Simulations of Membranes & Membrane Proteins

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Outline: Membranes

- Why are membranes interesting/important?
- Lipids - amphiphilic molecules
- Micelles, bilayers, vesicles, mixtures
- Bilayer phases (crystal, gel, liquid crystalline)
- Special difficulties for simulations
- Bilayer simulations & typical systems
- Membrane proteins, transmembrane helices
- Membrane protein simulations
Importance of Membranes

- Controls transport into/from cells
- 30% of eukaryotic proteins are associated with membranes (membrane proteins, receptors)
- 50% of current drugs target membrane proteins
- “Without membranes you die” (David van der Spoel, 2007)
Lipids

- Charged or strongly polar (zwitterionic) headgroups
- 1-2 Hydrophobic chain(s)
- Amphiphilic molecules
- Typical headgroups: Phosphocholine (PC) Phosphoethanolamine (PE)
- Glycerol link to acyl chain
- Typical chains: Palmitoyl (16 CH₂ groups)
Lipid names...

- **DPPC** - Dipalmitoylphosphatidylcholine
  1,2-Dipalmitoyl-sn-Glycero-3-phosphocholine

- **DOPG** - Dioleoylphosphatidylglycerol
  1,2-Dioleoyl-sn-Glycero-3-phosphoglycerol (negative charge!)

- **POPE** - Palmitoyloleoylphosphatidylethanolamine
  1-Palmitoyl-2-Oleoyl-sn-Glycero-3-phosphoethanolamine

- **DPC** - Dodecylphosphocholine
  (single chain only, forms micelles)

- **Cholesterol**
Lipid conformations

- Large headgroups, single chain: Lipids form micelles
- Higher concentration: hexagonal phases
- There are also inverted micelles and hexagonal phases
- Similar size headgroup/chains: Bilayers or vesicles
Bilayer phases

- Very low temperature: proper crystalline phase
- Low temperature: gel phase
- Room temperature: liquid crystalline phase (Lα)
Cellular membranes
A liquid crystalline bilayer
Lipid Bilayer analysis?

- Experimental techniques:
  - Neutron scattering
  - Liquid X-ray crystallography
  - NMR, EPR, fluorescence spectroscopy
- Average area/lipid
- Order parameters from spectroscopy
- Electron density over the bilayer
- Lipid diffusion
- Average properties -> great statistics!
Bilayer simulations

- Lipid parameters / force fields
- Topologies
- Getting initial conformations
- Simulation cell shapes
- Interaction parameters & setup
- Pressure coupling
- Center-of-mass motion
Lipid force fields

- Groups: phosphate, choline, glycerol, acyl
- Surprisingly hard to get accurate results
- Many force fields lead to lipid area & volumes that are 20% lower than experimental values!
- Do NOT use: Vanilla Gromacs FF, Gromos96, OPLS-AA/L, Amber, old Charmm FF
- Do USE: Berger lipid force field, Charmmm27
- Berger is available on Gromacs site, Charmmm27 in beta for Gromacs distributions
Lipid topologies

- No bilayer coordinates in PDB
- No standardized atom or chain names
- pdb2gmx does not work automatically
- Best option: Find existing topologies (Gromacs site, Tieleman, Lindahl, others)
- Alternative 1: Create RTP building blocks
- Alternative 2: Hack topologies manually
Example DPPC topology

; Topology for united-atom DPPC
; Written by Erik Lindahl, version 980624
; After a gromos version by Olle Edholm

; include our own forcefield - don't use gromacs parameters!
#include "ffDPPC.itp"

[moleculetype]
; Name nrexcl
DPPC 3

[atoms]
; nr type resnr residu atom cgnr charge ; Chiu charges!
1 C3N 1 DPPC  CN1 1 0.16
2 C3N 1 DPPC  CN2 1 0.16
3 C3N 1 DPPC  CN3 1 0.16
4 NL 1 DPPC  NTM 1 0.26
...
17 CH2 1 DPPC  C1B 6 -0.04
18 CH2 1 DPPC  C1C 7 0.0
19 CH2 1 DPPC  C1D 8 0.0
20 CH2 1 DPPC  C1E 9 0.0
1,4 interactions excluded
R-B/Kuwajima torsions

Example DPPC topology

...[dihedrals]
; ai aj ak al funct (func=1 is normal dihedrals, 3=R-B)
  1  4  5  6  1
  4  5  6  7  1
...
  17 18 19 20  3 ; R-B torsions for acyl chains
  18 19 20 21  3
...
[system]
; Name
DPPC membrane with 28 waters per lipid

[ molecules ]
; Compound #mols
DPPC  64
SOL  3000

R

Dihedral (degrees)

Energy (kJ/mol)

trans
gauche
United vs. all atom DPPC

- 50 vs 130 atoms per lipid
- 3x atom density in hydrophobic region
- ~9x interaction density in hydrophobic region
- Roughly 4-5x simulation speed difference
Bilayer structures

- Find pre-equilibrated conformations
- Ask authors of published papers (works great for Gromacs topologies too)
- Use genbox & genconf to change system size
- Repeat single lipid conformation on grid, equilibrate for a long time in vacuo
- Repeat single lipid(s), add random rotation and tilts, shorter equilibration
- Simulate bilayer formation ‘the natural way’
Cell shapes

- Rectangular membranes
- Easiest option when the simulation goal is to calculate wave-vector dependent properties (undulations, etc.)
Cell shapes

