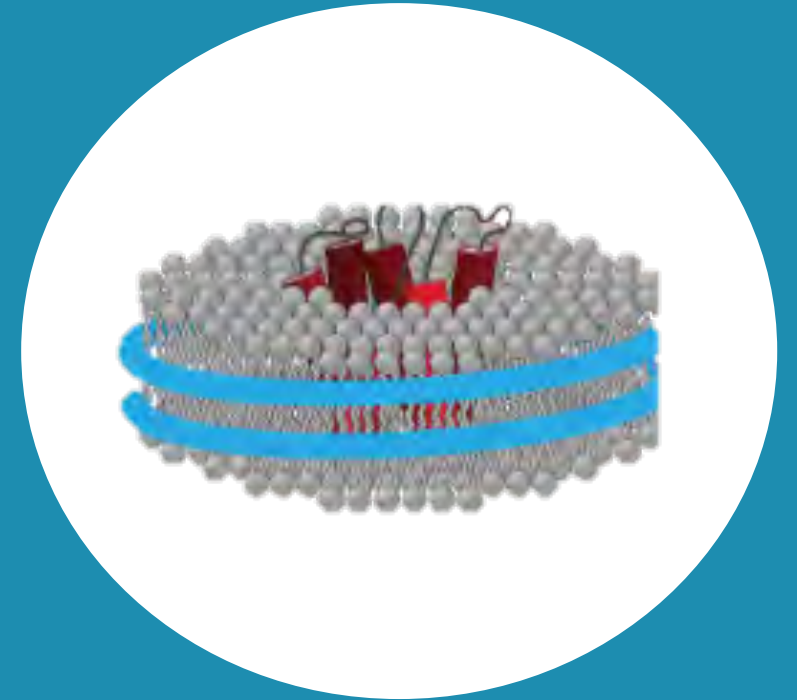# Eukaryotic and Prokaryotic xMALPs composition: a comparative study

Dr. Marta Barniol-Xicota

Laboratory of Chemical Biology
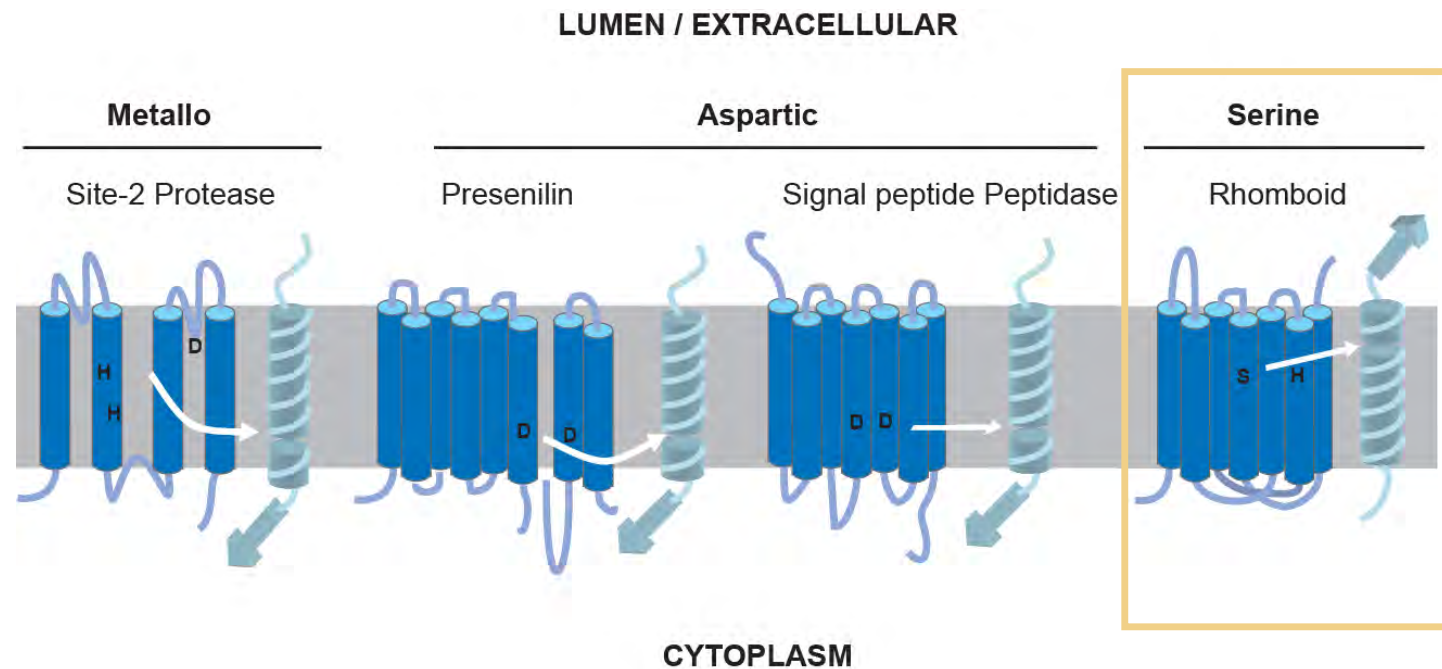
marta.barniolxicota@kuleuven.be

