



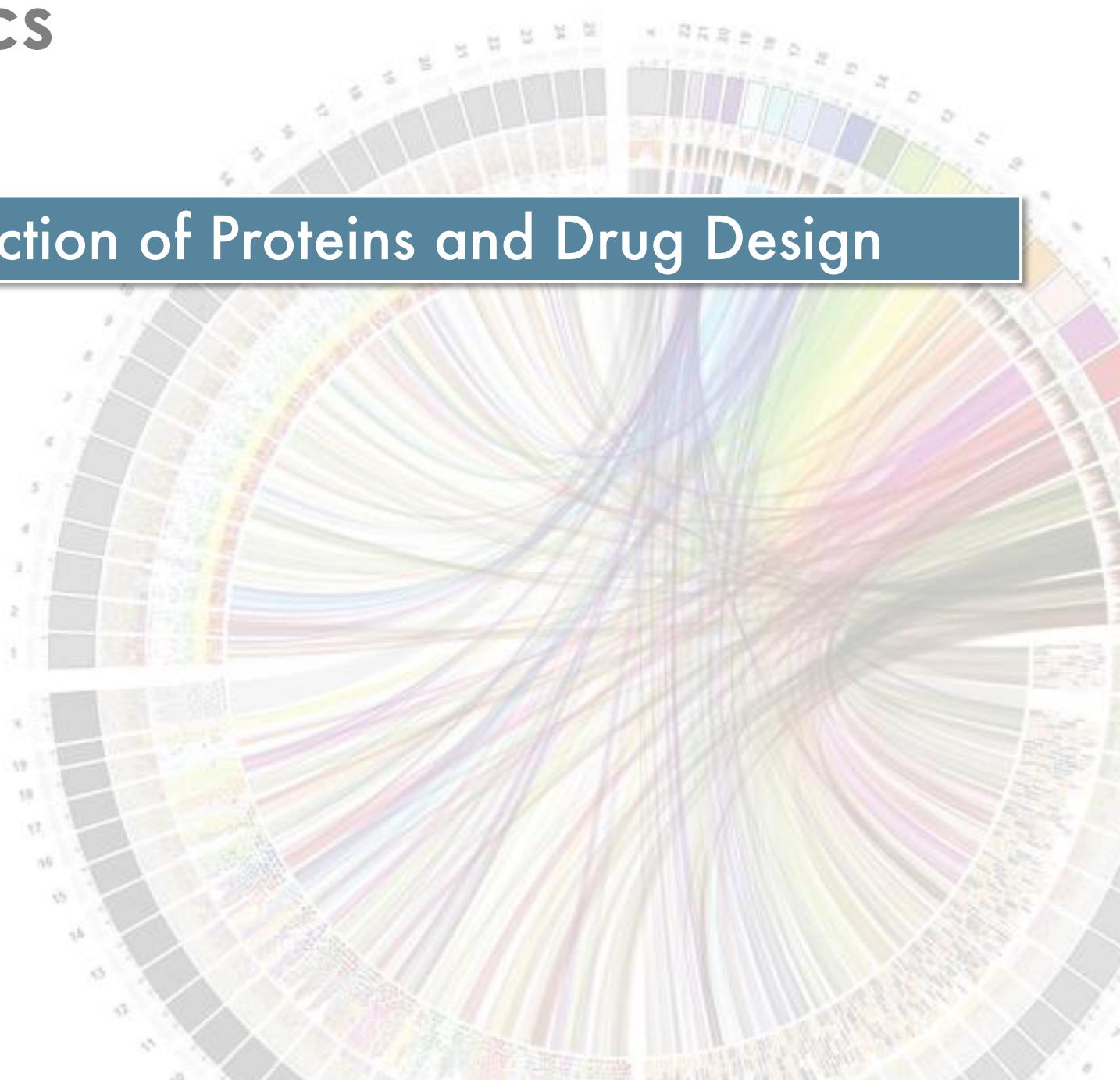
Module 4: Structure and Function of Proteins and Drug Design

Molecular Dynamics Simulations of Membrane Proteins

Nil Casajuana-Martin

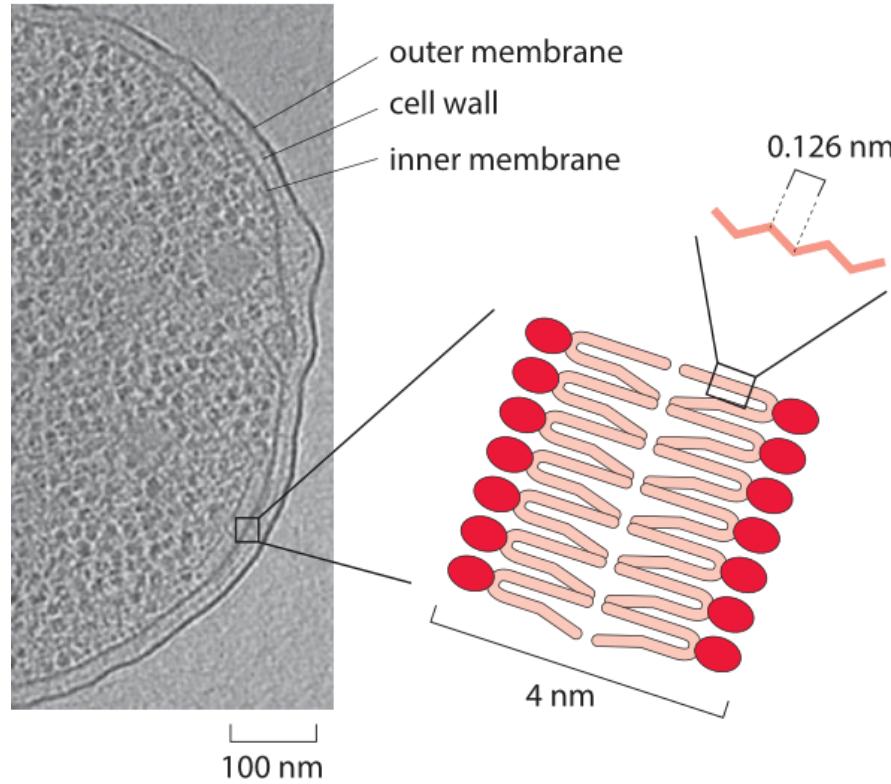
Laboratory of Computational Medicine

Nil.Casajuana@uab.cat

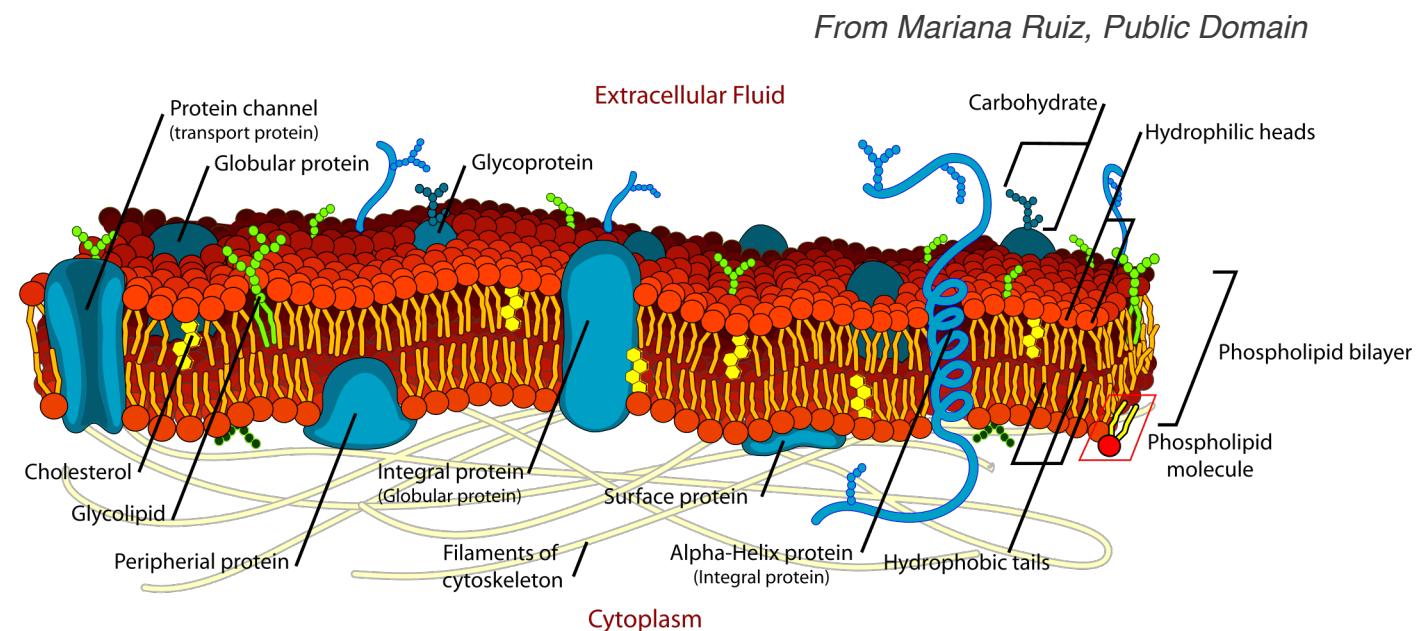


The cell membrane

- Separates the interior of all cells from the outside environment
- Phospholipid bilayer **with embedded proteins**

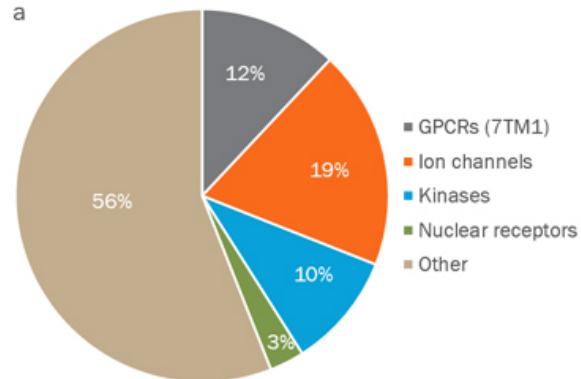


Adapted from A. Briegel et al. Proc. Nat. Acad. Sci., (2009)

