Insights into the mechanism of fengycin, an antimicrobial lipopeptide

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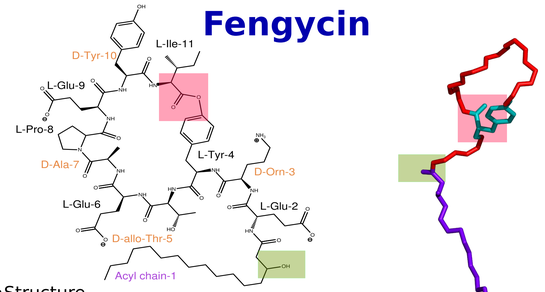
Abstract

Fengycins are a class of antimicrobial fungicides, synthesized by the bacterial family, Bacillus, which function by damaging the fungus' cell membrane. Fengycin is already in use as an agricultural fungicide and also has medical potential. This makes fengycin a potential drug candidate and comprehending its mechanism of action is a crucial step in drug development. Previously, it has been observed in coarse-grained simulations that fengycins form aggregates in model fungal membranes (POPC) but not in model bacterial membranes (POPE:POPG). Our hypothesis is that aggregate formation plays a crucial step for fengycin disrupting the fungal membranes. The results suggest that fengycin molecules interact with zwitter-ionic POPE and not effectively with the negatively charged POPG because of the electrostatic interactions between glutamates of fengycin and positively charged ammonium in POPE headgroup. Also POPE can facilitate hydrogen bonding while POPC cannot. So, fengycin attracts POPE more than POPG resulting in an accretion of similar lipids in the bilayer. This suggests that aggregates can be dissolved in POPE:POPG while they remain conglomerated in POPC. In addition, interactions between specific residues near the ring closing helps fengycin to remain as aggregates in POPC. Also, some of the residues, ILE, DTYR and GLU-9 can have different population of states based on their position along the membrane normal, which plays important role in packing of monomers in aggregates that in turn determines its stability. However, most recent all-atom simulation results suggest that preference for the aggregation process in one membrane over the other is not statistically significant, although this may be more due to slow convergence than a true lack of selectivity.

Fengycin



- Structure
  - --16-carbon acyl chain
  - --β-Hydroxyl group
  - --Cyclic ring with eight amino acids
  - --Ester bond between Tyr-4 and Ile-11
  - --4 D-amino acids
  - --Net charge = -2

Simulation Details

- Parameters derived using the Force Field toolkit extension to VMD J. Comput. Chem. 2013, 34, 2757-2770.
- Salt 100mM NaCl
- POPC, POPE:POPG (2:1) membrane models
- 90 lipids and 10 fengycins per leaflet
- 7,500 waters and 50,000 total atoms per system
- POPG has -1 charge
- Box size: 90 Å x 90 Å x 70Å
- 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

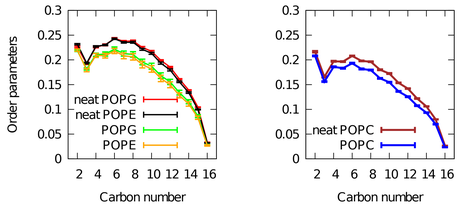
System	Time(μs)
POPC + Feng	3 X ~5μs
POPE:POPG(2:1) + Feng	3 X ~5μs
POPE:POPG(2:1)	4 X ~150ns
POPC	4 X ~150ns

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

Fengycin alters membrane bilayer

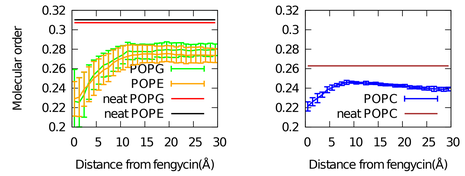
Fengycin disorders lipid chains

Order parameters of palmitoyl chain



-- Order parameters like ^{2}H quadrupolar splitting
-- $S_{CD} = \left\langle \frac{3}{2}(\cos^2\theta - 1) \right\rangle$
-- Reduces chain order

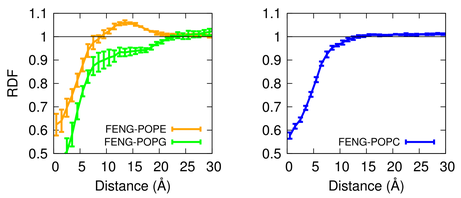
Average order parameters vs distance from fengycin



-- Average 2nd and 3rd principal axes for saturated chains
-- $S_{\mathrm{mol}} = \left\langle \frac{3}{2}(\cos^2\theta - 1) \right\rangle$
-- Chains significantly disordered relative to neat layer
-- Effect strongest at short range < 10 Å

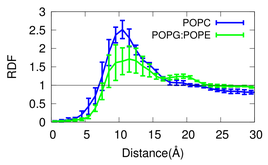
Fengycin attracts POPE lipids

RDF in membrane plane



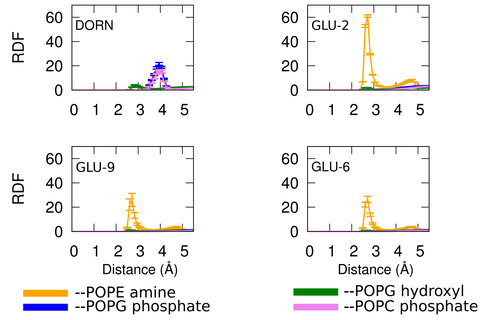
-- Fengycin prefers POPE more than other lipids
-- No interaction with POPC and POPG

Fengycin aggregation lipid dependent



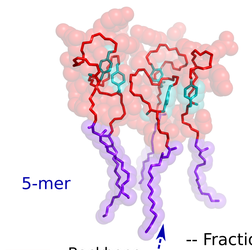
-- Two prominent shells in POPE:POPG
-- Aggregation stronger in POPC