Rhomboids are fragile intramembrane proteases

Undercharacterized group of intramembrane proteases
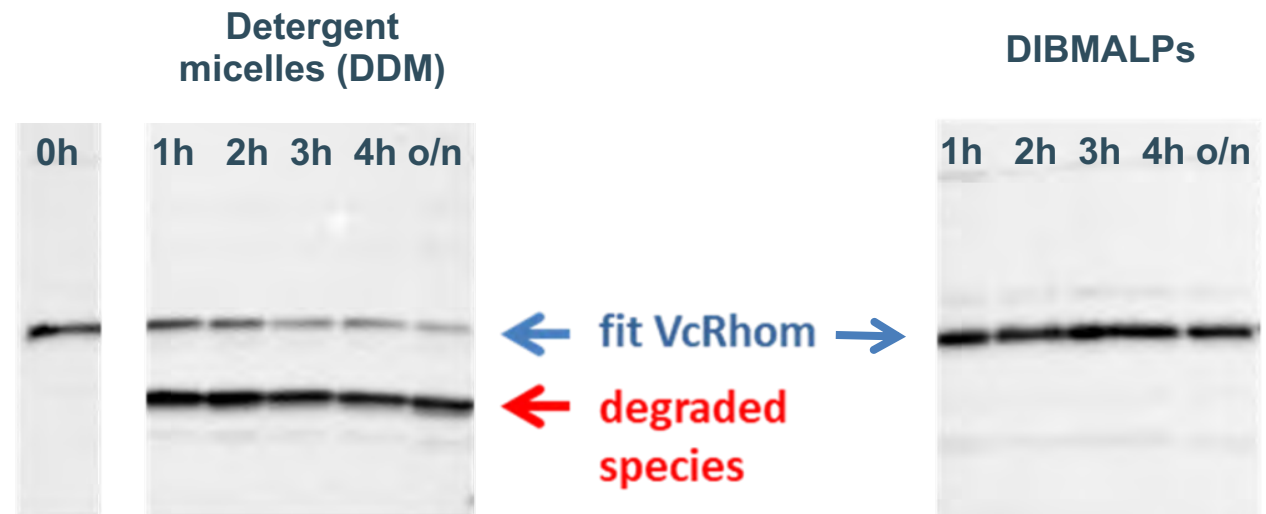
Substrate cleavage in the membrane

Linked to: Alzheimer´s disease, Malaria, Parkinson´s disease

xMA stabilize fragile intramembrane proteases

Challenging expression & purification:

- Self-process in detergent micelles

- Loss of activity

Barniol-Xicota, M. & Verhelst, S. H. L. JACS. **2018**, 140, 44, 14557

Faculty of Medicine, Laboratory of Chemical Biology

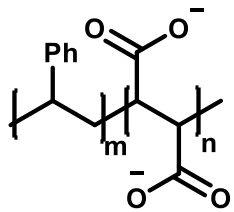KU LEUVEN

Activity level changes depending on xMA used

Activity measured using activity based probe TAMRA-FP + in gel resolution

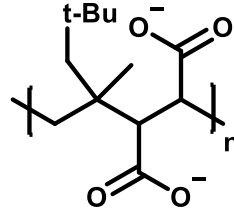Barniol-Xicota, M. & Verhelst, S. H. L. JACS. **2018**, 140, 44, 14557