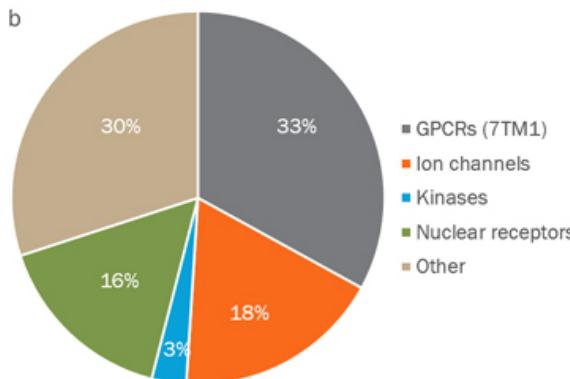


Membrane proteins

~30% of all genes



>50% of all drugs



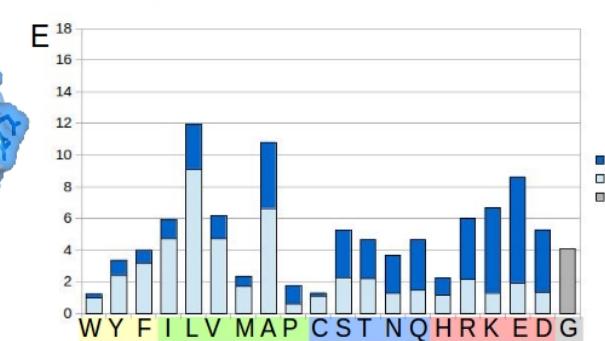
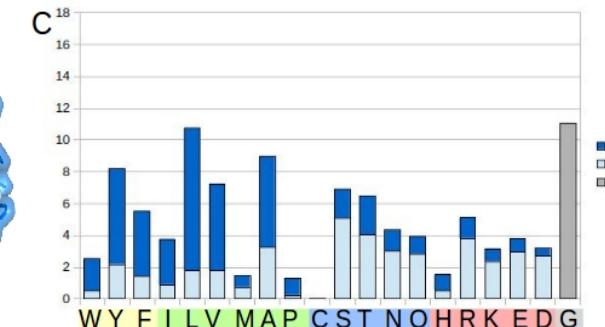
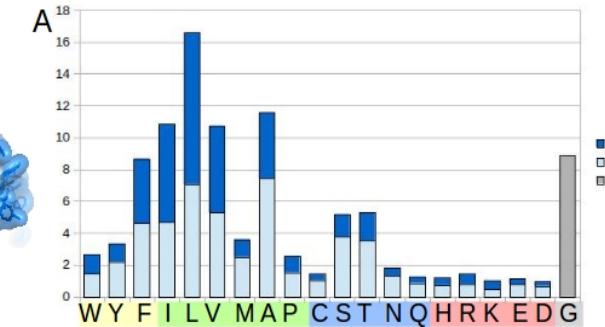
	Globular proteins	Membrane proteins
shape	spherical	helix bundle / β -barrel
need to	be soluble in water	cross the membrane
exterior	hydrophilic	hydrophobic
interior	hydrophobic	helix bundles: hydrophobic β -barrel: hydrophilic

Residue composition

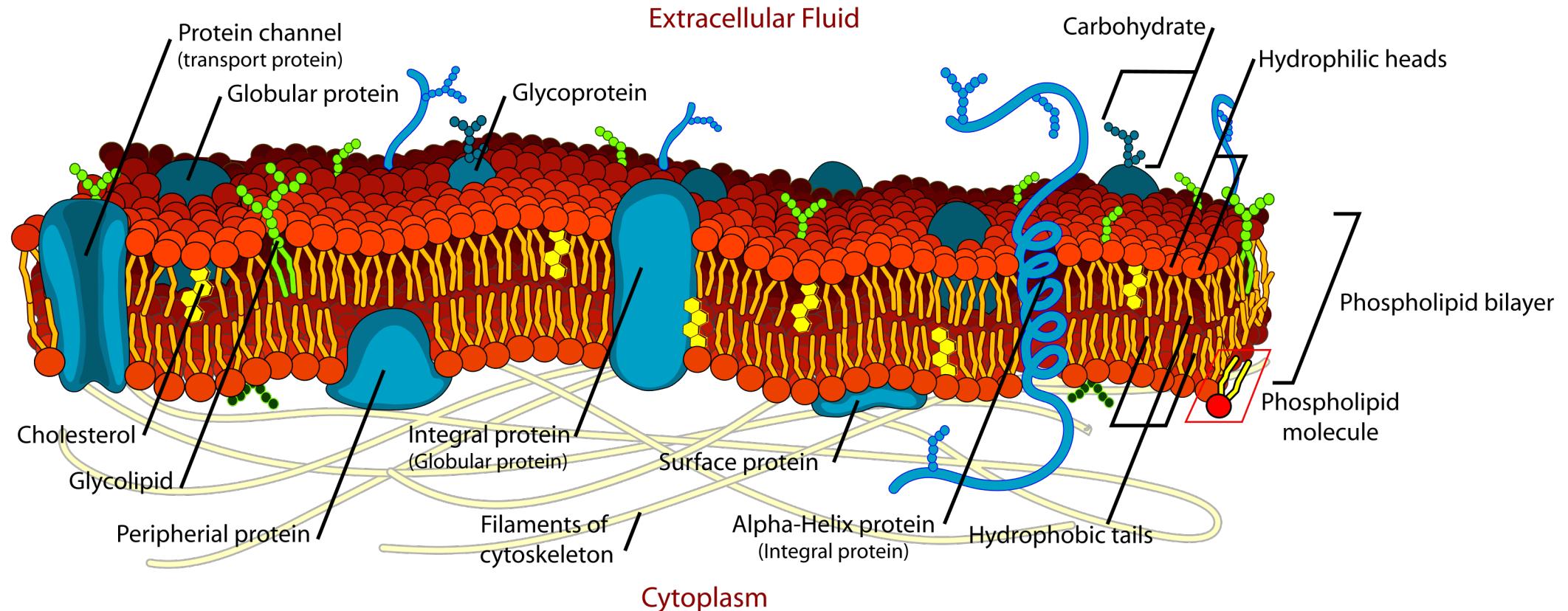
TM helix bundles

TM β -barrels

globular



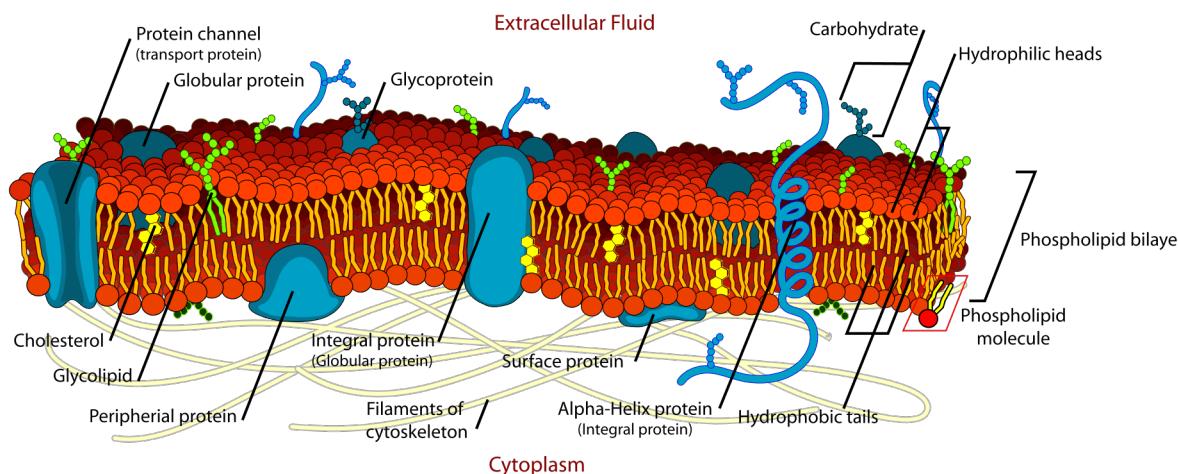
The cell membrane



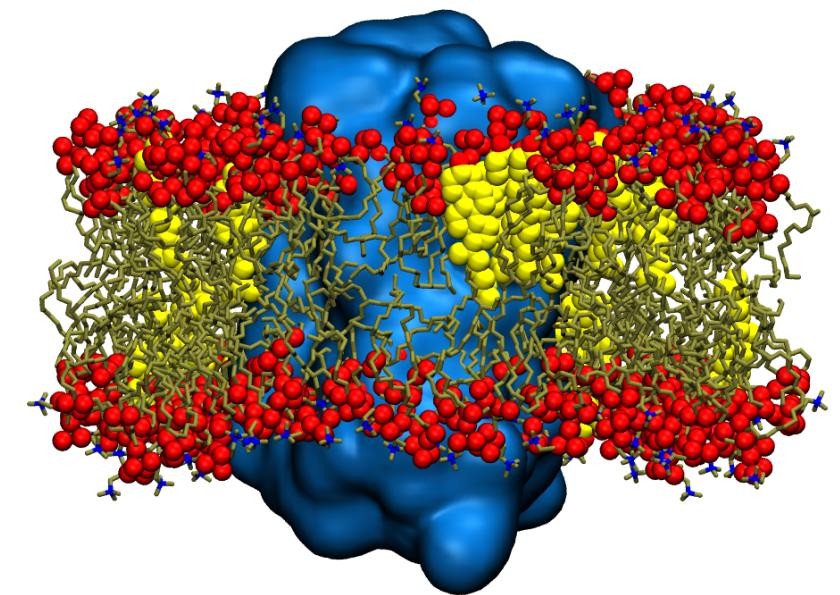
From Mariana Ruiz, Public Domain

From “reality”...

