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

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Abstract

Antimicrobial lipopeptides (AMLPs) are acylated cationic peptides with broad-spectrum antimicrobial activity and low hemolytic activity. We used microsecond-scale coarse-grained molecular dynamics simulations with the MARTINI force field to understand AMLPs' modes of action. Previous free energy calculation quantified the binding affinity and selectivity of a single AMLP to different membrane. Our data showed that the acyl chain of C16-KGGK, one of the AMLPs, is mainly responsible for its affinity to membrane while the peptide portion determines the selectivity towards different membrane lipid composition. Here we extend our free energy calculation to a micelle of C16-KGGK, which resembles the aggregated structure of C16-KGGK in solution. We found the hydrophobic contacts of C16 to C16 and C16 to lipid tails are robust reaction coordinates to characterize C16-KGGKs' micellization and their interactions with membranes. A total of about 300 microseconds of umbrella sampling simulations reveal that the barrier to entry of a C16-KGGK micelle to the mammalian membrane is much higher than that to the bacterial membrane. Our results provide biophysical insights into the mechanism of lipopeptides' antimicrobial action.

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 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
- Different perturbation to different membranes once bound?
- Difficult to probe by experiment

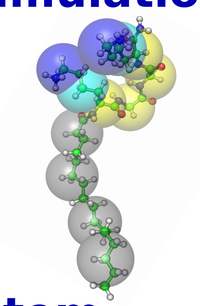
Molecular dynamics simulation

Coarse-grained (CG) MARTINI force field

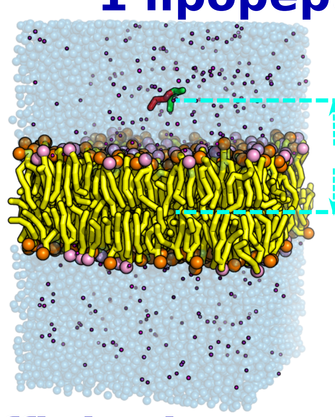
- Computationally efficient
  --Fewer DOF, 4 heavy atoms ⟶ 1 pseudo-atom
  --Allows larger time-step (10 - 20 fs)

Umbrella sampling and WHAM

- Calculate the potentials of mean force (PMFs) along a reaction coordinate
- Bias potential added to facilitate barrier crossing
- Recover unbiased PMFs by Weighted Histogram Analysis Method
- Kumar et al., J. Comp. Chem. 1992, 13, 1011

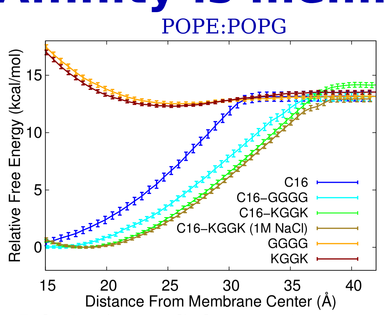


1 lipopeptide system

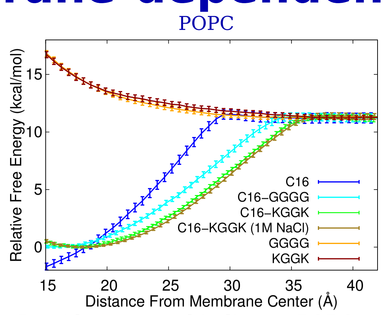


- 3 different molecules
  C16-KGGK, C16-GGGG, C16
- 2 types of membrane
  --Bacterial membrane model
  --320 POPE : 160 POPG
  --Mammalian membrane model
  --480 POPC
- Vary salt concentration
  --Always have neutralizing ions
  --100 mM NaCl (low)
  --1 M (high)
- Each system is 21,000 CG atoms
- Simulation time
  --30 to 35 windows/system
  --About 1 μs/window

