Computational simulation for structural stability and formation of DOPC and DPPC lipid vesicle using coarse-grained simulation with Martini force field

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Who we are…

INDUSTRIAL PHARMACY LABORATORY

Under the directorship of Dr. Pardeep Gupta, the Industrial Pharmacy Laboratory (IPHL) at Saint Joseph's University is a full-scale research facility serving the needs of the current drug pipeline, including small molecules and proteins.

A catalyst of scientific progress, IPHL is a GLP-compliant pharmaceutical research facility offering complete **drug delivery research services**. The laboratory is fully equipped to support analysis, preformulation, and formulation research. IPHL scientists collaborate to overcome formulation and manufacturing challenges, streamline processes to increase cost-effectiveness, and develop product specific analytical methods for an array of different clients.

Recent projects include complete preformulation and formulation of oral solid dosage forms, formulation and supply of preclinical samples, formulation of a liposome based IV product, lyophilization cycle development, and development of a filtration method for a difficult to filter product.

Funded by the Burroughs Wellcome Fund and the Philadelphia College of Pharmacy, IPHL occupies 4,500 square feet of state-of-the-art space in the historic Griffith Hall.

Our Team

Kamal Jonnalagadda, PhD
Professor
Director of Pharmaceutics Graduate Program

Jasmin D. Monpara
Assistant Director, IPhL
Pharmacy Practice and Administration

Rijo John
Research Scientist/ Postdoctoral Associate
Pharmaceutical Sciences

Equipment

- Single and multi-station tablet presses–B and D tooling
- V Blender
- High shear granulators
- Fluid bed coater and Vector pan coater
- Retsch mill, jet mill, and Quadro Comil for micronization and nano-sizing
- Buchi spray dryer
- Fully programmable VirTis lyophilizer
- 3-D printer
- Mini extruder
- Carver press
- Sonifiers and homogenizers
- Regular and ultra-centrifuges
- Ultra low freezers for sample storage

Characterization

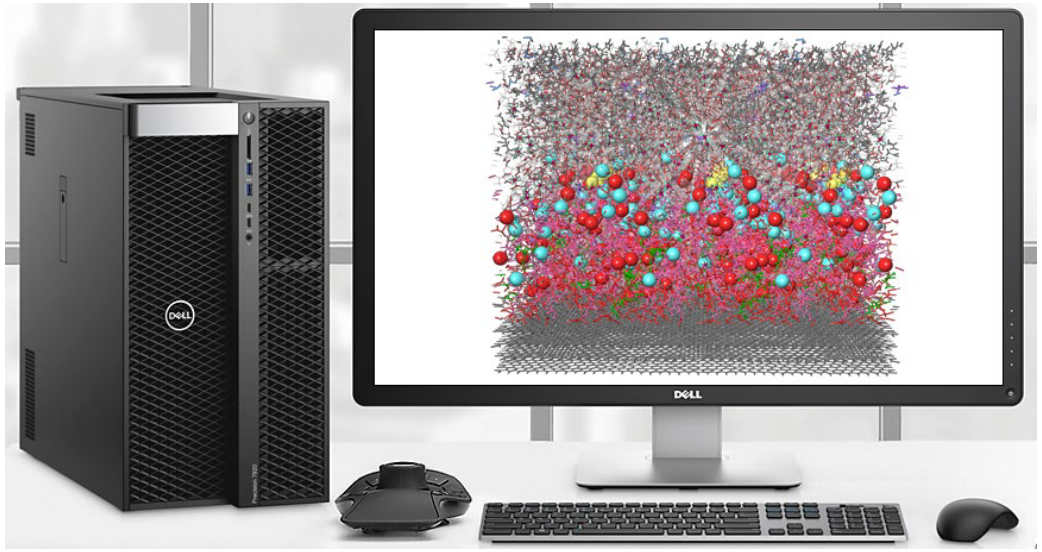
- FTIR
- Brookfield viscometers
- Malvern Zetasizer and Mastersizer
- VanKel tap density
- Autograph for tensile, compression, impact, and hardness measurement
- Tablet hardness, friability, and disintegration
- Microscopy and scanning electron microscopy (SEM)

Analysis

- Agilent, Waters and Shimadzu HPLC systems with DAD and fluorescence detectors
- LCMS with triple quad MS capability
- Automated ELISA equipment
- SDS-PAGE electrophoresis
- Dissolution studies
- Stability studies
- KF and IR moisture analysis

