Elucidating Antimicrobial Lipopeptide Action via Combined Coarse-grained and All-atom Molecular Dynamics

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Abstract

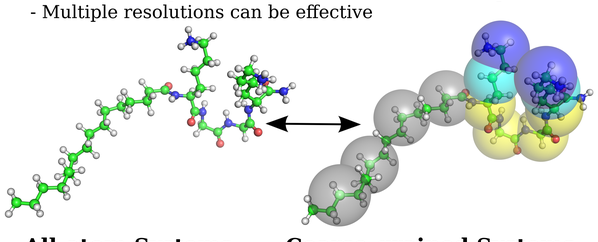
The advent of multiple drug resistant bacterial strains and the lack of novel antibiotic therapeutics to respond remains one of the pressing medical concerns of our time. Recent work has highlighted a class of synthetic compounds, known as antimicrobial lipopeptides, which show great promise as a scaffold for future drug design. One such compound, palmitoyl-Lys-Gly-Gly-DLys (C16-KGG**K**), has micromolar minimum inhibitory concentrations against a variety of bacterial and fungal species. Here we have used coarse-grained and all-atom molecular dynamics in tandem to probe the biophysical mechanism of action behind this lipopeptide with varying detail and time-scales. Our results are validated to experimental results and suggest a possible mechanism by which lipid bilayers are disrupted.

Lipopeptides of Interest

- Synthetic antimicrobial lipopeptide C16-KGG**K**
  - KGG**K** peptide sequence
  - Last lysine is a D-amino acid (in red)
  - Palmitoyl chain linked via peptide bond
- Physical features bestow selectivity/potency
  - Net positive charge
  - Amphipathic
- Investigated by the Shai lab
  - *Makovitzki, Avrahami, and Shai, PNAS, 2006, 103, 15997-16002*

Multiscale Modeling Approach

- Full atomic detail in a simulation is computationally expensive
- Abstracted detail trades accuracy for sampling
- Multiscale modeling
  - Choose the representation that best answers the question
  - Multiple resolutions can be effective



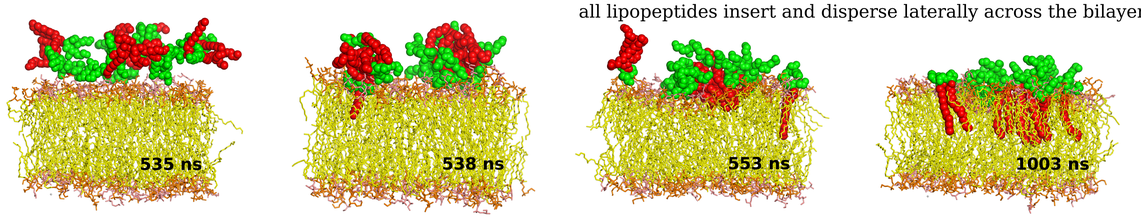
All-atom Systems	Coarse-grained Systems
• CHARMM27 force field - 2 fs timestep	• MARTINI force field - 10 fs timestep
• 20 lipopeptides - Free in solution above bilayer - Simulates binding	• 48 lipopeptide micelle - Equilibrated in solution
• 180 lipids in bilayer - 2:1 POPE:POPG (bacteria-like)	• 480 lipids in bilayer - 2:1 POPE:POPG (bacteria-like)
• 12,000 water molecules	• 24,000 water beads
• 100 mM NaCl	• 100 mM NaCl
• 60,850 atoms	• equivalent to 120k atoms
• 15 ns/day on BG/L - 2048 cores	• 100 ns/day on cluster - 8 cores
• 3 simulations - 6 μs total	• 4 simulations - 20 μs total (80 μs effective time)

For reference, use the code to the left to access a digital copy of this poster. To learn more about this work or to contact the authors, visit us at:

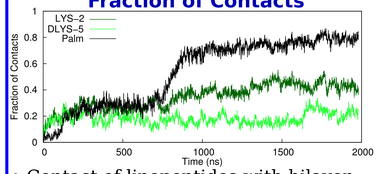
http://membrane.urmc.rochester.edu

All-atom Simulations

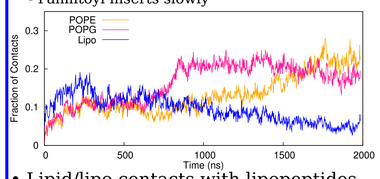
In three all-atom trajectories, the lipopeptides rapidly aggregate in solution while binding to the bilayer. Eventually all lipopeptides insert and disperse laterally across the bilayer.



Fraction of Contacts

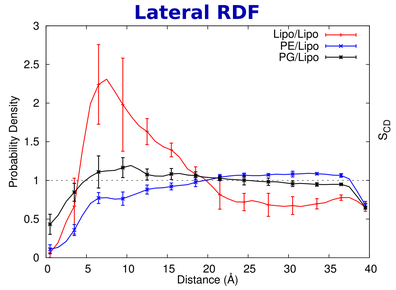


- Contact of lipopeptides with bilayer
  - Lysines make rapid contact
  - Palmitoyl inserts slowly



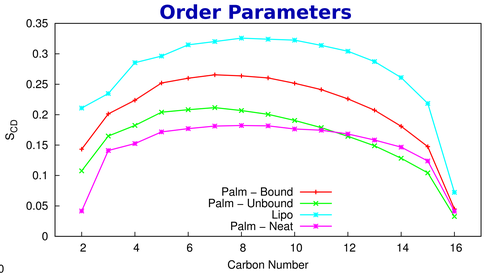
- Lipid/lipo contacts with lipopeptides
  - Lipopeptide peak indicates early aggregation
  - Similar POPE/POPG contact, despite 2:1 POPE:POPG concentration ratio