Affinity is membrane-dependent



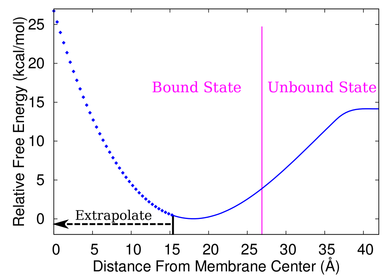
- Polarity (neutral) does not increase affinity to POPE:POPG bilayer

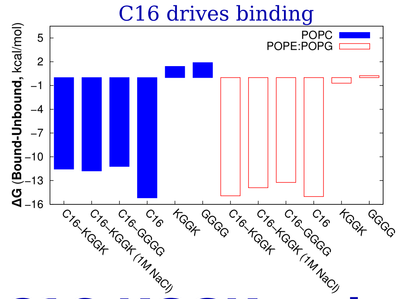


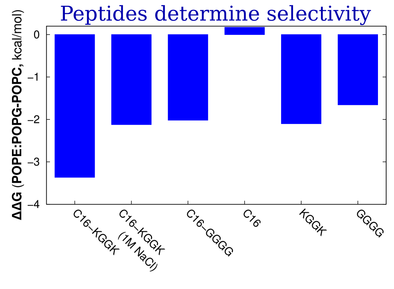
- Peptides oppose binding to POPC
- C16 insertion is always preferred

Quantification of the PMFs

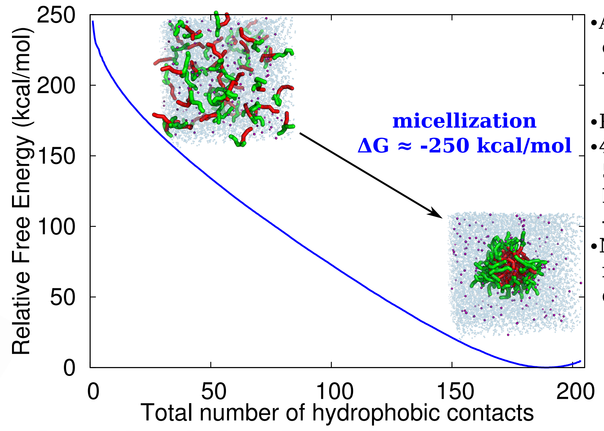
- Estimate ΔG between the bound and unbound state
- Calculate ΔΔG between the 2 membrane systems
- High barriers expected when peptides buried in membrane
  --Difficult to get sufficient samples of the orthogonal DOFs
- Extrapolate the PMFs to z = 0 by fitting each to a polynomial function (except for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





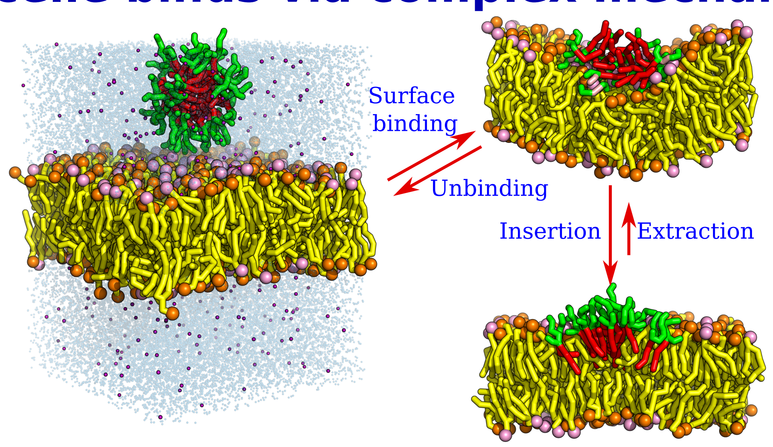


C16-KGGKs micellize in solution



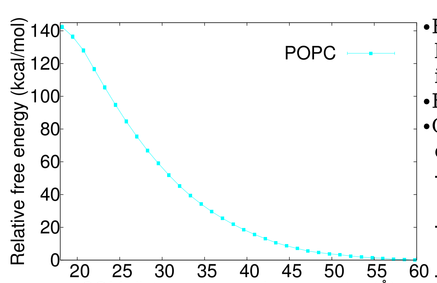
- Aggregation was seen in experiment
  --Important to biological activity
- PMFs of C16-C16 contacts
- 48 C16-KGGK solvated in 5132 polarizable MARTINI waters
  --Concentration ≈ 130 mM
- Need orders of magnitude more water to simulate dilute solution

