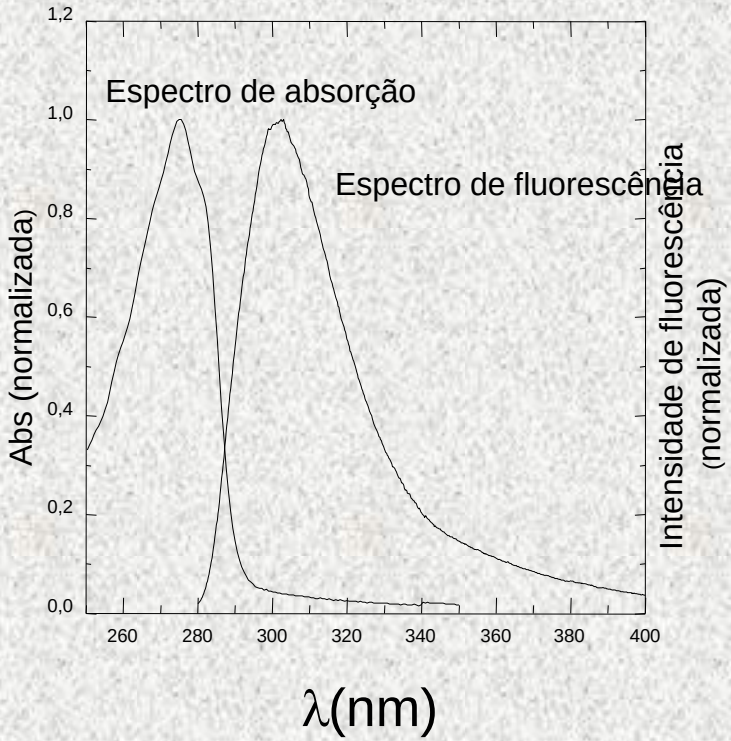


Diagrama de Jablonski. Os estados eletrônicos fundamental, primeiro e segundo estados excitados são representados por S_0, S_1 e S_2, respectivamente. Em cada nível de energia eletrônico existe uma série de níveis de energia vibracionais. Os espectros hipotéticos de absorção e emissão de fluorescência e fosforescência, correspondentes às transições observadas no diagrama, encontram-se à esquerda.



Espectros de absorção e de emissão de fluorescência para a tirosina.

Em seguida à absorção de luz, $S_0 + h\nu_a \rightarrow S_n$, diversos processos ocorrem:

Transições radioativas

$S_1 \rightarrow S_0 + h\nu_f$ ($\sim 10^{-8}$ a 10^{-9} s)

$T_1 \rightarrow S_0 + h\nu_p$ (ms a s)

Relaxações não radioativas

$S_n \rightarrow S_1$ ($\sim 10^{-12}$ s)

$S_1 \rightarrow T_1$

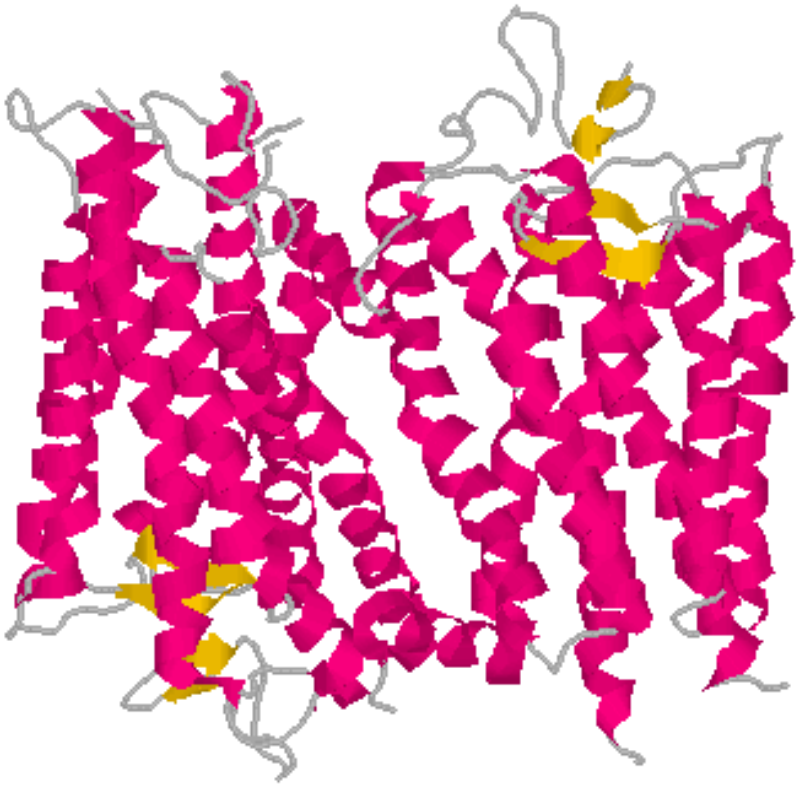
$S_1 \rightarrow S_0$

$T_1 \rightarrow S_0$

Super-family of G protein coupled receptors (GPCR)
Represent a large proportion of membrane-bound receptors

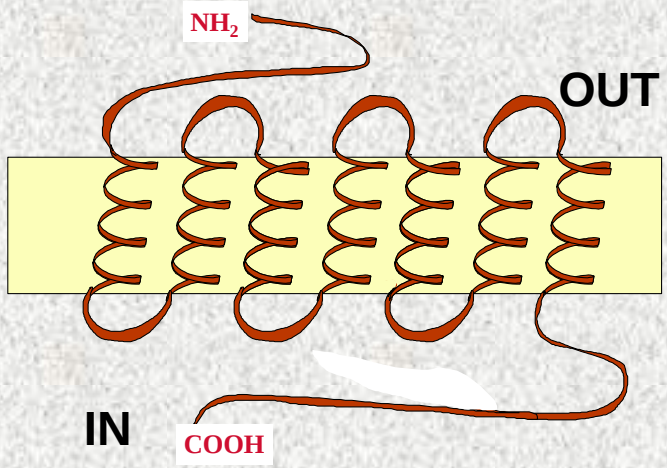
GPCR are targets of a large number of drugs

However, due to the difficulties in using high resolution techniques X-ray crystallography, NMR for membrane proteins, the only GPCR whose structures have been resolved are rhodopsin and the β-adrenergic receptor (2007)

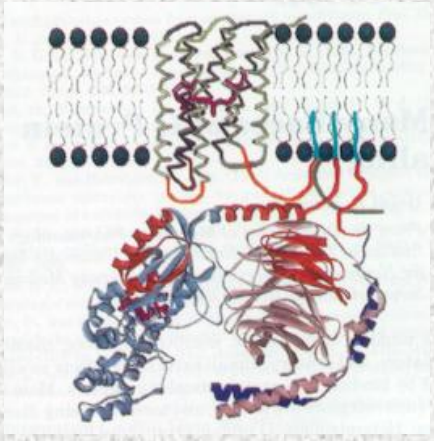


Palczewski et al (2000) Science, 289, 739

By analogy with rhodopsin ,
The accepted model for GPCR consists of seven transmembrane helices (TM) connected by three extracellular (EL) and three intracellular (IL) loops
The N- and C-termini are outside and Inside the cell, respectively



Mechanism of signal transduction



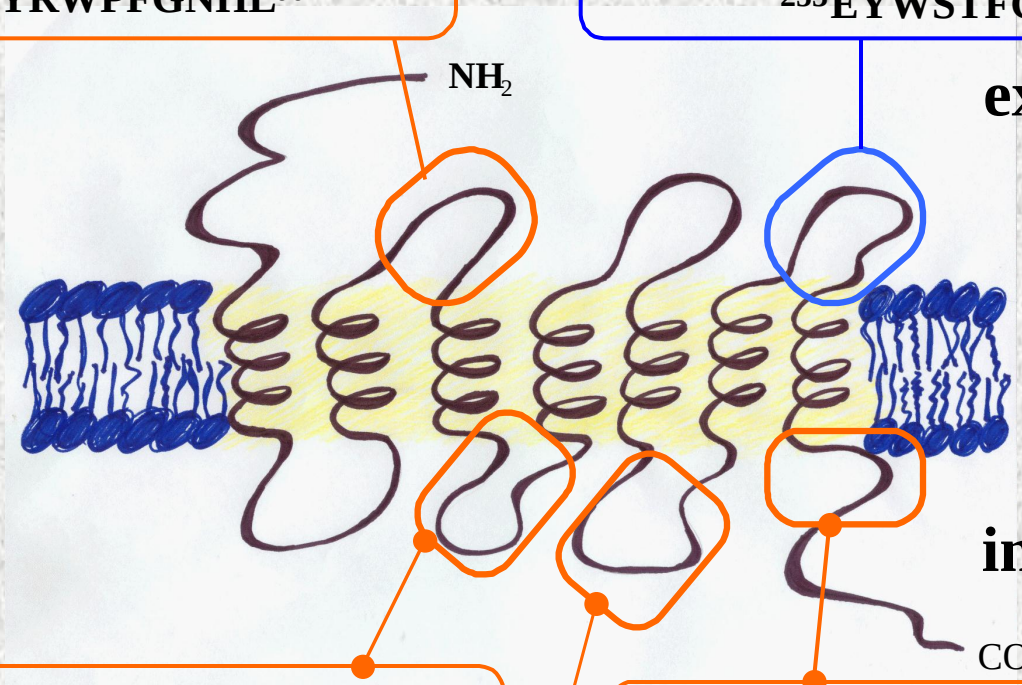
Hamm, J Biol Chem 1998 273: 89

Ligand binding induces conformacional changes in the EL that propagate through the TM, causing a rearrangement of the helices relative orientation
Such reorganization induces conformational changes in the IL, activating the G protein

*Due to the difficulties to obtain conformational information about the whole protein, **fragments** have been studied*
In several cases, the fragments present conformations similar to those predicted or found in the whole protein

Use of spectroscopic techniques
CD, fluorescence, EPR, NMR
to study the conformation and dynamics of peptides
whose sequence is present in GPCR
EL, IL, or TM, in the absence and presence of
model membranes - micelles and bilayers

Laboratory of Structural



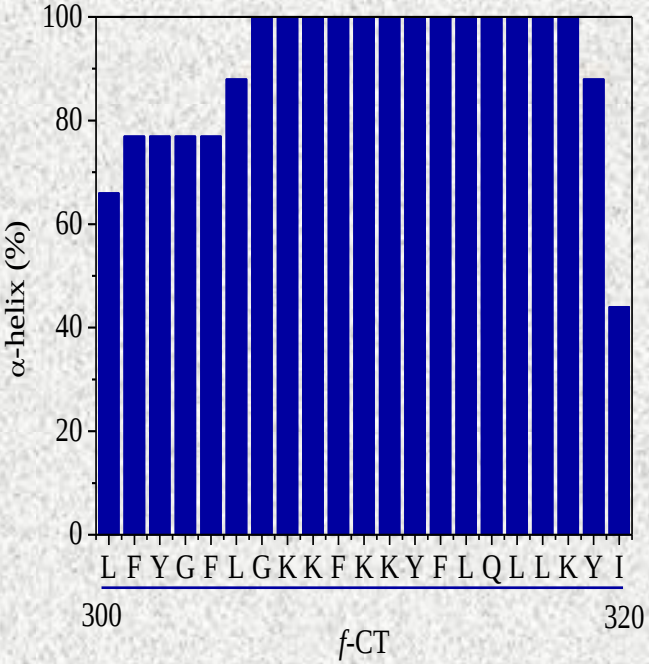
Laboratório de Biologia Estrutural

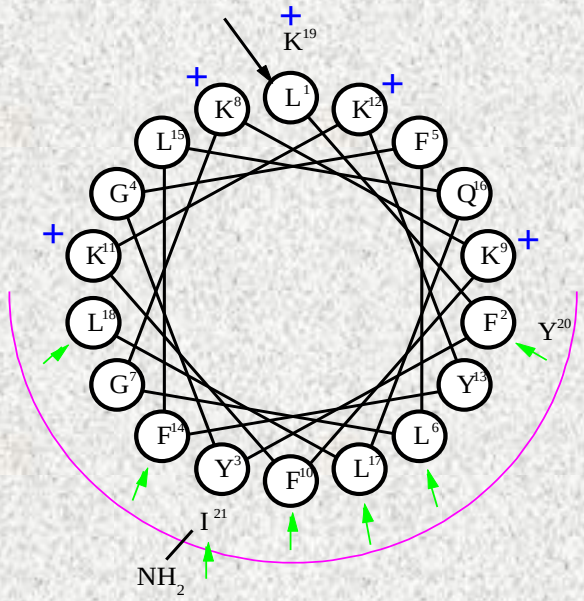
Location and sequence of extra-membranous domains (loops)
(EL1 and EL3, extra-cellular, and IL2, NIL3, CIL3, and *f*CT, intracellular)

