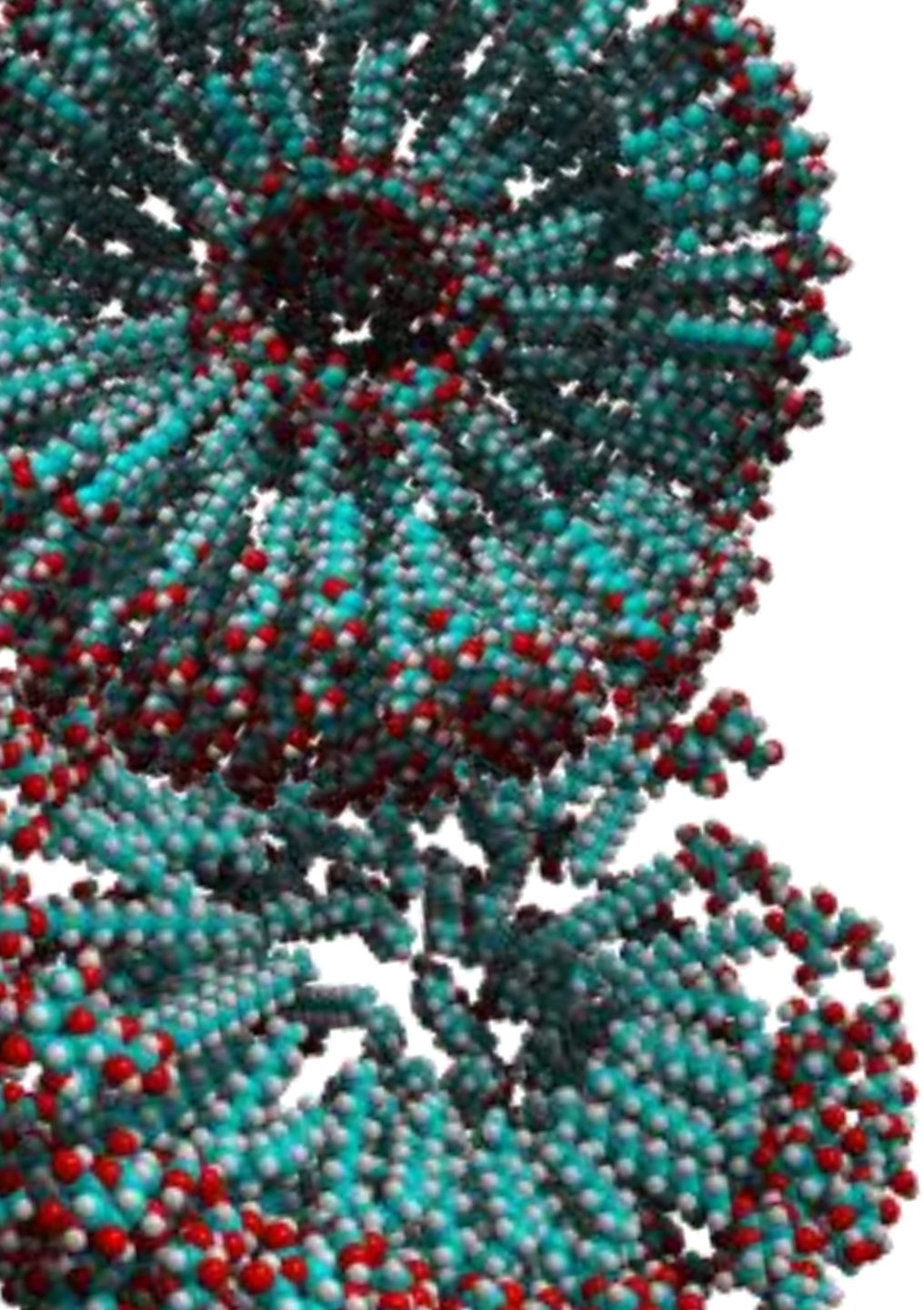


2018 IMAGINENANO Bringing together Nanoscience & Nanotecnnology

NanoSpain Conf 2018

Nanosystems as biomimetic interfaces: a new strategy to predict drug candidate biophysical profile

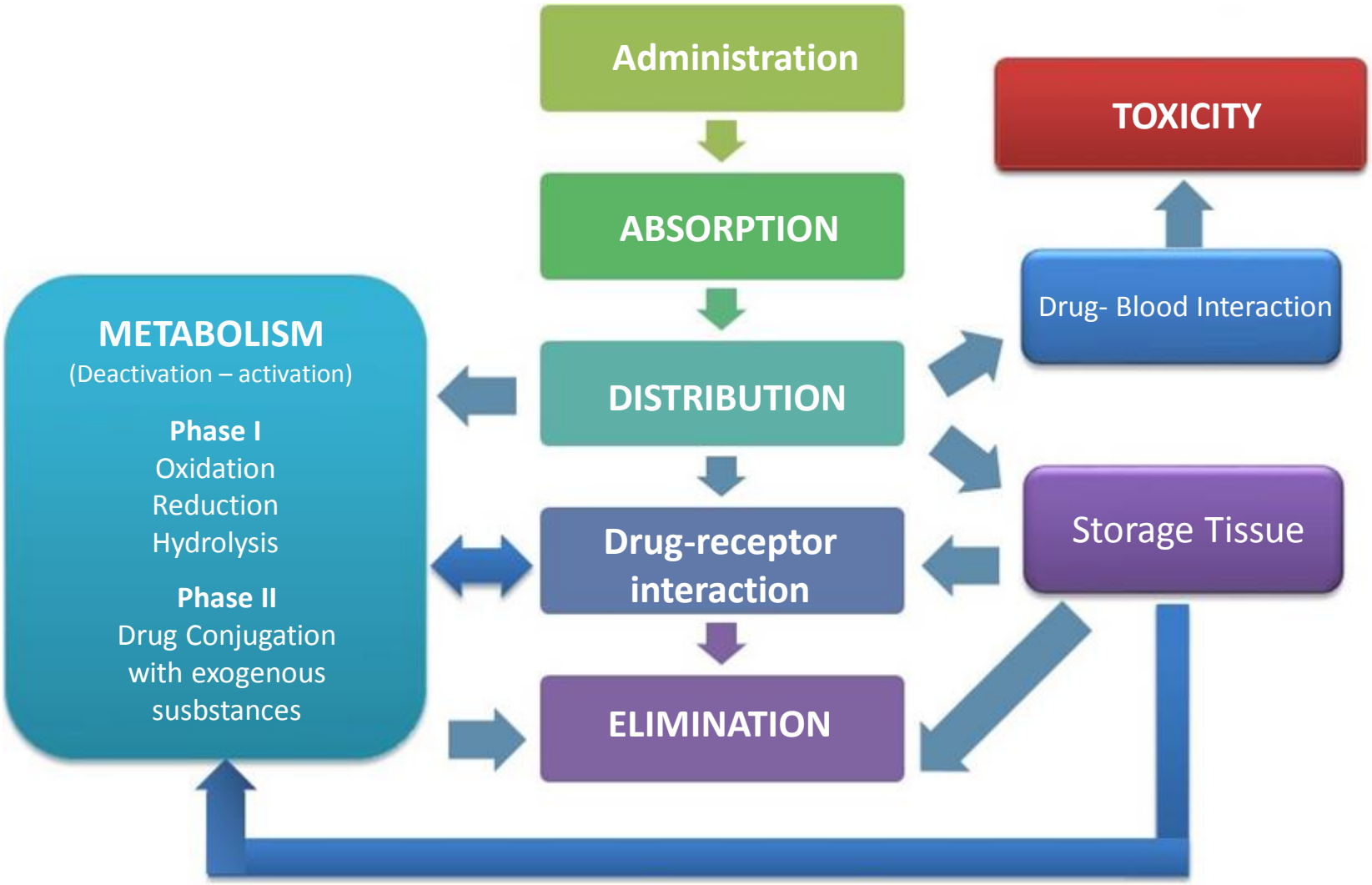
EDUARDA BARBOSA FERNANDES

MSc in Biophysics and Bionanosystems

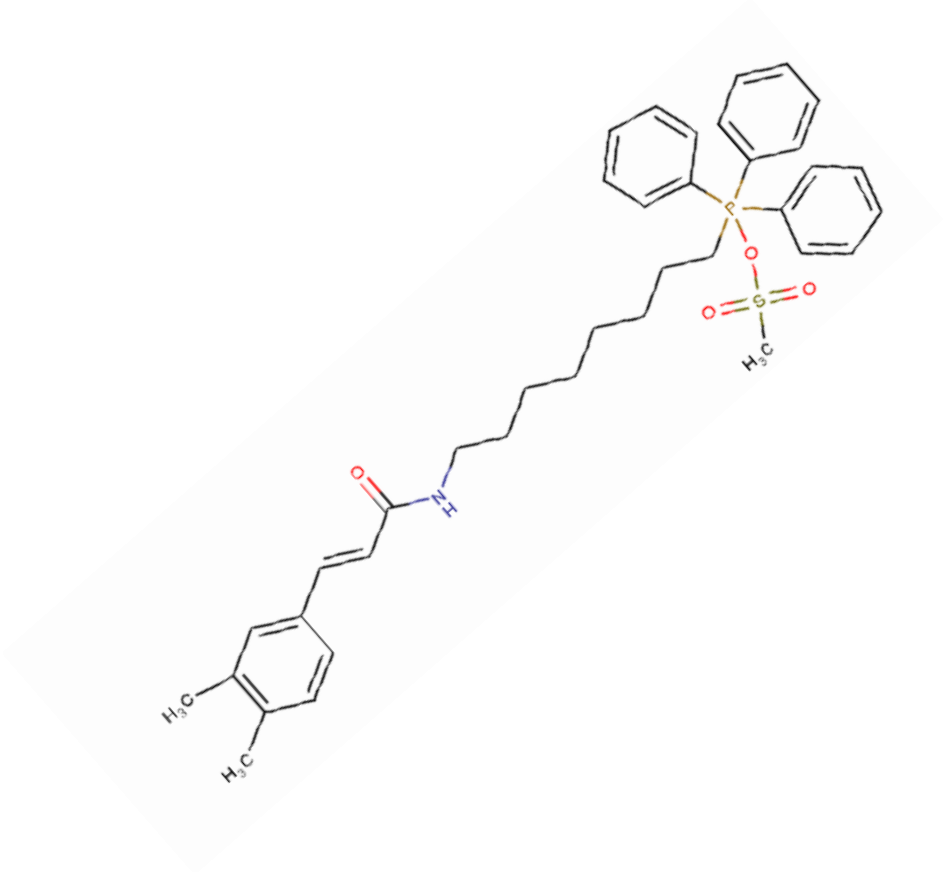
Cofinanciado por:

COMPETE 2020 PORTUGAL 2020 UNIÃO EUROPEIA Fundo Europeu de Desenvolvimento Regional IAPMEI Parcerias para o Crescimento