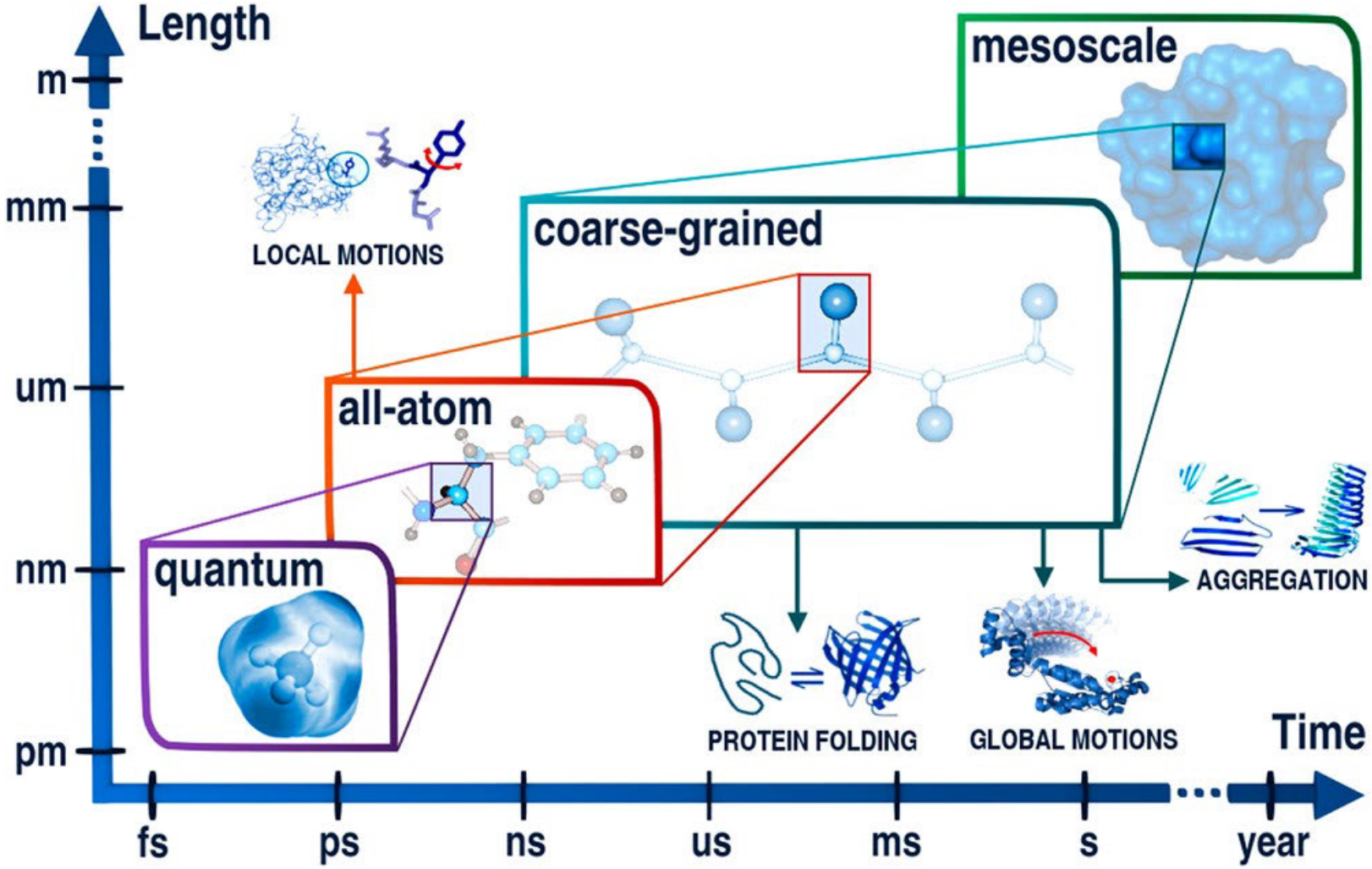
- DSC and mDSC
- TGA
- ITC
- Hot stage microscopy

- Biotek Plate reader
- Circular dichroism spectroscopy
- UV spectrophotometer



- 2X Nvidia A6000 (48GB GDDR6) (10,752 NVIDIA® CUDA® Cores)
- 2X Intel Xeon (40 CPU cores)
- Schrodinger® Materials Science Suite

Computational approach to understand physical systems



Ref: *Chem. Rev.* 2016, 116, 14, 7898−7936

Available tools for in-silico studies

Software packages

FAST. FLEXIBLE. FREE.
GROMACS

NAMD
Scalable Molecular Dynamics

LAMMPS
Large-scale Atomic/Molecular Massively Parallel Simulator

Schrödinger

Force-fields

- GROMOS
- OPLS-AA
- OPLS-3
- CHARMM
- AMBER
- CMM-CG (used by LAMMPS)
- MS-CG (Used by Schrodinger)
- MARTINI (General Purpose)

Objectives of the current study

- ❑ To simulate different lipid-based compositions
  - Using two model drugs (Amphotericin and Doxorubicin)
  - With three different lipids with varying characteristics (DPPC, DOPC and DSPG)
- ❑ To understand the potential for self assembly of the systems and effect of charge-based interactions in self-assembly
- ❑ Coarse-grained simulation of microsecond timescale to capture self-assembly events

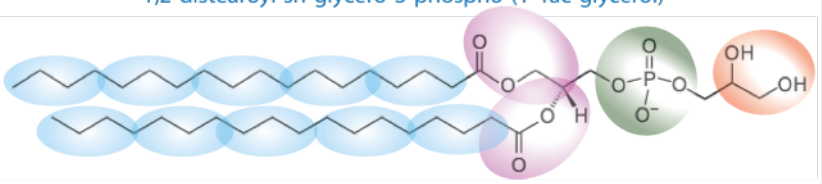
Application

- ❑ Validated model can potentially be used in early stage of drug development where availability of material is scarce

Coarse graining of molecules using Martini force field

DSPG

1,2-distearoyl-sn-glycero-3-phospho-(1'-rac-glycerol)

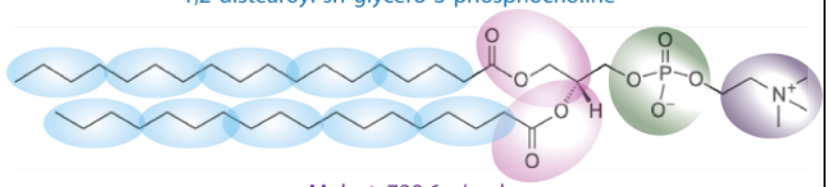


Mol wt: 800.5 g/mol
Tg: 55 °C

C1	$CH_3-(CH_2)_2-CH_3$	butane (C1)
Na	$CH_3-C(=O)-O-CH_3$	methyl-acetate (N4a)
Q_1	Charged particle	
P1	$(CH_3)_2CH-OH$	isopropanol (P1)