AT_1 receptor peptides

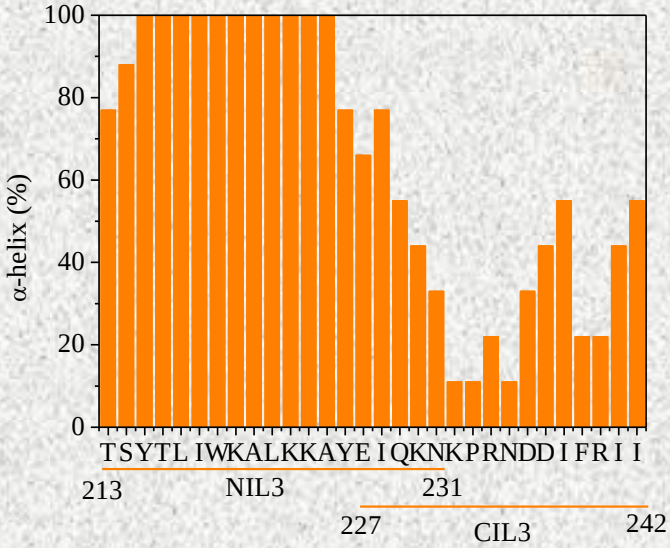
Prediction of α-helical content and amphipathic helical wheel

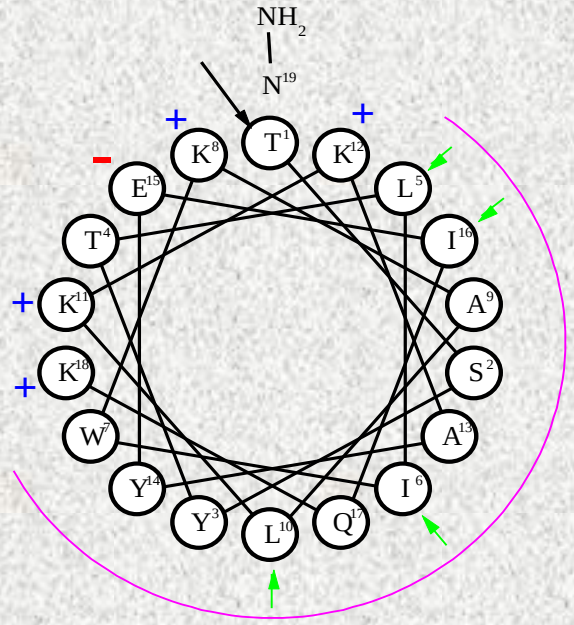
fCT





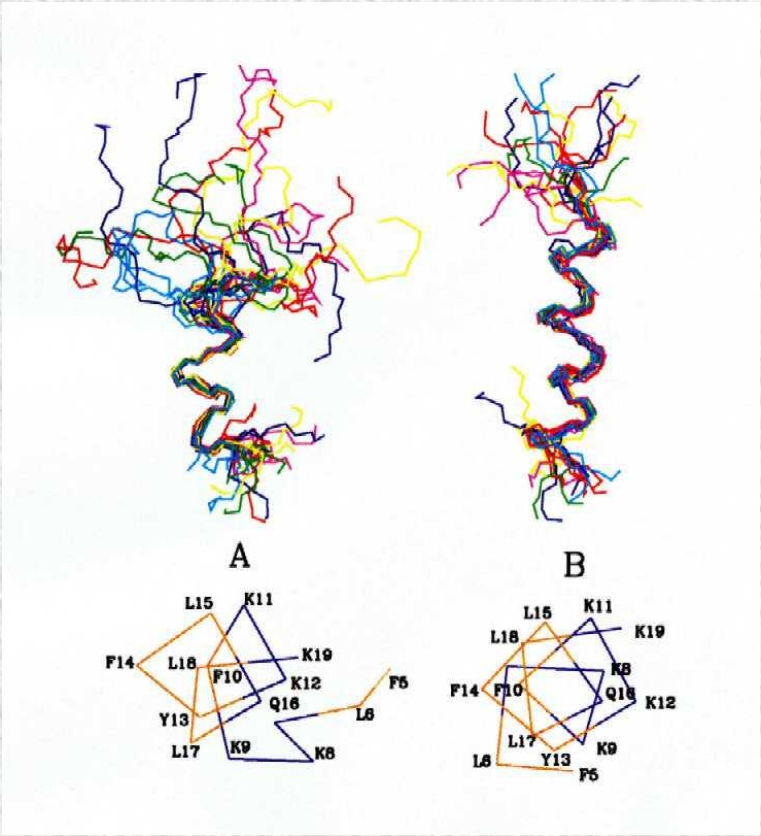
NIL3

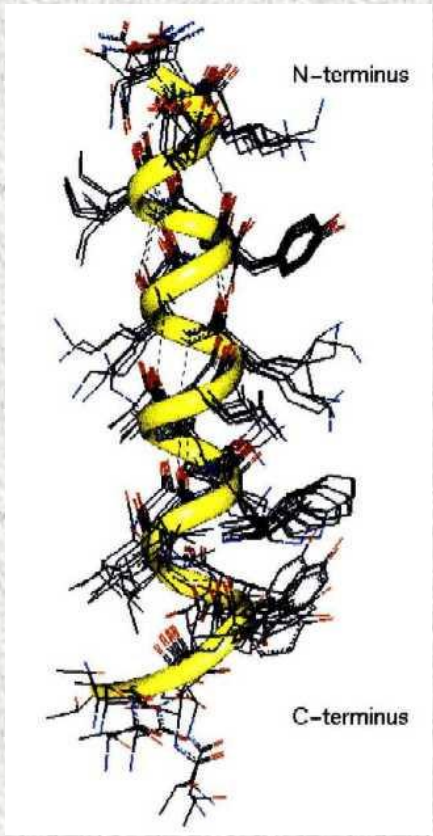




Conformations obtained by NMR

in 30% TFE





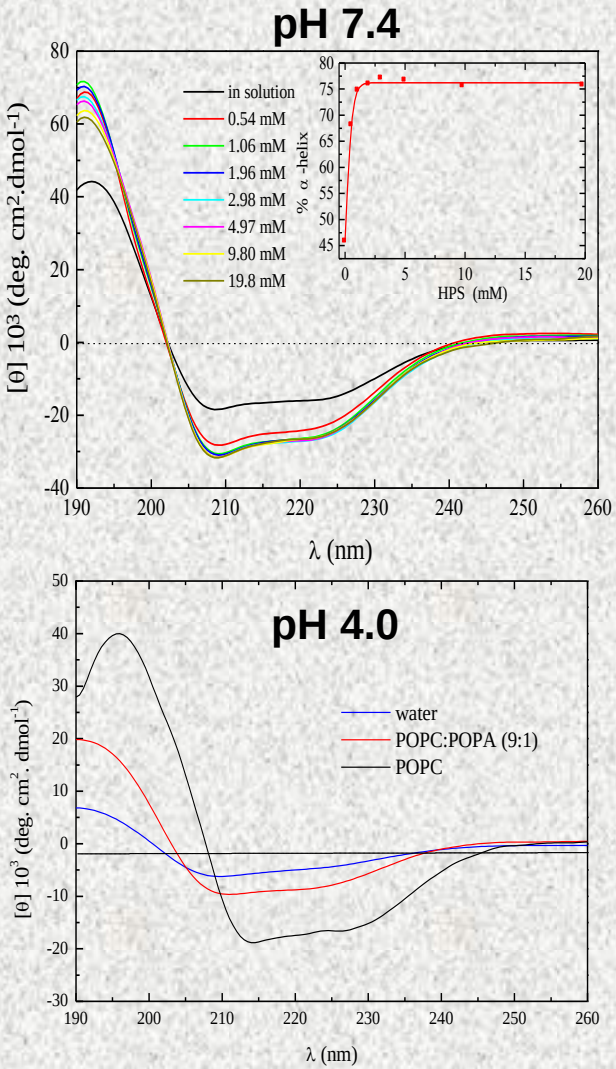
***f*CT** ***f*CT** **NIL3**

28°C **5°C** **25°C**

Franzoni *et al* J. Biol. Chem. 1997
Franzoni *et al*, J. Biol. Chem. 1999

BINDING OF INTRACELLULAR LOOPS *f*CT, NIL3, AND IL2 TO MICELLES AND BILAYERS CAUSES INCREASE IN FLUORESCENCE AND IN α-HELICAL CONTENT

*f*CT - *Binding to micelles and bilayers*

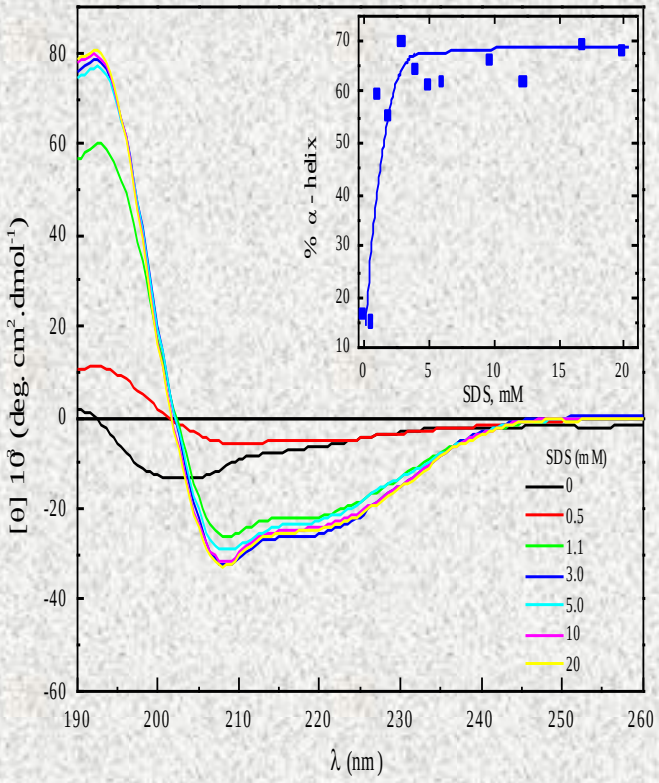


Effect of binding to model membranes on *f*CT fluorescence and α-helical content

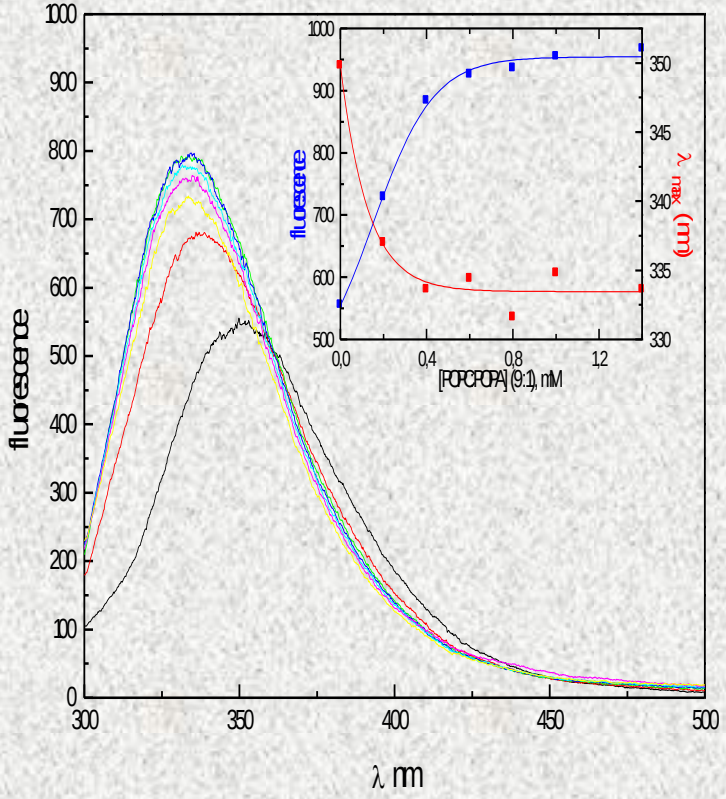
	pH 4.0		pH 7.4	
	F/F_0[a]	% α-helix	F/F_0	% α-helix
H_2O	-	1	-	10
POPC	1.42	56	1.97	50
POPC:POPA (9:1)	1.92	19	2.52	25
HPS	3.19	79	1.94	77
SDS	4.34	72	1.89	71
TFE		37		-

NIL3 - *Binding to micelles and bilayers* (pH 7.4)

SDS



POPC:POPA (9:1)



NIL3 binds to negatively charged and zwitterionic micelles, but ONLY to negatively charged bilayers

Upon binding NIL3 acquires *α-helical* conformation

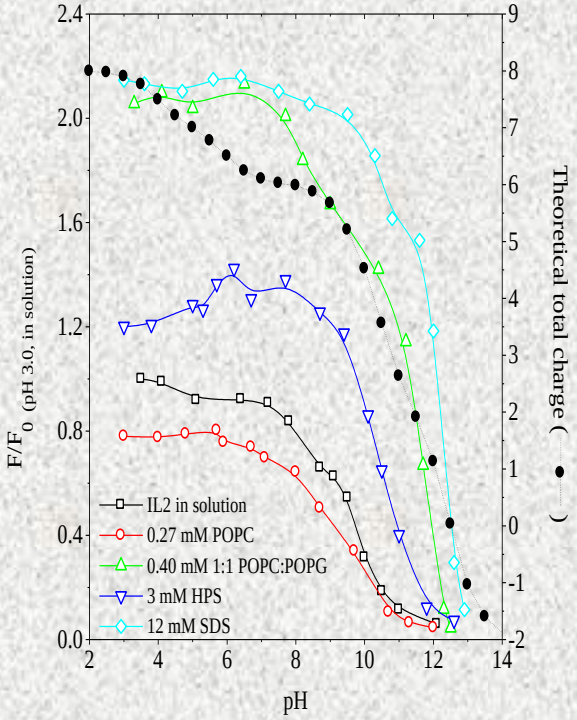
Laboratory of Structural Biology

Effect of binding to model membranes on NIL3 *fluorescence and α-helical content*

	pH 4.0			pH 7.4		
	F/F_0	λ_{max} (nm)	α-helix (%)	F/F_0	λ_{max} (nm)	α-helix (%)
H_2O	-	351	10	-	353	10
POPC	-	351	21	1.13	345	20
POPC: POPA (9:1)	1.27	339	17	1.67	334	16
HPS	2.69	337	27	3.39	337	50
SDS	1.59	331	65	1.38	337	68
TFE			48			-

