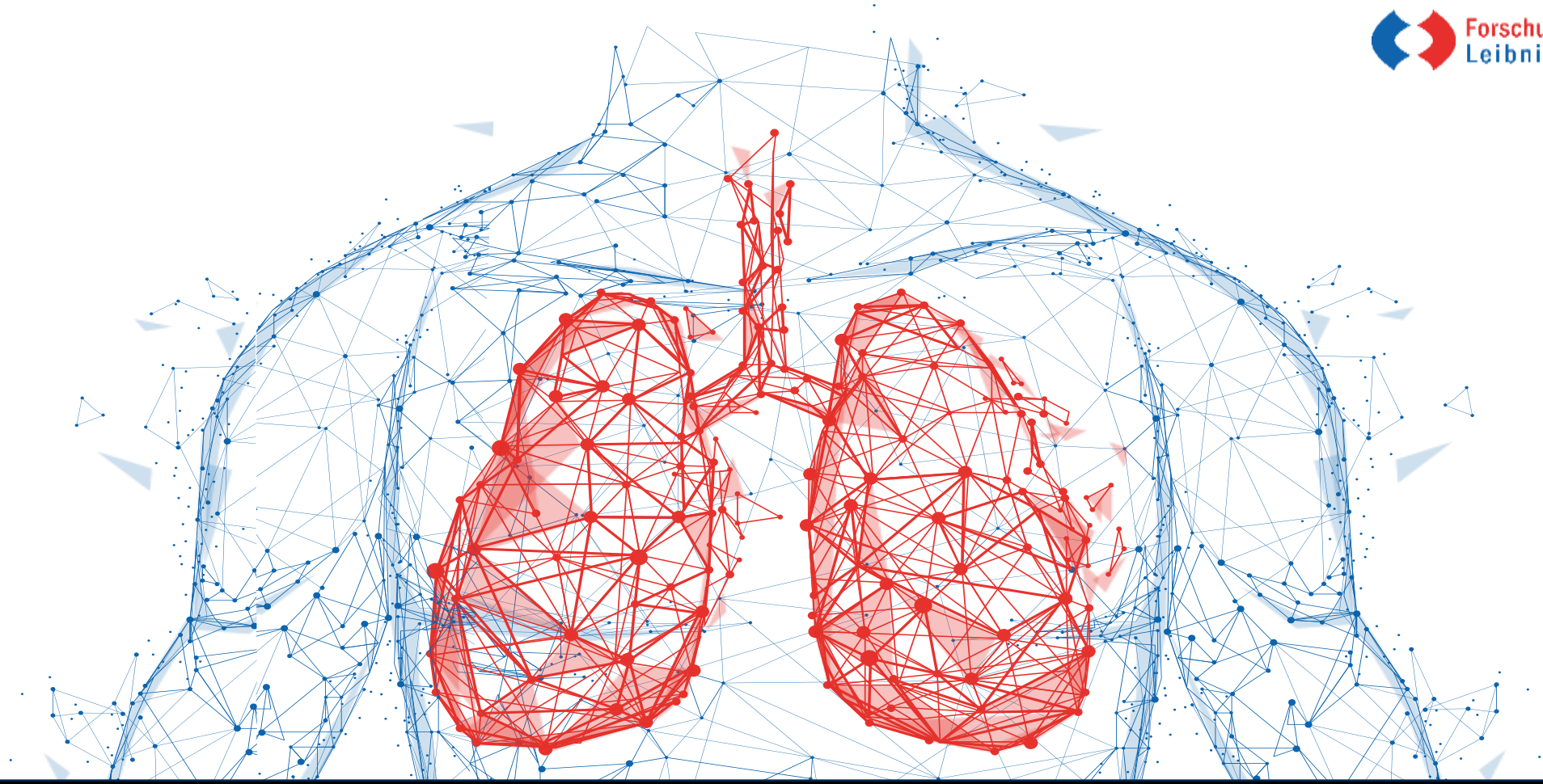
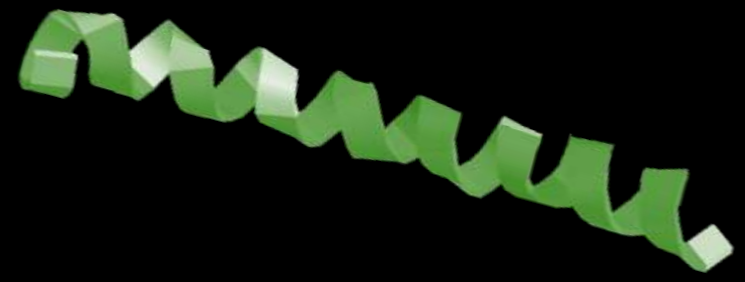


The Use of Liposomes in the Assessment of Antimicrobial Activity of Membrane-Active Compounds

Christian Nehls

PRC-Webinar 10.12.2025

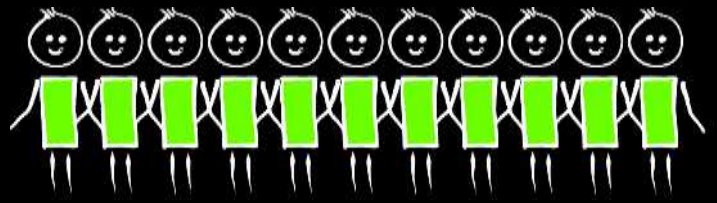


Antimicrobial peptides with therapeutic potential

Estimated deaths due to antimicrobial resistance

Research Center Borstel
Leibniz Lung Center

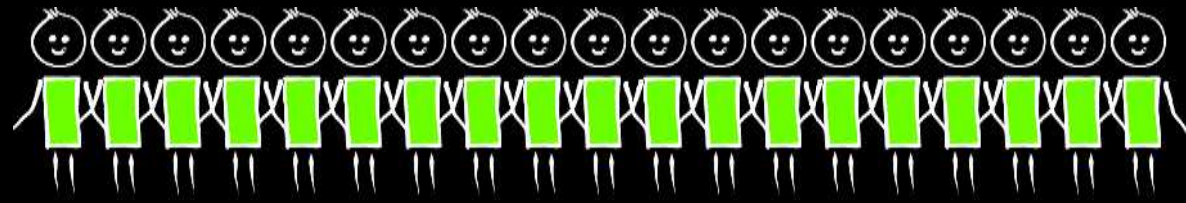
2021



1.14 Mio.

(4.71 Mio. associated)

2050



1.91 Mio.

(8.22 Mio. associated)

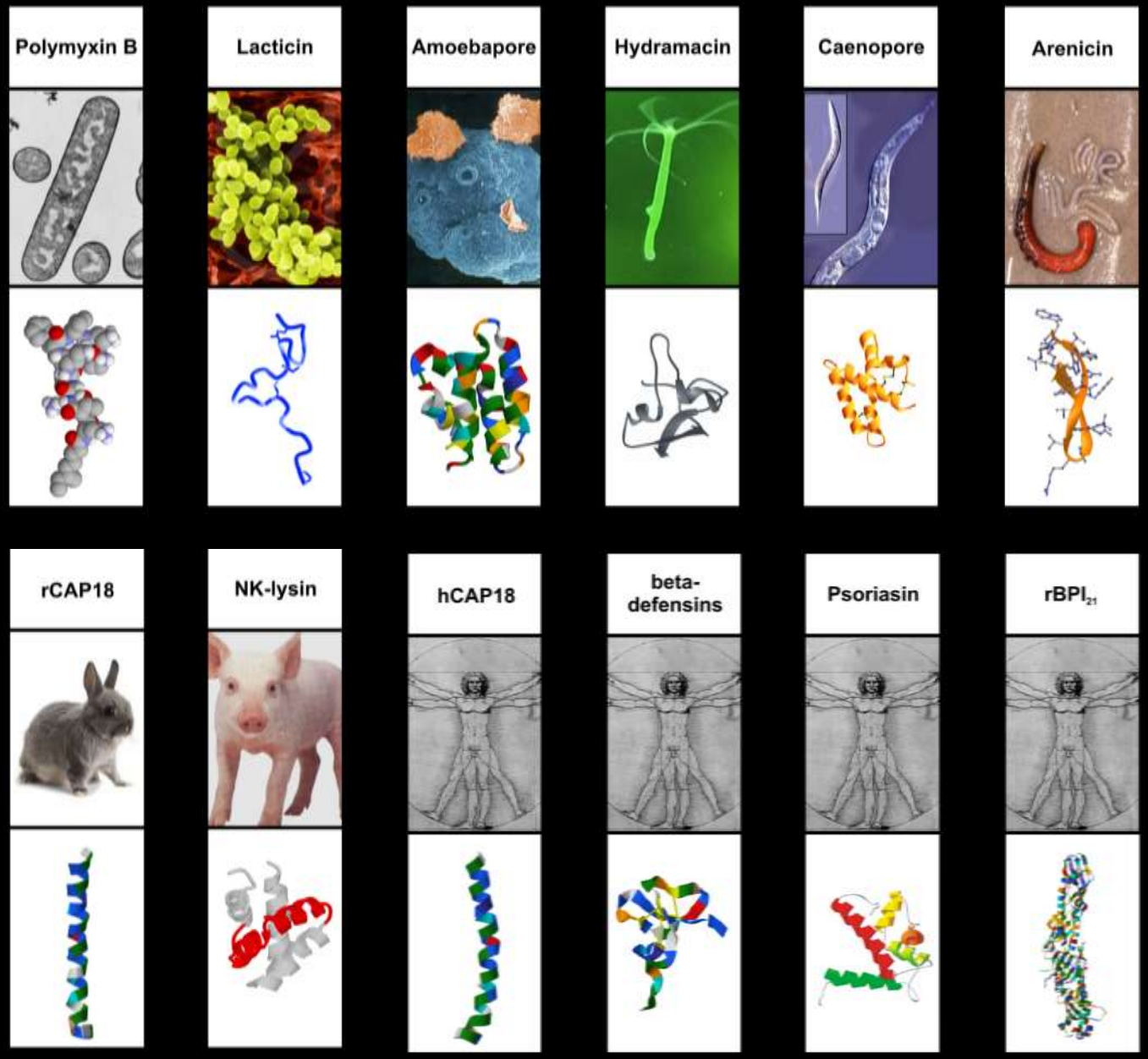
New treatment options are needed

= 100 000 estimated annual deaths

Naghavi, Mohsen, et al. "Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050." The Lancet 404.10459 (2024)

Occurence of antimicrobial peptides (AMPs)

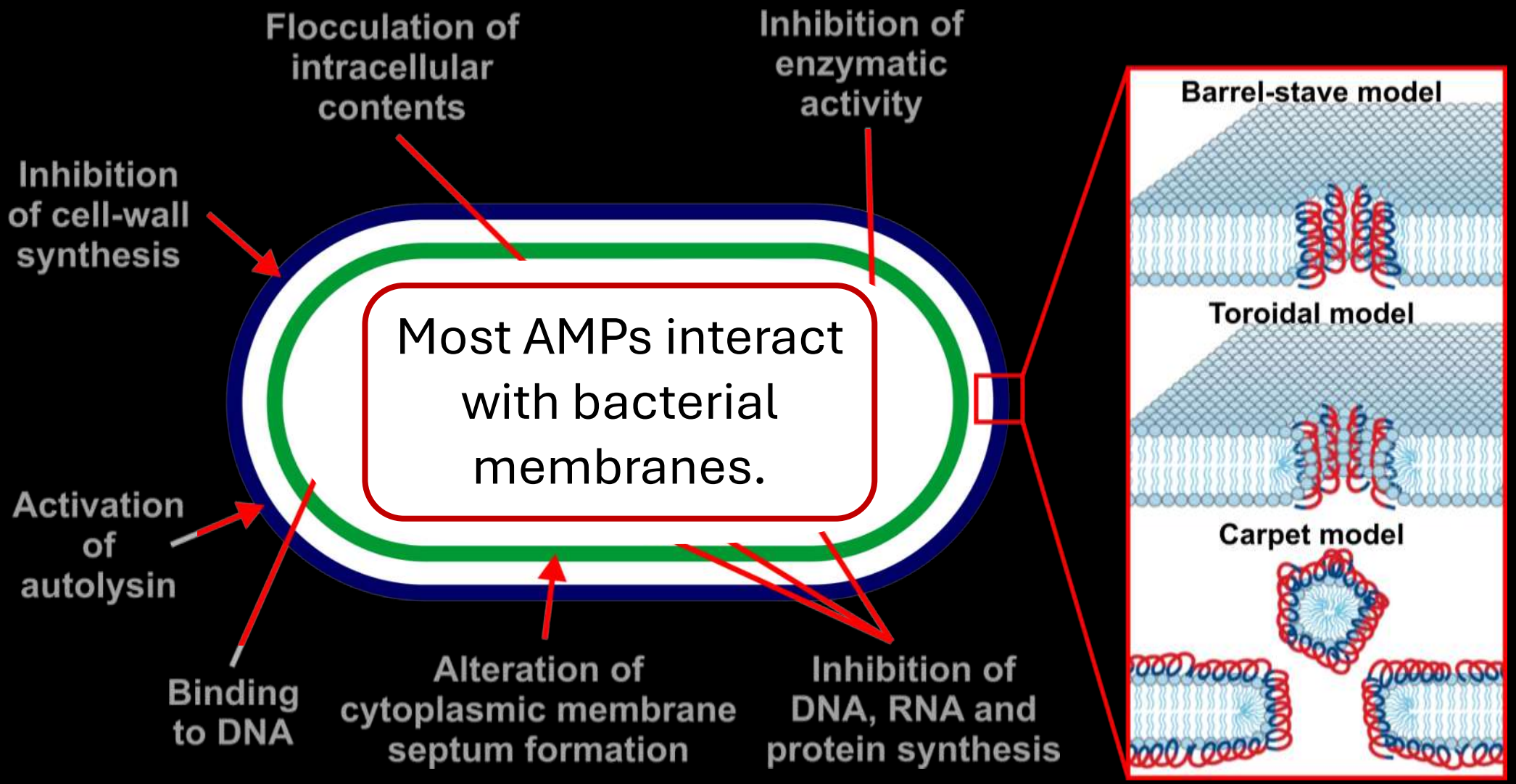
Research Center Borstel
Leibniz Lung Center



- Size
- Sequence
- Charge
- Dipole moment
- Flexibility
- Hydrophobicity
- Amphipathicity
- Conformation and structure

AMPs occur in a large diversity

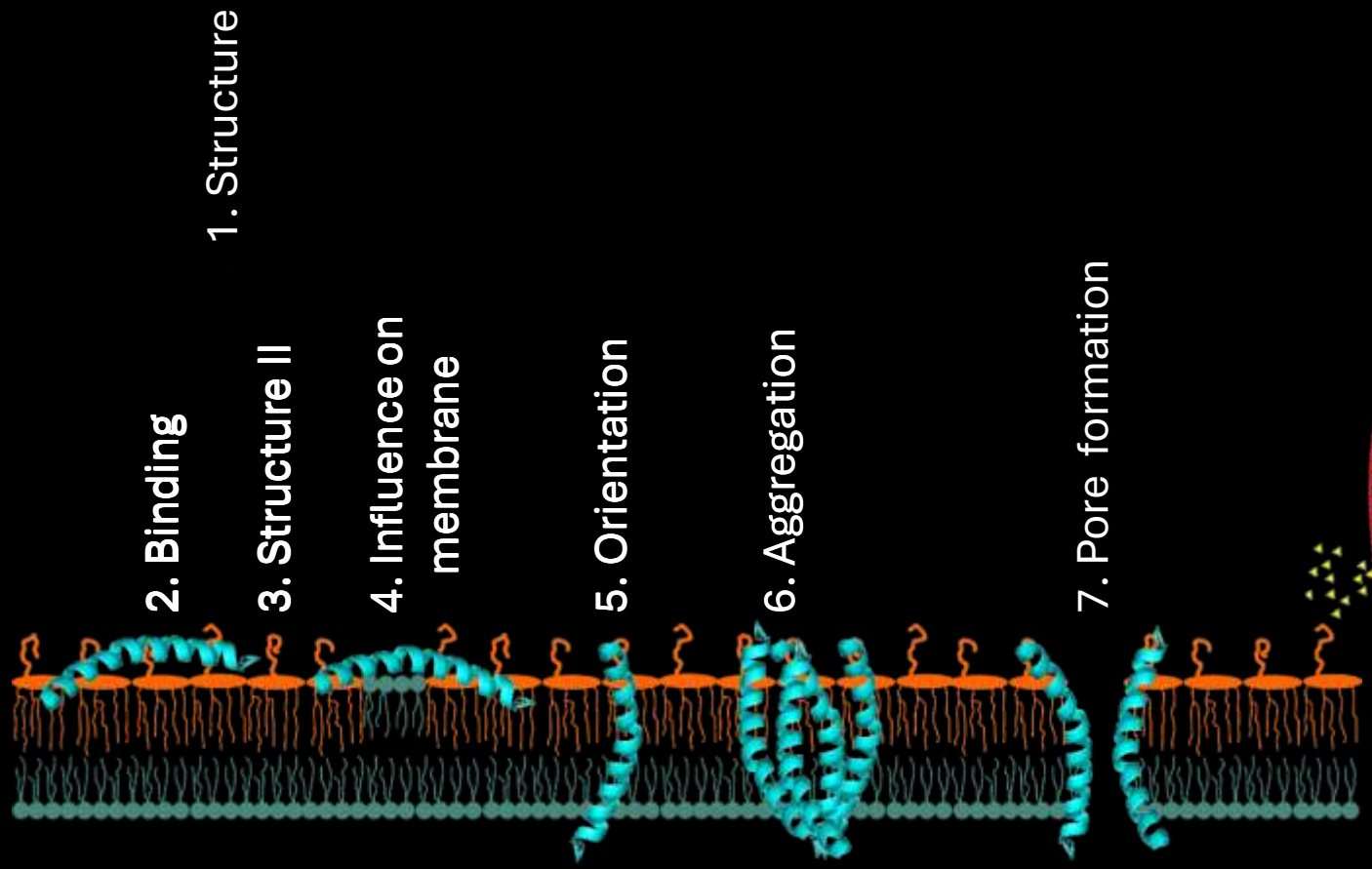
Modes of Action of AMPs

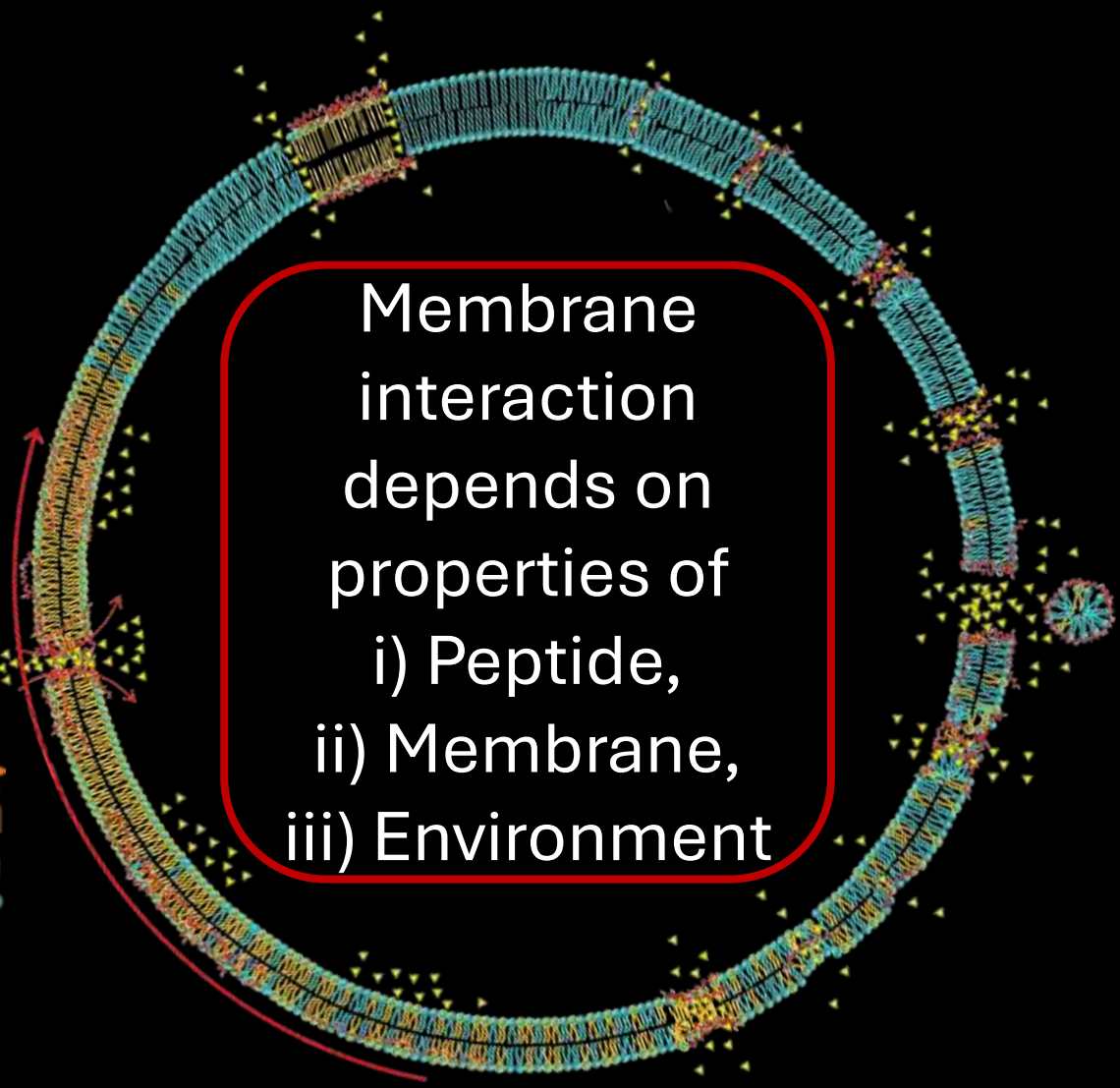


Adapted from Bogden, Nat Rev Micro, 2005; Oren et al., Biopolymers, 1998; Huang et al., Phys Rev Lett, 2004

