LACTOFERRICIN PEPTIDES CHARACTERIZED USING ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS AND SOLID STATE NMR

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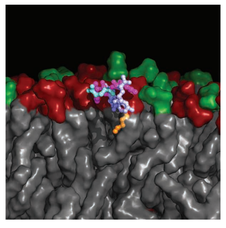
Poster PDF

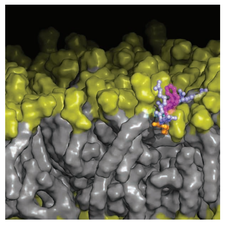
http://tinyurl.com/4b5jbqo

Abstract

Lactoferricin B is a cationic antimicrobial peptide with broad-spectrum effectiveness. A small hexapeptide (LfB6, RRWQWR-NH_2) extracted from this peptide has similar antimicrobial properties that can be enhanced by attaching a short fatty acid to the N-terminus (C6-LfB6). The mechanism for interaction between the antimicrobial peptide and the bacterial cell membrane is not well understood, but it is hypothesized to depend on lipid composition. Bacterial membranes generally contain a significant (20-25%) fraction of negatively charged lipids, in contrast with the zwitterionic mammalian membranes. In the case of LfB6, the presence of the tryptophans and arginines is thought to promote selective interactions with the negatively charged bacterial membranes. Here, we investigate the interactions of both LfB6 and C6-LfB6 with lipid bilayers using all-atom molecular dynamics simulations in concert with solid state ^{2}H NMR. In particular, we investigated the peptide interactions with a model bacterial membrane (3:1 POPE:POPG) and a model mammalian membrane (POPC), and compared our results to solid state ^{2}H NMR data. The results show subtle changes in the membranes and conformational substates of the lipopeptides, elucidating the effects of antimicrobial peptide binding.

Systems





3:1 POPE:POPG

- 100 Lipids per leaflet
  - POPE in Green, POPG in Red
- 7,900 waters (50% w/w)
- 50 mM salt (6 Cl^-, 50 Na^+)
- 49,172 total atoms

POPC

- 90 Lipids per leaflet
- 7,850 waters (50% w/w)
- 50 mM salt (21 Cl^-, 15 Na^+)
- 48,022 total atoms

- 2 Peptides per simulation (one shown)
  - C6 (orange)
  - Arg (magenta)
  - Trp (blue)
  - Gln (cyan)
- Minimal interaction between peptides

- CHARMM27 forcefield
- Electrostatics using PME
- 10 Å vdW cutoff
- NPγT at 50°C
- Tension: γ = 32.5 dyn/cm
- 2 fs time step, RATTLE
- NAMD-2.6 for BlueGene/P

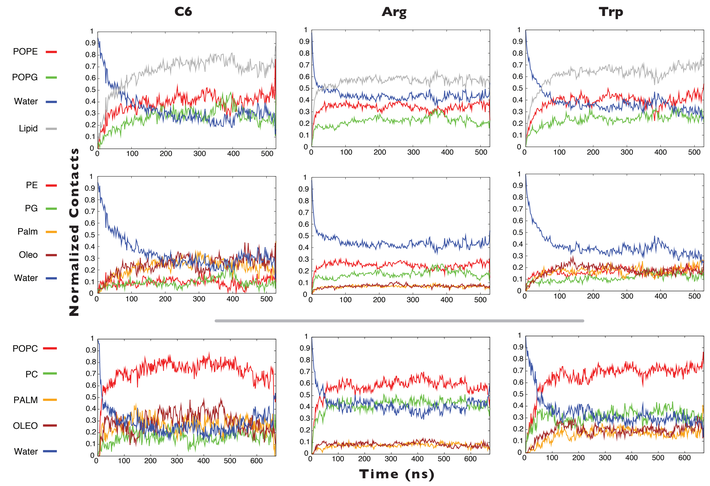
The first 100ns of each simulation is considered equilibration and excluded from area calculations