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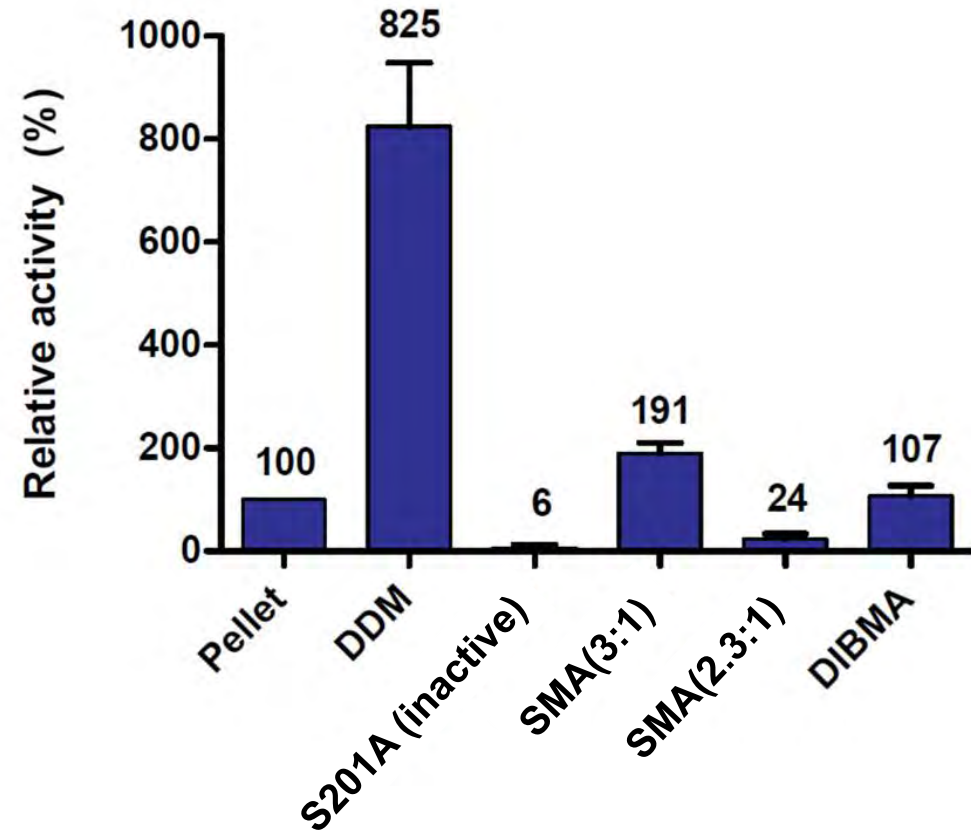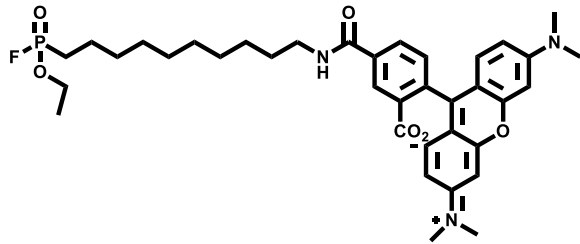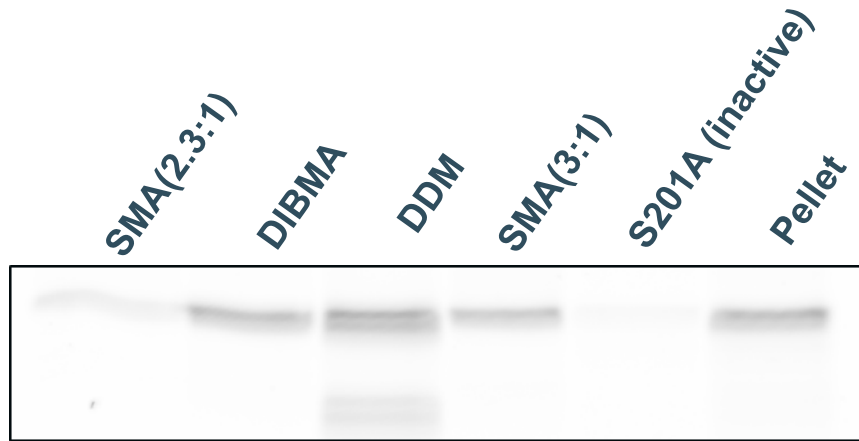
Do xMALPs ressemble the native mebrane?
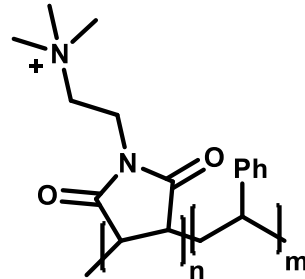
xMAs used in this work:

SMA
m = 2.3 or 3
n = 1

DIBMA
n = 1

SMA-QA
m = 2.3
n = 1

Membranes solubilized:

