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Czech Advanced Technology and Research Institute

Model Complexity in All-Atom Plasma Membrane Simulations

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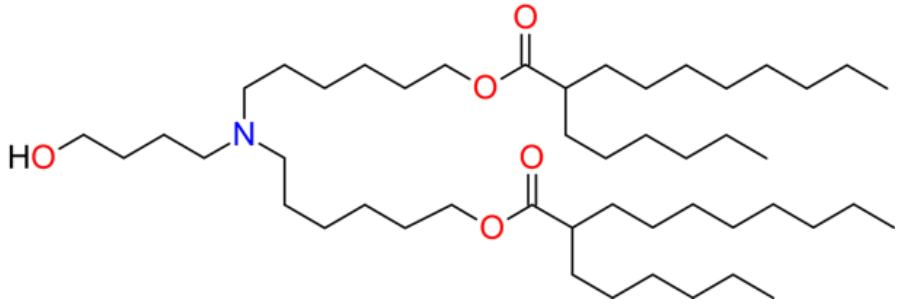
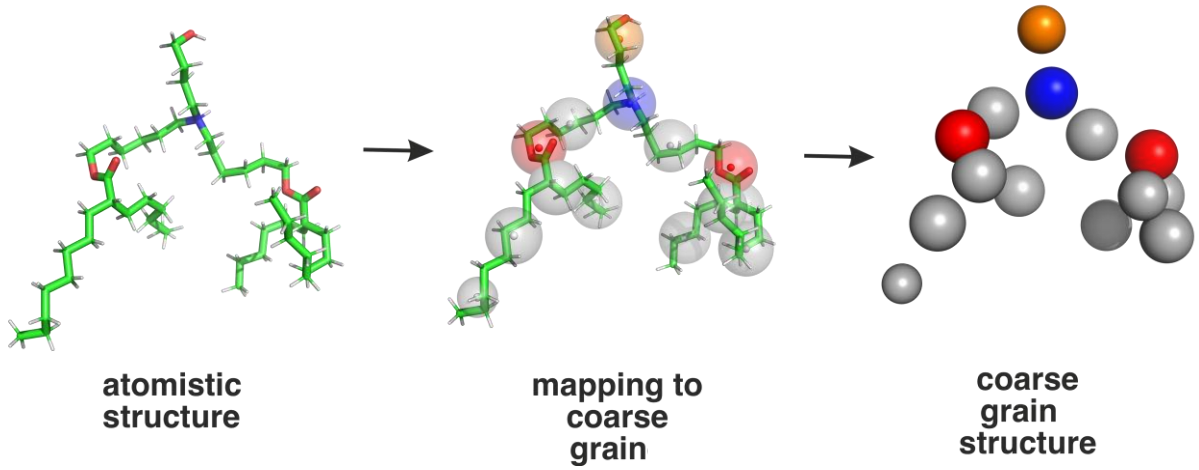
Part 1: All atom simulations

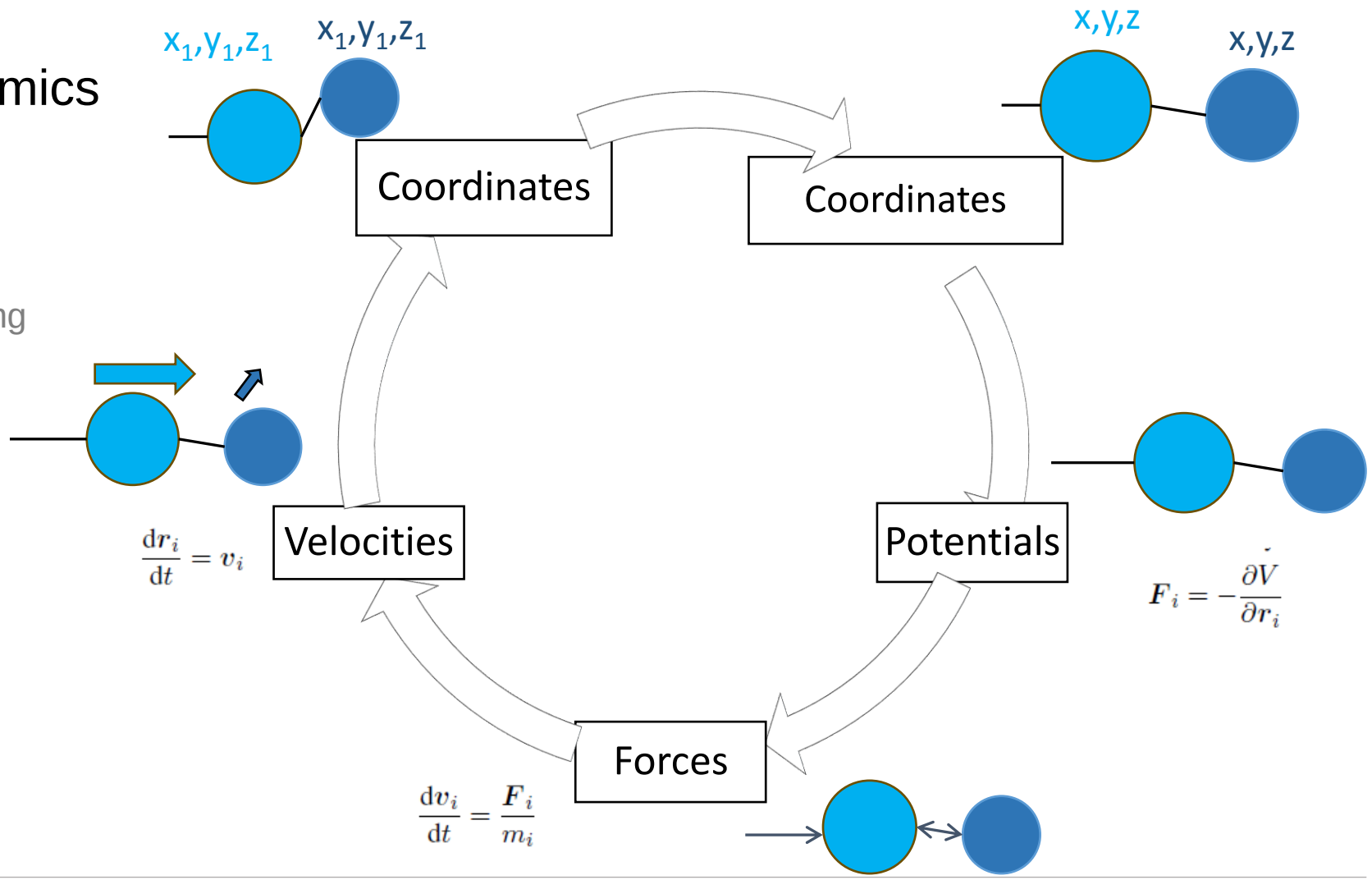
- Term used for molecular dynamics simulations
- A description of model complexity
- Every atom of the chemical structure is represented in the model
- In contrast, coarse-grained method use “beads” of multiple atoms
- Different computational requirements





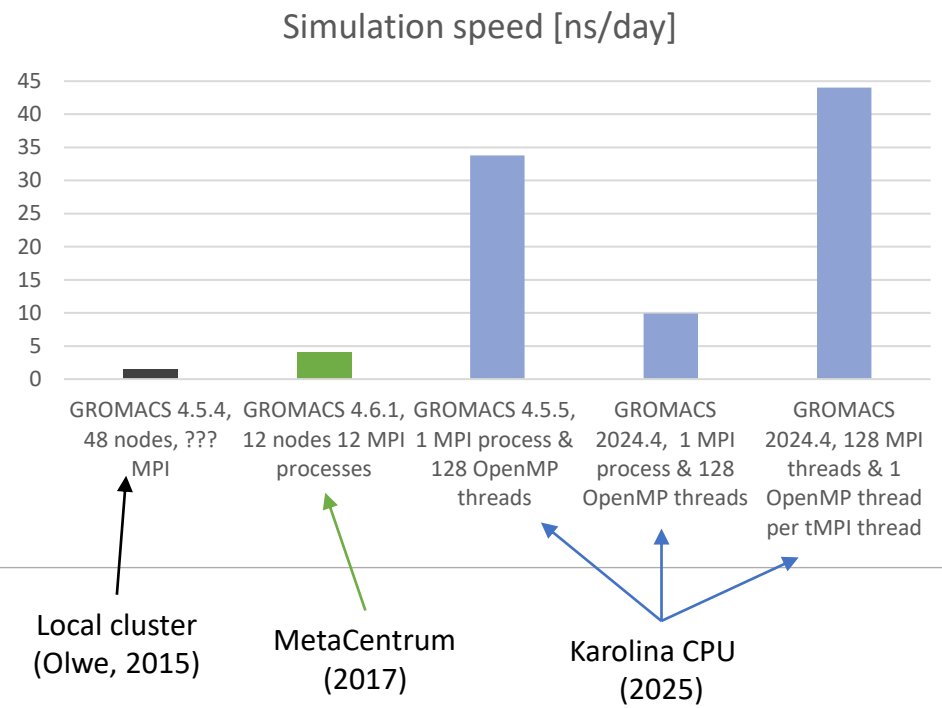
Part 1: Molecular dynamics

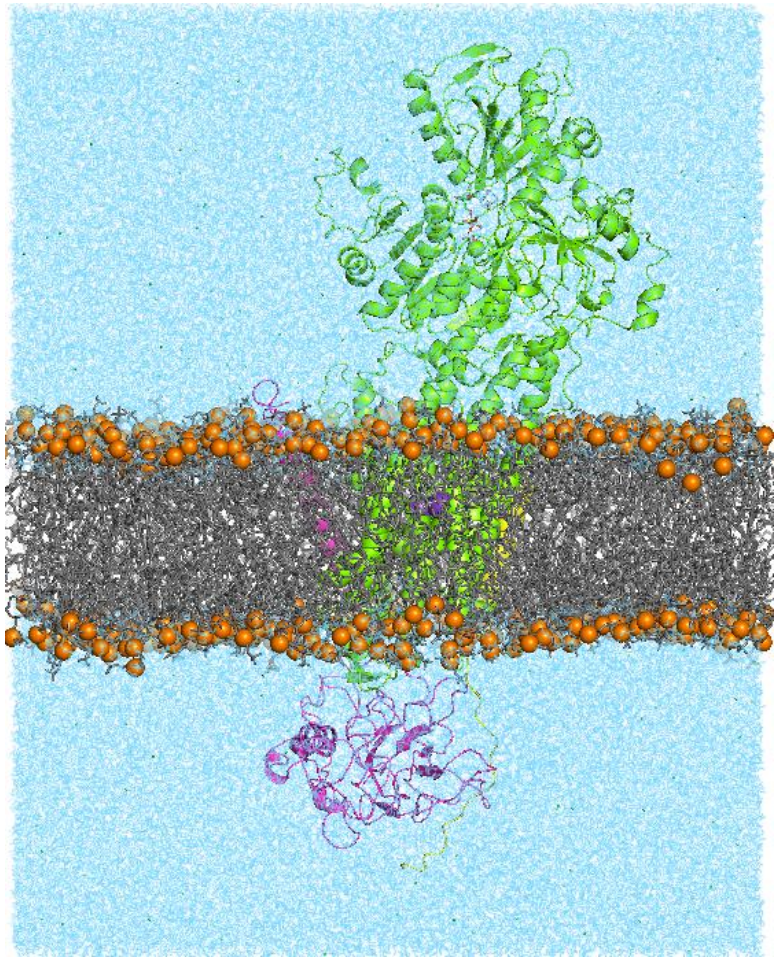
- Method used to simulate movements of atoms in a system
- A series of equations describing a stepwise evolution of a system
- Molecules are described by “force fields” of different resolutions
  - All atom
  - Coarse grain
- Tens to hundreds thousand atoms and millions to billions steps



Part 1: Computational Example

- The old-system reruns show that for MD (on CPUs), the number of cores is not the only important part
  - MPI/OpenMP decomposition
  - Software preferences
- The inputs were too old for GPU

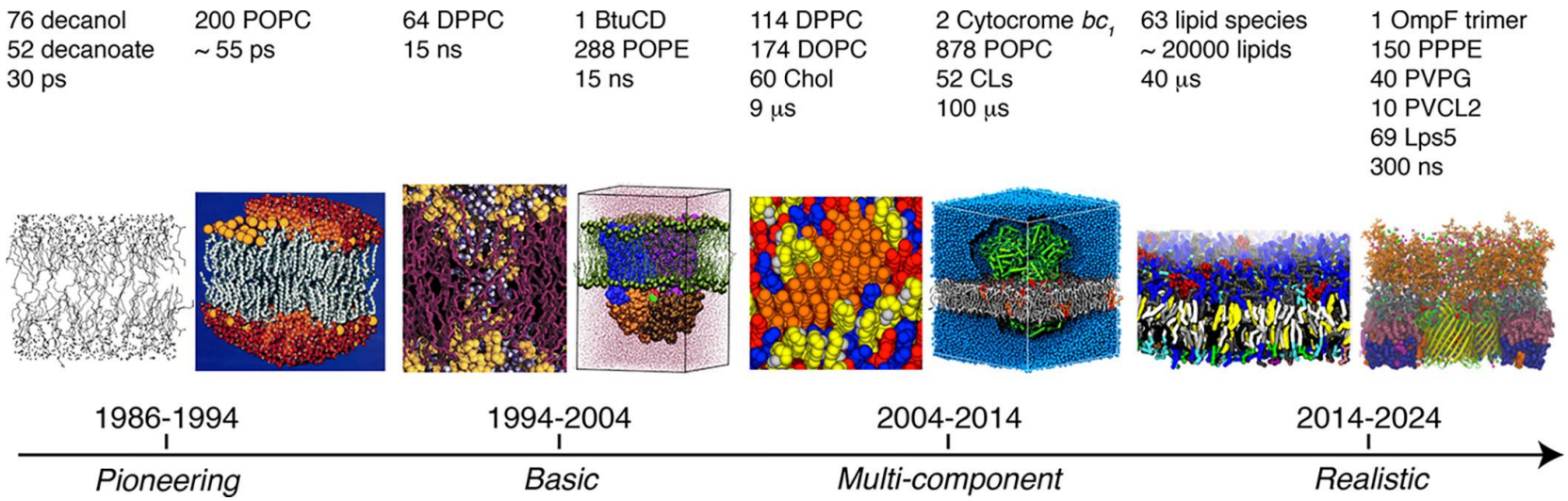




Na^+/K^+ ATPase in water, 3 subunits (1500 residues), 530 lipids (DOPC), UA forcefield, 314 000 atoms, 100 ns

