Membrane selectivity of an antimicrobial peptide using molecular dynamics

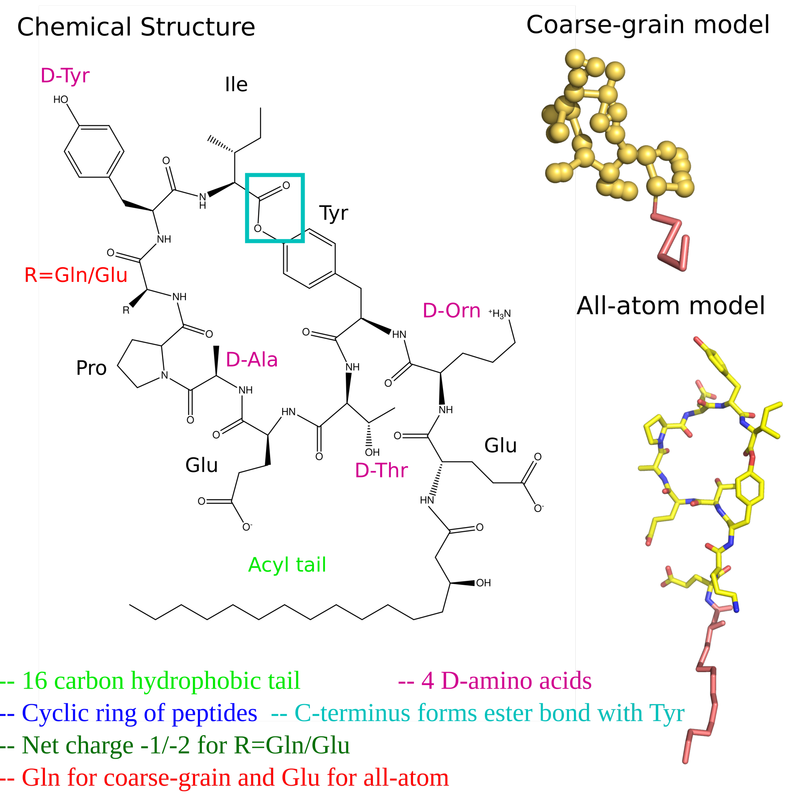
Sreyoshi Sur[1], Tod D. Romo[1], Alan Grossfield[2]
[1]University of Rochester, NY, USA
[2]University of Rochester Medical School, Rochester, NY, USA

Poster PDF http://bit.ly/2CiNv9V

Abstract

Fengycin is a cyclic lipopeptide synthesized by bacteria in the Bacillus family as an immune response against fungus, where it attacks and disrupts the cell membrane. Although it is in use as an agricultural funcide, its mechanism is not well understood. One hypothetical mechanism suggests that lipopeptide aggregation disorders lipids and ultimately results in membrane rupture. Here, we use weighted ensemble molecular dynamics simulations coupled to all-atom and coarse-grained models to quantitate these effects. We show that electrostatic and hydrophobic interactions between lipid head groups and fengycin's peptide part play a key role in the formation of aggregates. This may in turn determine fengycin's membrane selectivity. We also demonstrate that cholesterol slows the binding of fengycin to zwitterionic membranes and alters its propensity to aggregate once bound. This suggests a pair of mechanisms by which fengycin distinguishes between fungal and mammalian membranes.

Fengycin



-- 16 carbon hydrophobic tail -- 4 D-amino acids
-- Cyclic ring of peptides -- C-terminus forms ester bond with Tyr
-- Net charge -1/-2 for R=Gln/Glu
-- Gln for coarse-grain and Glu for all-atom

