# COMPARISON OF MEMBRANE INTERACTIONS OF ACYLATED AND NON-ACYLATED LACTOFERRICINS BY SOLID-STATE NMR SPECTROSCOPY AND MOLECULAR DYNAMICS


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## Abstract

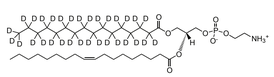

LfB6 (RRWQWR-$NH_2$) is a tryptophan- and arginine-rich antimicrobial peptide with broad spectrum effectiveness derived from bovine lactoferrin. Membrane binding occurs via electrostatic interactions between arginines and negative charges on the bacterial cell membrane and intercalation of the tryptophans at the membrane interface. N-terminal acylation ($CH_3(CH_2)_4CO$-RRWQWR-$NH_2$; C6-LfB6) can enhance the antimicrobial activity (Greathouse et al. (2008) J. Pept. Sci 14:1103). Solid-state $^2H$ and $^{31}P$ NMR spectroscopy combined with all-atom and coarse-grained molecular dynamics (CG-MD) simulations have confirmed subtle differences between 1:100 (peptide to lipid) LfB6 and C6-LfB6 in bilayers composed of 3:1 POPE:POPG (anionic, bacterial-like) and POPC (zwitterionic, mammalian-like). MD simulations reveal that the arginines of C6-LfB6 make first contact with POPE:POPG; whereas the C6 tails are first to contact POPC. LfB6 shows no sequence preference. Additionally, C6-LfB6 inserts more deeply than LfB6 into both membranes. Tryptophan emission fluorescence spectra suggests the tryptophans in LfB6 and C6-LfB6 are more water exposed in neutral compared to anionic membranes, while CG-MD simulations reveal that LfB6 comes off the POPC membrane, exposing the tryptophans to water. Acylation, therefore, increases the "stickiness" of the peptide for lipid bilayers. Although both peptides at 1:100 show significant membrane effects during short range simulations, C6-LfB6 has less influence on lipid order. We now compare experimental and molecular dynamics results for LfB6 and C6-LfB6 at 1:25 peptide to lipid. Solid-state $^2H$ NMR spectra indicate that C6-LfB6 has a greater effect on the lipid acyl chain order at 1:25 compared to 1:100; whereas the effects of LfB6 are similar at both concentrations. Molecular dynamics simulations will be presented for comparison.


## Experimental Methods

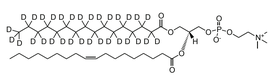
**LfB Peptides**

+$H_3$N-Arg-Arg-Trp-Gln-Trp-Arg-Amide

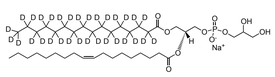
$CH_3(CH_2)_4CO$-Arg-Arg-Trp-Gln-Trp-Arg-Amide



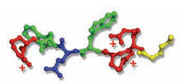
**POPE-d31 (neutral)**



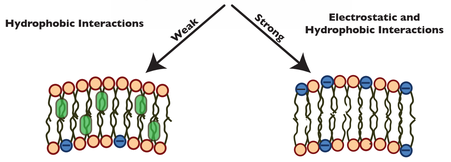
**POPC-d31 (neutral)**

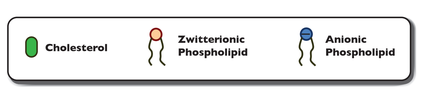


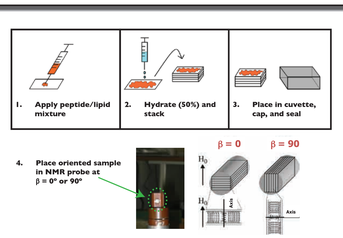
**POPG-d31 (anionic)**



**Acylated Lactoferricin Peptide**

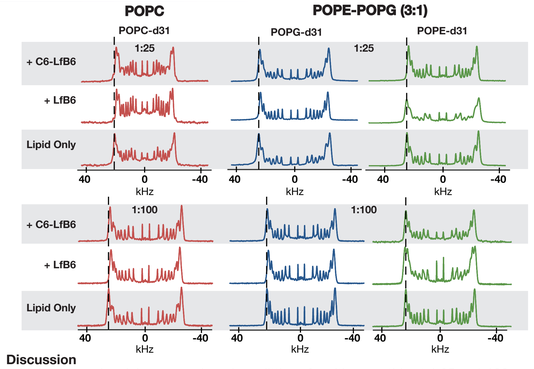



## Experimental Results

### $^2H$ NMR

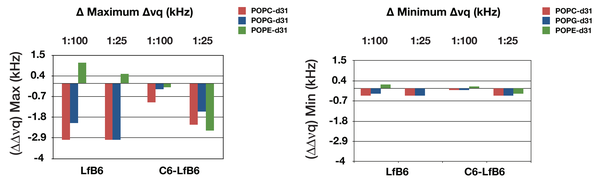



**Discussion**

- Little change in minimum quadrupolar splittings for either peptide at 1:25 or 1:100
- Larger changes in maximum splittings compared to minima for both peptides
  - Changes greater at 1:25 compared to 1:100 for C6-LfB6