PHARMACOKINETIC DRUG-PROFILING



MITOCIN-3 PROFILE

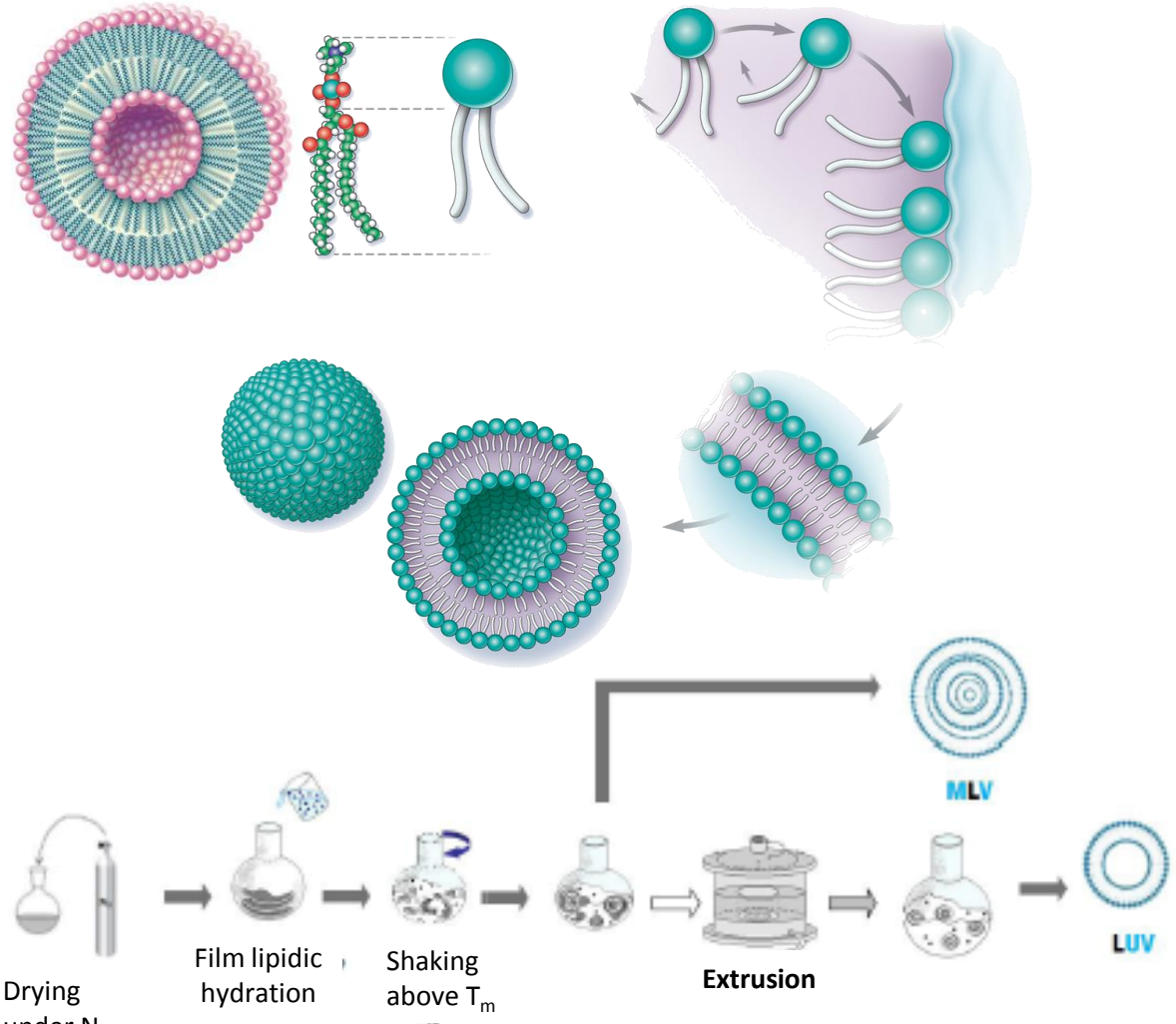


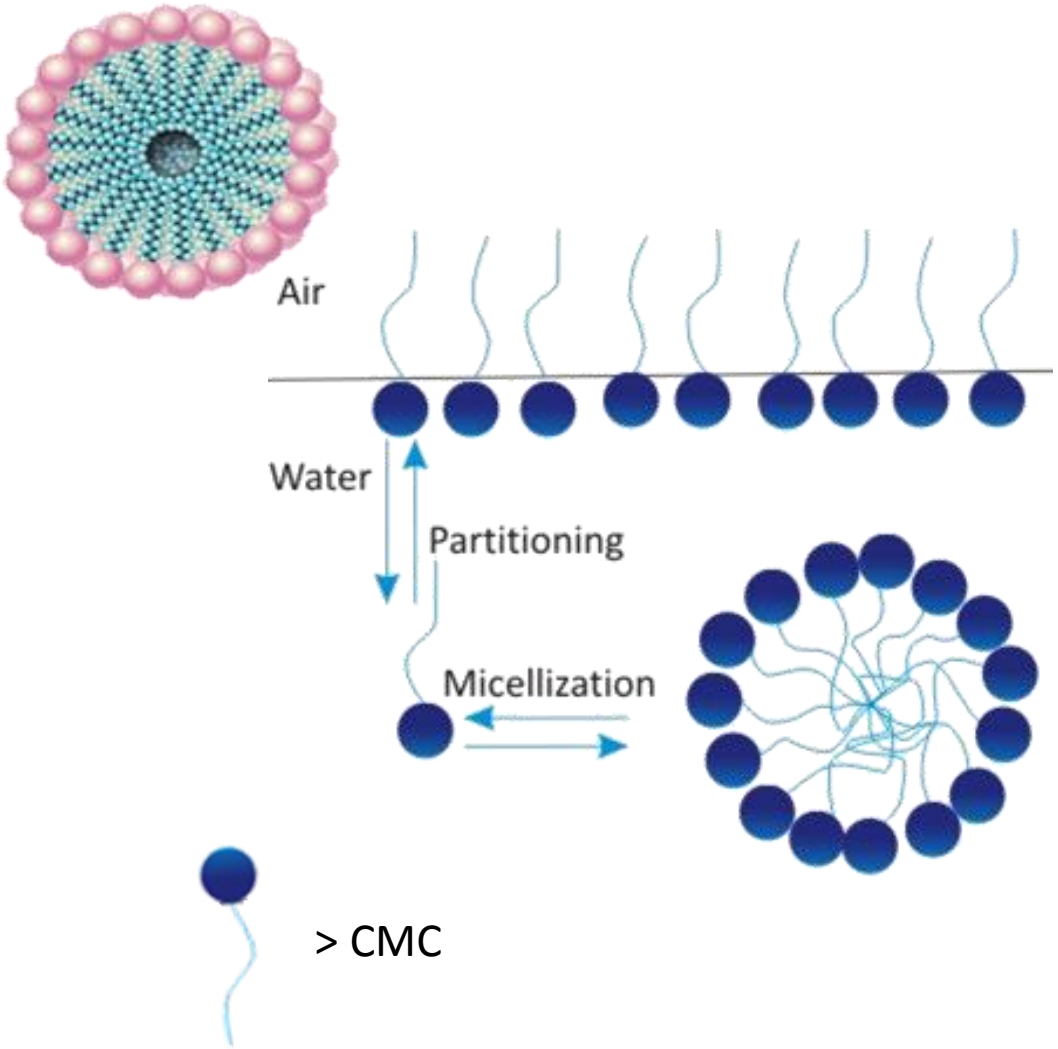
Oral Administration

Blood Brain Barrier Penetration

Non-toxic to mitochondrial membranes

LIPID-BASED NANOSYSTEMS



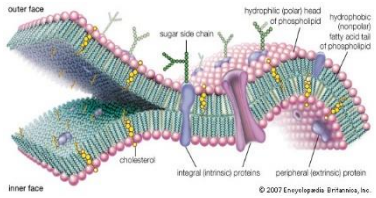


> CMC

CMC – Critical Micelar Concentration

CELL MEMBRANE

What happens *in vivo*?

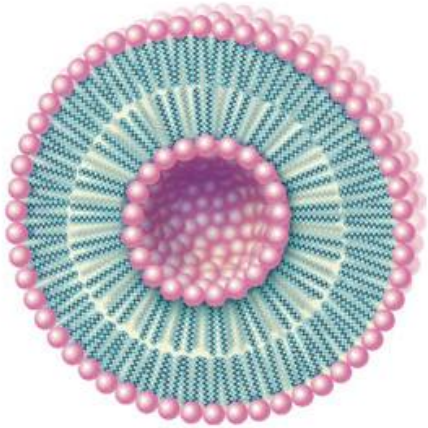


Composition:
PL (40-70%; inner: PS, PE; outer: PC, SM)
CHOL (0-50 %)
GL (0-26%)
RAFTS:PC:SM:CHOL (1:1:1)

Lipid phase:
L_α : L_d phase + L_O (≈ L_β phase in rafts)
L_β phase in oxidized membranes

pH = 7,4

How we mimic it?



LUVs made of DMPC

What we measure?

ABSORPTION

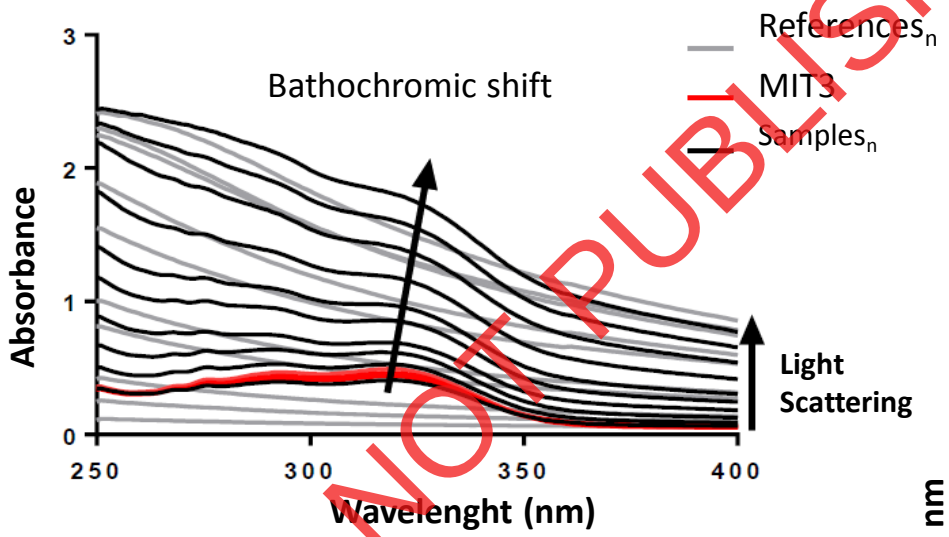
Lipophilicity
Membrane Permeability

How we measure?