Membrane	Type	Tension (dyn/cm)	Length (ns)	Avg Length (ns)	Avg Area/Lipid (Å^2)	Avg Area (Å^2)
POPE:POPG	Neat	32.5	241.8 236.5 238.0	239.15	65.4 64.9 66.8	65.2 ± 0.4
		35	244.8 243.0	242.2	66.1 67.4	66.8 ± 0.5
		37.5	243.0 241.8 243.8	242.8	66.8 68.2 68.4	68.3 ± 0.1
POPE:POPG	C6-LfB6	32.5	535.7 531.7 530.2 529.8 350.2 344.7 333.4 280.8	429.6	65.5 66.3 65.6 65.4 65.5 65.1 65.4 65.3	65.5 ± 0.4
POPC	Neat	32.5	347.6 344.7	346.2	70.4 68.3	69.4 ± 1.5
POPC	C6-LfB6	32.5	584.6 672.1 663.6 651.8	634.0	71.1 71.1 71.1 71.1	71.1

Lipopeptide Binding Mechanism

Methods

- Peptide components: C6, Arg, and Trp.
  - 5Å radius sphere is probed
  - Count atoms within the sphere
  - Fractional contribution of fatty acid, lipid head group, and water
- Time series averaged across all simulations
  - Multiple trajectories needed to capture mechanism



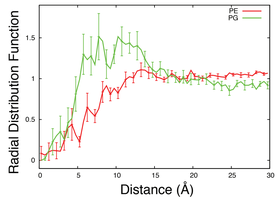
POPE:POPG

- Order of membrane association:
  - Arginines (~25 ns)
  - Tryptophans (~50 ns)
  - C6 Tails (~75 ns)
- Contacts made with POPG are nearly equal that of POPE, even though there are 3x as many POPEs
- C6 tails contact acyl chains more than head groups
- Arginines make more contacts with the POPE and POPG head groups
- Tryptophans contact all components equally

POPC

- Order of membrane association:
  - C6 Tails (~25 ns)
  - Arginines & tryptophans (~50 ns)

Radial Distribution



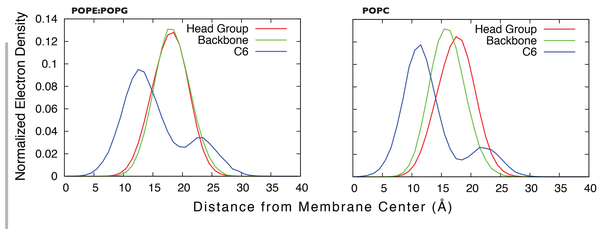
Methods

- Radial distribution function in the plane of the membrane for the PE and PG head groups after binding (~200 ns)
- Error bars are standard error considering each peptide as one sample

Discussion

- Local enrichment of anionic PG about cationic lipopeptide

Electron Density



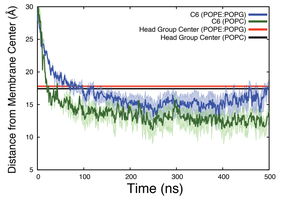
Methods

- Electron density is computed for the lipid head groups, peptide backbone atoms, and the C6 tail
- Density is symmetrized about membrane center
- Density for each component is then normalized

Discussion

- Peptide backbone and tail reside ~2Å deeper in the POPC membrane than POPE:POPG
- Tail can reside at the membrane-water interface in both systems
- Tail is 3x as likely to be buried

C6 Location



Methods

- Centroid for the C6 tail of each peptide
- Distance from the membrane center is averaged over all 8 simulations (thick line)
- Wide bands show the average distance for C1 and C6

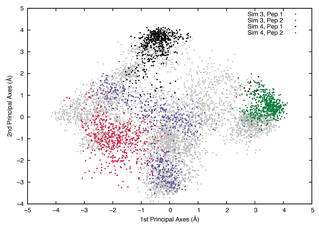
Discussion

- C6 tail enters the membrane faster in POPC and buries more deeply
- Tail generally oriented vertically within the membrane

