CHARACTERIZATION OF MEMBRANE INTERACTIONS WITH LACTOFERRICIN PEPTIDES BY ALL-ATOM AND COARSE-GRAINED MOLECULAR DYNAMICS SIMULATIONS, SOLID-STATE NMR, AND FLUORESCENCE SPECTROSCOPY

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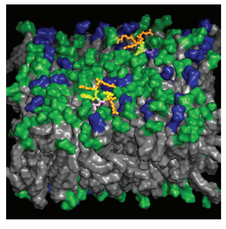
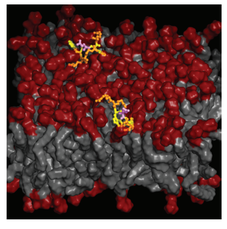
http://tinyurl.com/6t92pab

Abstract

LfB6 (RRWQWR-NH_2) is a small cationic antimicrobial peptide with broad spectrum effectiveness that is derived from bovine lactoferrin. The mechanism for interaction between the antimicrobial peptide and the bacterial cell membrane is hypothesized to depend on lipid composition. Bacterial membranes generally contain a significant fraction of negatively charged lipids in contrast with zwitterionic mammalian membranes. Previously, we characterized the interactions of an acylated LfB6 (C6-LfB6) with a model bacterial membrane (3:1 POPE:POPG) and a model mammalian membrane (POPC). Here, we investigate the interactions of the non-acylated LfB6 peptide with the same model membranes, using over 17 μs of all-atom molecular dynamics as well as 53 μs of coarse-grained simulations, and we compare our results to solid-state ^{2}H NMR and fluorescence spectroscopy. Molecular dynamics simulations reveal that the LfB6 peptide backbone does not penetrate as deeply in the model membranes as C6-LfB6 and that there is no preference in order of side-chain binding, unlike C6-LfB6. Further, molecular dynamics indicates the LfB6 tryptophans are more deeply buried in the membrane than C6-LfB6, yet fluorescence spectroscopy suggests they are more water-exposed. Coarse-grained molecular dynamics reveals that LfB6 comes off the membrane more easily than C6-LfB6, explaining the tryptophan membrane location and water exposure. The results also show subtle changes in the membranes' structure between the acylated and non-acylated peptides.

System Construction





All-Atom

3:1 POPE:POPG
- 2 peptides
- 100 lipids per leaflet
  - POPE in green, POPG in blue
- Solvated to 50% w/w (7,900 waters)
- 50 mM salt (plus neutralizing)
- ~49,000 atoms

POPC
- 2 peptides
- 90 lipids per leaflet
  - POPC in red
- Solvated to 50% w/w (7,850 waters)
- 50 mM salt (plus neutralizing)
- ~48,000 atoms

- CHARMM 27 forcefield
- Electrostatics using PME
- 10 Å vdW cutoff
- NPγT at 50°C

- γ = 32.5 dyn/cm
- 2 fs time step, RATTLE
- NAMD-2.6 for BlueGene/P

Coarse Grained

3:1 POPE:POPG
- 2 peptides
- 100 lipids per leaflet
- 2,000 waters
- 50 mM salt (plus neutralizing)
- NPT at 50°C

POPC
- 2 peptides
- 90 lipids per leaflet
- 2,000 waters
- 50 mM salt (plus neutralizing)
- NPT at 50°C

MARTINI forcefield v2.1 with GROMACS 4.5.3 and 4.5.4

Simulations

All-Atom

Membrane	Type	Tension (dyn/cm)	Length (ns)	Avg Length (ns)	Area / Lipid (Å^2)	Avg Area / Lipid (Å^2)
POPE:POPG	Neat	32.5	242	239	65.4	65.7
			237		64.9	
			238		66.8	
POPE:POPG	C6-LfB6	32.5	536	430	65.5	65.5
			532		66.3	
			530		65.6	
			530		65.4	
			350		65.5	
			345		65.1	
			333		65.4	
			281		65.3	
POPE:POPG	LfB6	32.5	862	1332	65.2	64.9
			1006		64.5	
			1661		64.9	
			1799		64.8	
POPC	Neat	32.5	348	381	70.4	69.9
			345		68.3	
			479		70.6	
			351		70.5	
POPC	C6-LfB6	32.5	585	643	71.1	71.1
			672		71.1	
			664		71.1	
			652		71.1	
POPC	LfB6	32.5	1145	996	70.9	70.8
			839		70.7	
			840		70.8	
			1160		70.8	