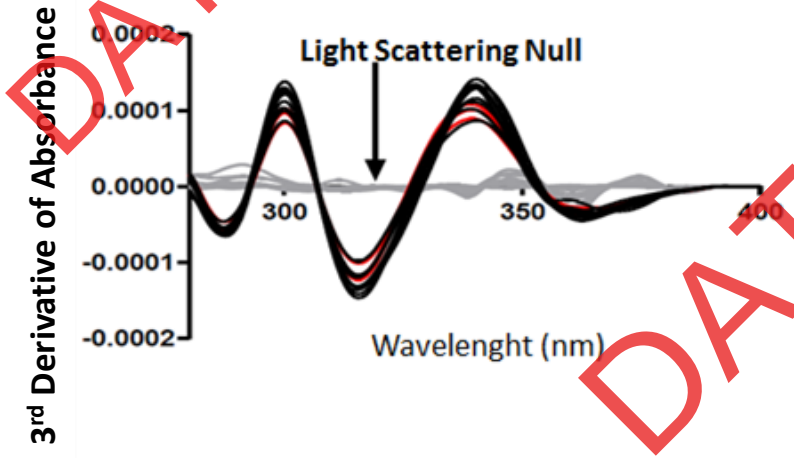
Derivative Spectroscopy

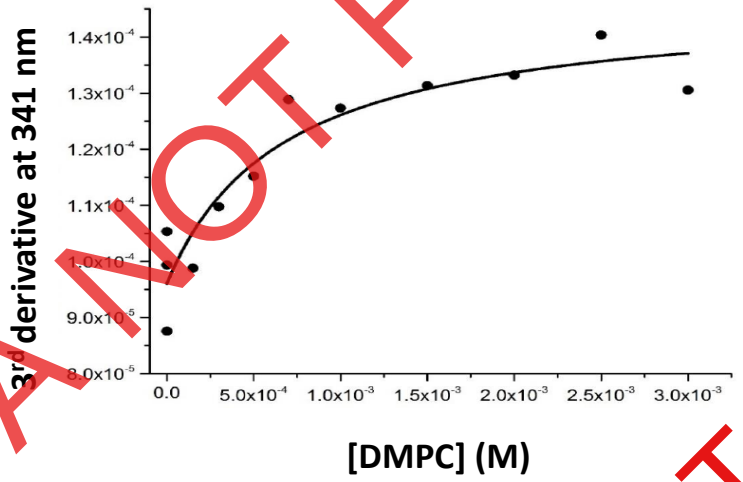
$$D = D_w + \frac{(D_m - D_w)Kp[L]V\phi}{1 + Kp[L]V\phi}$$

PARTITION COEFFICIENT IN CELL MEMBRANE MODEL



$$D = D_w + \frac{(D_m - D_w)Kp[L]V\phi}{1 + Kp[L]V\phi}$$



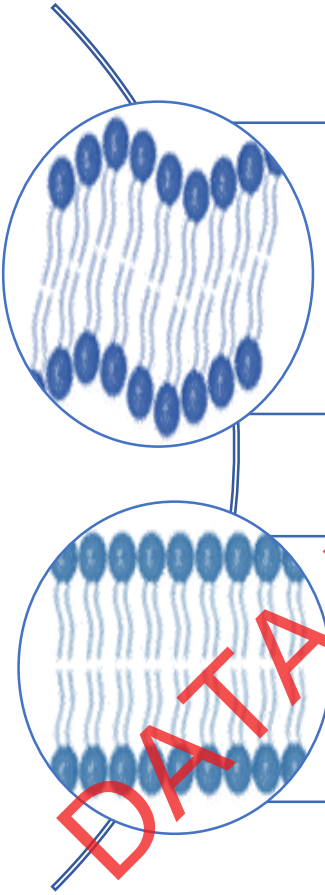


Log Kp = 3.34 ± 0.02

Moderate to high lipophilicity
Good balance between solubility and permeability

Good oral absorption
Absorption by **transcellular route**
Greater distribution in peripheral tissues than in liver

THERMODYNAMIC PARAMETERS OF PARTITION



ΔH = -14.9 KJmol^{-1}

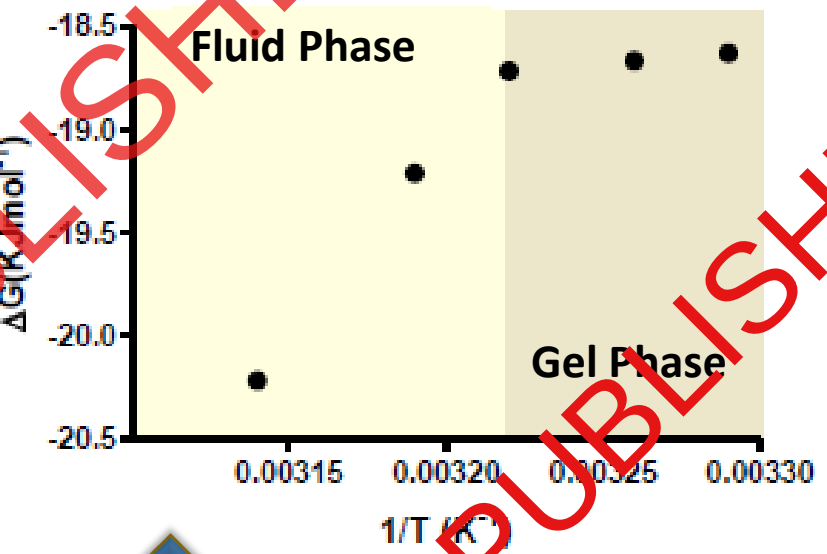
T$\Delta S_{average}$ = 3.68 KJmol^{-1}

$\Delta G_{average}$ = - 19.72 KJmol^{-1}

ΔH = 43.9 KJmol^{-1}

T$\Delta S_{average}$ = 63.1 KJmol^{-1}

$\Delta G_{average}$ = - 18.67 KJmol^{-1}



Temperature
Greater drug penetration

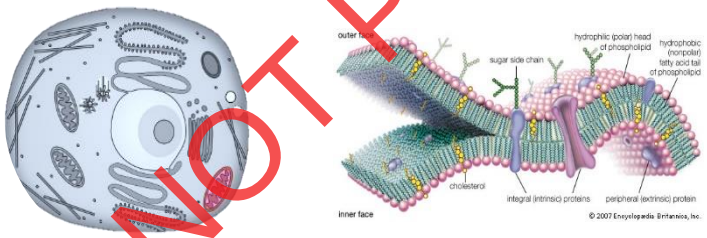
$$\ln(Kp) = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

↑ ΔS , ΔG, ΔH

Van der Waals interactions
Spontaneous process

CELL MEMBRANE

What happens *in vivo*?



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CHOL (0-50 %)
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RAFTS:PC:SM:CHOL (1:1:1)

Lipid phase:
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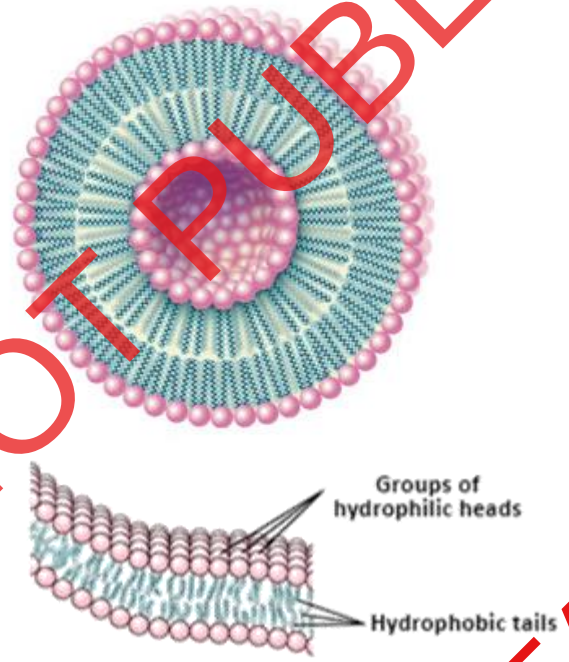
L_β phase in oxidized membranes

pH = 7,4

How we mimic it?



LUVs made of DMPC
(labelled with *n-AS* probes)

What we measure?

DISTRIBUTION

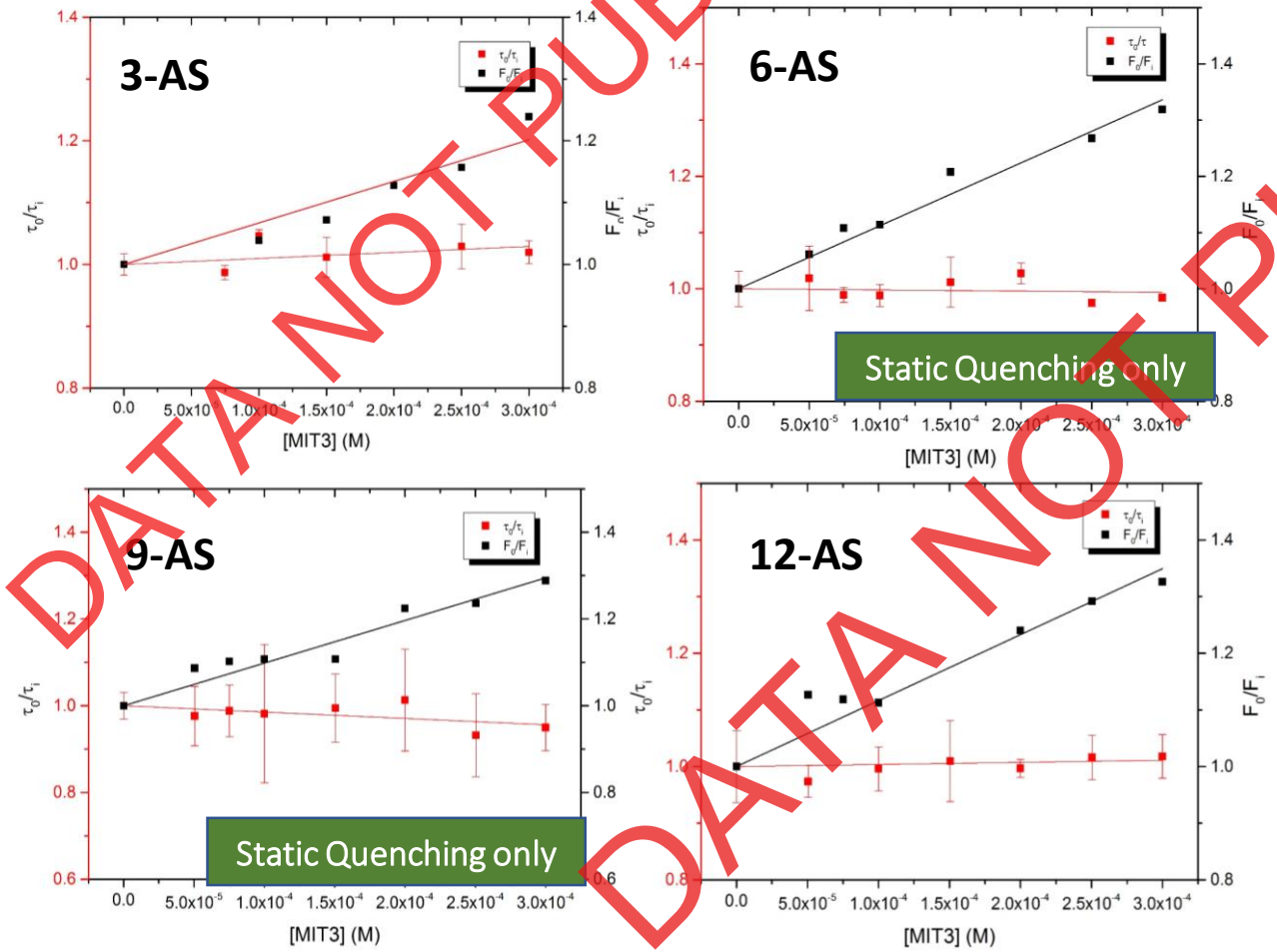
Membrane Location

How we measure?

