Understanding the function of the cyclic antifungal lipopeptide fengycin using all-atom simulation

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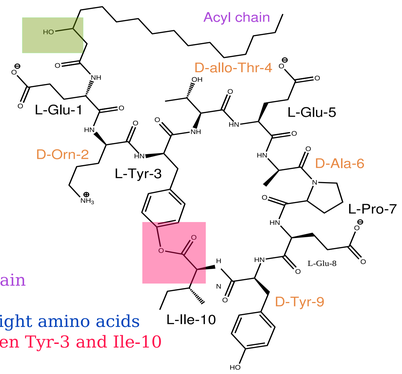
Poster PDF
http://tinyurl.com/fengycin

Abstract

Fengycin is one of a class of cyclic lipopeptides synthesized by the bacterial genus *Bacillus*. Many bacteria synthesize similar cyclic peptides, some of which have antifungal or even antibacterial properties, so studying how they interact with membranes is a promising path for drug development. Previously, we ran a series of coarse-grained molecular dynamics simulations using MARTINI force field exploring the interactions of fengycin with models for bacterial and fungal membranes. The results suggested that the peptide's ability to aggregate and deform the membrane depends on the nature of the surrounding lipid headgroups, and that these interactions might be the origins of its selectivity. However, coarse-grained models, by definition, lack atomic-level resolution, so all-atom simulations are needed to confirm and expand on these results. First, we developed parameters for several unusual chemical moieties found in fengycin, such as the cyclization between the C-terminus and a Tyr side chain, as well as the amide linkage between Glu and β-hydroxy palmitic acid. We validated these parameters via simulations of isolated and clustered fengycin molecules in water, as well as simulations of it bound to two membrane compositions: POPC (model mammalian/fungal membranes) and 2:1 POPE:POPG (model bacterial membrane). The results show that the specificity of fengycin aggregation and its ability to deform lipid bilayers is not based only on electrostatic interactions. Moreover fengycin distorts the POPE:POPG membrane more than the POPC model bilayer.

Fengycin



- Structure
  - --16-carbon acyl chain
  - --β-Hydroxyl group
  - --Cyclic ring with eight amino acids
  - --Ester bond between Tyr-3 and Ile-10
  - --4 D-amino acids
  - --Net charge = -2

Simulation Details

- Parameters developed with the Force Field toolkit VMD extension
  -- C.G. Mayne et al. J. Comput. Chem. 2013, 34, 2757-2770. DOI: 10.1002/jcc.23422.
- Physiological 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: CHARMM27
- Ensemble: NPγT
  -- γ = 30 dyn/cm
- Thermostat: Langevin
  -- 310 K 1 bar
- Electrostatics: PME
- VDW cutoff: 10 Å
- Timestep: 2 fs
  --RATTLE
- Software: NAMD 2.8
- Computer resource: BlueGene/Q

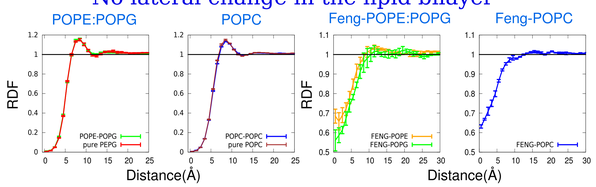
Simulation	Time
Fengycin	
POPE:POPG	3 x ~1.3 μs
POPC	3 x ~1.1 μs
Water	3 x 0.6 μs, 1.5 μs
Neat membrane	
POPE:POPG	3 x ~150ns
POPC	3 x ~150ns

Work done in LOOS (Lightweight Object Oriented Structure analysis library), an open source C++ library designed and maintained by the Grossfield lab. LOOS provides a concise, adaptable framework for designing analysis tools that interfaces with native file formats of most simulation packages.