Micelle binds via complex mechanism



- Surface binding involves dehydration
- Insertion involves exchanging hydrophobic contacts with membranes

COM distance is not an effective Reaction Coordinate

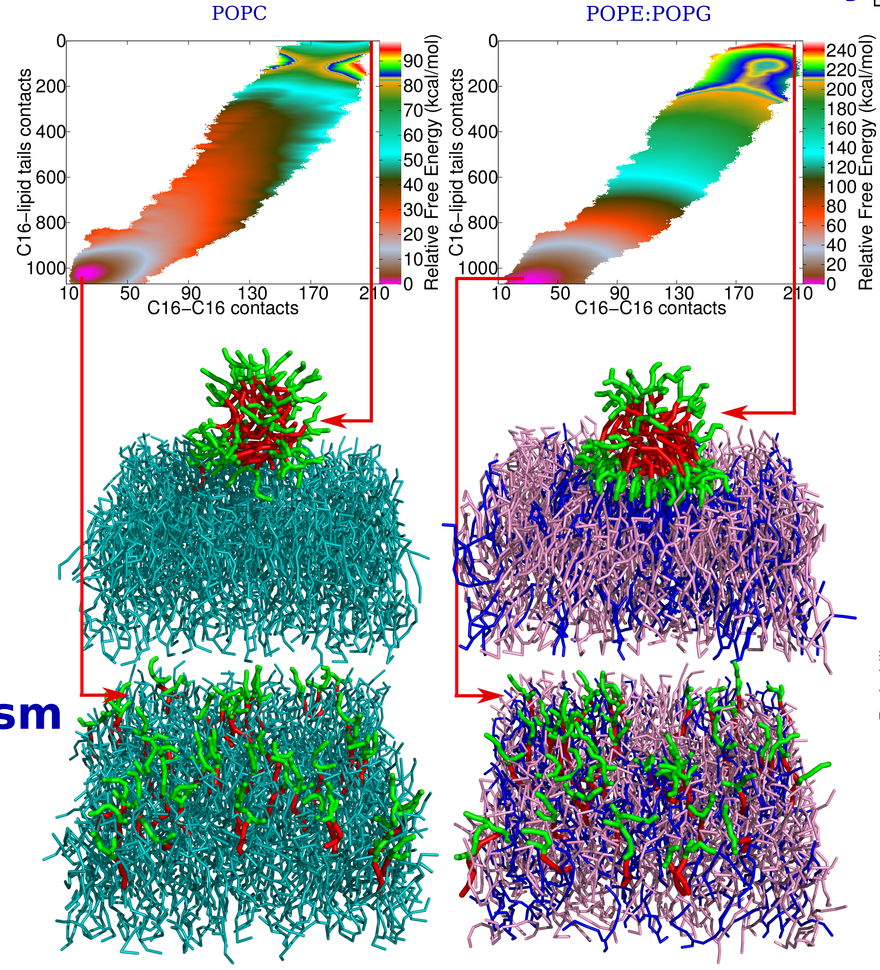


- Exchanging hydrophobic contacts between micelle and membranes is slow
- Essentially doing "brute-force" sampling
- COM distance as RC gives counter-intuitive results
  --Inserted state is predicted to be unfavorable
  --Hidden barrier orthogonal to COM distance hinders convergence
  --Can not be resolved by Hamiltonian exchange sampling