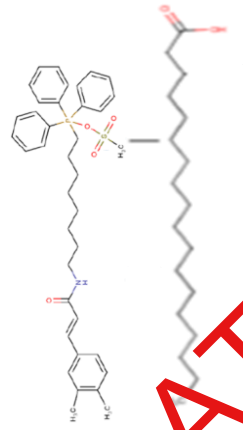
Time resolved fluorescence (TCSPC)
Steady state fluorescence

MEMBRANE LOCATION

$$\frac{F_0}{F} = 1 + k_q \tau_0 [MIT3] = 1 + k_D [MIT3]$$



Constant Probe	K_D (M^{-1})	K_S (M^{-1})	K_q ($M^{-1} s^{-1}$)
3 - AS	97,48	575,1	1,03x10[illegible]
6 – AS	0	1120,4	1,42x10[illegible]
9 – AS	0	983,1	1,28x10^{11}
12 - AS	37,00	1127,4	1,17x10^{11}

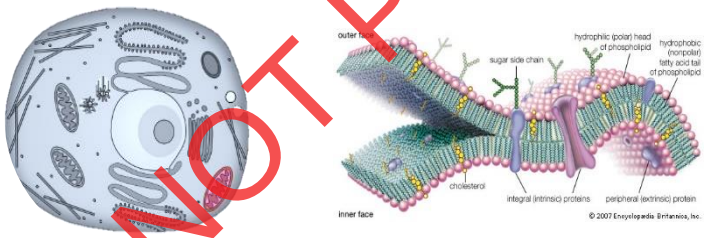


Kq > 1,[illegible]x10^{10} $M^{-1}s^{-1}$ for all probes
Greater quenching in C6

Ubiquitous location in the lipid bilayer
Deactivating groups near C6

CELL MEMBRANE

What happens *in vivo*?



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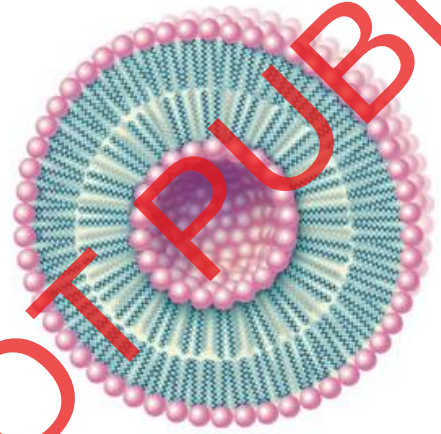
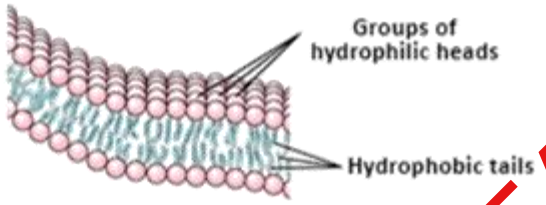
L_β phase in oxidized membranes

pH = 7,4

How we mimic it?





LUVs made of DPPC

What we measure?

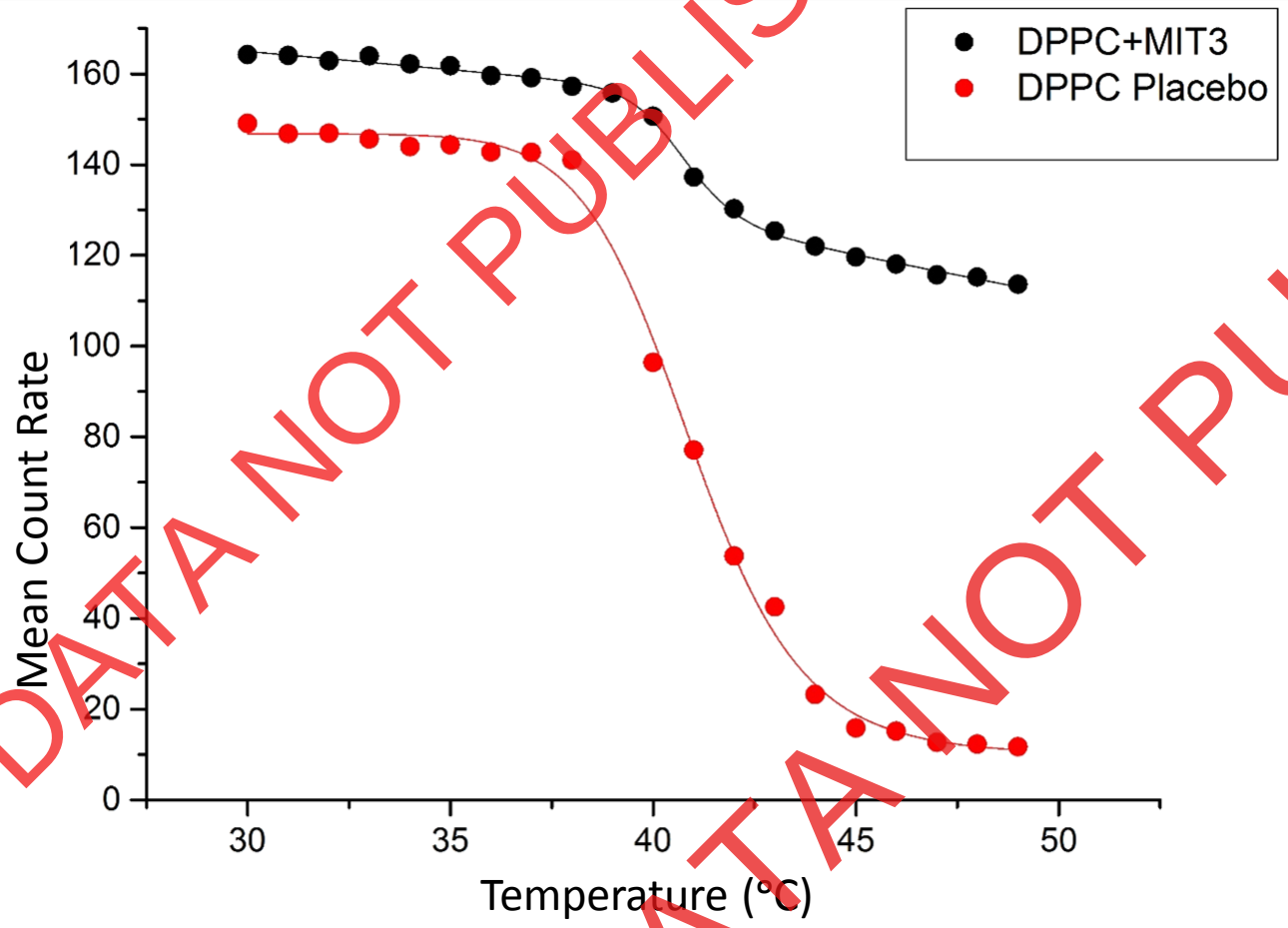
Toxicity

Biophysical Changes in Biomembranes

How we measure?

Differential Scanning Calorimetry
Small-angle X-Ray Scattering
Wide-angle X-Ray Scattering
Dynamic Light Scattering

BIOPHYSICAL CHANGES IN BIOMEMBRANES



$$MCR = r_{s1} + p_1 T + \frac{r_{s2} - r_{s1} + p_2 T - p_1 T}{1 + 10^{B\left(\frac{1}{T} - \frac{1}{T_m}\right)}}$$

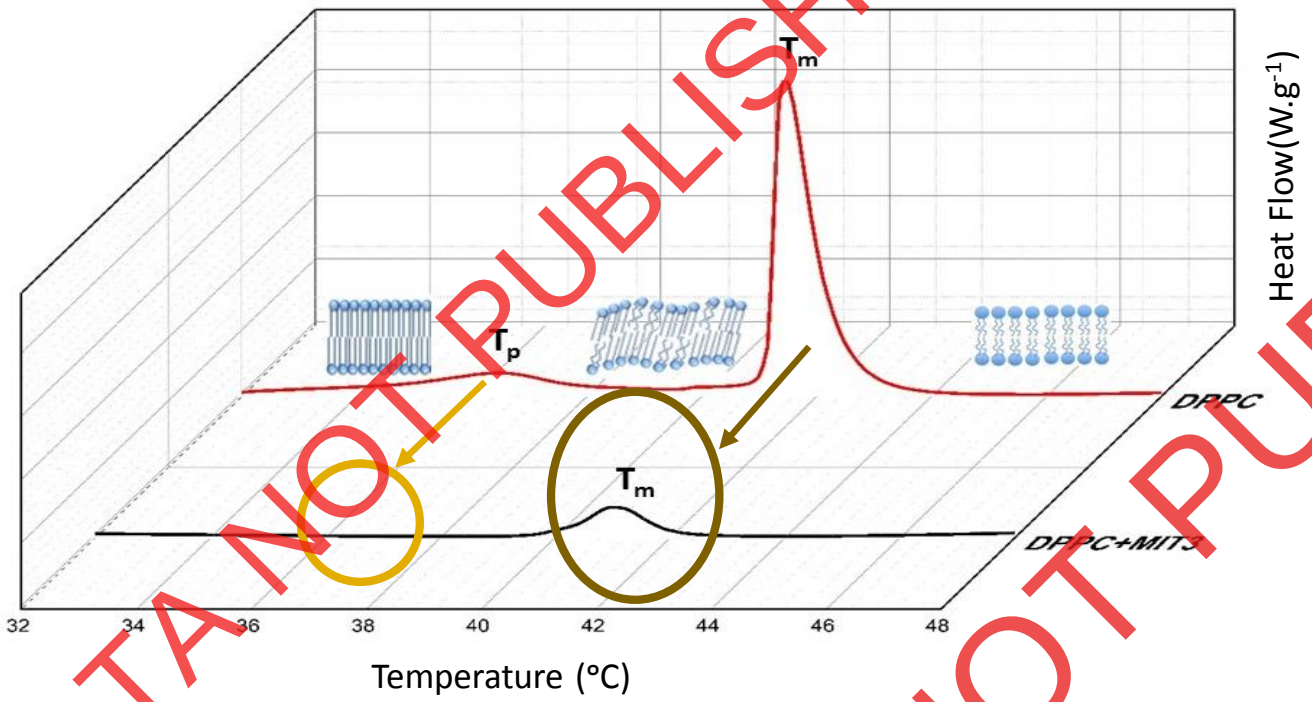
System	Cooperativity	T_m (°C)
DPPC placebo	855.82	41.28
DPPC+MIT3	661.52	41.12