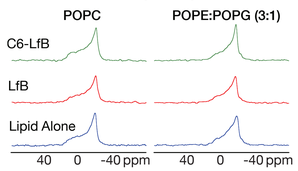
**Changes in Minimum and Maximum Quadrupolar Splittings (Δνq) for LfB Peptides**

| | 1:100 | | 1:25 | |
|---|---|---|---|---|
| | Minimum (kHz) | Maximum (kHz) | Minimum (kHz) | Maximum (kHz) |
| **POPC-d31** | 5.6 | 55.8 | 4.4 | 47.2 |
| + LfB Native | 5.2 (-0.4) | 52.8 (-3.0) | 4.0 (-0.4) | 44.2 (-3.0) |
| + C6-LfB Native | 5.5 (-0.1) | 54.8 (-1.0) | 4.0 (-0.4) | 45.0 (-2.0) |
| **POPE:POPG-d31** | 5.4 | 54.2 | 5.7 | 56 |
| + LfB Native | 5.1 (-0.3) | 52.1 (-2.1) | 5.3 (-0.4) | 53 (-3.0) |
| + C6-LfB Native | 5.3 (-0.1) | 53.9 (-0.3) | 5.3 (-0.4) | 54.5 (-1.5) |
| **POPE-d31:POPG** | 5.2 | 54.0 | 5.7 | 56.4 |
| + LfB Native | 5.4 (+0.2) | 55.1 (+1.1) | 5.7 (0) | 56.9 (+0.5) |
| + C6-LfB Native | 5.3 (+0.1) | 53.8 (-0.2) | 5.3 (-0.3) | 53.9 (-2.5) |




### $^{31}P$ NMR

**Multi-Lamellar Vesicles**
**1:50 Peptide:Lipid**

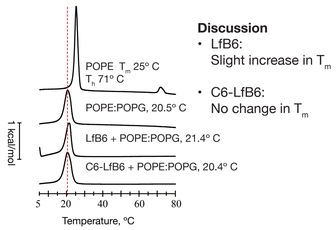



**Discussion**

- Minimal changes in $^{31}P$ CSA for POPC or POPE:POPG in presence of LfB6 or C6LfB6 (1:50)

### Differential Scanning Calorimetry

**POPE:POPG (3:1)**

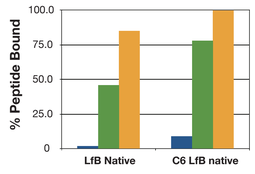



**Discussion**

- LfB6:
  Slight increase in $T_m$
- C6-LfB6:
  No change in $T_m$

### Partitioning Assays

**MLVs, 1:50,10 mM NaCl**
**10 mM Tris, pH 7.5**





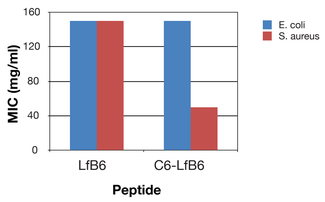


**Discussion**

- Minimal binding for LfB and C6-LfB6 in neutral lipid (POPC)
- Acylation enhances binding
- Even 10% DOPG (anionic) increases binding
- DOPE:DOPG (3:1) 80-100% bound

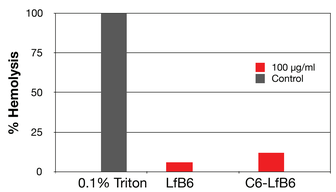
### Minimum Inhibitory Concentration

**LfB Peptides**




### Hemolytic Assay

**hRBCs**

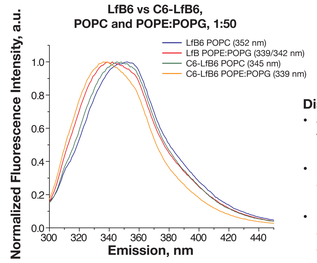



**Discussion**

- Little hemolytic activity
- C6-LfB6 slightly more hemolytic than LfB6

### Trp Fluorescence Spectroscopy

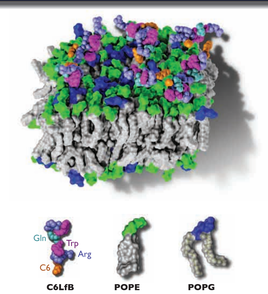
**LfB6 vs C6-LfB6,**
**POPC and POPE:POPG, 1:50**