... to a model



From Mariana Ruiz, Public Domain

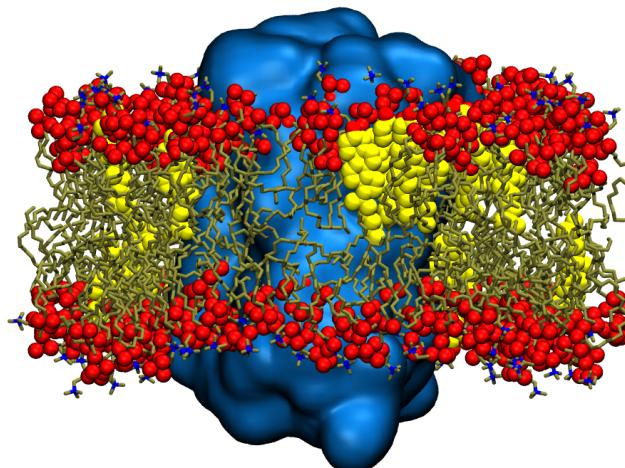
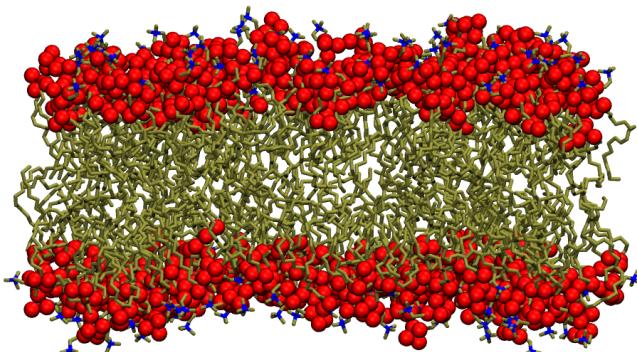


One (or few) component phospholipid bilayer

simpler = less sampling

Meet the components

Phospholipid



Usually PC based

- Representative lipid
- Desired membrane properties
- Well studied experimentally

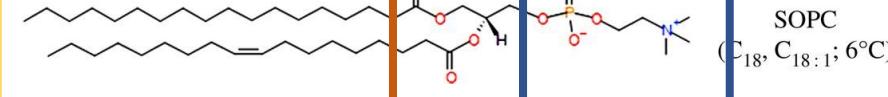
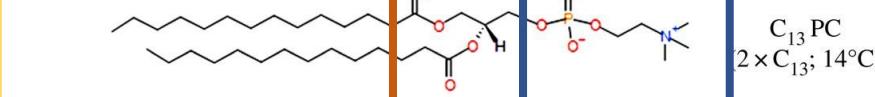
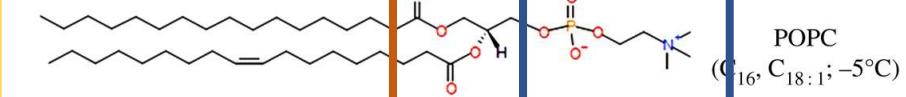
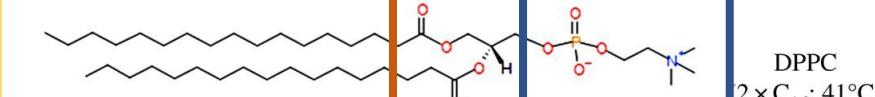
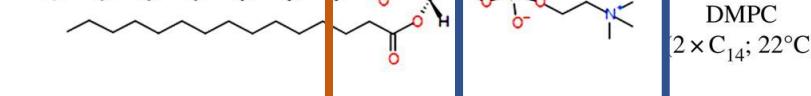
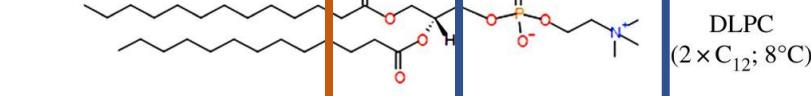
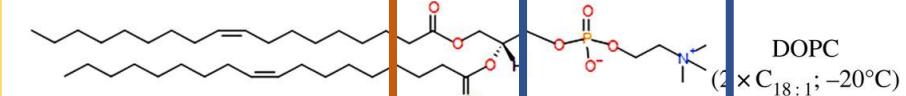
The most typical phospholipids in a membrane model

DOPC: 1-oleyl-2-oleyl-sn-glycero-phosphatidylcholine

DPPC: 1-palmitoyl-2-palmitoyl-sn-glycero-phosphatidylcholine

POPC: 1-palmitoyl-2-oleyl-sn-glycero-phosphatidylcholine

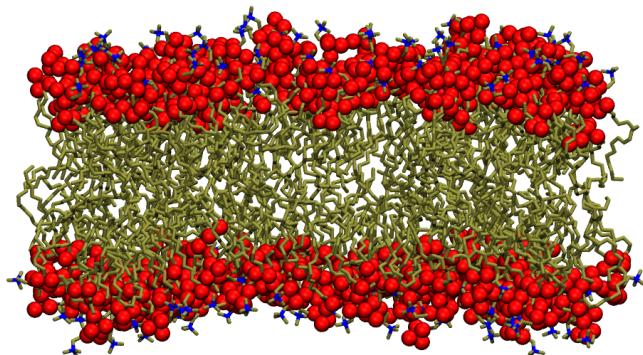
2x fatty acids glycerol PC



Extracted from C. Duncan et al. J R Soc Interface, (2014)

Meet the components

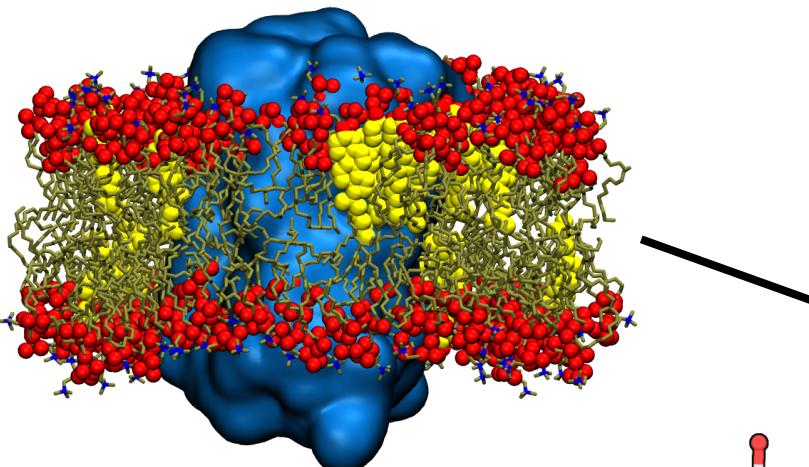
Phospholipid



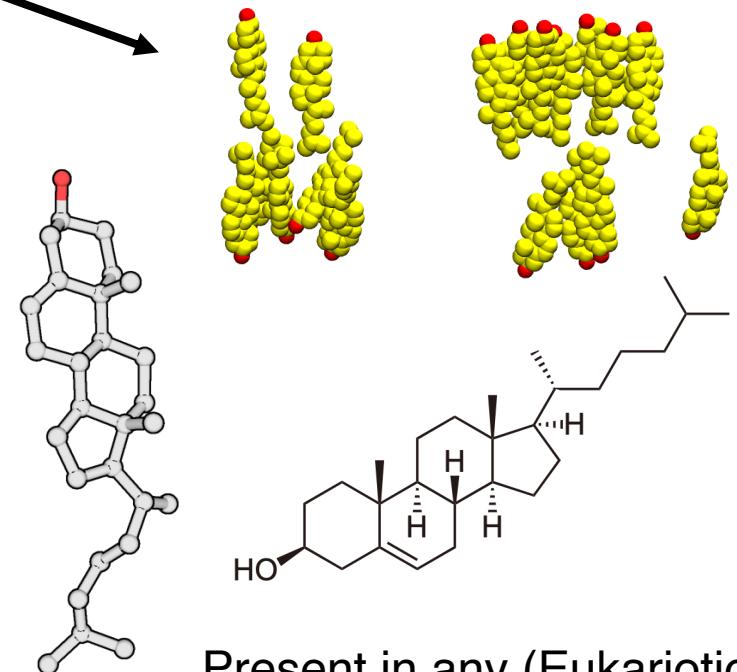
Usually PC based

- Representative lipid
- Desired membrane properties
- Well studied experimentally

POPC or DOPC/DPPC most typical



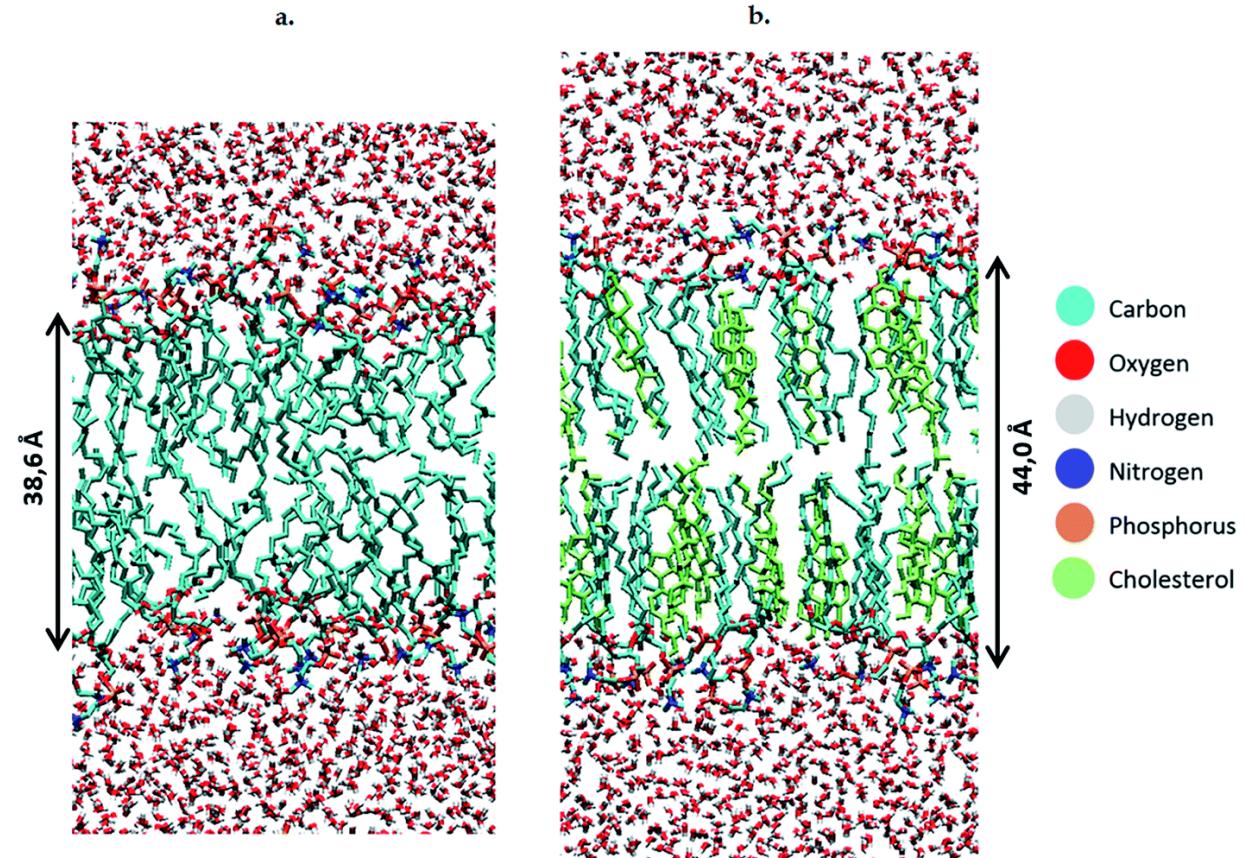
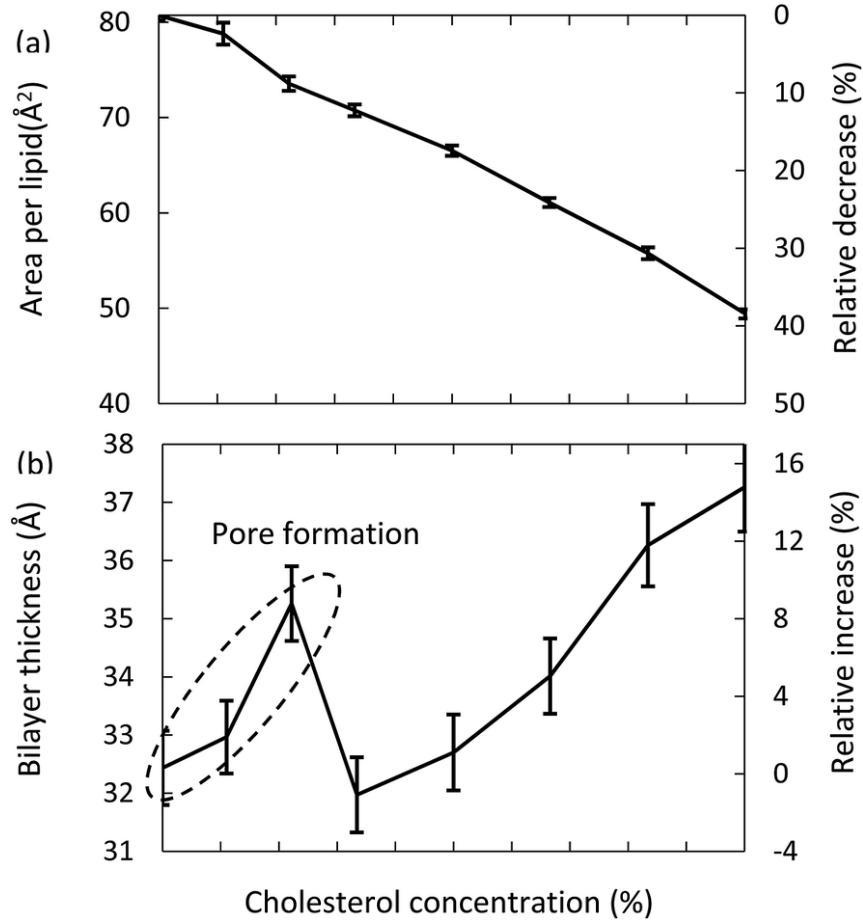
Cholesterol



