

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



UNIVERSITY OF
ROCHESTER

Dejun Lin, Alan Grossfield
Department of Biochemistry and Biophysics, University of Rochester Medical Center



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 its 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 mixture of C16-KGGK, which resembles the aggregated structure of C16-KGGK in solution. We found the hydrophobic contacts of C16 and C16 to lipid tails are robust reaction coordinates to characterize C16-KGGK's 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

Origin of selectivity

- Different binding affinity to human and microbial membranes?
- Need to know the ΔG of binding or insertion to different membranes

Molecular dynamics simulation

All-atom (AA) force field

- Computationally expensive
- Large number of degrees of freedom (DOF)
- Small time-step (2 fs)
- Coarse-grained (CG) MARTINI force field
- Computationally efficient
- Fewer DOF, 4 heavy atoms \rightarrow 1 pseudo-atom
- Allows larger time-step (10 - 20 fs)

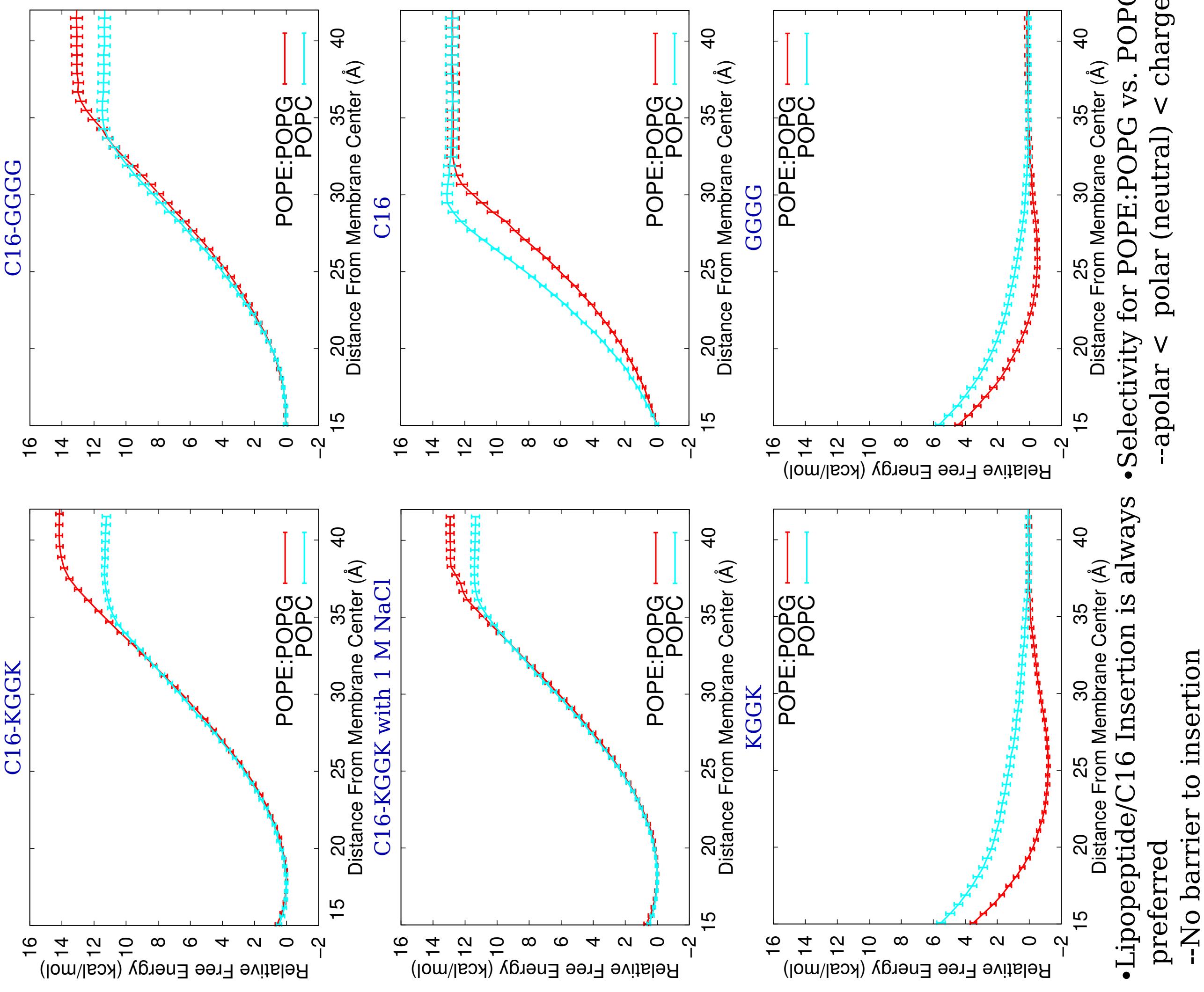
Umbrella sampling and WHAM

- 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 et al., J. Comp. Chem. 1992, 13, 1011

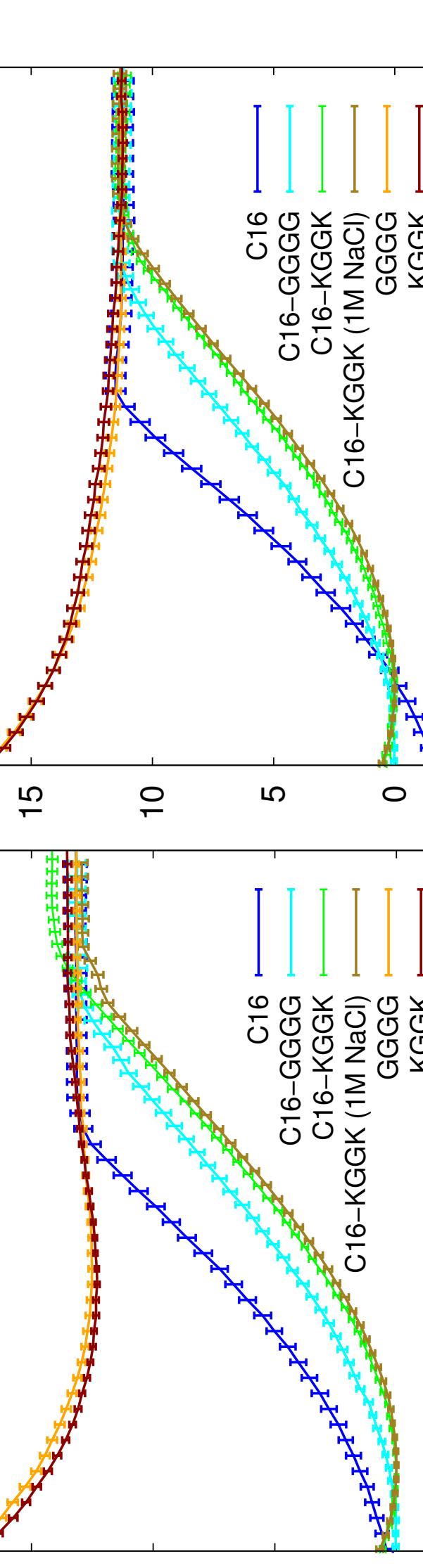
1-lipopeptide systems

- 3 different ligands
- 1 C16-KGGK
- 1 C16-GGG
- 1 C16
- 2 types of membrane
- Bacterial membrane model
- Mammalian membrane model
- Vary salt concentration
- Always have neutralizing ions
- 100 mM NaCl (low)
- 1 M (high)
- Reaction coordinate
- Ligand center of mass (COM) to membrane center along Z-axis
- NPT, P = 1 bar, T = 300 K
- Simulation time
- 30 to 35 windows/system
- About 1 μ s/window