Part 1: Membranes, Membrane Proteins and HPC

- Increasing HPC computational power allows the creation of larger systems



Marrink S. et al, Computational Modeling of Realistic Cell Membranes, Chemical Reviews, 2019

Part 1: Membranes, Membrane Proteins and HPC

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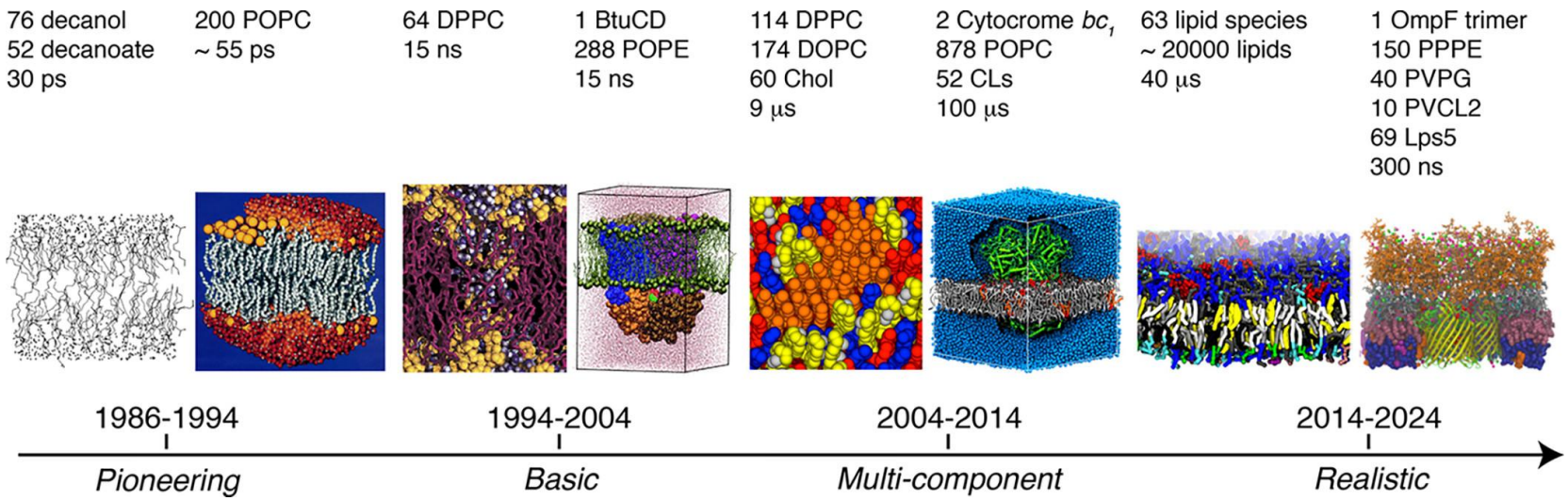
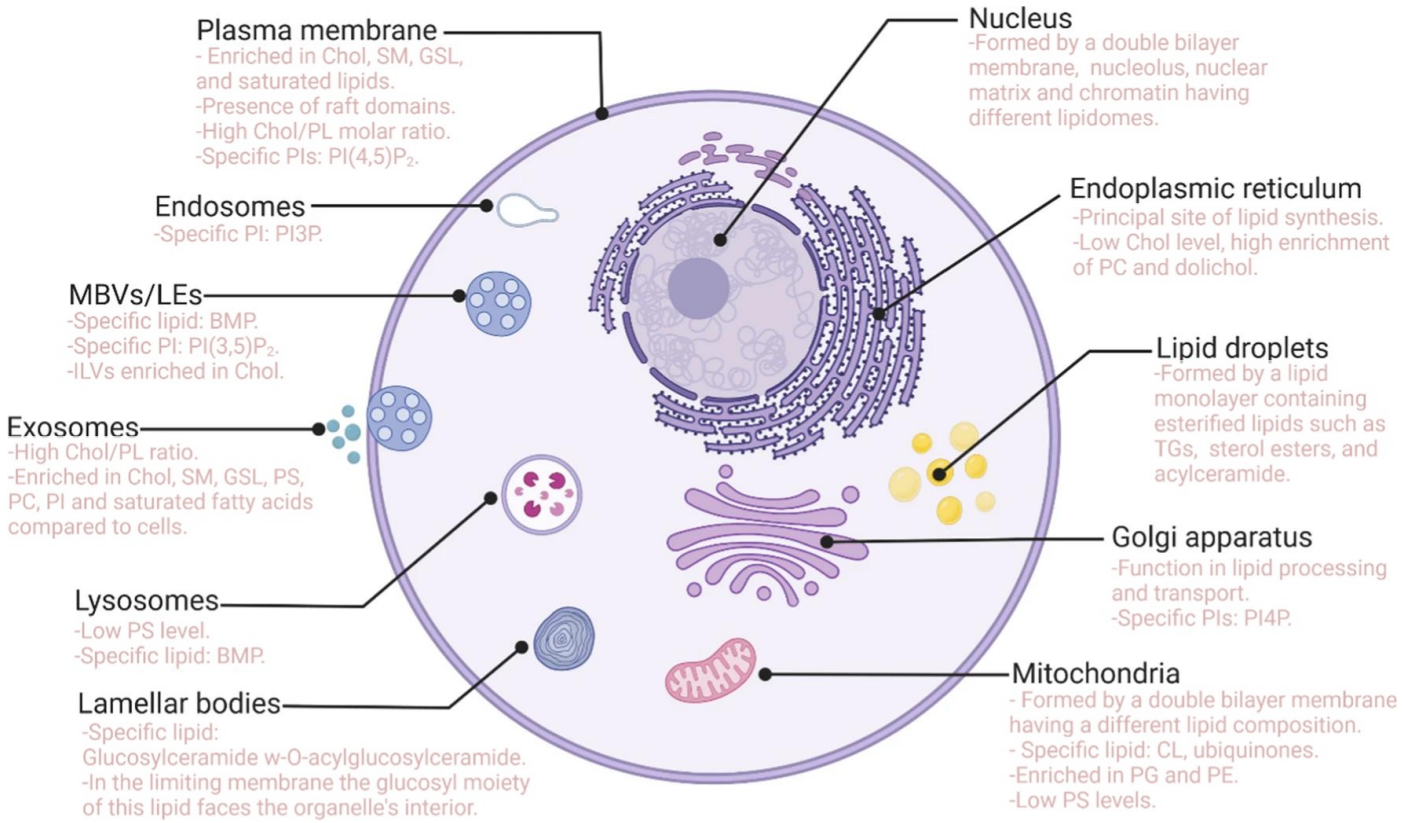
- The question changes from “What systems are we able to build?” to “How should we set up the system to efficiently make use of the computational capacities?”



Marrink S. et al, Computational Modeling of Realistic Cell Membranes, Chemical Reviews, 2019

Part 2: Biological Membranes – where they are and how they behave

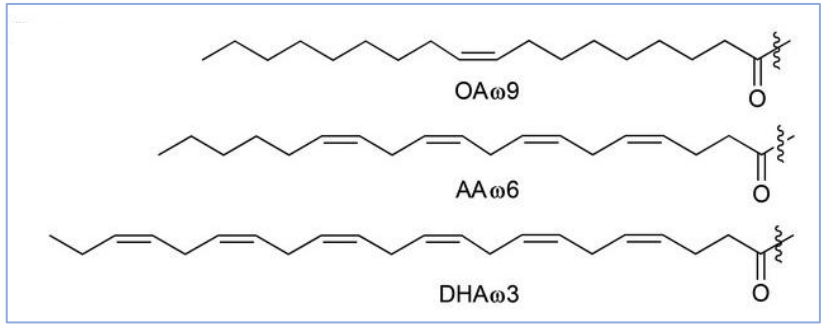
- Compartment walls
- Trafficking
- Metabolism
- Signalling
- Various composition and properties



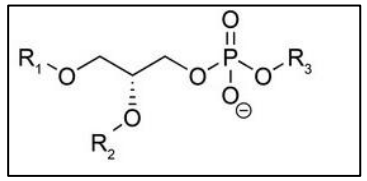
Sarmento et al, The expanding organelle lipidomes: current knowledge and challenges, Cellular and Molecular Life Sciences, 2023

Part 2: A quick intro into phospholipids

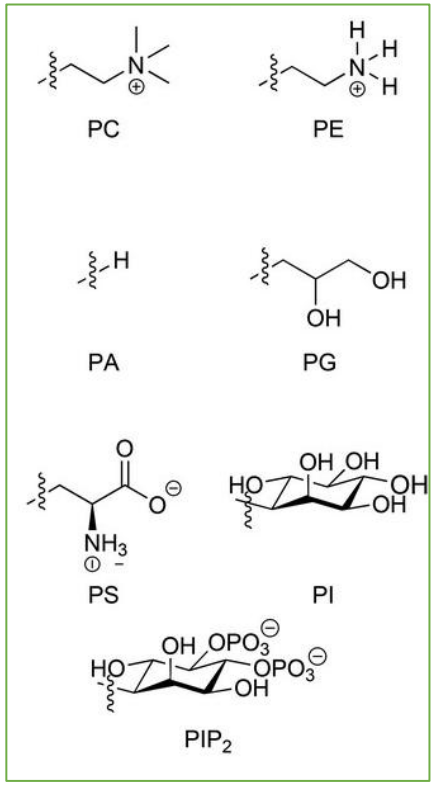
- A typical biological lipid consists of head and tail groups attached to a glycerol phosphate



Tail
- 12-24 C long
- Hydrophobic
- (Un)saturated
- The core of the membrane

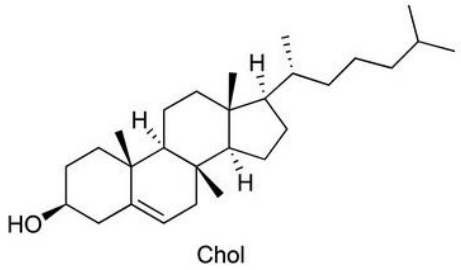


Glycerol phosphate
- Connects everything
- Very polar



Head
- Different functional groups
- Hydrophilic
- Can be charged
- Outer layer of the membrane

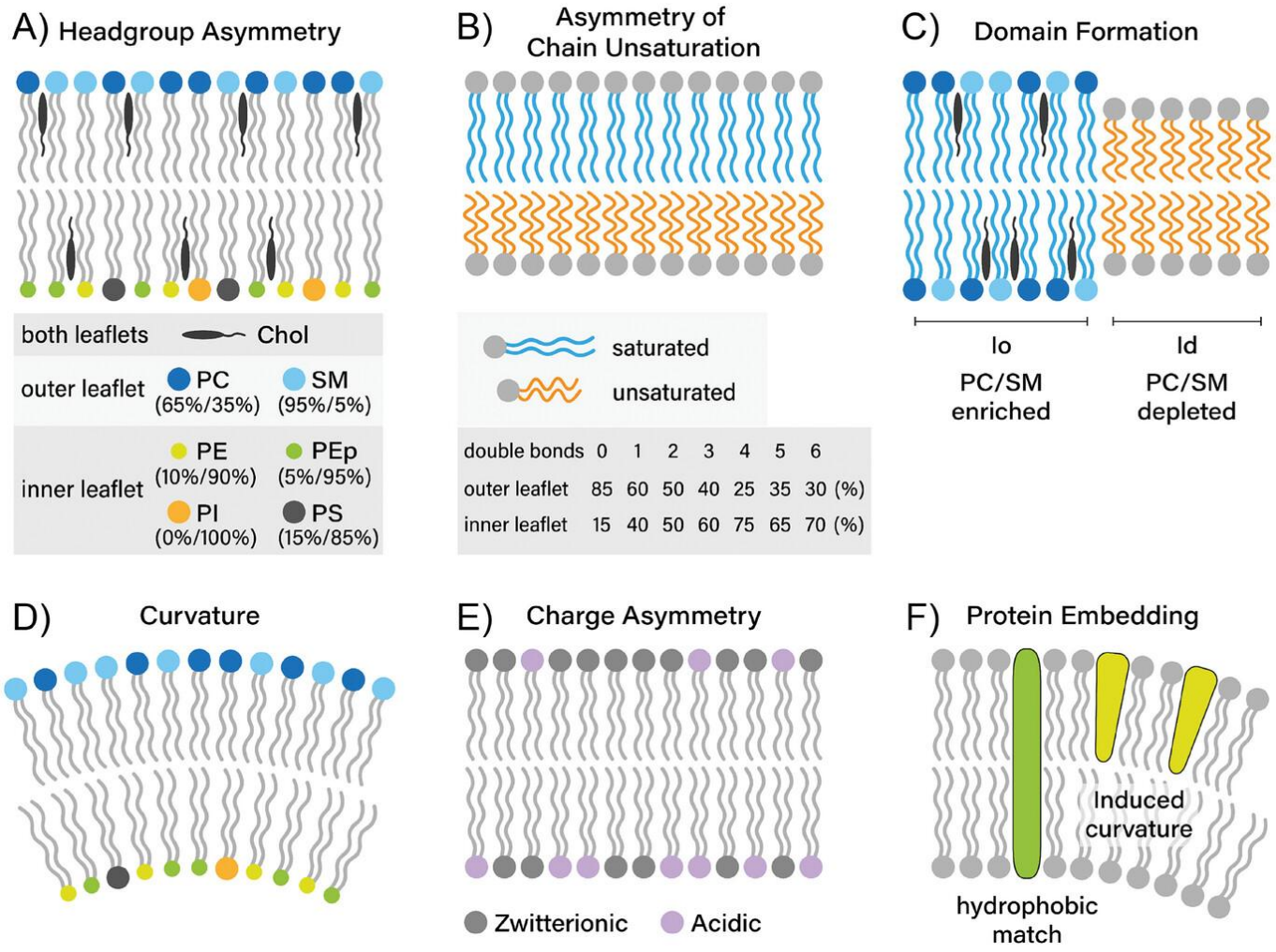
Cholesterol
- hydrophobic rings and polar OH group
- Makes membrane thicker and stiffer



