Investigation of the Mechanism of Antimicrobial Lipopeptides Using Coarse-Grained Molecular Dynamics Simulations

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Abstract

Antimicrobial lipopeptides (AMLPs) are a series of acylated cationic peptides with broad-spectrum antimicrobial activity and low hemolytic toxicity. We used microsecond-scale coarse-grained molecular dynamics simulations with the MARTINI force field to understand AMLPs' modes of action. Rigorous free energy calculations have been performed to probe the mechanism for their selectivity for different membranes. Although these studies provided useful insights, artifacts arising from the coarse representation of electrostatics in MARTINI force field complicated further interpretation of the simulations. To address this deficiency, we are developing a new coarse-grained force field for AMLPs and lipids based on elliptical Gay-Berne van der Waals potential and electric multipoles. This force field will retain much of the computational efficiency of current coarse-grained models while the detailed representation of electrostatics and molecular shape in this force field will provide more realistic descriptions of molecular interactions among AMLPs and membrane lipids.

Antimicrobial Lipopeptides

- Tetrapeptides with 2 Lys conjugated to a fatty acid tail
- Resistant to degradation due to D-amino acids in the peptide portion
- Inexpensive to synthesize
- Broad-spectrum antimicrobial activity
- Minimal Inhibitory Concentration (MIC) in micromolar range
- Presumably act by permeabilizing membrane
- Makovitzki, A. et al.,PNAS 2006, 103,15997

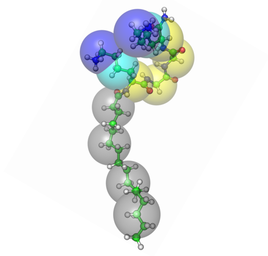
Origin of Selectivity

- Different binding affinity to human and microbial membranes?
- Need to know the ΔG of binding or insertion to different membranes
- Short-range interaction between AMLPs and lipids once bound?
- Difficult to probe by experiment

Molecular Dynamics Simulation

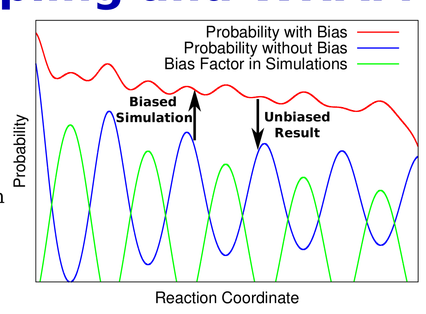
All-atom (AA) force field
- Computationally expensive
  - --Large number of degrees of freedom (DOF)
  - --Small time-step (1 ~ 2 fs)

Coarse-grained (CG) MARTINI force field
- Computationally efficient
  - --Fewer DOF, 4 heavy atoms ⟶ 1 pseudo-atom
  - --Allows larger time-step

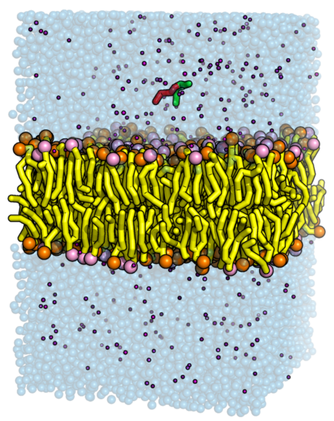


Umbrella Sampling and WHAM

- High free-energy barriers create "gaps" in brute-force sampling
- Umbrella sampling bridges the gaps
  - --Bias potential added to facilitate barrier crossing
  - --Recover unbiased probability distribution by Weighted Histogram Analysis Method
- Kumar, S. et al., J. Comp. Chem. 1992, 13, 1011