Present in any (Eukariotic) cell membrane

Modifies membrane's features

Cholesterol modifies membrane's features



From J. Van der Paal et. al. Chem. Sci. (2016)

From “reality”...

... to a model

From experiments

(x-rays, neutron scattering, solid state NMR)



to Force Fields

(dihedrals, Lennard-Jones, charges...)

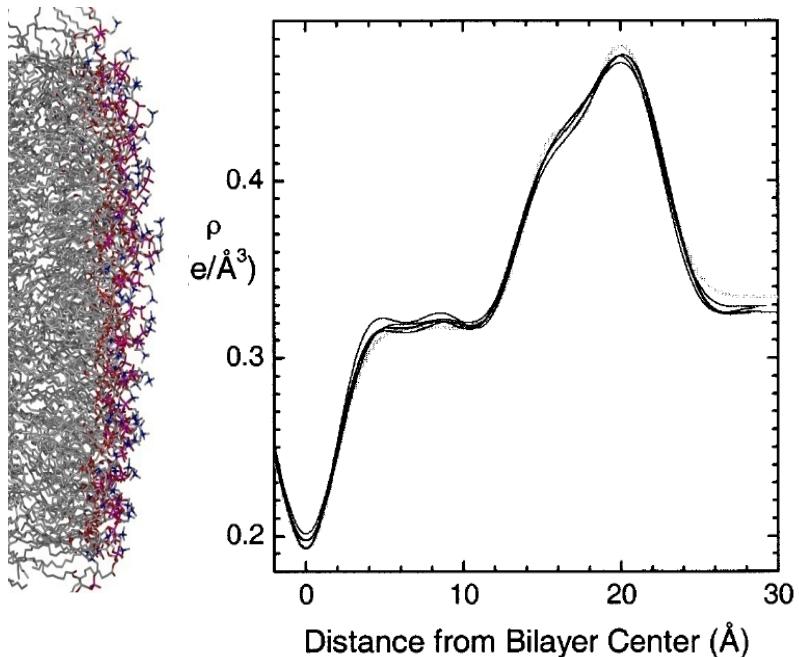


FIGURE 8 Electron density profile obtained from the fit in Fig. 7 (broad gray curve) and by Fourier reconstruction (narrow black curves) for several D spacings in range A with 10 measured orders of diffraction. The Fourier phases are those in Fig. 7. By using the scaling factors K_m obtained from the model, the Fourier profiles are placed on an absolute scale.

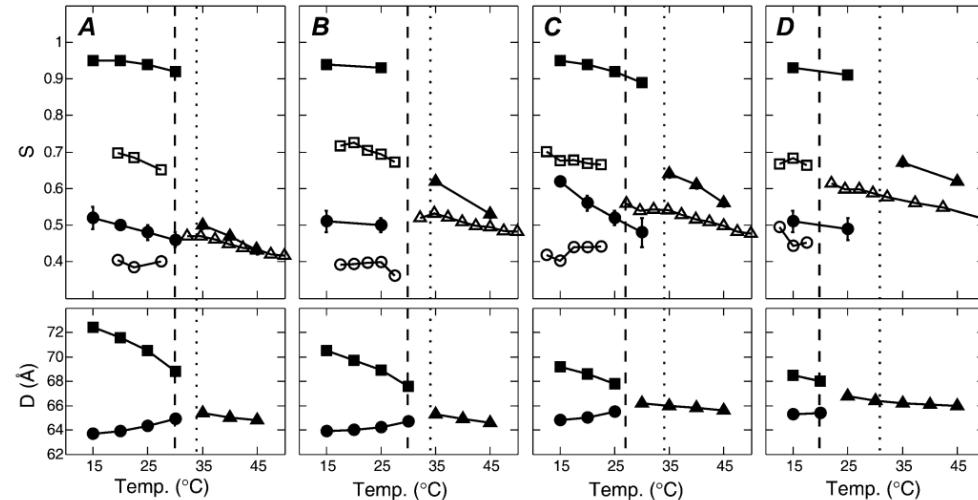


FIGURE 6 Top plots show $S_{x\text{-ray}}$ (solid symbols) or S_{NMR} (open symbols), and bottom plots show lamellar repeat D (for MLV samples) versus temperature for 1:1 DOPC/DPPC + varying amounts of cholesterol: (A) 15%, (B) 20%, (C) 25%, and (D) 30%. Different shapes represent different phases: squares, Lo; circles, Ld; triangles, single phase. The dashed vertical line shows the miscibility transition temperature $T_{\text{mix}}^{\text{NMR}}$ detected by NMR (10), and the dotted line shows $T_{\text{mix}}^{\text{Fluor}}$ detected by fluorescence microscopy (32). An offset of $+2.5^{\circ}\text{C}$ has been added to the ^2H -NMR temperatures, reflecting the lower melting temperature of DPPC-d62 compared with DPPC. For many of the $S_{x\text{-ray}}$ data points, the error bars (determined from uncertainties in the fits) are smaller than the symbols. Uncertainties in lamellar repeats are $\pm 0.5 \text{ \AA}$.

Lipid (All-atom) Force Fields

Developing and Testing of Lipid Force Fields with Applications to Modeling Cellular Membranes

Alison N. Leonard,^{‡,§} Eric Wang,^{†,§} Viviana Monje-Galvan,[†] and Jeffery B. Klauda^{*,†,‡,§}

[†]Department of Chemical and Biomolecular Engineering and [‡]Biophysics Graduate Program, University of Maryland, College Park, Maryland 20742, United States

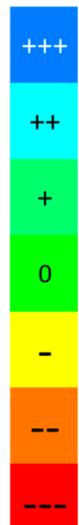
Table 1. Summary of Lipid Force Fields^a

grouping	lipid FF	years published	community
all atom	CHARMM22 (C22) ^{101,102}	1996, 1997	CHARMM
	CHARMM27 (C27) ¹⁰³	2000	CHARMM
	CHARMM27r (C27r) ^{104,105}	2005	CHARMM
	GAFF ^{106,107}	2007, 2008	AMBER
	CHARMM36 (C36) ^{71,108,109}	2010	CHARMM
	Slipids ^{110,111}	2012, 2013	Self/AMBER
	Lipid14 ¹¹²	2014	AMBER
	OPLS-AA ¹¹³	2014	OPLS
	GROMOS (Berger) ⁴²	1997	GROMOS

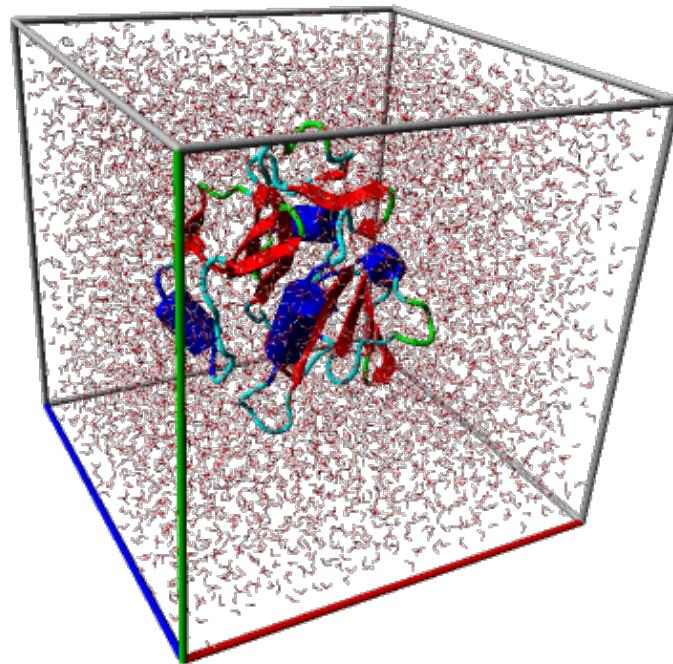
A Critical Comparison of Biomembrane Force Fields: Structure and Dynamics of Model DMPC, POPC, and POPE Bilayers