AMP membrane interaction

Research Center Borstel
Leibniz Lung Center

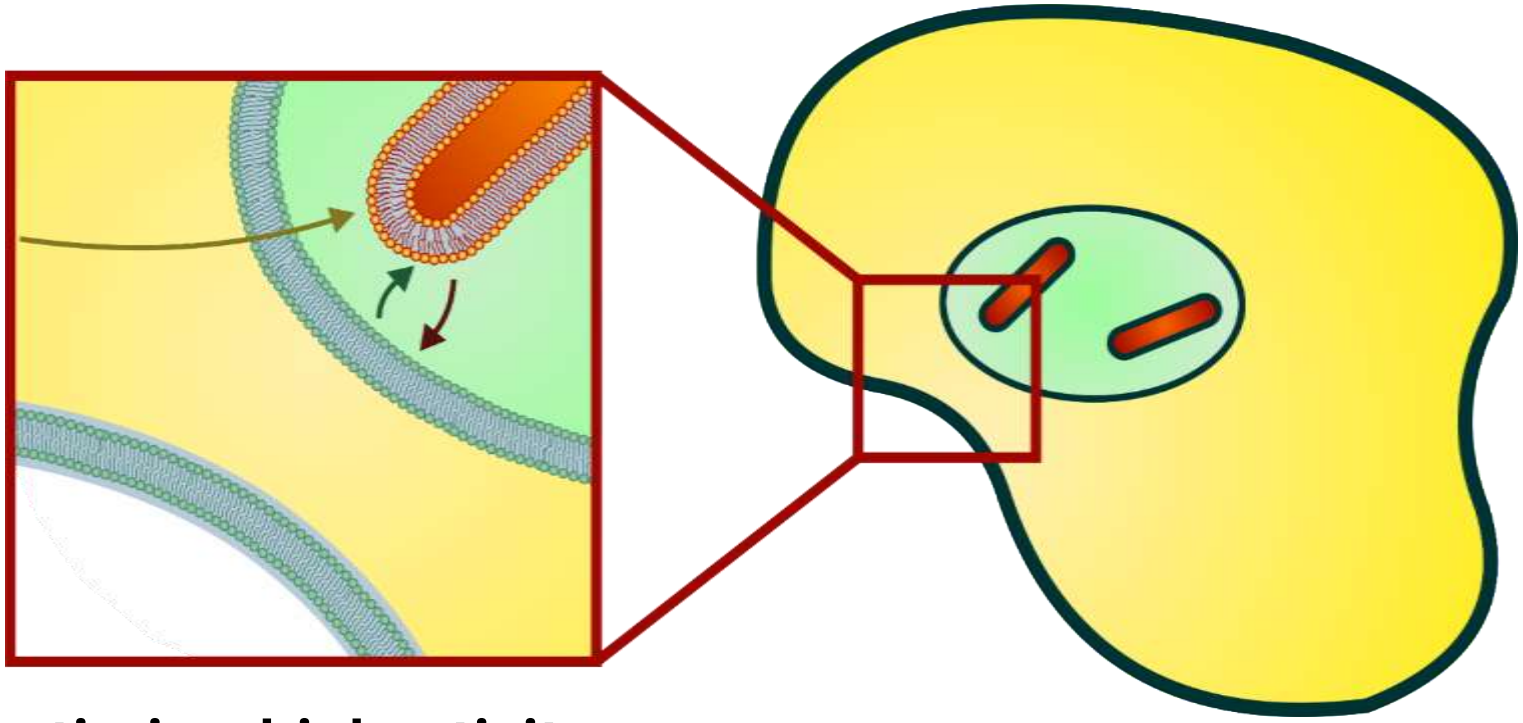




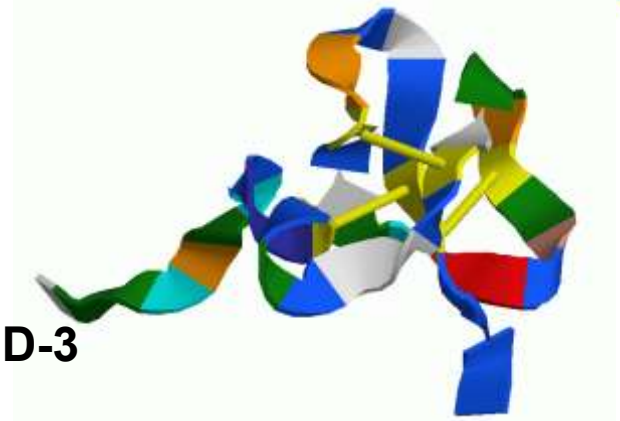
Membrane interaction depends on properties of
i) Peptide,
ii) Membrane,
iii) Environment

Adapted from Guha et al., Chem. Rev., 2019

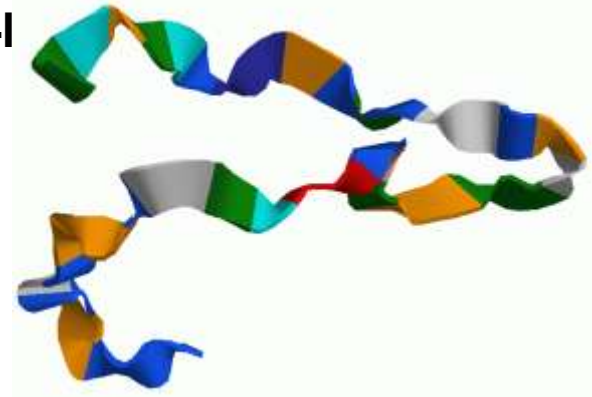
Intracellular context of AMP antimicrobial activity



LL32



hBD-3

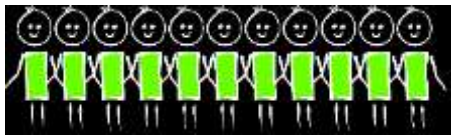


hBD-3-l

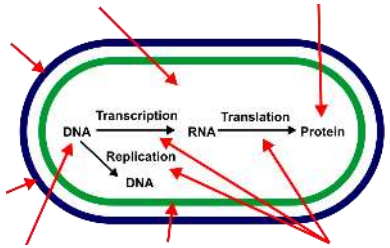
Antimicrobial activity:

I.	Bacteriostatic:	Lactoferrin	(Nutrient deprivation)
II.	**Bactericidal:**	**Defensins**	**(Membrane permeabilization)**
		Cathelicidins	**(Membrane permeabilization)**
III.	Hydrolysis:	Lysozyme, …	(Carbohydrates, …)

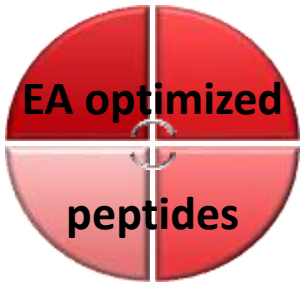
Take-homes



AMPs are a promising alternative to classical antibiotics with a much lower tendency for resistance induction.



Membrane interaction is prerequisite for any AMP-pathogen interaction.



Specificity of AMPs can be narrowed using directed optimization.

Where, who and what

Research Center Borstel, Leibniz Lung Center

Manor house from 1751

New NRC building from 2022

New lab building from 2024

75

1947 - 2022

Center for Structural Systems Biology (CSSB)

Research Center Borstel
Leibniz Lung Center

CSSB
Centre for Structural
Systems Biology

BNITM
Bernhard-Nocht-Institut für Tropenmedizin

Research Center Borstel
Leibniz Lung Center

MHH

DESY.

HZI HELMHOLTZ
Zentrum für
Infektionsforschung

UKE
HAMBURG

EMBL

LIV Leibniz-Institut für Virologie

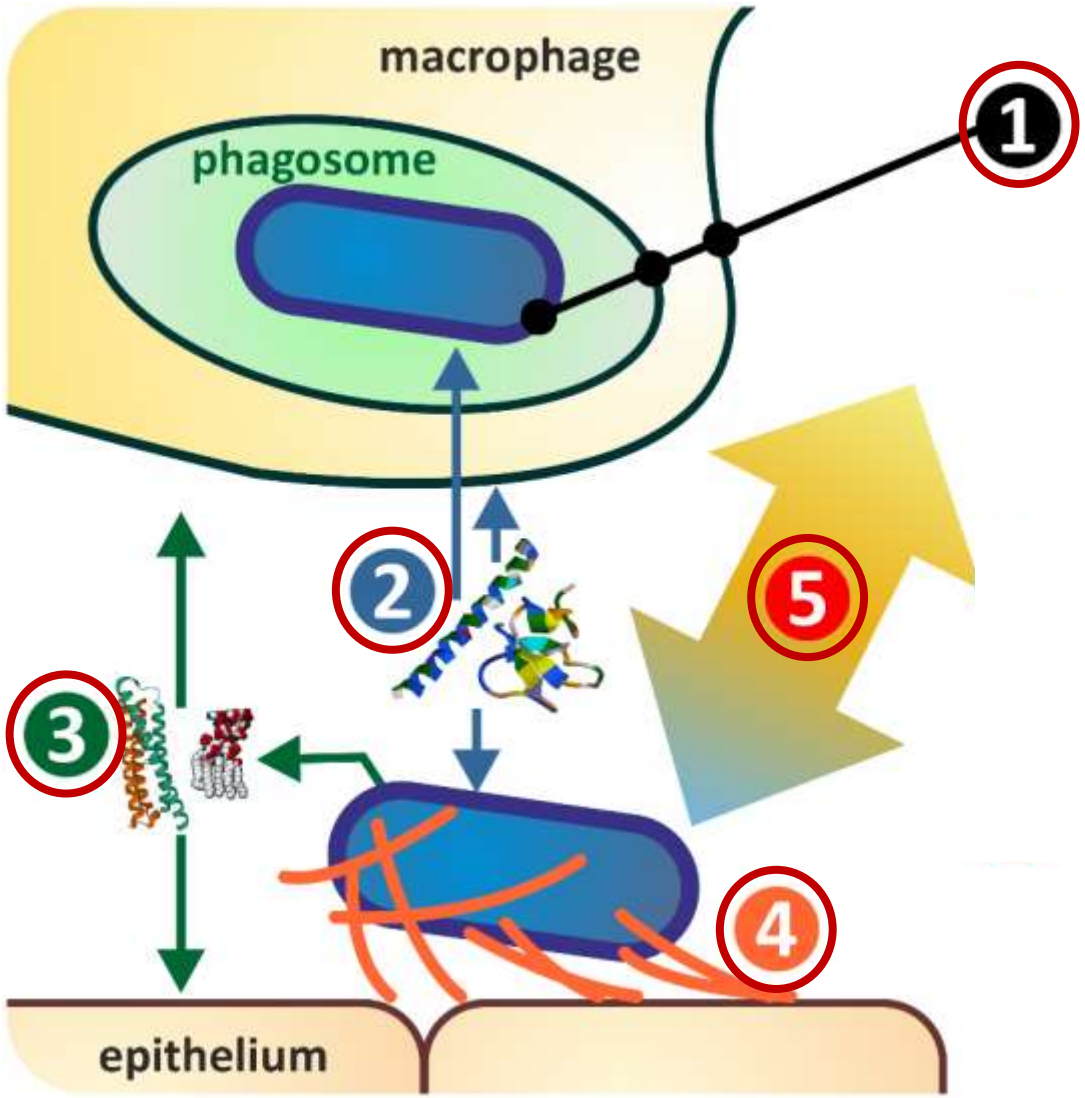
UHH

First CSSB Symposium 2015

Gutsmann lab @ Research Center Borstel

Team Microfluidics

Research focus



To develop new therapies, we

investigate membrane properties and interactions in the context of lung disease.

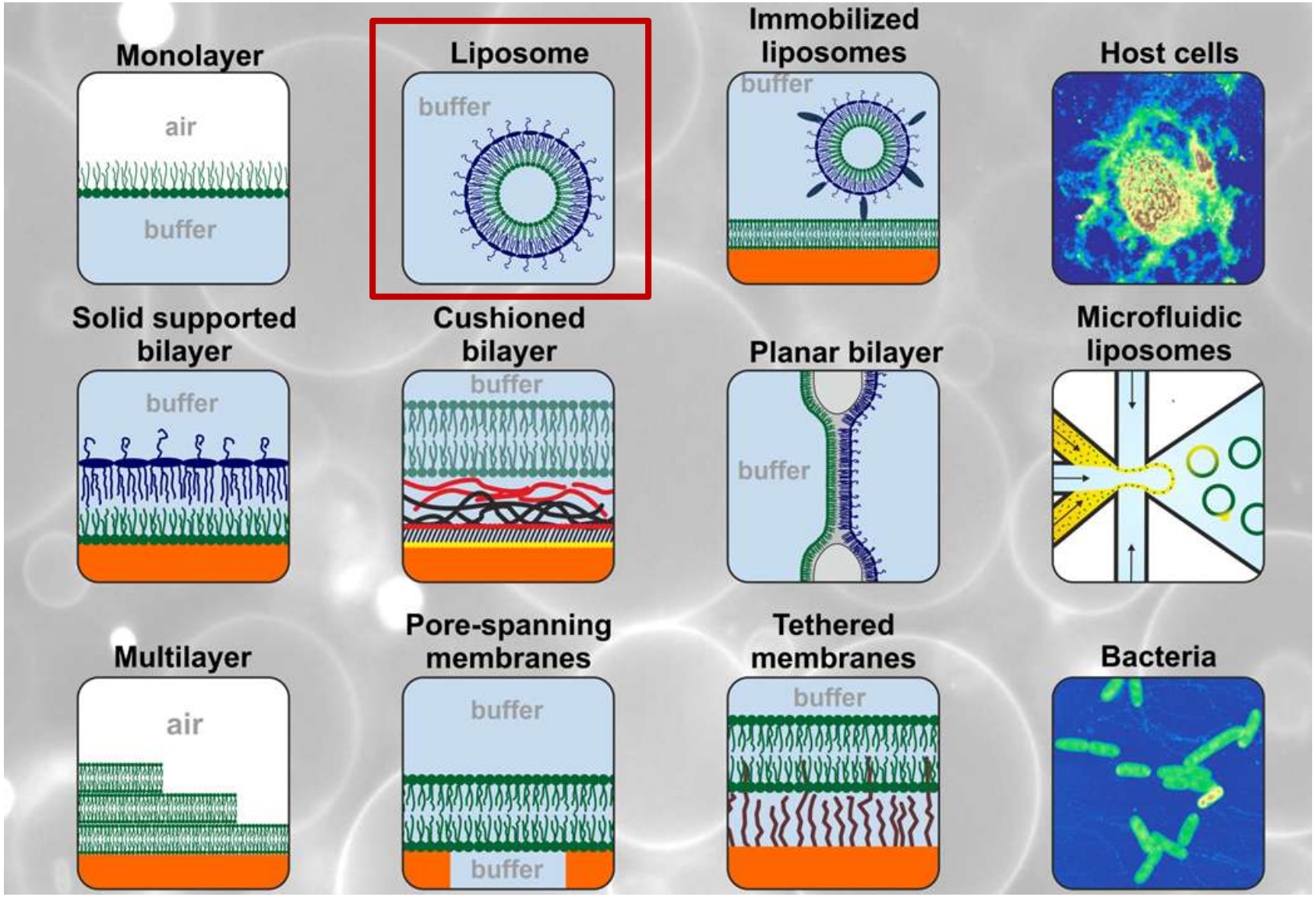
develop antimicrobials to combat antimicrobial resistance.

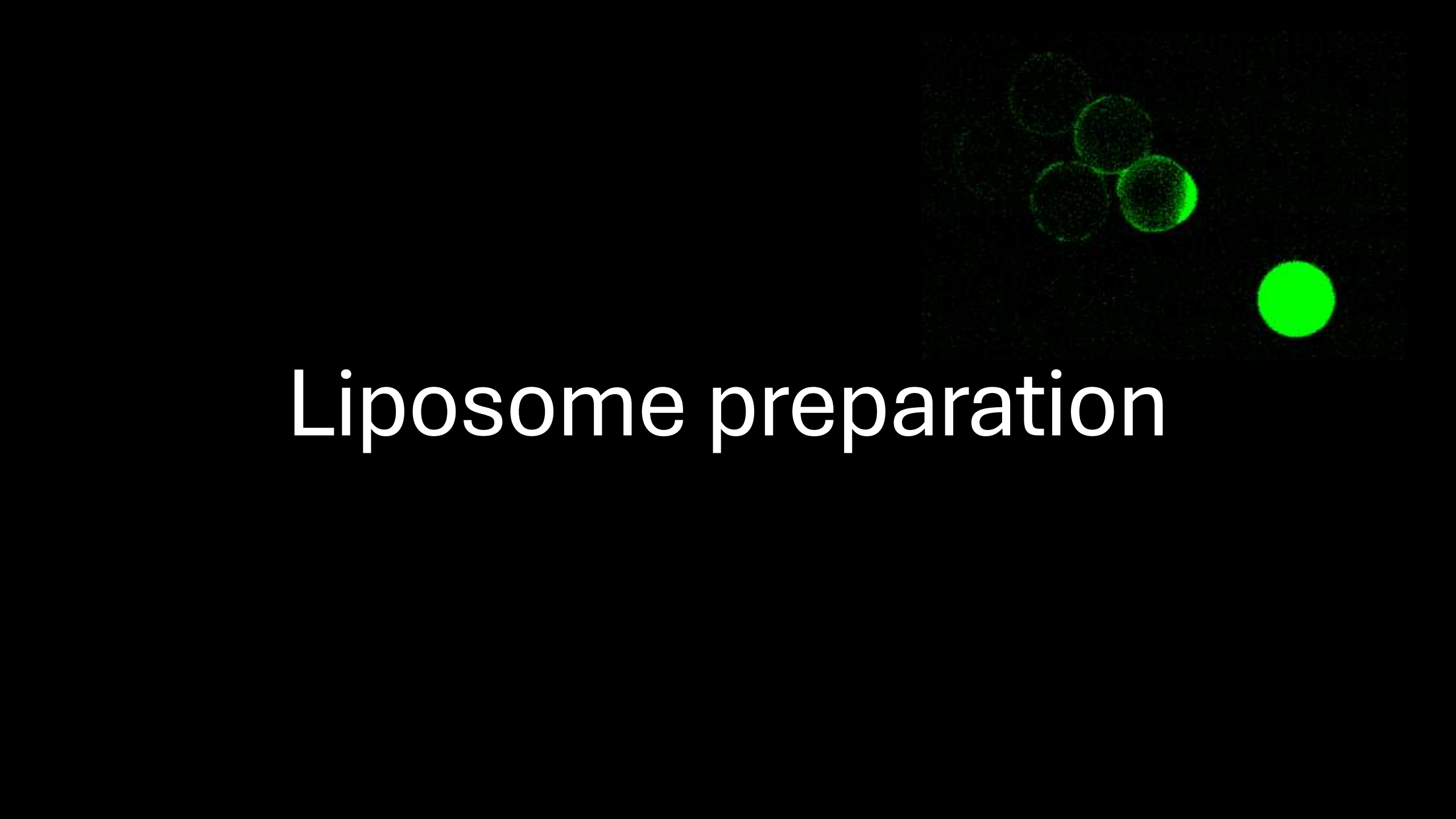
explain mechanisms of microbial toxin membrane activity.

probe molecular adhesion mechanisms that underlie pathogen persistence.

generate, manipulate, characterize and detect bioactive aerosols.