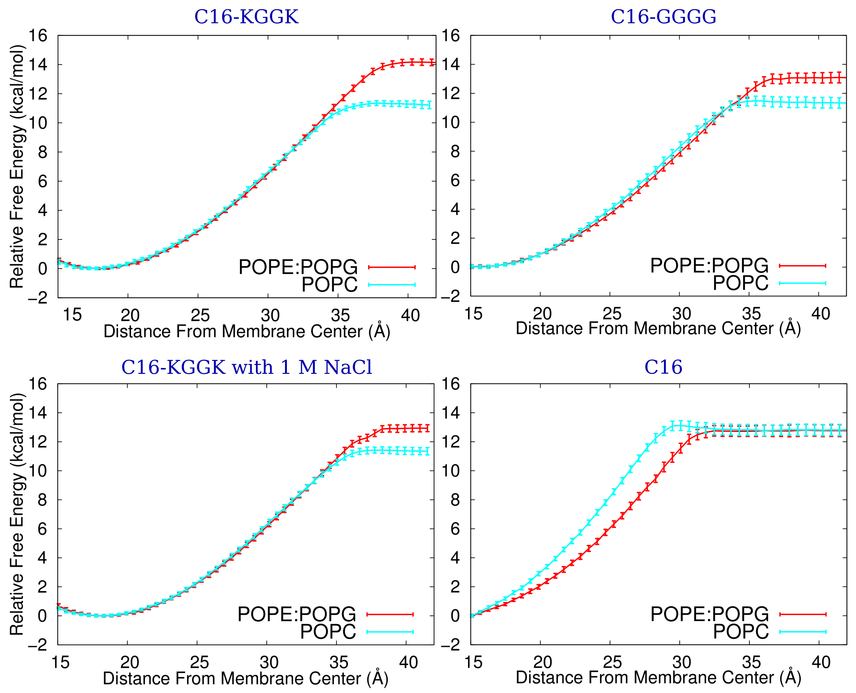


System Setup



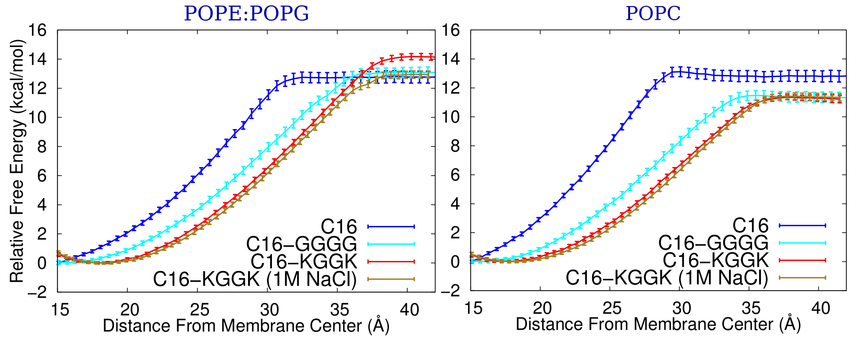
- 3 different ligands
  - 1 C16-KGGK
  - 1 C16-GGGG
  - 1 C16
- Bacterial membrane model
  - -- 320 POPE : 160 POPG
- Mammalian membrane model
  - -- 480 POPC
- Physiological salt concentration
  - -- 0.1 M NaCl (plus neutralizing)
- High salt concentration
  - -- 1 M NaCl (plus neutralizing)
- Reaction coordinate
  - -- Ligand center of mass (COM) to membrane center along Z axis
- Simulation time
  - -- 30 to 35 windows each system
  - -- About 1 μs/window

Polarity Enhances Selectivity



- Insertion is always preferred
  - --No barrier to insertion
- Selectivity
  - --Apolar < polar (neutral) < charged

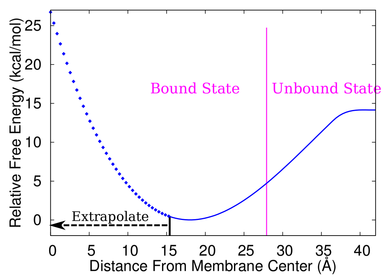
Affinity is Membrane-Dependent

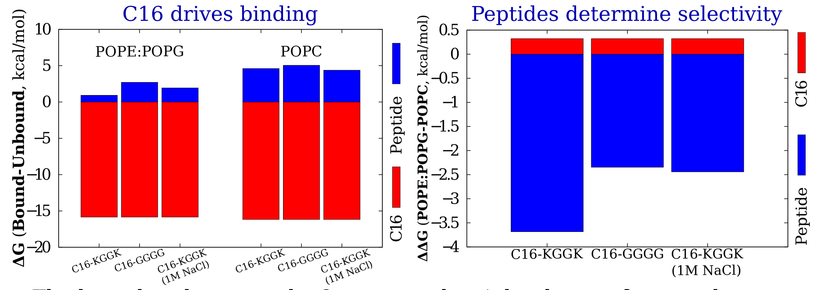


- Polarity (neutral) does not increase affinity to POPE:POPG bilayer
- Peptides oppose binding to POPC

Quantification of the PMFs

- Estimate ΔG between the bound and unbound state
- Calculate ΔΔG between the 2 membrane systems
- High barriers expected when the peptides buried in membrane
  - --Difficult to get sufficient samples of the orthogonal DOFs
- Extrapolate the PMFs to z = 0 by fitting each to a polynomial function (except for C16 system)