$\Delta T \approx K$

↘ Cooperativity

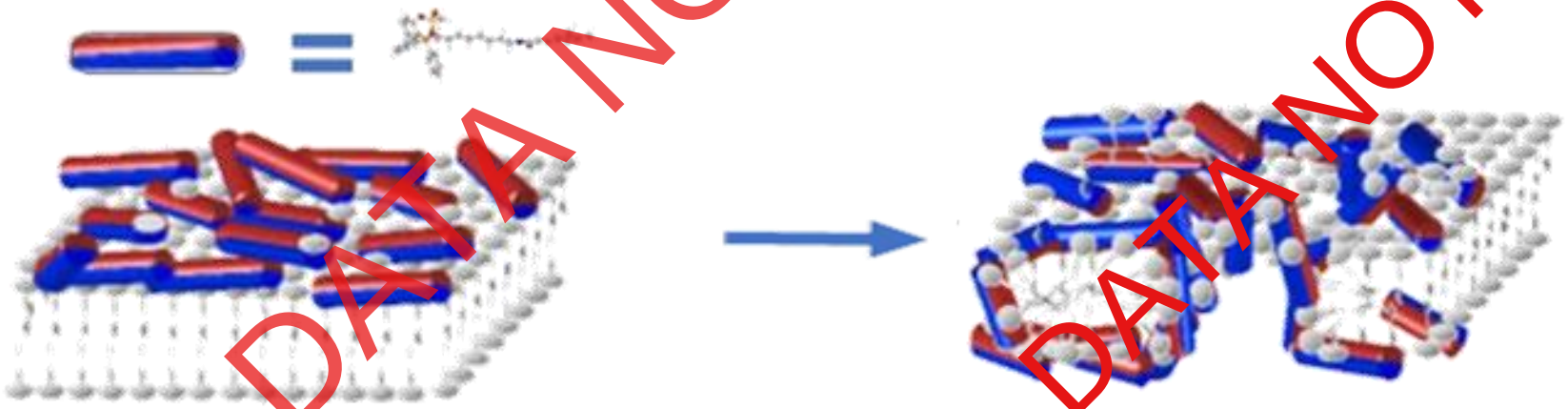
No membrane disruption at low concentrations

DIFFERENTIAL SCANNING CALORIMETRY STUDIES (DSC)

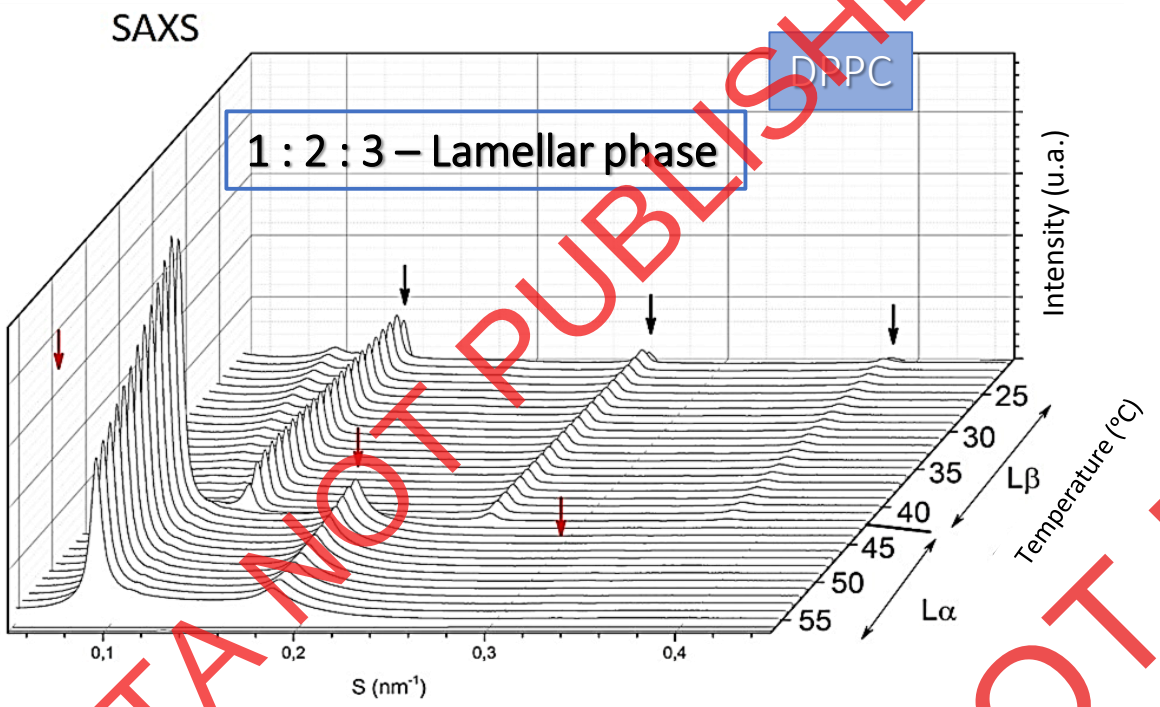


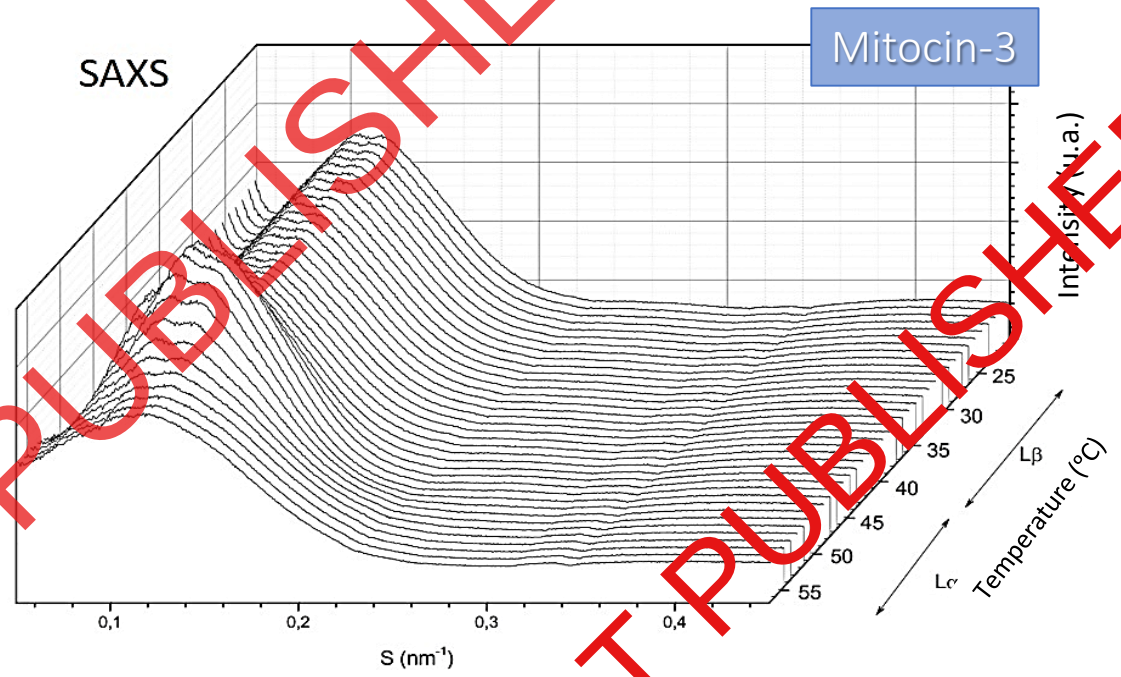
Tm ≈ K
↓ Cooperativity
↓ Δ*H*

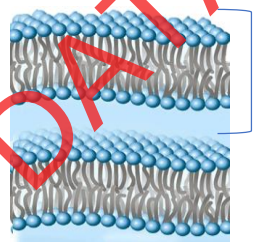
Influence at headgroup region (C1 to C8)
Major perturbations of biophysical parameters
Hydrophobic Interactions



SMALL-ANGLE X-RAY SCATTERING (SAXS)







d_L

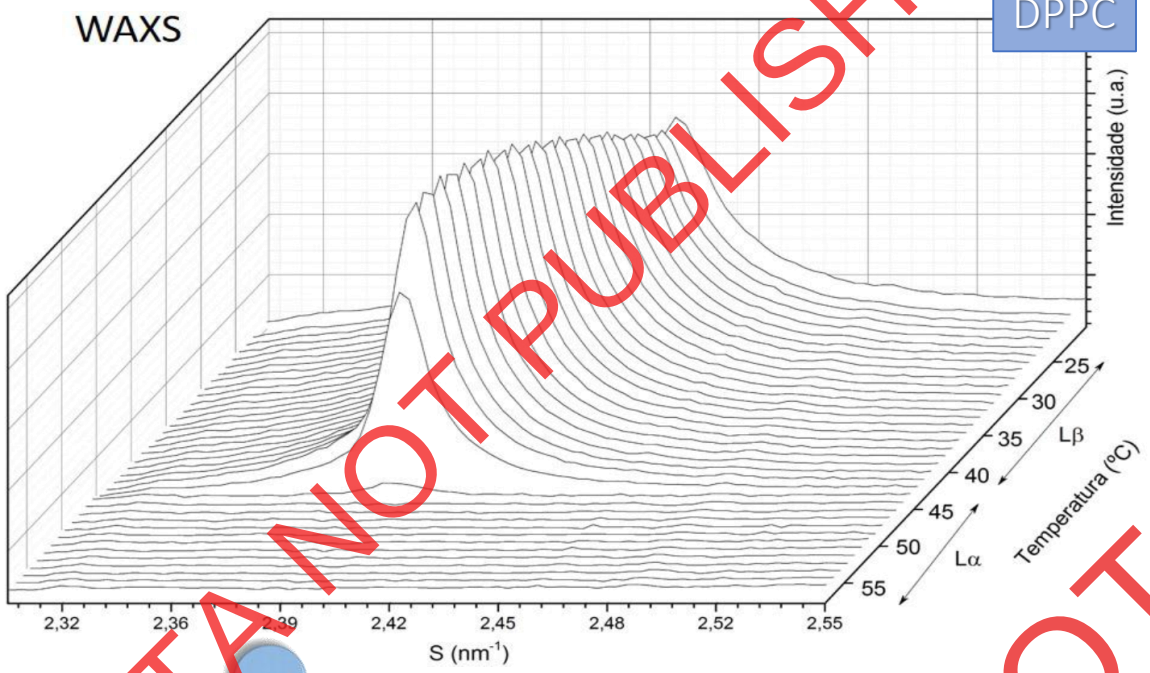
	DPPC PLACEBO		DPPC+MIT3	
	d_L (Å)	ξ (Å)	d_L (Å)	ξ (Å)
GEL PHASE	76.52	10971	90.24	249
FLUID PHASE	77.60	11170	91.24	449