Krist
and J
†Com
Germ
‡Lehrs
Staud

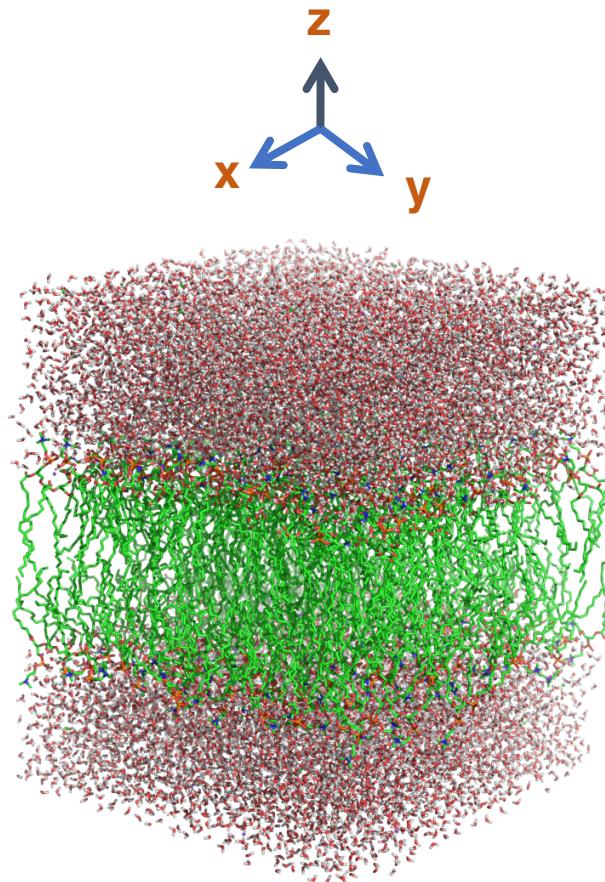
	CHARMM36		GROMOS54a7		Slipids		Lipid14	
	PC	PE	PC	PE ^a	PC	PE	PC	PE
Lipid volume	2.3%	0.7%	2.4%		3.8%	2.1%	4.6%	4.1%
Bilayer thickness	1.0%	8.3%	1.5%		3.7%	2.8%	1.9%	8.0%
Acyl chain order parameters	0.029	0.030	0.035		0.010	0.010	0.011	0.027
³¹ P- ¹³ C dipolar coupling [Hz]	31.9		32.7		30.7		33.5	
Lipid diffusion coefficients	0.9%	11.7% ^b	86.8%		4.2%	25.8% ^b	5.1%	39.8% ^b
Phase transition characteristics	T _m	FWHM	T _m	FWHM	T _m	FWHM	T _m	FWHM
	8.6%	+++	6.7%	-	4.8%	++	9.2%	+++
Computational efficiency	rel speed	rel parall.	rel speed	rel parall.	rel speed	rel parall.	rel speed	rel parall.
	120%	100%	205%	41%	100%	78%	188%	35%



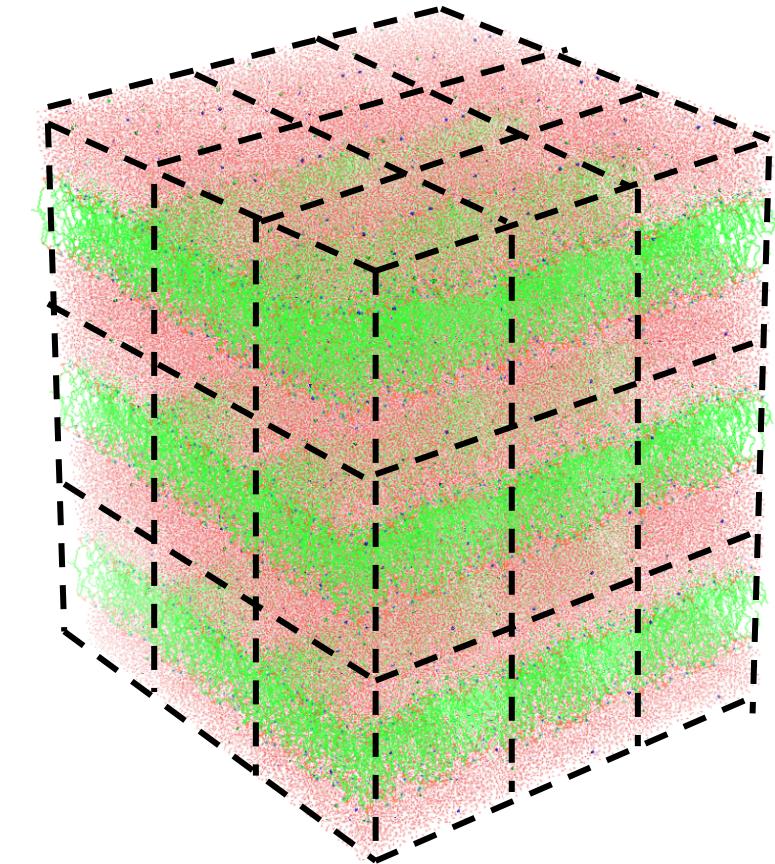
Considerations



isotropic



semi-isotropic / anisotropic



Today...

- **Membrane-only system**
 - Just POPC
 - POPC+CHL
 - Comparative analysis
- **Protein-membrane system**
 - CB_2 in a POPC+CHL membrane

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 - CB₂ in a POPC+CHL membrane

The software: PACKMOL-memgen

The builder

PACKMOL-Memgen: A Simple-To-Use, Generalized Workflow for Membrane-Protein–Lipid-Bilayer System Building

Stephan Schott-Verdugo^{†,‡} and Holger Gohlke^{*,†,§}^{id}

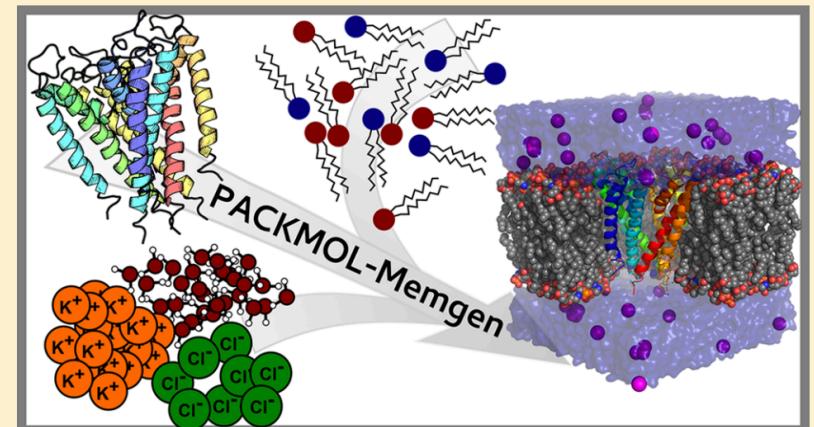
[†]Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany

[‡]Centro de Bioinformática y Simulación Molecular (CBSM), Faculty of Engineering, Universidad de Talca, 1 Poniente 1141, Casilla 721, Talca, Chile

[§]John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC) & Institute for Complex Systems—Structural Biochemistry (ICS 6), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

Supporting Information

ABSTRACT: We present PACKMOL-Memgen, a simple-to-use, generalized workflow for automated building of membrane-protein–lipid-bilayer systems based on open-source tools including Packmol, memembed, pdbremix, and AmberTools. Compared with web-interface-based related tools, PACKMOL-Memgen allows setup of multiple configurations of a system in a user-friendly and efficient manner within minutes. The generated systems are well-packed and thus well-suited as starting configurations in MD simulations under periodic boundary conditions, requiring only moderate equilibration times. PACKMOL-Memgen is distributed with AmberTools and runs on most computing platforms, and its output can also be used for CHARMM or adapted to other molecular-simulation packages.