Hydrophobic contacts are robust reaction coordinates

- Can resolve slow exchanging of hydrophobic contacts
- Switching function of inter-group distances:
  $s(d) = (1 + (\frac{d}{r_0})^6)^{-1}$
  --d is the inter-particle distance
  --r_0 is the normalizing factor
- Pair-wise sum of s(d) over:
  --C16 (r_0 = 1.0 nm)
  --C16 and lipid tails (r_0 = 2.0 nm)
- Implemented in GROMACS-4.6.3 (in-house)
  --Force-decomposition parallelization
  --Optimized for computational performance
  --Compatible with GROMACS replica-exchange (REMD)
- Characterize a distinct and reversible path of C16-KGGK insertion into membrane
- Each system is about 51,300 CG atoms
- Simulation time
  --808 windows/system * 1.2 μs/window

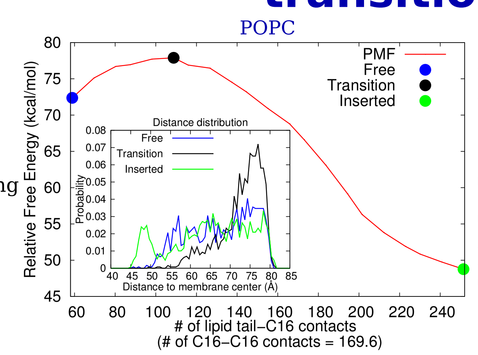
Micellization enhances selectivity



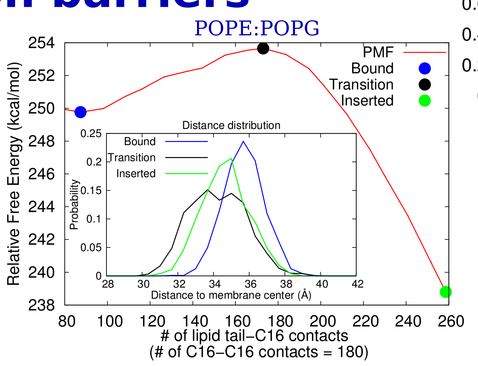
- Interfacial binding is unstable
  --C16 partially exposed to water
  --No specific binding to lipids
- Transition to insertion is steep
  --High barrier $\Delta G_{tran} \approx 35$ kcal/mol
- Insertion is the most favorable state
  --$\Delta G_{ins} \approx -45$ kcal/mol

- Interfacial binding is stable
  --$\Delta G_{bind} \approx -40$ kcal/mol
  --Lys binds to POPG head groups
- Transition to insertion is smooth
  --Low barrier, $\Delta G_{tran} \approx 5$ kcal/mol
- Insertion is the most favorable state
  --$\Delta G_{ins} \approx -248$ kcal/mol