Huster D, et al,Phospholipid Membranes as Chemically and Functionally Tunable Materials, Advanced Materials, 2024

Part 2: Lipids in the membranes

- The variability of lipid heads and tails and their combinations can lead to different local properties
- Lipid structures can take up several lamellar or non-lamellar phases



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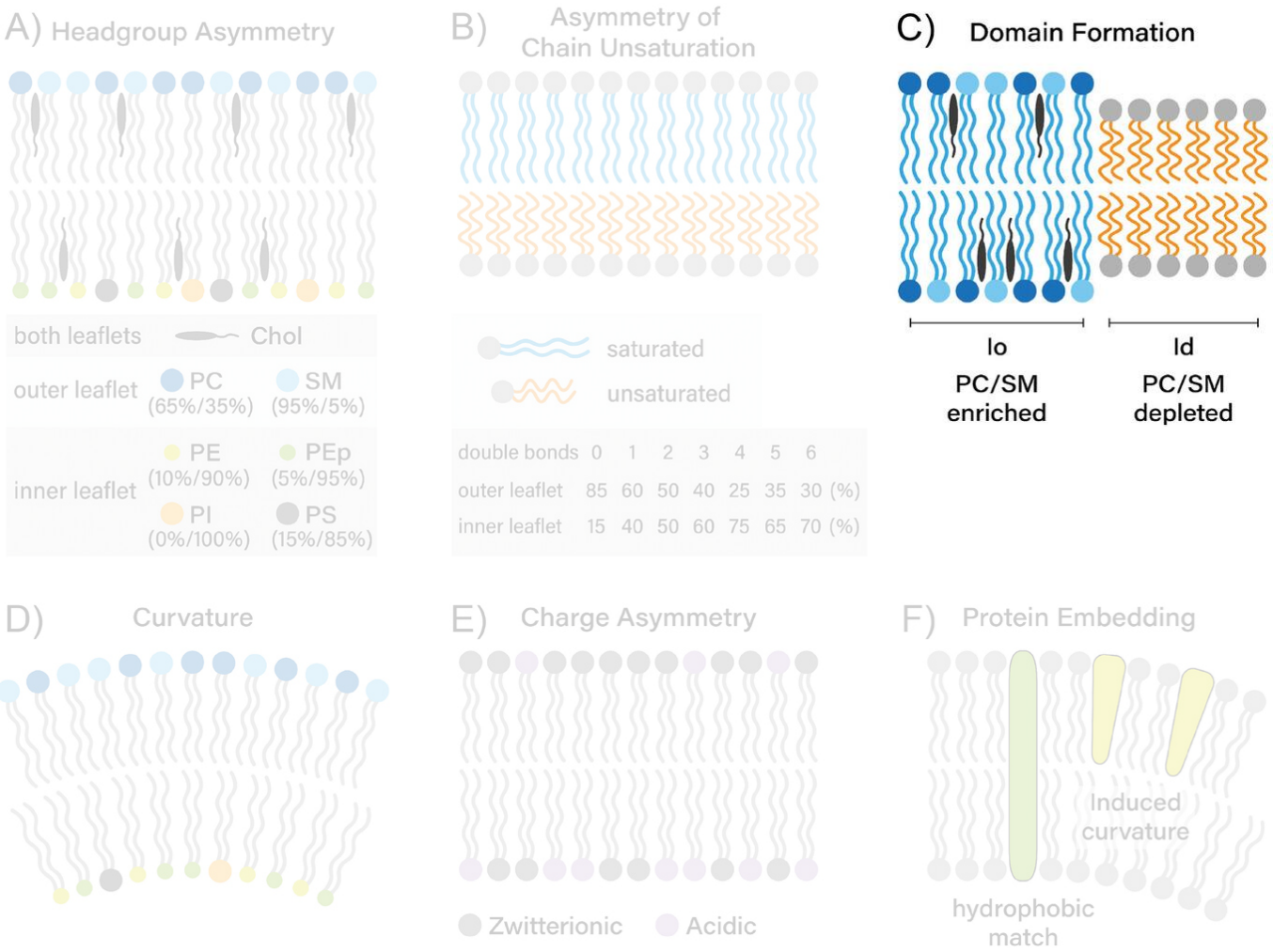
  - Most common are liquid ordered (Lo) and liquid disordered (Ld) phases



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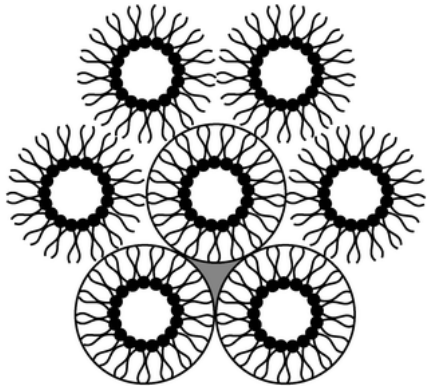
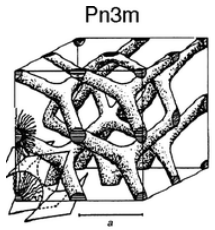
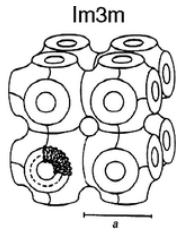
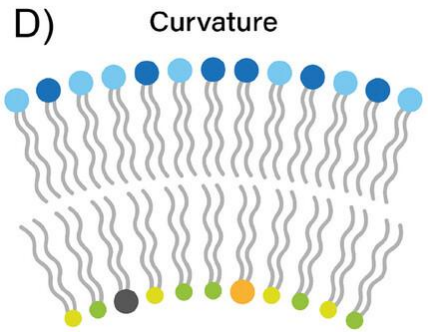
- Lipid structures can take up several lamellar or non-lamellar phases
  - Most common are liquid ordered (Lo) and liquid disordered (Ld) phases
  - Lipid nanoparticles can form hexagonal, inverted hexagonal or cubic phases





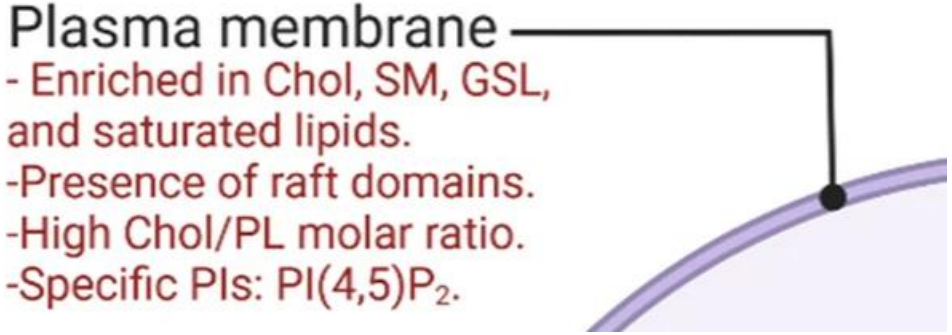


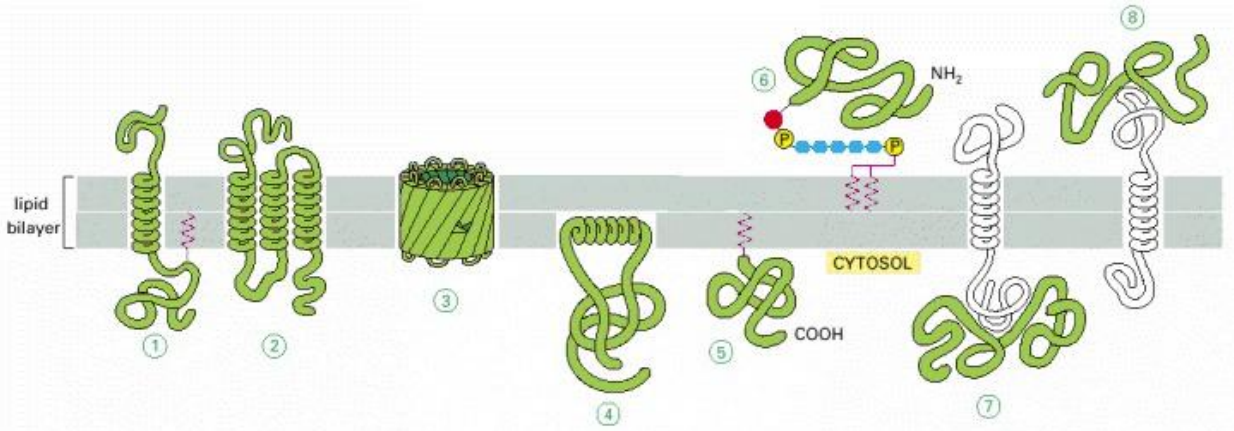


Huster D, et al,Phospholipid Membranes as Chemically and Functionally Tunable Materials, Advanced Materials, 2024

Part 1: Plasma Membrane

- The outer layer of the cell
- Keeps the cell together and controls what goes in and out
- Asymmetrical
  - Different lipids (heads and tails)
  - Different charge
- Notable specialised lipids are
  - PIP inside (cell signalling)
  - Glycolipids outside (cell recognition)
  - Sphingosines (stiffness)
- Largest ratio of cholesterol and subsequent stiffness
- Covered in proteins – about 50% by mass

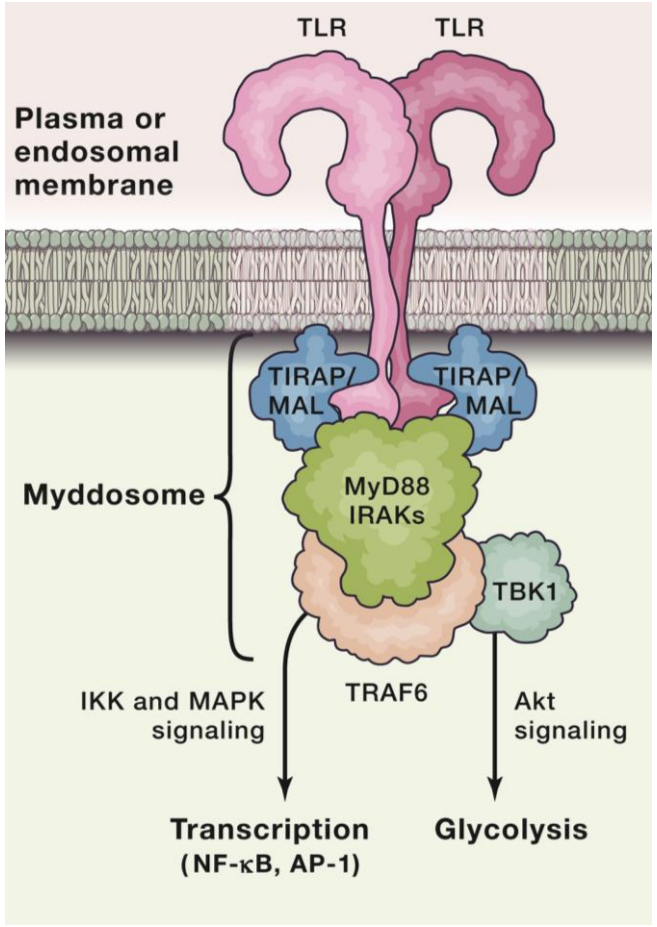


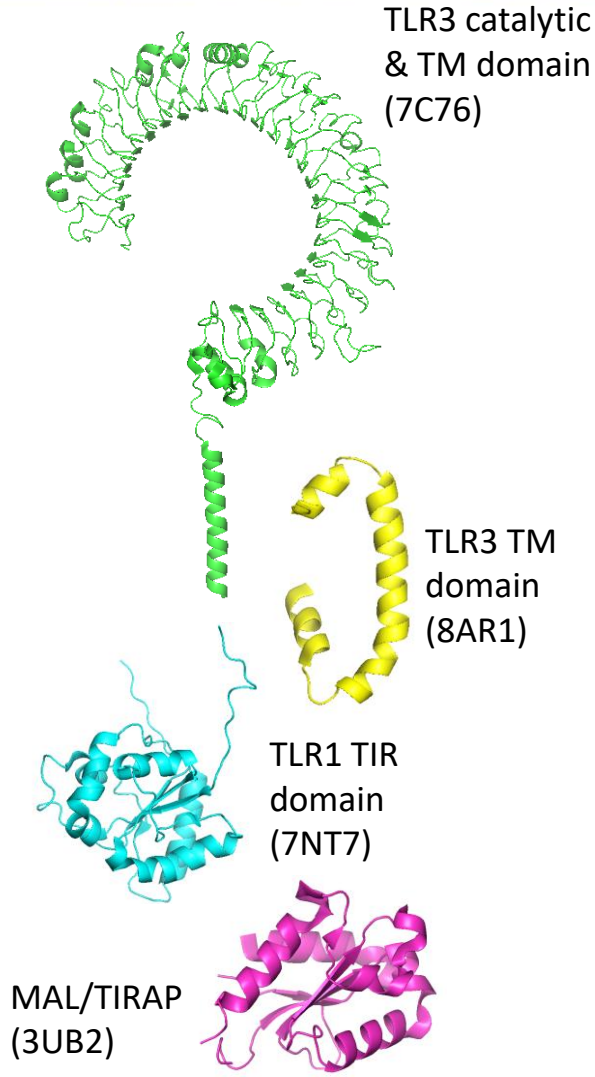


Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. Membrane Proteins. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26878/