- Prokaryotic : *E. coli*

- Eukaryotic **: Jurkat cells**

> **Solubilization efficiency**

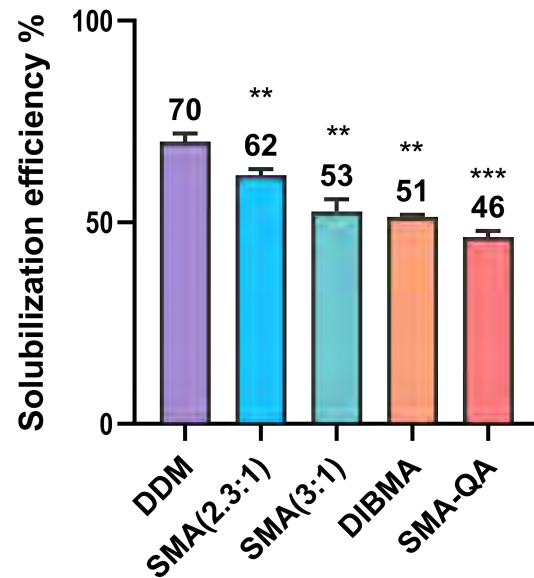   compared to non ionic detergent DDM

> **Protein content**
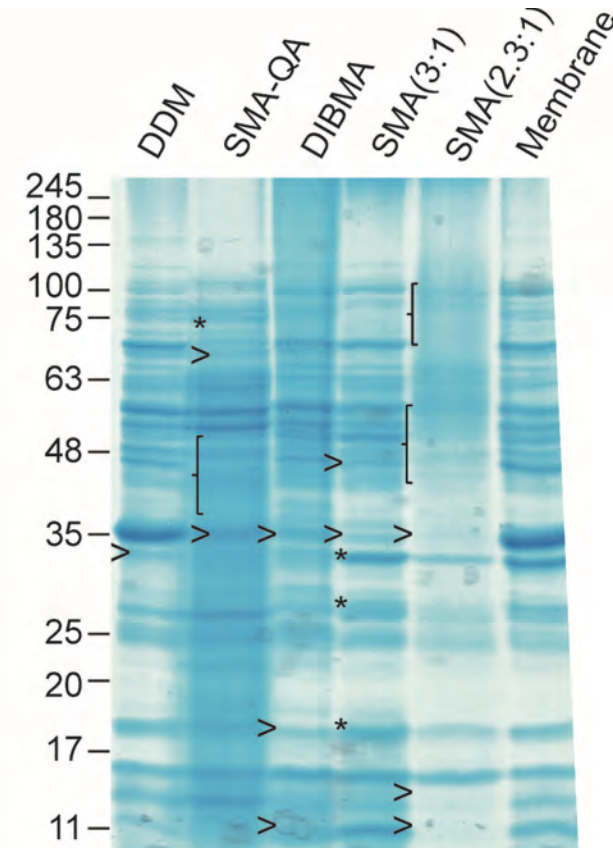
   SDS-PAGE

> **Lipid content:**

   lipidomics

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xMA are efficient solubilizing agents ***

E. coli solubilization efficiency

In **Jurkat** membranes SMAs and DIBMA

as efficient as DDM
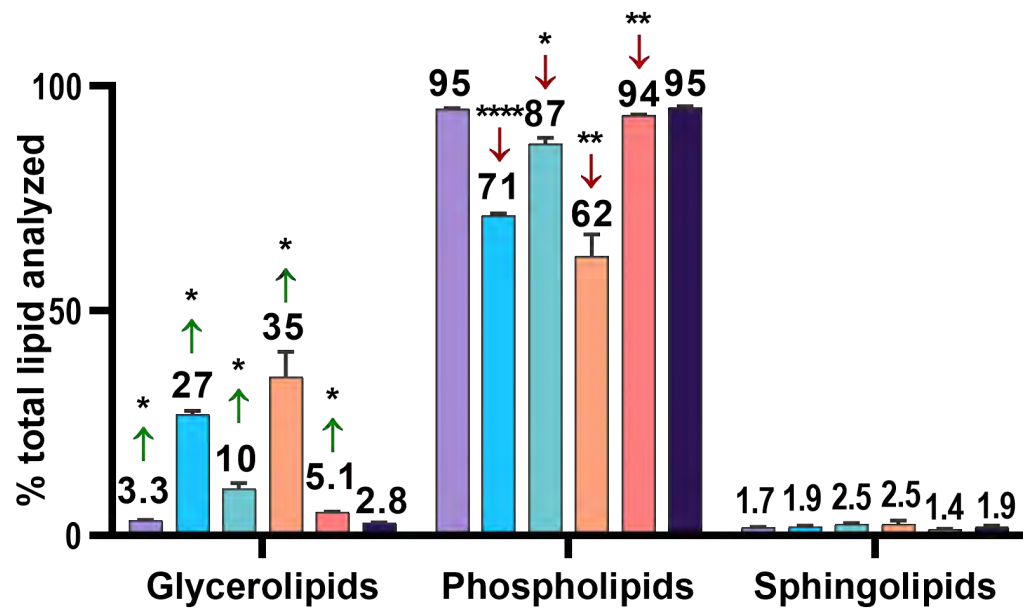
E. coli prot content:

Poor solubilization of high MW proteins

by all xMAs in **Jurkat** membranes

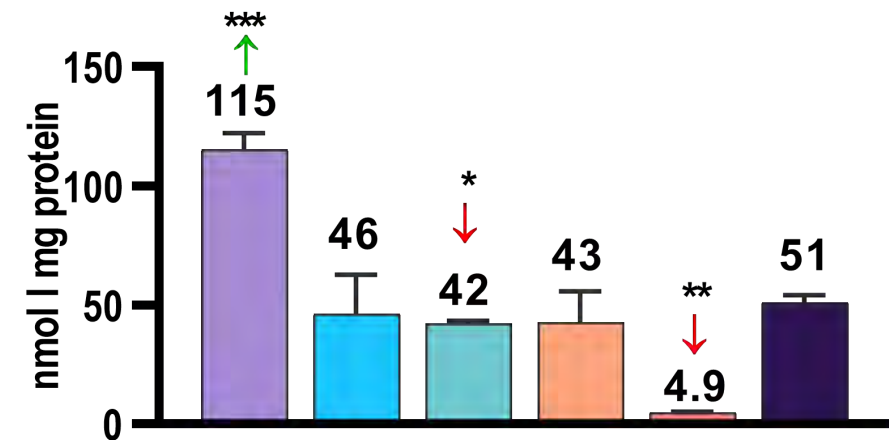# xMA preferentially solubilize lipid species

Lipid composition (Jurkat)