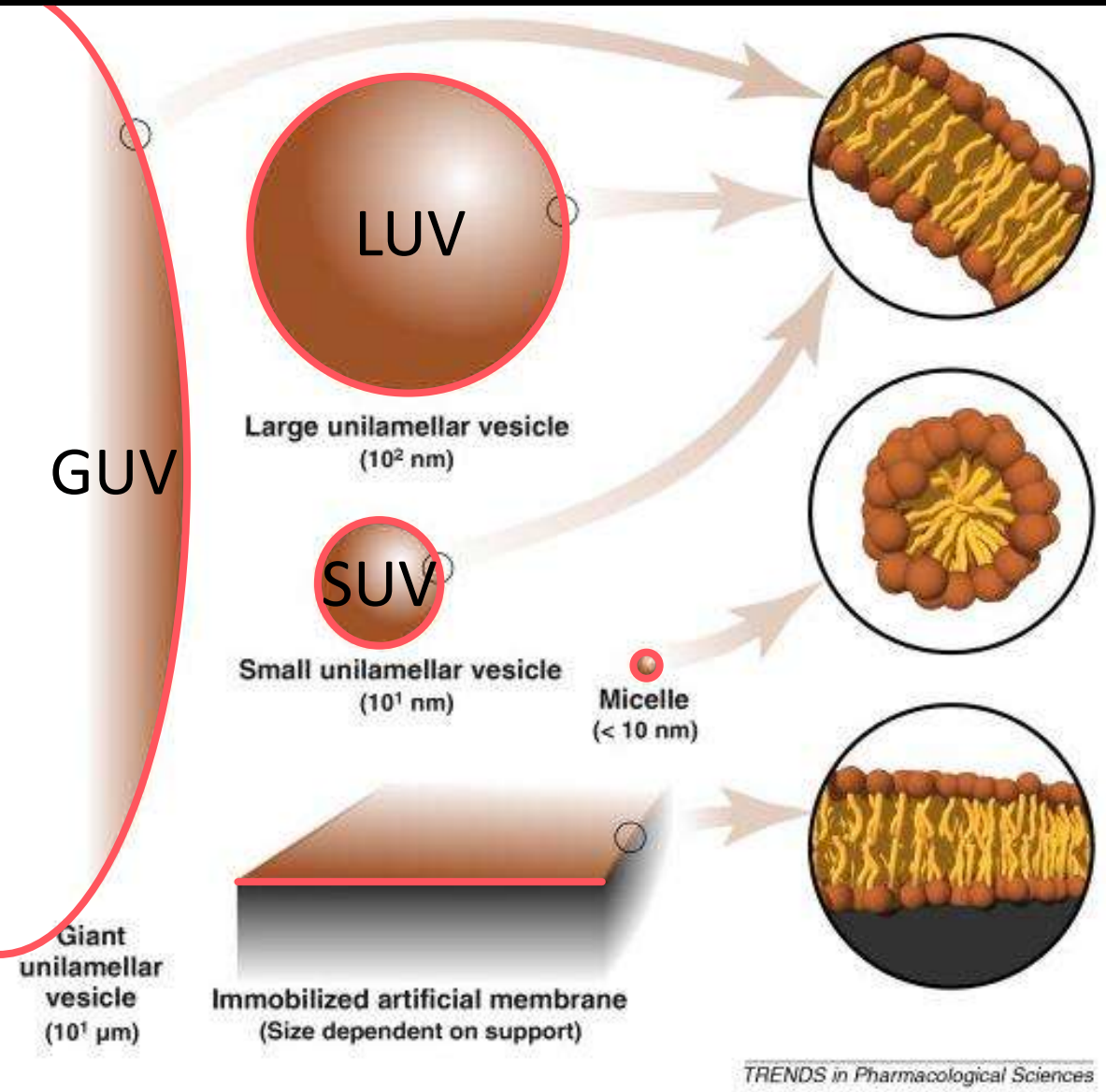
Model membrane systems

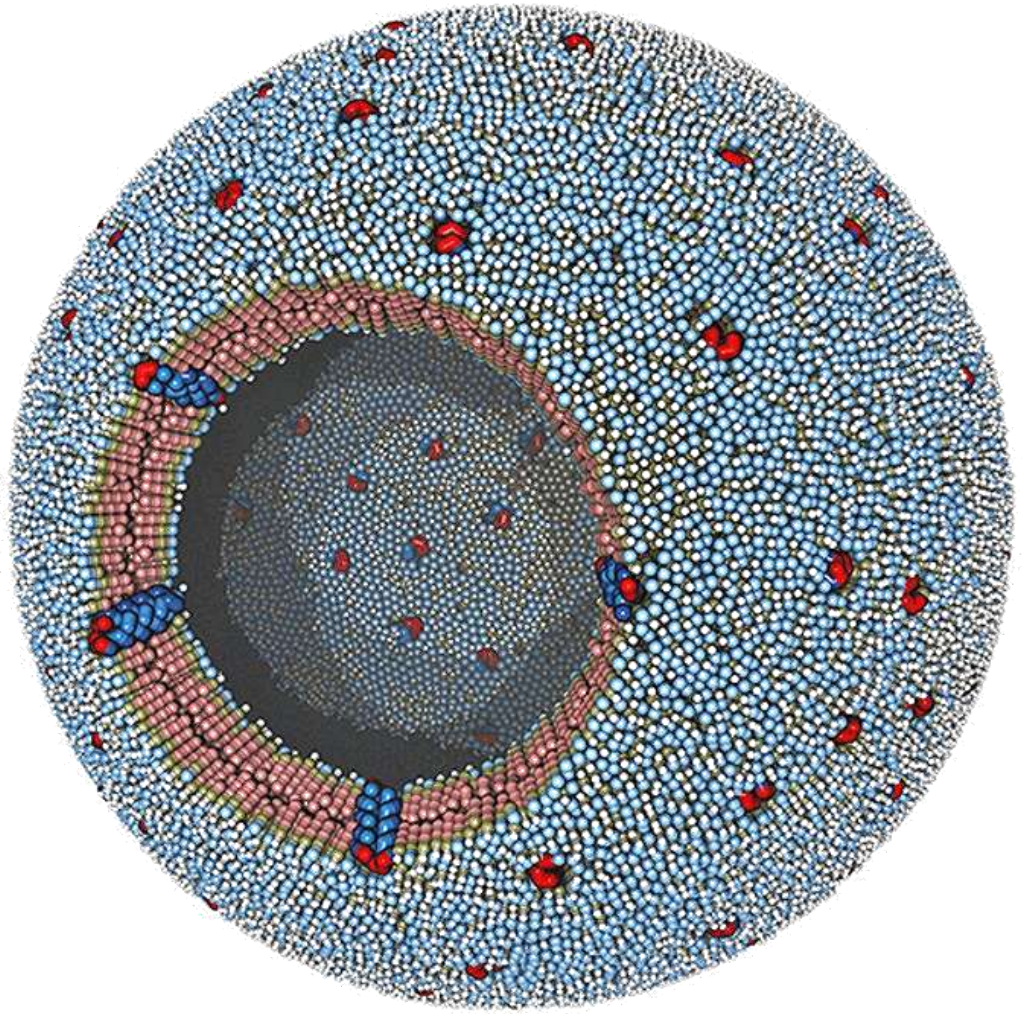




Liposome preparation

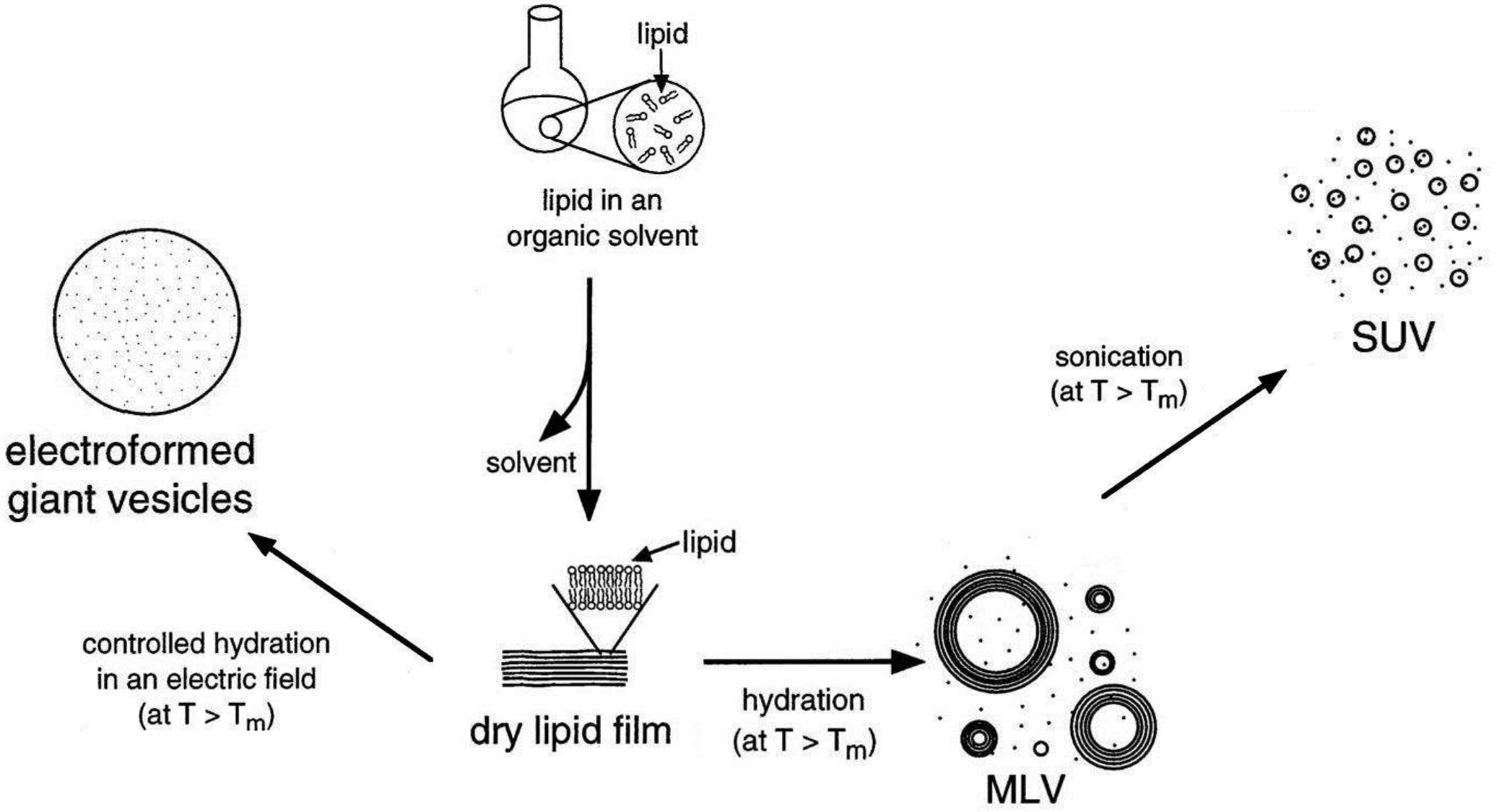
Size order of liposomes





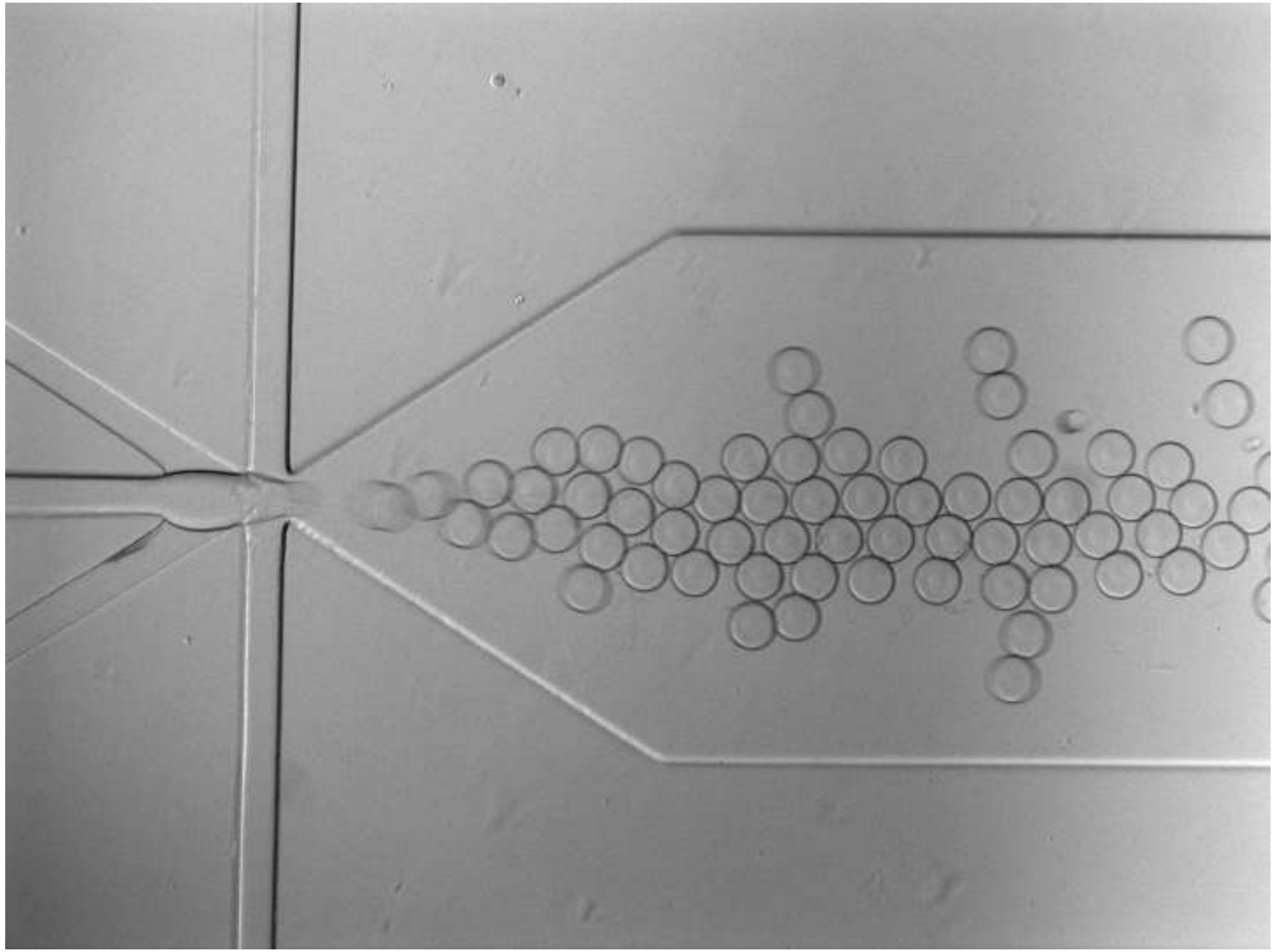
d = 34 nm, ≈ 40.000 lipids

Conventional preparation of liposomes

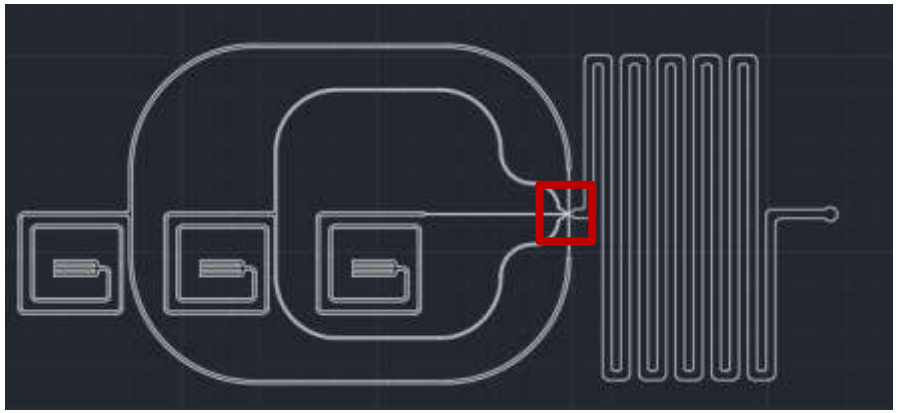


adapted from: Walde, Encyclopedia of nanoscience and nanotechnology 9, 2004

Formation of giant liposomes on microfluidic chip

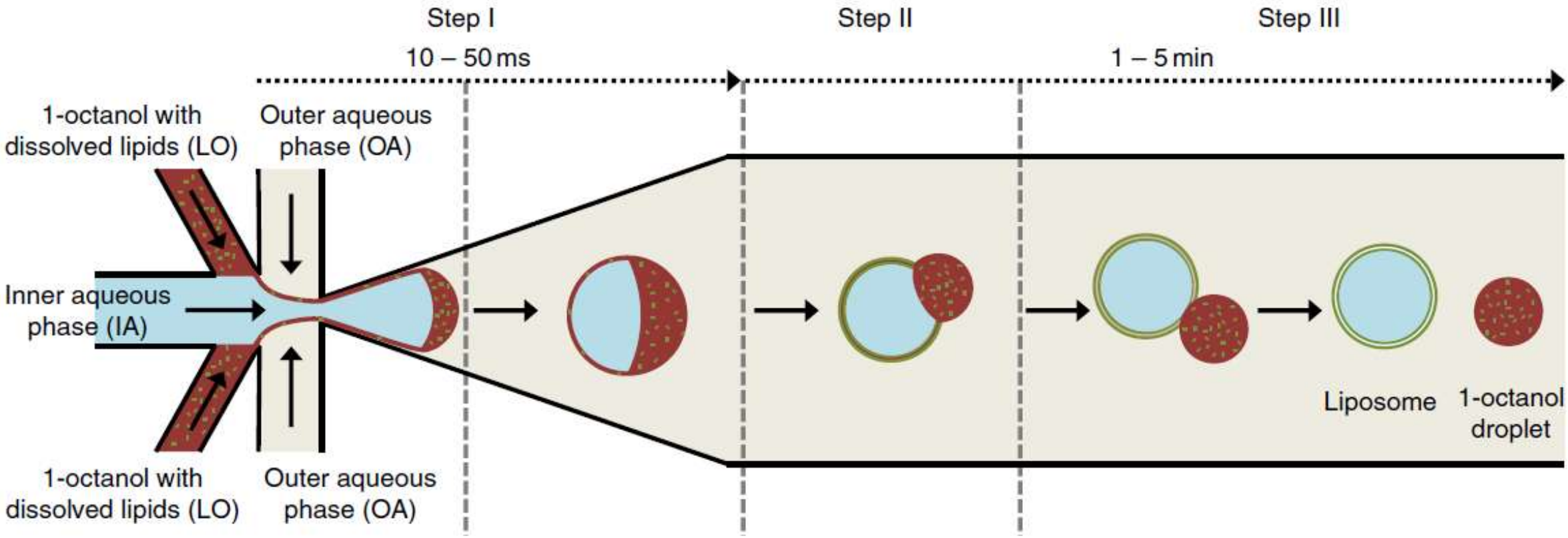


- High throughput
- Uniform size
- High encapsulation efficiency



Bruns, Nandy, Nehls - unpublished

Octanol-assisted liposome assembly on chip

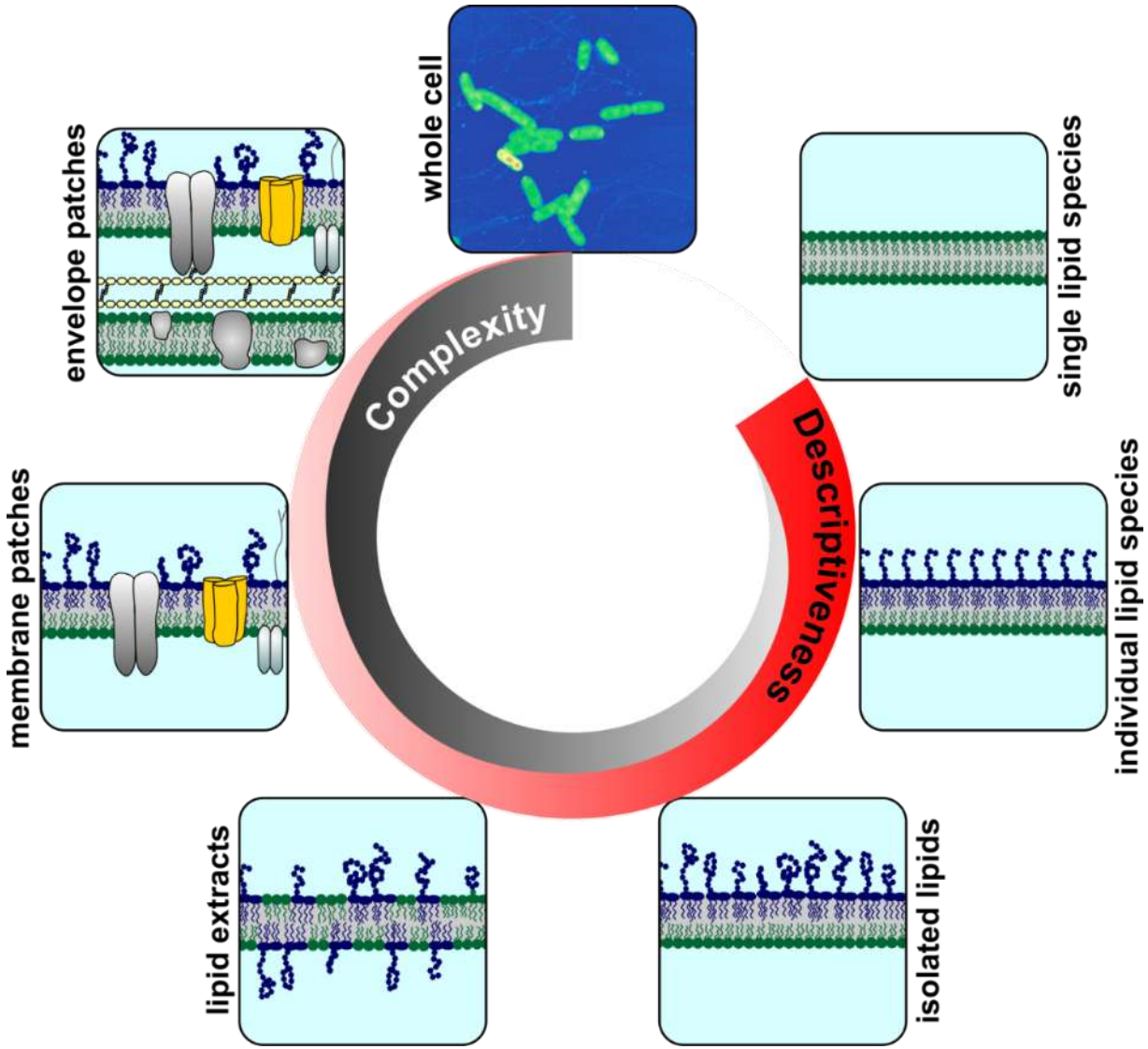


Double emulsion droplet formation

Oil pocket formation

Oil pocket detachment

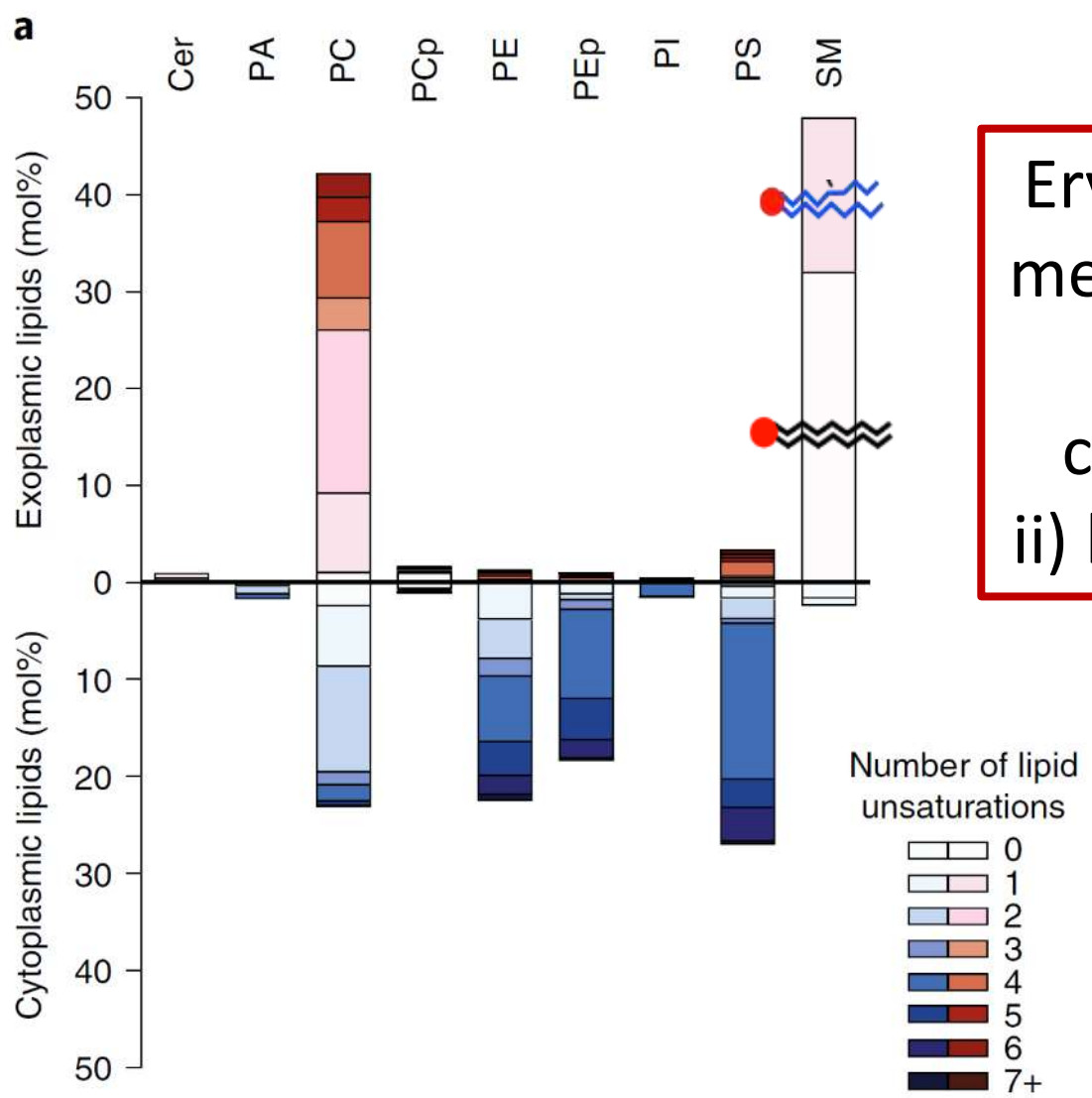
How complex should we make the system?



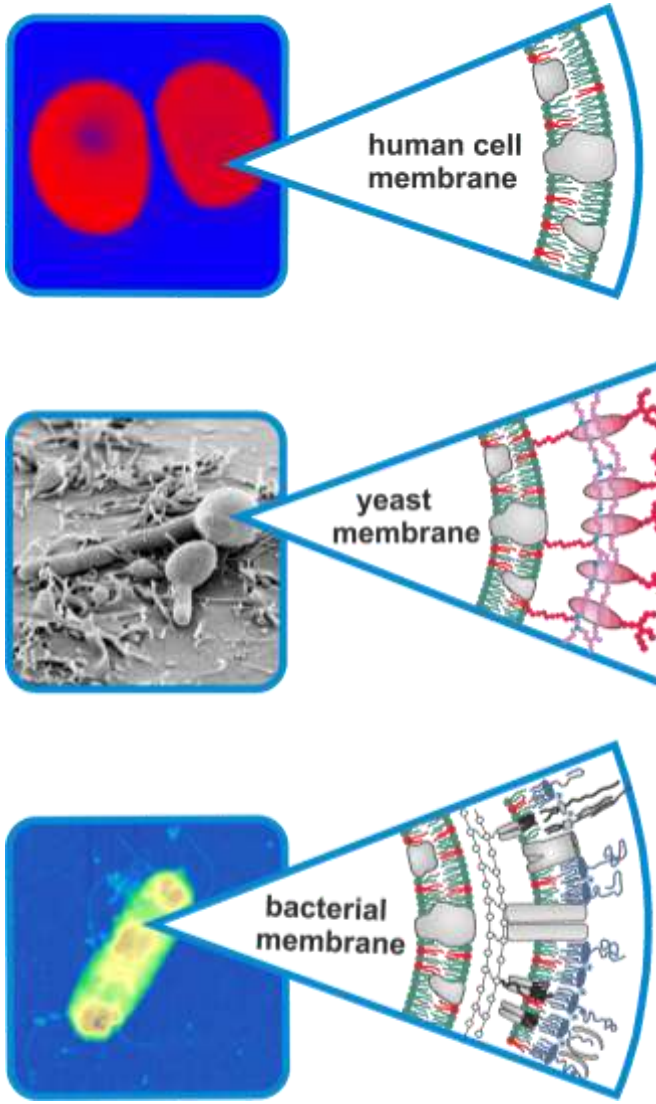
Successive complexity increase (bottom-up) adds factors for unspecific membrane interaction