Part 2: Plasma Membrane Proteins

- Specific in their structure in comparison to soluble proteins in blood or cytoplasm
  - Catalytic domain
  - Signalling domain
  - Transmembrane domain
- Recent rise in experimental methods allows for better capture
- Many protein structures are still known as fragments





Fitzgerald K. , et al. Toll-like Receptors and the Control of Immunity, Cell, 2020

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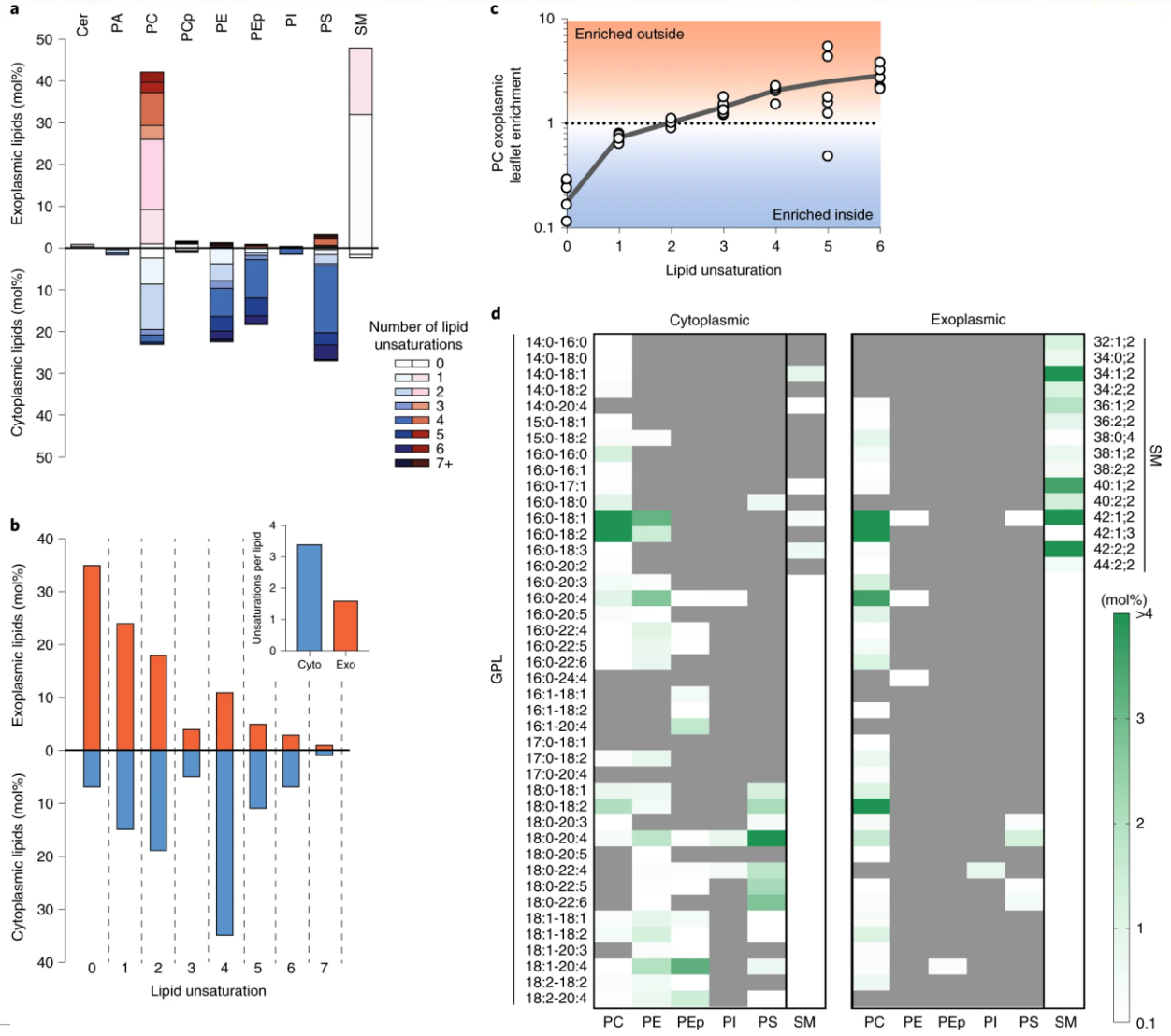
Part 2: Plasma Membrane In Experiment

- Asymmetry and the lipid variability makes experimental study challenging
- More than 1000 lipid in a cell

- ‘ Using enzymatic digestion, the asymmetric distribution of ~400 lipid species was defined for human RBC PMs and compiled into a detailed model for the compositions of PM leaflets. ‘



Lorent JH, et al. Plasma membranes are asymmetric in lipid unsaturation, packing and protein shape, Nature Chemical Biology, 2020

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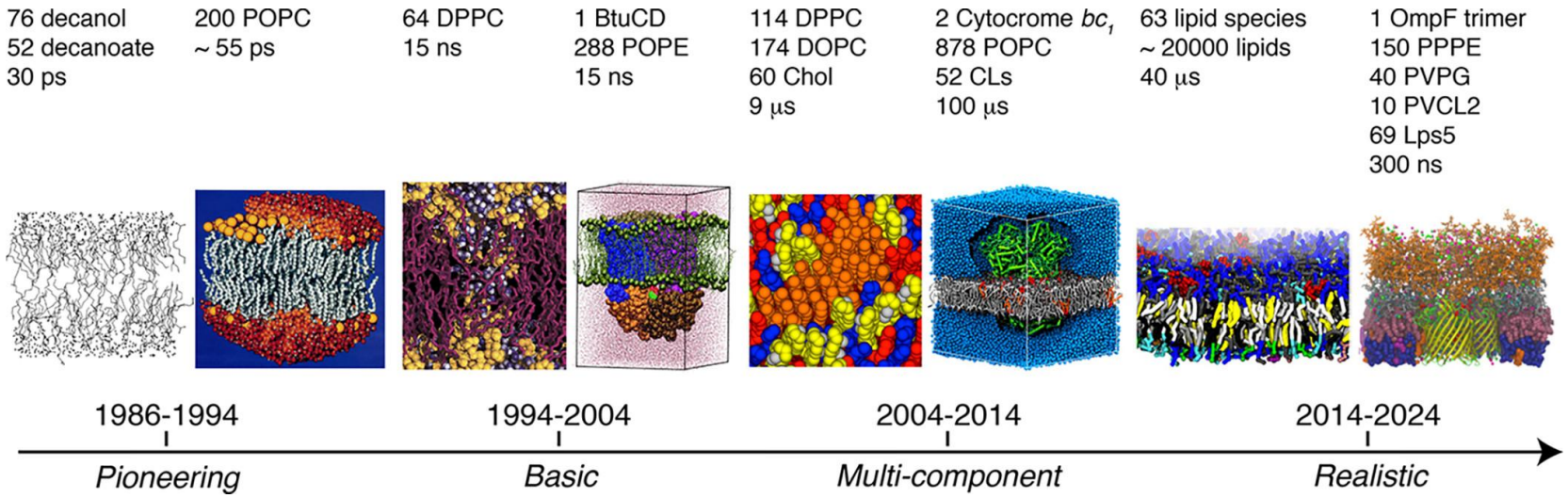
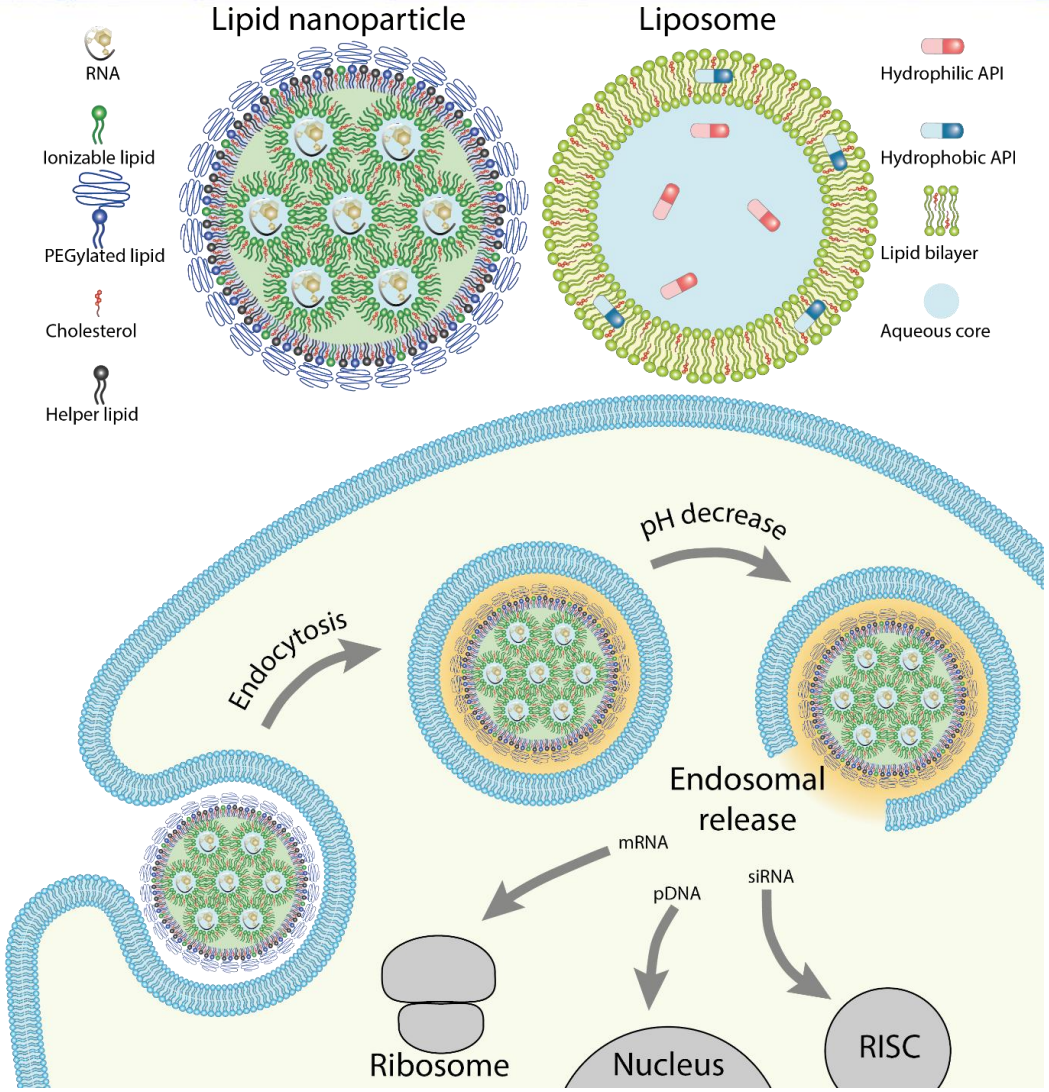


Marrink S. et al, Computational Modeling of Realistic Cell Membranes, Chemical Reviews, 2019

