Effects of cholesterol on fengycin, an antimicrobial lipopeptide, using weighted ensemble method

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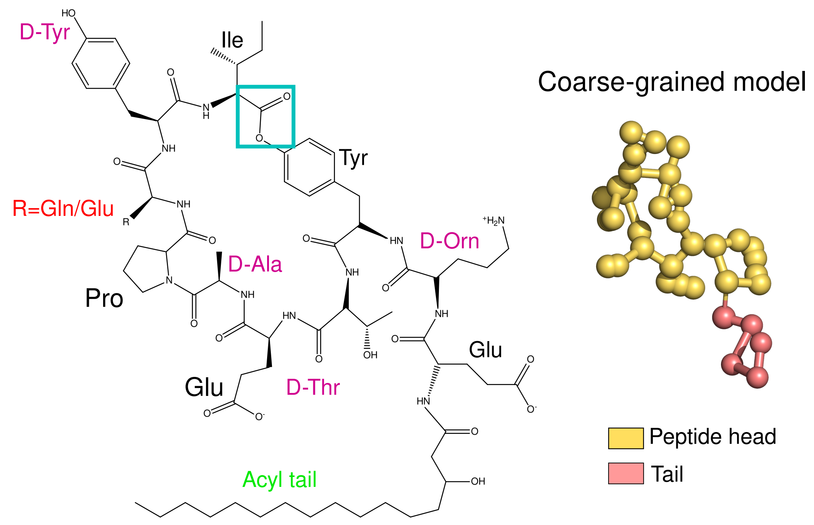
Abstract

Fengycin is an antimicrobial lipopeptide synthesized by the bacteria *Bacillus subtilis* and commercially available as an agricultural fungicide. One of the ways fengycin kills fungal cells is by binding and damaging their cell membranes. Previous all-atom simulations suggested aggregation of lipopeptides is the first step that leads to membrane deformation. Here we attempt to explain fengycin's selectivity for fungal over mammalian cells by examining the effects of cholesterol on its aggregation. Using a weighted ensemble path sampling method, we found that cholesterol compacts the aggregate size distribution. Even though we observed disordering of lipids right next to fengycins, cholesterol-rich membranes rebound to a more ordered structure away from the lipopeptides. We also found that cholesterol does not affect binding of a single fengycin molecule to the membrane. Our preliminary results of fengycin micelle binding to membrane show intermediate states for cholesterol-rich membranes. The results will help us understand the implications of cholesterol on fengycin's fungicidal properties and possibly indicate whether fengycin can be a potential drug candidate.

Fengycin

Chemical Structure



Coarse-grained model

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

Research questions

1) How does cholesterol affect a single fengycin binding to the membrane?
2) Does cholesterol hinder micelle binding to the membrane?
3) How does cholesterol alter the aggregation of fengycins on membrane surface?

System details

Systems	Simulation	# of atoms	Time	Fengycins	Membrane	Replicates	CPU time
1	Weighted ensemble	~42000	~50 iterations	10 as micelle	POPC and POPC:CHOL	1 each (running)	3 months
2	Weighted ensemble	~20000	~200 iterations	1 in water	POPC and POPC:CHOL	1 each	8 months
3	Weighted ensemble	~30000	~400 iterations	15 membrane bound	POPC and POPC:CHOL	3 each	14 months
4	Brute force	~20000	~90 µs	10 as micelle	POPC and POPC:CHOL	4 each	6 months

Simulation details

-- 150 mM NaCl
-- POPC (fungal), POPC:CHOL(5:1) (mammalian) membrane models
-- ~144 lipids per leaflet and 15/10 fengycins on one leaflet
-- ~13000 waters per system (Polar water model)
-- 4:1 bead mapping
-- Box size: 115 Å x 115 Å x 115 Å
-- Forcefield: MARTINI version 2.0
-- Ensemble: NPT
-- V-Rescale thermostat 300 K
-- Parrinello-Rahman barostat 1 bar
-- Electrostatics & VDW: Reaction field & Verlet
-- VDW cutoff: 10 Å
-- Timestep: 20 fs (LINCS)
-- Software: Gromacs 2016.3 on BlueHive 2

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.
https://github.com/GrossfieldLab/loos

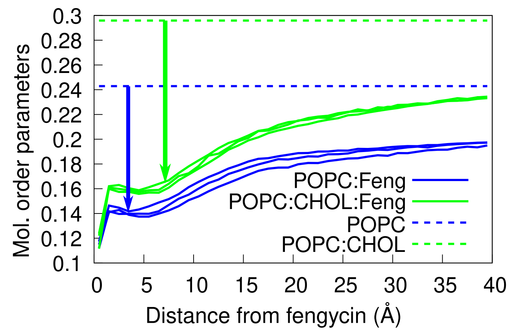
Does cholesterol change aggregation of fengycins?