All-atom simulation Details

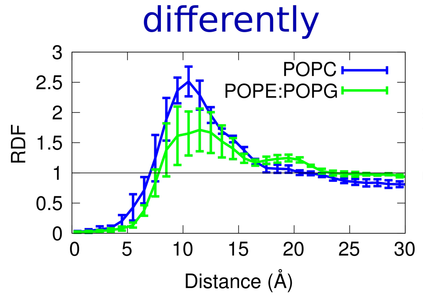
- 100 mM NaCl
- POPC(fungal), POPE:POPG (2:1) (bacterial) membrane models
- 90 lipids and 10 fengycins per leaflet
- 7,500 waters (TIP3P water) and 50,000 total atoms per system
- POPG has -1 charge
- Box size: 90 Å x 90 Å x 70 Å
- Forcefield: CHARMM36
- Ensemble: NPT
- Langevin 310 K, 1 bar
- Electrostatics: PME
- VDW cutoff: 10 Å
- Timestep: 2 fs (RATTLE)
- Software: NAMD 2.9 on BlueGene/Q

System	# of atoms	Time(μs)
POPC	~49000	~4.5x4
POPE:POPG	~49500	~5x4

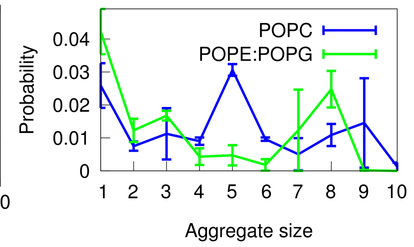
Analysis done in LOOS (Lightweight Object Oriented Structure analysis library), an open source C++ library designed and maintained by the Grossfield lab for designing analysis tools.
http://loos.sourceforge.net
https://github.com/GrossfieldLab/loos

Lipid-dependent aggregation

Fengycin aggregates differently



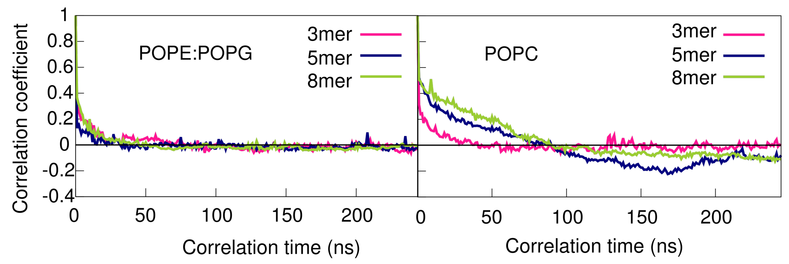
Aggregate size probability distribution



-- Aggregation stronger in POPC -- 5mers and 9mers found in POPC
-- Second peak in POPE:POPG -- 8mers mostly in POPE:POPG

Apparent contradiction?

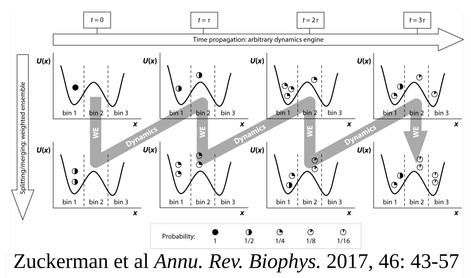
Lifetime of aggregates



-- POPE:POPG: all aggregates are short lived
-- Large aggregates survive longer in POPC
-- Higher order structures in POPE:POPG are not real aggregates
-- Collision between small aggregates shows up as larger aggregates in POPE:POPG

Sur et al. *J. Phys. Chem. B*, 2018, in Press

Weighted Ensemble (WE) Dynamics



Zuckerman et al *Annu. Rev. Biophys.* 2017, 46: 43-57

x = Progress coordinate
Here:
Fengycin-fengycin contacts (C_{AB})

$$C_{AB} = \sum_{i \in A} \sum_{j \in B} Sij$$

Number of contacts between center of mass distance r_{ij} (S_{ij})