Successive complexity decrease (top-down) can reveal causality for specific interaction

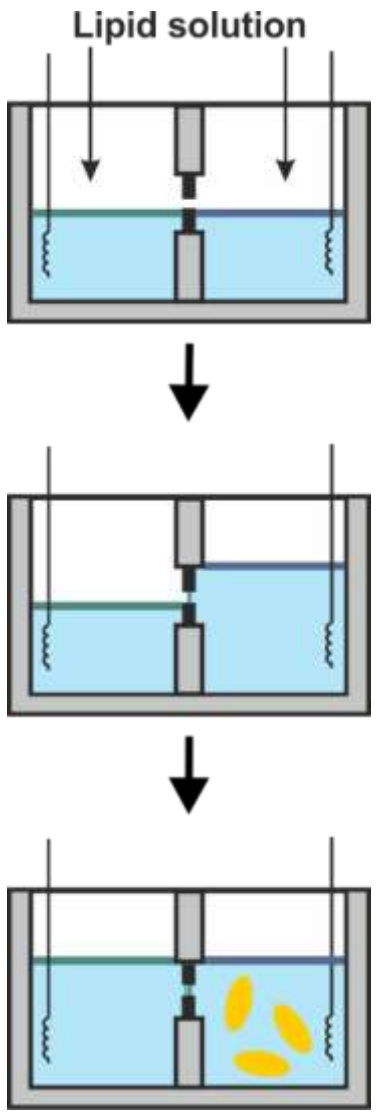
Which natural membranes are asymmetric?

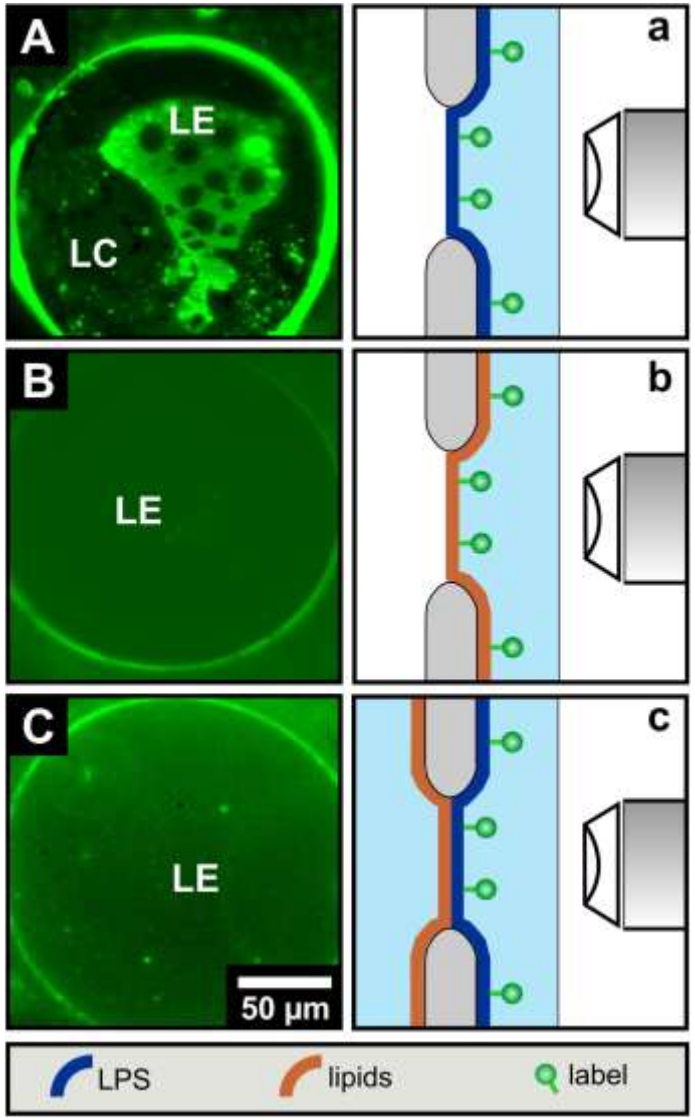


Erythrocyte plasma membranes differ in
i) headgroup composition and
ii) lipid unsaturation



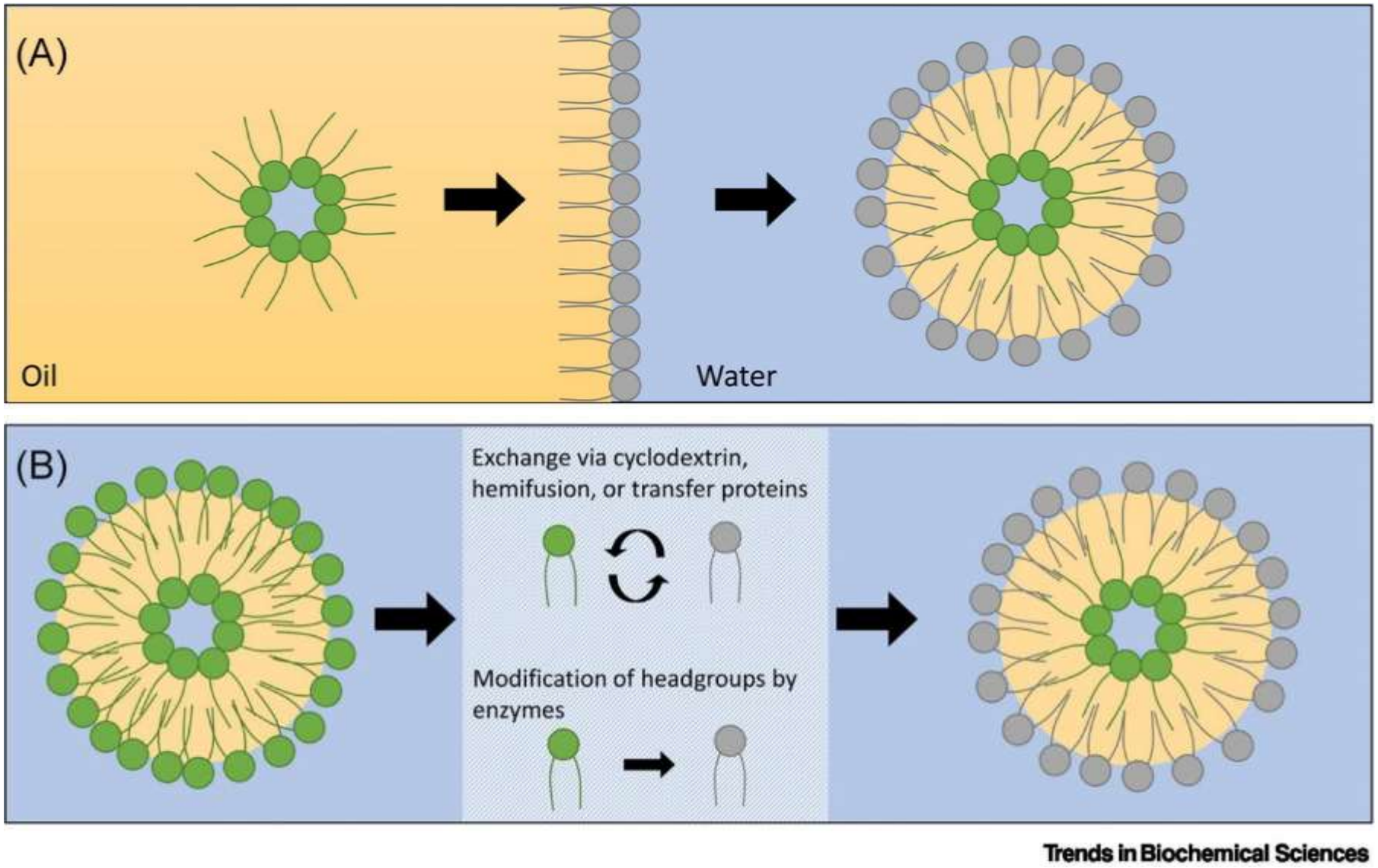
Montal-Mueller technique for asymmetric membranes





Asymmetric membranes are more than addition of two monolayers

Formation of asymmetric liposomes

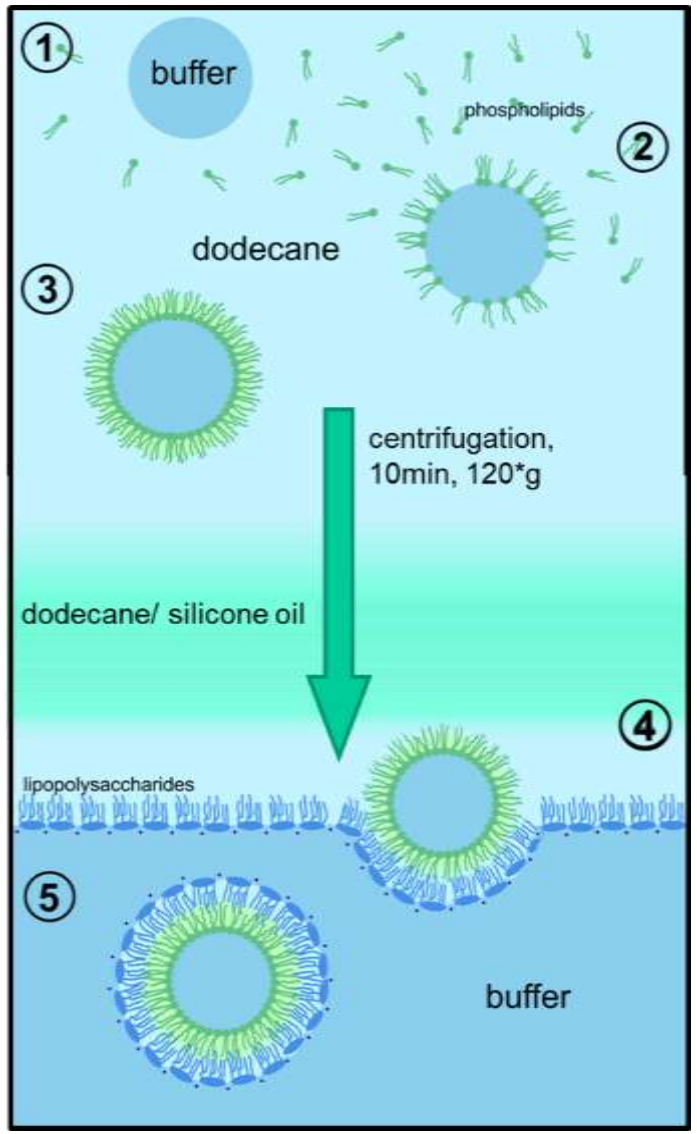


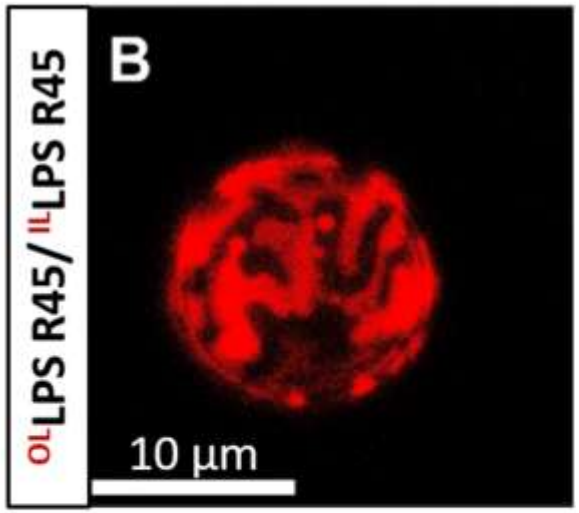
Layer-by-layer assembly

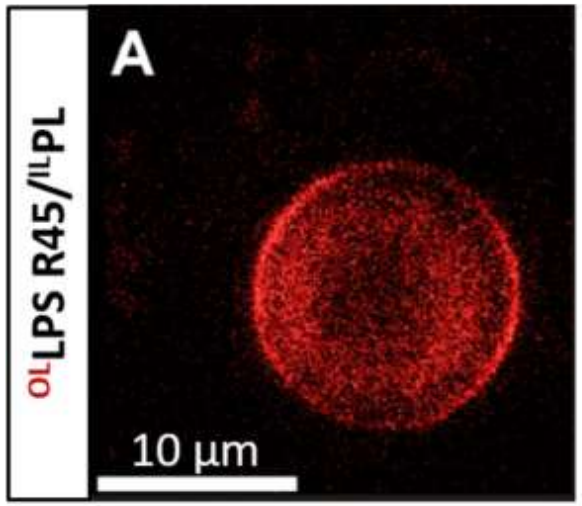
Outer leaflet exchange

Pabst, Keller, Trends Biochem. Sci. 49(4), 2024

Formation of asymmetric liposomes by phase transfer



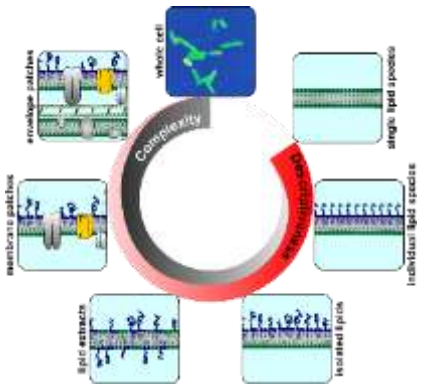




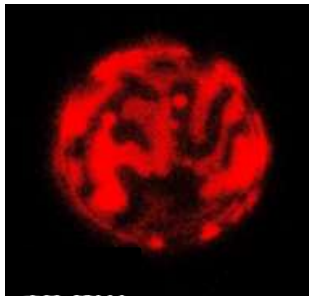
Phase transfer derived liposomes confirm relevance of asymmetry