http://loos.sourceforge.net

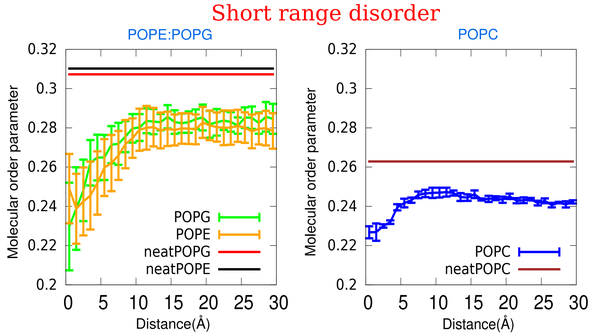
Fengycin in lipid

No lateral change in the lipid bilayer



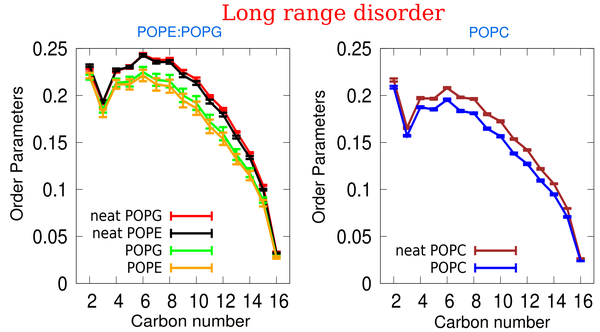
- Radial distribution in membrane plane
  --no change in the lipid-lipid packing
- Fengycin packs tighter against POPE and POPG

Fengycin decreases chain order
Short range disorder



- Molecular order parameter for lipid palmitoyls
  -- Average 2nd and 3rd principal axes
  -- $S_{MOL} = \left\langle \frac{3}{2}(cos^2\theta - 1) \right\rangle$
  -- Chains significantly disordered relative to neat bilayer
  -- Effect strongest at short range, < 10 Å

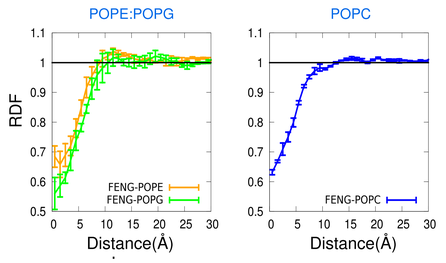
Long range disorder



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

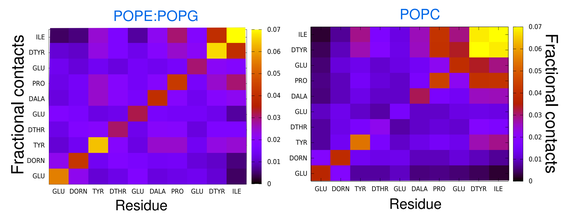
Fengycins form aggregates

Fengycin - Fengycin RDF

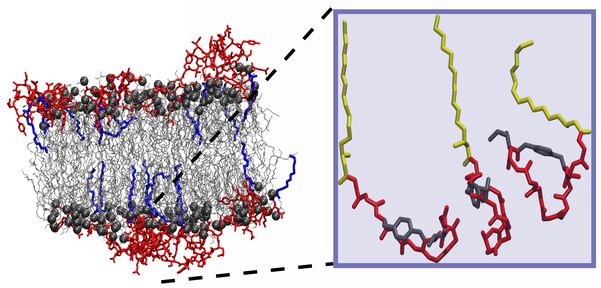


- Aggregation stronger in POPC

Specific interactions drive formation of aggregates

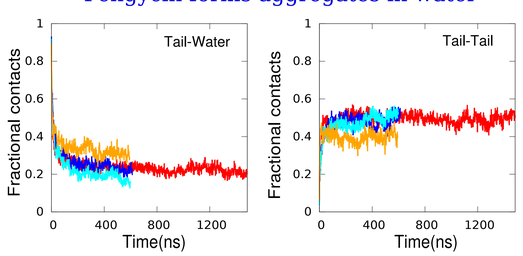


- Fengycin rings tend to pack in parallel
- Strongest contacts are Ile and Tyr
- Glu - Glu interaction is high but charge repulsion also present
- Specific interactions
  --Residues proximal to hydrocarbon tail