The first 100ns is considered equilibration and excluded from calculations

Coarse Grained

Membrane	Type	Length (ns)	Avg Length (ns)	Area / Lipid (Å^2)	Avg Area / Lipid (Å^2)
POPE:POPG	C6-LfB6	3100	3055	63.6	63.6
		3417		63.6	
		2701		63.6	
		3002		63.6	
POPE:POPG	LfB6	3428	3209	63.5	63.5
		3406		63.5	
		3001		63.4	
		3002		63.4	
POPC	C6-LfB6	3549	3368	67.8	67.8
		3620		67.8	
		3102		67.8	
		3202		67.8	
POPC	LfB6	4300	3679	67.7	67.6
		4400		67.6	
		3003		67.6	
		3012		67.7	

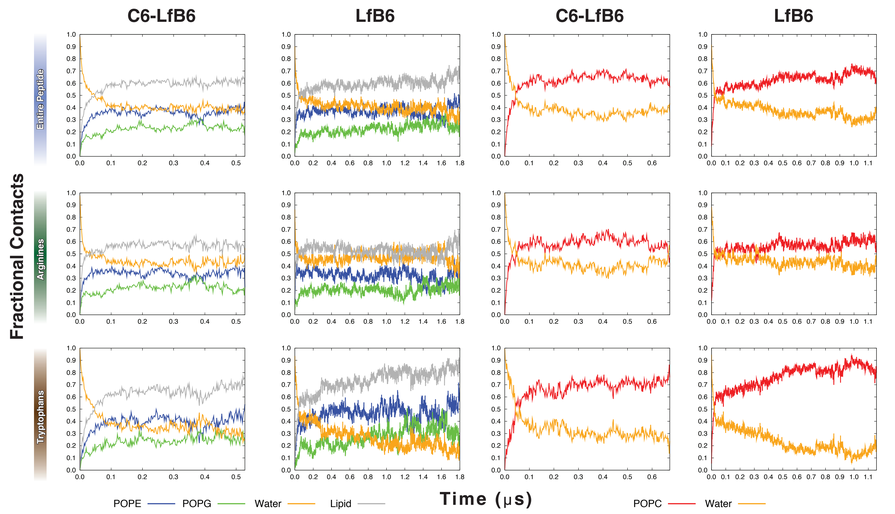
The first 500ns is considered equilibration and excluded from calculations

Lipopeptide Binding Mechanism

Methods
- 5Å probe radius
- Count atoms within the sphere
- Fractional contribution by different components
- Peptide heavy atoms probed for entire peptide, all arginine atoms, all tryptophan atoms, and all C6 tail atoms (not shown)
- Time series averaged across all simulations

POPE:POPG
- *Acylated:*
  - Arg touches first, followed by Trp and then C6
  - Trp has slightly more lipid contacts than Arg
  - POPG contacts nearly equal POPE despite 3:1 ratio in membrane.
- *Non-Acylated:*
  - No order seen in contact
  - POPG contacts nearly equal POPE despite 3:1 ratio in membrane
  - Trp makes more lipid contacts than acylated

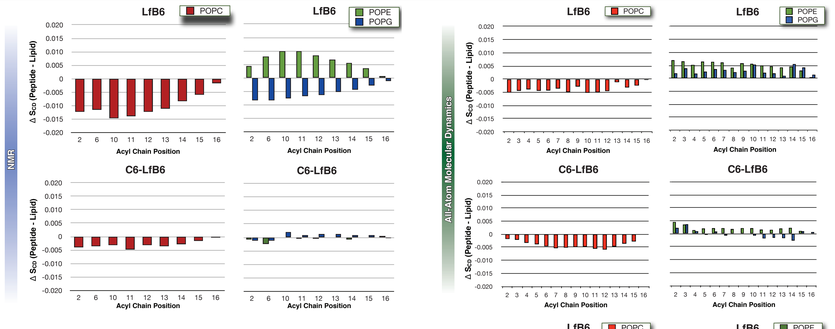
POPC
- *Acylated:*
  - C6 tails touch first (not shown), followed by Arg and Trp
  - Trp makes slightly more lipid contacts than acylated POPE:POPG
- *Non-Acylated:*
  - No preference in contact order
  - Trp makes more lipid contacts than acylated and non-acylated POPE:POPG



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

Methods
- Simulation order parameters calculated using LOOS
- 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

Discussion
- Subtle changes in membrane order for acylated peptide.
- Relative pattern of membrane order agrees between MD and NMR, despite differing absolute order parameters:
  - POPC < POPG < POPE