$\uparrow d_L$, $\downarrow \xi$

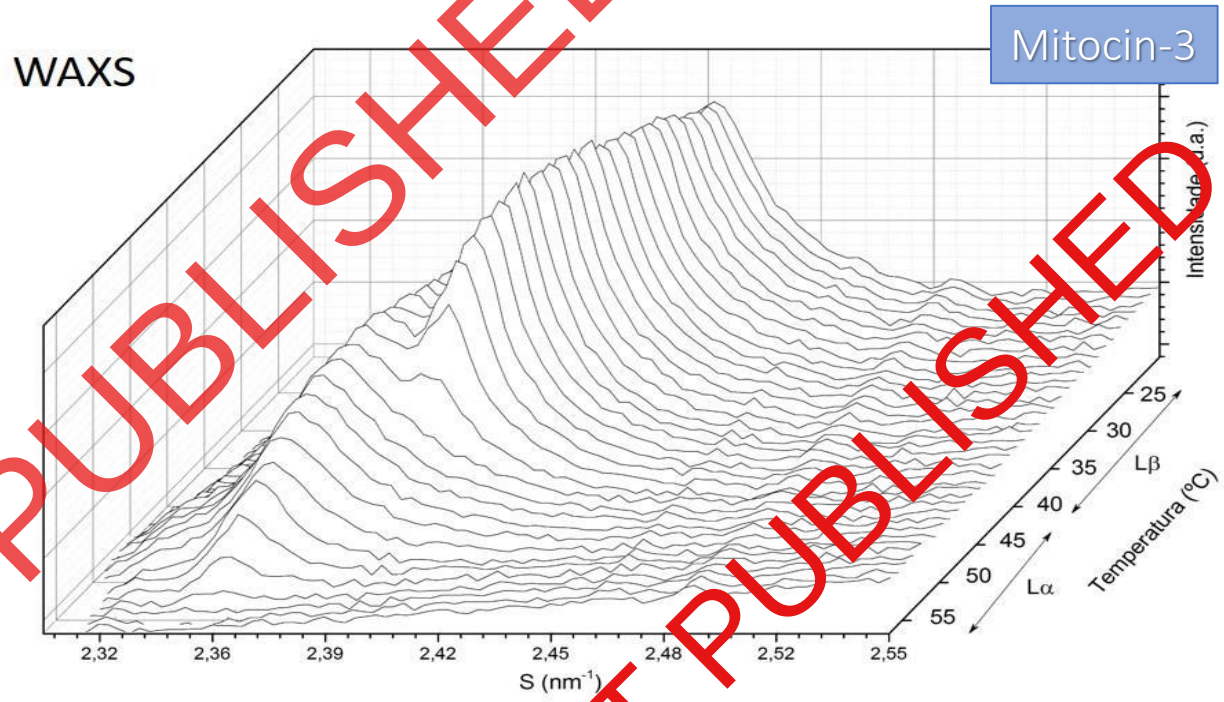
Increased hydration layer

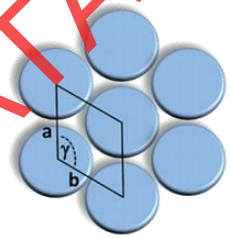
WIDE-ANGLE X-RAY SCATTERING (WAXS)

DPPC



Mitocin-3





	DPPC PLACEBO	DPPC+MIT3
	d_C (Å)	d_C (Å)
GEL PHASE	4.2 4.1	4.2 4.1
FLUID PHASE	-	4.2

Pseudohexagonal Packing

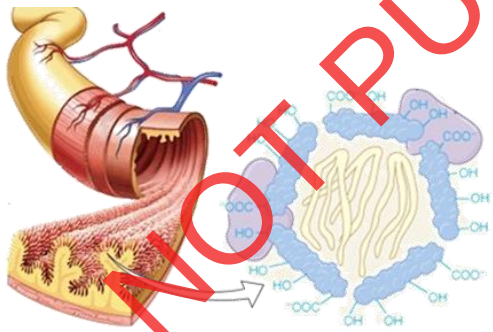
Hexagonal Packing

$d_c \approx K$

No disruption of lipid packing

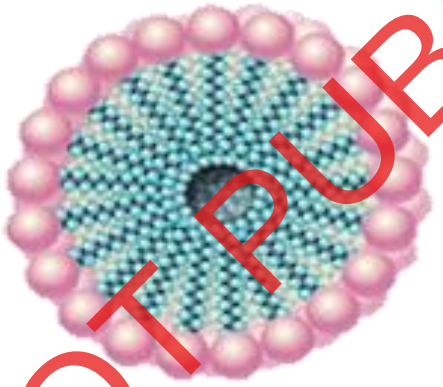
BILIAR SALTS

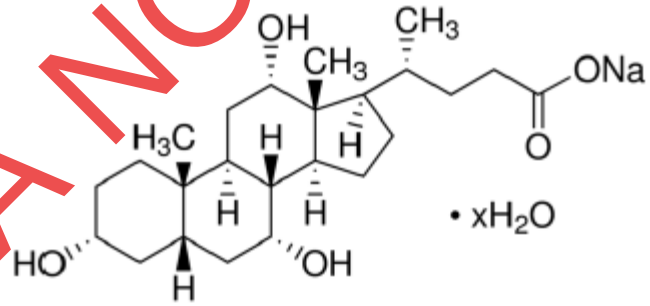
What happens *in vivo*?



Composition:
Bile salts (glycholate, deoxycholate)
Lipase
Trygliceride

How we mimic it?





Micelles made by sodium cholate

What we measure?

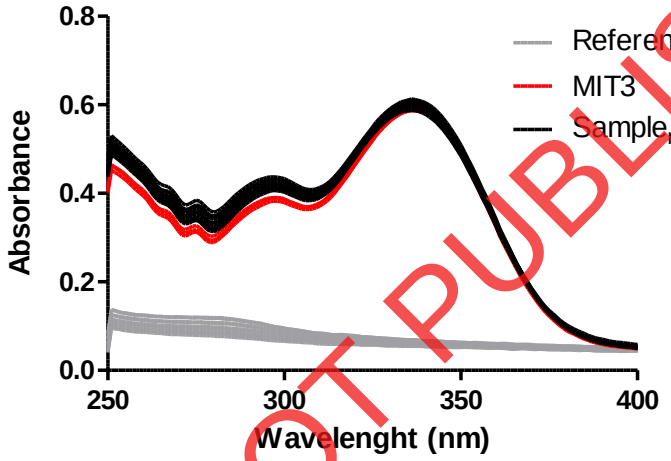
Absorption

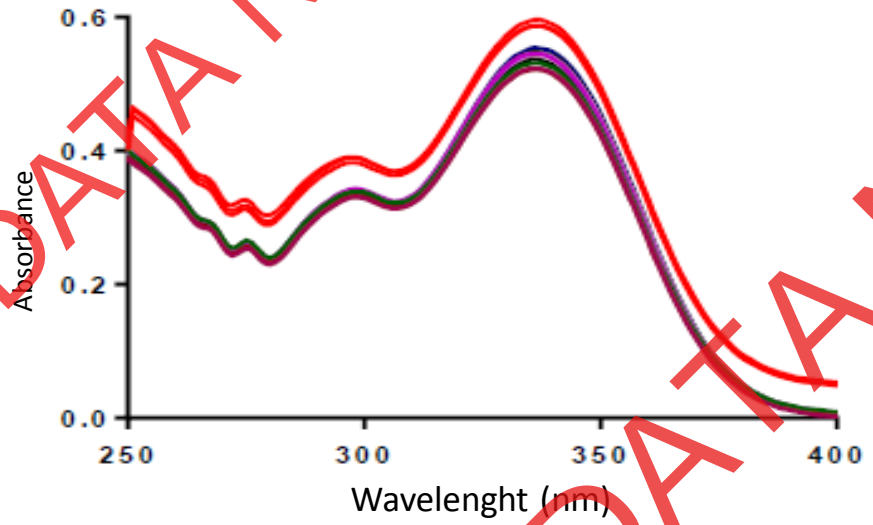
Lipophilicity
Intestinal Permeability

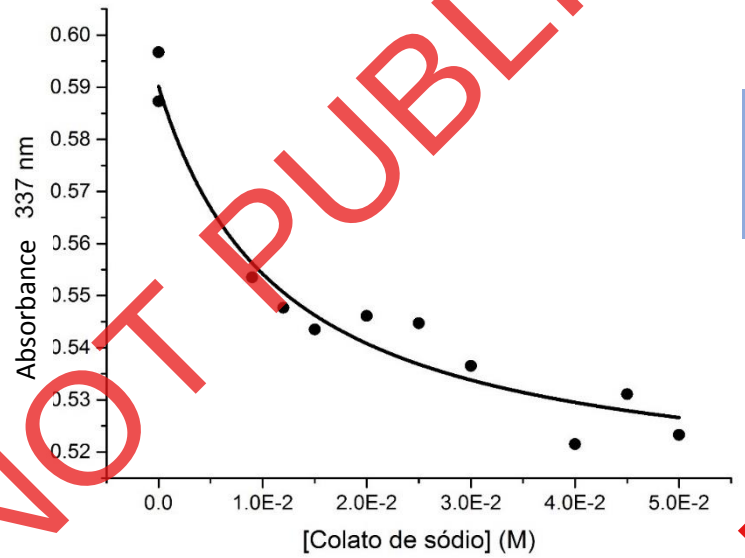
How we measure?

Spectrophotometry

PARTITION COEFFICIENT IN BILIAR SALTS MICELLES







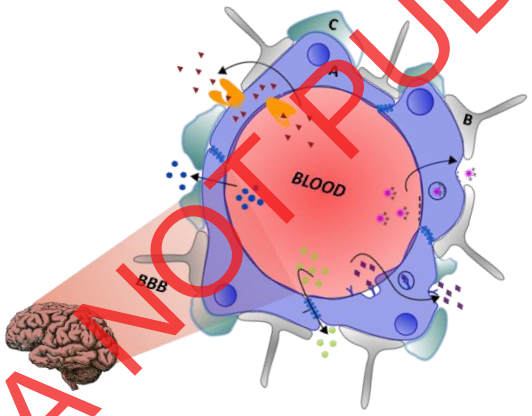
LogKp = 2.31 ± 0.08

Log P > 0
Permeation by transcytosis

Tendency for **good intestinal absorption** of the compound

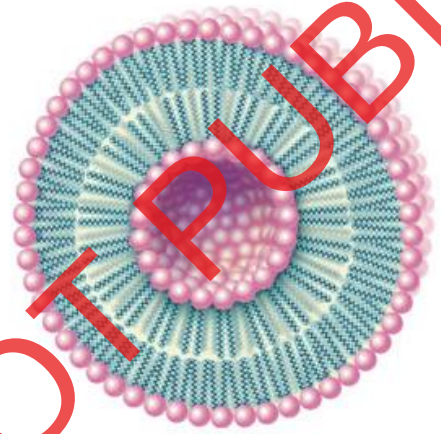
BLOOD-BRAIN BARRIER

What happens *in vivo*?



Composition:
PI (4,8 – 5,5%)
PE 22,8 – (25,2%)
PC 28,7 – (33,2%)
PS (6,8 – 10,7 %)
SM (33,4%)
CHOL (20,8%)

How we mimic it?



LUVs made by Porcine Brain Extract

What we measure?