- Hexagonal membranes
- Maximizes periodic separation distance
- Can be viewed compact/triclinic/rectangular

Compact
Triclinic (actual periodicity)
Rectangular & whole (default output)
Coulomb interactions

- Very large dipoles & parts with low dielectric screening (hydrophobic core)
- Cut-offs are bad
- Reaction-field would assume an isotropic and homogeneous system (not the case)
- Always use PME
- PME works fine with triclinic cells in Gromacs
Van der Waals stuff

• One reason for the difficulties in simulating membranes is the sensitivity to nonbonded Van der Waals parameters

• Small changes will affect packing of chains, and thus the pressure and area/lipid

• Normal cut-off or switch around 1.0nm is OK, but turn on long-range dispersion correction to get correct pressure
Pressure coupling

- Two-dimensional liquid crystals
- Bilayer should be able to deform in XY-plane
- Anisotropic pressure coupling should be enabled throughout production runs!
  - Neat trick: Semiisotropic coupling (XY+Z)
- Berendsen coupling does not provide a true NPT ensemble - Parinello-Rahman is better
- Slow relaxation (10ps) to avoid oscillations
Compressibility

- Water value works fine as approximation
- Compressibility is really a 3x3 tensor
  Gromacs: 3 diagonal + 3 off-diagonal elements
- Off-diagonal elements zero: only scale cell along the box vectors
- Off-diagonal elements same as diagonal: Enable cell distortion/shear transformations
- You can also force the cell to distort by setting off-diagonal reference pressure to non-zero
Thermostats

- Polar and non-polar parts of the system are not always strongly coupled.
- Bond constraints remove kinetic energy: anisotropic temperature in acyl chains!
- Use separate thermostats for water & lipids, and also protein(s) if present.
Center-of-mass motion

- Center-of-mass motion of entire system normally removed in simulations
- Weak z-coupling in bilayers:
  - Water moves right, lipids left
  - Upper layer right, lower left
- Remove center-of-mass motion separately for: water, upper layer, lower layer

```
comm_grps = upper lower SOL
```
Lipid diffusion

- Frequently overestimated by an order of magnitude due to layer c-o-m motion!
- Can be corrected after simulations too, though

MSD (10x slower than H2O)
**Electron density**

- `g_density`

You need to provide electrons.dat with number of electrons/atom, see `-h` flag.

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The figure shows a graph of electron density (nm\(^3\)) against distance from the bilayer center (nm). The peaks in the graph indicate regions of higher electron density, which could correspond to areas of lipid packing or other dense molecular structures within the membrane. The x-axis represents the distance from the center of the bilayer, while the y-axis plots the electron density. The data is plotted with two curves, possibly indicating different conditions or phases of the membrane.
Order parameters

- **g_order** (requires index file with i-1, i+1)

\[ S_{CD} = \frac{3 \langle \cos^2 \theta \rangle - 1}{2} \]
Undulations

- Assign lipid z-coordinates to a grid
- Perform 2D Fourier transforms
- Plot amplitudes vs. wave vector magnitude
Example: cholesterol

(Cells can control membrane stiffness with cholesterol)
Undulations

Collective Membrane Motion

Single Lipid Motion
Membrane proteins

- Prepare structure without membrane
- Solvate protein in bilayer, e.g. with genbox
- Add solvent water
- Freeze protein for relaxation
  
  ```
  freezeigrps = protein
  freezeedim = y y y
  ```
- Avoid water entering between lipids and protein by restraining the water z coordinates
Water restraints

- Open the topology in a text file, and after the water #include line add:

```plaintext
; Position restraint for each water oxygen
[ position_restraints ]
;  i funct  fcx  fcy  fcz
  1   1   0   0   1000
```

- Waters are free in XY-plane, but z-restrained
- Works for lipids too in really bad system
- Equilibrate lipids ~10ns, then water too
- If water enters the membrane, remove it
- Multiple equilibration cycles can be necessary
Mixed lipid & protein FFs

- Berger force field does not include proteins. However, based on OPLS, so it mixes nicely with OPLS-AA/L (all-atom) for proteins!
- Charmm27 OK too (but 4-5x more expensive).
- Pure Gromos96, OPLS-AA/L, Amber, etc: constrain cell dimensions for reasonable lipid density & area - can still be OK if lipids are mainly passive solvent for your protein.
- The Berger force field performs equally well, if not better, compared to Charmm27.
- Hydrogens on chains only have low charge. No net charge - extremely weak dipoles!
- In principle the weak dipoles could aid the solvation of polar/charged groups.
- However, in practice the Berger force field mixed with OPLS-AA reproduce these experimental values very well too!
If anything, the solvation cost is slightly low compared to in vivo values!
United vs. All-atom - 2

- Nothing wrong with all-atom force fields
- For proteins in water the difference is negligible
- But not for membranes - significant performance boost with united atom model
- “Proof is in the pudding”
- Hard to justify 4x increased cost without any indication the results would be better
- Sampling still worse issue than FF details!
Summary

- Easier than proteins: 256-1024 lipids provide way better statistics
- Harder than proteins: Quite sensitive to force field details, less work done
- Very slow processes (>100ns)
- Interesting non-equilibrium biology: ion transport, insertion, vesicle formation, etc.
- Membranes scale great (>1000 CPUs in CVS)