DSPC

1,2-distearoyl-sn-glycero-3-phosphocholine

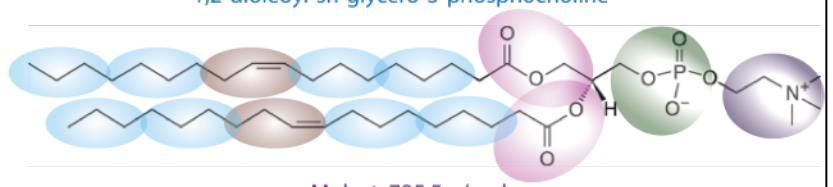


Mol wt: 789.6 g/mol
Tg: 55 °C

C1	$CH_3-(CH_2)_2-CH_3$	butane (C1)
Na	$CH_3-C(=O)-O-CH_3$	methyl-acetate (N4a)
Q_0	Charged particle	
Qa	Charged particle	

DOPC

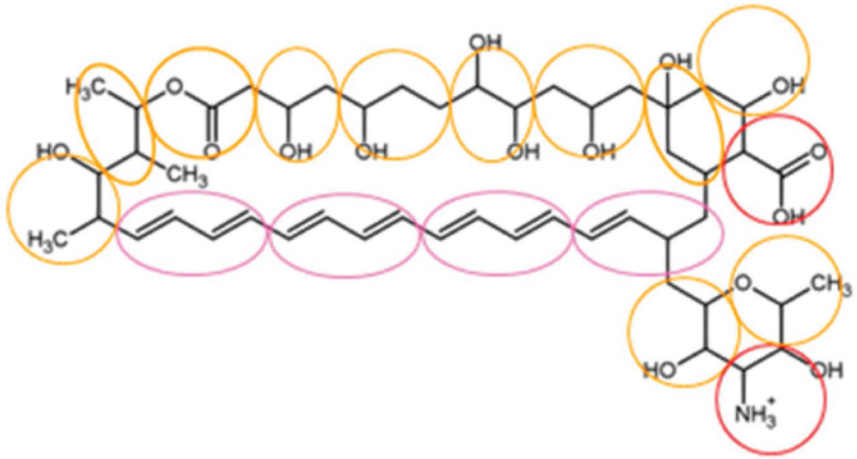
1,2-dioleoyl-sn-glycero-3-phosphocholine

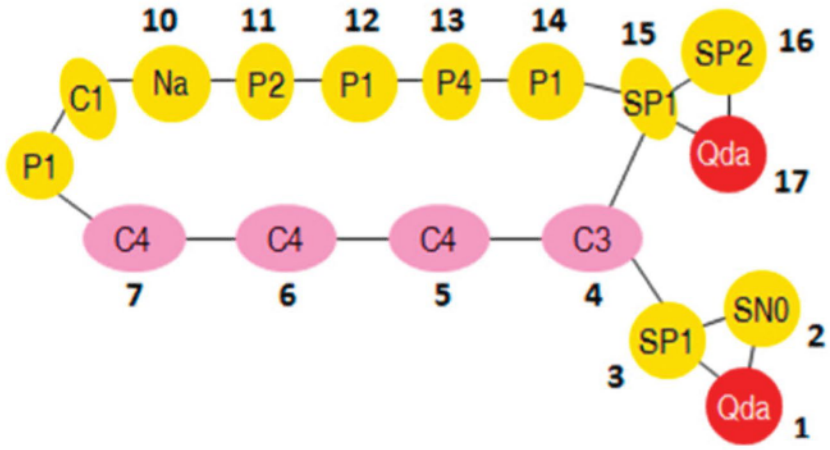


Mol wt: 785.5 g/mol
Tg: -17 °C

C1	$CH_3-(CH_2)_2-CH_3$	butane (C1)
C4	$CH_3-CH=CH-CH_3$	1-butene (C4)
Na	$CH_3-C(=O)-O-CH_3$	methyl-acetate (N4a)
Q_0	Charged particle	
Qa	Charged particle	

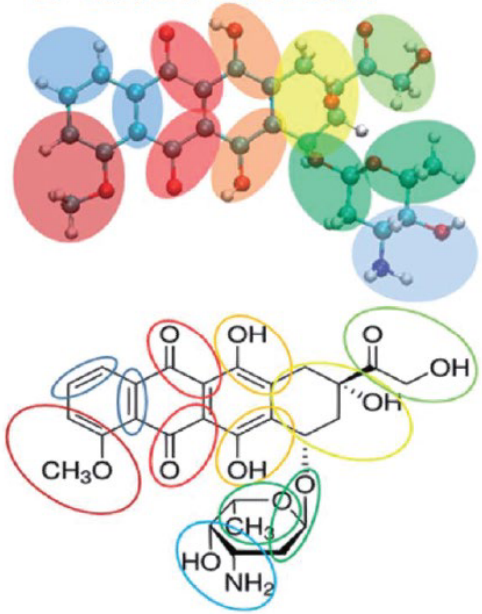
Amphotericin-B





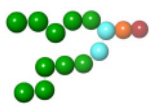
Ref: Mobasheri, Meysam, et al. "Solubilization behavior of polyene antibiotics in nanomicellar system: insights from molecular dynamics simulation of the amphotericin B and nystatin interactions with polysorbate 80." Molecules 21.1 (2015): 6.