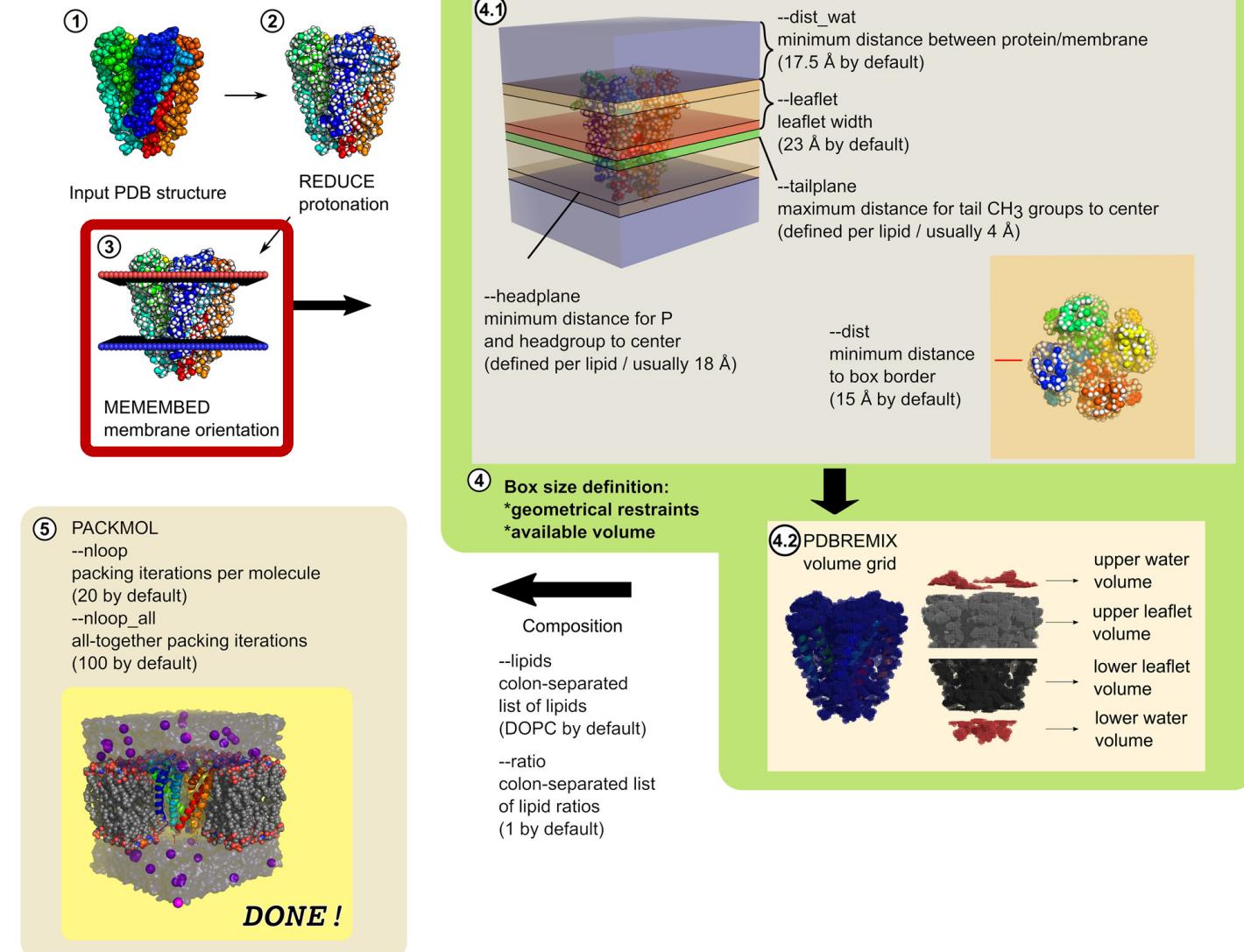
The software: PACKMOL-memgen

The builder

- Membrane-only systems
- Protein-membrane systems

orientations of (OPM) database
proteins in membranes

<https://opm.phar.umich.edu/>



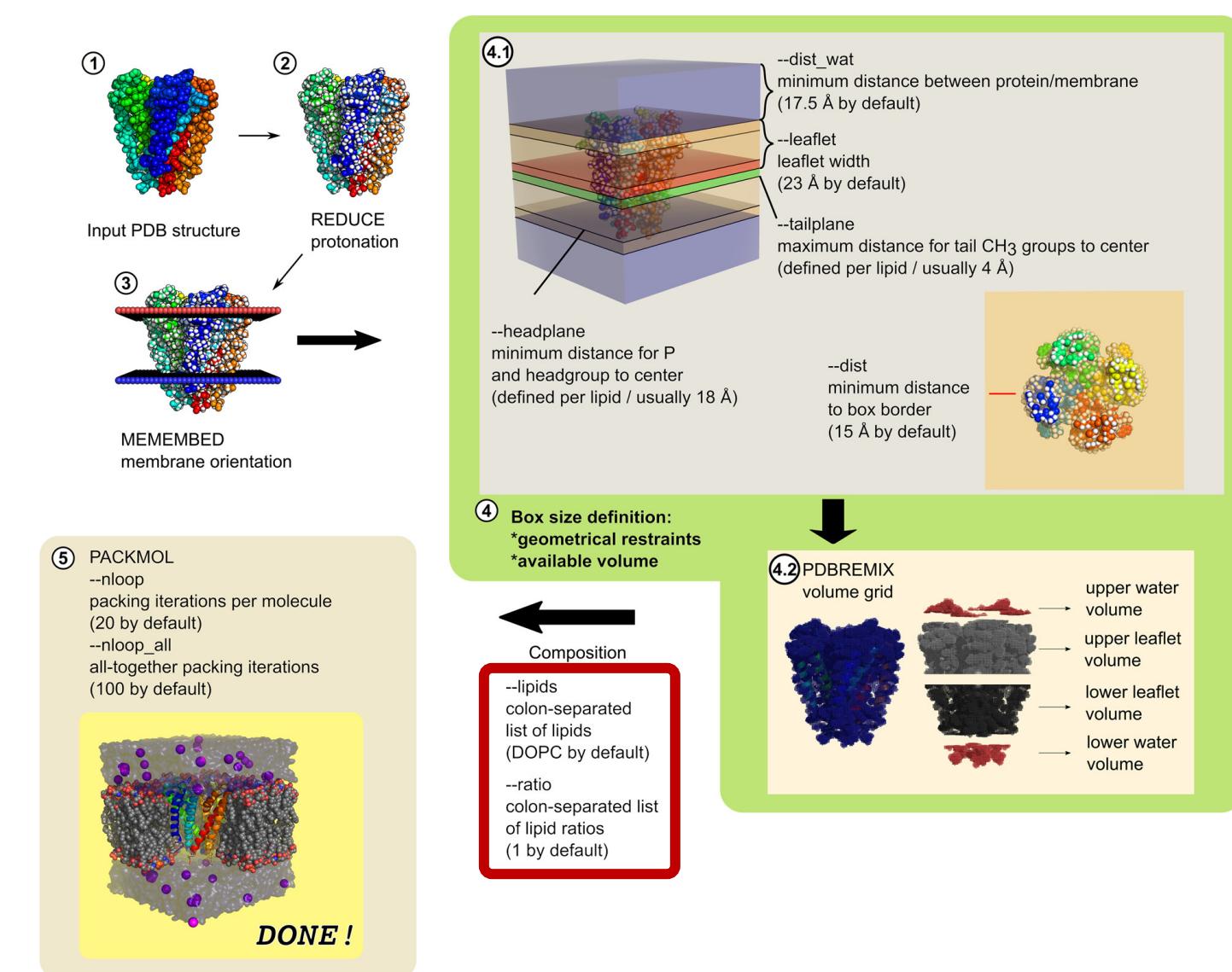
The software: PACKMOL-memgen

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Table 1. Lipids and Cholesterol Available in PACKMOL-Memgen[a](#)

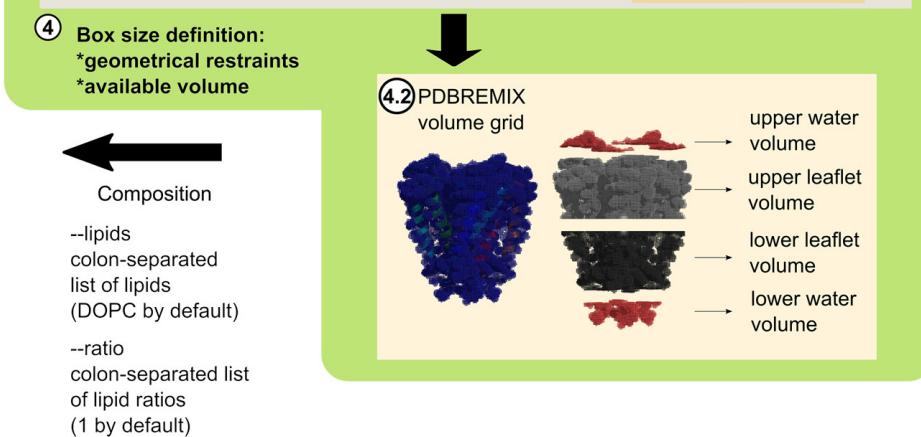
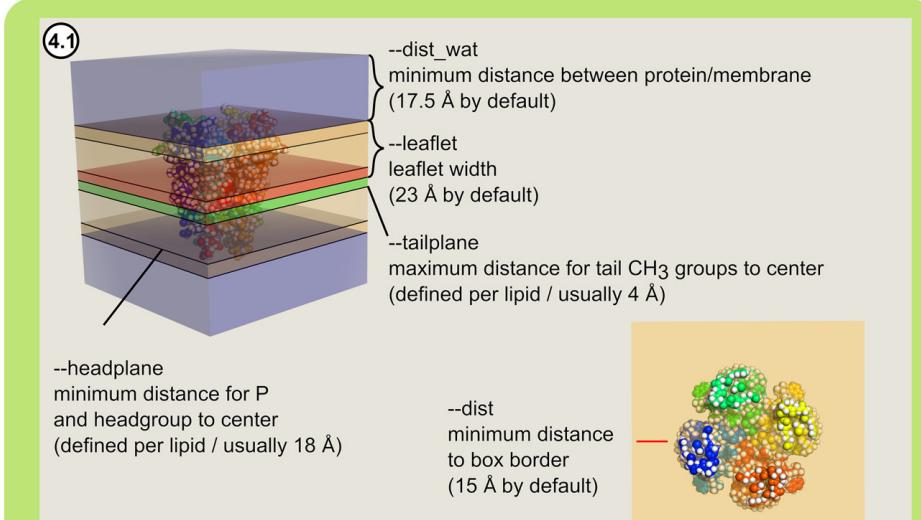
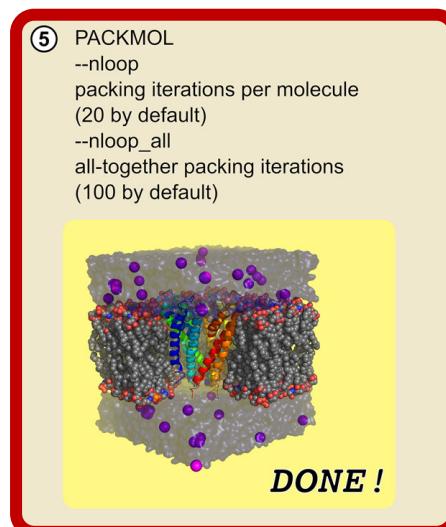
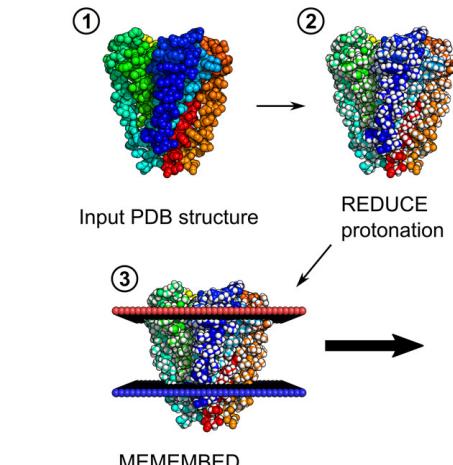
DAPA	DMPA	DPPA	PLPA	SDPA
DAPC	DMPC	DPPC	PLPC	SDPC
DAPE	DMPE	DPPE	PLPE	SDPE
DAPG	DMPG	DPPG	PLPG	SDPG
DAPS	DMPS	DPPS	PLPS	SDPS
DLPA	DOPA	DSPA	POPA	PSM
DLPC	DOPC	DSPC	POPC	SSM
DLPE	DOPE	DSPE	POPE	CHL1
DLPG	DOPG	DSPG	POPG	
DLPS	DOPS	DSPS	POPS	