Laboratory of Structural

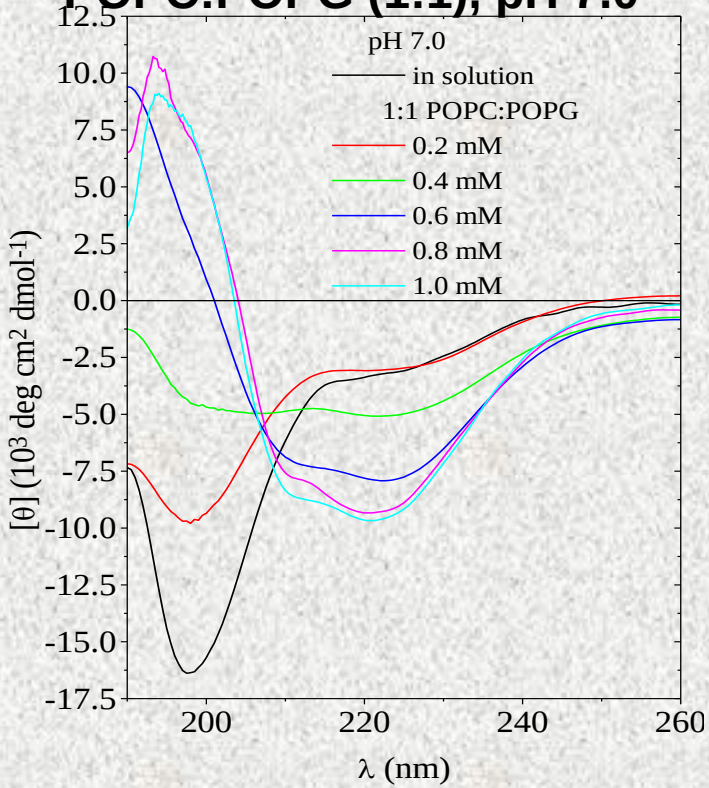
IL2 - *Binding to micelles and phospholipid bilayers*



Stern-Volmer constants for fluorescence quenching by acrylamide.

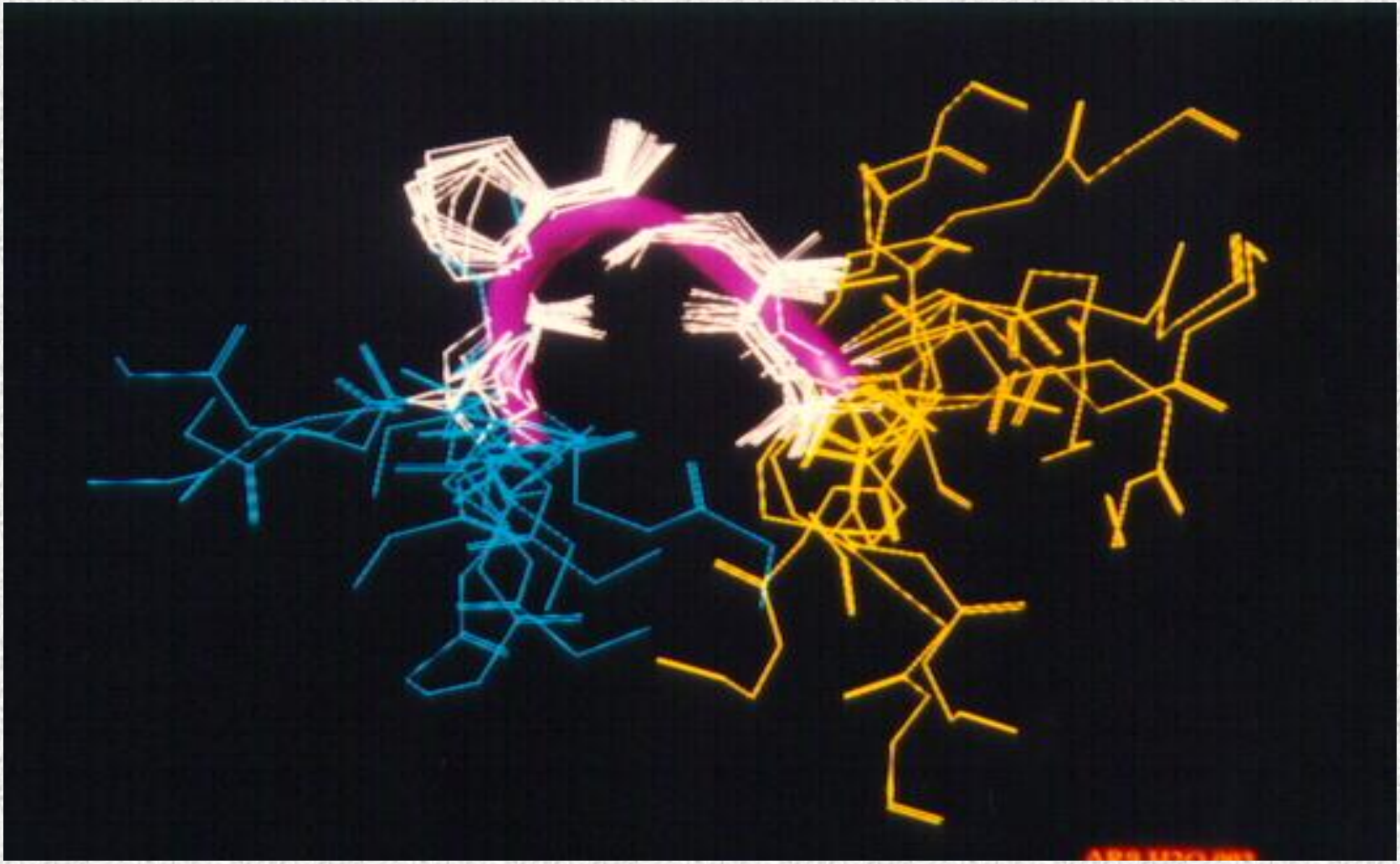
	K_{SV} (M^{-1})			
pH	**in solution**	**SDS Micelles (12 mM)**	**HPS Micelles (2.5 mM)**	**POPC: POPG vesicles (0.4 mM)**
4.0	16 ± 3	6 ± 1	6.4 ± 0.5	0
7.0	16 ± 3	3 ± 1	3.0 ± 0.3	0
10.0	13 ± 2	3 ± 1	1.5 ± 0.5	0.6 ± 0.3
12.0		4 ± 1		

POPC:POPG (1:1), pH 7.0



IL2 binds to negatively charged and zwitterionic micelles, but ONLY to negatively charged bilayers
Upon binding IL2 acquires *α-helical* conformation

EL1 - *NMR-derived conformation*

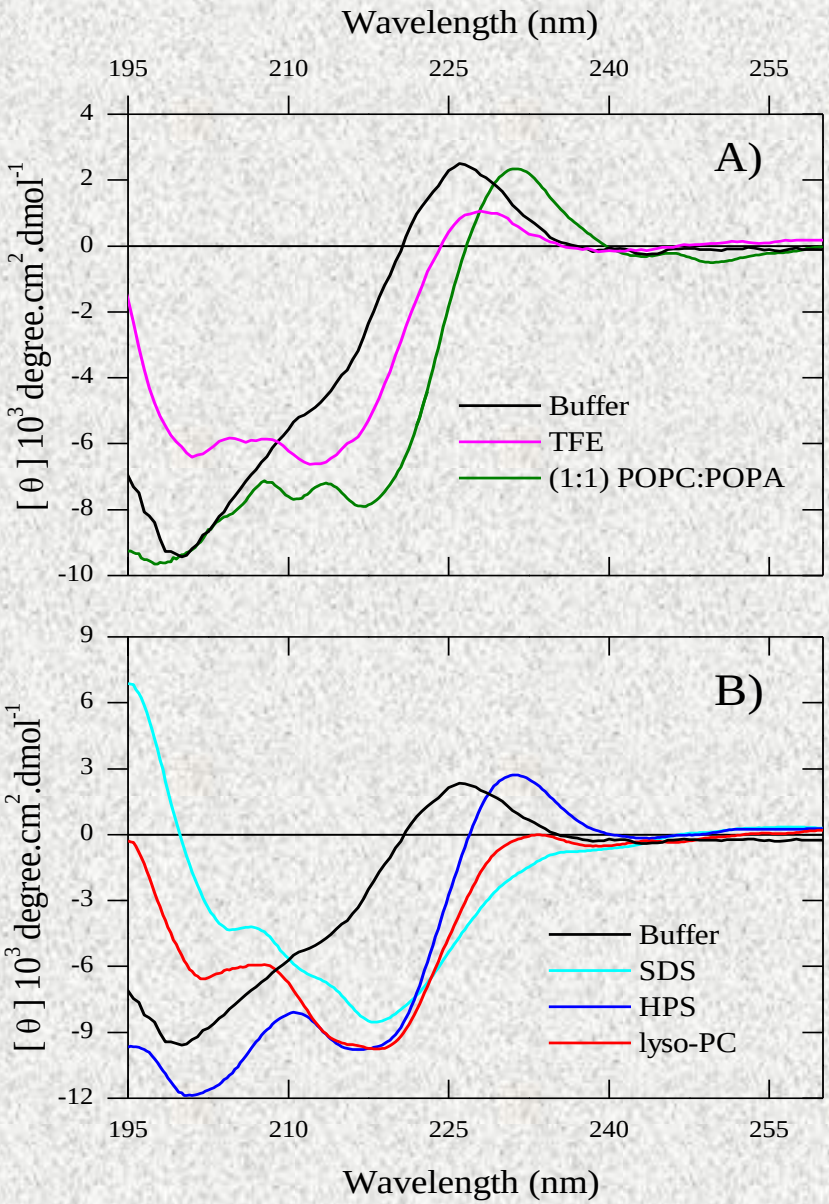


EL1 *trans*-Pro isomer (ca. 75%)

90% H_2O/10% D_2O, pH 4.0, 27°C.

Laboratory of Structural Biology

EL1 - *Binding to micelles and bilayers*



λ_{max}, relative fluorescence intensity, and anisotropy (*r*) values for the spectra of *f*EL1 in buffer and in the presence of micelles and bilayers.

*f*EL1 in	pH	λ_{max}	F/F_0	*r*
Buffer	4.0	348	1	0.03
	7.5	349	1	0.02
	9.0	349	1	0.02
SDS	4.0	334	0.49	0.09
	7.5	334	0.54	0.07
	9.0	335	0.75	0.09
lyso-PC	4.0	334	1.4	0.07
	7.5	333	1.5	0.07
	9.0	333	1.7	0.06
HPS	4.0	336	3.9	0.08
	7.5	336	3.8	0.04
	9.0	337	3.7	0.08
POPC:POPA	4.0	332	1.5	0.07
	7.5	335	1.6	0.07
	9.0	333	1.5	0.07

Salinas et al, Biopolymers 2002

Peptide penetration in the bilayer

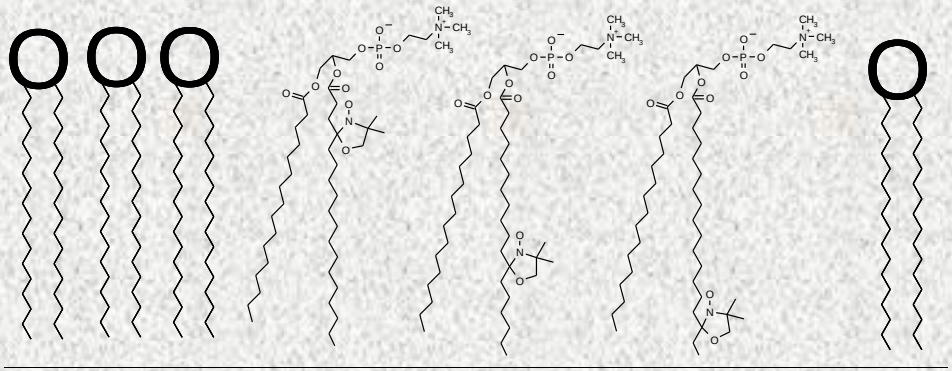
Determination by intrinsic fluorescence quenching by nitroxides at different positions along the acyl chain