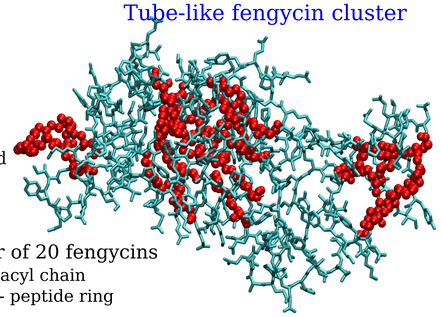
Fengycin in water

Fengycin forms aggregates in water



- Fraction of contacts between fengycin hydrocarbon tails
  --Tail-tail
  --Tail-water
- Initial aggregation is fast
- Peptide portion remains hydrated

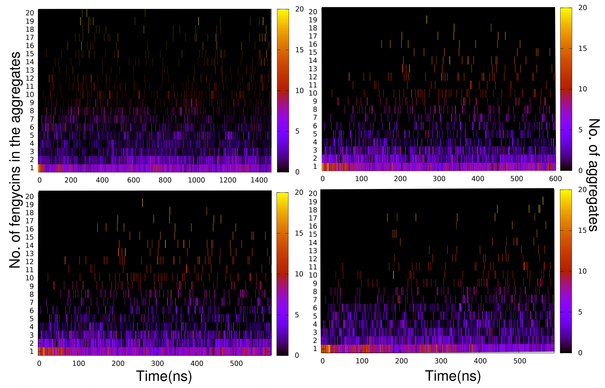
Tube-like fengycin cluster



Cluster of 20 fengycins
--Red - acyl chain
--Cyan - peptide ring

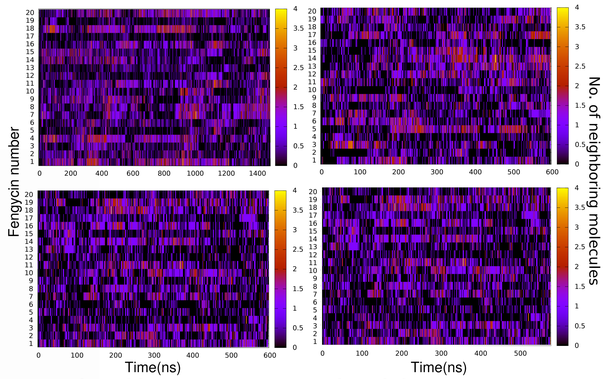
Fengycin in water

Cluster formation in Fengycin

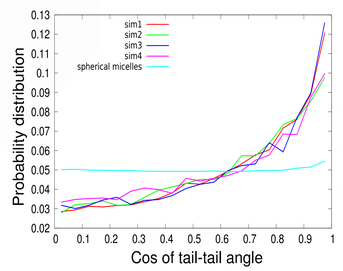


- Population of fengycins in different sized aggregates
- Aggregate defined by 15 contacts within distance 3.5 Å
- One or two large aggregates per simulation
- All 4 trajectories very similar

Fengycin forms tube-like structures



- Number of fengycins in contact with each fengycin
  -- equilibrates rapidly usually having 2-3 neighbors
  -- not spherical micelle



-- Crossing angle between adjacent fengycin tails
-- Cosine distribution flat for random spherical micelles
-- Fengycin chains strongly prefer parallel orientation

Conclusion

- Fengycins don't alter headgroup packing
- They disorder the hydrocarbon region
- Fengycin-Fengycin aggregate specific but flexible
  -- Oligomerize in membrane within 100 ns
  -- Stronger in bacterial model membrane

Future directions

- Compute the free energy of fengycin binding to model membranes
- Effects of cholesterol, ergosterol