Phase transfer allows formation of pure lipopolysaccharide liposomes

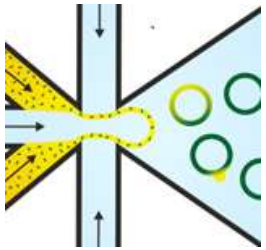
Take-homes



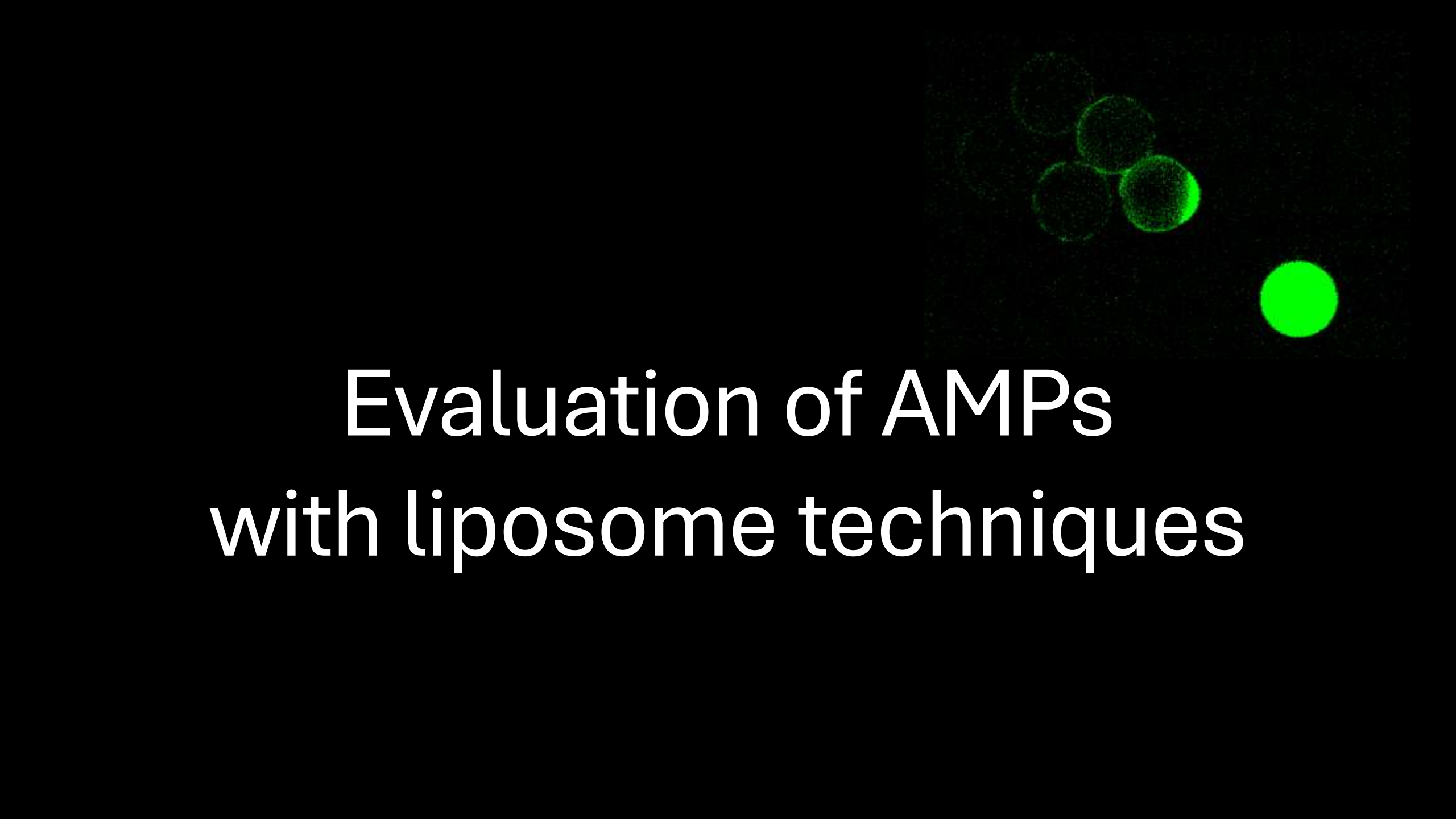
To understand interaction processes at membranes, the natural membrane complexity has to be reduced.



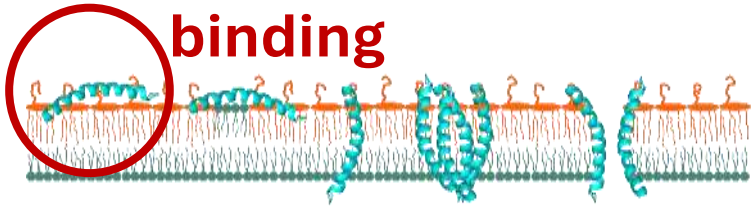
Membrane asymmetry is directly linked to biological function and has to be considered for model systems.



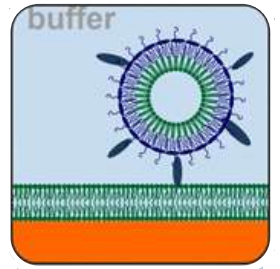
Microfluidic techniques allow homogeneous GUV generation with exceptional encapsulation efficiency.

