Characterization of Potent Antimicrobial Lipopeptides Via All-atom and Coarse-grained Molecular Dynamics

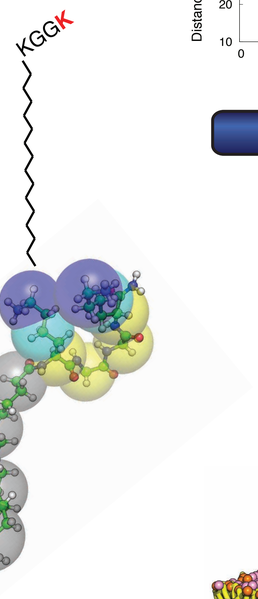
Joshua N. Horn, Jesse D. Sengillo, Tod D. Romo, Alan Grossfield

University of Rochester Medical School, Rochester, NY, USA

Abstract

The emergence of antibiotic-resistant pathogens is one of the major medical problems of the 21st century, prompting renewed interest in the development of novel antimicrobial compounds. Previous work utilized microsecond-scale all-atom molecular dynamics to characterize the structure and dynamics of a synthetic antimicrobial lipopeptide (AMLP), C16-KGGK. Here we use the MARTINI coarse-grained forcefield to abstract detail from our system in order to increase sampling. Our results show that the AMLPs preferentially bind to negatively charged bilayers and locally alter membrane organization and dynamics.

Lipopeptides

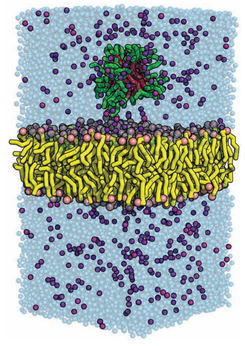


- Mimic natural antimicrobial peptides
- Short peptide attached to palmitic acid at N-terminus
  - KGGK
  - D-amino acid labeled in red
  - Peptide linkage
  - Acyl chain improves membrane affinity
- Makovitzki, Avrahami, and Shai.
  - *PNAS. 2006, 103, 15997-16002*

Coarse-Graining

- MARTINI forcefield
- Faster than all-atom molecular dynamics
  - Fewer interactions to compute
  - Smoother energy surface
  - Larger masses allows larger timestep
  - 100 ns/day on 8 core Linux cluster
- Marrink SJ et al.
  - *J Phys Chem B. 2007, 111, 7812-24.*

Simulation Details



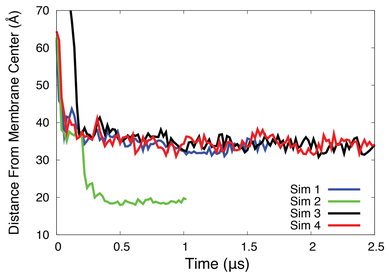
- Two systems of interest
  - POPC ("mammalian")
  - 2:1 POPE/POPG ("bacterial")
  - POPG is negatively charged
- 480 lipids
- 24k water beads (96k effective waters)
- Neutralizing Na^+ and Cl^- ions
  - additional 100 mM concentration
- 10 fs timestep
- 9 Å switch, 12 Å cutoff
- 300K, Nosé-Hoover thermostat
- 1 atm, Parrinello-Rahman barostat
- 48 AMLPs in micelle conformation
  - Spontaneously form in pure water

System	Type	AMLPs	Total Time (μs)
POPE/POPG	AA	0	2x0.1
		20	3x1.9
		60	1.2, 2x0.4
POPE/POPG	CG	48	2x1.5, 2x2.7
		96	2x1.0, 2x2.5
		144	2x1.0, 2x2.1
POPC	CG	48	2x1.6, 2x4.8
		96	2x2.5, 2x4.5
		144	2x0.7, 2x2.3
POPE/POPG Neat	CG	0	2x1.2
POPC Neat	CG	0	2x1.1
All-atom			7.5
Coarse-Grained			55

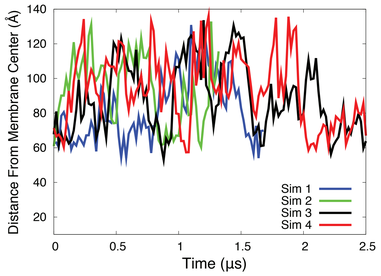
- CG allows for greater sampling
  - 100 ns/day vs 15 ns/day
- CG systems are much larger
  - 480 lipids vs 180 lipids
  - 48/96/144 AMLPs vs 20 AMLPs
- For 1 microsecond
  - 3.5 months on BlueGene/L (AA)
  - 10 days on Linux cluster (CG)

Micelle Binding Depends on Membrane Composition

POPE/POPG

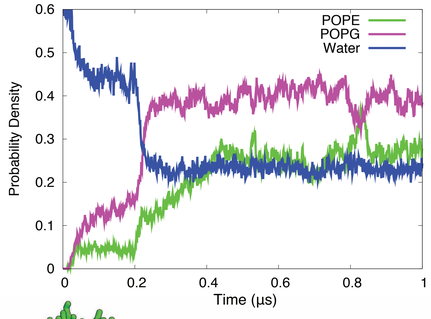


POPC

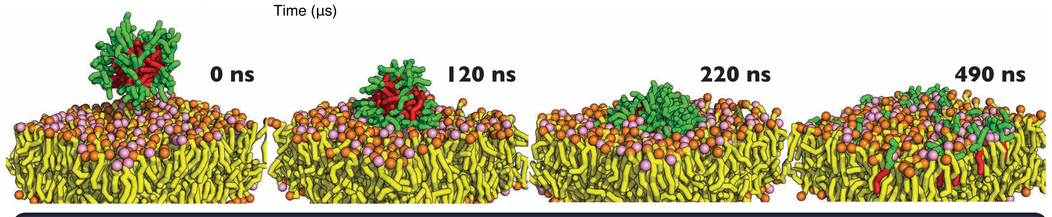


- Distance from bilayer center to micelle center of mass
  - Scales on Y-axes differ
- Single micelle simulations
- POPE/POPG: binding is rapid
  - Simulation #2 inserts
- POPC: micelle never binds to membrane surface