Doxorubicin



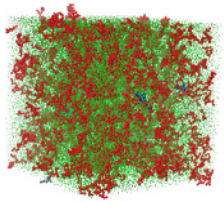
Ref: RSC Adv., 2020, 10, 3233

Approach



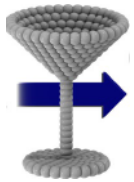
Mapping

- Beads were mapped to structures of lipids and Drugs
- 3-4 heavy atoms were mapped to a single bead following Martini-3 protocol



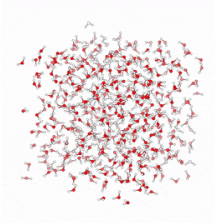
Disordered system

- Disordered system was prepared using
  - 5 drug molecules
  - ~901 lipid molecules
  - 60311 molecules of water (containing 10% anti-freeze water)



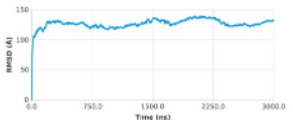
Force field assignment

- Martini Force field was assigned to the mapped particles in disordered system



Simulation

- Brownian minimization followed by Martini MD simulation
- NPT ensemble, Temperature 300K, Pressure 1 bar
- Time step 10 fs, Simulation time 3000 ns

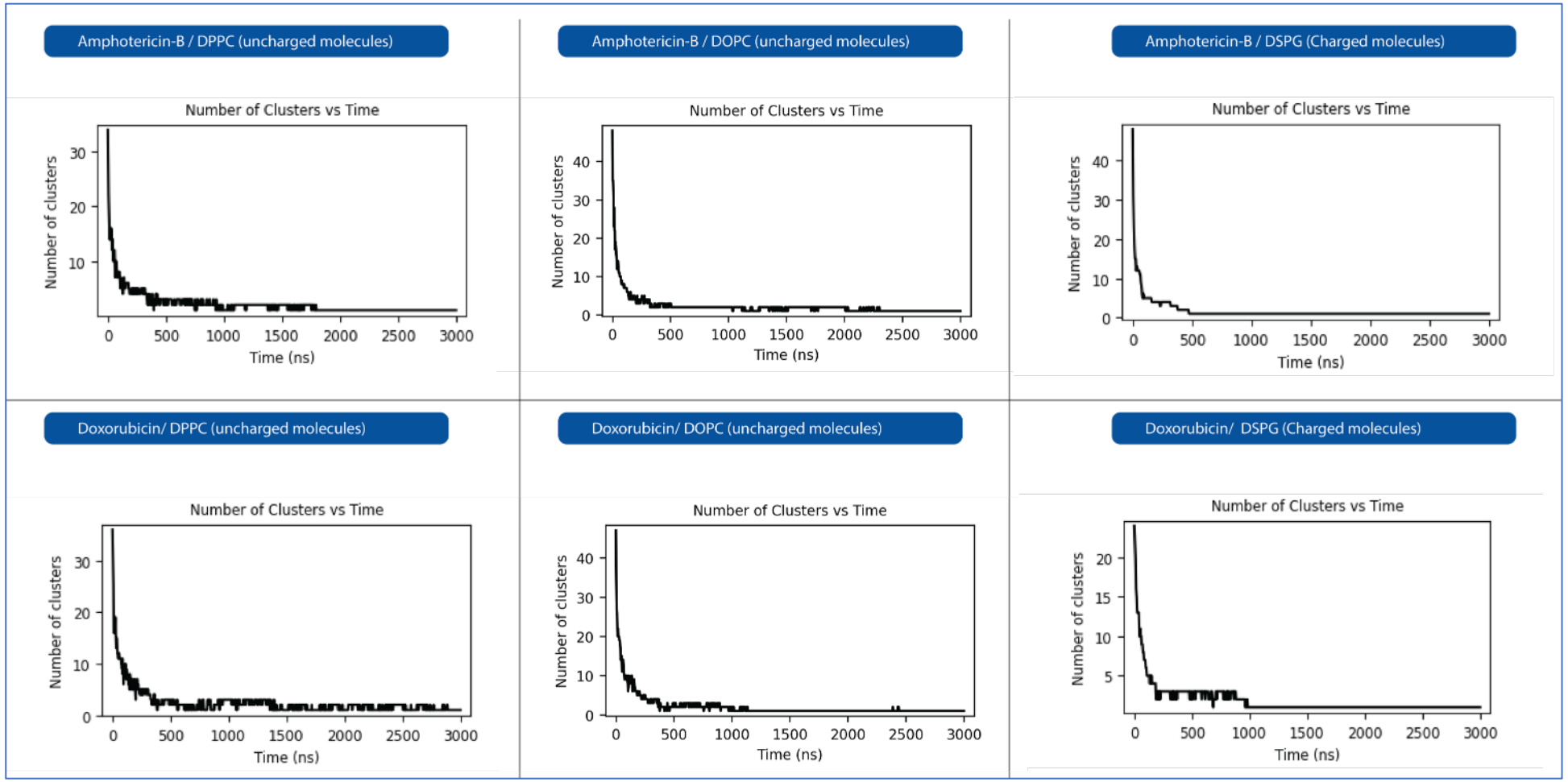


Trajectory analysis

- Analysis of Root mean squaredeviation, Radius of gyration, Cluster analysis and Radial distribution function