Parallax method

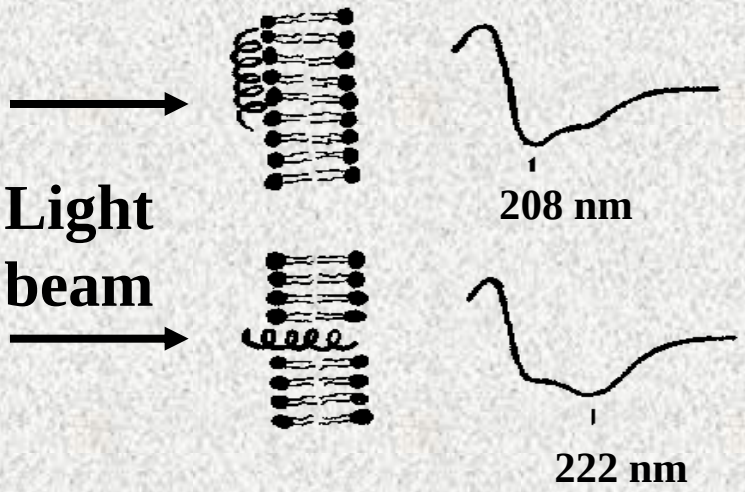
Peptide orientation

Determination by oriented CD

Fluorescence Quenching experiment

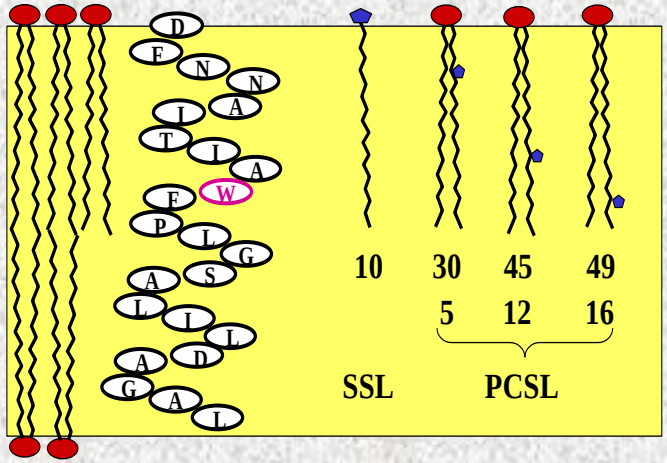


Oriented CD experiment



% Quenching of TM2 fluorescence

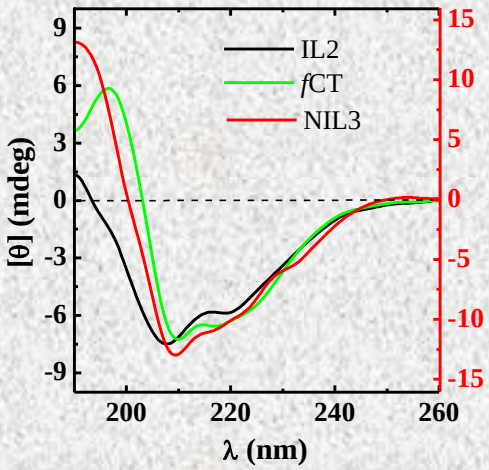
Location of W^{15} and TM2 in the bilayer

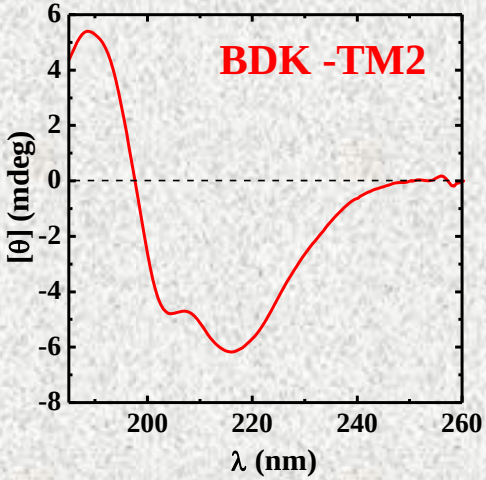


Fluorescence quenching shows EL1, IL2, *f*CT, and NIL3 at the bilayer-water interface

TM2 crosses the bilayer.

Oriented CD





The peptides are oriented as expected for their location in the receptors

*f*CT orientation in agreement with that observed in rhodopsin crystal structure

CONCLUSIONS

Conformations with higher secondary structure content are stabilized in TFE and upon binding to model membranes

The lower polarity of the solvent and of the interface favors formation of intramolecular hydrogen bonds

The peptide fragments tend to present conformations compatible with their conformation in the whole protein

IMPLICATIONS FOR RECEPTOR FUNCTION

The function of GPCR requires them to have dynamic structures

Local pH, polarity, and interactions with the lipid phase can modulate peptide conformation

Moreover, subtle changes at the interface can stabilize different conformational states

Changes in the environment, as well as ligand binding, could induce conformational changes similar to those observed for the extra- and intracellular receptor loops during signal transduction

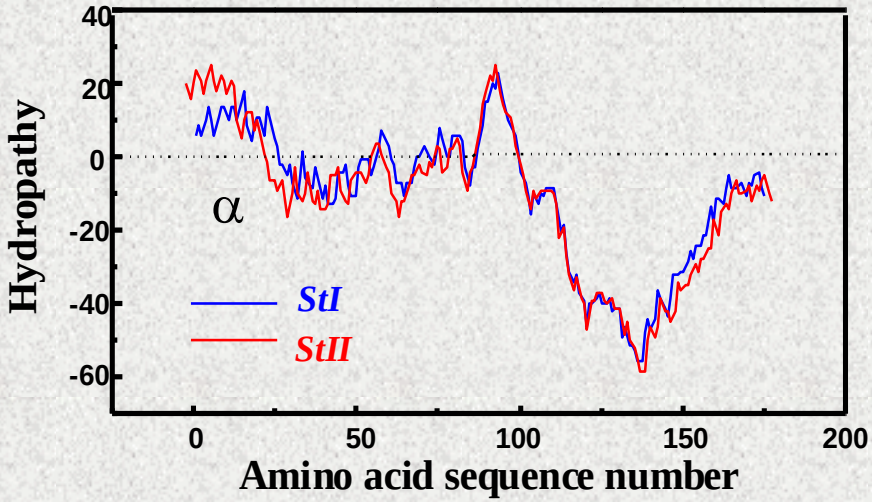
Sticholysines I and II - sea anemone pore-forming toxins

Stychodactyla helianthus

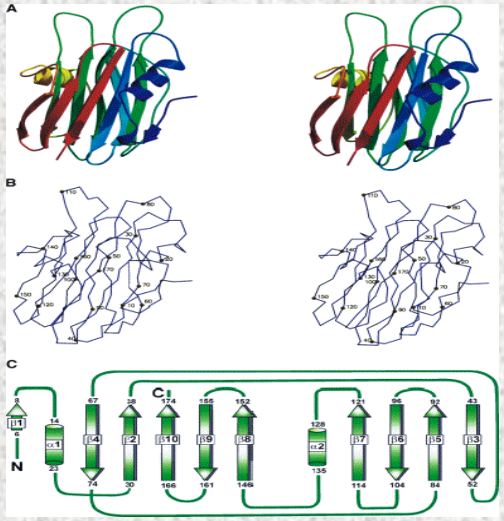
Sequence alignment of St I and St II

```
St I   SELAGTIIDGASLTFEVLDKVLGELGKVSRKIAVGIDNESGGTWTALNAYF
St II  ALAGTIIAGASLTFQVLDKVLEELGKVSRKIAVGIDNESGGTWTALNAYF

St I    RSGTTDVILPEVVPNTKALLYSGRKSSGPVATGAVAAFAYYMSNGNTLGV
St II   RSGTTDVILPEFVPNTKALLYSGRKDTGPVATGAVAAFAYYMSSGNTLGV

St I    MFSVPFDYNWYSNWWDVKIYPGKRRADQGMYEDMYYGNPYRGDNGWYQKN
St II   MFSVPFDYNWYSNWWDVKIYSGKRRADQGMYEDLYYGNPYRGDNGWHEKN

St I    LGYGLRMKGIMTSAGEAKMQIKISR
St II   LGYGLRMKGIMTSAGEAKMQIKISR
```

Hydropathy plot for St I and St II according to Kyte and Doolittle



St II structure determined by X ray crystallography



Mancheño et al, Structure 11 (2003)

Percent change in order parameter (S) and in h_{+1}/h_o

Alvarez et al, Chem. Phys. Lipids, 2003

label	temperature (°C)	measured parameter	StI	StII
5-SASL	10	S	2.3	0.78
	22	S	6.5	5.6
	47	S	5.4	-1.9
7-SASL	10	S	12.4	11.2
	22	S	16.3	16.6
	47	h_{+1}/h_0	-11.9	-14.4
12-SASL	10	h_{+1}/h_0	-9.9	-18.2
	22	h_{+1}/h_0	-10.6	-15.7
	47	h_{+1}/h_0	-5.8	-5.8
16-SASL	10	h_{+1}/h_0	-0.88	-3.2
	22	h_{+1}/h_0	-2.6	-4.7
	47	h_{+1}/h_0	-8.2	-3.2

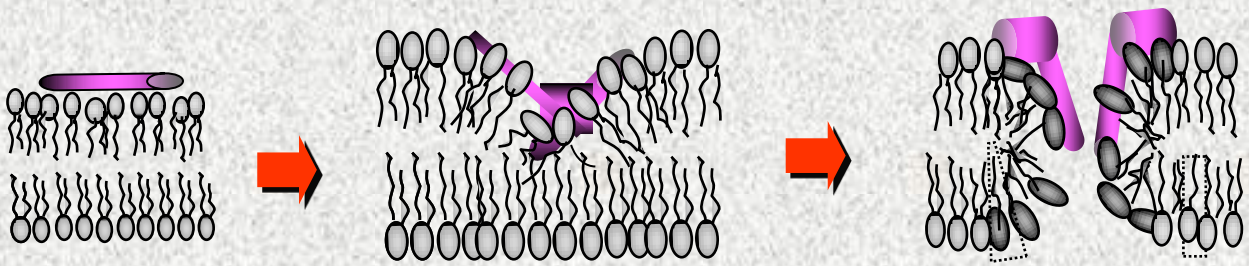
1. **The effect of St II is seen more deeply in the bilayer**

2. **The effect of both toxins at C16 is very small, in agreement with models that propose that the bulk of the protein sits at the bilayer-water interface**

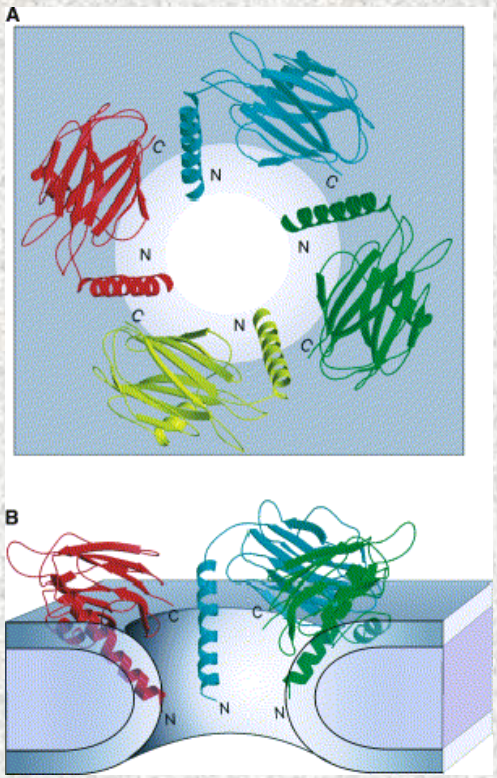
3. **The differences between St I and St II are in agreement with differences in activity**

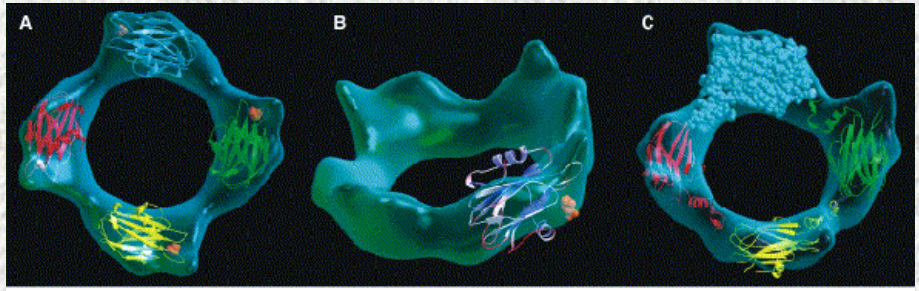
Poster N47, Iris

Model for peptide-membrane interaction and pore formation



Toroidal pore formation - positive curvature
The pore lined by the polar face of the peptide (tetramer) and by lipids polar heads





Mancheño et al, Structure 2003.