Aggregation causes membrane disorder

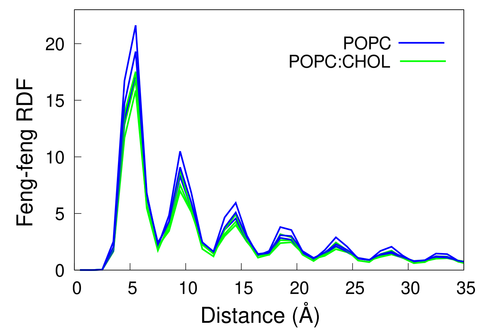
-- Molecular order parameter

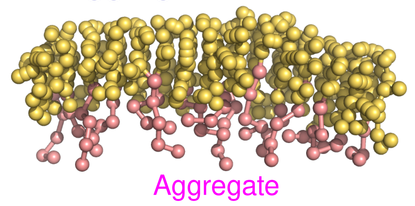
$$S_{mol} = \left\langle \frac{3}{2}(cos^2\theta - 1) \right\rangle$$

-- θ = avg. angle between 2nd and 3rd principal axes w.r.t membrane normal
-- $S_{mol} \propto$ Chain order
-- Fengycins disorder the chains
-- Order rebounds for cholesterol-rich membranes



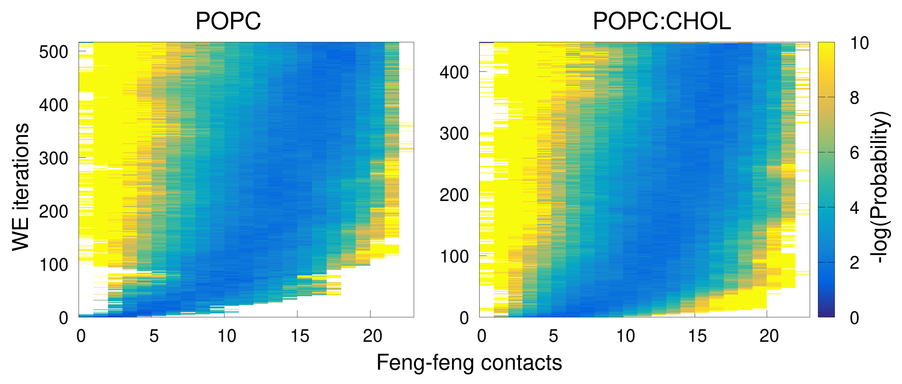
Fengycin forms linear aggregates





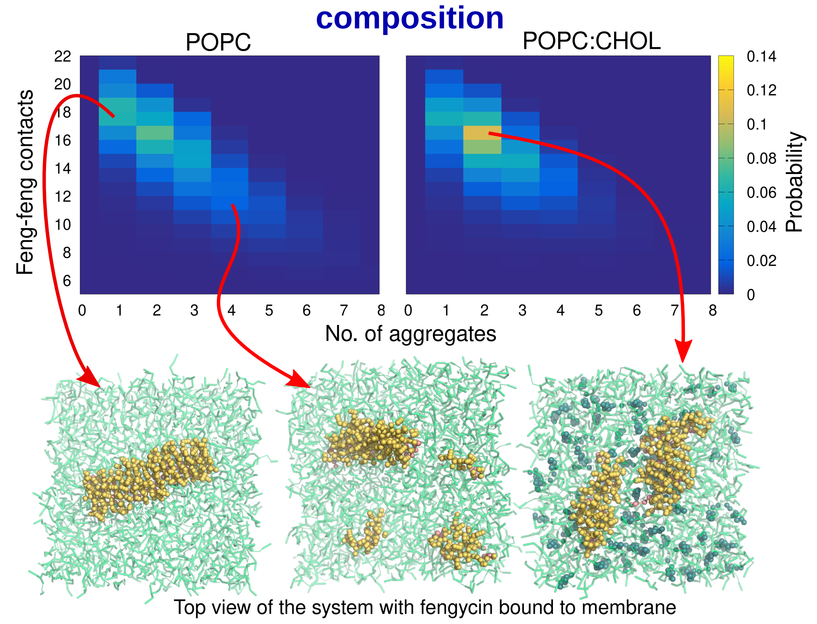
-- **R**adial **d**istribution **f**unction is probability distribution where 1-> random distribution
-- Equidistant peaks suggest linear structure
-- Linear aggregates not seen in all-atom simulations
-- Aggregation reduced in cholesterol-rich membrane