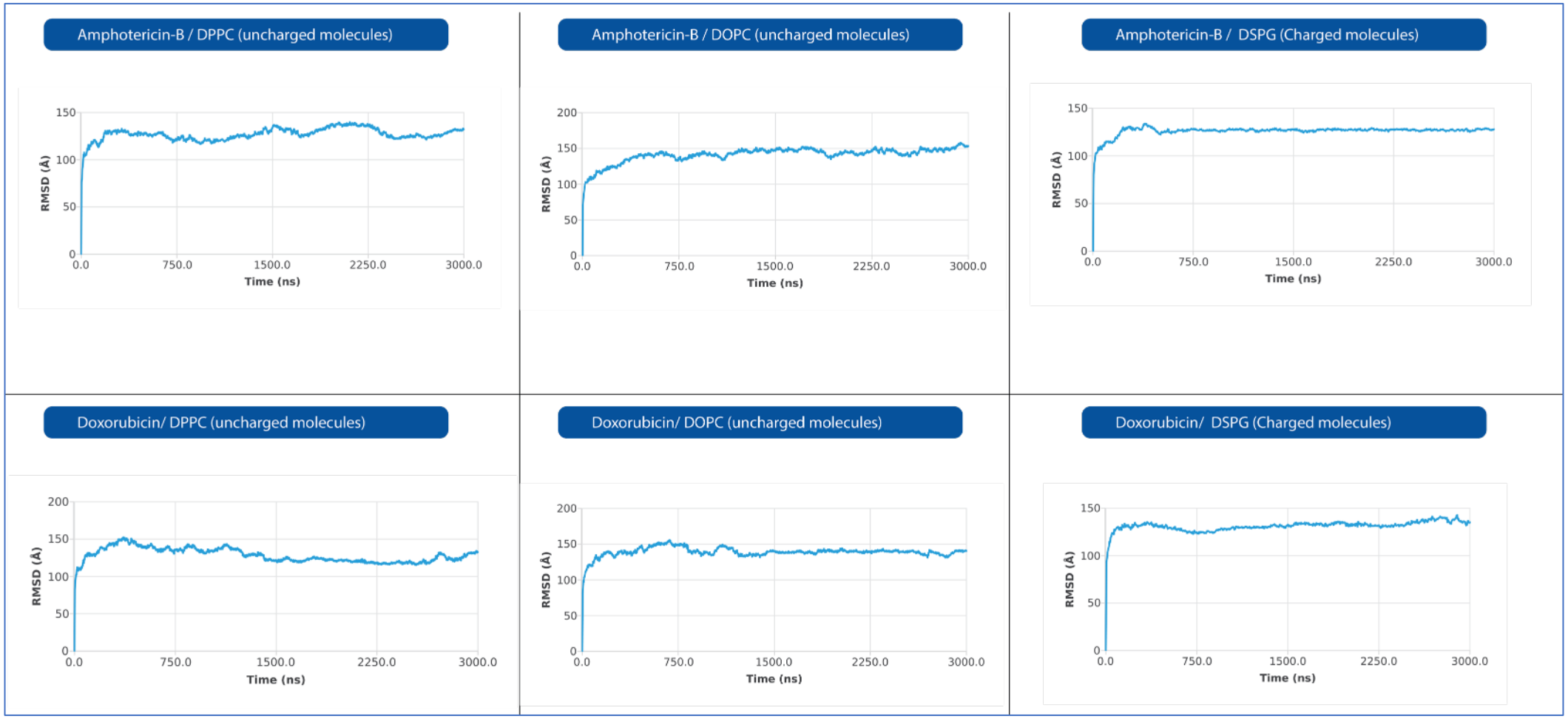
Cluster Analysis

- The number of clusters of liposome model structure indicates the self-assembly and aggregation of the lipids.
- Initial high clusters are due to disordered lipids which are merged to make a closed liposome model at the end of the simulation
- DPPC/DOPC/DSPG lipids spontaneously aggregated, and then they collide together and form plate-like and disc-shaped membranes.
- Aggregation to form a single large cluster of lipid vesicle was at different time scale for DPPC/DOPC/DSPG lipids.