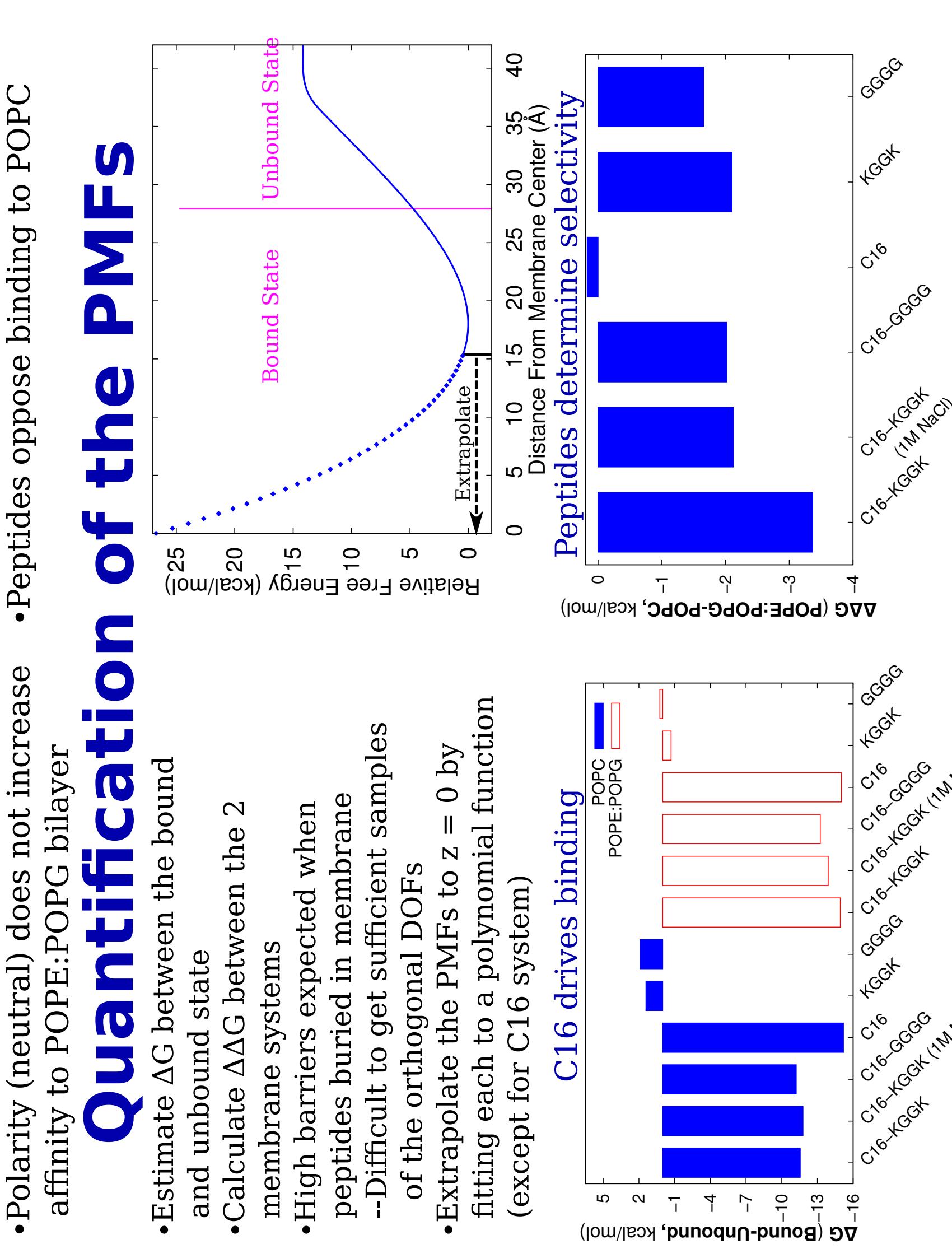
Polarity enhances selectivity