Part 3: Our project and its motivation

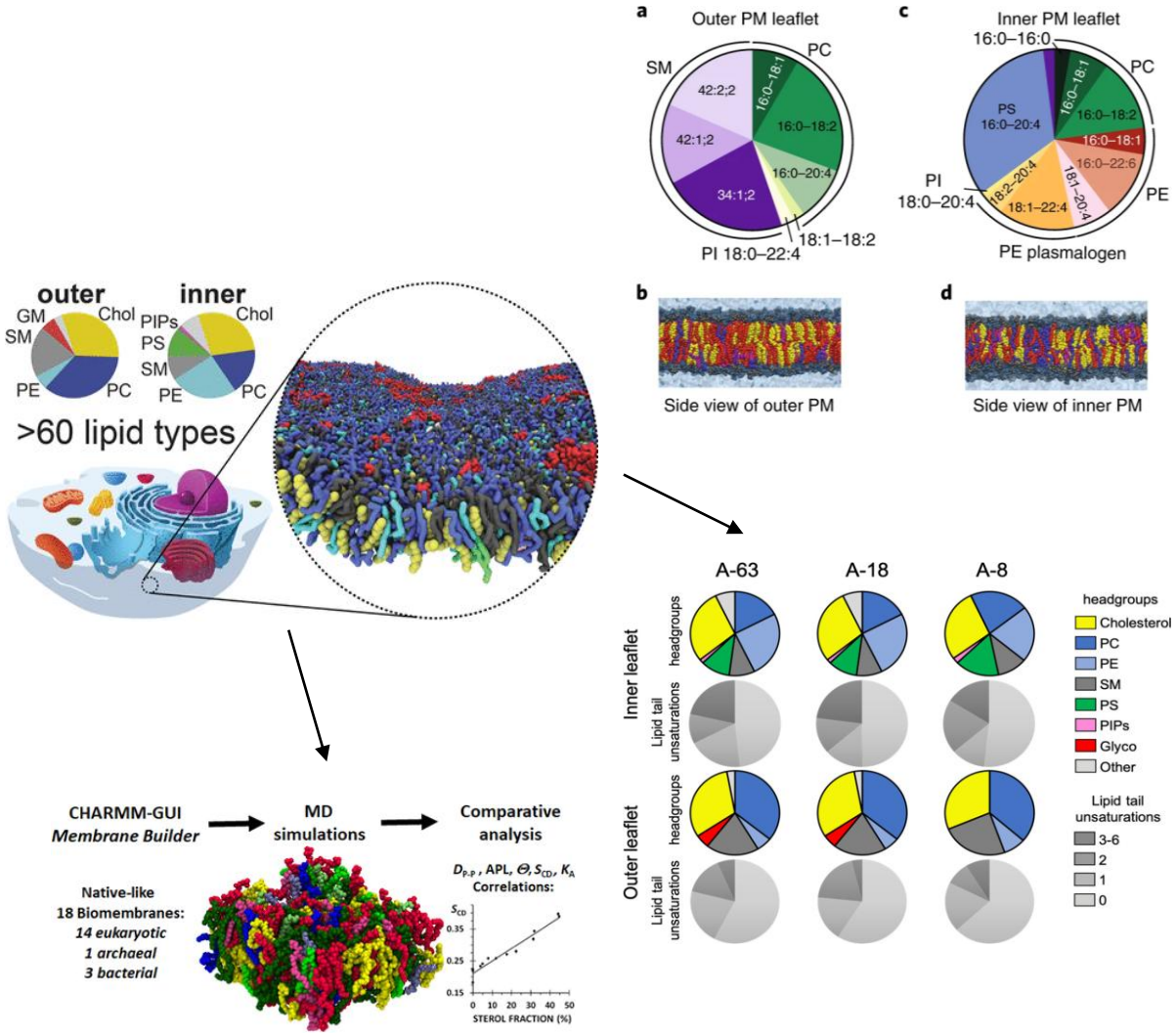
- Lipid-based vesicles are increasingly used as delivery systems for a range of medical drugs
- It is generally agreed that they enter the cell via endocytosis
- The mechanism of the entire pathway is not clear – and it causes losses in cargo delivery process
- MD simulations can provide computational hypotheses on the steps of this process
- To do that, we need to have a model of the membranes involved in the process



Paloncýová et al, Computational Methods for Modelling Lipid-Mediated Active Pharmaceutical Ingredient Delivery, Molecular Pharmaceutics, 2025

Part 3: Existing Plasma Membrane Models

- Different groups built plasma membrane models based on different experiments in different resolutions with different approximations
- The most notable are
  - Lorent et al (2020 experiment) – all atom symmetric
  - MARTINI forcefield group (Ingólfsson et al, 2014, 2020) – coarse grained (M2) asymmetric
    - Subsequent simplifications
  - CHARMM-GUI group (Pogozheva et al, 2022) – all atom asymmetric
    - Starts with the 60+ lipid MARTINI CG model
    - Simplify the membrane in a different manner
- Plasma membrane protein models frequently use a symmetric single-lipid (POPC) membrane



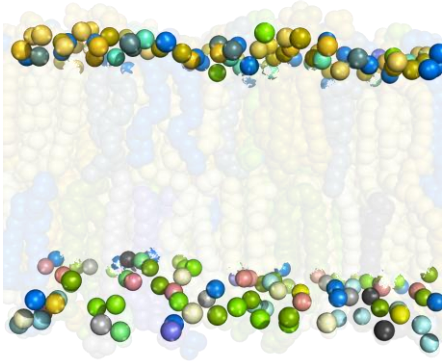
Ingólfson H. et al, Capturing Biologically Complex Tissue-Specific Membranes at Different Levels of Compositional Complexity, The Journal of Physical Chemistry B, 2020

Pogozheva I. et al, Comparative Molecular Dynamics Simulation Studies of Realistic Eukaryotic, Prokaryotic, and Archaeal Membranes, Journal of Chemical Information and Modeling, 2022

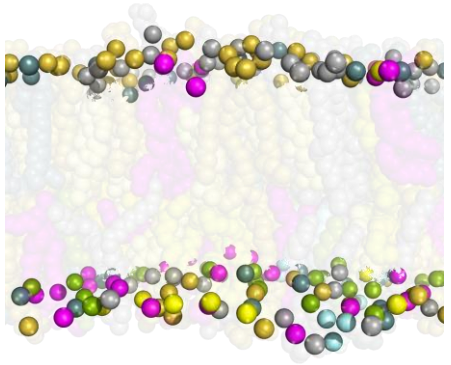
Part 3: Our membranes - complex mimics

- Three membrane models
  - Asymmetric
  - 8 – 18 lipids
  - Different head and tail properties
  - All include cholesterol
  - All include some sphingosines

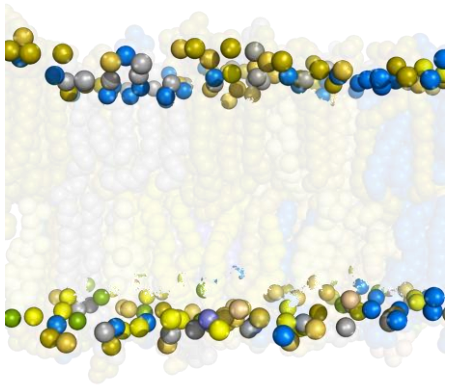
L18



PMm11



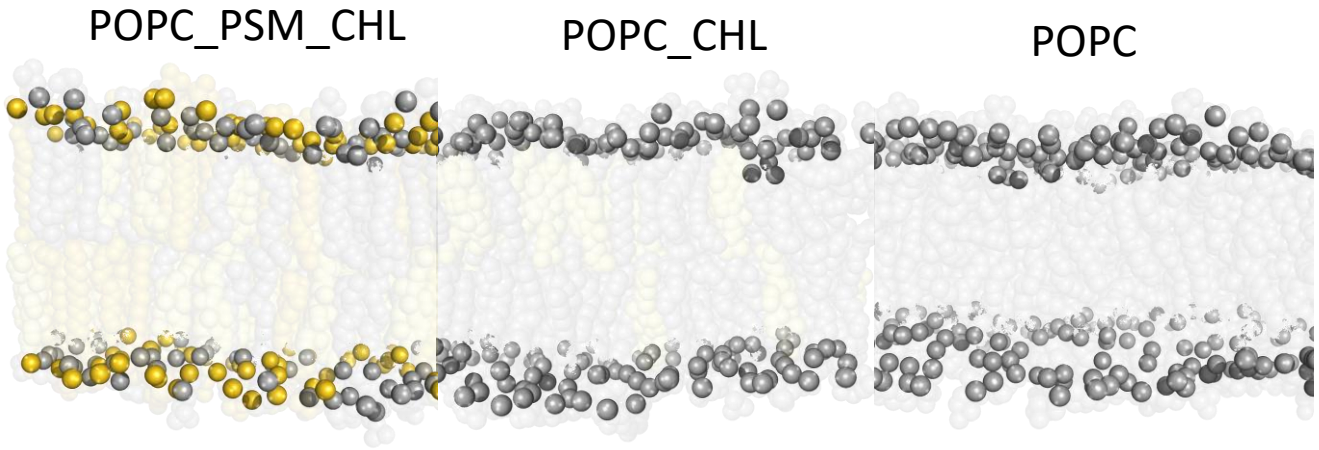
A8



System	PC (%)	PE (%)	SM (%)	PS (%)	PI/PIP (%)	Glyco-lipids (%)	CHL (%)	Other (%)	Average tail unsaturation	Average tail length [#C]	Number of lipid species
L18_outer	29.5	0.0	30.8	1.3			38.5		0.9	18.6	18
L18_inner	24.3	17.6	1.4	13.5	2.7		40.5		1.9	18.2	
PMm11_outer	35.8	5.0	21.4			3.8	34.0		0.8	17.8	11
PMm11_inner	17.3	25.3	10.7	10.0	5.3		30.0	1.3	1.1	17.7	
A8_outer	37.0	8.0	24.0				31.0		0.9	17.6	8
A8_inner	21.4	21.5	10.8	16.1	2.2		28.0		1.4	17.8	

Part 3: Our membranes - simple membranes

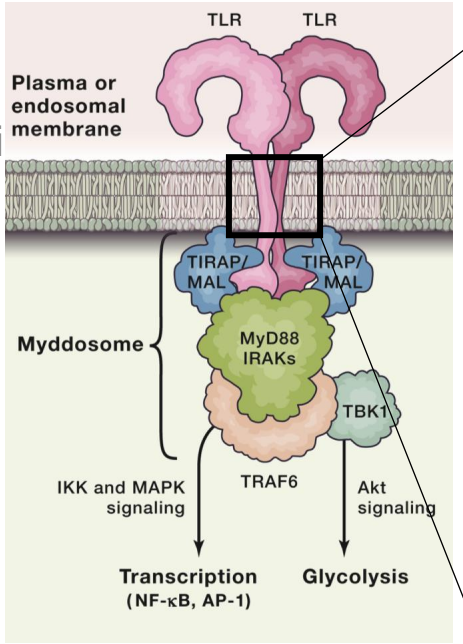
- Three simple models
  - POPC
  - POPC+CHL
  - POPC+CHL+PSM

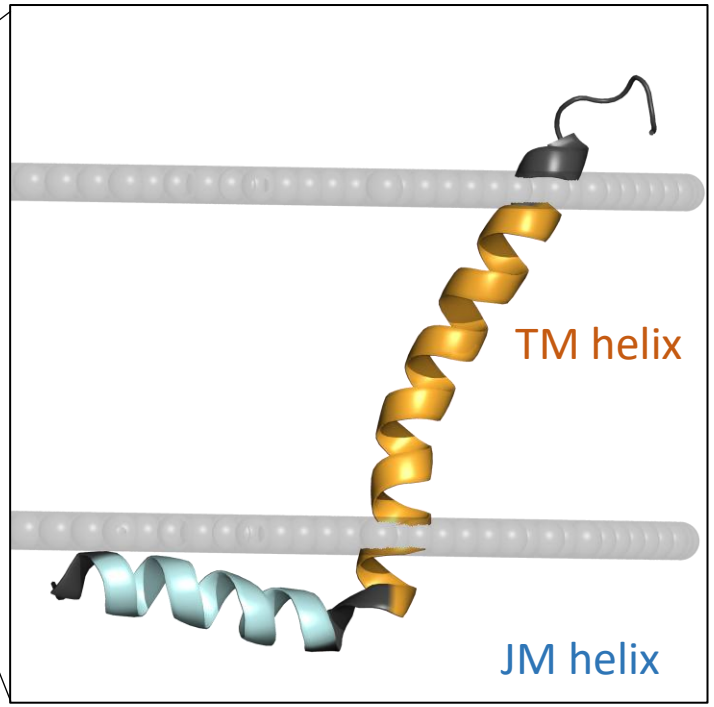


System	PC (%)	PE (%)	SM (%)	PS (%)	PI/PIP (%)	Glyco-lipids (%)	CHL (%)	Other (%)	Average tail unsaturation	Average tail length [#C]	Number of lipid species
POPC_PSM_CHL	33.3		33.3				33.3		0.5	17	3
POPC_CHL	66.6						33.3		0.5	17	2
POPC	100.0								0.5	17	1
DMPC_DMPG	80.0							20.0	0	14	2

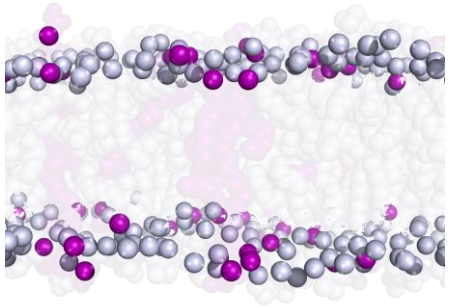
Part 3: Our protein (and one more membrane)

- TRL2 protein
- Immune response protein – thought to react to stimuli by moving between liquid ordered and liquid disordered cell membrane patches
- Transmembrane segment with two helices
- Experimentally measured in a lipid bicelle
  - Seventh membrane model