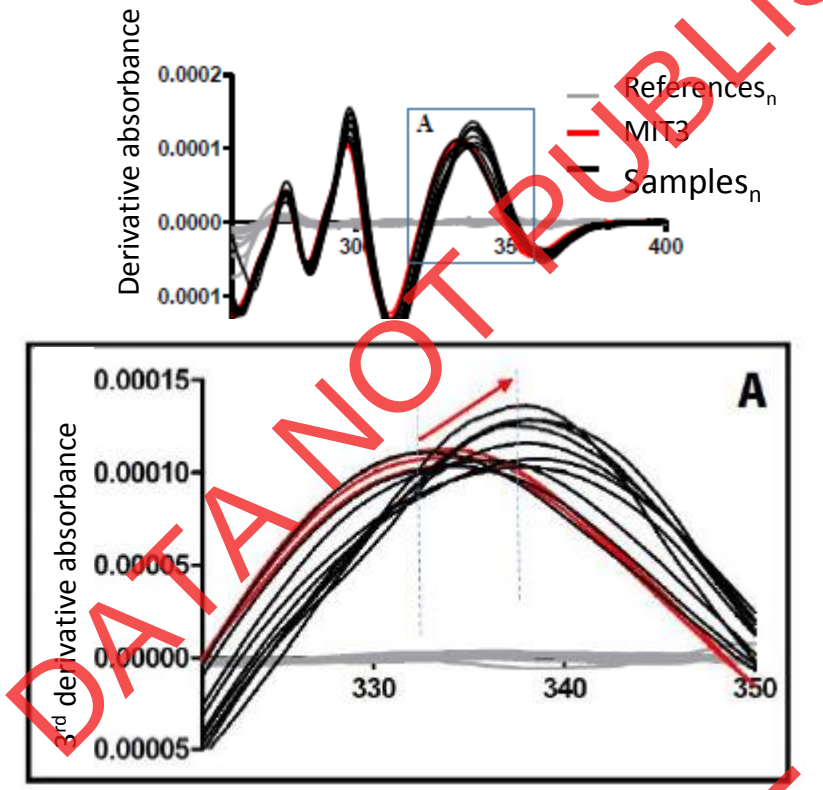
Distribution

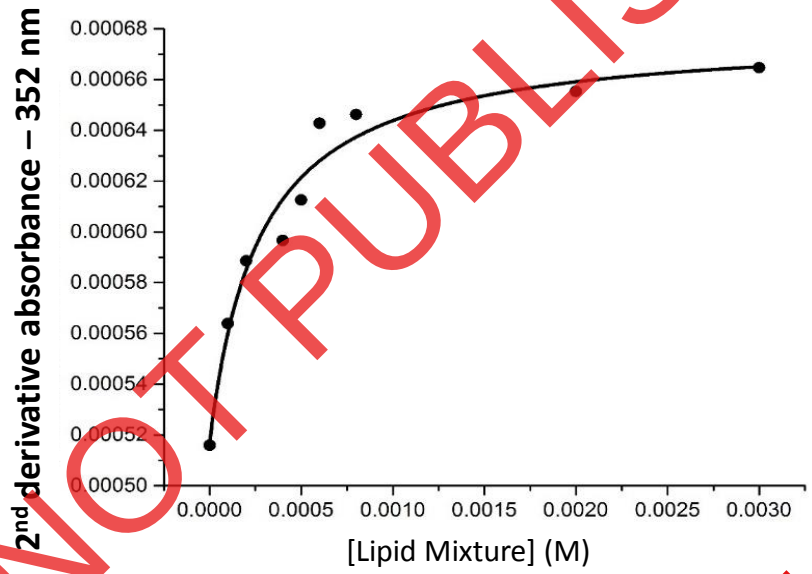
BBB Permeability

How we measure?

Derivative Spetroscopy

BBB PERMEABILITY





Log Kp = 3.64 ± 0,25

$$LogBB = \log\left(\frac{C_{brain}}{C_{blood}}\right)$$

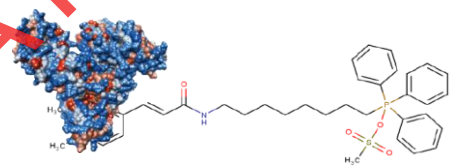
Log BB = 2.77

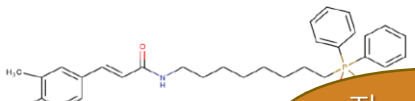
Good penetration through BBB

PLASMA PROTEIN BINDING

What happens *in vivo*?

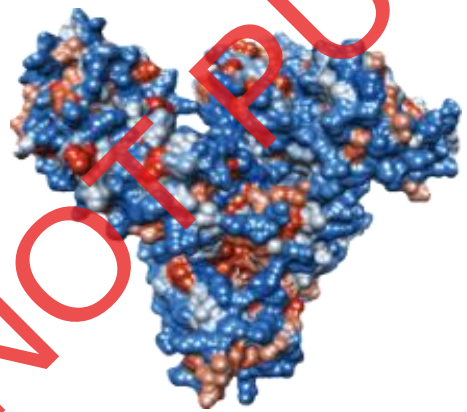
Composition:
Human Plasma 55%
- 7% proteins (HSA - 60%)





Therapeutic effect

How we mimic it?



What we measure?

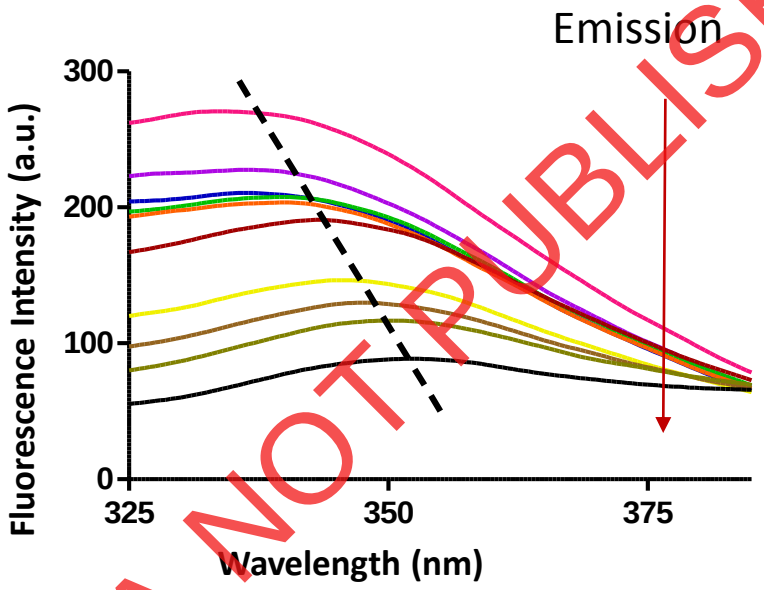
Distribution/Excretion

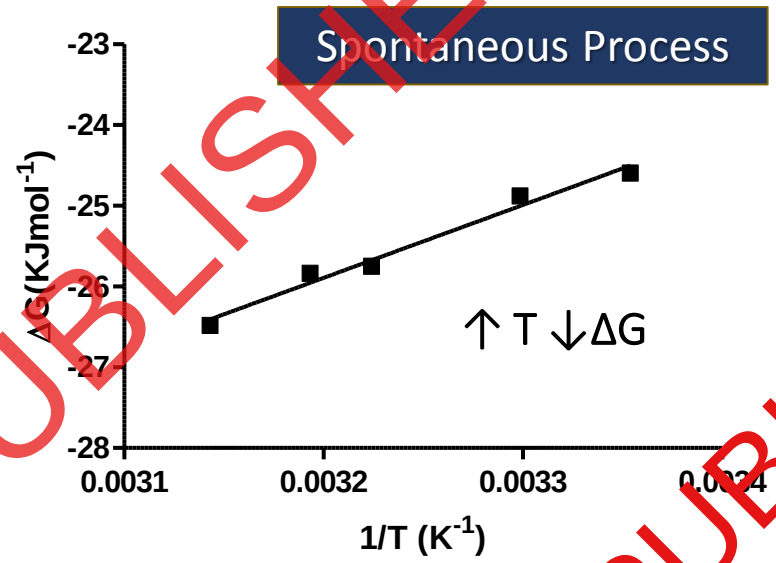
Plasma Protein Binding

How we measure?

Fluorescence
Synchronous Fluorescence
DLS
ELS

FLUORESCENCE QUENCHING





T (°C)	n	K_D (M)
25	1	4.91 x10^{-5}
30		5.17 x10^{-5}
37		4.61 x10^{-5}
40		4.90 x10^{-5}
45		4.49 x10^{-5}