Molecular mechanism of pore formation not yet clarified

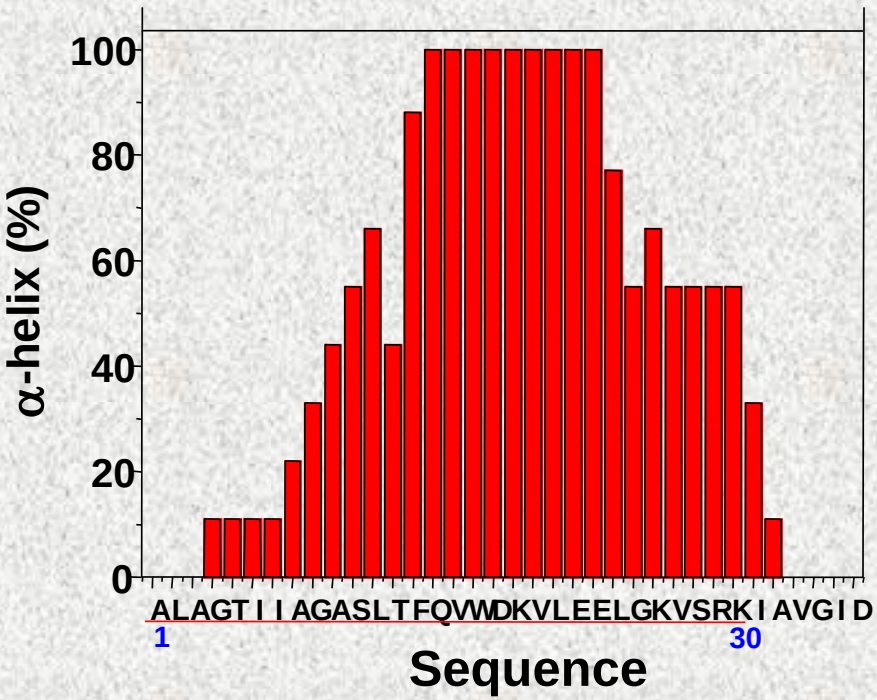
N-terminal α-helix postulated in membrane insertion

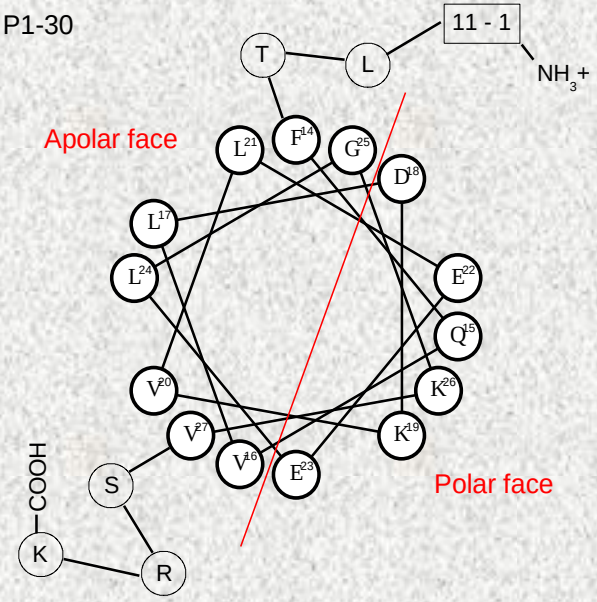
Peptides corresponding to St II N-terminus were synthesized

Peptide	Sequence	Residues
P1-30	ALAGTIIAGASLTFQVLDKVLEELGKVSRK	1-30
PW2	AWAGTIIAGASLTFQVLDKVLEELGKVSRK	1-30
PW7	ALAGTIWAGASLTFQVLDKVLEELGKVSRK	1-30
PW12	ALAGTIIAGASWTFQVLDKVLEELGKVSRK	1-30
PW17	ALAGTIIAGASLTFQVWDKVLEELGKVSRK	1-30
PW21	ALAGTIIAGASLTFQVLDKVWEELGKVSRK	1-30
PW24	ALAGTIIAGASLTFQVLDKVLEEWGKVSRK	1-30
P11-30	SLTFQVLDKVLEELGKVSRK	11-30
P16-35	VLDKVLEELGKVSRKIAVGI	16-35

PREDICTION OF SECONDARY STRUCTURE, HEMOLYSIS, AND PERMEABILIZING ACTIVITY

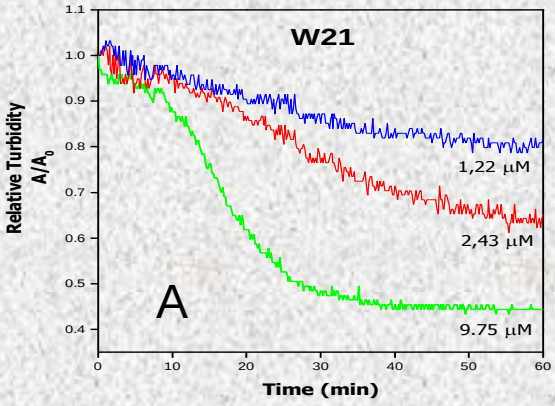
α-helix probability for P1-30

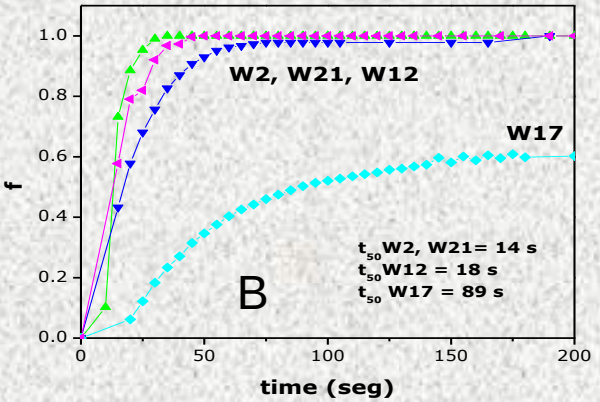




Helical wheel, amphipathic α-helix

High propensity to acquire α-helical conformation in the region 14 to 24



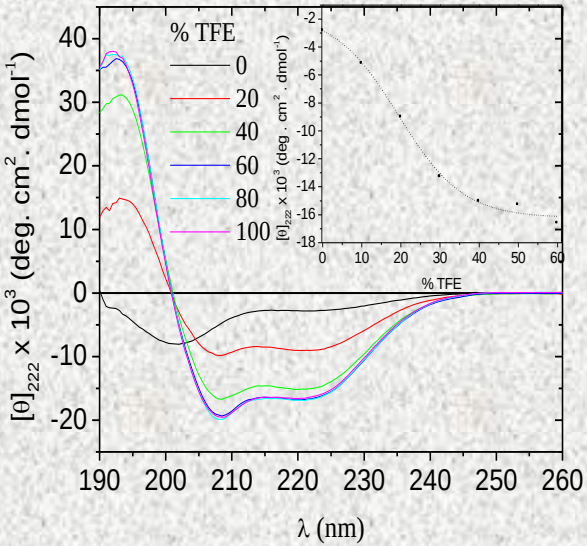


Peptides cause hemolysis (A) and permeabilize model membranes (B)

EFFECT OF TFE AND INTERACTION WITH MODEL MEMBRANES

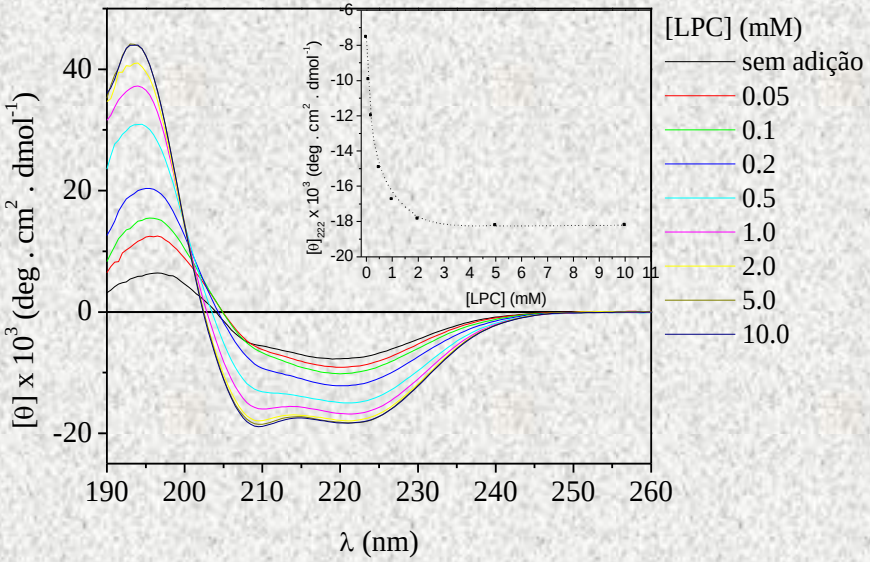
P1-30 - TFE

Casallanovo et al, Biopolymers, 2006

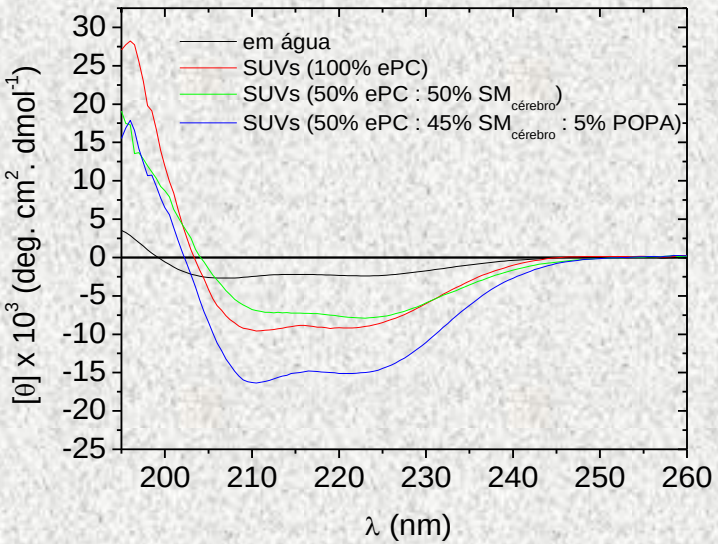


In TFE and upon binding to model membranes, the peptides acquire α-helical conformation

P1-30 - micelles



PW12 - bilayers



α-helical content and number of residues in α-helical conformation (in parenthesis) in different media*

Medium Peptide	100% TFE	20 mM SDS	10 mM HPS	10 mM LPC
P1-30	0.75 (22.5)	0.26 (7.9)	0.33 (9.8)	0.55 (16.4)
PW2	0.80 (24)	0.34 (10.3)	0.46 (13.8)	0.69 (20.6)
PW7	0.65 (19.6)	0.37 (11)	0.45 (13.6)	0.61 (18.3)
PW12	0.61 (18.3)	0.40 (12)	0.60 (18)	0.67 (20)
PW17	0.56 (16.8)	0.36 (10.7)	0.38 (11.5)	0.52 (15.7)
PW21	0.63 (19)	-----	0.50 (15)	-------
P11-30	0.35 (7)	0.33 (6.6)	0.33 (6.6)	0.38 (7.6)
P16-35	0.43 (8.7)	0.30 (6)	0.30 (6)	0.39 (7.7)

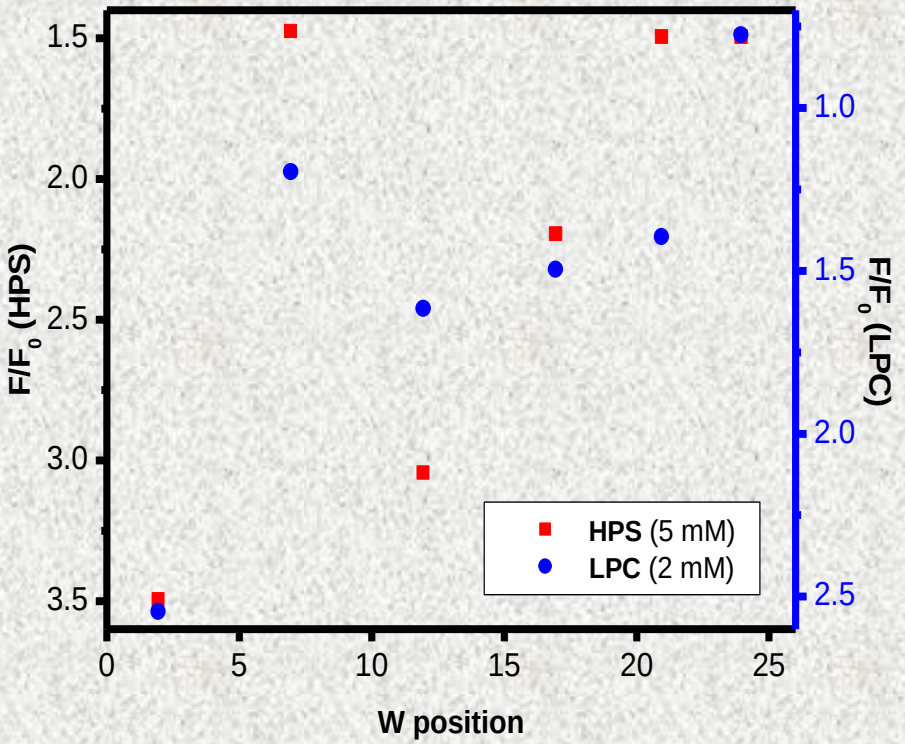
- Higher α-helical content (compared to crystal) for membrane-bound 30-residue peptides could be due to membrane-induced increase in helix length as a step in the mechanism of pore formation

  α-helical content of St II increases by 5% (9 residues) upon binding to membrane (Menestrina et al, 1999)

  This could correspond to the increase of α-helical content found for the N-terminal peptides

Peptide Topography

Differential increase in fluorescence intensity as a function of W position



Increase of fluorescence intensity

Decrease of λ_{max}

Fluorescence quenching by acrylamide

shows that the 1-12 segment is located more deeply

in the hydrophobic core of micelles and bilayers

(PW7 – *exception*, *probably due to aggregation*)

amphipathic α-helix resides close to the interface

In membrane-mimetic environments, peptides containing residues 1-30 of St II N-terminus are able to acquire conformation similar to that found in whole protein

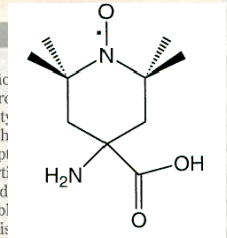
Data suggest that 1-12 segment plays a role in anchoring of the amphipathic α-helix (residues 14-24) to the interface

P. N23, Joana
P. N142, Gustavo

science/technology

A NEW SPIN: PEPTIDE PROBES

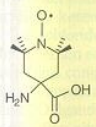
Unnatural amino acid TOAC, a novel spin label used in EPR studies, helps illuminate peptide structure



Elizabeth K. Wilson
C&EN West Coast News Bureau

In this golden age of biotechnology, proteins and their abbreviated siblings, peptides, are under remarkable scrutiny. And as with proteins, the folding and structure of peptides are of immense interest to biochemists. They want to know precisely what drives a peptide to adopt a certain structure and how peptides interact with other peptides, proteins, and cell membranes—this knowledge will generate innumerable and invaluable leads for therapeutic drug design and other biological applications.