$$S_{ij}(r_{ij}) = \frac{1}{1 + (\frac{r_{ij}}{r_0})^6}$$

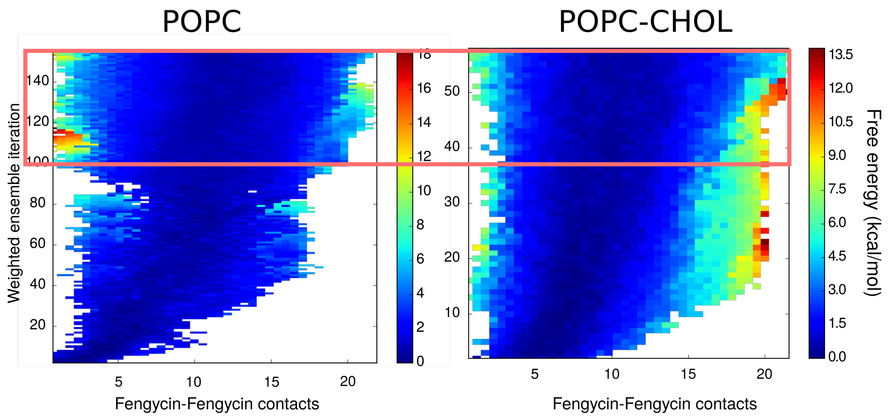
-- Multiple trajectories started with equal weights
-- Reaction coordinate/s selected and divide it's range into bins
-- Every τ seconds analyze the reaction coordinate values
-- Determine position of trajectories in the bin space
-- Trajectories are split or merged and their weights equally distributed if the total trajectories in a bin exceed certain threshold
-- Unbiased molecular dynamics

System	# of CG beads	Cumulative Time(μs)
POPC	30243	~88
POPC:CHOL	29955	~58

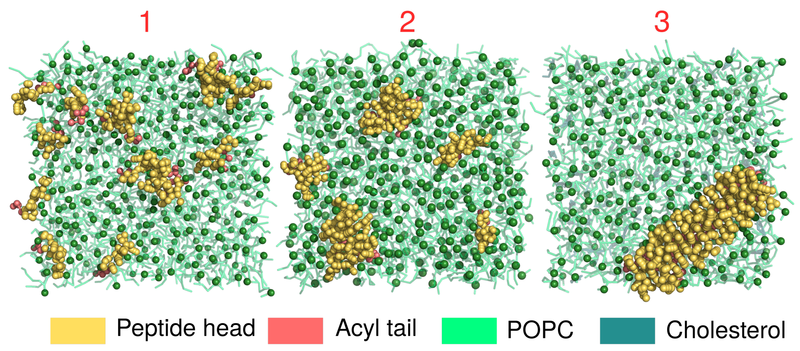
WE parameters	Values
τ	5 ns
# of trajectories	12
No. of bins	25

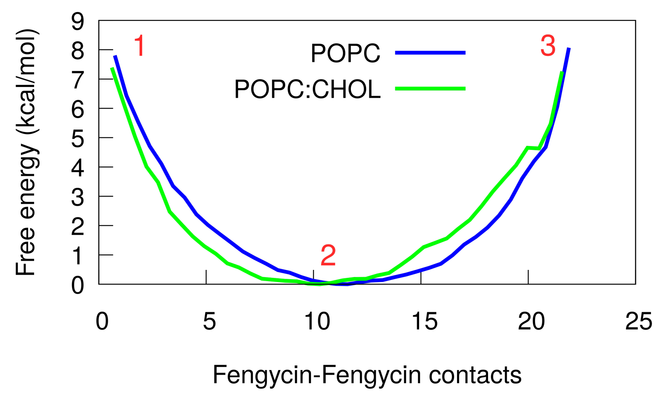
Weighted ensemble simulations are done using WESTPA (The Weighted Ensemble Simulation Toolkit with Parallelization and Analysis) :
https://github.com/westpa/westpa

Aggregation Free energy



-- Fengycin bound to POPC/POPC:CHOL membrane
-- Fengycin contacts ↑ Aggregation ↑
-- Minima: moderate number of aggregates