Analyzed by LC-MS/MS

Cholesterol content

Data from colorimetric assay
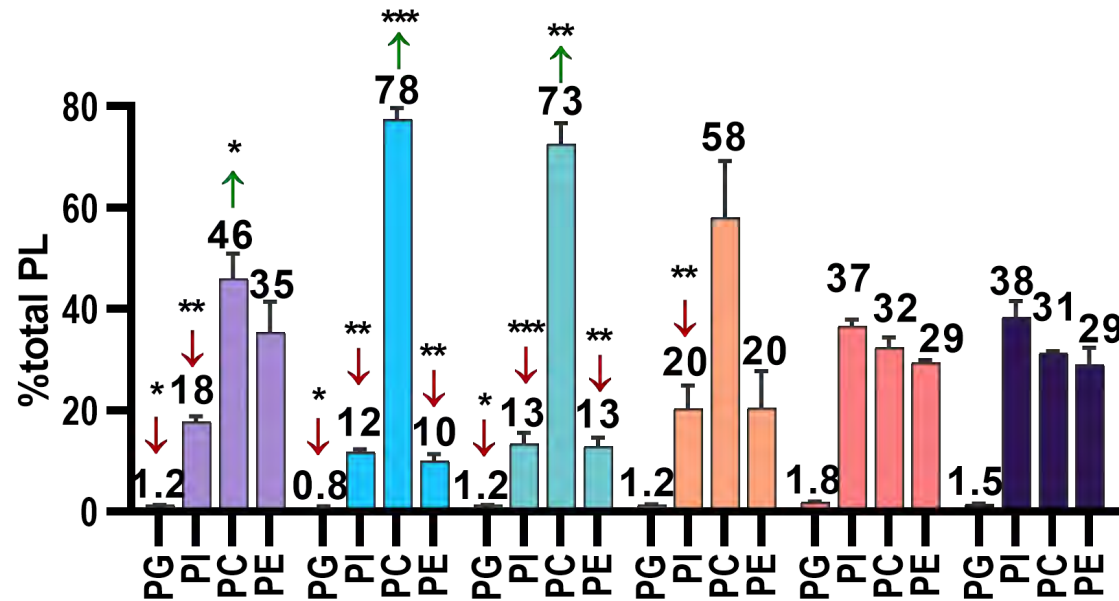

DDM SMA(2.3:1) SMA(3:1) DIBMA SMA-QA Membrane

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Lipid charge does not influence solubilization

Saturation has little effect on xMA solubilization

Fatty acid chain saturation in total lipid content (Jurkat)