Scientists, of course, have numerous tools at their fingertips with which to study peptides and proteins, including nuclear magnetic resonance spectroscopy and X-ray crystallography. And thanks to recent advances in technology, electron paramagnetic resonance (EPR) spectroscopy, sometimes also referred to as electron spin resonance (ESR), has moved alongside these tried-and-true methods.



TOAC

EPR has a number of powerful advantages. Like NMR, it measures the energy required to flip the spin of a particle in the presence of an electromagnetic field, except that the particle that has its spin flipped is an unpaired electron rather than a nucleus. Pairs of electrons interact much more strongly than nuclei, and so the EPR technique is more sensitive over longer distances. EPR experiments can be done in solution and in solids, and one can obtain spectra in real time at a resolution of 1 millisecond.

Although any chemical entity with an unpaired electron (such as a radical) can give rise to an EPR spectrum, the source of the unpaired electron used in EPR investigations often is a so-called spin label—a moiety such as a nitroxide or a metal that chemists affix to a molecule.


From left, São Paulo spin label researchers Schreier, Nakaie, and Paiva.

Nitroxide spin labels typically used in peptide and protein studies are attached through flexible chains. For example, a common flexibly linked spin label consists of a disulfide group with methylene groups on either side, attached to a cysteine residue. But because these chains flop around, the resulting EPR spectra tend to be muddy, making it difficult to obtain accurate information.

But now a unique spin label that's particularly well suited for peptides is making a splash with some biochemists. The label is a little-known unnatural amino acid dubbed TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid). TOAC has the advantage of being rigid and so yields extremely accurate information about the twists and turns of a peptide's backbone. It is also a strong helix stabilizer—that is, its presence in a peptide chain helps form α- and 3_{10}-helices, two common secondary structures in polypeptides.

The molecule actually has been around since the 1960s. In the early 1980s, then-graduate student Clovis R. Nakaie, guided by biophysics professor Antonio C. M. Paiva at the Federal University of São

chemistry pr
the Universit
how to attach
ends of a pep

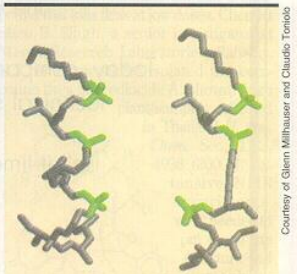
But insert
into the midd
difficult probl
bels, TOAC is
and of itself, and so it can't be simply added to a peptide side chain. In order to insert TOAC, it must be worked into the peptide synthesis itself.

Unfortunately, the solid-phase technique used to synthesize peptides, in which amino acids are sequentially added onto a strand attached to a resin, employed treatments with trifluoroacetic acid (TFA) that irreversibly neutralized the vital nitroxide group.

In 1993, Nakaie, who is now a biophysics professor at the Federal University of São Paulo, and graduate student Reinaldo Marchetto found a solution that involved, in part, using hydrofluoric acid instead of TFA during the synthesis, which preserved the nitroxide.

Several groups now are finding TOAC very useful for peptide EPR studies, and they say interest in the label is growing.

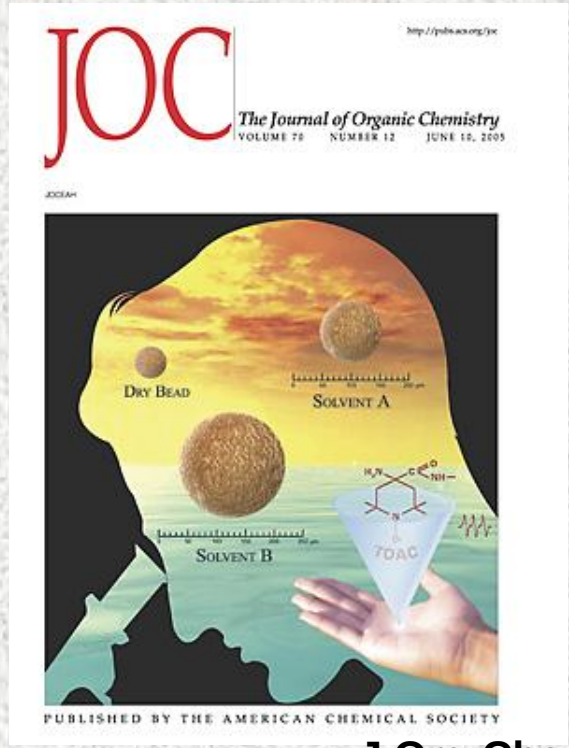
"This is a really wonderful reporter of


Courtesy of Glenn Millhauser and Claudio Toniolo

Two conformers of the fungal peptide trichogin. ESR measurements using TOAC spin labels show that the predominantly α-helical conformer at left and the mixed helical and unfolded conformer at right exist in nearly equal proportions. TOAC labels are green and the remaining backbone and side chain atoms are gray.

1981 Nakaie et al publish in the Braz. J. Med. Biol. Res. the first paper describing the use of the paramagnetic amino acid TOAC. to synthesize peptides

At first, it was only possible to bind TOAC at the N-terminus.
In 1993 we reported a procedure to insert TOAC in internal positions (Marchetto et al, J Am Chem Soc 1993)



Chem & Eng News 78, 54 (2000)

J Org Chem, 2005

AII, BK and their labeled analogues in the presence of TFE

EPR and CD

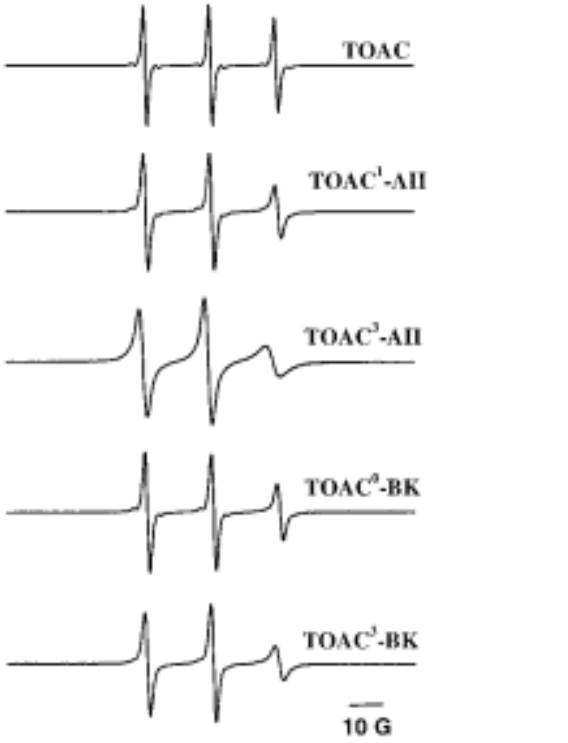


FIGURE 1 EPR spectra of (from top to bottom) 0.1 m*M* TOAC, TOAC[1]-AII, TOAC[3]-AII, TOAC[0]-BK, and TOAC[3]-BK in 100% TFE.

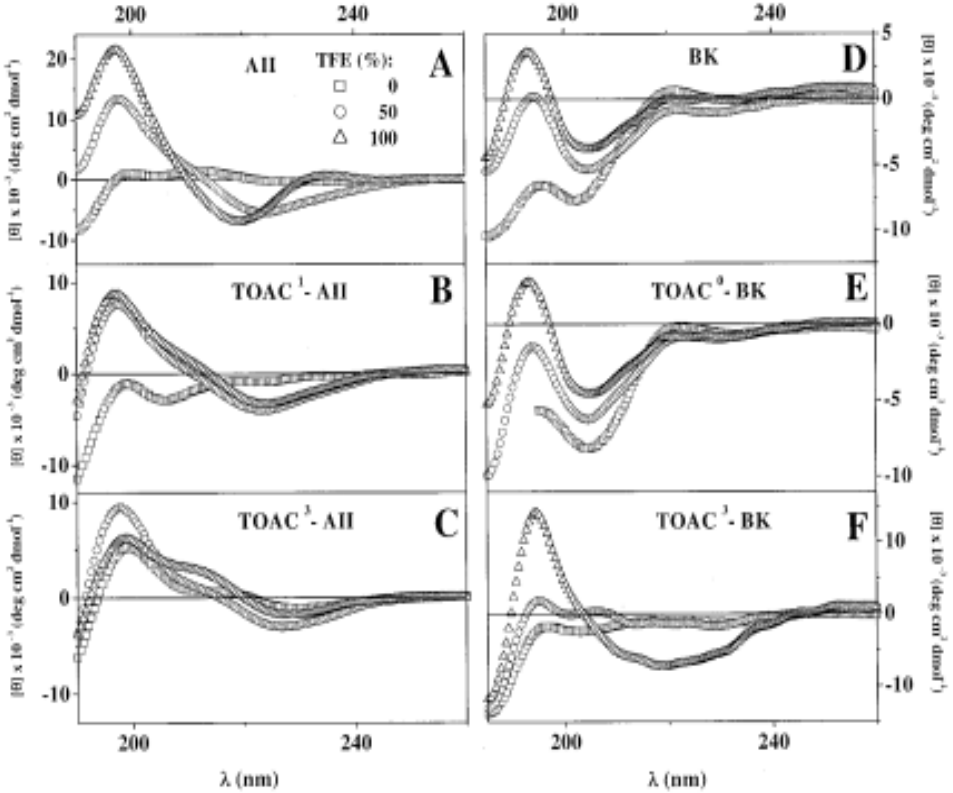


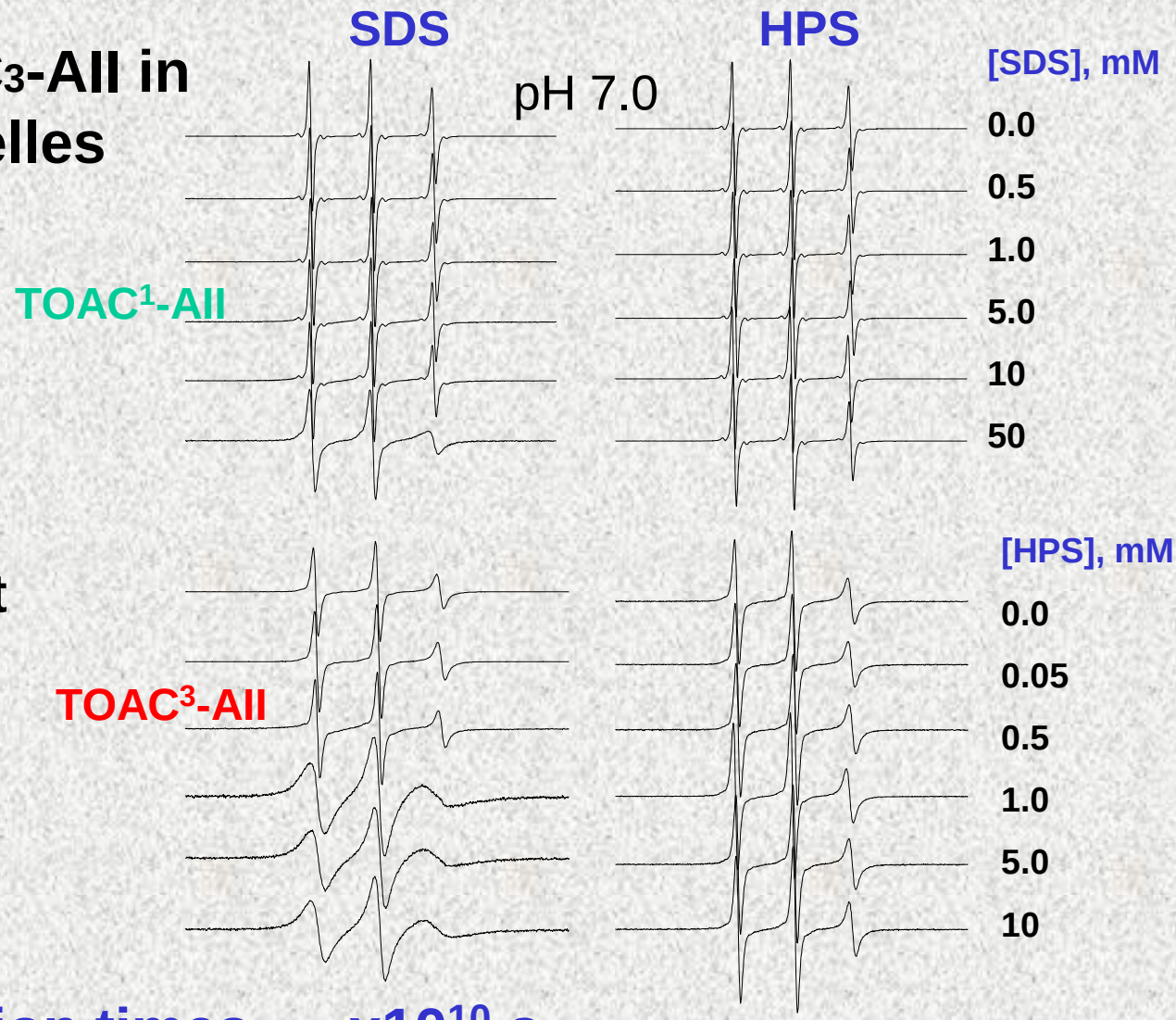
FIGURE 4 CD spectra of AII (A), TOAC[1]-AII (B), TOAC[3]-AII (C), BK (D), TOAC[0]-BK (E), and TOAC[3]-BK (F) in the absence (□) and presence of 50 (○) and 100% (△) TFE. The aqueous solution was at pH 4.0.