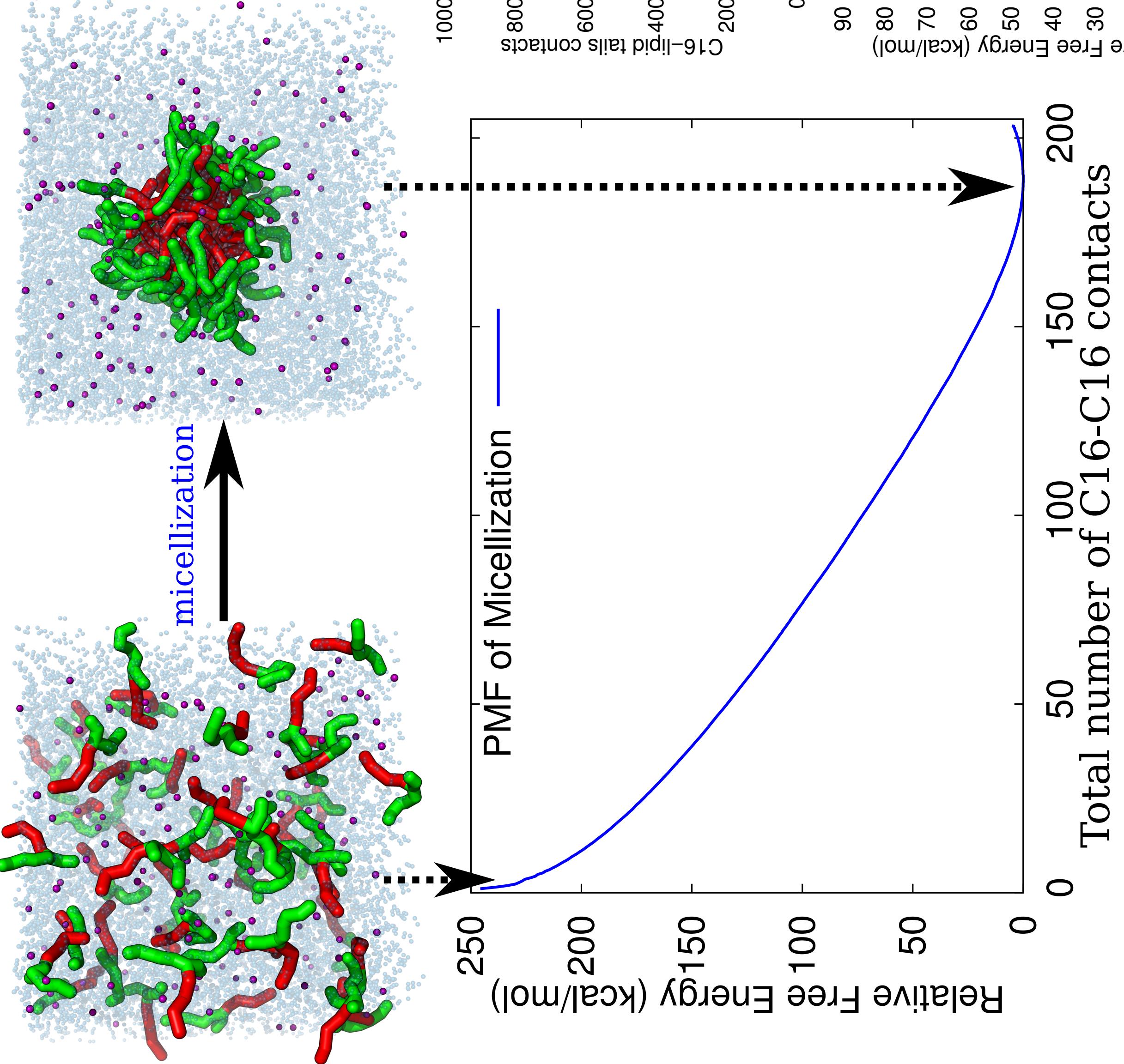
Affinity is membrane-dependent