The software: PACKMOL-memgen

The builder

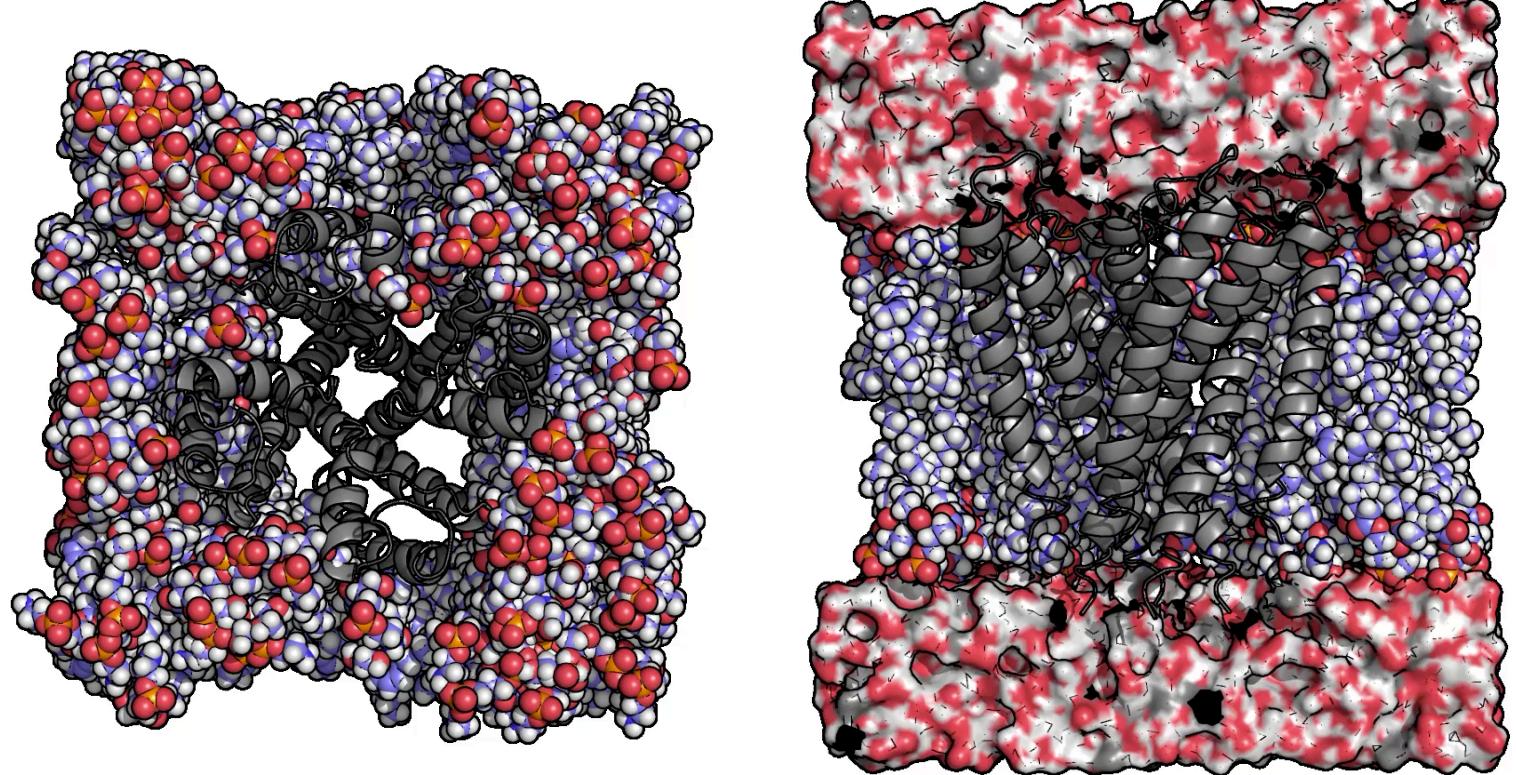
- Membrane-only systems
- Protein-membrane systems