Native peptides and those labeled at the N-terminus have similar conformations

Internally-labeled peptides have a TOAC-imposed bend

Schreier et al, Biopolymers 2004

$TOAC_1$-AII and $TOAC_3$-AII in the presence of micelles



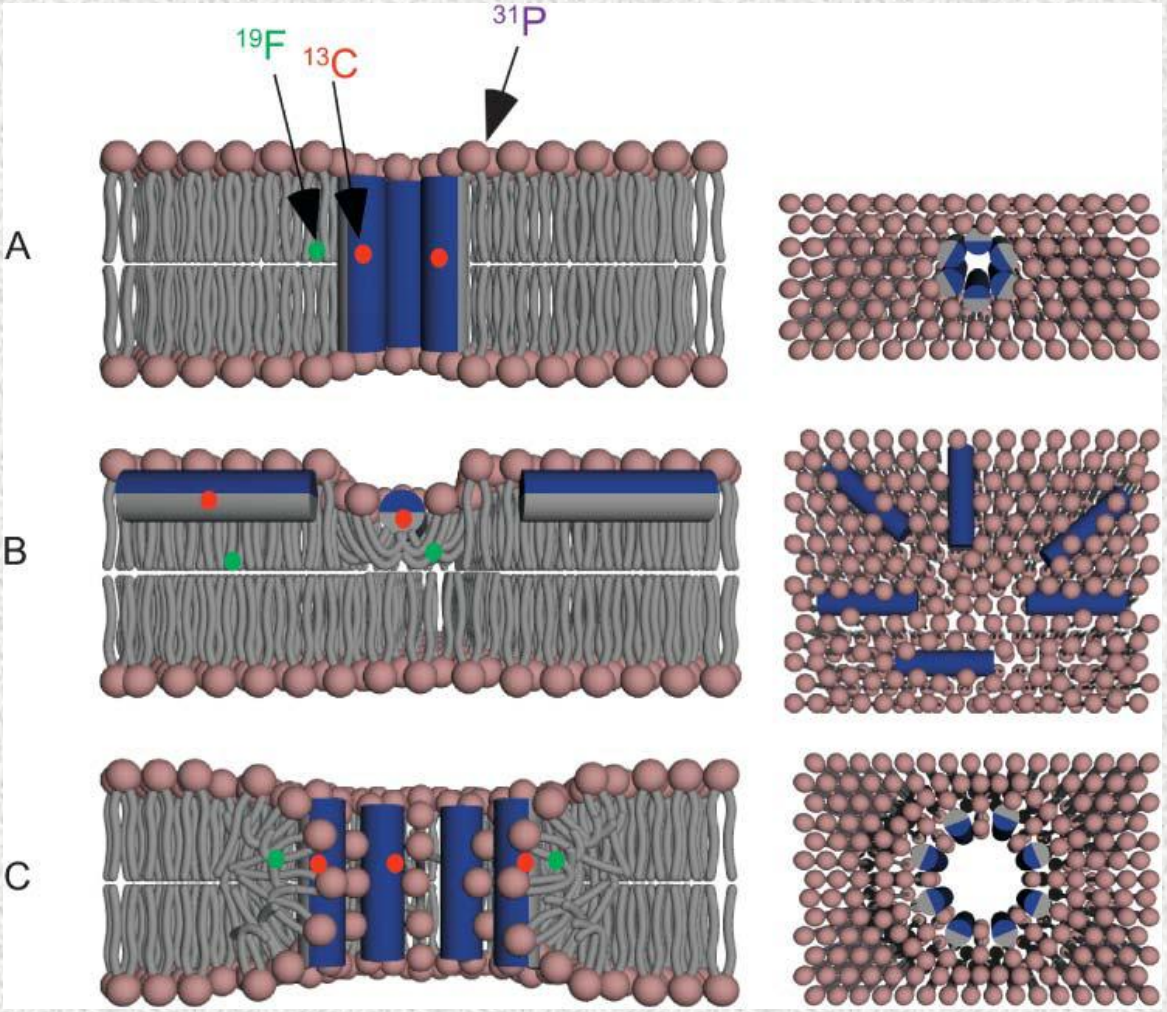
Both TOAC¹-AII and TOAC³-AII bind to negatively charged SDS, but not to zwitterionic HPS

Rotational correlation times, τ_C x10^{10} s

	TOAC¹-AII	TOAC³-AII
no SDS	1.65	6.13
+ 50 mM SDS	12.5	36.1

Vieira el al, Biopolymers, 2009

MECHANISMS OF ACTION PROPOSED FOR ANTIMICROBIAL PEPTIDES



barrel stave

carpet

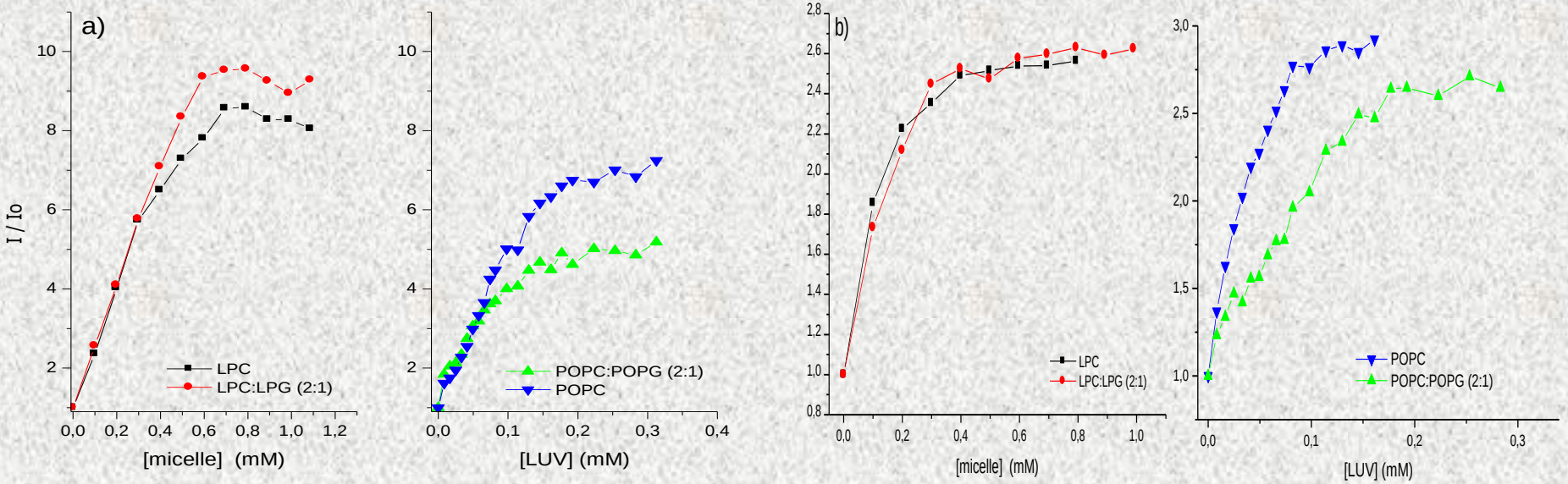
toroidal pore

Toke et al. Biophys. J. (2004) 87, 662-674

Antimicrobial peptide

Indolicidin - ILPWKWPWWPWRR-NH_2

IND **TOAC⁰-IND**



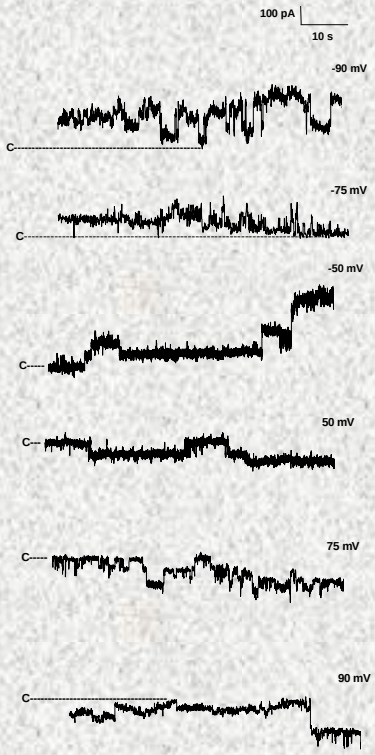
The peptides bind to micelles and bilayers, zwitterionic and negatively charged

λ_{max} for IND and TOAC⁰-IND fluorescence in the absence and presence of micelles and LUVs

	solution	LPC	LPC:LPG	POPC	POPC:POPG
IND	348	343	342	342	341
TOAC⁰-IND	349	344	344	343	344

Antimicrobial peptide Tritrpticin (TRP3)

VRRFPWWWPFLRR



Salay et al, FEBS Lett 2004

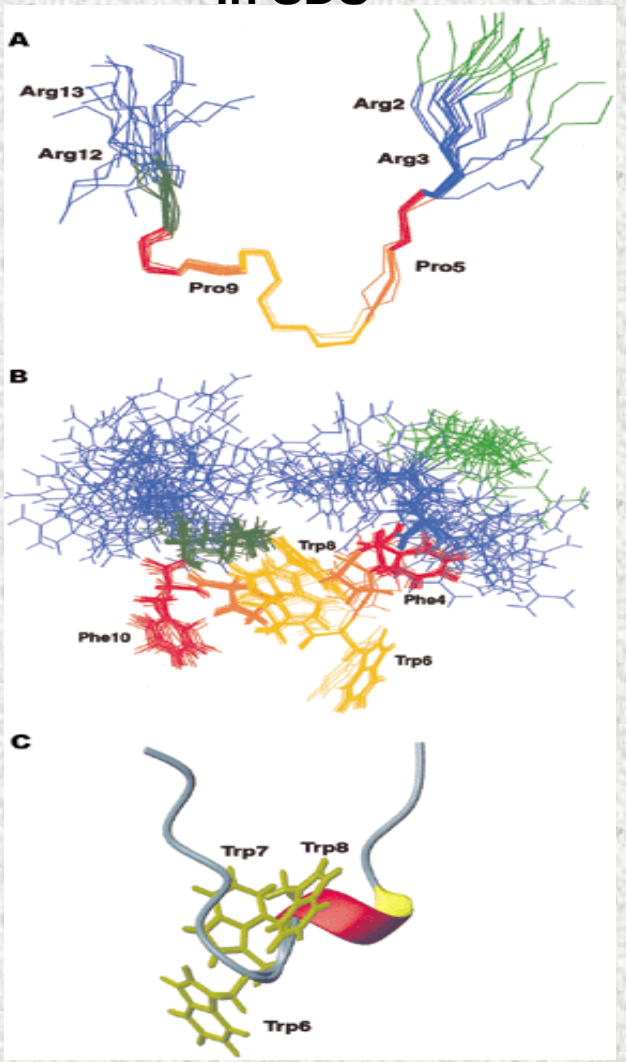
TRP3 in azolectin (negatively charged) planar lipid bilayers generates ion channel activity

TRP3 pore is cation selective

In planar bilayers of a zwitterionic lipid Trp3 also showed ion channel activity, but in a much less frequent and less prominent way

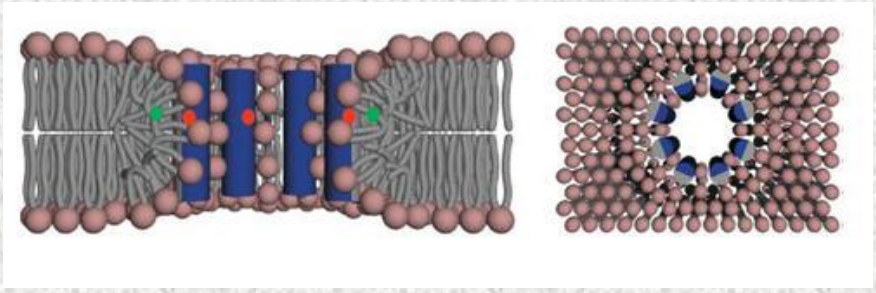
But, TRP3 size and conformation could not give rise to a transmembrane pore