Analyzed by LC-MS/MS

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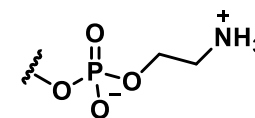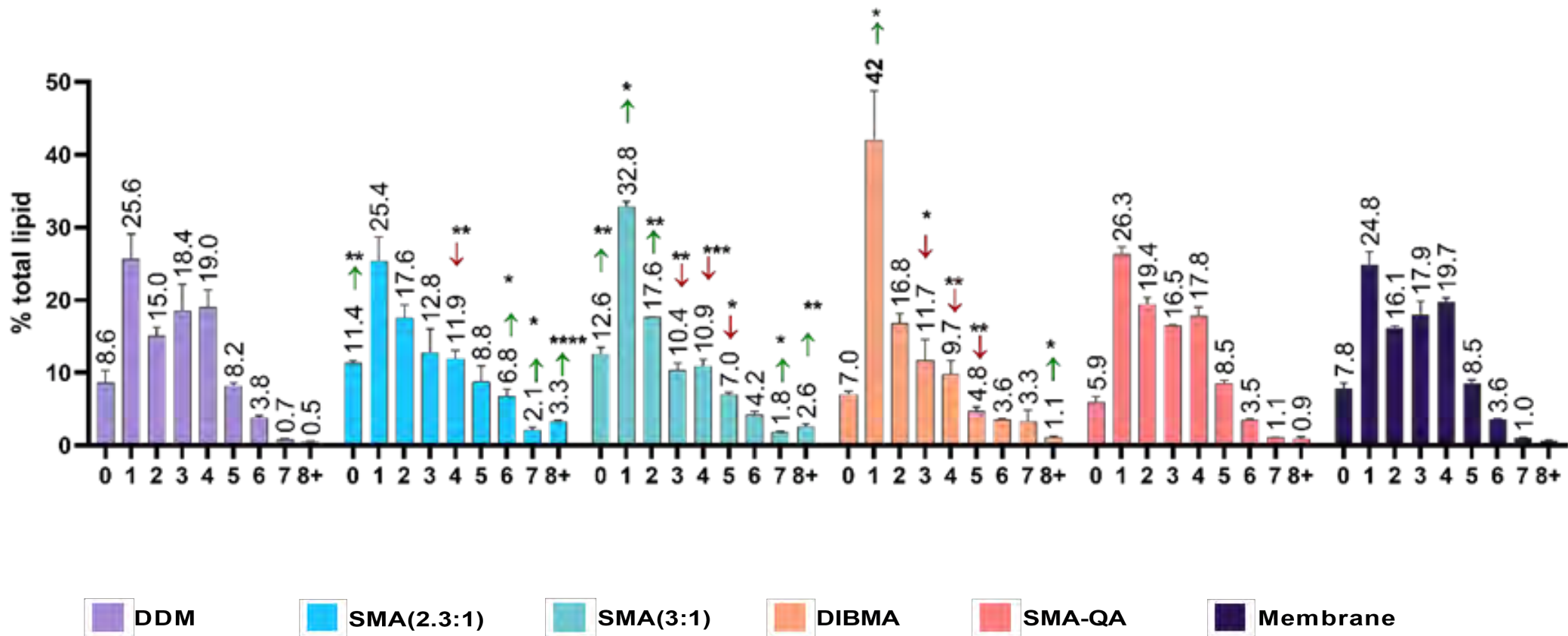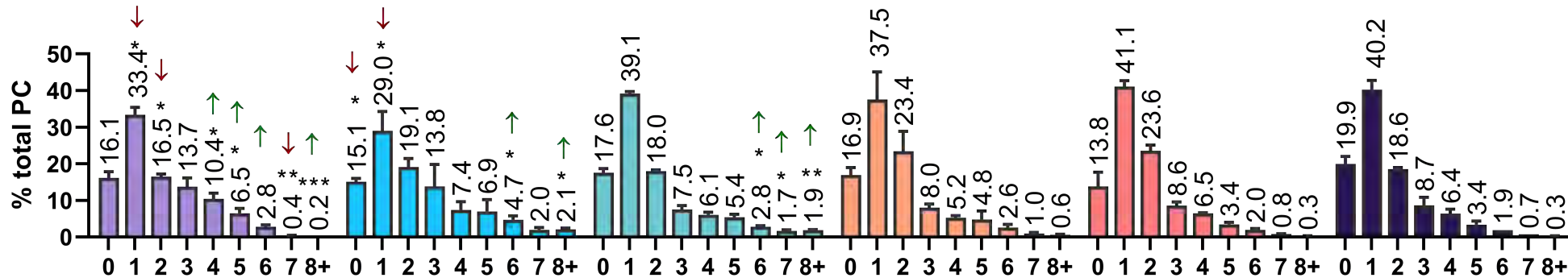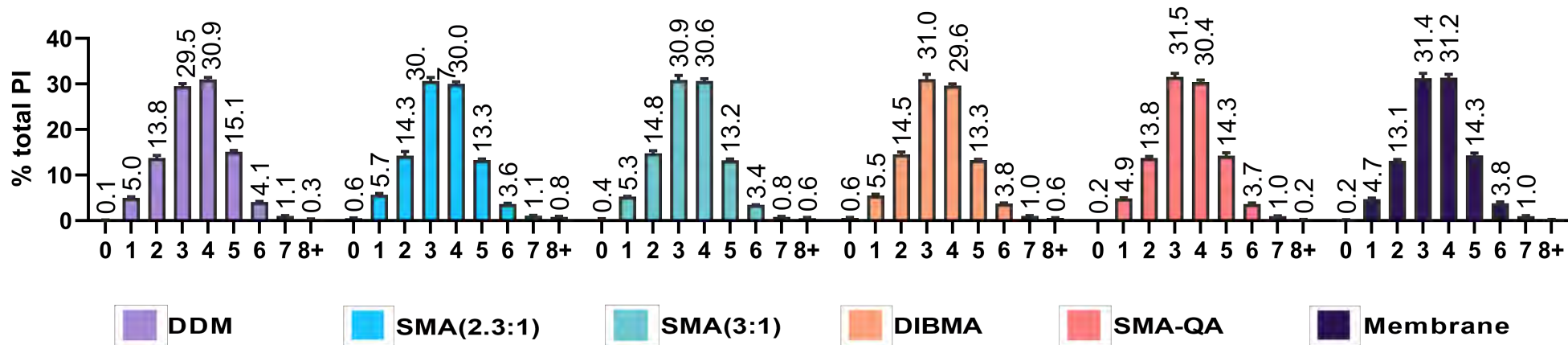# Saturation has little effect on xMA solubilization

