Concentration-Dependent Mechanisms of Melittin-Induced Membrane Destabilization: an Interdisciplinary Approach

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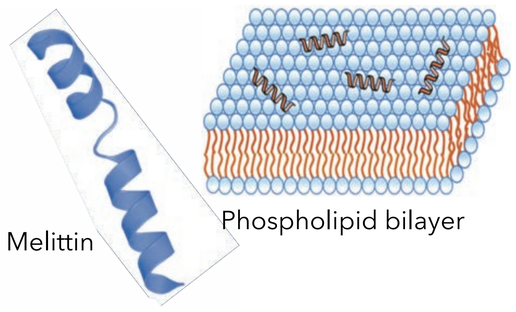
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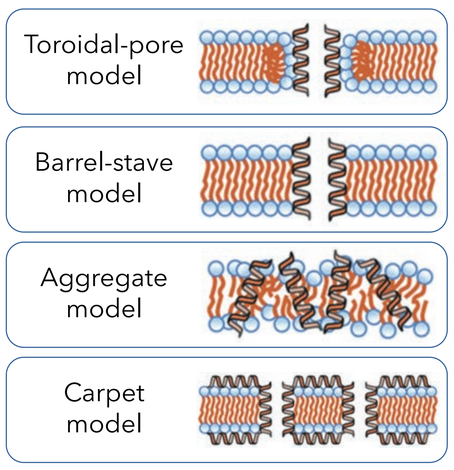
Introduction

Peptide – cell membrane interaction

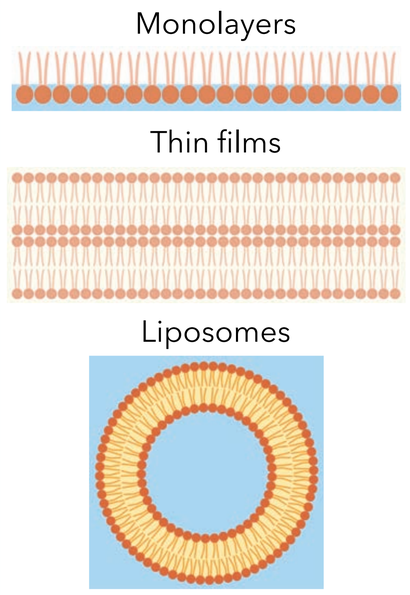


Antimicrobial peptides (AMPs) are promising alternatives to conventional antibiotics with different mechanisms of action

Possible mechanisms of action



Methods

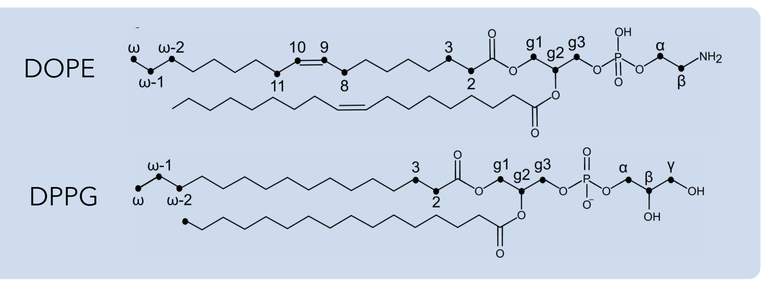


Complementary techniques that allow to obtain a full view of the mechanism of action of a peptide

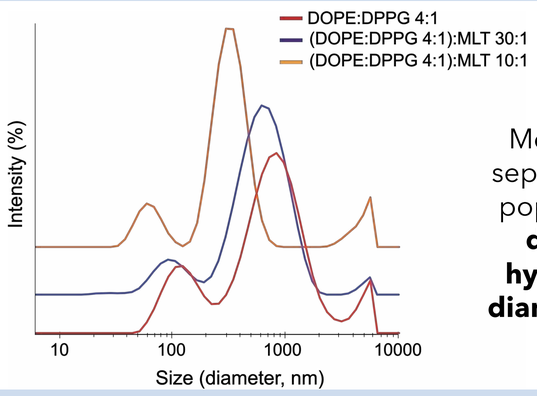
- Micro level: Langmuir-Blodgett (BAM, PM-IRRAS); DLS
- Nano level: AFM
- Atomic level: ss-NMR

Results

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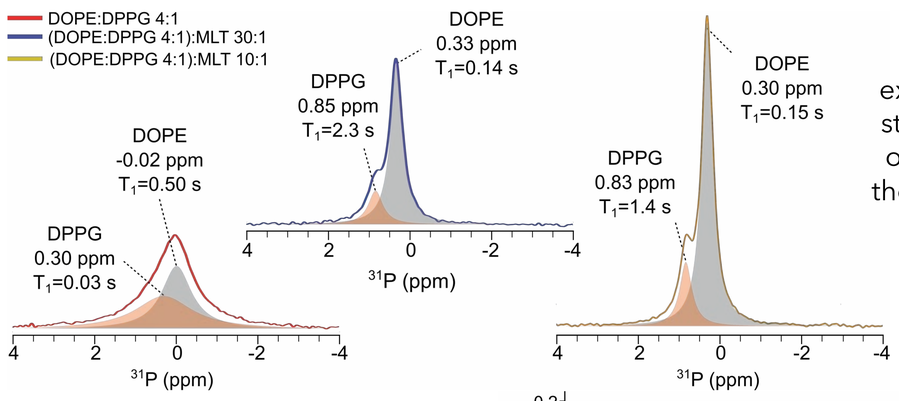
Dynamic light scattering (DLS)