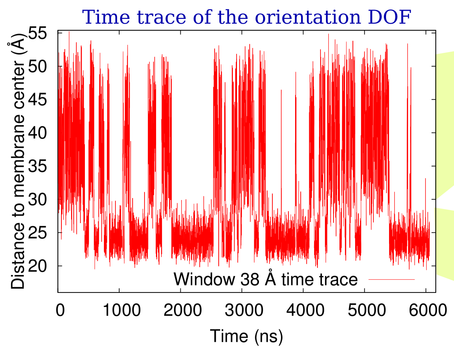


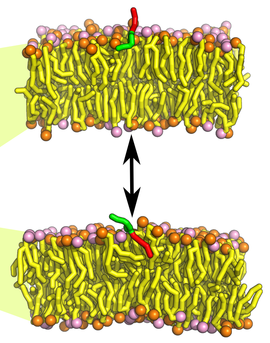
- The boundary between the 2 states and weighted sum of squared residuals of each fit (WSSR):

	C16-KGGK/ POPE:POPG	C16-KGGK/ POPC	C16-GGGG/ POPE:POPG	C16-GGGG/ POPC	C16/ POPE:POPG	C16/ POPC	C16-KGGK (1 M NaCl)/ POPE:POPG	C16-KGGK (1 M NaCl)/ POPC
Boundary (Å)	40.5	38.5	37.5	35.5	31.5	30.5	40.5	38.5
WSSR (kcal/mol)^3	0.0122	0.0731	0.0755	0.0658	N/A	N/A	0.0266	0.0642

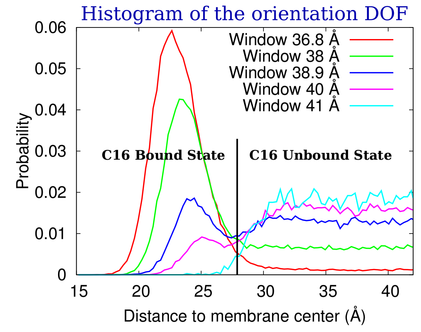
Convergence

- Orthogonal slow DOF must be well-sampled
  - --Restrain COM and sample ligand's orientation

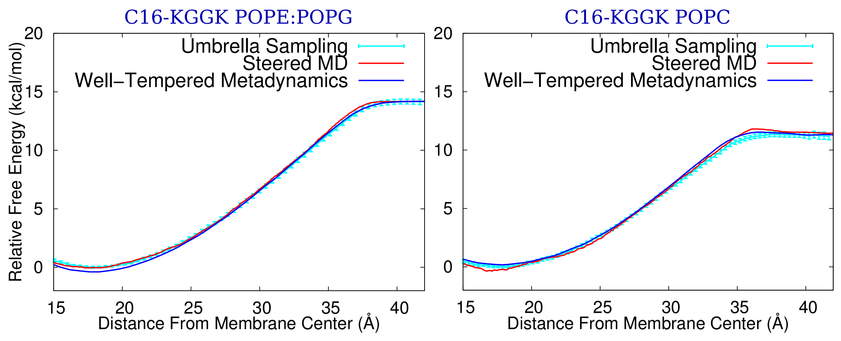




- Distance of the last hydrocarbon bead to membrane center
- A C16 bound state and an C16 unbound state
- The bound state dominates the windows below 36 Å
- The unbound state dominates the windows above 41 Å
- PMFs converge at about 1 μs/window of simulation



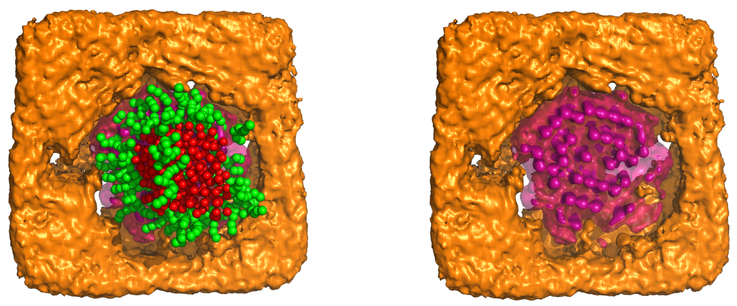
Steered MD and Metadynamics Validate the Results



Conclusions

- Selectivity arises mostly from peptide-membrane repulsion
  - --Peptides oppose binding to mammalian membrane
  - --Neutral peptides don't bind bacterial membrane
- Positive charges on peptides enhance selectivity
- Acyl chains greatly enhance binding
- Lipopeptides micellize in solution
  - --More stable in solution
  - --Peptides aggregate on micelle surface
  - --Much higher selectivity
  - --High barrier to insertion due to the breaking of hydrophobic interaction
  - --Difficult to get sufficient sampling