Fatty acid chain saturation in <u>specific</u> lipid classes (Jurkat) - Analyzed by LC-MS/MS
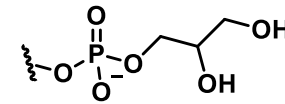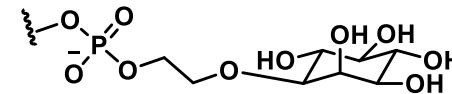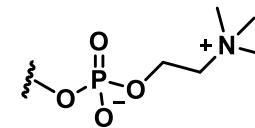
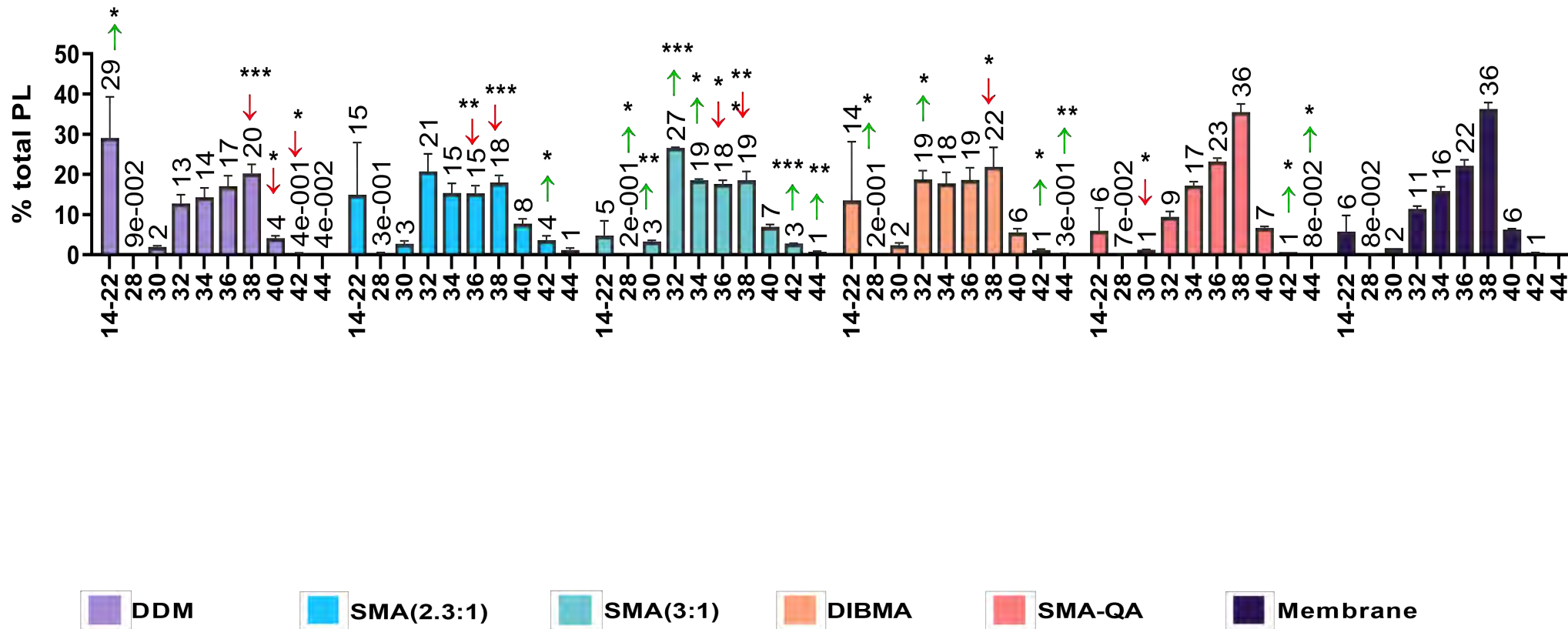Phosphatidylcholine (PC)

Phosphatidylinositol (PI)

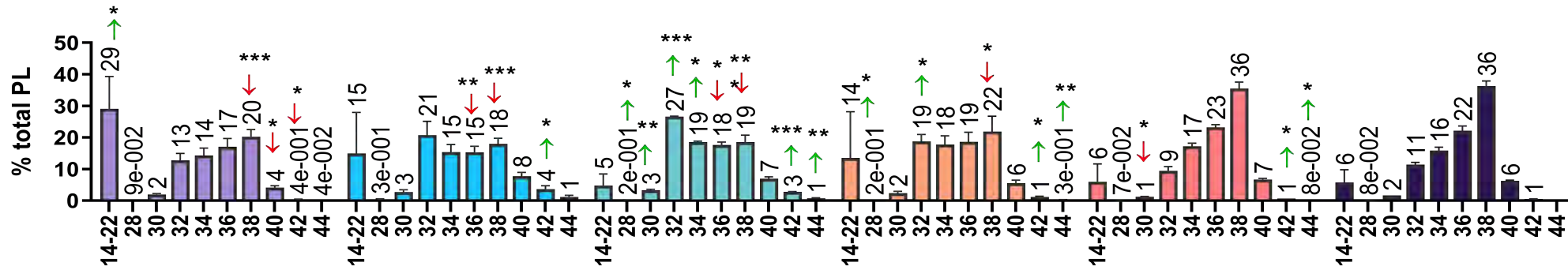Legend: DDM, SMA(2.3:1), SMA(3:1), DIBMA, SMA-QA, Membrane

FA chain length does not guide xMA solubilization

Fatty acid chain length total carbons in total phospholipid (Jurkat) Analyzed by LC-MS/MS
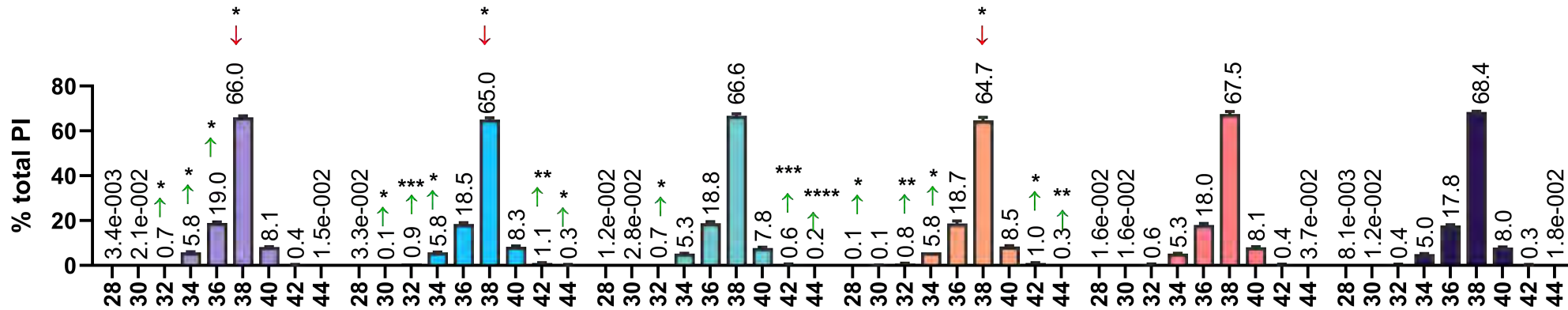
FA chain length does not guide xMA solubilization