Peptide Conformation

Methods

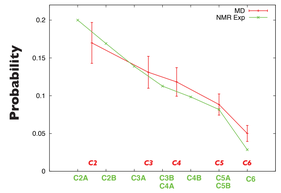
- C6-LfB6 extracted from all 8 simulations
  - 16 total peptides
- Principal component analysis (PCA) performed in LOOS
  - PCA on heavy atoms



Discussion

- Principal axes form a reduced basis "phase space" of C6-LfB6 conformations
- Projects conformations onto first two principal axes for classification
- Clusters indicate conformations that are structurally similar
- Each peptide is sampled for ~0.5 μs, yet clustering indicates little overlap between simulations
- Multiple, long simulations required to adequately sample configuration space of peptide

C6 Order (POPE:POPG)

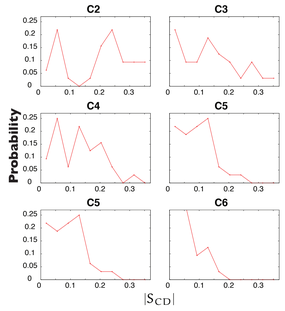


Methods

- Order parameters for C6 tail in POPE:POPG after binding
  - First 200 ns excluded
  - Standard error per peptide
- NMR shows evidence of additional peaks
  - Could be long-lived configurations

Discussion

- Simulation is consistent with experiment



Methods

- Time-averaged S_{CD} for each proton
- Probability distribution of $|S_{CD}|$ shown for each carbon

Discussion

- Evidence for long time-scale heterogeneity in order parameters
- Consistent with electron density plots

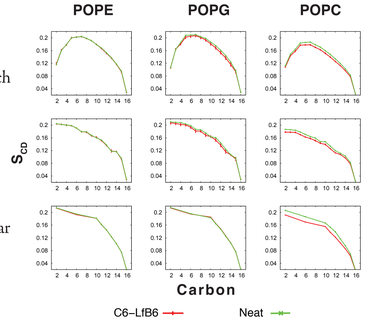
Effects on Membrane Structure: ^{2}H Order Parameters

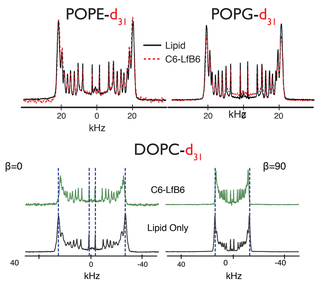
Methods

- Order parameters computed using LOOS
- Top panels show simulation order parameters
- Middle panels show simulation data sorted to match experimental data
- Lower panels show experimental data

Discussion

- Acyl C-H bond orientation relative to membrane normal: $S_{CD} = -\frac{1}{2}\langle 3\cos^2\theta_{CD} - 1\rangle$
- Experimentally measured by deuterium quadrupolar splitting in solid state NMR
- Slight decrease in order for POPG palmitoyls
- Experimental changes are subtle





Experimental Methods

- 0.25 μmol peptide: 25 μmol lipid (1:100)
- 50% hydration (by weight)
- 50°C
- Lipids (POPE:POPG [3:1], and POPC):
  - POPE-d_{31}:POPG
  - POPE:POPG-d_{31}
  - POPC-d_{31}
- Supported lipid bilayers

Conclusions

POPE:POPG

- C6-LfB6 rapidly associates with membrane
- Arginines lead the binding, followed by tryptophans and then the C6 tail
- C6 tail inserts into the membrane
- Slight decrease in POPG order
- Evidence of long-lived conformational heterogeneity
- Matches NMR experiment

POPC

- C6-LfB6 rapidly associates with membrane
- C6 tail leads the binding, with no preference between tryptophan and arginine
- Entire peptide inserts more deeply into the membrane
- Greater decrease in POPC order (though still subtle)
- Matches NMR experiment

See Posters B687 (today) and #3456 (B561) on 3/9 for related work

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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