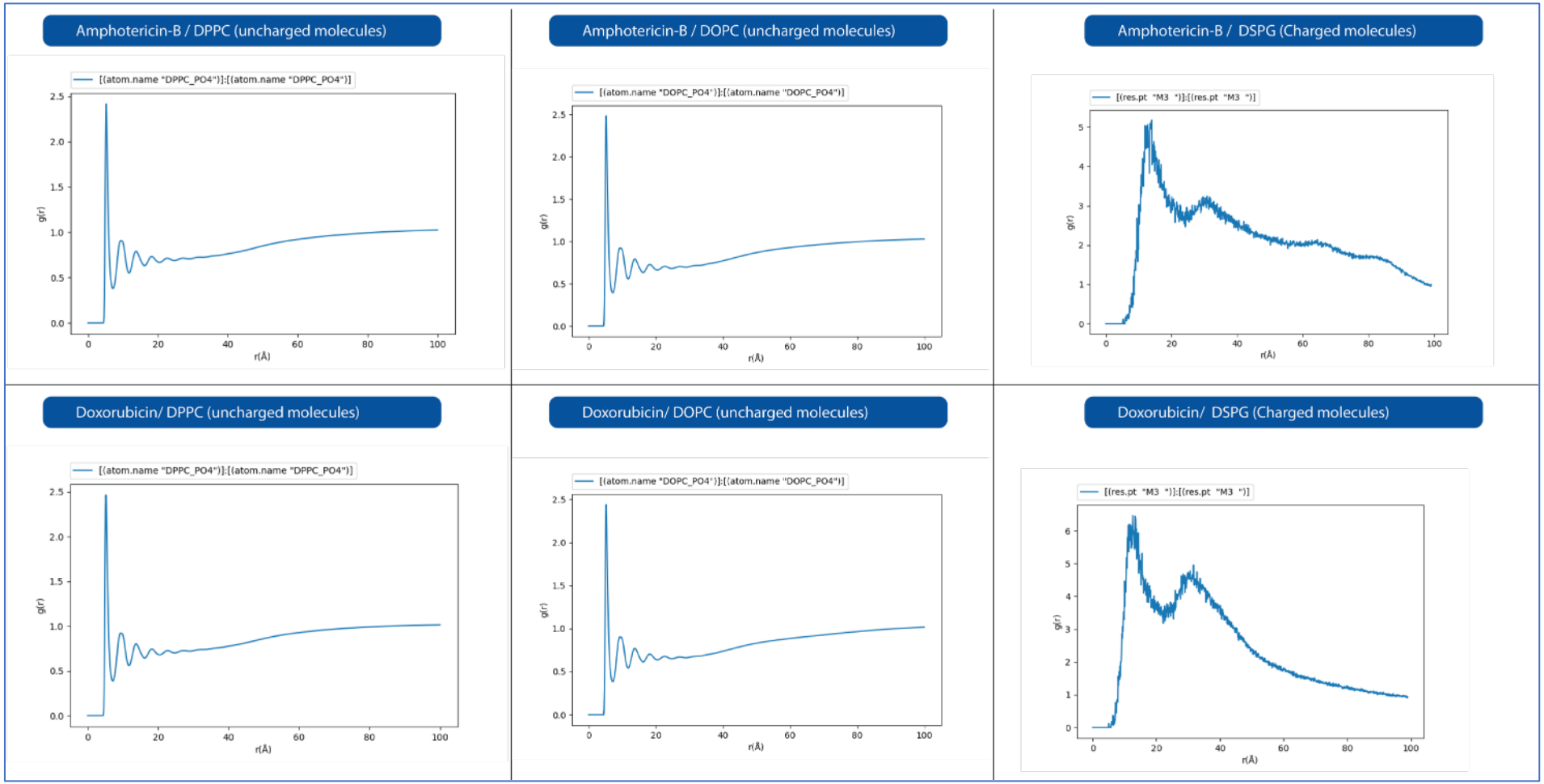
Root Mean Square Deviation (RMSD)

- RMSD value describes the average distance between each atom in the system at a specific time
- A molecule with an RMSD value that does not change much over time indicates a stable molecular conformation.
- The structural stability of the systems during simulation is suggested by the converging curve of RMSD
- It is evident from the simulation that the time required for attaining stable structural formations is around 1 nanosecond, after which RMSD reaches a constant value of 100–150 Å ensuring stable structural formation for the lipids



Radial Distribution Function (RDF) (-PO4 group of the lipids)

- RDF is calculated around a (set of) atom(s) or around the center of a mass of a set of atoms or molecules
- The radial distribution function (RDF) calculated for lipids around the center of mass confirmed that the 3000 ns time scale is appropriate for the formation and equilibration of the liposomal model for DOPC/DPPC phospholipids, while RDF for the charged DSPG does not attained equilibrium



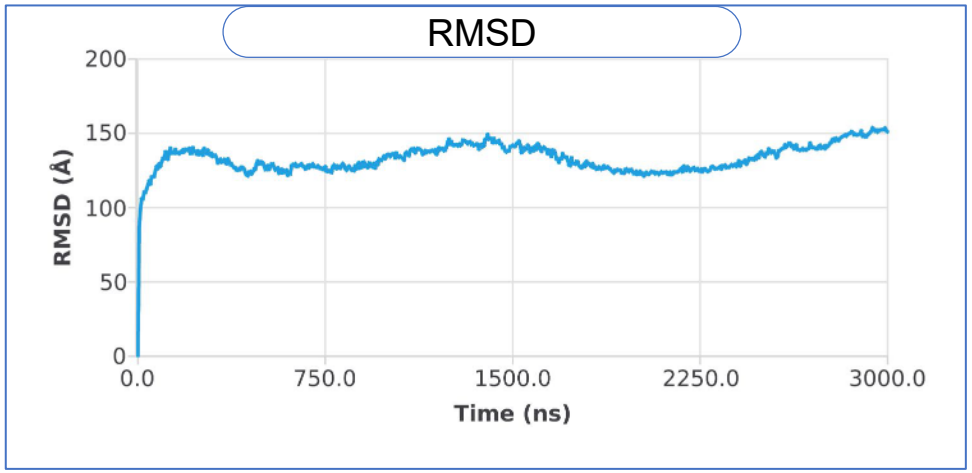
Radius of Gyration

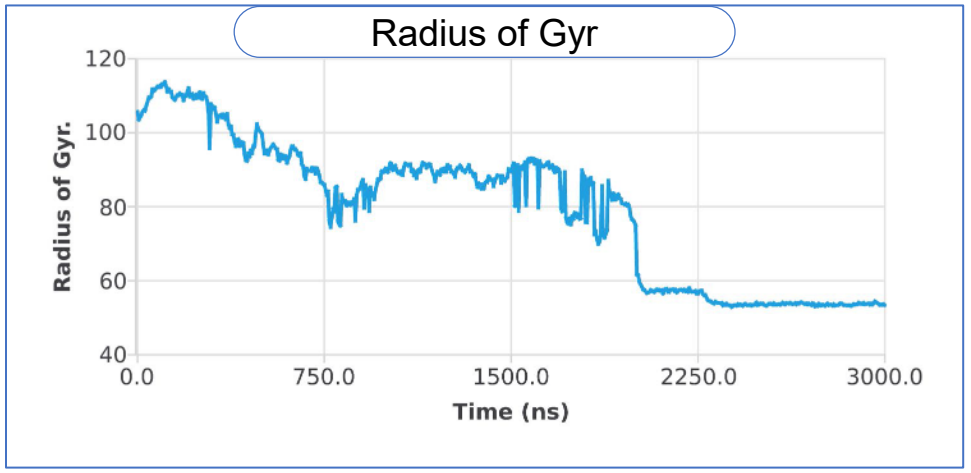
- The radius of gyration of phospholipid molecules is the square of mean distance of all atoms of the DOPC/DPPC/DSPG molecules from the axis of rotation of phospholipid molecule
- The decrease in Rg confirms the formation of elliptical and closed liposomal models with both lipids. Gyration radius of the DPPC/DOPC depicted a sudden decrease, suggesting structural transformation, whereas such a sharp drop was not observed with the DSPG lipids.

