ESTIMATING THE FREE ENERGY TO BIND A POTENT ANTIMICROBIAL LIPOPEPTIDE TO A MODEL MEMBRANE BILAYER

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Abstract

A series of synthetic antimicrobial lipopeptides (AMLPs) based around a common architecture of 4 amino acids (2 lysines), with a saturated fatty acid conjugated to the N-terminus, have been shown to have broad-spectrum antimicrobial activity and low hemolytic activity. Previous all-atom and coarse-grained molecular dynamics simulations from our group have shown that these molecules form micelles in solution and readily bind to model lipid bilayers. Here, we used microsecond-scale coarse-grained molecular dynamic simulations with the MARTINI force field to explore the thermodynamics governing the binding process, considering both isolated lipopeptides molecules and the micellar state. Using a combination of equilibrium umbrella sampling and non-equilibrium Jarzynski-style calculations, we estimate the binding free energy and explore the mechanism of entry. Our results provide biophysical insights into the mechanism of lipopeptides' antimicrobial action.

Antimicrobial Lipopeptides

- Tetrapeptides (2 lysines) 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

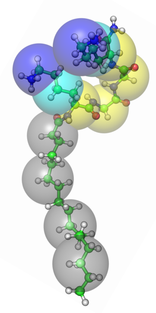
Origin of Selectivity

- 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?
- Computer simulation is an apt tool to use

Molecular Dynamics Simulation

All-atom Model
- Obtain trajectory of motions of all atoms in the system governed by classical mechanics
- Provide atomic and femto-second resolution
- Computationally expensive

CG model based on MARTINI force field
- Reduce the number of degrees of freedom in the system
  -- 4 heavy atoms ⟶ 1 pseudo-atom
- Computationally efficient
  -- Allow larger time-step in simulation



Umbrella Sampling and WHAM

- Biased samplings along a reaction coordinate *s* so that:

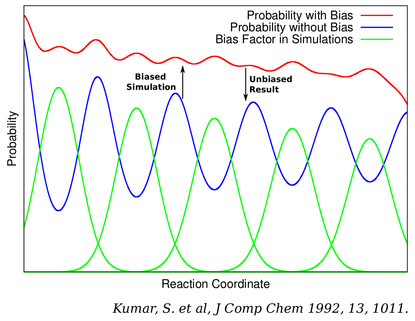
$$P_{bias,i}(s) = A_i C_i(s) P_0(s)$$

- Equilibrium distributions from biased simulations are unbiased and combined using Weighted Histogram Analysis Method:

$$A_i^{-1} = \sum_s C_i(s) P_0(s) \quad P_0(s) = \frac{\sum_{i=1}^{M} n_i(s)}{\sum_{i=1}^{M} N_i A_i C_i(s)}$$

- $P_0(s)$ and A_i are solved iteratively to get the potentials of mean force, Φ:

$$\Phi(s) = -k_B T \ln P_0(s)$$



Kumar, S. et al, J Comp Chem 1992, 13, 1011.

Steered Molecular Dynamics

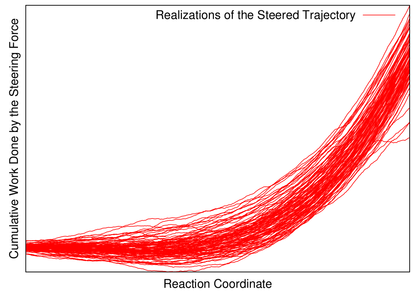
- Non-equilibrium simulation based on Jarzynski's Equality:

$$< e^{-\beta W} >= e^{-\beta \Delta G}$$

where W is the cumulative work done by external forces
- Stiff harmonic potential is used to steer the system from one state to another along a given reaction coordinate
- Potentials of Mean Force are estimated as:

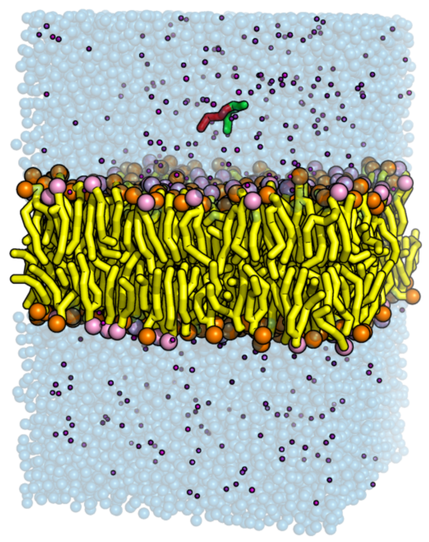
$$\Delta\Phi(\lambda(t)) = \Delta G(\lambda(t)) = -\frac{1}{\beta} \ln < e^{-\beta W(t)} >$$