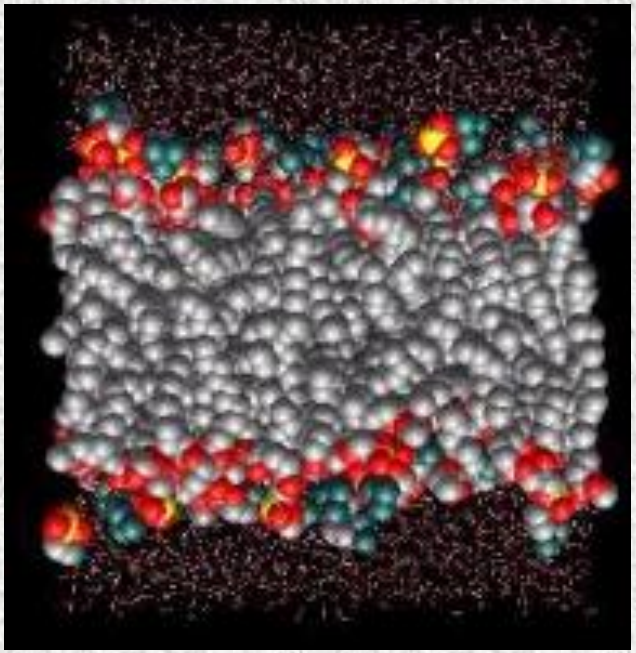
In SDS



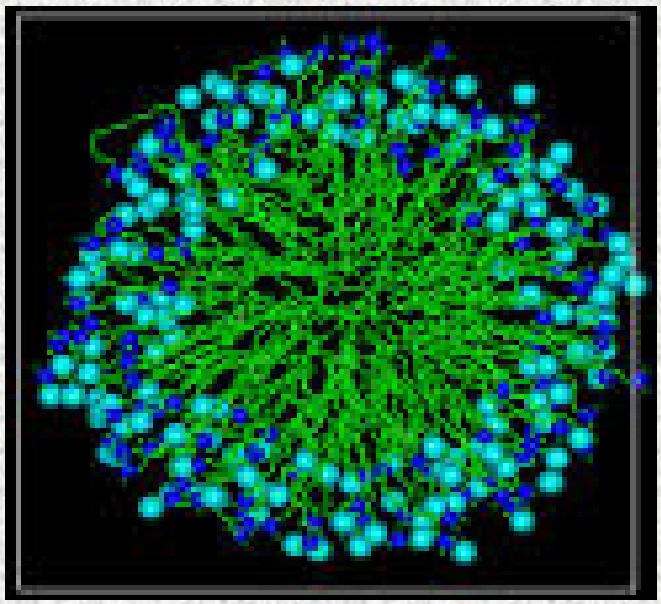
Schibli et al, Biochemistry 38 (1999) 16749-16755

We proposed that TRP3 forms a toroidal pore



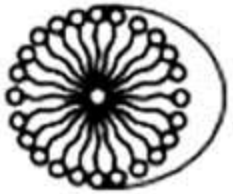
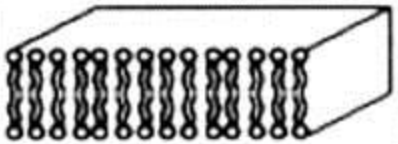
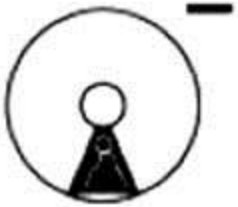


In a toroidal pore, the lipids display positive curvature, as in a micelle



Due to the different geometry – micelles possess a high curvature, the molecular packing is looser in micelles than in bilayers

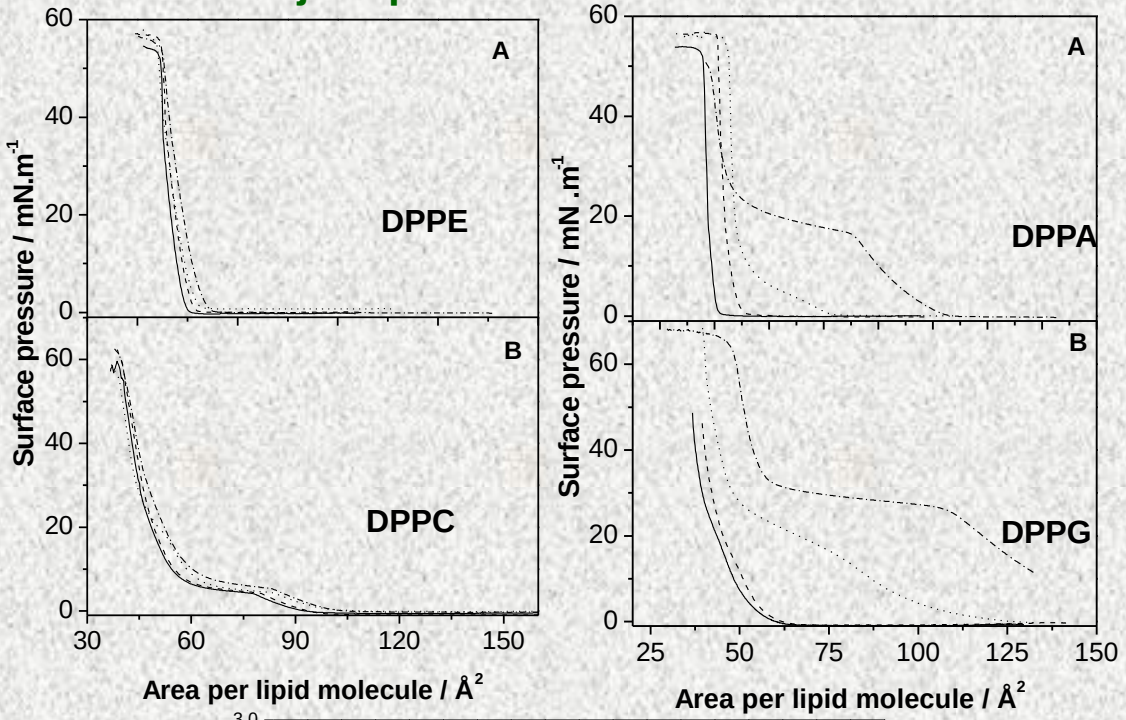
INFLUENCE OF MOLECULAR SHAPE ON THE TYPE OF AGGREGATE FORMED

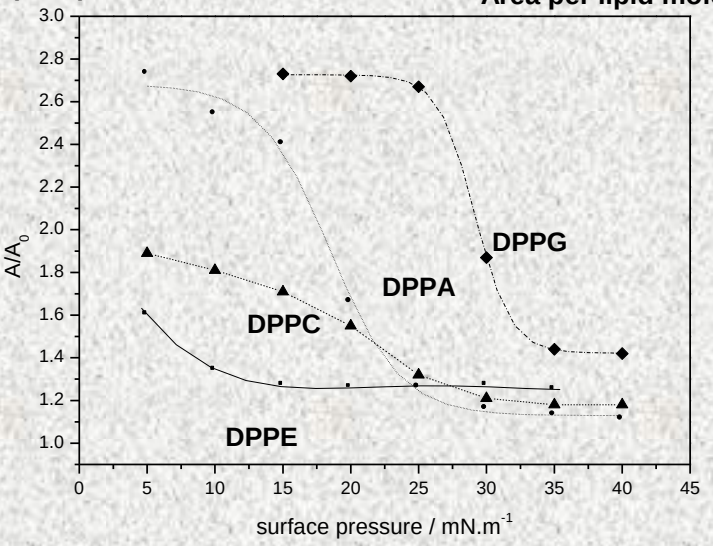
Lipid Species	Molecular Shape	Lipid Phase
Lysophospholipids Detergents	Type-I  Inverted Cone / Wedge	 Micellar
Phosphatidylcholine Sphingomyelin Phosphatidylserine Phosphatidylinositol Phosphatidylglycerol Phosphatidic acid (PA) Cardiolipin (CI) Digalactosyldiglyceride	Bilayer-preferring  Cylinder	 Bilayer
Phosphatidylethanolamine CI + Ca^{2+} PA + low pH + Ca^{2+} / Mg^{2+} Monogalactosyldiglyceride Unsaturated fatty acids Ceramide Diacylglycerol Cholesterol	Type-II  Cone	 Hexagonal-II

TRP3 INTERACTION WITH MONOLAYERS

DPPC, DPPE – zwitterionic DPPA, DPPG – anionic

DPPG major lipid in bacteria





Surface pressures of the order of 25-35 mN/m are correlated with the pressure in bilayers, while 15 mN/m corresponds to the pressure in micelles. The sigmoidal behavior of the curves could correspond to a transition analogous to a bilayer-to-micelle transition.

The molecular organization of TRP3-anionic phospholipid systems (especially DPPG) at low surface pressures could resemble the arrangement in micelles, which, in view of their positive curvature, resemble the lipid organization in toroidal pores.

P. S12 José Carlos

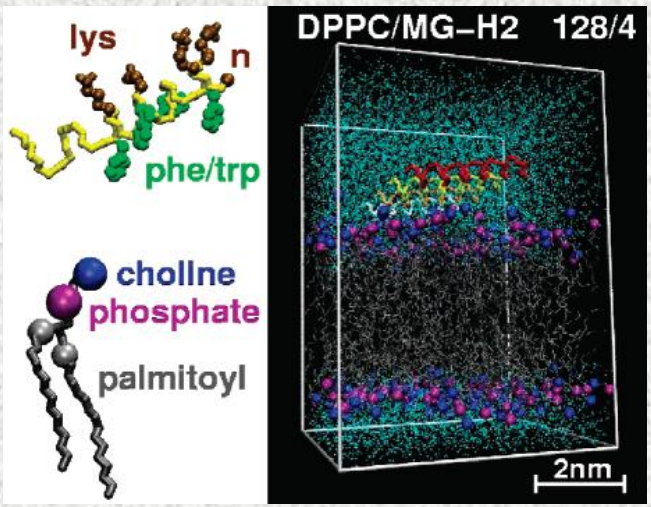
CONCLUSIONS

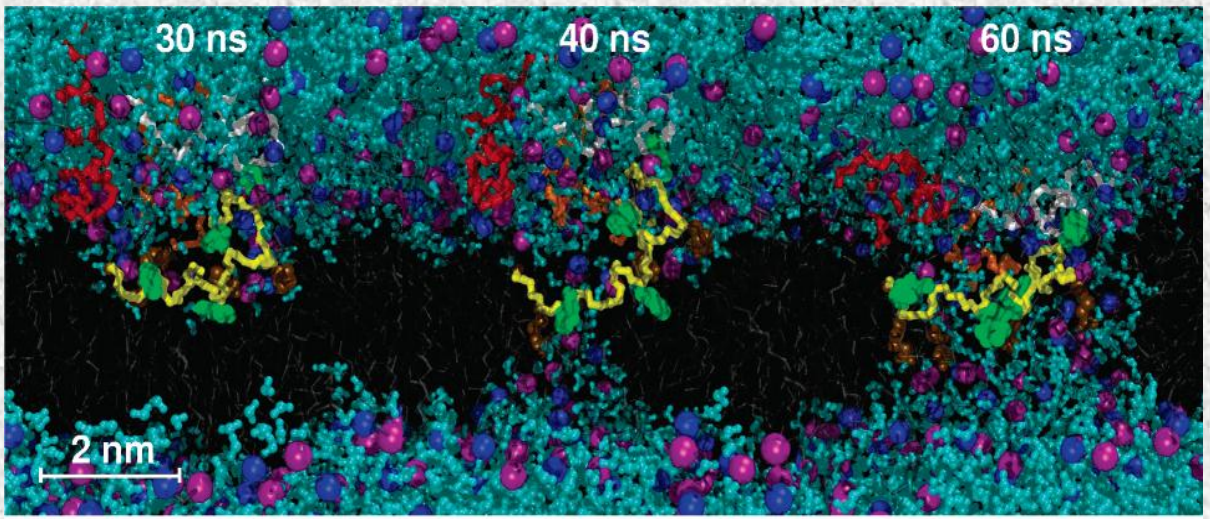
Binding of peptides to bilayers and micelles is qualitatively and/or quantitatively modulated by different factors.

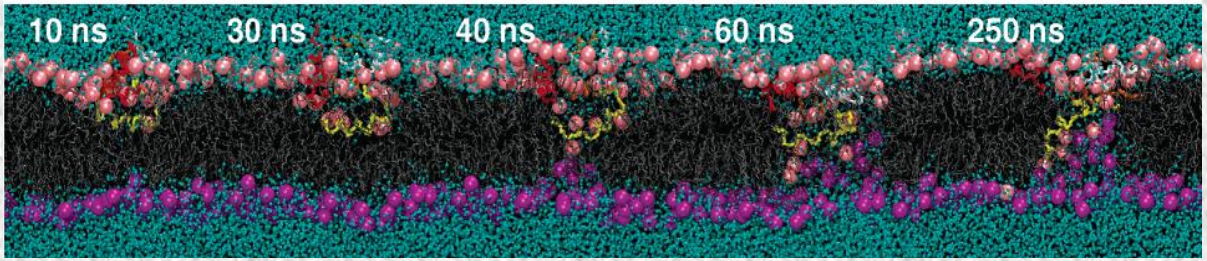
In several cases, electrostatic interactions are required for binding to the bilayer, while such interactions play a lesser role in binding to micelles.

These differences are due to the different geometry of both aggregates, micelles presenting a much more pronounced positive curvature. As a result, the molecular packing in micelles is much looser than in bilayers.

Different forces play a role in peptide-membrane interaction: electrostatic interactions, hydrophobic effect, hydrogen bonding, van der Waals interactions







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Financiamento: FAPESP, CNPq, CAPES, USP

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