DMPC_DMPG



Part 3: Simulation setup and methodology

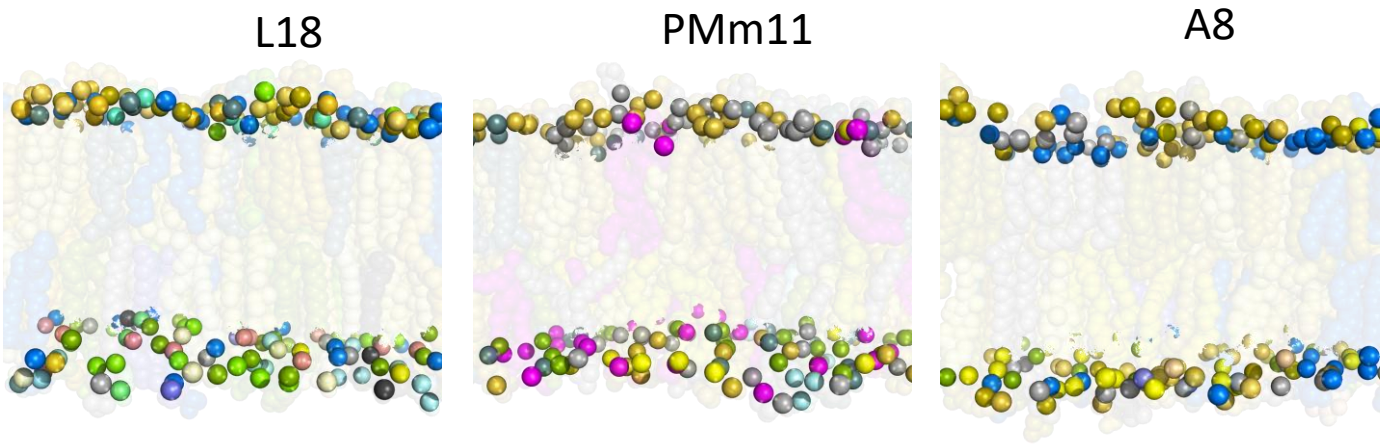
- CHARMM-GUI / CHARMM36+TIP3P forcefield
- Roughly 300 lipids
- 200 ns of membrane only simulation
- 1000 ns of membrane-protein simulation
  - 5 replicas
- 310 K/318 K
- Karolina CPU
  - GROMACS (2018.1, 2024.1)
  - 128 MPI process, 1 OpenMP threads
  - About 77 ns/day
- LUMI-C
  - GROMACS (2024.0)
  - 32 MPI processes, 4 OpenMP threads
  - About 78 ns/day
  - (40 ns/day with 1 MPI process, 128 OpenMP threads)

- **Different setup for a similar system**
- Membrane with 700 lipids
- Karolina GPU
  - AMBER 22
  - 104 ns/day
- LUMI-G
  - AMBER 24
  - 91 ns/day

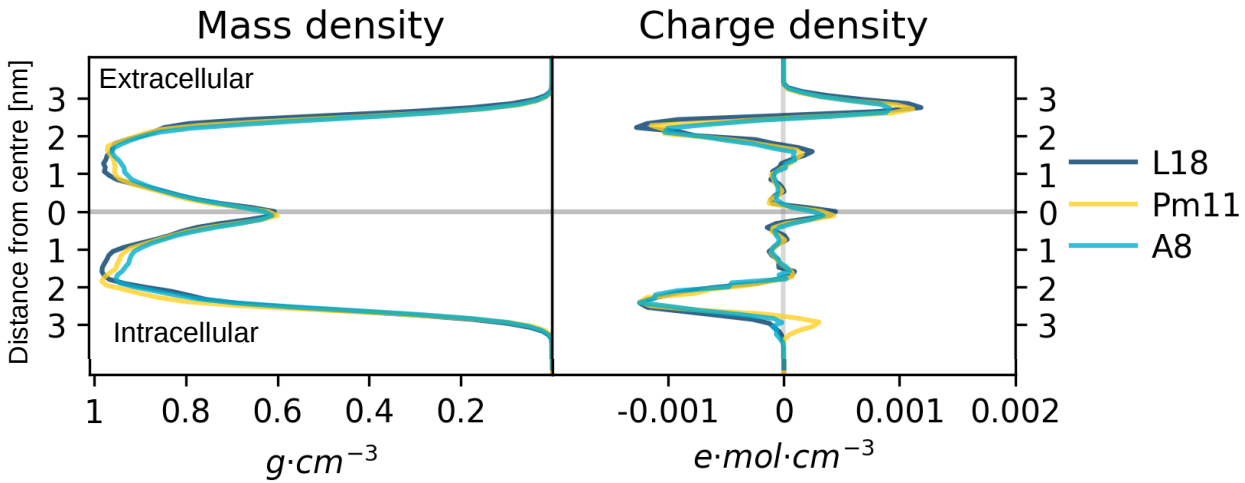
- **Analysis tools**
  - GROMACS tools in an interactive job
  - Python (jupyter notebook)
    - MDAnalysis, LiPyphilic and about 20 dependencies)

Part 4: Results PM Mimics

- Three membrane models
  - Asymmetric
  - 8 – 18 lipids
  - Different head and tail properties
  - All include cholesterol
  - All include some sphingosines



System	Membrane thickness [nm]
L18	4.25 ± 0.03
Pm11	4.23 ± 0.03
A8	4.19 ± 0.04



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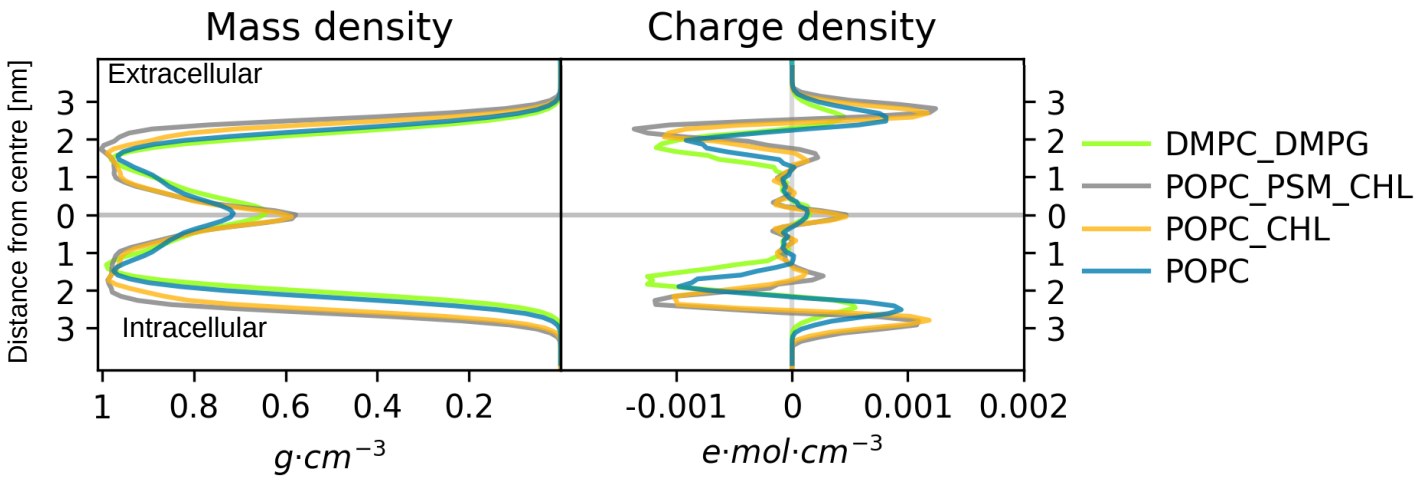
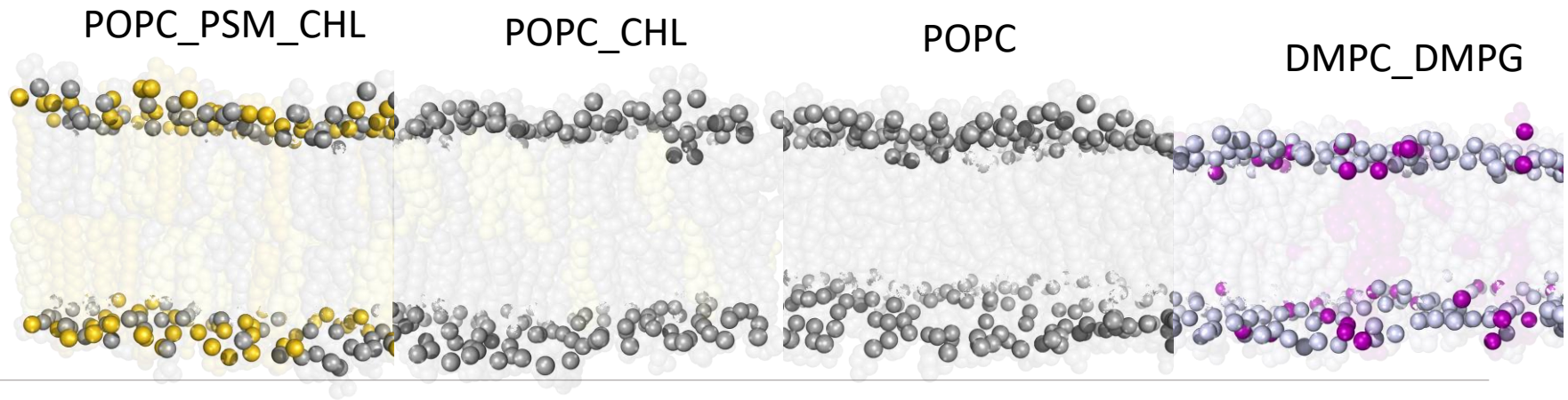
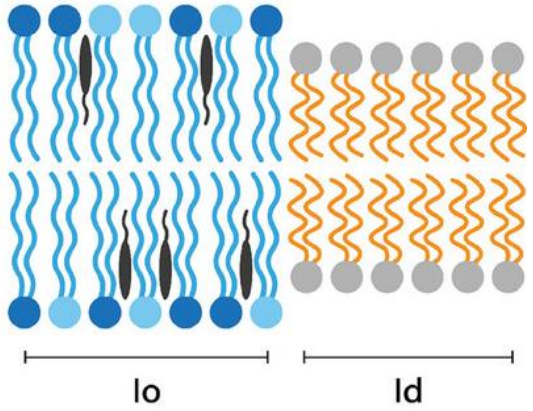


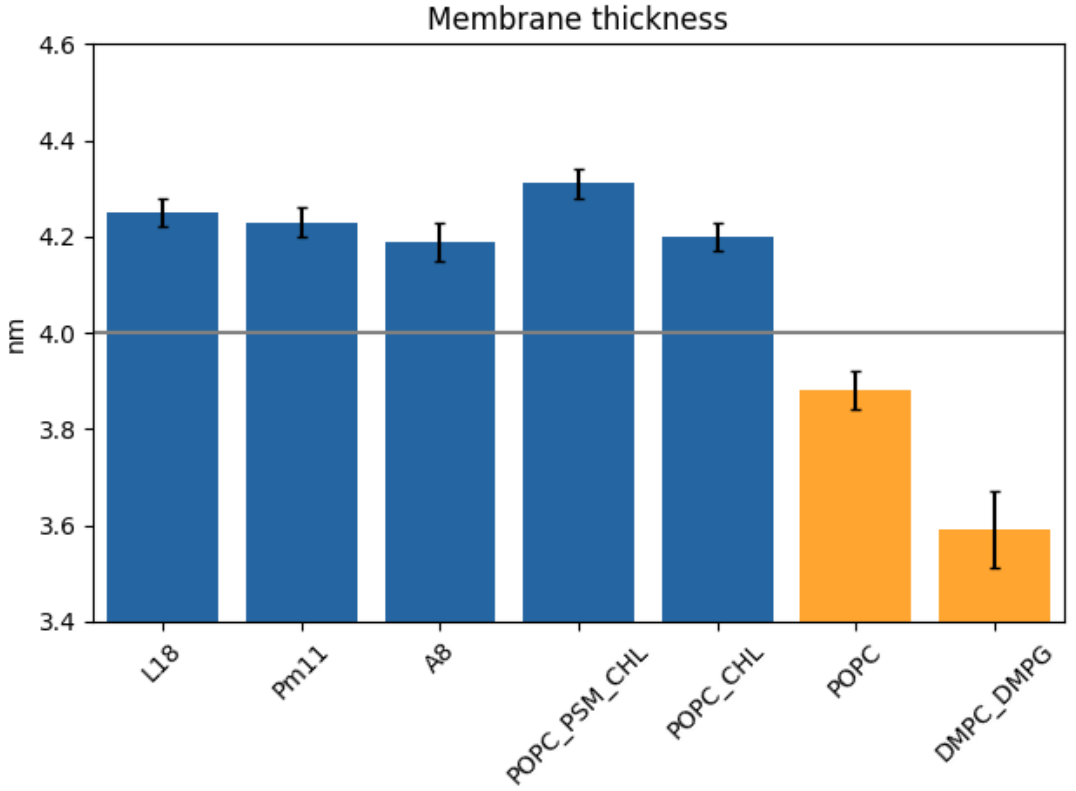
System	Membrane thickness [nm]
POPC_PSM_CHL	4.31 ± 0.03
POPC_CHL	4.20 ± 0.03
POPC	3.88 ± 0.04
DMPC_DMPG	3.59 ± 0.08