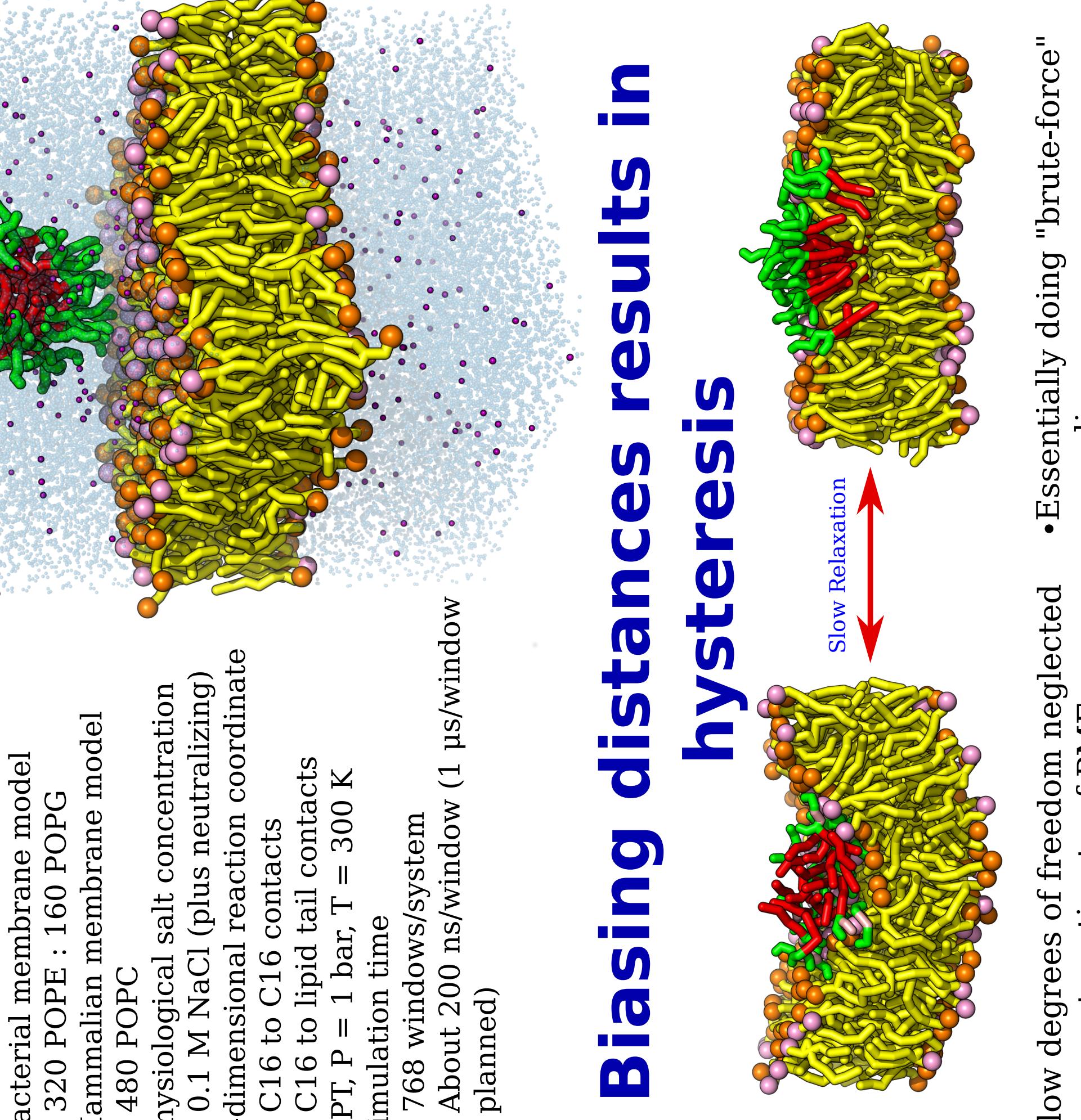
Biassing distances results in hysteresis