The software: PACKMOL-memgen

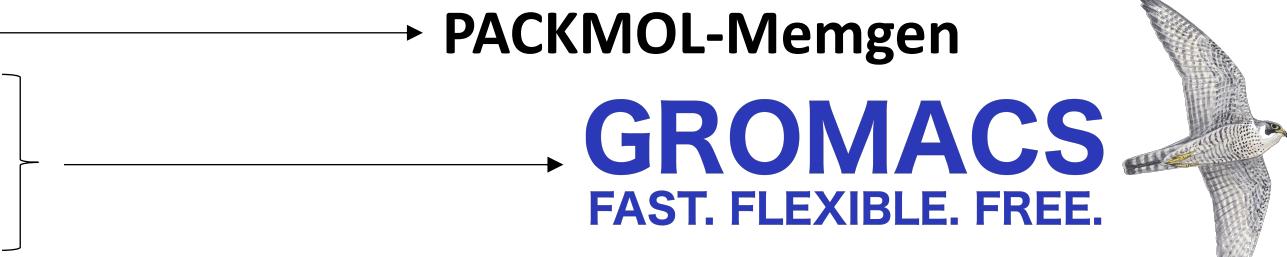
The builder

- Membrane-only systems
- Protein-membrane systems



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The software: GROMACS

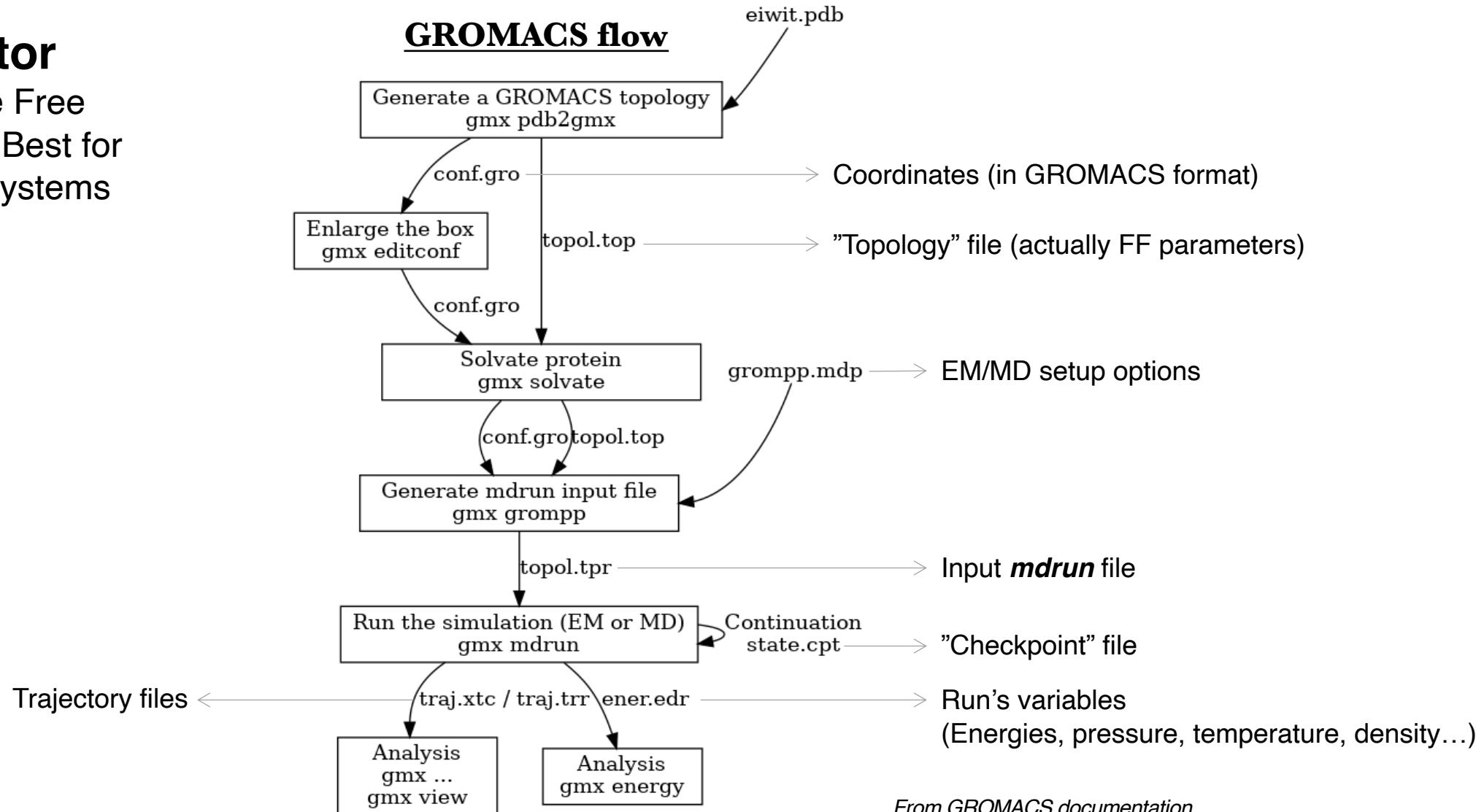
The **simulator**

- Fast Flexible Free
- (One of the) Best for membrane systems

The software: GROMACS

The simulator

- Fast Flexible Free
- (One of the) Best for membrane systems



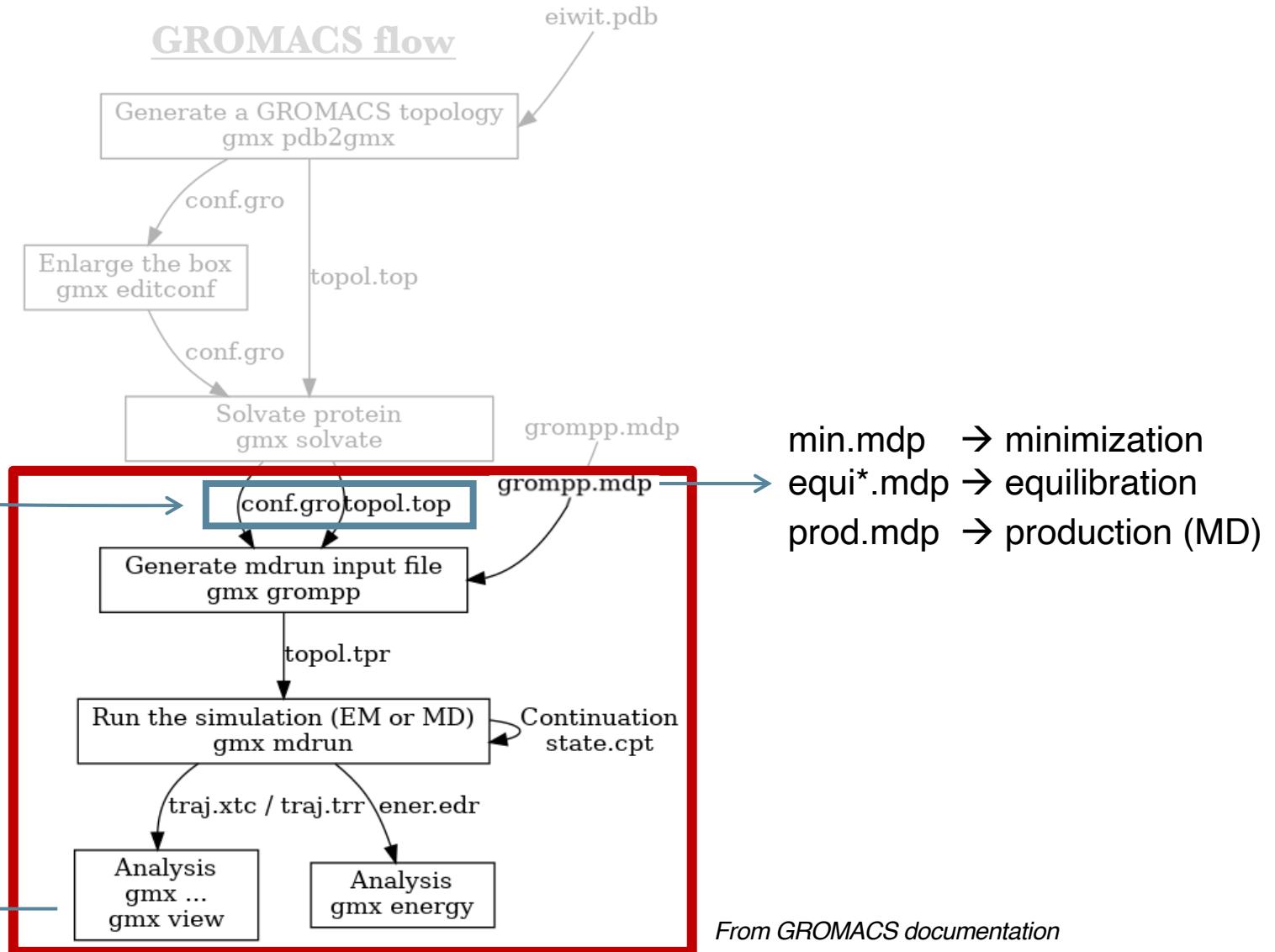
The software: GROMACS

The simulator

- Fast Flexible Free
- (One of the) Best for membrane systems

PACKMOL-memgen

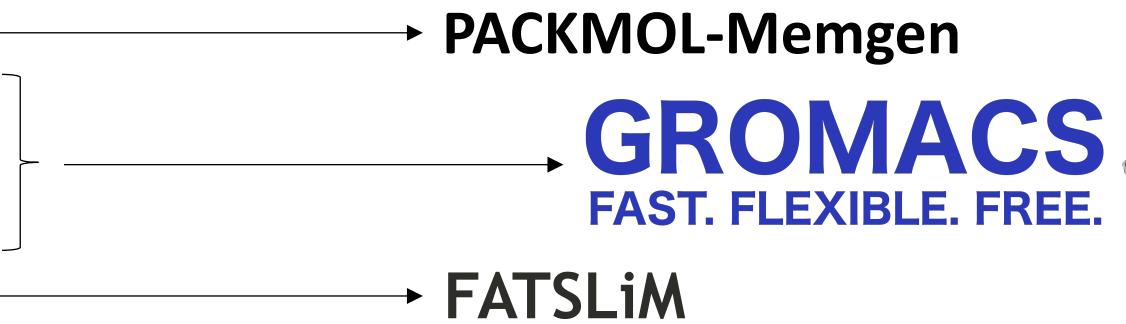
transformation
(from AMBER to GROMACS)



Also with **FATSLiM**...

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The software: **FATSLiM**

A fast and robust toolbox for the analysis of your MD simulations of lipid membranes!

FATSLiM

license

GPLV3+

release v0.2.1

coverage

100%

build

passing

DOI

10.5281/zenodo.158942

FATSLiM stands for “**F**ast **A**nalysis **T**oolbox for **S**imulations of **L**ipid **M**embranes” and its goal is to provide an efficient, yet robust, tool to extract physical parameters from MD trajectories.

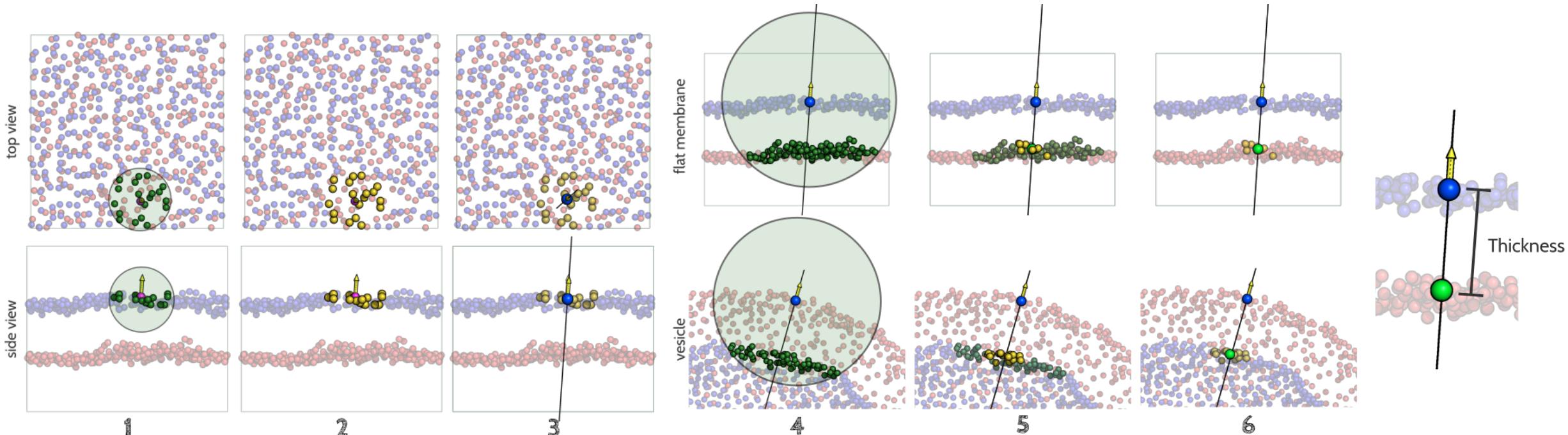
The main objective of FATSLiM is to decrease as possible the amount of time needed to analyze MD trajectories: the ultimate goal is to process a several-gigabyte-big file is just a few minutes or less. This is why a rather important part of FATSLiM’s development is focused on code optimization and simplification in order to maximize its efficiency.

The software: FATS LiM

The membrane analyzer

- Membrane thickness

```
$ fatslim thickness -c conf.gro -t traj.xtc -n index.ndx --plot-thickness thickness.xvg
```



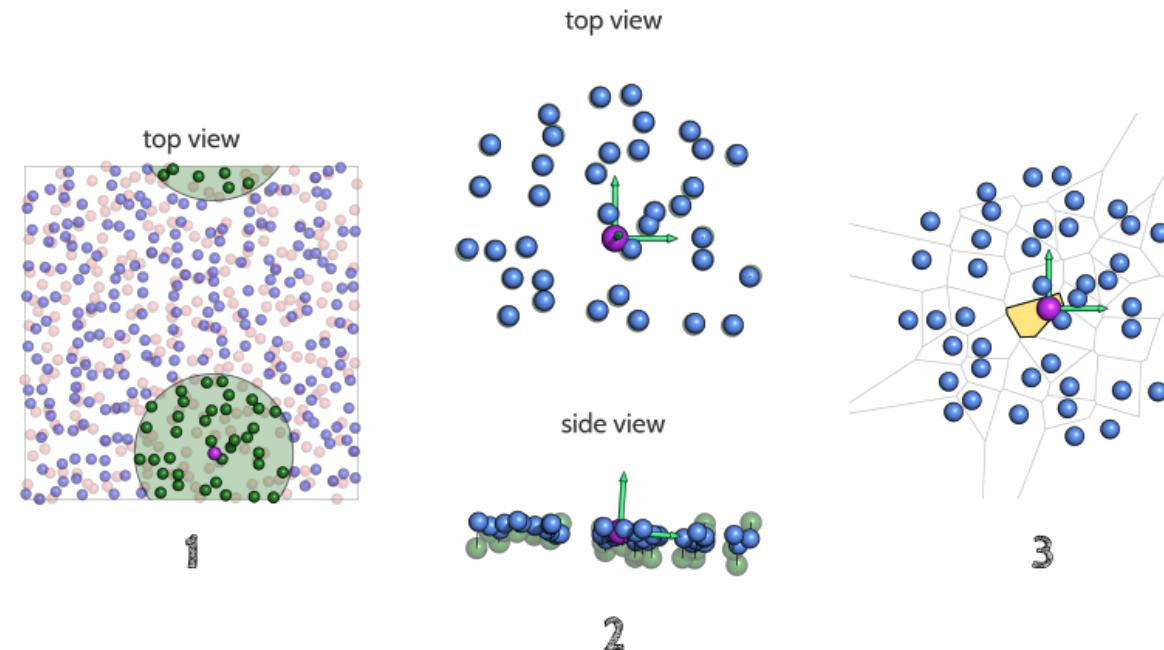
<https://pythonhosted.org/fatslim/documentation/thickness.html>

The software: FATS LiM

The membrane analyzer

- Membrane thickness
- **Area per lipid (APL)**

```
$ fatslim apl -c conf.gro -t traj.xtc -n index.ndx --plot-thickness thickness.xvg
```



<https://pythonhosted.org/fatslim/documentation/apl.html>

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PACKMOL-Memgen

GROMACS
FAST. FLEXIBLE. FREE.

FATSLiM



Force Fields:

- **Lipid14** for membrane
- **TIP3P** for water
- **Amber14ILDN** for protein

TO DO

1. Pick **ONE** simulation from an online repository:

CHARMM-GUI

Effective Simulation Input Generator and More

<http://www.charmm-gui.org/?doc=archive>



<http://gpcrmd.org/>

2. Write a report (**2 PAGES MAX!**) with the analysis of:

- Membrane thickness
- APL
- RMSD
- RMSF
- SS conservation

3. Send it to Nil.Casajuana@uab.cat with "MD_membrane_env" as the **Subject**.