Part 4: Membrane behaviour

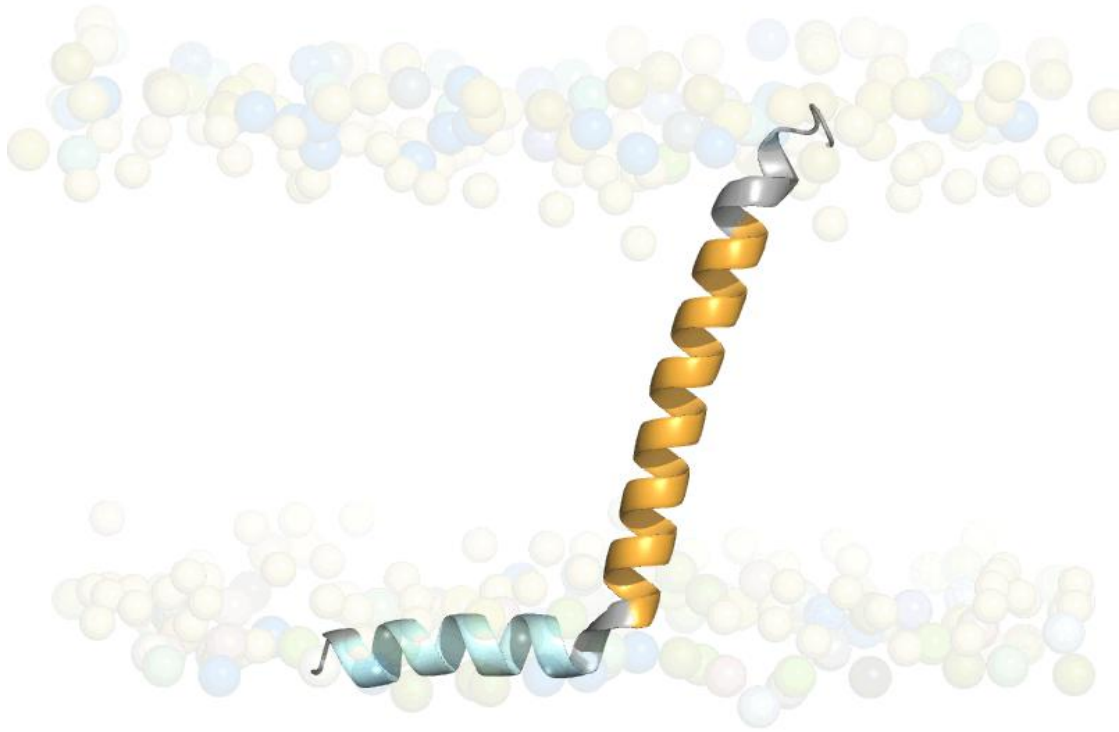
- Properties of our membrane models do not primarily depend on the fine details of composition
- The main feature guiding the properties is the presence or absence of cholesterol and the subsequent phase
- Liquid ordered and disordered membranes are significantly different in their properties





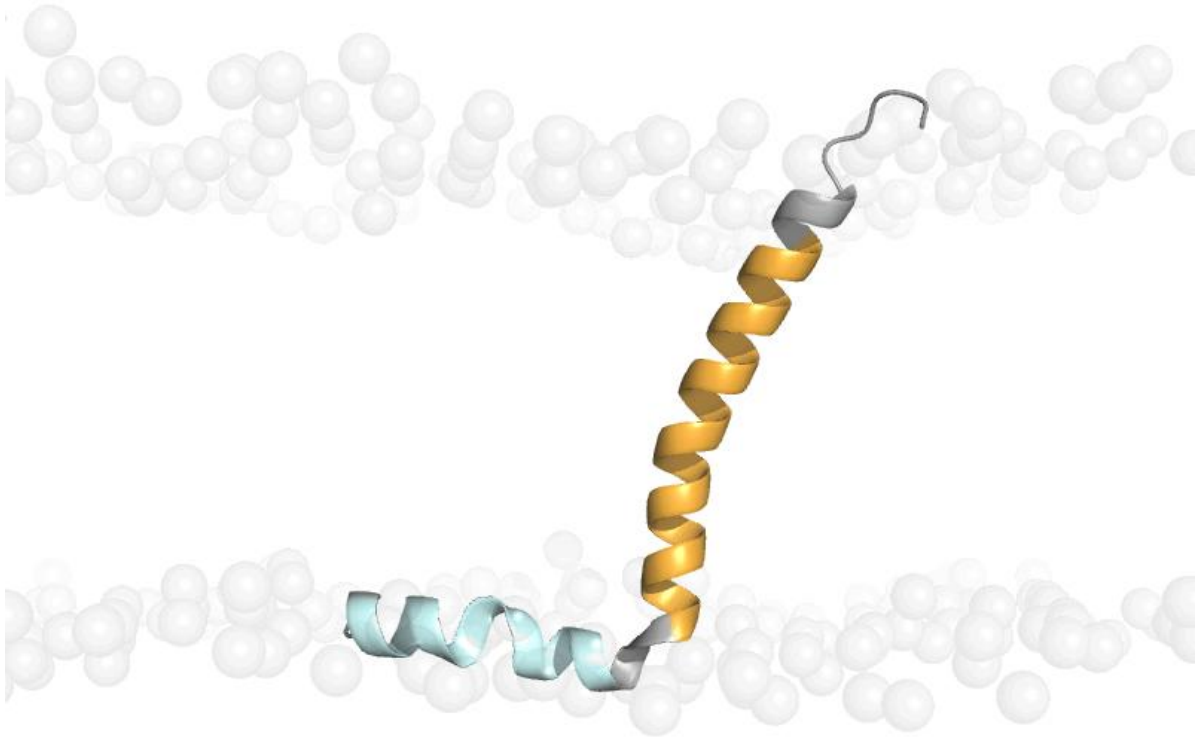
Part 4: Results Case 1

- The protein conformation remains similar to the starting structure
- It flexes within the membrane
- It transiently straightens or tilts in the membrane



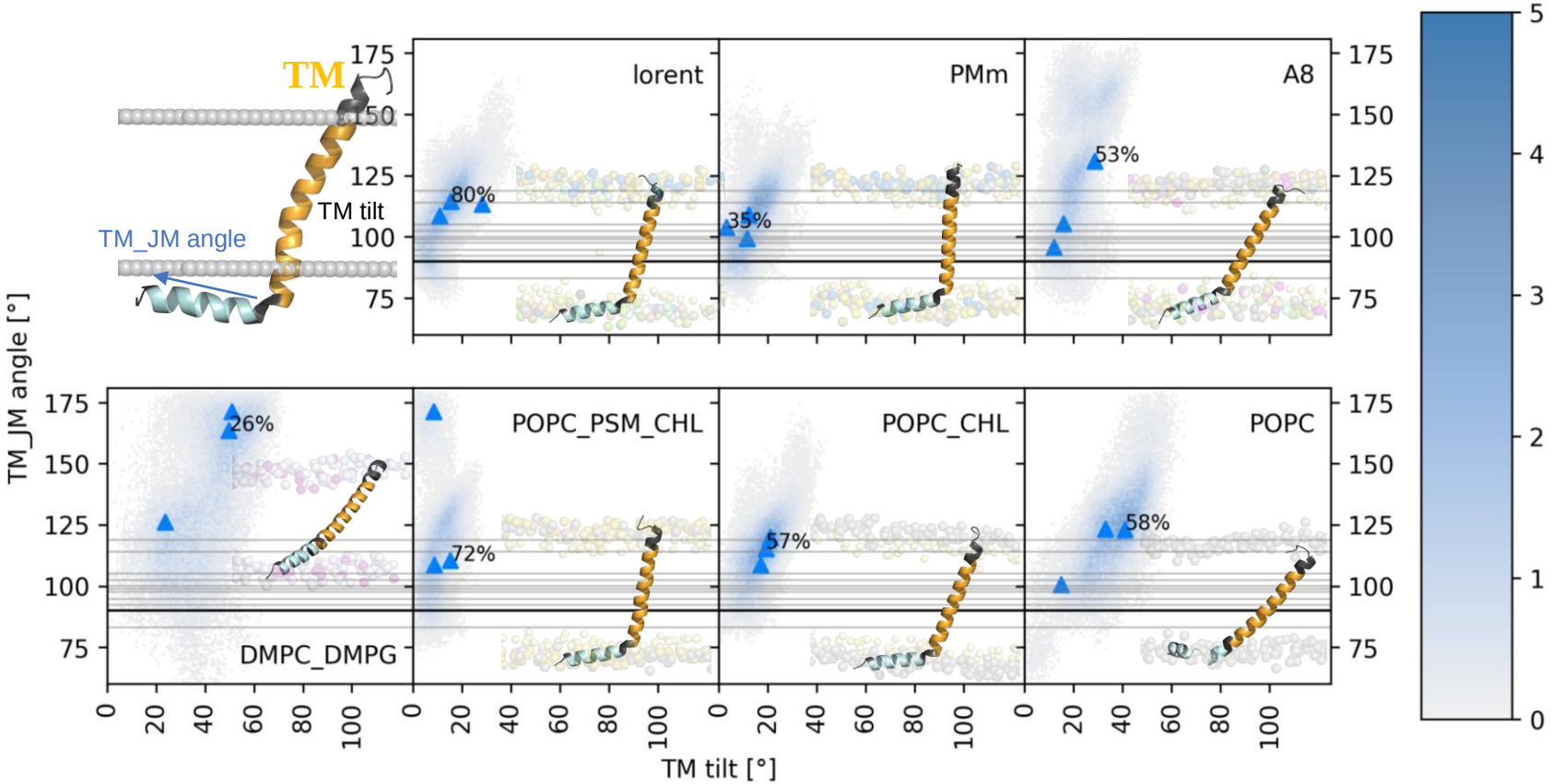
Part 4: Results Case 2

- The protein conformation changes from the starting structure
- It flexes within the membrane
- It transiently unwinds at the JM helix



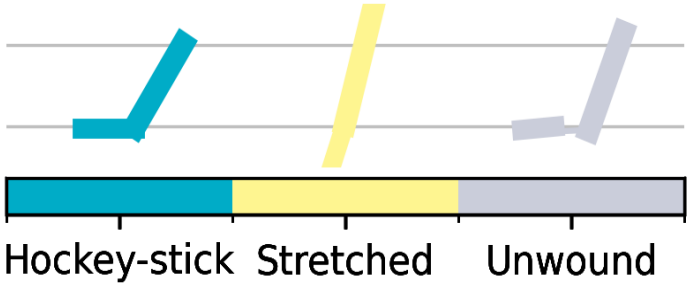
Part 4: TM-JM angle ranges

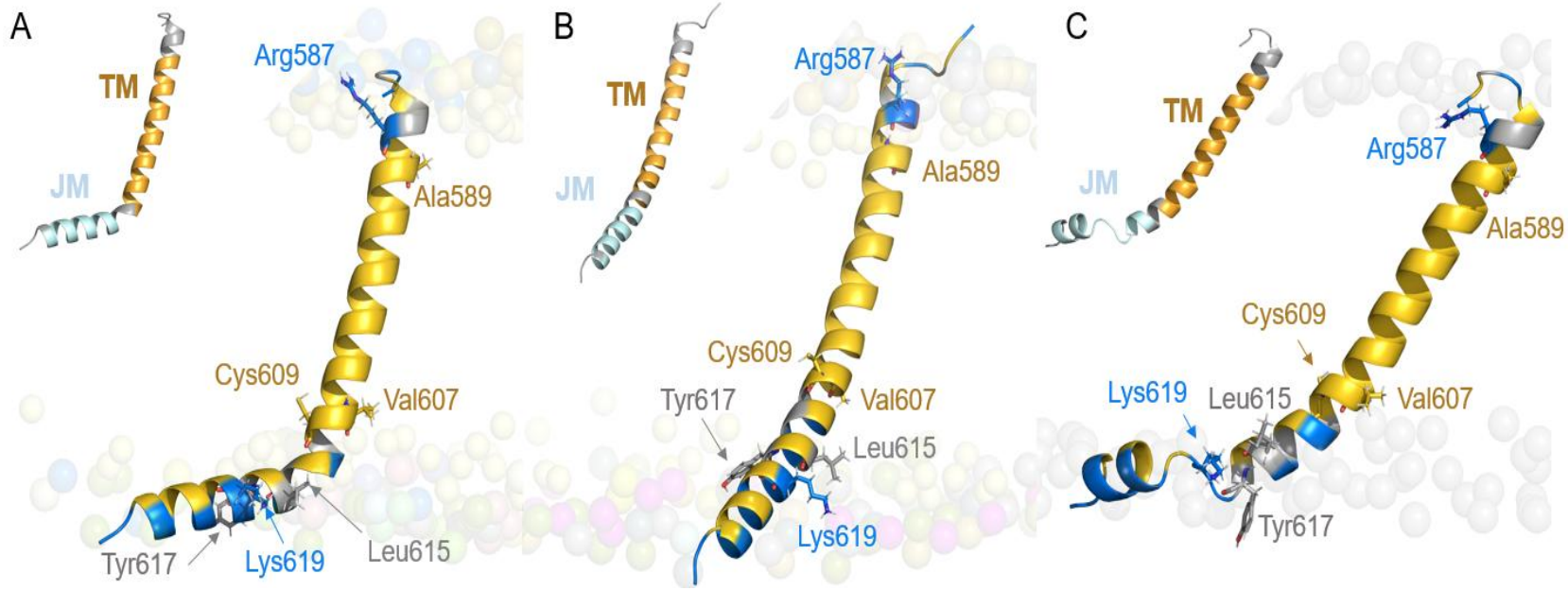
System	TM Tilt [°]	TM_JM Angle [°]
L18	14±8	115±11
Pm11	14±7	110±11
A8	21±7	120±15
POPC_PSM_CHL	8±4	119±12
POPC_CHL	18±7	115±10
POPC	38±10	124±13
DMPC_DMPG	49±19	164±9