Melittin causes separation of size populations and **decrease in hydrodynamic diameter** of MLVs

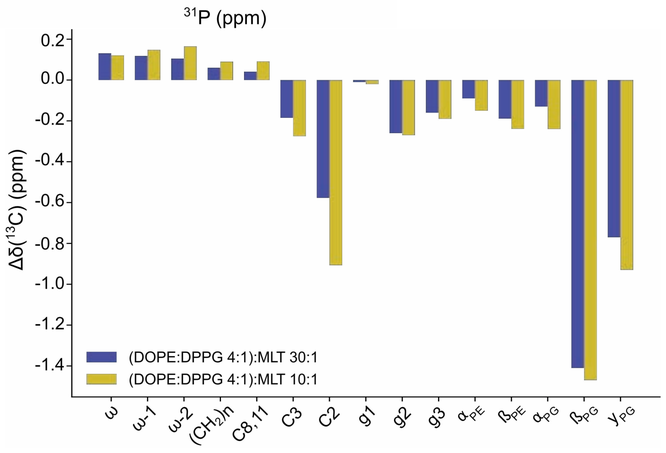
Solid-state NMR (ssNMR)

^{31}P @ MAS freq. of 10 kHz on a 700 MHz spectrometer



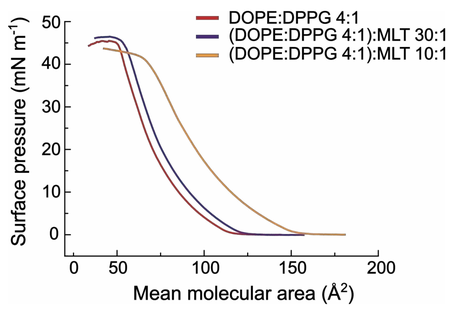
The ^{31}P-NMR experiments show a stronger interaction of the peptide with the **anionic group** of DPPG

The change in ^{13}C chemical shifts when melittin is introduced shows a favored interaction with the **C=O and DPPG headgroups**

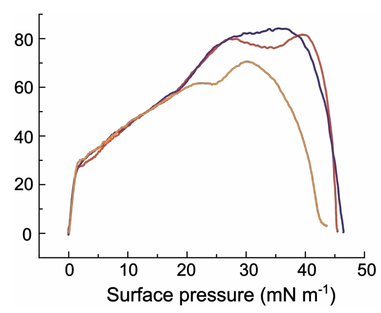


Langmuir-Blodgett (LB) technique

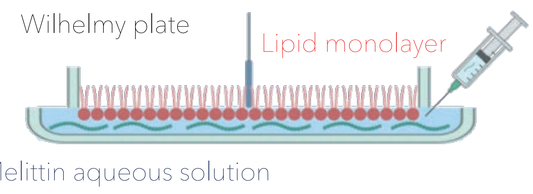
LB confirms the expansion of the lipid monolayer when melittin is present, as well as a decrease in the overall rigidity of the system



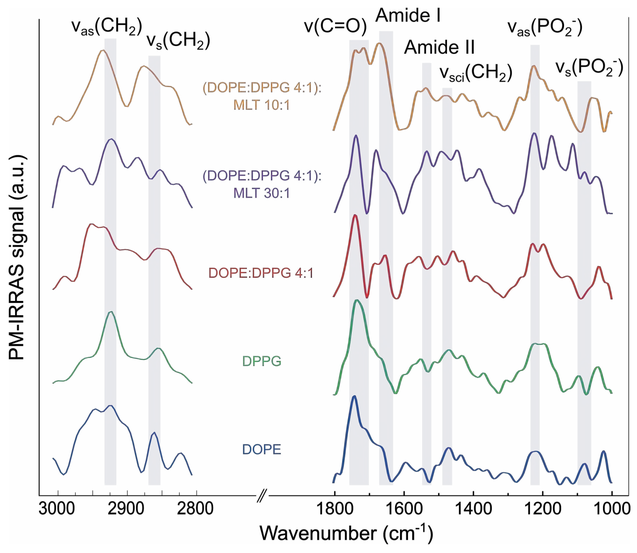
Surface pressure – Area isotherms



Compressibility modulus



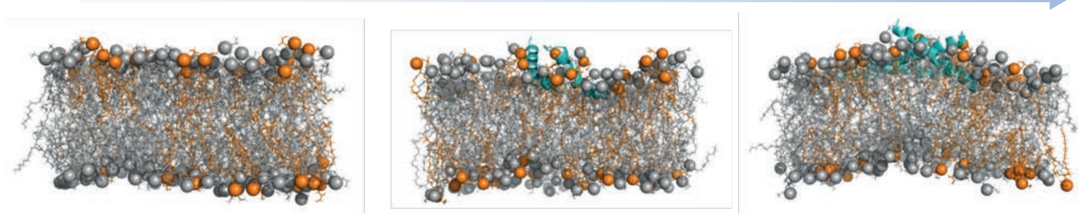
PM-IRRAS shows an increase in the **disorder of the acyl chains** proportional to the melittin concentration, as well as **higher hydration of the C=O and PO_2^- groups**