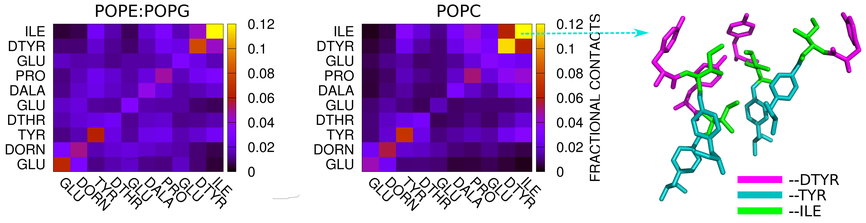
Charged residues attract heagroups



-- Mainly electrostatic interaction between charged residues and lipid headgroup
-- Negative glutamates interact with POPE ammonium group
-- Positive Orn attracts phosphates

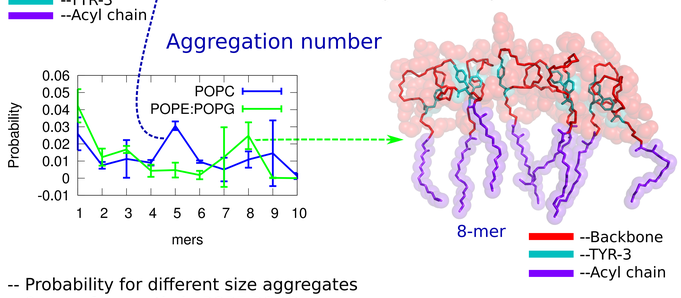
Aggregation via specific contacts





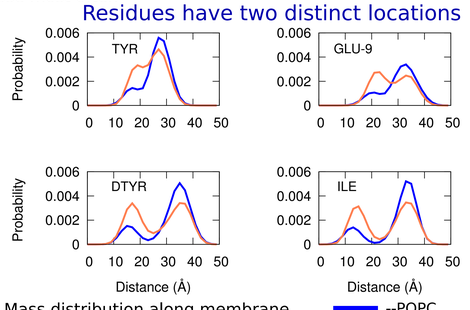
-- Fractional contacts between neighboring fengycin's residues
-- Residues near the ring closure at Tyr-3 and Ile-10 come close most often

Aggregation number



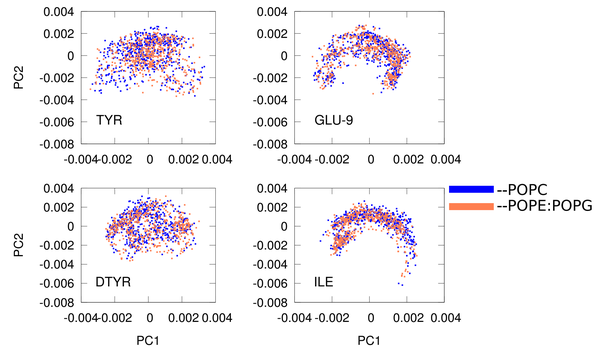
-- Probability for different size aggregates
-- 8-mers frequently in POPE:POPG
-- 5-mers customarily observed in POPC
-- Higher likelihood of monomers in POPE:POPG compared to POPC

Residues have two distinct locations



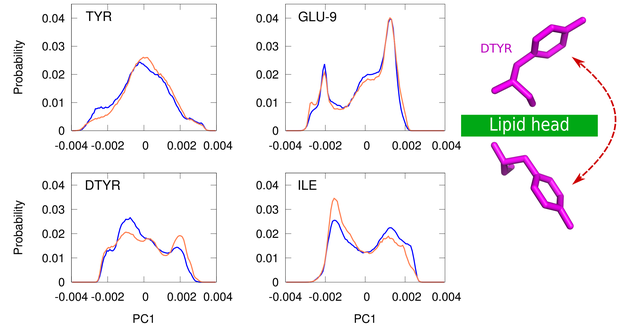
-- Mass distribution along membrane normal for certain residues
-- Some residues have two distinct peaks

Principal Component Analysis

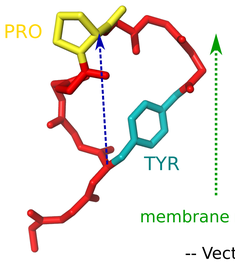


-- Align fengycin backbone to isolate side-chain motion
-- TYR moves with backbone
-- Other residues GLU-9, DTYR and ILE move separately

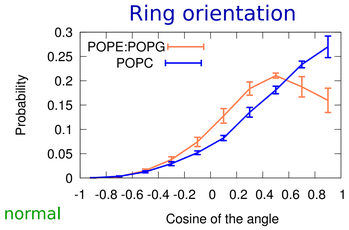
Histogram of PC1



-- GLU-9, ILE and DTYR observe many different states
-- Residues interact with water or with hydrophobic lipid interior



Ring orientation



-- Vector between Cα of TYR and PRO
-- Angle between vector and membrane normal
-- Ring orientation has single peak

Conclusions

- -- Fengycin disorders the lipid chains
- -- Fengycin attracts POPE over POPG
- -- Aggregation depends on lipid
- -- Linkage residues interact more in aggregates
- -- Residues show different conformations along z-axis
- -- Ring has preferred orientation towards the membrane normal

Future directions

- -- Determine the free energy of aggregation of fengycins in the two different membrane.
- -- Coarse grained simulations
  - Effect of sterols
  - Fengycin micelle binding

References : J. Horn, A. Cravens , A. Grossfield, Biophysical Journal, 1612-1623, 2013