K_D < 100 µM

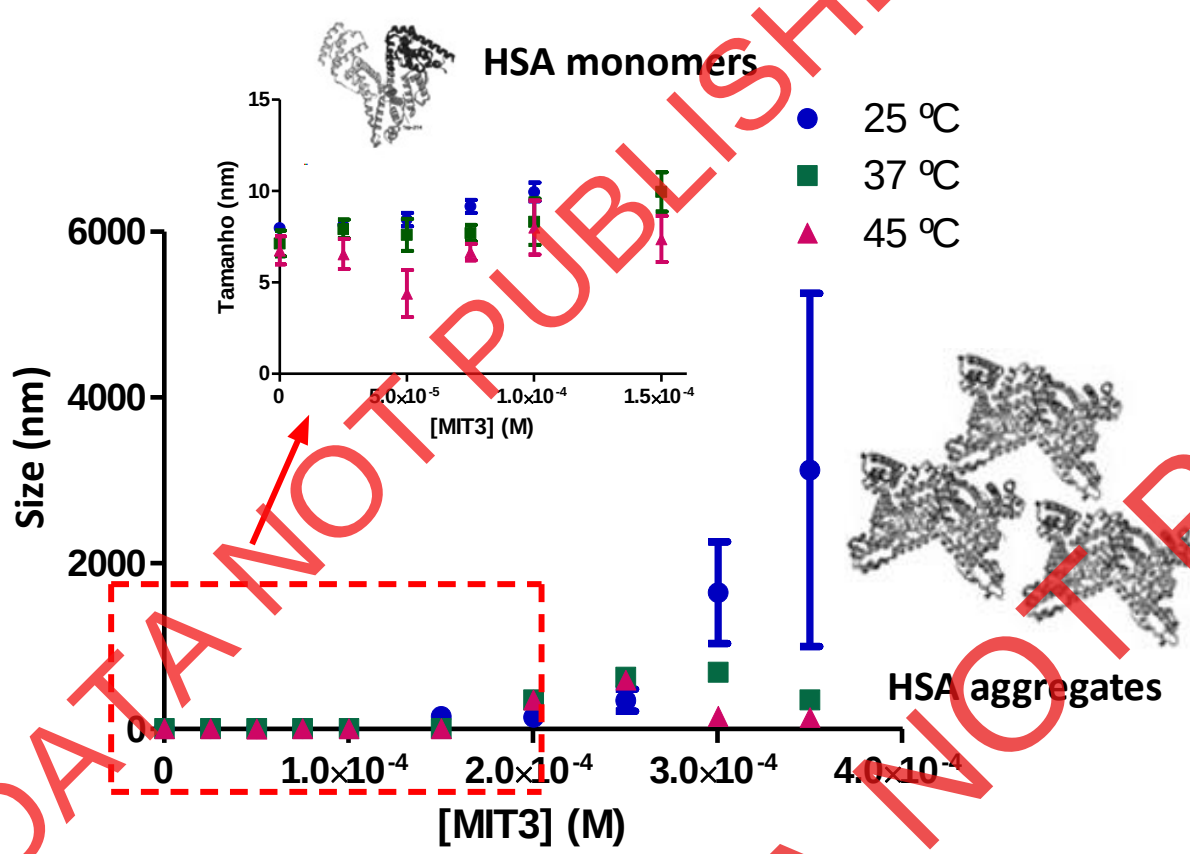
$\Delta H > 0$
$\Delta S > 0$

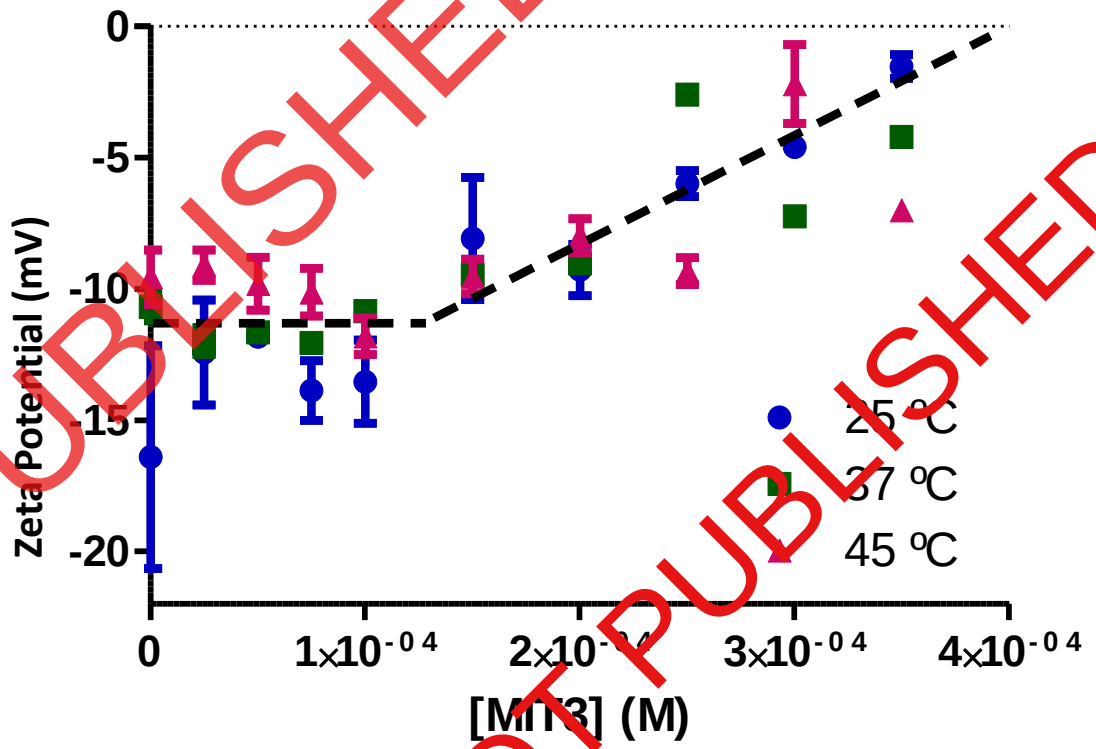
$\Delta G < 0$
K_D < 100 µM

Hydrophobic Interaction

Spontaneous and Stronger interaction

DLS E ELS





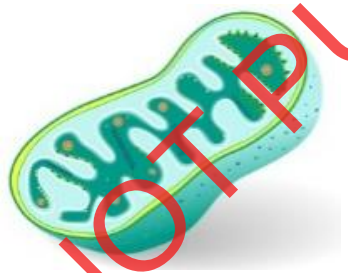
Saturation at HSA $2x10^{-4}$ M

Formation of aggregates and neutralization of surface charge

Interaction confirmed

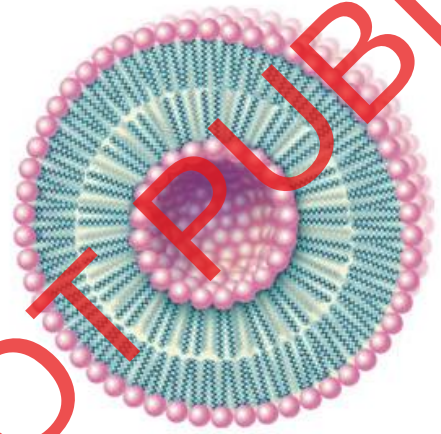
INNER MITOCHONDRIAL MEMBRANE

What happens *in vivo*?



Composition of i.m.m.
PC:PE:CL (1:1:1)
Lipid phase:
$L_\alpha + H_{II}$
pH = 7,4

How we mimic it?



MLVs made by mixture:
DSPC:DOPE:CL

What we measure?

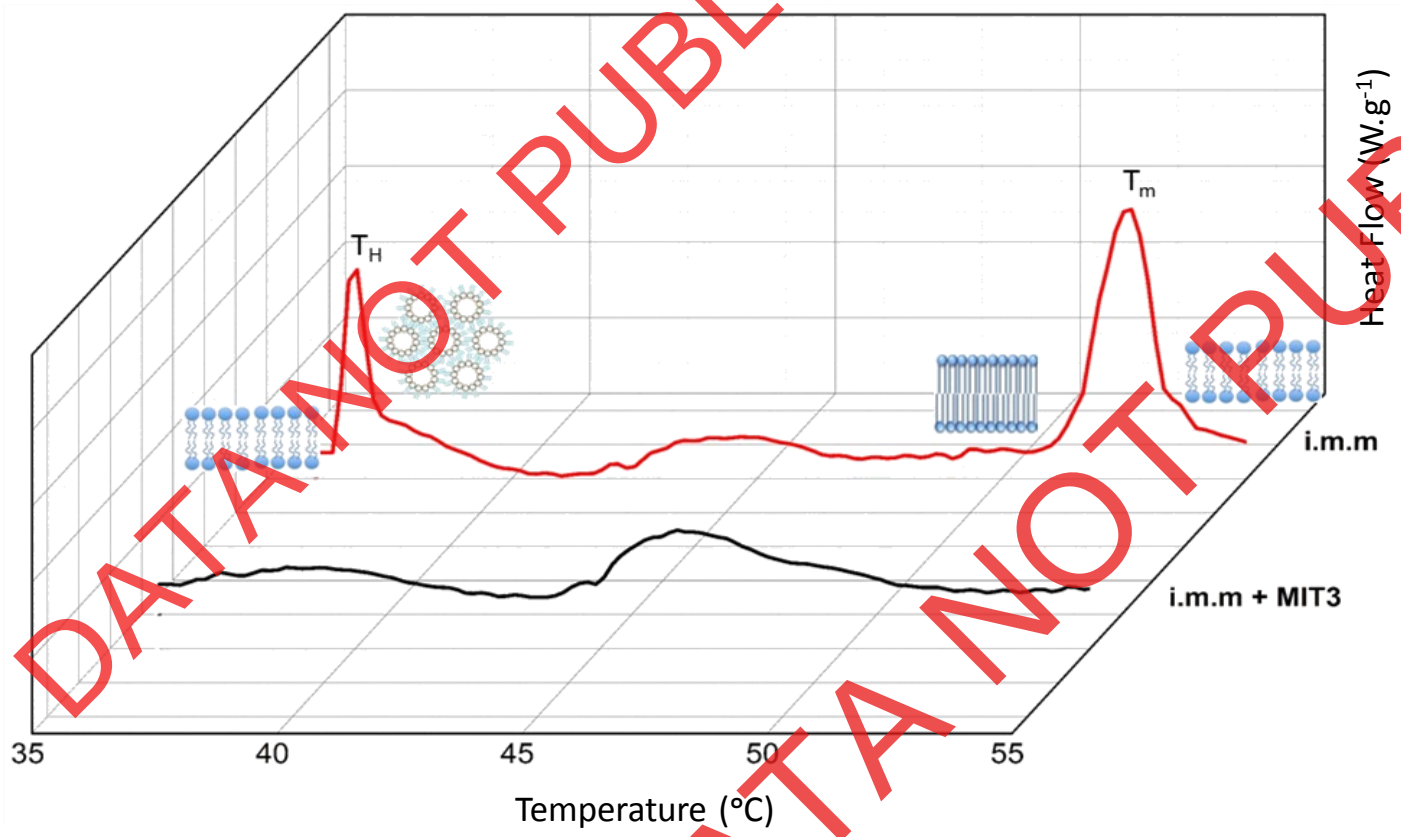
Toxicity

Biophysical Changes in Biomembranes

How we measure?

DSC

DIFFERENTIAL SCANNING CALORIMETRY STUDIES (DSC)

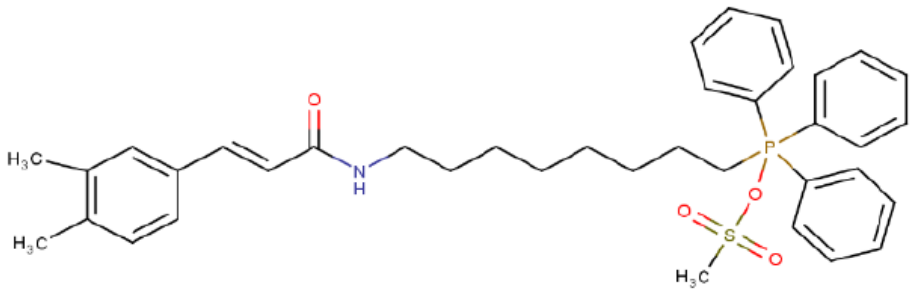


Single Endotherm
No thermodynamic changes

Homogenization of lipid components

Not correlated with i.m.m toxicity

CONCLUSIONS



MITOCIN-3

- GOOD MEMBRANE PERMEABILITY
- OFF-TARGET DISTRIBUTION
- MEMBRANE TOXICITY IN HIGH CONCENTRATIONS
- GOOD INTESTINAL ABSORPTION
- BIOACCUMULATION
- GOOD PENETRATION AND DISTRIBUTION AT THE TARGET

AKNOWLEDGEMENTS

Universidade do Minho

Eduarda Fernandes
CFUM – University of Minho

Marlene Lúcio
CFUM – University of Minho

U.PORTO

Sofia Benfeito
CIQUP – University of Porto

Fernando Cagide
CIQUP – University of Porto

Fernanda Borges
CIQUP – University of Porto

Cofinanciado por:

COMPETE 2020 PORTUGAL 2020 UNIÃO EUROPEIA Fundo Europeu de Desenvolvimento Regional IAPMEI Parcerias para o Crescimento Elettra Sincrotrone Trieste | Elettra and FERMI lightsources Elettra nº20155321/2016

FCT Fundação para a Ciência e a Tecnologia MINISTÉRIO DA CIÊNCIA E DO ENSINO SUPERIOR

UID/FIS/04650/2013
IF/00498/2012

QREN QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL PORTUGAL 2007.2013

UNIÃO EUROPEIA Fundos Estruturais

EYE On DRUG

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Thank you for your attention!

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