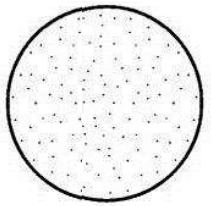

Evaluation of AMPs
with liposome techniques

Confocal microscopy on immobilized liposomes

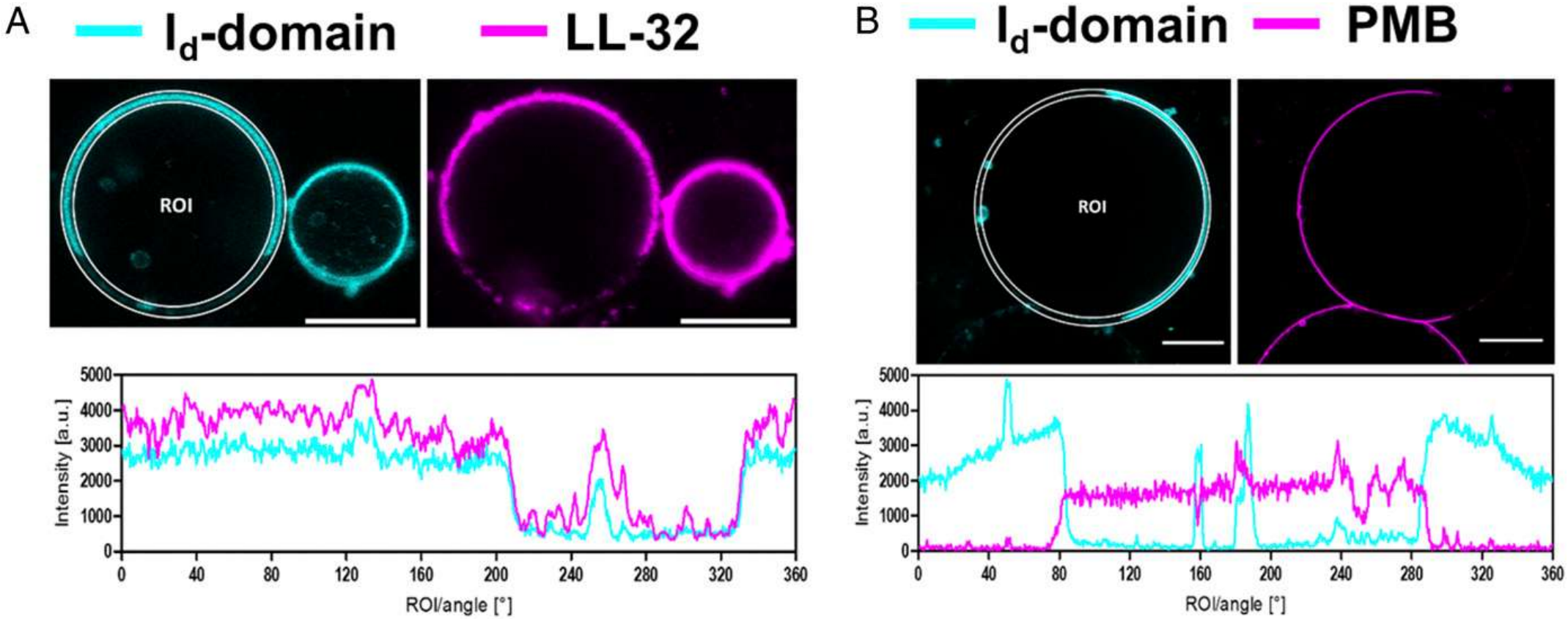


Specific binding to lipid domains determines AMP mode of action

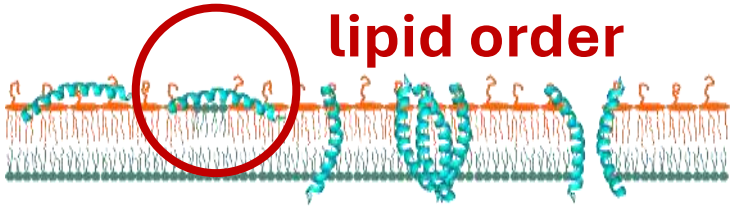


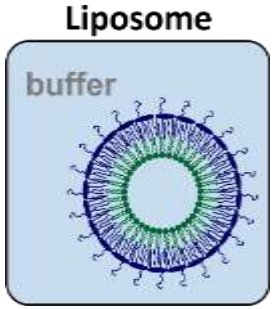


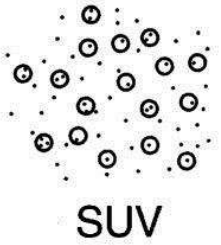
electroformed giant vesicles

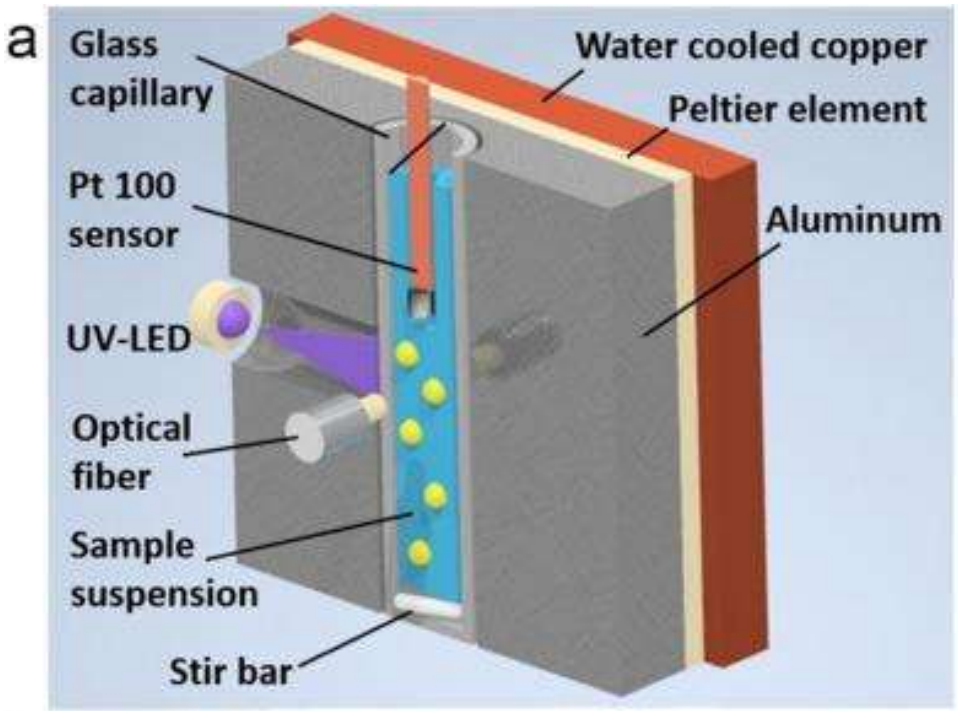


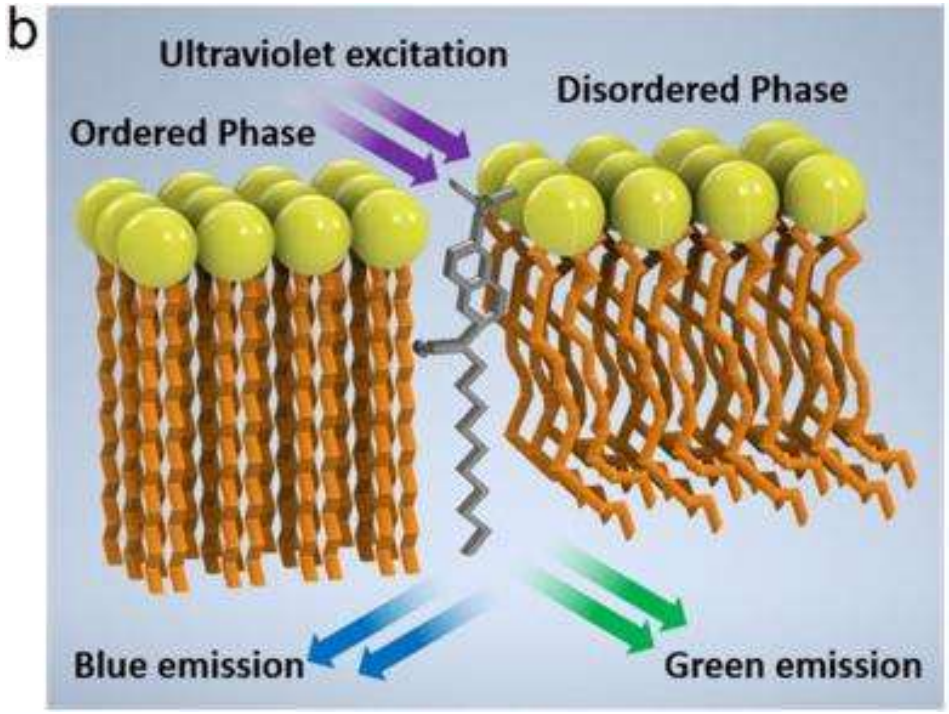
Lipid State Observer LISO on SUVs/LUVs



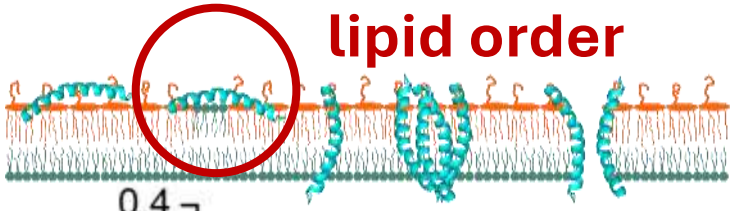


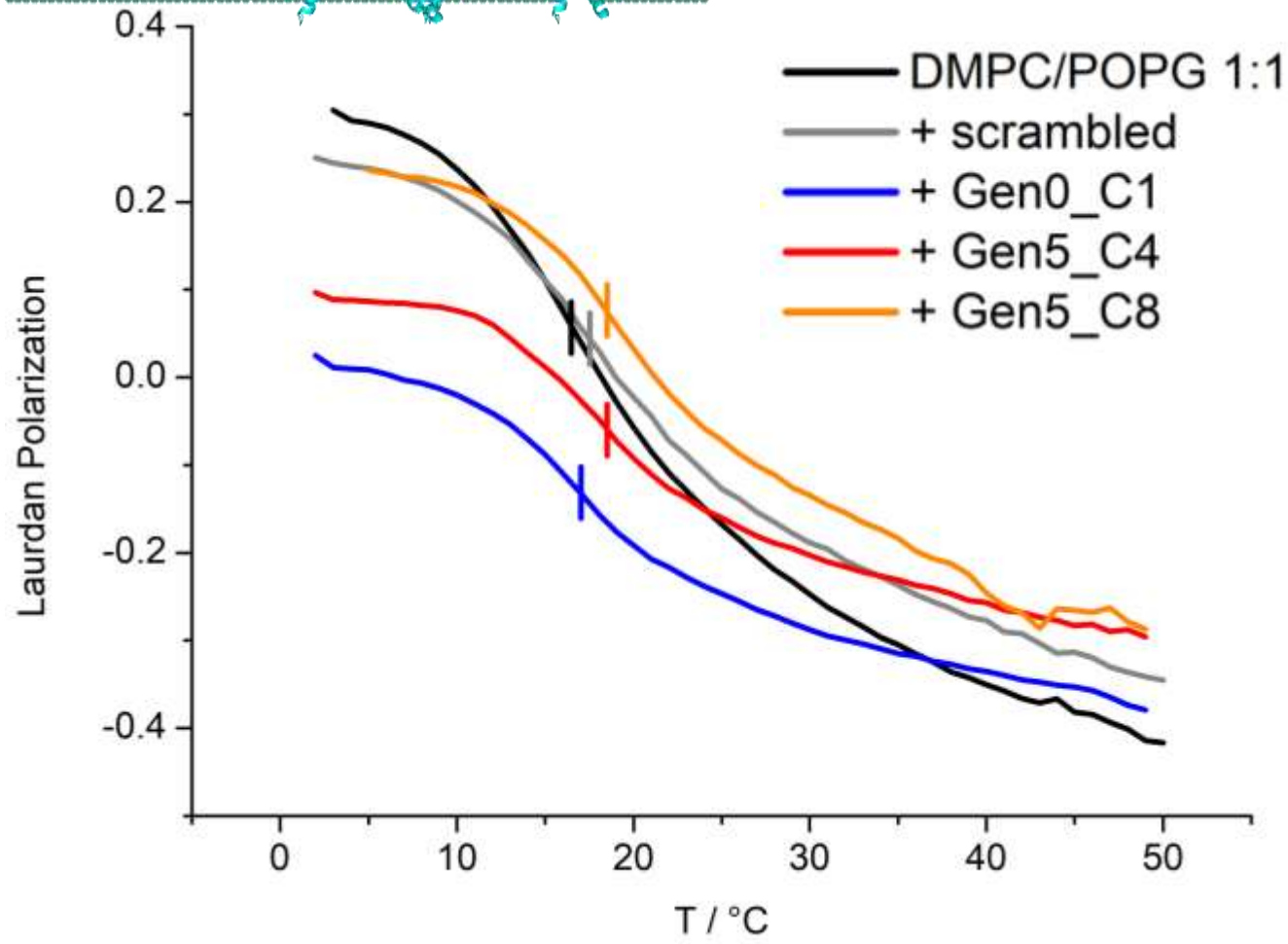


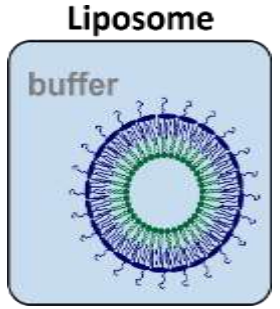


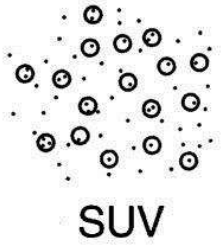


Lipid order and phase transition: LISO – Lipid State Observer



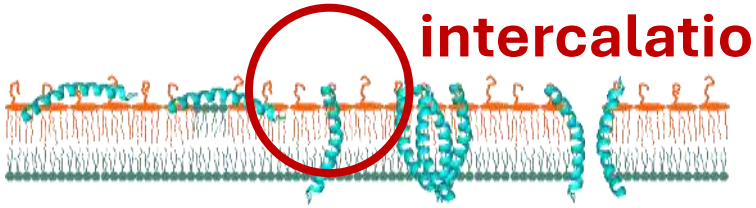


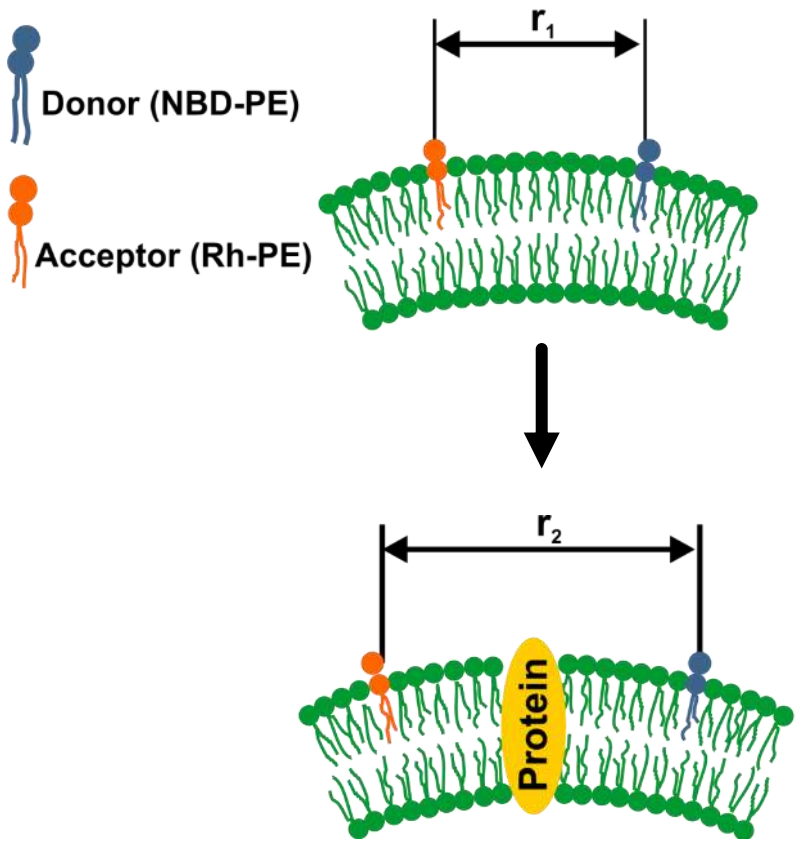


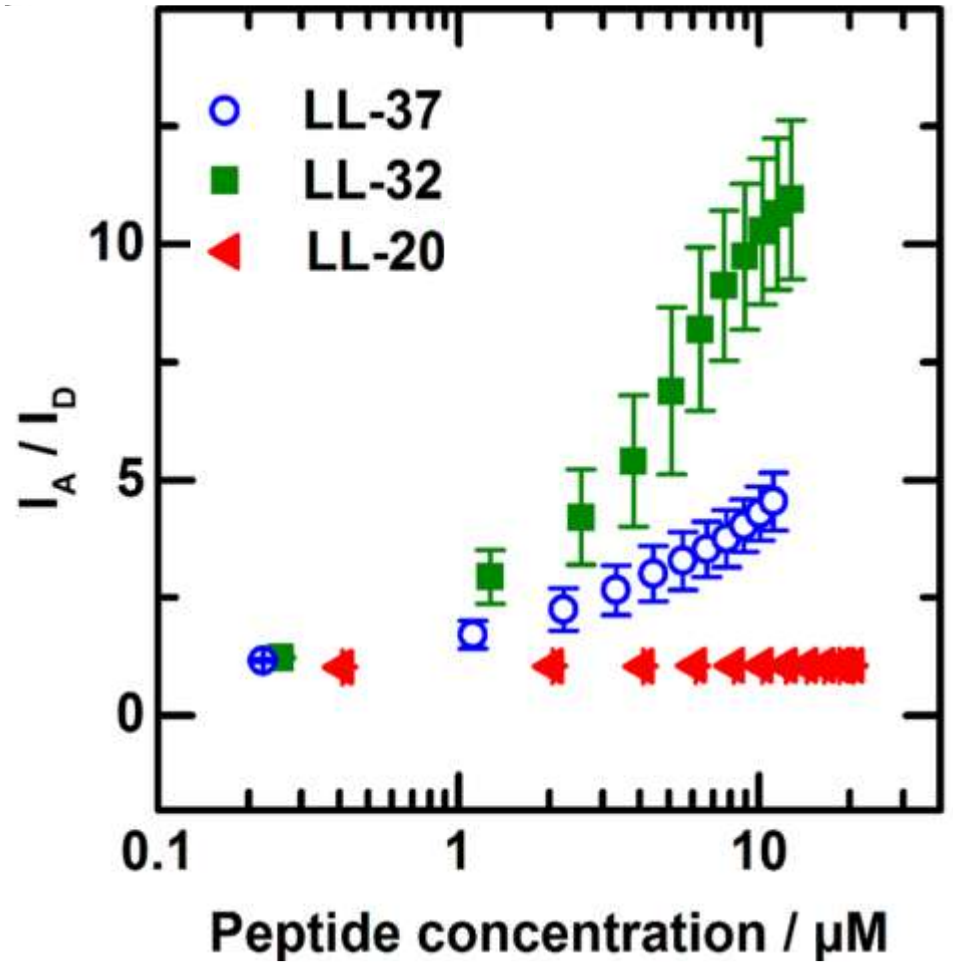


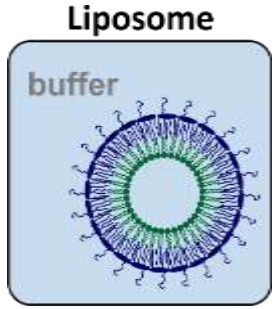
More successful EA AMPs (Gen5)
i) level lipid order and
ii) shift phase transition to higher T

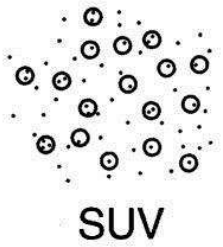
FRET spectroscopy on SUVs/LUVs





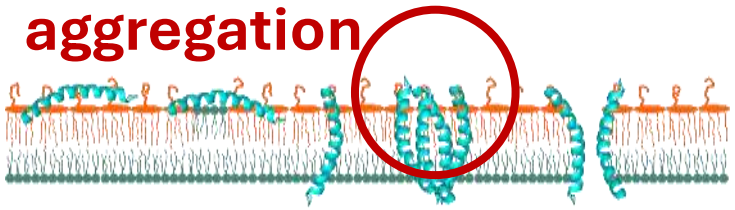


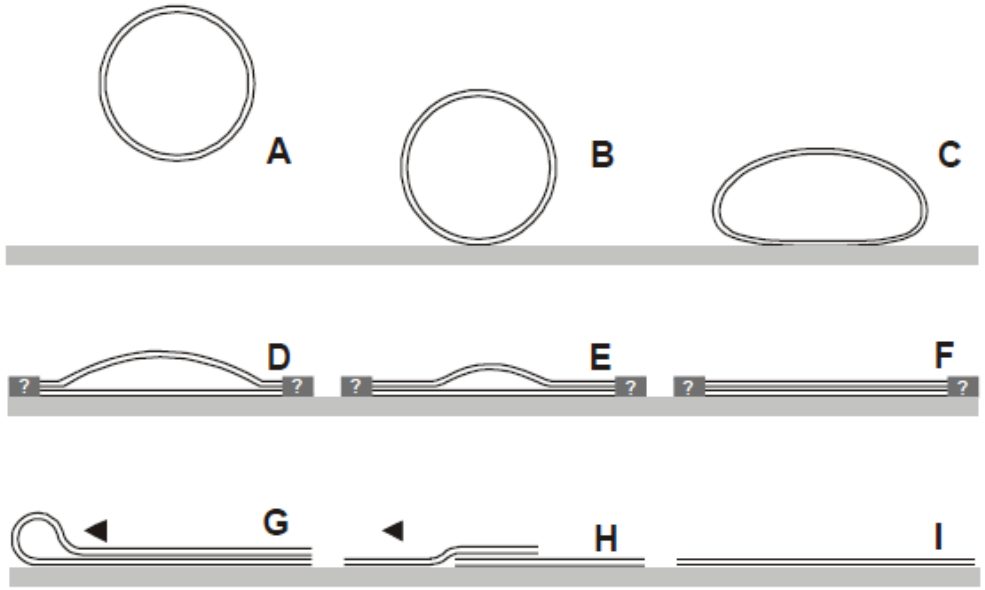


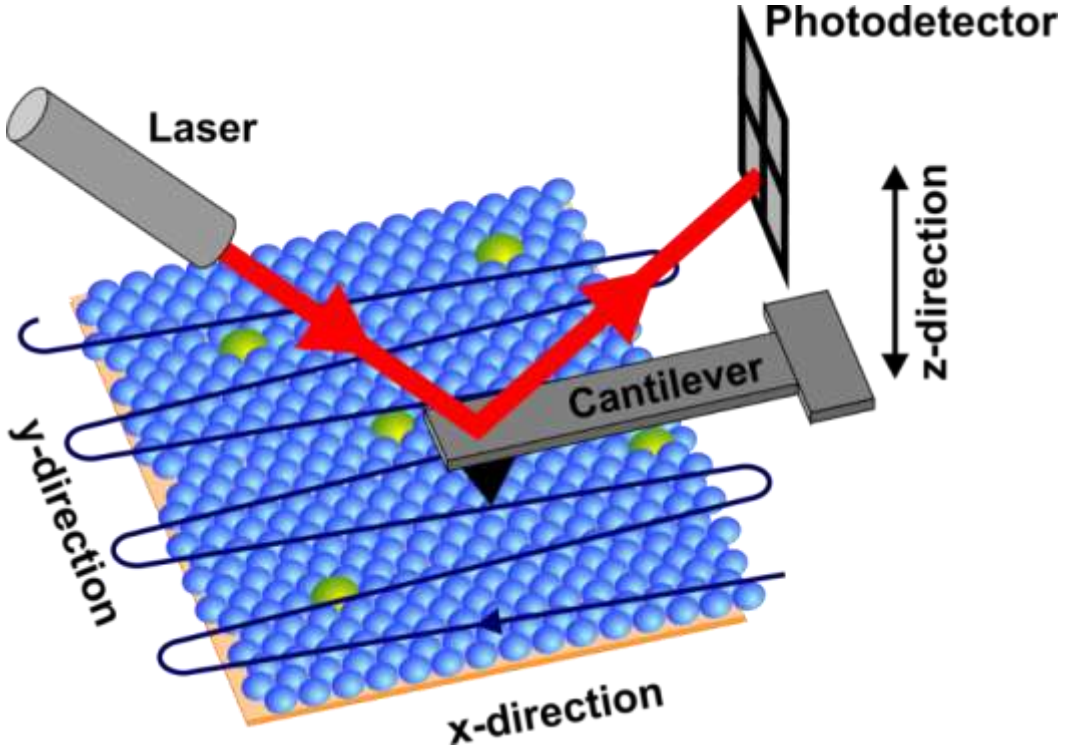


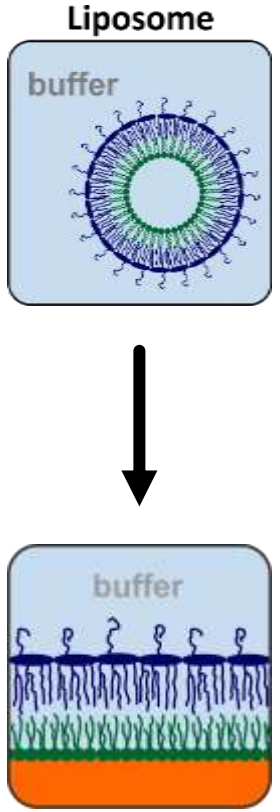
Partitioning between bacterial lipids depends on VPRTES tail of LL37

Atomic force microscopy on solid supported membranes









LL-32 and hBD3-l on PE+PG+TDM (1:1:2) bilayer

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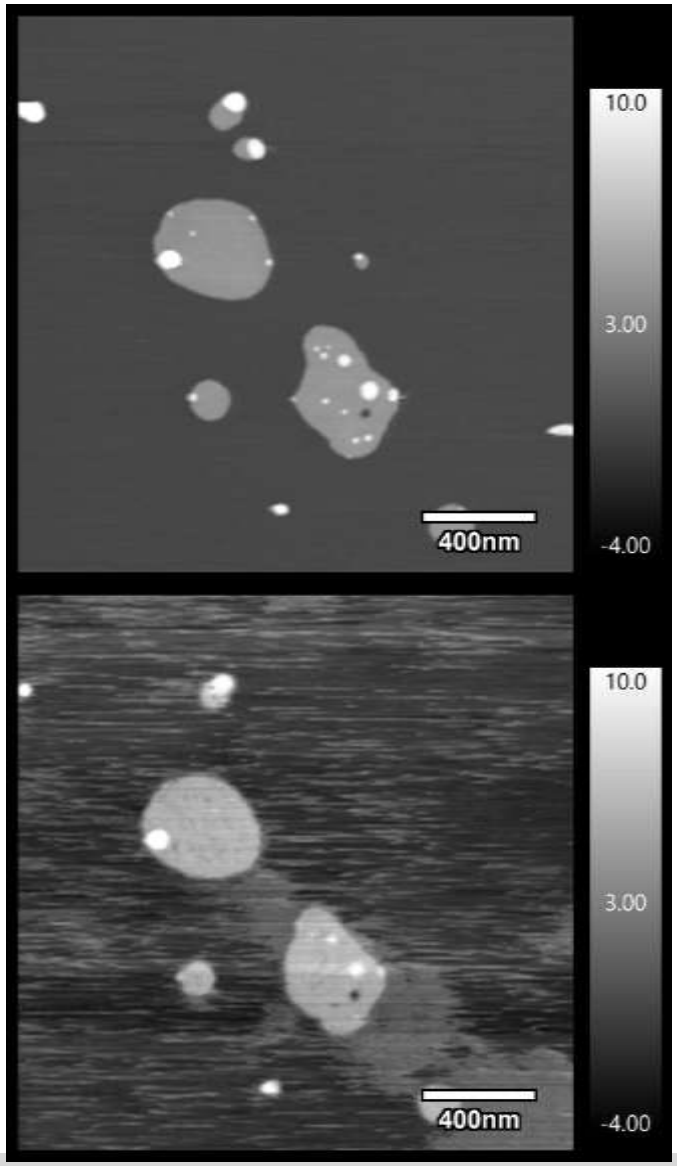

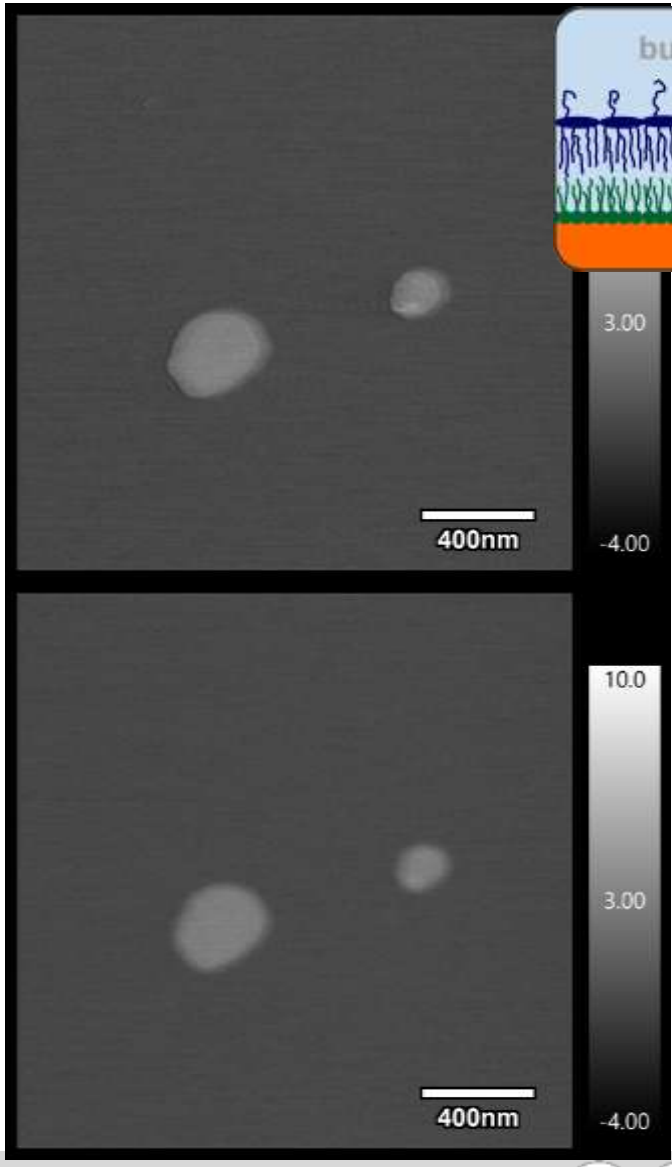

Nehls et al.: manuscript in preparation

TDM: Trehalose dimycolate

Potassium iodide fluorescence quenching assay

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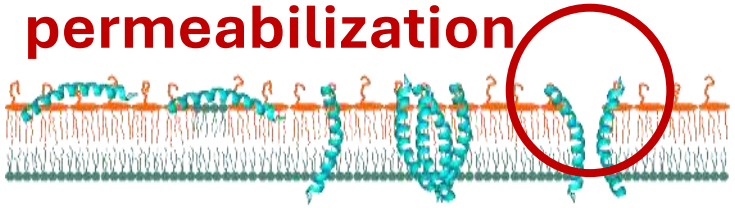
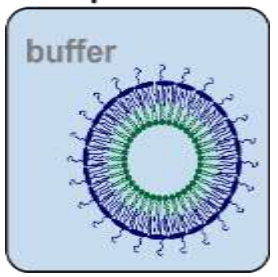
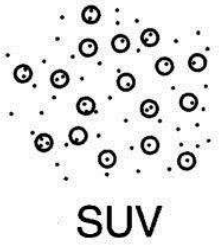
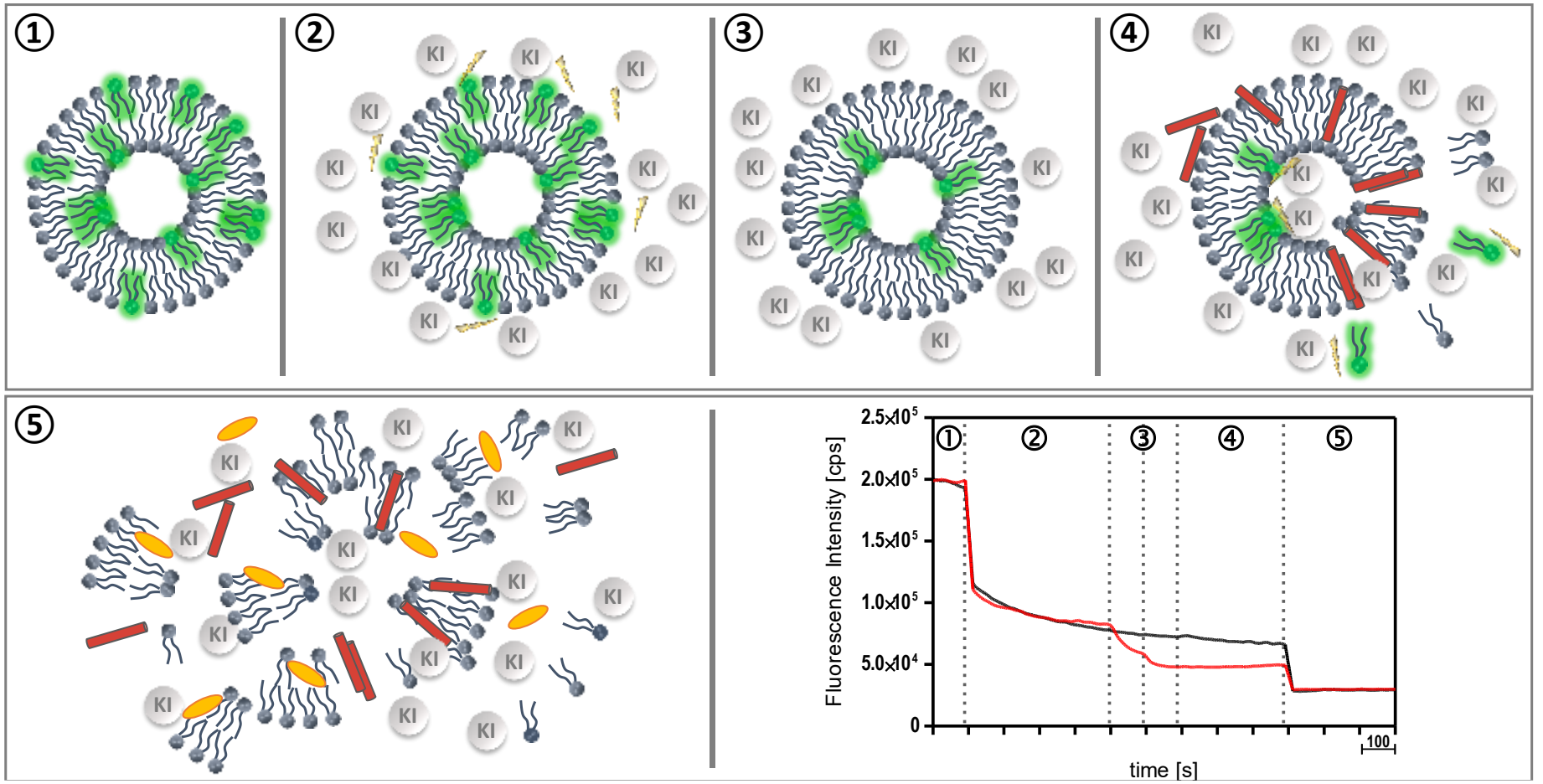
permeabilization