Crystallization Artifact

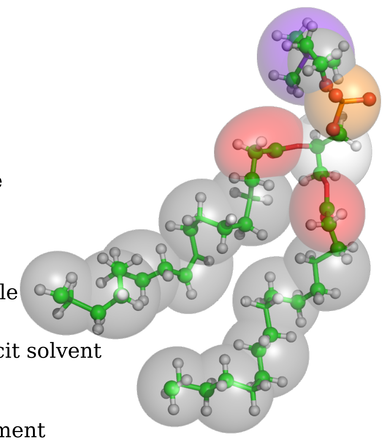


- A micelle of 48 C16-KGGK interacts with POPE:POPG bilayer
- Lysine side chains co-crystallize with POPG phosphates
- Artifact arises from coarse electrostatics of MARTINI force field
  - --Complicate the interpretation of the binding event
  - --Need a force field that provides more detailed electrostatics

Horn, J. N. et al. Biochim. Biophys. Acta, 2012, 1818, 212

Gay-Berne Electric Multipole Coarse-Grained Force Field

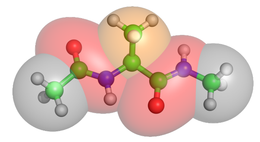
- Gay-Berne vdw potential
  - --Parametrized by fitting CG potential to that of all-atom AMOEBA FF
  - --Refined by fitting to experimental thermodynamics data
- Electric multipole moments
  - --Multipole expansion up to quadrupole
  - --Parameterized by fitting to gas-phase QM (MP2/aug-cc-PVTZ) EMP
  - --Half of the induced dipole in liquid phase is added to the permanent dipole
- Elliptical rigid-body particle
- Explicit or Generalized Kirkwood implicit solvent
- Implementation
  - --Serial version in Tinker (in-house)
  - --LAMMPS MPI version under development

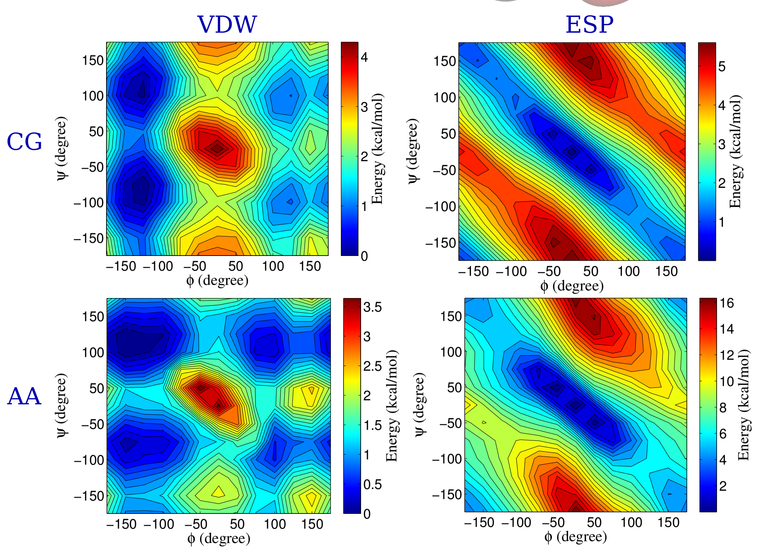


Wu J., et al. J Chem Phys 2011, 135, 155104

Preliminary Peptide Data

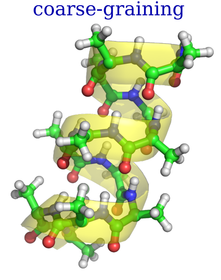
- Alanine dipeptide
- CG vs. AA (OPSLAA) Gas-phase van der Waal's and electrostatic potential

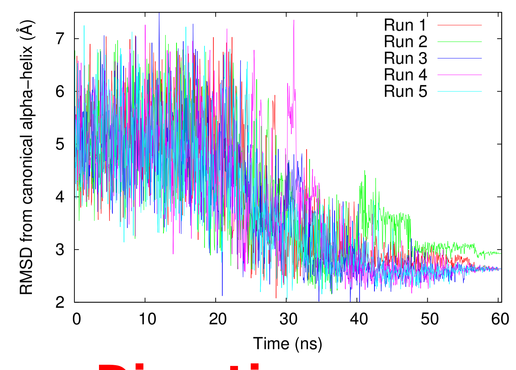




- Folding a 12-mer polyalanine
- 5 independent runs of simulated annealing

Final structure by reverse coarse-graining





Future Direction

- Optimize peptide parameters
- Polarizability and implicit solvents for elliptical multipoles
- Parametrize for lipids and lipopeptides
- Complete implementation in LAMMPS
- Simulations using the new force field

The umbrella sampling data is analyzed using WHAM (Weighted Histogram Analysis Method) implemented by Alan Grossfield. It's available at: http://membrane.urmc.rochester.edu/content/wham

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