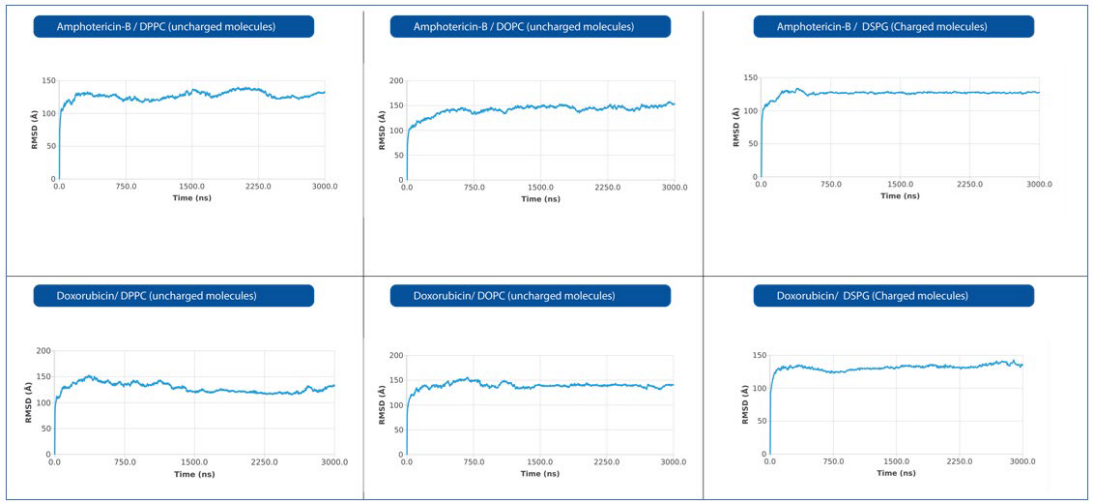
Ambisome® Formulation

System composition	
Component	# of molecules
DSPG$^{(-)}$	230
HSPC	560
Cholesterol	111
Amphotericin B$^{(+)}$	5
Water	66342