Fatty acid chain total carbons in **total** phospholipid (Jurkat) - Analyzed by LC-MS/MS

Fatty acid chain total carbons in total phosphatidylinositol (Jurkat) Analyzed by LC-MS/MS

Legend: DDM, SMA(2.3:1), SMA(3:1), DIBMA, SMA-QA, Membrane
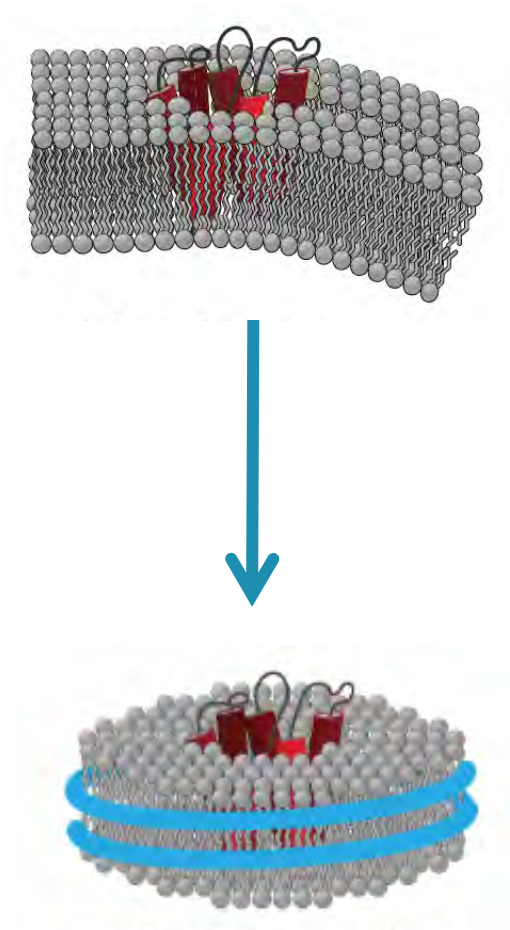
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Conclusions

- xMA **efficient** solubilizing agents

- **Membrane structure** seems to influence solubilization preferences

- Select xMA depending on **protein** of interest and **expression system**

- Not all xMAs are membrane like.

  SMA(3:1) most *membrane like*.

- Caution when determining lipid **environment/activity** of xMALPed proteins

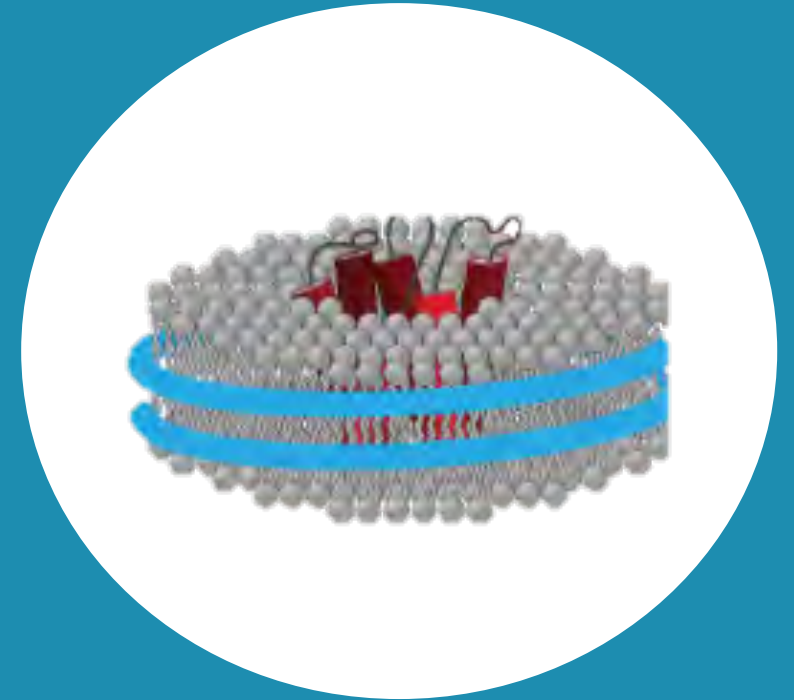- Future: **novel polymers** with improved membrane disruption properties

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Acknowledgements

- Prof. Steven Verhelst

- Lab of Chemical Biology

Preprint available in Chemrxiv

https://chemrxiv.org/s/6cccbf5fdacc5887a89d

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