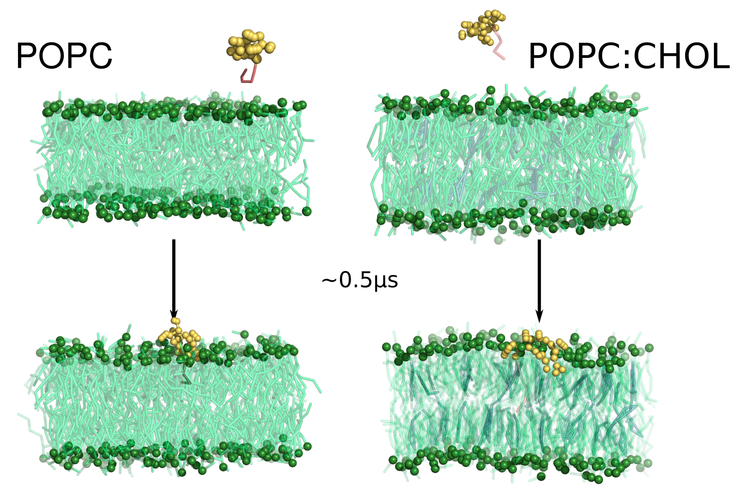
-- Suggests cholesterol favors less aggregation
-- Results are not converged at high contact values

Coarse-grain simulation Details

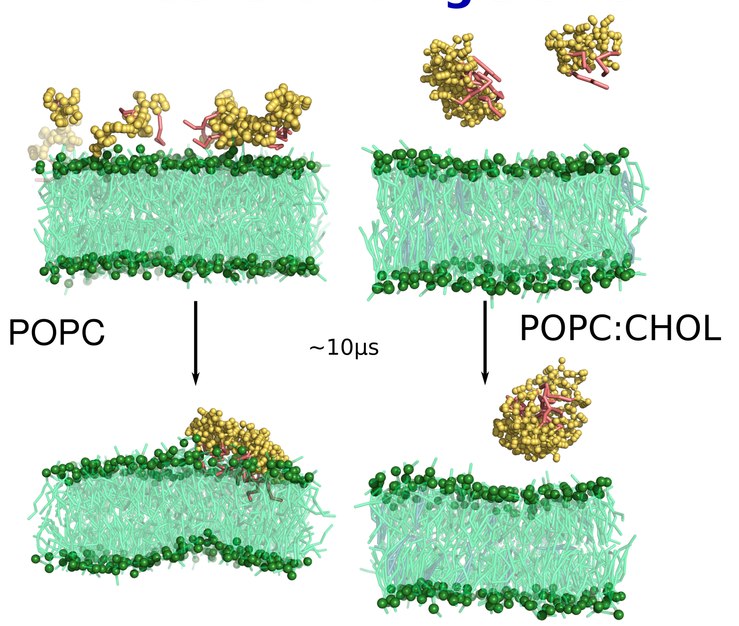
- 150mM NaCl
- POPC (fungal), POPC:CHOL(5:1) (bacterial) membrane models
- ~144 lipids per leaflet and 10 fengycins on one leaflet
- ~4500 waters per system (Polar water model)
- 4 atoms: 1 bead mapping
- Box size: 90 Å x 90 Å x 110 Å
- Forcefield: MARTINI version 2.0
- Ensemble: NPT
- Nose-Hoover thermostat 300 K
- Parrinello-Rahman barostat 1 bar
- Electrostatics & VDW: Shift function
- VDW cutoff: 10 Å
- Timestep: 20 fs (LINCS)
- Software: Gromacs 5.0.6 and 2016.1 on BlueHive 2

System	# of CG beads	Time(μs)
POPC	21076	~27 x 6
POPC:CHOL	16756	~39 x 6

Monomer binds spontaneously



Micelle binding slower



-- Binding occurs in POPC at multi-microsecond scale
-- Bends POPC
-- Remains in aggregated state after binding
-- Fengycin induces positive curvature
-- Cholesterol prevents fengycin micelle binding
-- Membrane bends to avoid binding
-- Fengycin induces negative curvature

Conclusions

-- Aggregation depends on membrane composition
-- Larger aggregates more stable in POPC
-- All aggregates short lived in POPE:POPG
-- Fengycin does not bind to cholesterol rich membrane
-- Single fengycin bind to membrane with or without cholesterol
-- Free energy curves vary with lipid in membrane

Future directions

-- Micelle binding free energy
-- Comparison of binding energy of surfactin and fengycin
-- Free energy of micellization using all-atom weighted ensemble simulations

Acknowledgements

Department of Chemistry
University of Rochester
Grossfield Laboratory