where <> denotes the average over an ensemble of trajectories



Park, S. et al. J Chem Phys 2004, 120, 5946

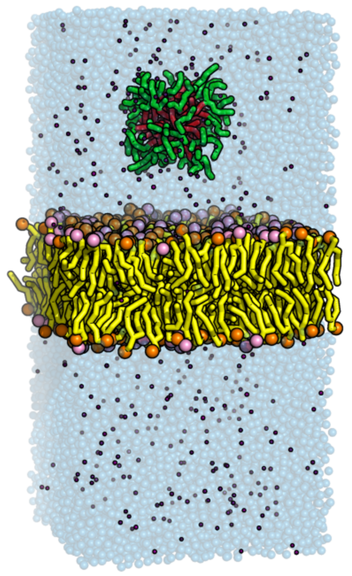
Single AMLP System



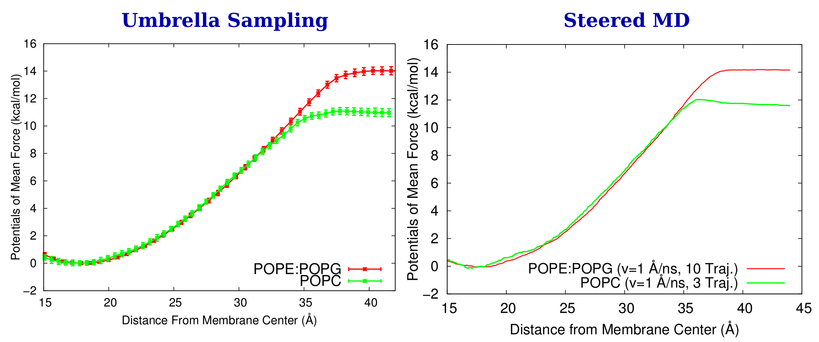
- Lipopeptide
  1 C16-KGG**K**
- Bacterial membrane model
  -- 320 POPE : 160 POPG
- Mammalian membrane model
  -- 480 POPC
- Physiological saltconcentration
  -- 109 NaCl Ions (plus neutralizing)
- High salt concentration
  -- 1090 NaCl Ions (plus neutralizing)
- 14757 water beads
- Typical force constants in umbrella samplings
  -- 2.39 kcal/(mol*Å^2)
- Total simulation time
  -- 81,325 ns

AMLP Micelle System

- Lipopeptide
  48 C16-KGG**K** in Micellar State
- Bacterial membrane model
  -- 320 POPE : 160 POPG
- Mammalian membrane model
  -- 480 POPC
- Physiological salt concentration
  -- 109 NaCl Ions (plus neutralizing)
- 24000 water beads
- Typical force constants in umbrella samplings
  -- 2.39 kcal/(mol*Å^2)
- Total simulation time
  -- 104,740 ns