C16-KGGKs micellize in solution



C16-KGGK micelle-membrane system

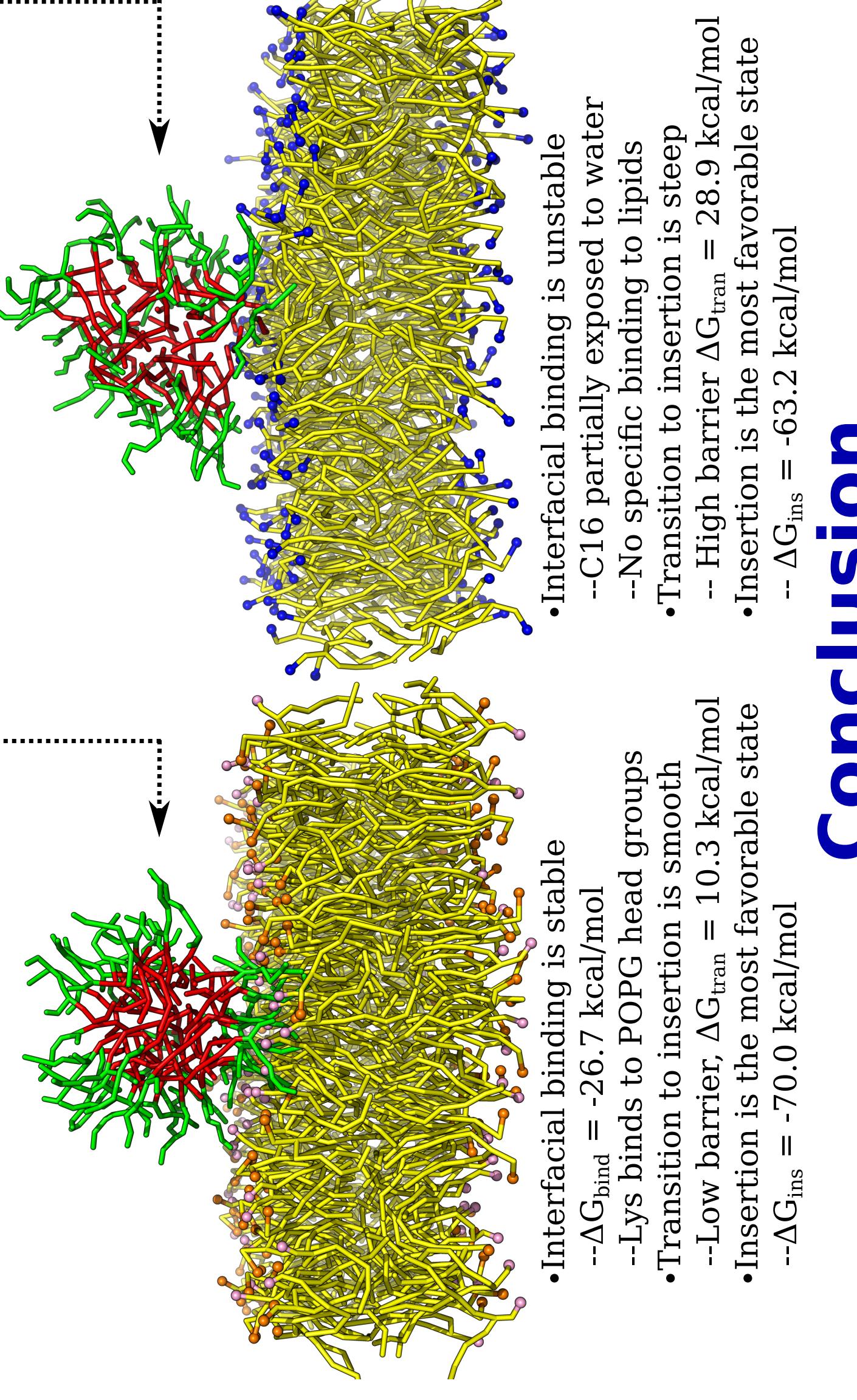
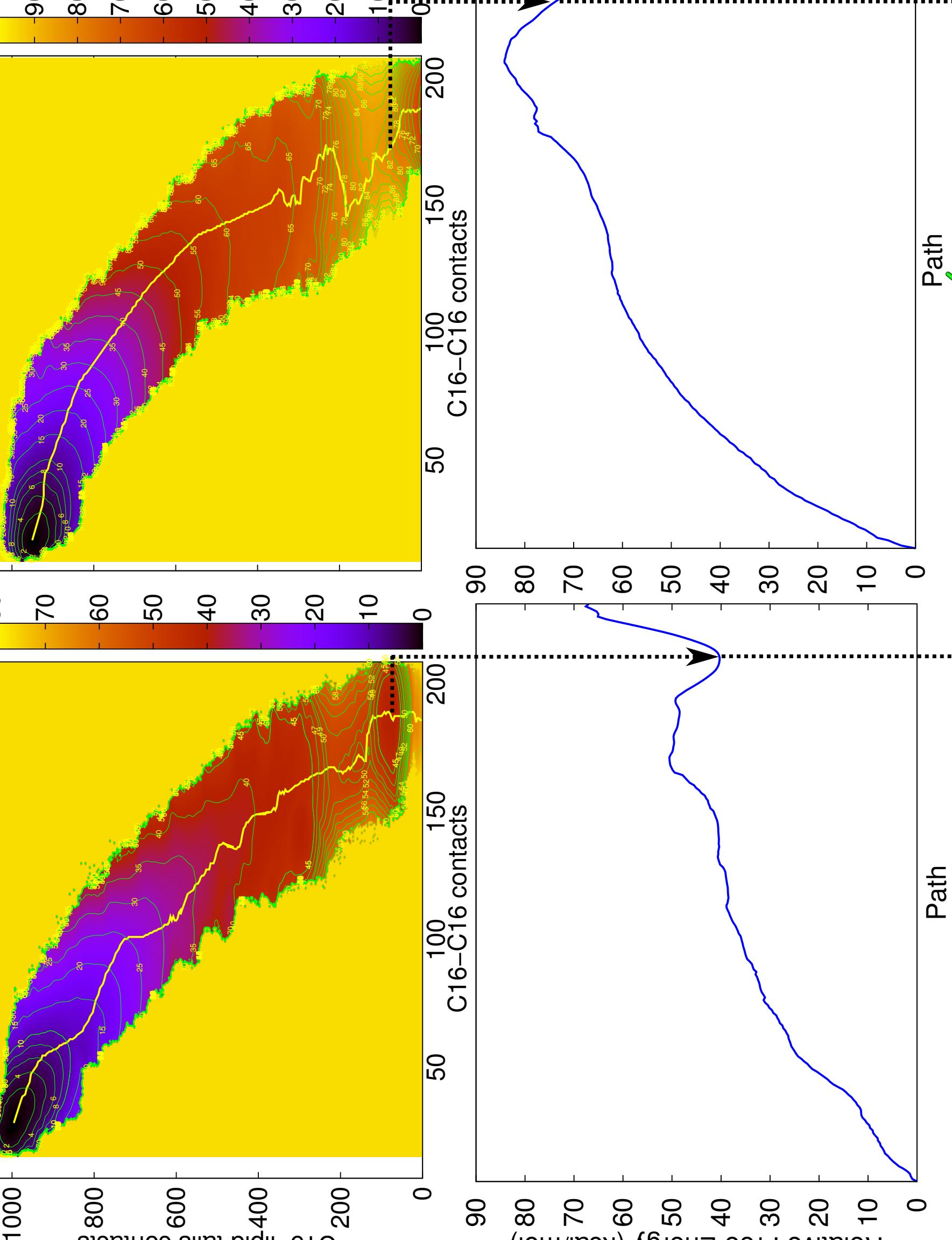


Hydrophobic contacts are robust reaction coordinates

- 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
 - No obvious hysteresis in steered MD runs
- Switching function of inter-group distances:

$$s(d) = (1 + \frac{d}{r_0})^{-1}$$
 - 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)

C16-KGGKs prefer binding to bacterial membranes



Conclusion

- Hydrophobic contacts characterize micellization and membrane insertion
- Lipopeptides oppose binding to mammalian membrane
- Neutral peptides don't bind bacterial membrane
- Acyl chains enhance binding
- Positive charges on peptides enhance selectivity
- Slow relaxation
- The Umbrella Sampling data is analyzed using WHAM (Weighted Histogram Analysis Method) implemented by Alan Grossfield.
- It's available at: <http://membrane.urnl.rochester.edu/content/wham>
- LOOS (Lightweight Object Oriented Structure analysis library) is a project of the [LOOS](http://loos.sourceforge.net) LightLab 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>