The position of melittin's amide I and amide II bands confirms its **α-helical** conformation when in contact with the lipid monolayer

Molecular Dynamics

Melittin concentration



DOPE:DPPG 4:1

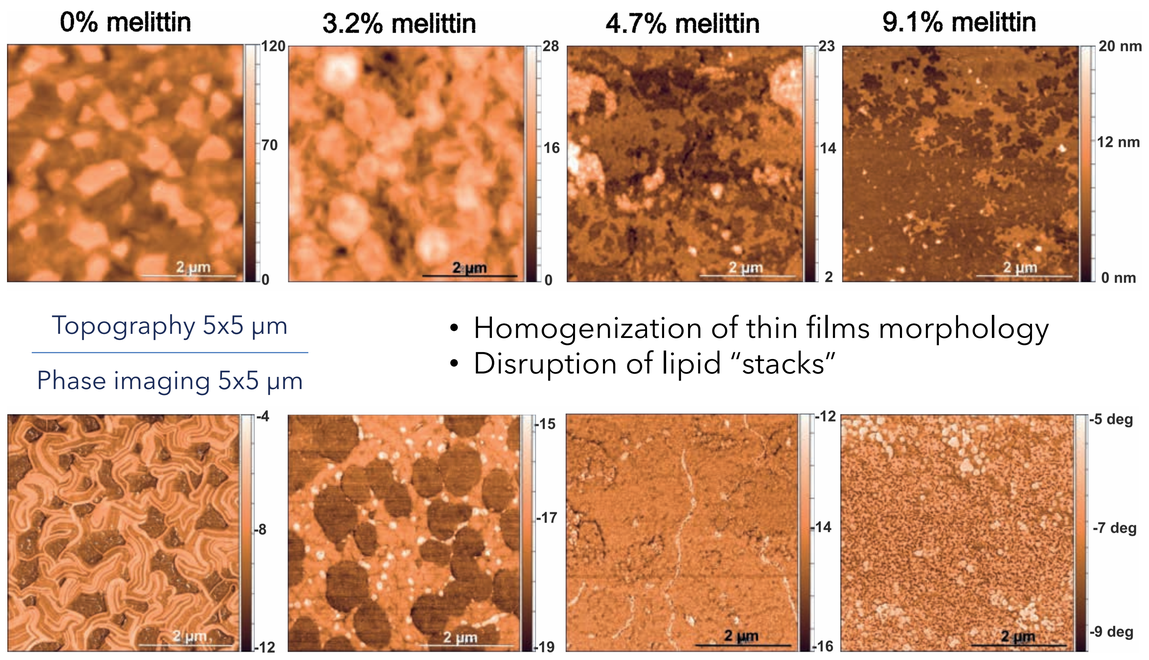
DOPE:DPPG 4:1
2 melittin chains

DOPE:DPPG 4:1
4 melittin chains

Membrane thickness - Order parameters

Area per lipid - Diffusion coefficient

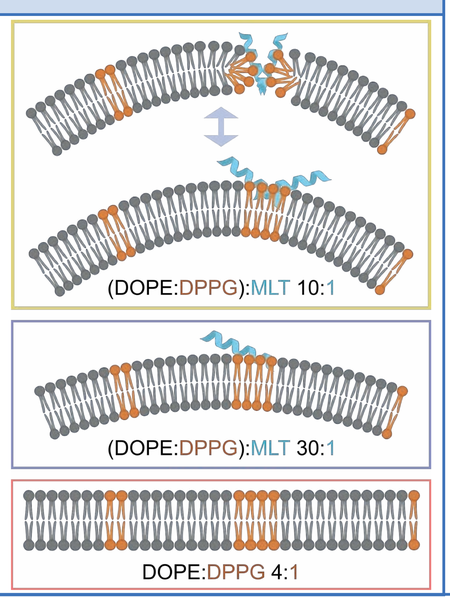
Atomic force microscopy (AFM)



- Homogenization of thin films morphology
- Disruption of lipid "stacks"

Conclusions

- Increase in melittin content increases **acyl chain disorder and water penetration.**
- Predominant interaction with the **carbonyl groups** of phospholipid bilayer.
- Predominant interaction with the **anionic phosphate group.**
- Melittin causes acts via a **concentration-dependent mechanism**: nonspecific permeabilization at low concentrations, and kinetic defects at higher concentrations.



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