Distance-Based Order Parameters

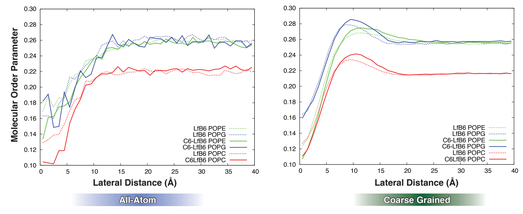
Methods
- Only use lipids on same leaflet as peptide
  - Lipids must be within 10 Å of a peptide in the plane of the membrane
- Order parameters calculated using LOOS as above

Discussion
- Peptide significantly decreases chain order at short range
- Slightly larger decrease in short range chain order for acylated peptide
- Larger decrease in POPC order, followed by POPG, and then POPE

Molecular Order Parameters

Methods
- Calculate principal components for lipids
- Use 2nd and 3rd principal components in lieu of C-H bond
- "Molecular Order Parameter" calculated as above
- Consider only lipids on the same leaflet as peptide
- "Molecular Order Parameter" binned based on lateral distance to nearest peptide

Discussion
- Significant short-range effect on membrane order in all systems and peptides
- Acylated peptide decrease short-range order more than non-acylated
- POPC < POPG < POPE
- Good agreement between all-atom and coarse-grained molecular dynamics
- Origin of "hump" in coarse-grained data unclear

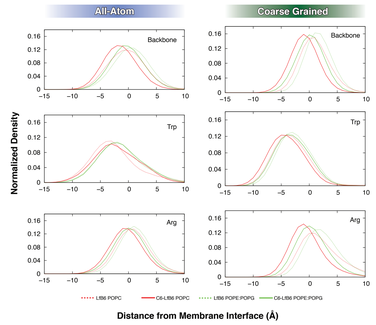


Peptide Water Exposure

Electron Density

Methods
- Electron density along the membrane normal is calculated using LOOS
- Membrane interface is defined as Z-axis location of peak lipid head group density
- Electron density for peptide backbone, tryptophans, and arginines plotted relative to membrane interface
- Density is normalized for visualization purposes

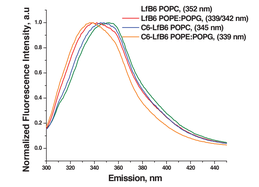
Discussion
- Backbone of acylated peptide resides deeper in both membranes than the non-acylated
- Backbone is buried more deeply in POPC than in POPE:POPG
- Trp is more deeply buried in POPC
- Arg remains near the membrane interface
- The acylated Arg is more buried than the non-acylated



Fluorescence Spectroscopy

Methods
- Trp emission fluorescence spectroscopy
- 1:50 peptide:lipid ratio

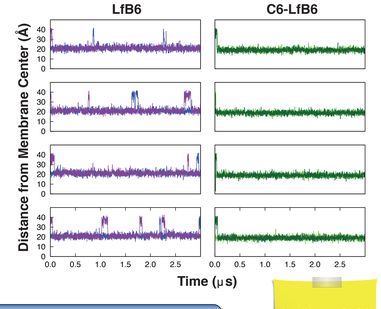
Discussion
- Non-acylated Trp in POPC is blue-shifted suggesting greater water exposure
- Lipid/water contacts and electron density plots indicate Trp is more deeply buried in the POPC membrane



Coarse Grained MD

Methods
- POPC membrane
- Distance along the membrane normal from the center of mass of the peptide to the membrane center plotted
- Representative simulations shown

Discussion
- Non-acylated peptides come off the membrane and rebind
- Acylated peptides do not leave the membrane during 12 μs of simulation in aggregate
- Unbinding explains greater water exposure of tryptophans despite deeper burial



Conclusions

- The acylated and non-acylated peptides both have subtle effects on the membrane
- The relative order is consistent between MD and NMR:
  - POPC < POPG < POPE
- Both peptides show significant membrane effects at short range
- Unlike the acylated peptide, the non-acylated peptide shows no preference in binding sequence
- The acylated peptide binds deeper in the membrane than the non-acylated one
- Tryptophans reside deeper in POPC membranes than POPE:POPG
  - Fluorescence suggests greater water exposure for Trp in POPC
- Coarse-grained MD shows that the non-acylated peptide comes off the POPC membrane, exposing the Trp to water
- Combining all-atom and coarse-grained MD reconciles the seemingly contradictory fluorescence and MD data
- Acylation increases the "stickiness" of the peptide

See Poster B174 on Feb. 26th for Related Work...

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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