**Discussion**

- Spectral shift indicates water exposure of Trp
- POPC:
  - LfB6 > C6-LfB6
- POPE:POPG
  - Less than POPC
  - LfB6 > C6-LfB6

## Molecular Dynamics




### Simulation Details

- 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 on BlueGene
- *~10.5 µs aggregate simulation time*
- All analyses performed using LOOS

See Poster B599 today for information about LOOS

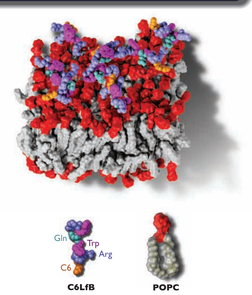
### System Construction

**3:1 POPE:POPG**

- 8 peptides
- 100 lipids per leaflet
  - POPE in green, POPG in blue
- Solvated to 50% w/w (7,092 waters)
- 50 mM salt (plus neutralizing)
- ~48,000 atoms

**POPC**

- 8 peptides
- 90 lipids per leaflet
  - POPC in red
- Solvated to 50% w/w (7,105 waters)
- 50 mM salt (plus neutralizing)
- ~47,000 atoms




## Molecular Dynamics

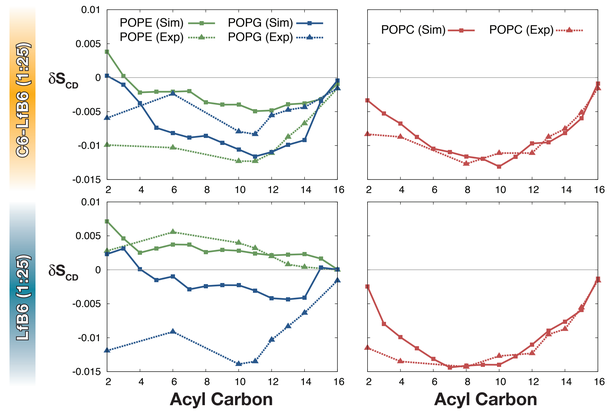
### Membrane Order Parameters

**Methods**

- Acyl C-H bond orientation relative to membrane normal
- Experimentally measured by deuterium quadrupolar splitting in solid state NMR
- $\delta S_{CD}$ is the difference in order parameter between the membrane with peptide and the neat membrane

**Discussion**

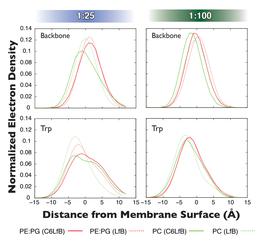
- Order decreased for POPC by both C6-LfB6 and LfB6
- Order decreased for POPE and POPG by C6-LfB6
- Order increased for POPE by C6-LfB6, but POPG is decreased




### Water Exposure (Electron Density)

**Methods**

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




**Discussion**

- Backbone is buried more deeply in POPC than in POPE:POPG
- Trp is more deeply buried in POPC
- Trp density broadened for acylated peptide
- Appears to contradict fluorescence data
- Coarse-grained umbrella sampling indicates peptide is less "sticky"
- Umbrella sampling with coarse-grained MD gives ΔΔG estimates for POPE:POPG vs POPC:
  -2.9 kcal/mol (C6-LfB6)
  -2.8 kcal/mol (LfB6)
- Estimated ΔΔG from acylation:
  -1.5 kcal/mol (POPC)
  -1.7 kcal/mol (POPE:POPG)

## Conclusions

- LfB6 and C6-LfB6 peptides have minimal effects on the lipid head groups of neutral and anionic lipids by $^{31}P$ NMR
- $^2H$ NMR Spectra reveal that:
  - Both peptides have little effect on the order of the terminal methyl groups of POPC, POPE, or POPG at 1:100 or 1:25
  - Both peptides decrease the order of all lipids in vicinity of head groups, except for LfB6 in POPC which increases the order
  - The effects on lipid order for C6-LfB6 at 1:100 and 1:25 are similar
  - C6-LfB6 results in larger decrease in order at 1:25 compared to 1:100
  - Broadening of POPE-$d_{31}$ $^2H$-line widths is observed in the presence of LfB6, but not C6-LfB6
- Simulations show same trend in membrane order as experiment for 1:25
  - Reduced order is a short-range effect
- Trp residues in both peptides are more water-exposed in neutral POPC membranes than in anionic mixture POPE:POPG
- Coarse-grained umbrella sampling indicates peptides associate less tightly with POPC membranes than POPE:POPG despite going deeper when bound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