Ambisome® Formulation

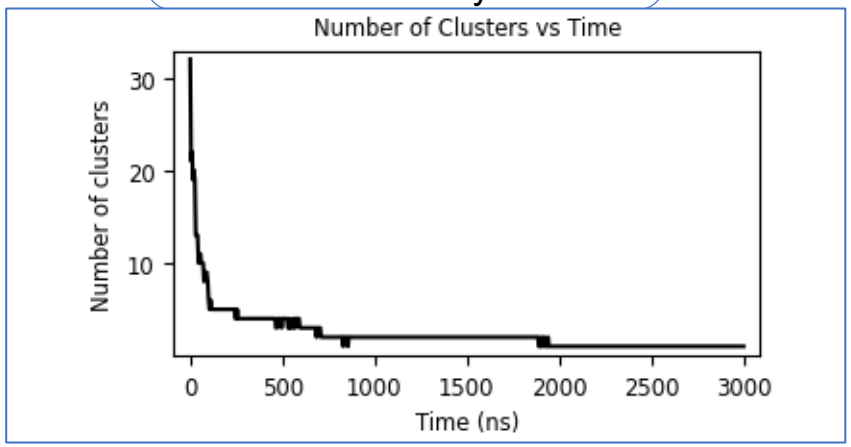




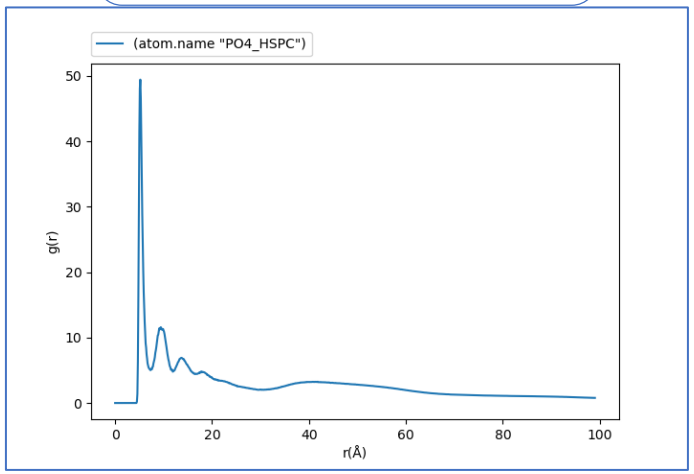


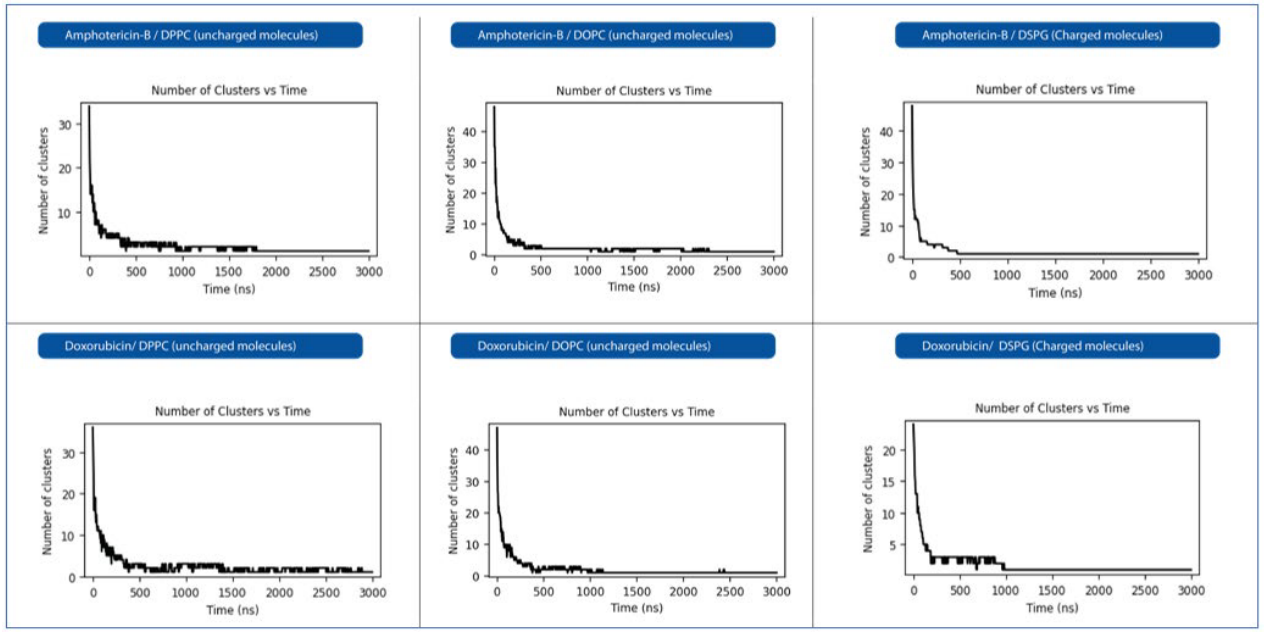
Ambisome® Formulation

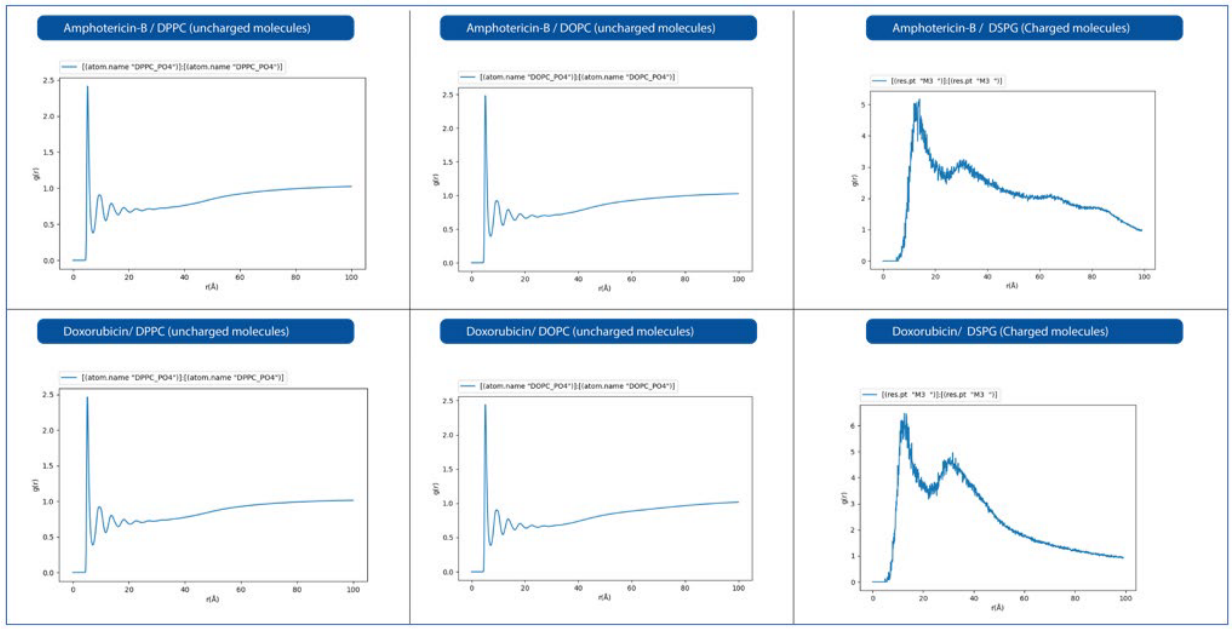
Cluster analysis



RDF







Key observations

- Coarse-grained MD simulation demonstrated the formation of stable self-assembled structures at 3000 ns with the phospholipids
- DPPC containing systems self-assembled into lamella but did not form a vesicle
- DOPC-containing systems self-assembled into vesicles of ~6-7 nm radius
- DSPG-containing systems showed drug-dependent behavior, the one with Amphotericin-B self-assembled into a worm-like lamellar structure while the one with Doxorubicin exhibited disc-like bilayer at the equilibrium
- The analysis of RDF, gyration radius, cluster analysis, and RMSD analysis indicates clear vesicle formation with DOPC compared to DPPC/DSPG.

Conclusion

- These simulations serve as a quantitative application of mathematical principles and provide a complementary approach for investigating the structural mechanisms and stability of lipid vesicles.
- The future studies need to include complete formulation components (i.e. helper lipids, cholesterol, buffers etc. for better understanding of the formulation.)

Thanks for your time and patience !!

Q&A