COM distance does resolve the transition barriers



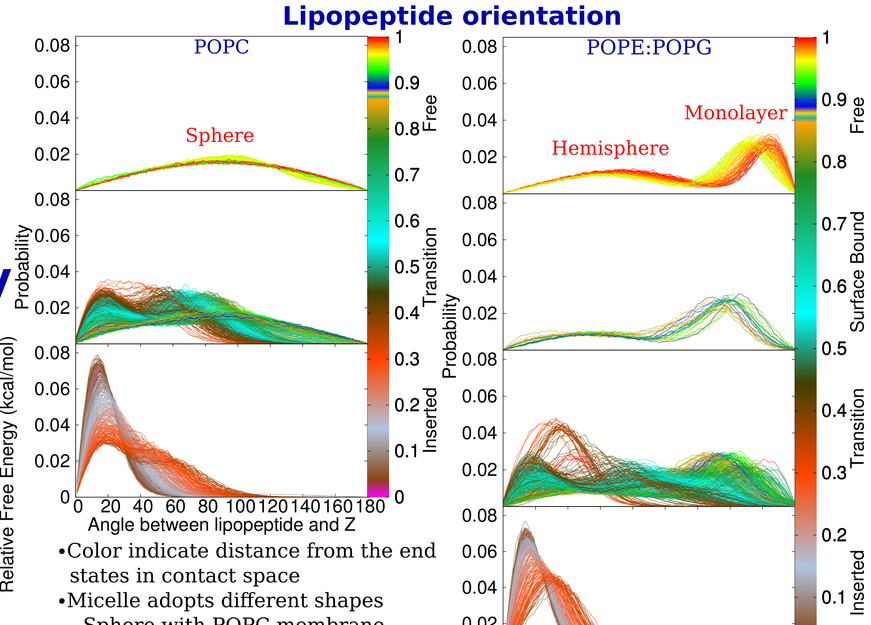
- 1D PMF around transition barrier



- States differing by 20 kcal/mol have very similar COM distance distribution

Binding Mechanism depends on membrane composition

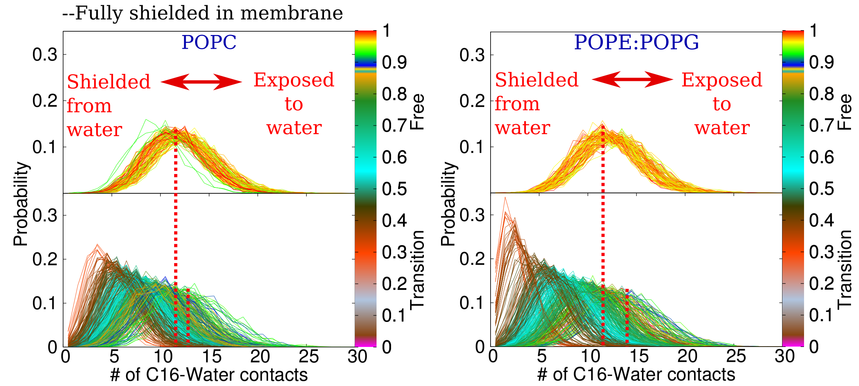
Lipopeptide orientation



- Color indicate distance from the end states in contact space
- Micelle adopts different shapes
  --Sphere with POPC membrane
  --Hemisphere-monolayer with POPE:POPG membrane

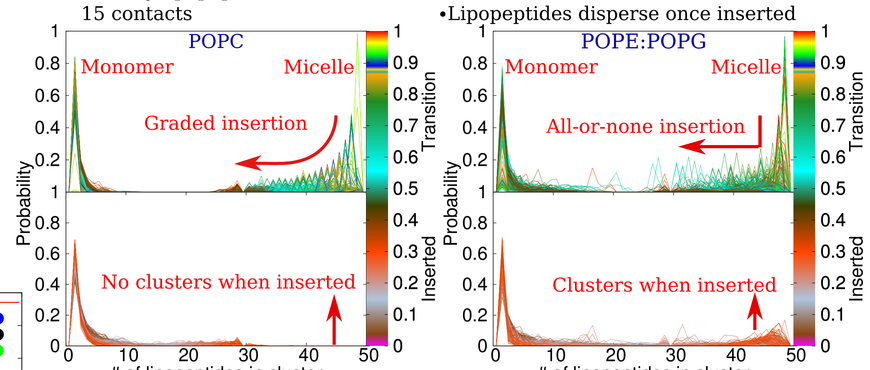
C16-Water contacts

- Hydrophobicity drives binding
  --Partially shielded in micelle
  --Fully shielded in membrane
- Exposure increases during insertion
  --More dramatic in POPE:POPG



Lipopeptide clusters

- Distribution of cluster sizes reports insertion mechanism
  --Define contacts by a 1 nm cutoff
  --Identify lipopeptides with at least 15 contacts
- Insertion mechanism varies with membrane composition
  --Graded insertion in POPC
  --More all-or-none in POPE:POPG
- Lipopeptides disperse once inserted



Conclusion

- Selectivity arises mostly from peptide-membrane repulsion
  --Peptides oppose binding to mammalian membrane
  --Neutral peptides don't bind bacterial membrane
- Acyl chains enhance binding
- Positive charges on peptides enhance selectivity
- Hydrophobic contacts characterize micellization and membrane insertion
- Lipopeptides micellize
  --More soluble in solution
  --Peptides aggregate on micelle surface
  --Much higher selectivity
  --High barrier to insertion due to the breaking of hydrophobic interaction
  --Mechanism of entry depends on membrane composition