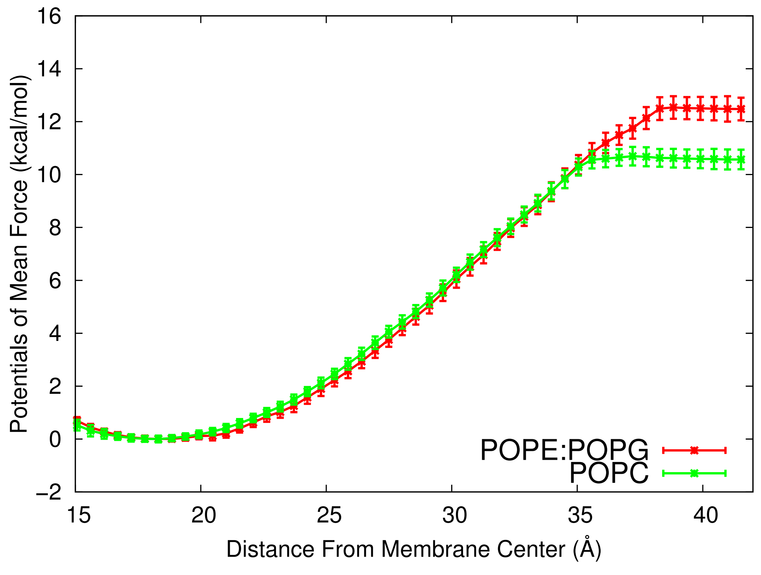


PMFs with 0.1 M NaCl



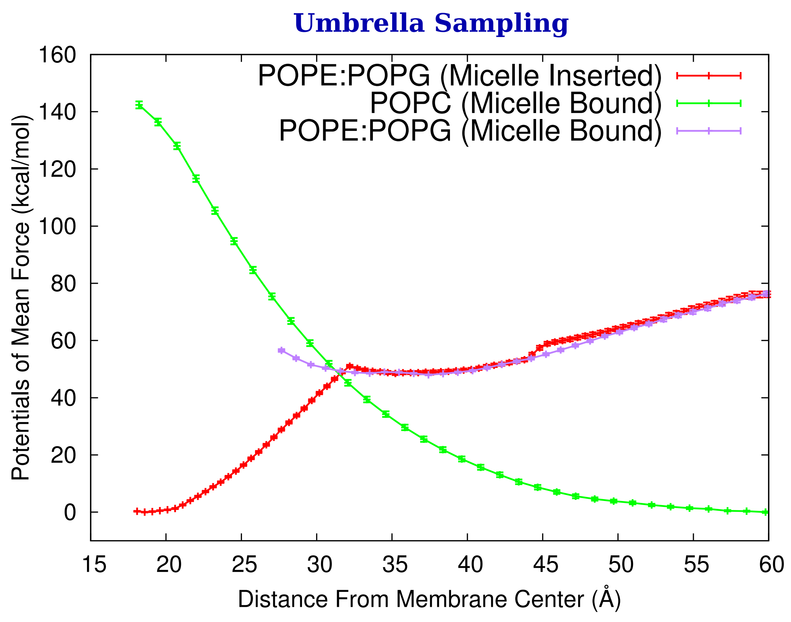
- Insertion is always preferred
- ΔΔG (POPE:POPG - POPC) is about 2.6 kcal/mol
- No barrier to insertion
- Results from the 2 methods matches closely

PMFs with 1M NaCl



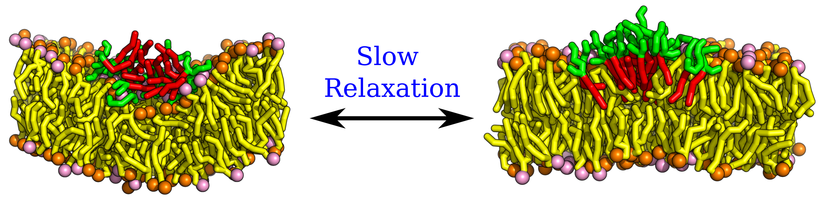
- ΔΔG is decreased to 1.9 kcal/mol
- Electrostatic interactions contribute a significant portion to binding

PMFs



- Binding to POPE:POPG is favorable
- ΔΔG of binding is 71.2 kcal/mol
- Binding to POPC is unfavorable
- Insertion is rarely observed

Problem: Hysteresis



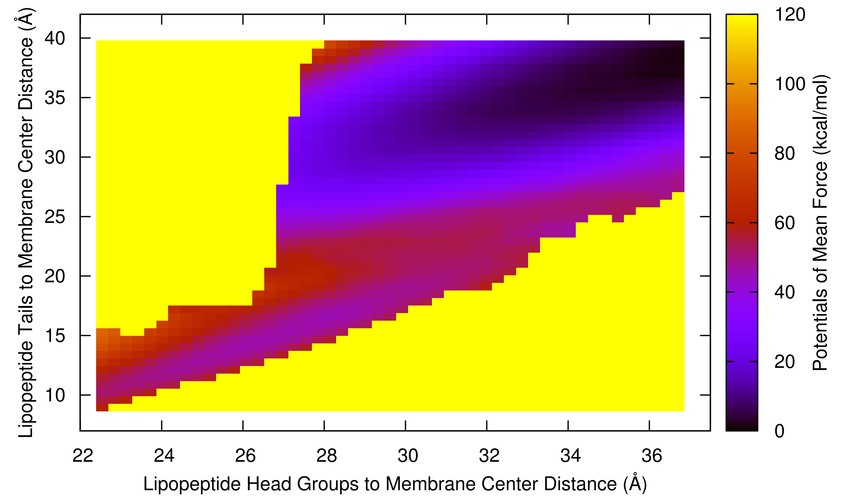
- The reaction coordinate is degenerate
  -- Slow degrees of freedom orthogonal to the reaction coordinate neglected
- Essentially doing "brute-force" samplings
- Inaccurate estimate of PMF

Possible Solutions:
- Allow sufficient relaxation time
- Bias other degrees of freedom

2-Dimensional Umbrella Sampling

- To control the opening of the micelle
- Reaction coordinate **x**: Distance between AMLP head groups and membrane center
- Reaction coordinate **y**: Distance between AMLP tails and membrane center
- Estimate the PMF as a function of (x, y)

2D PMF



- Bound state and insertion state are clearly distinguished
- Need very stiff harmonic potentials and thus large number of windows
- The barrier between the 2 states is roughly 20 kcal/mol
- Computationally expensive
- The answers are not converged

Conclusions

- For 1 lipopeptide, binding/insertion to membrane is always favorable compared to being free in solution
- The binding/insertion to bacterial (anionic) membrane (POPE:POPG) is even favored over that to mammalian (neutral) one (POPC)
- The results suggest that the selectivity of AMLPs arises from electrostatic interaction with membrane
- Better reaction coordinates are necessary to characterize the interaction between membrane and AMLPs micelle

Future Directions

- Find better reaction coordinates
- Calculate the PMFs under different conditions, e.g., salt concentration, other lipid and AMLP species
- Try other free energy calculation techniques such as Multi-Canonical Ensemble Method

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