Liposome







Decreasing the PC content as for resistant strain

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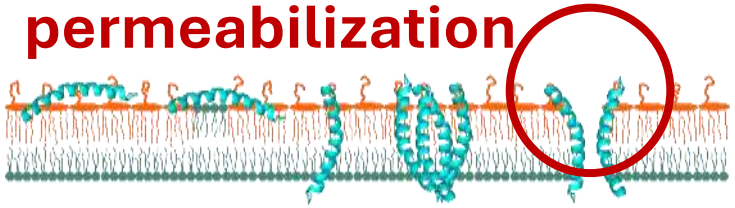
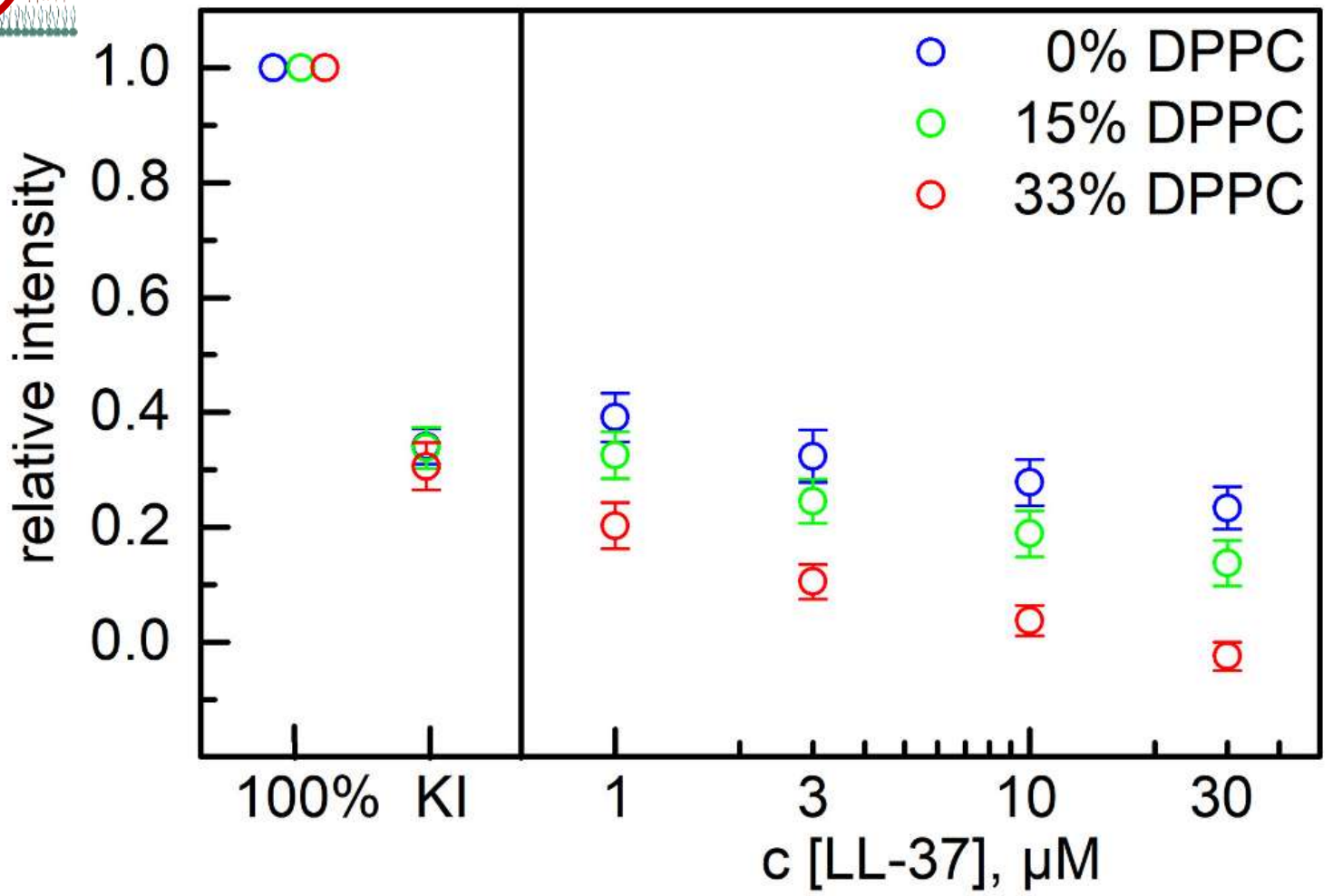
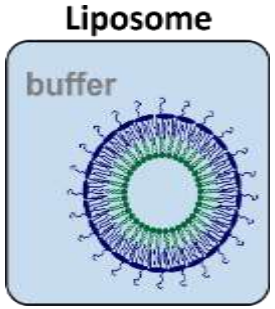
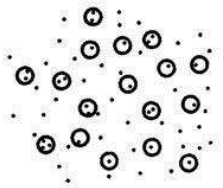


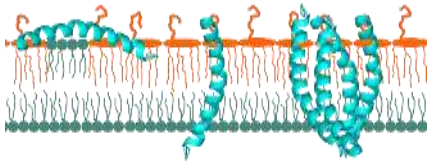
Resistance of *Legionella micdadei* towards LL-37 depends on PC content of membrane



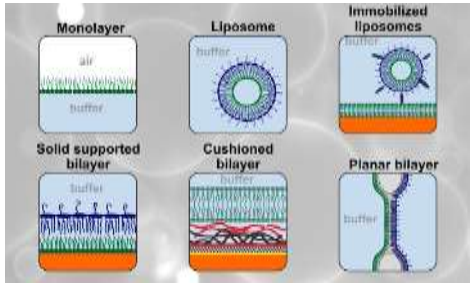




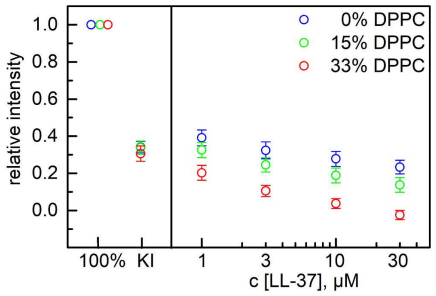
Take-homes



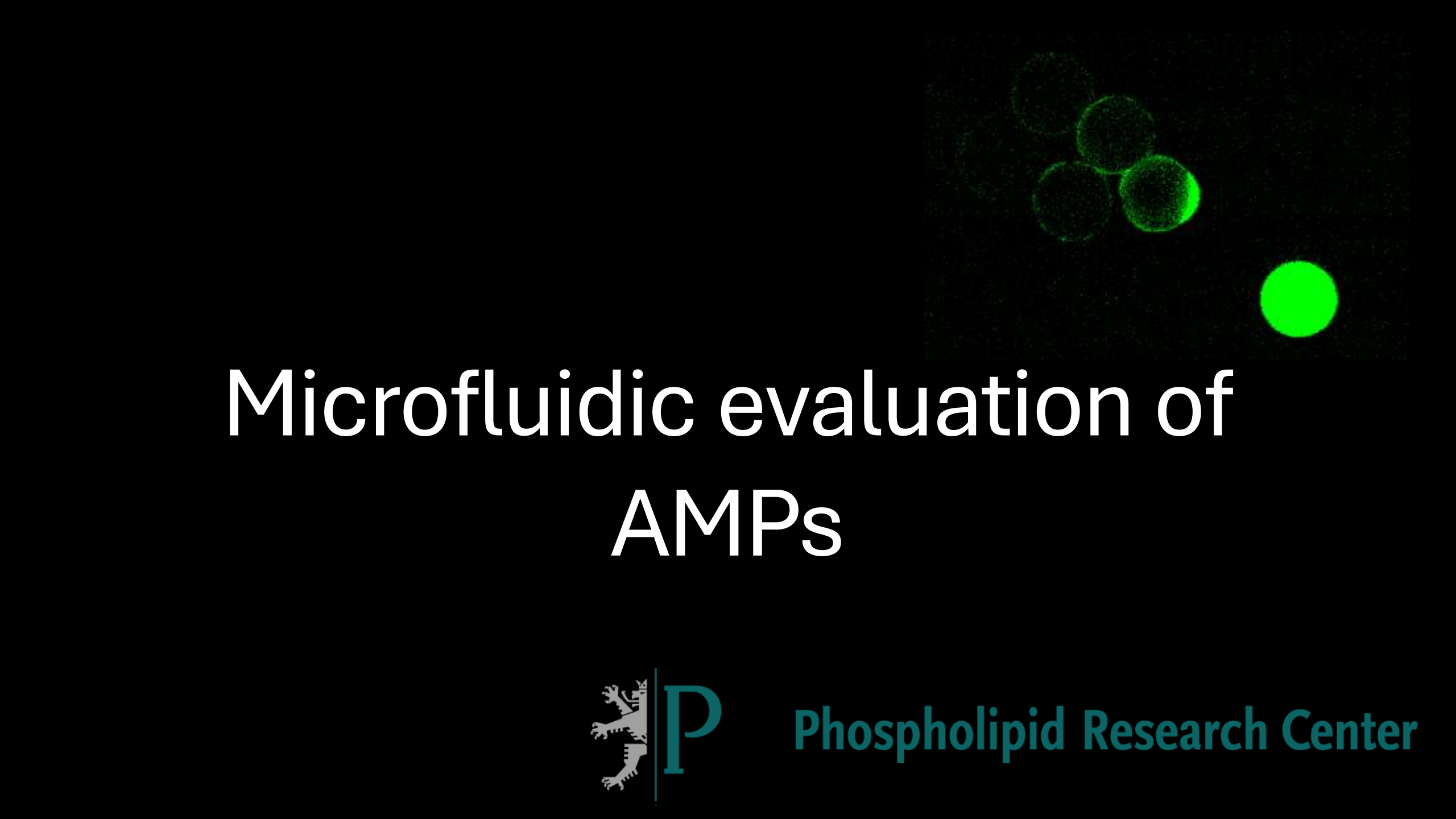
All critical membrane interaction steps can be analyzed using liposome techniques.



Only complementary comparison of different techniques and model systems provides full understanding.



Changes in lipid composition may dramatically modify the membrane interaction.



Microfluidic evaluation of AMPs

Phospholipid Research Center

Team Microfluidics

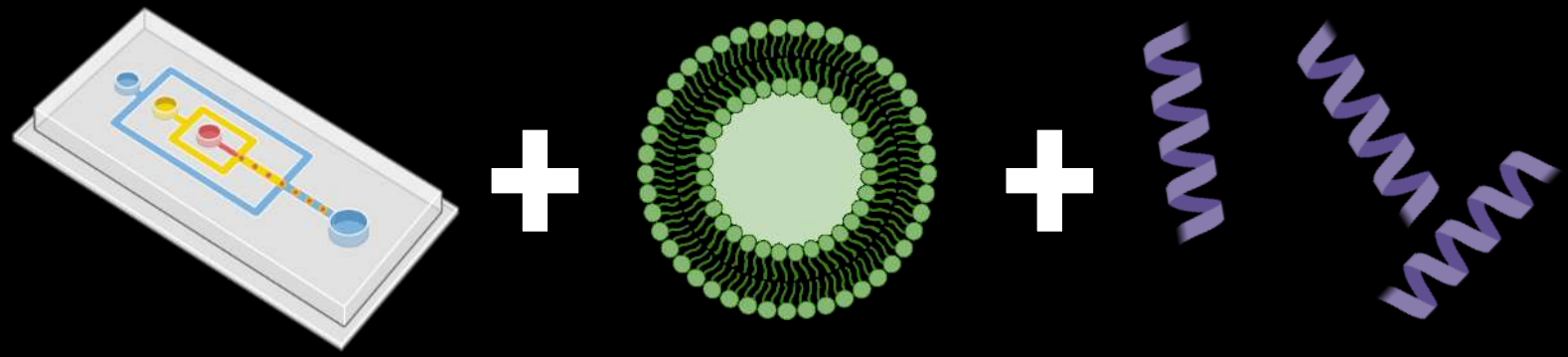
Research Center Borstel
Leibniz Lung Center

Janina Nandy
Research Center Borstel

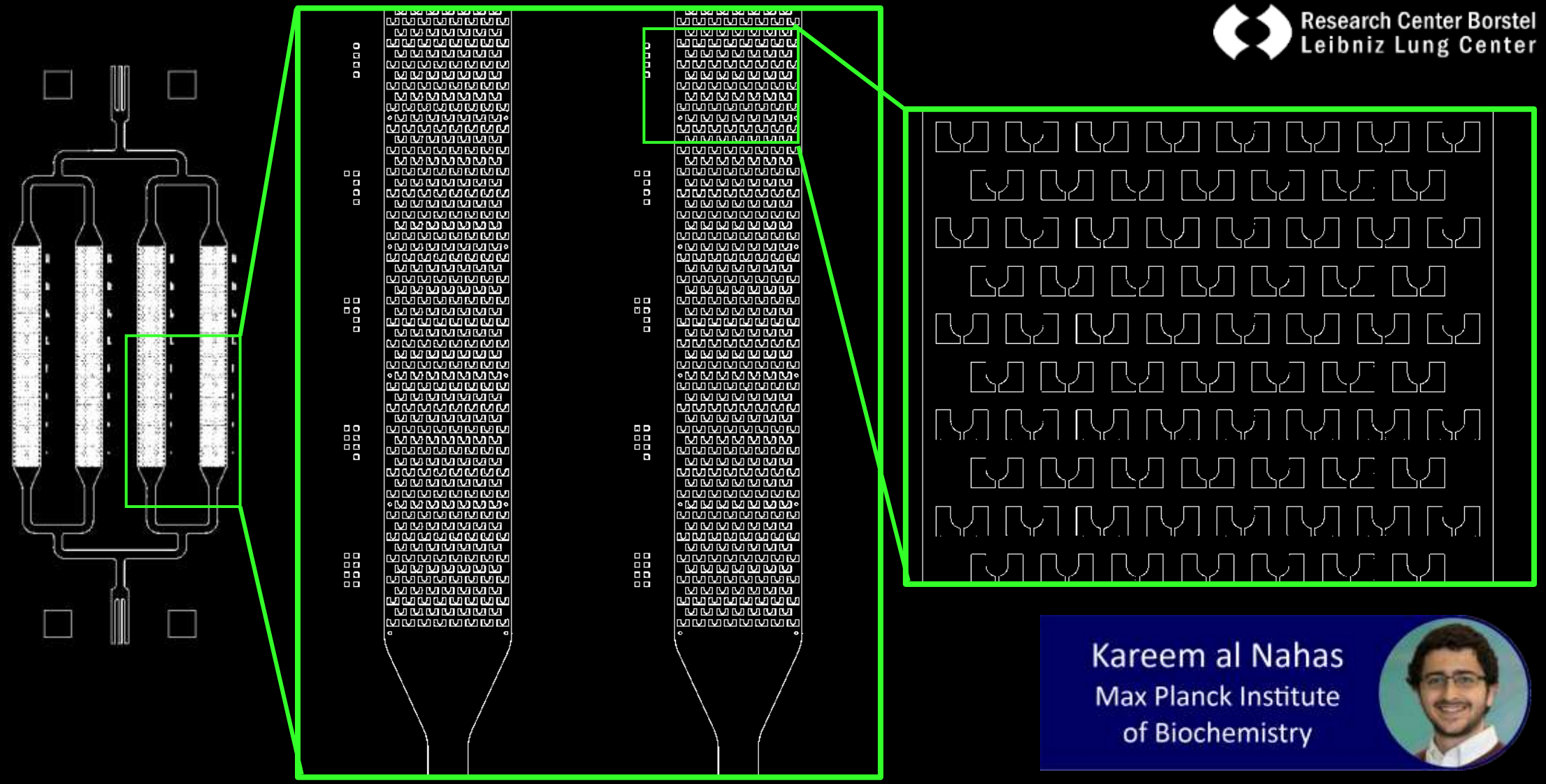
Rieke Bruns
Research Center Borstel

Marie Wetzel
Research Center Borstel

Franziska Kiesow
Research Center Borstel



Standardized prediction of antimicrobial activity



Kareem al Nahas
Max Planck Institute
of Biochemistry

Marcus Fletcher
Imperial College London

Al Nahas et al. Lab on a Chip, 2019

Trapping hundreds of liposomes on chip

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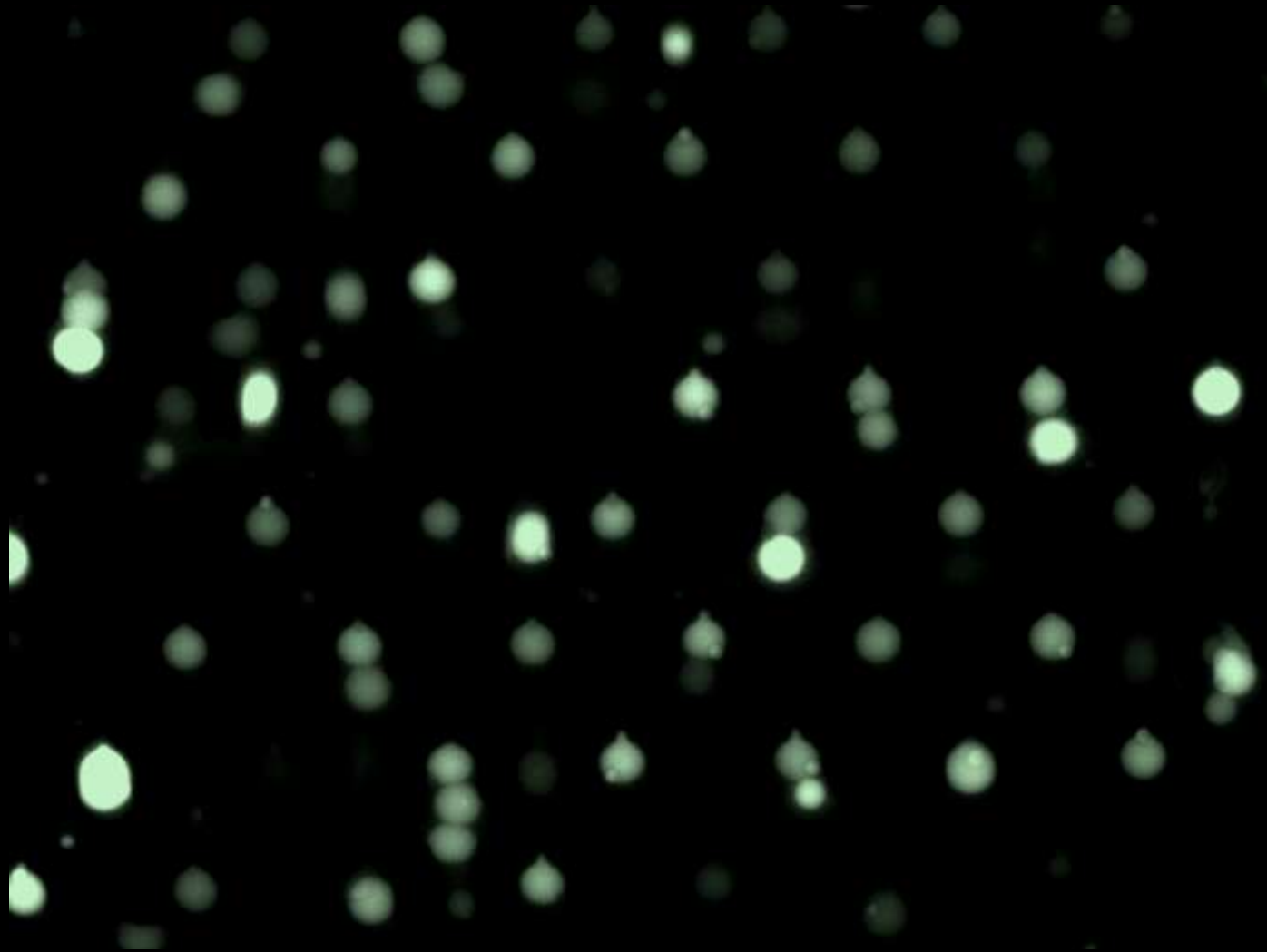
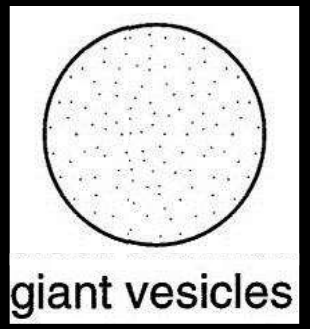
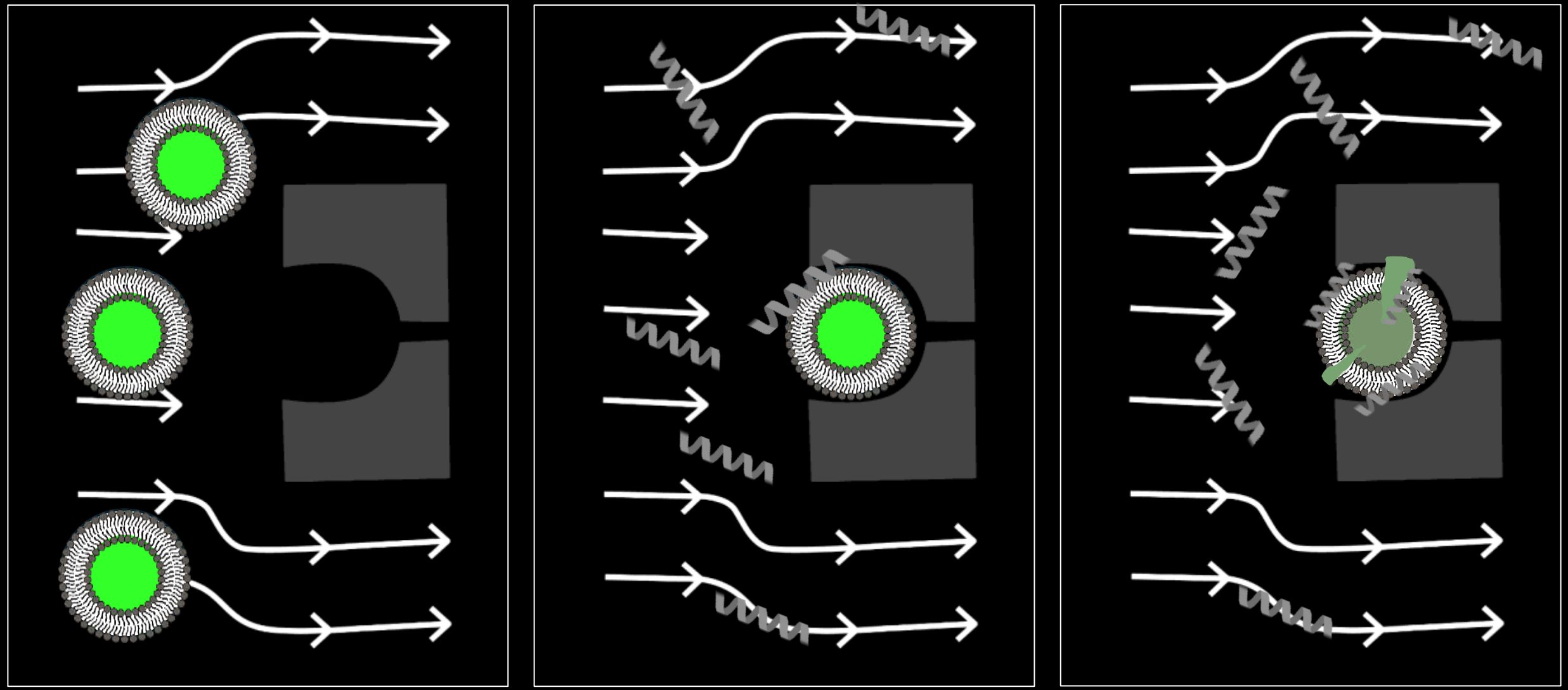
Leibniz Lung Center