Problem: System not yet converged



-- Free energy distribution w.r.t each WE iteration
-- Minima for free energy still changing in POPC
-- Slow convergence for progress coordinate

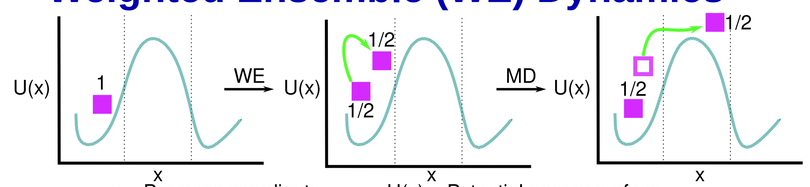
Distribution of aggregates depend on membrane composition



Top view of the system with fengycin bound to membrane

-- Pairwise contact comparison determine whether two lipopeptides are part of an aggregate
-- The number of aggregates and fengycin-fengycin contacts together estimate the size of aggregates
-- Cholesterol restricts the aggregate distribution

Weighted Ensemble (WE) Dynamics



-- Atom-atom contacts between fengycins/lipids

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

-- Fengycin-fengycin/lipid contacts

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

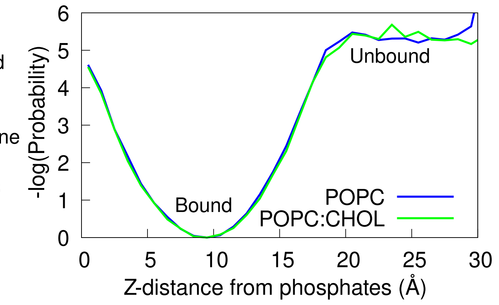
-- Trajectories are split or merged and their weights redistributed

Adapted from Zuckerman et al Annu. Rev. Biophys. 46;43-57

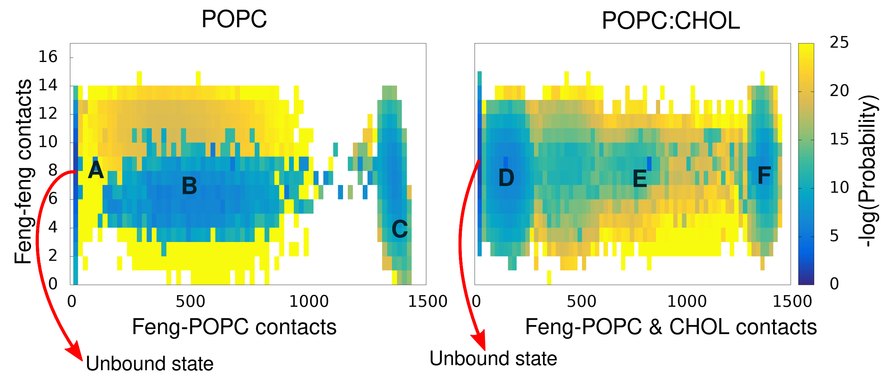
System	Fengycin bound to membrane	Micelle binding to membrane
τ(ns)	5	4
Max # of traj in bin	5	6
Progress coordinates	Feng-Feng contacts	Feng-feng contacts Feng-lipid contacts
No. of bins	50	1600

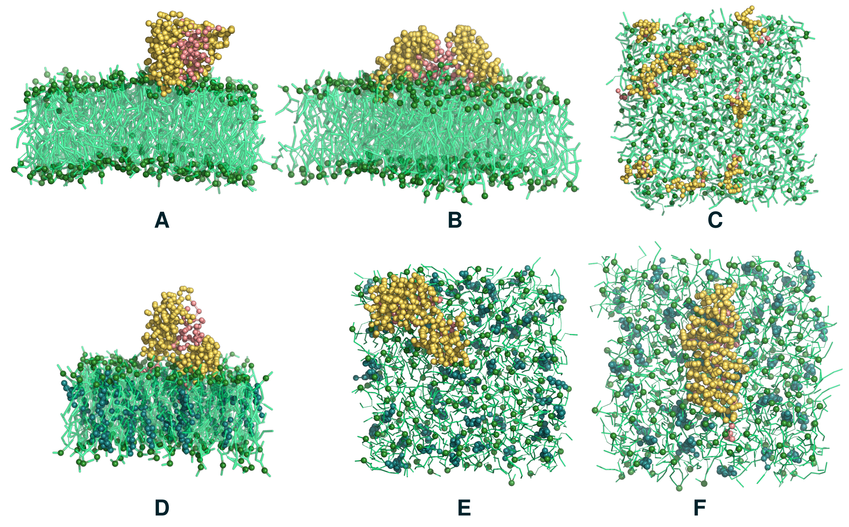
Monomer binds non-selectively

-- Distance between fengycin's peptide head and phosphates
-- 10Å -> Bound state
-- Binding is favored irrespective of membrane composition
-- Single fengycin has no selectivity towards membrane composition