Lateral RDF



- Radial distribution function of lipopeptides to each other bilayer component in the plane of the bilayer
- Lipo/Lipo peak result of aggregation
  - Decays over time as lipopeptides disperse
- Preference for POPG lipids at short range
  - More pronounced later in simulation

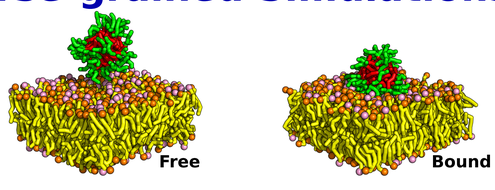
Order Parameters

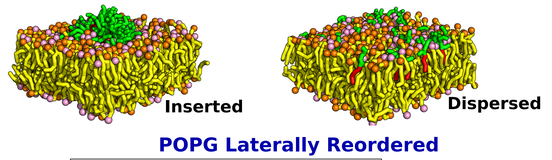


- Acyl C-H bond orientation
- θ, angle between C-H bond and membrane normal $S_{CD} = -\frac{1}{2}\langle 3\cos^2\theta_{CD} - 1\rangle$
- Measured via deuterium quadrapolar splitting in solid state NMR
- Bound - leaflet with bound lipopeptides
- Unbound - leaflet opposite of bound leaflet
- Lipopeptides increase bilayer order

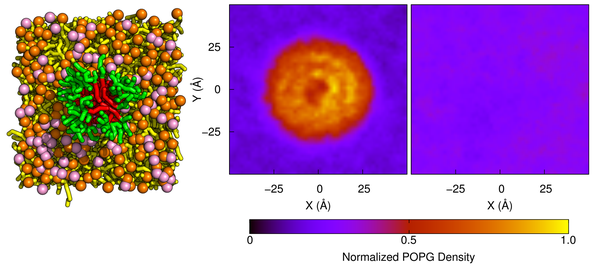
Coarse-grained Simulations

Of four CG trajectories, only saw complete insertion in one. In the remaining three trajectories, the lipopeptide micelle remained attached to the bilayer in a "bound" state.



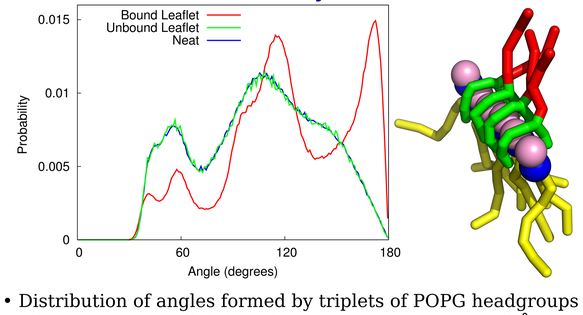


Bound Micelle Recruits POPG



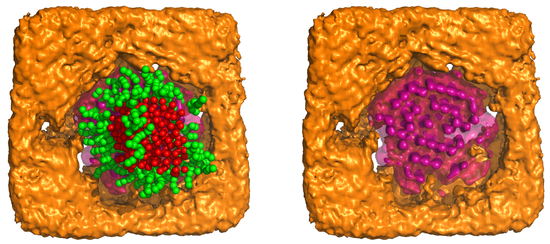
- Density map of POPG lipids in the plane of the bilayer
  - One trajectory shown, normalized by area and frames
  - Left image shows bound leaflet, right shows unbound
  - 0,0 represents position of the micelle center of mass
- High concentration of POPG directly beneath micelle
- Bilayer reorganizes laterally

POPG Laterally Reordered



- Distribution of angles formed by triplets of POPG headgroups
  - For every set of 3 lipids with headgroups less than 8 Å apart
- Neat bilayer and unbound leaflets have identical distribution
  - Diffuse, with very wide peaks
- Drastic change in bound leaflet, sharp peaks at 100° and 180°
  - Shows ordered headgroup packing
- Image shows a row of lipids ordered by lipopeptide action
  - Direct interaction between lysines and anionic headgroups

Limitations of MARTINI



- Left shows the micelle above an average 3D density map of the two lipid components, right shows the same with micelle removed
  - Green is peptide and red is palmitoyl tail of lipopeptide
  - Orange and pink are bulk densities of POPE and POPG, respectively
  - Darker pink in right image indicates regions of higher POPG density
- Bilayer crystallization is a cause for concern when working with the MARTINI forcefield
  - Consider using the published polarizable model in the future

Multiscale Summary

- C16-KGG**K** tends to micellize in solution
  - Structurally, the lipopeptide resembles a detergent
- All-atom simulations demonstrate that:
  - Free lipopeptides will bind and rapidly insert
  - Lysines drive initial interactions with the bilayer
  - Fully inserted lipopeptides change lipid order parameters
- Coarse-grained simulations demonstrate that:
  - Lipopeptides spontaneously form large micelles
  - Micelles are pseudo-stable and do not readily insert
  - Bound micelles demix the bilayer

Proposed Model of Action

- C16-KGG**K** action is likely a combination of effects
- At high concentrations
  - All lipopeptides insert and affect bilayer stability
- At lower concentrations
  - Available lipopeptides micellize and bind to the bilayer
  - On longer timescales, micelle demixes lipids

Future Plans

- High concentration all-atom simulations
  - Currently in progress
  - Quantify C16-KGG**K** effects on bilayer stability
- Mixed coarse-grained/all-atom sampling
  - Take advantage of CG speed to sample binding process
  - Reconstruct atomistic details at snapshots along trajectory
  - Run all-atom simulations to gather detailed information
  - Can parallelize long timescale all-atom simulations
- Implement polarizable water into simulations
  - Expect crystal artifacts to vanish

Some simulation details and results are in press: Horn et al., Biochim Biophys Acta, 2012 Feb; 1818(2):212-8.

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

http://loos.sourceforge.net