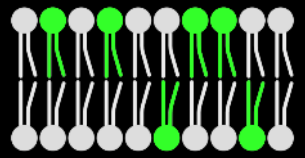




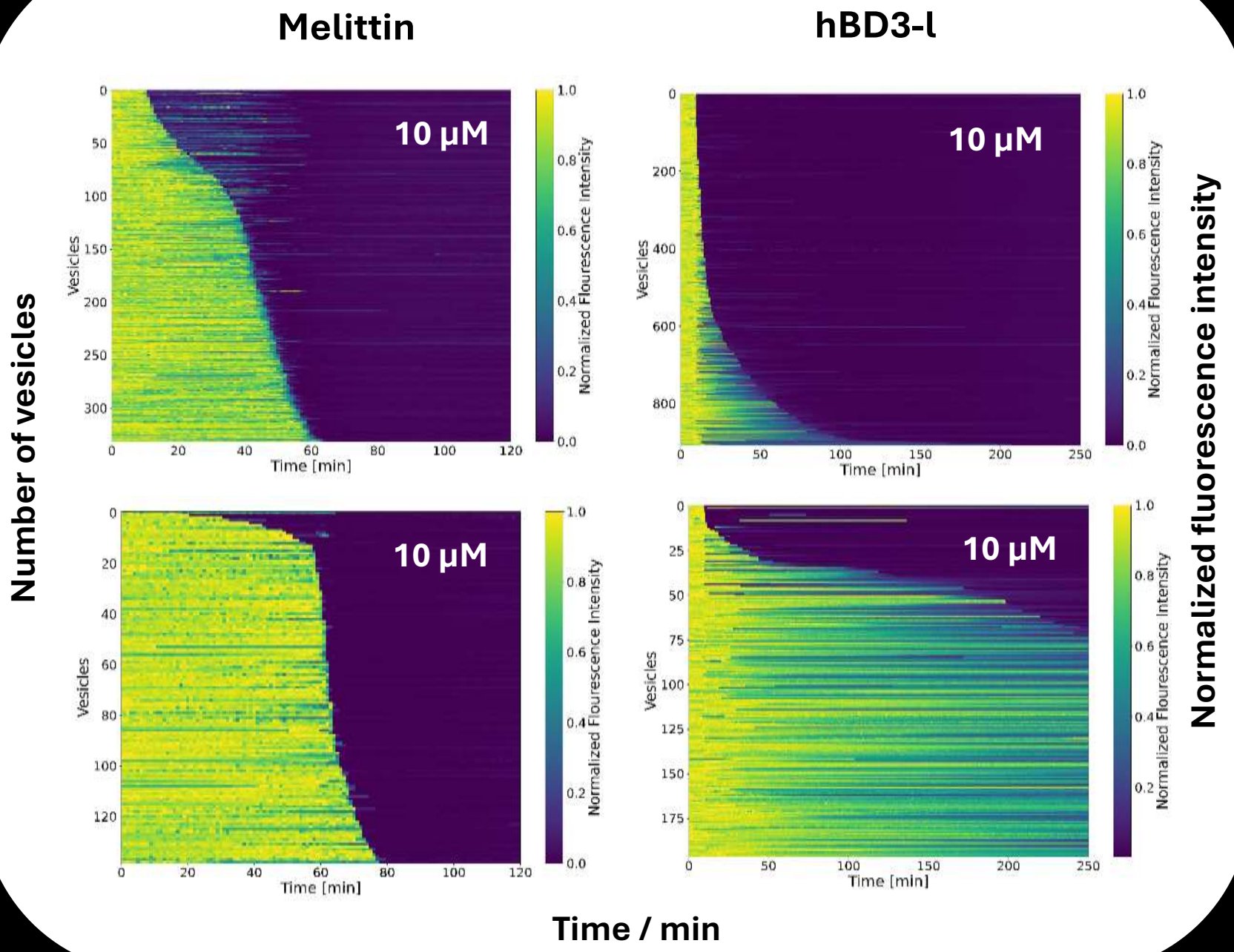
Time

Proof of concept

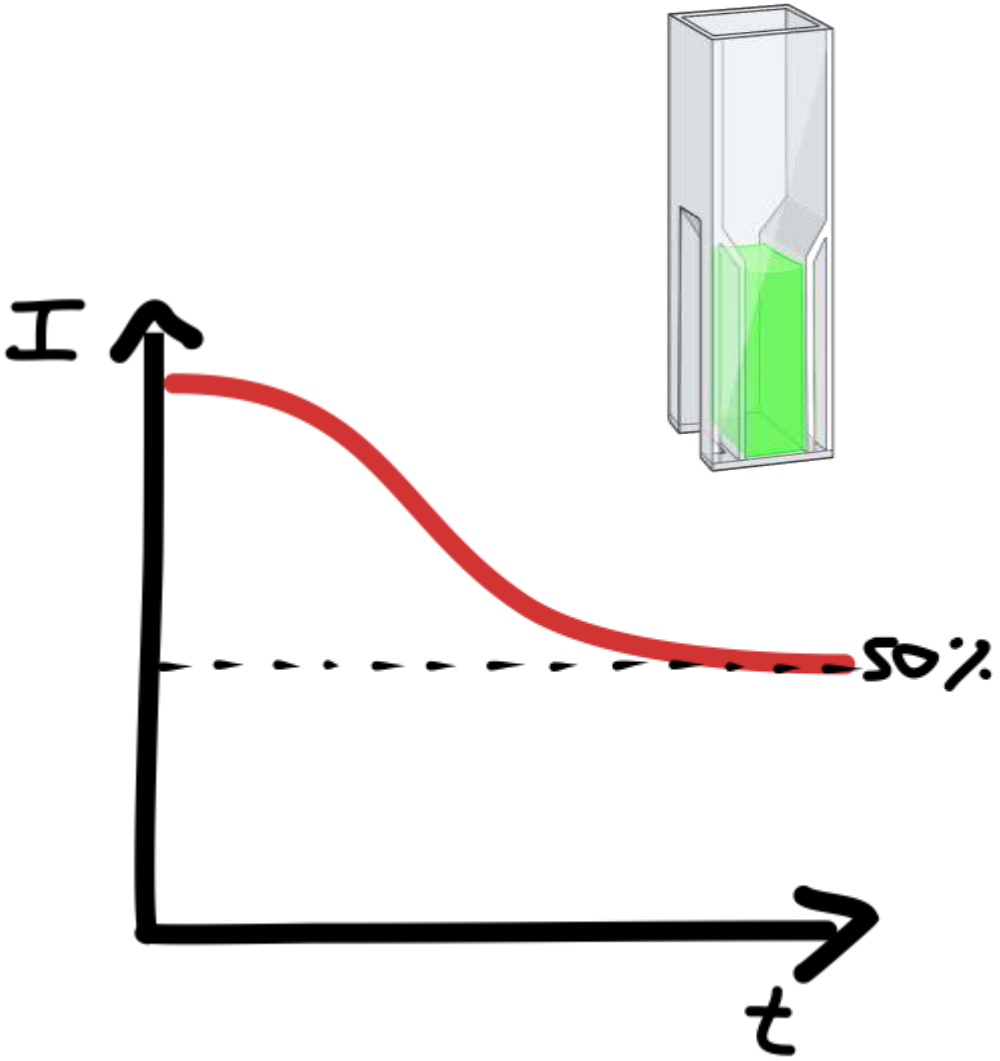
Bacterial Model
DOPC:DOPG 1:1



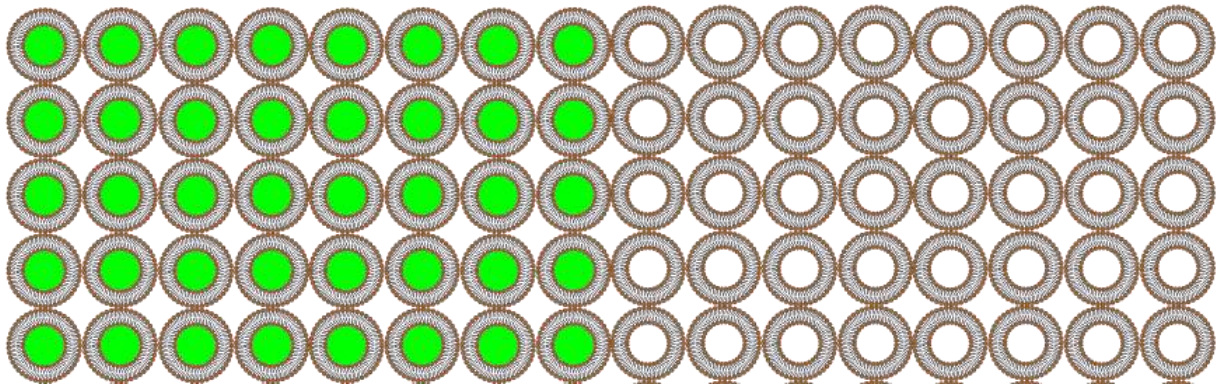
Dye release of microfluidic trapped liposomes provides reasonable results



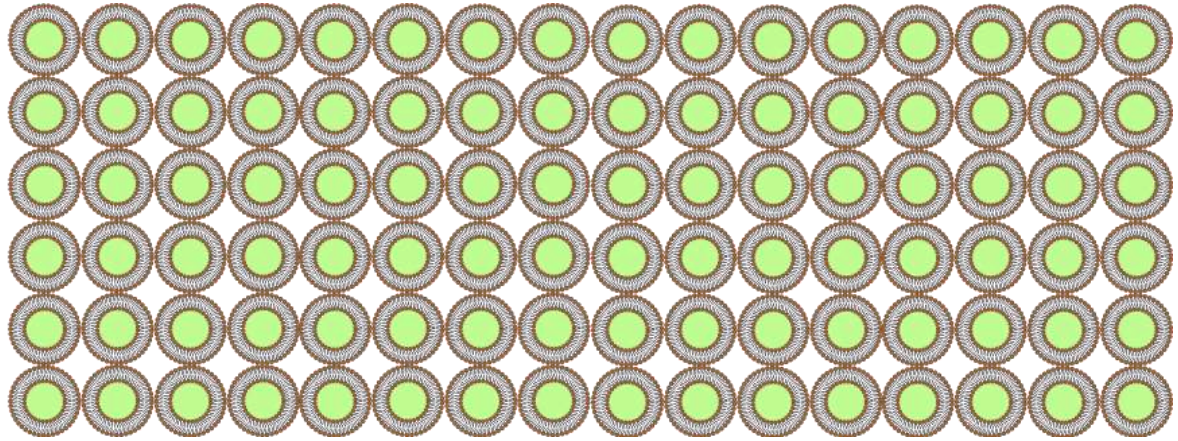
Our technique provides additional information...



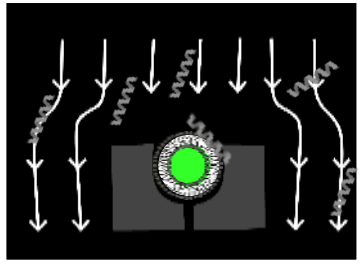
Half empty – half full **All-OR-NONE**



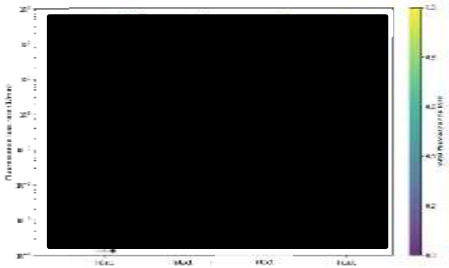
Partial release of dye **GRADED**



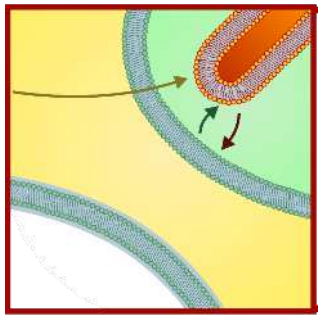
Take-homes



Microfluidic trapping bridges single liposome and bulk analysis.



Quantitative evaluation enables classification of mode of action continuum of membrane active compounds.



Membrane interaction is key to predict antimicrobial potential of AMPs (and other compounds).

Acknowledgement

Research Center Borstel
Leibniz Lung Center

Biophysics Group
Thomas Gutsmann
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Klaus Brandenburg

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Niels Röckendorf / Andreas Frey
Andra Schromm

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MAX PLANCK INSTITUTE — Naresh Yandrapalli

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RCSI ROYAL COLLEGE OF SURGEONS IN IRELAND — Marc Devocelle, John Connolly

Phospholipid Research Center

Federal Ministry of Education and Research

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Research Center Borstel
Leibniz Lung Center

International Membrane Biophysics Meeting

Membranes as Biological Barriers

Drübeck Monastery I March 23th to 26th 2026

Confirmed Invited Speakers

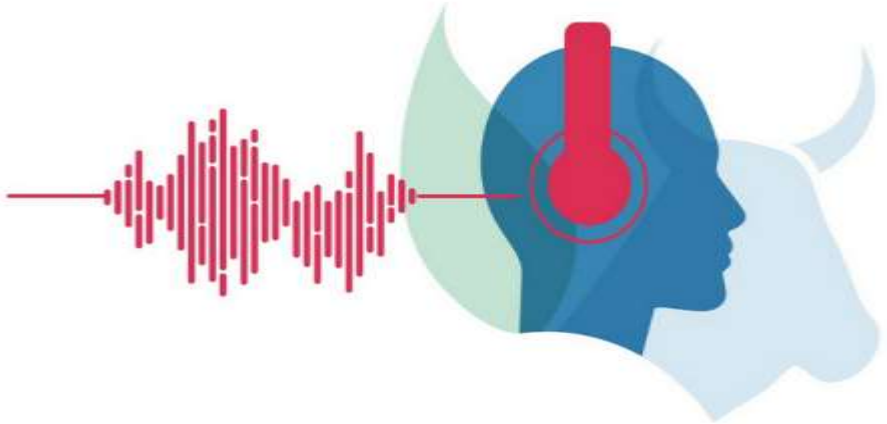
Elina Ikonen, Helsinki, FI
Erdinc Sezgin, Karolinska, SE
Katia Cosentino, Modena, IT
Matti Javanainen, Tampere, FI
Luca Monticelli, Lyon, FR
Jesus Perez-Gil, Madrid, ES
Gerard Wong, Los Angeles, USA
Radek Šachl, Prague, CZ
Cecilia Clementi, Berlin, DE
Anna Taubenberger, Dresden, DE
James Saenz, Dresden, DE
Alf Honigmann, Dresden, DE
Frauke Gräter, Mainz, DE
Hans-Joachim Galla, Münster, DE

Registration

Early Bird Deadline: December 16, 2025
Registration and abstract submission will be possible:
November 03, 2023 - January 16, 2025

Scientific organizers:
Christian Nehls, Borstel
Fabio Lolicato, Heidelberg
Ilpo Vattulainen, Helsinki

dgfb.org



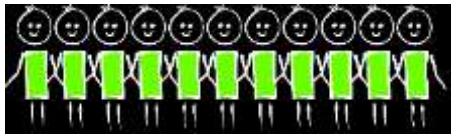
MIKROBEN IM VISIER

INFEKTIONEN VERSTEHEN, RESISTENZEN BESIEGEN

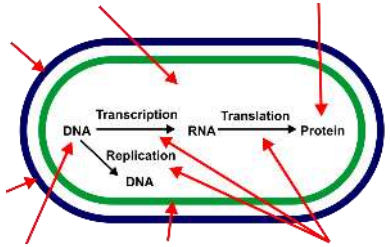
Wrap-up

Research Center Borstel
Leibniz Lung Center

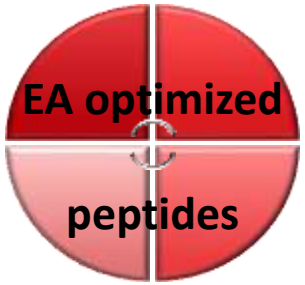
AMR



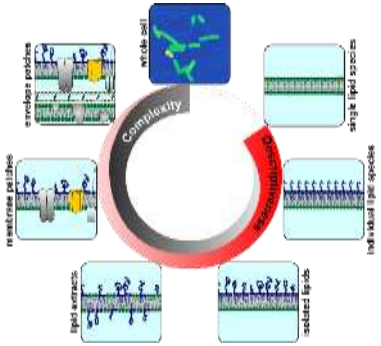
AMPs



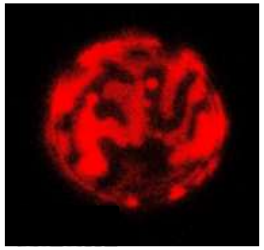
AMP optimization



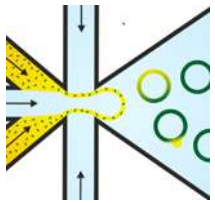
Model complexity



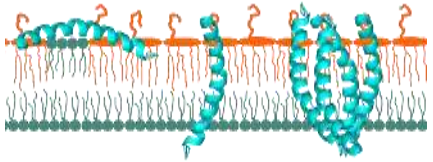
Asymmetriy



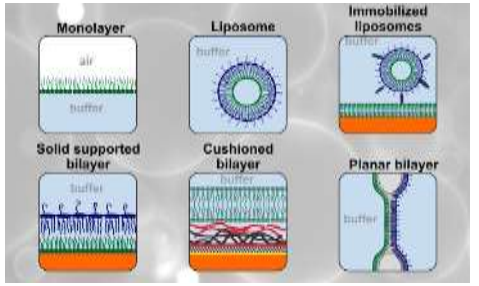
Microfluidics



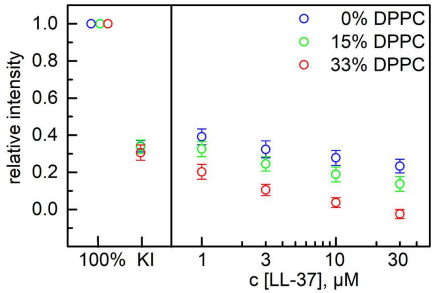
Membrane interaction



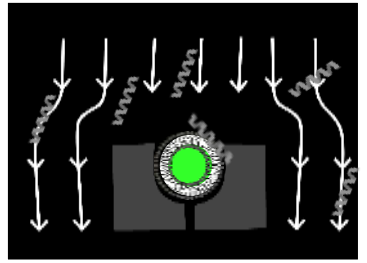
Membrane geometry



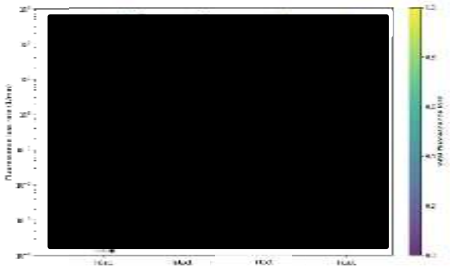
Resistance determinants



Microfluidic trapping



Graded - quantification



Membrane interaction