Cholesterol changes micelle binding pathway

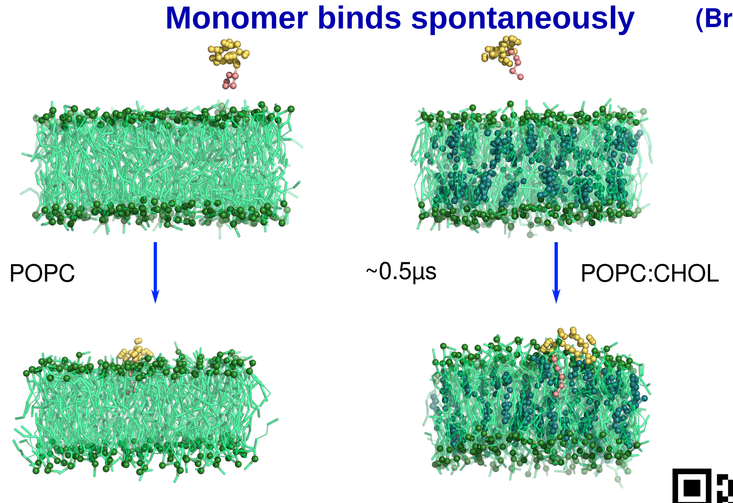




-- Cholesterol alters stability of: unbound, surface-bound (A, B, D) and fully bound states (C, E, F)
-- Binding pathway different in the presence of cholesterol
-- In cholesterol-rich membranes, micelles show higher propensity to be in the surface-bound state
-- Cholesterol increases surface-bound population
-- Cholesterol condenses membrane and makes micelle entry difficult
-- Systems not yet converged

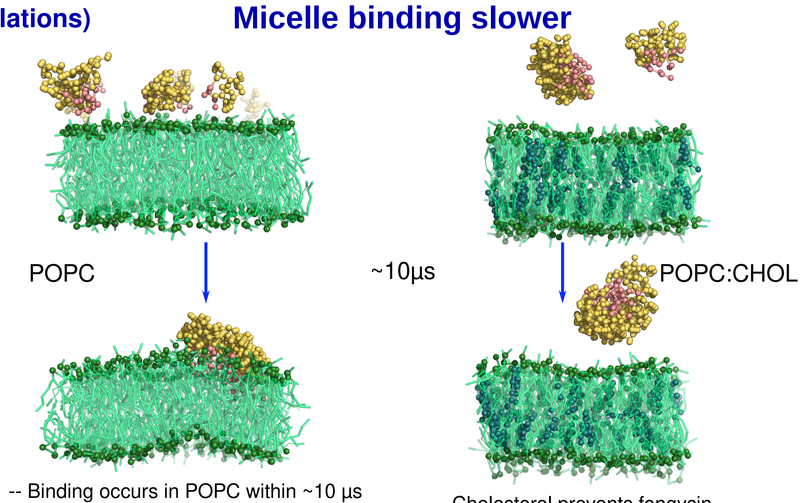
Does cholesterol impact fengycin binding to membrane?

Monomer binds spontaneously (Brute force simulations)



Weighted ensemble simulations are run using WESTPA:
https://github.com/westpa/westpa

Micelle binding slower



-- Binding occurs in POPC within ~10 µs
-- Bends POPC
-- Remains in aggregated state after binding
-- Fengycin induces positive curvature

-- Cholesterol prevents fengycin micelle binding even after 85 µs
-- Membrane bends to avoid binding
-- Fengycin induces negative curvature

Conclusions

-- Cholesterol affects fengycin aggregation
  -- Lipids more ordered
-- Cholesterol alters fengycin binding
  -- Monomer affinity is unchanged
  -- Slows micelle binding
  -- Changes free energy of intermediate states

Future directions

-- Micelle binding to membrane need more time to converge
-- Comparison of binding energy of surfactin and fengycin
-- Free energy of micellization using all-atom simulations for different fengycin types

Acknowledgements

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