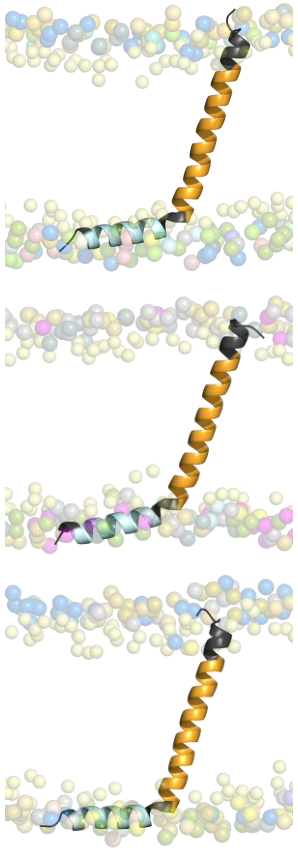
Part 4: Three major conformations

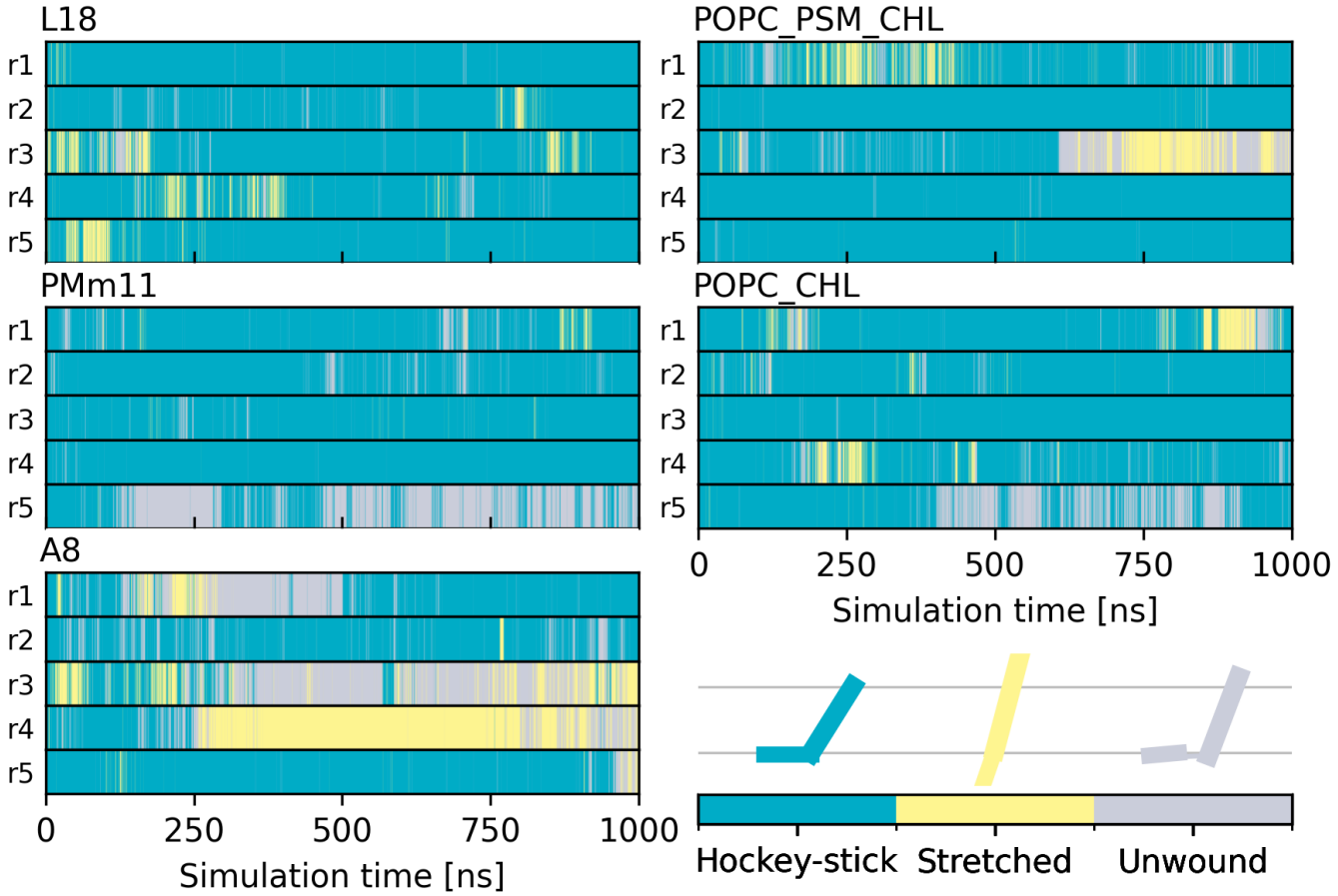
- Hockey stick (starting structure)
- Stretched
- Unwound – a group word for a range of options

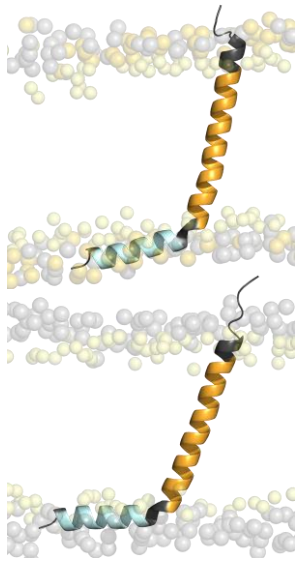


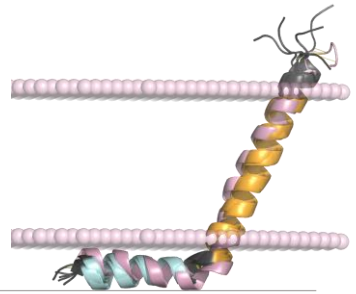


Part 4: Protein in membrane – liquid ordered phase

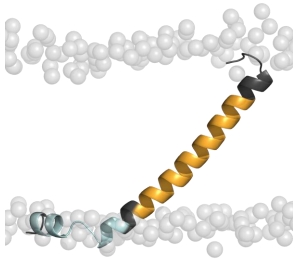


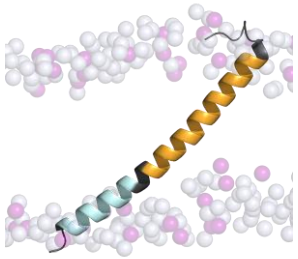


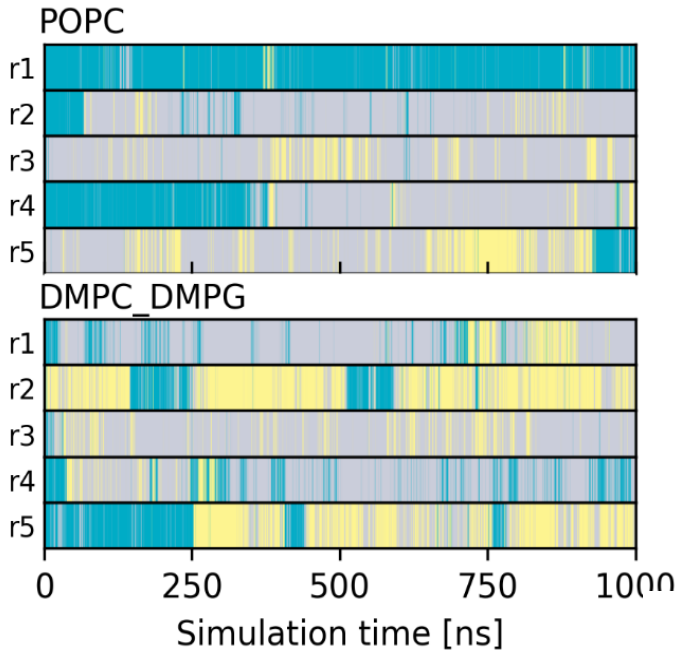


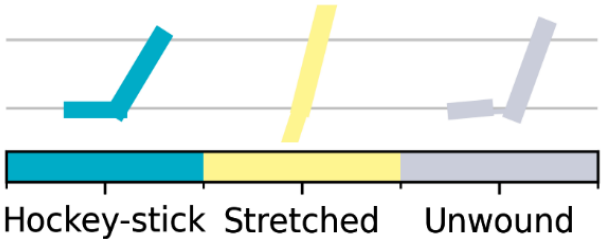


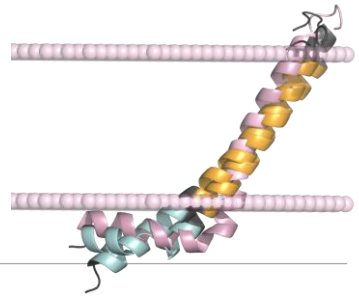
Part 4: Protein in membrane – liquid disordered phase





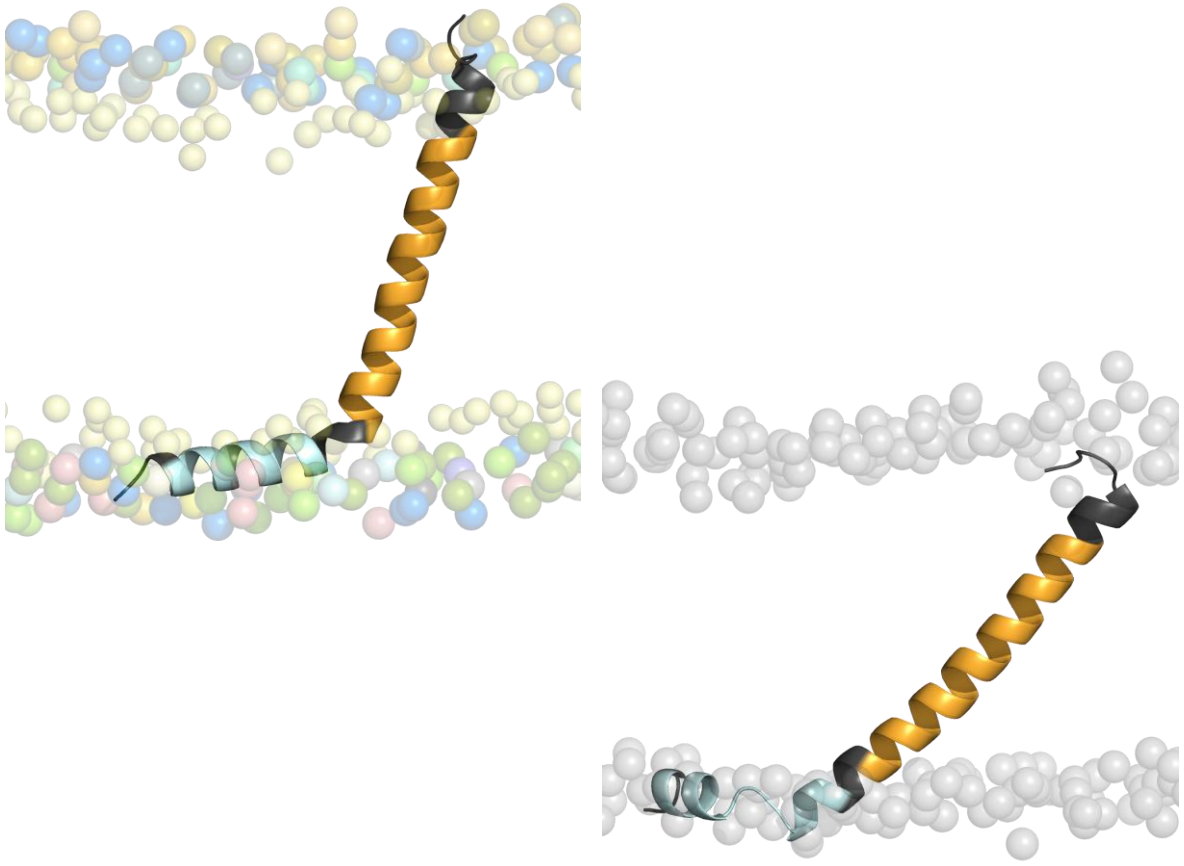






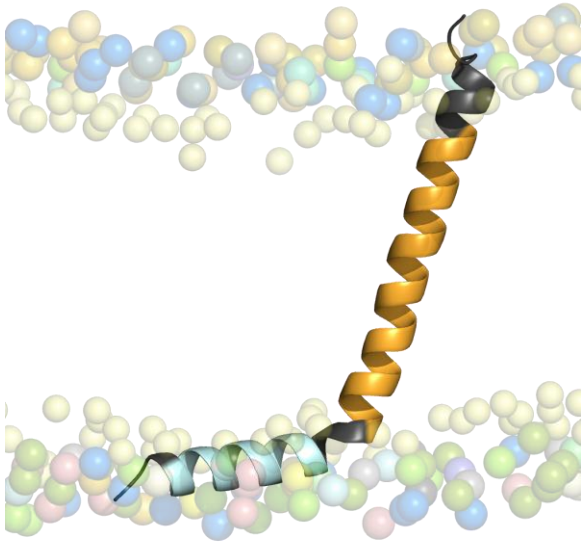
Summary

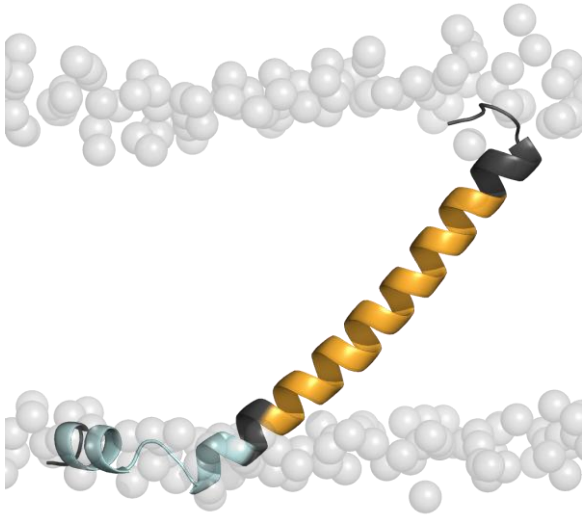
- Cholesterol content and subsequent membrane phase play major role in membrane properties
- More so than the precise lipid composition
- Liquid ordered membranes retain TLR2m in its experimental conformation



Conclusions

- Cholesterol content and subsequent membrane phase play major role in membrane properties
- More so than the precise lipid composition
- Liquid ordered membranes retain TLR2m in its experimental conformation





- Models of plasma membrane proteins should contain cholesterol
- Unless specific lipid interaction are expected, very simple model is sufficient to capture phase-related properties
- Multiple replicas show (lack of) statistical significance

Thank you for your attention!

- Many thanks to
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COST ACTION

CATRIN
Czech Advanced Technology and Research Institute

TECHSCALE

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