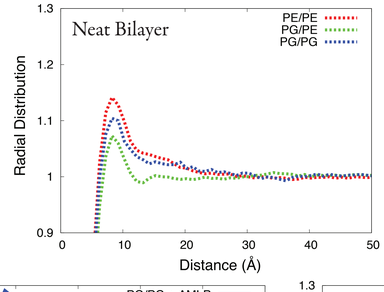
Binding and Insertion of an AMLP Micelle



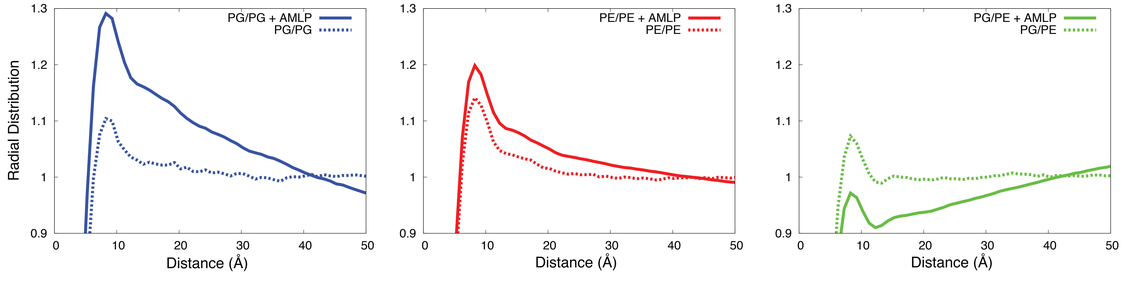
- POPE/POPG Simulation #2
- Fraction of contacts between AMLPs and lipids
  - Count atoms within 8 angstroms
  - Report fractional contribution
- Water displacement and lipid contact in first 50 ns
  - Rapid association with bilayer
- Micelle inserts at 200 ns
  - Lipid contacts increase, water decreases
- POPG contacts 2x higher than POPE
  - Bulk bilayer is 2:1 POPE/POPG



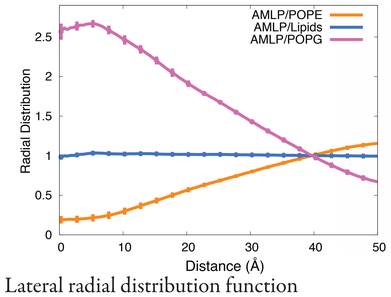
AMLPs Demix Lipid Bilayer

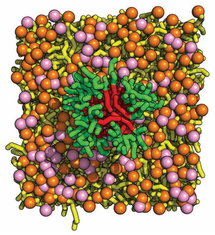


- Lateral lipid-lipid radial distribution function
  - Probability density as a function of distance in the membrane plane
  - Dotted lines: neat
  - Solid lines: bound micelle
  - 3 simulations, micelle bound but not inserted
- POPG/POPG enriched
- POPE/POPG depleted, POPE/POPE enriched
- AMLPs demix the membrane

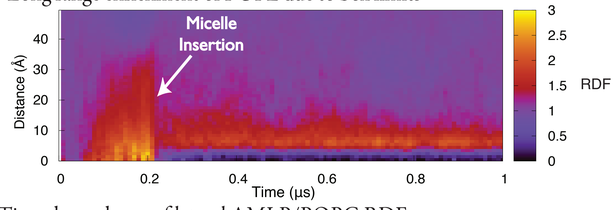


AMLP Recruits POPG





- Lateral radial distribution function
  - 3 simulations, micelle bound but not inserted
  - AMLP-lipid probability (POPE, POPG and total lipid)
- Short range enrichment of POPG
- Short range depletion of POPE
  - Long range enrichment of POPE due to box limits



- Time dependence of lateral AMLP/POPG RDF
  - Insertion changes mixing properties
  - Surface binding recruits POPG
  - After insertion, single shell of POPG around each AMLP

Conclusions

- Lipid composition drives binding
  - Anionic POPG lipids favor binding
- AMLPs preferentially interact with POPG
- AMLPs demix bilayer
- Possible mechanisms of action:
  - Local alteration of membrane composition
    - Epand RM and Epand RF. *Mol Biosyst. 2009, 5, 580-587.*
  - Change balance of ions locally
  - Post-insertion membrane perturbation
  - Poration

Future Plans

- Characterization of insertion process
  - More replicas of 1 micelle POPE/POPG system
- Large-scale all-atom systems
  - 4x larger than previous all-atom simulations
  - Compare and validate CG to all-atom simulations
  - Compare to solid-state NMR
- Couple rapid CG sampling to all-atom accuracy
  - Utilize CG model to sample
  - Reintroduce all-atom detail at key points in simulation
- See B767 for more AMLP simulation

Link to Poster (PDF)

http://tinyurl.com/6f2xslc

LOOS

LOOS (Lightweight Object Oriented Structure analysis library) is a project of the Grossfield Lab and is an open-source library using C++ and BOOST to provide an easy to use and easy to extend framework for rapidly developing analytical tools for molecular simulations. LOOS is available through SourceForge at:

http://loos.sourceforge.net