Self-organized peptide lipid complexes:

Peptide induced transmembrane water pores.

Cell Membranes

• Membrane structures highly dynamic.
• Transformations primarily driven by lipid physics
• Modulated by proteins
Cell Membranes

DMPC
2,3-dimyristoyl-sn-phosphatidylcholine (C14:0)

Continuous lipid matrix
However partial breakdown required for:

• transport of ions,
• transport small molecules
• membrane fusion etc.
Pore formation in lipid bilayers.

Pores formed:
- spontaneously
  *(how primitive cells could take up ions)*
- during aggregation
- after application of lateral tension
- electroporation
- Membrane curved at the openings
- Local perturbation of lipids.
- Hour-glass shape or toroidal pore.

S.J. Marrink
Pore Forming Peptide Toxins: Models of Membrane Protein Assembly

- Released into the environment
- Soluble in water
- Recognize and bind specifically to membranes
- Assemble spontaneously into functional complexes

Models for peptide induced pore formation/stabilization.

barrel stave         carpet                    toroidal pore

Are these true?
What is known:
1. bind to membrane.
2. aggregate.
3. form pore (release ions).

Proposed General mechanism

- Short cationic peptides from the skin of *Xenopus Leavis* (African clawed frog).
- Permeabilize the lipid matrix -> cell death.
- Bind preferentially to anionic lipids i.e. outer membrane of the bacterial cells.
- Broad antibacterial and anticancer activity (not hemolytic).
- Suggest formation of a toroidal pore (Neutron scattering).

**Magainin H2 (MG-H2)**

**sequence:**

```
ILE ILE  LYS  LYS  PHE LEU  HIS  SER  ILE  TPR  LYS
PHE  GLY  LYS  ALA  PHE  VAL  GLY  GLU  ILE  MET
ASN  ILE  +q   +q   +q   +q   +q   +q   +q   +q
```

**properties:**
- amphipatic
- binds to zwitterionic lipids
- structure: 50% α-helical (CD spectra in the presence of PCs)
- forms pores by cooperative action
- pore diameter ~ 2nm
- proposed to form a toroidal pore
Proposed Mechanism of Toroidal Pore Formation


Magainin H2 (MG-H2)

- Migrates to interface
- Embeds
- Partial helical structure
- No pore

1 copy placed randomly in solution (no tension)

Hari Leontiadou
Magainin H2 (MG-H2)

- Migrates to interface
- Embeds
- Significant helical structure
- PORE FORMATION

2 copies placed in solution P/L 1:32 (tension 20mN/m)

Leontiadou H, Mark AE, Marrink SJ 2006 Antimicrobial peptides in action JACS 128, 12156-12161

One peptide, nothing happens …

Two peptides, still nothing happens.

Four peptides, however …

LYS

PHE/TRP
**Magainin H2 (MG-H2)**

- Aggregates
- Migrates to interface
- Embeds

Spontaneous PORE FORMATION

4 copies, initially in the solution P/L 1:16
no tension

Leontiadou H, Mark AE, Marrink SJ 2006 Antimicrobial peptides in action JACS 128, 12156-12161

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The final structures of five independent simulations

Highly disordered yet still compatible with most of the available experimental data.

Leontiadou H, Mark AE, Marrink SJ 2006 Antimicrobial peptides in action JACS 128, 12156-12161
Antimicrobial Peptides: Case 2 Melittin

Principle component of Bee venom
26 amino acids.
+6 charge at pH 7.0
Acts by forming toroidal pores
Gly-Ile-Gly-Ala-Val_leu_lys-Val-Leu-Thr-Thr-Gly-
Leu-Pro- Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-
Gln-Gln-NH2

Effect of Melittin on Lipid Bilayers

Effect of Melittin on Lipid Bilayers

A. Below a critical concentration pores did not form.
B. Pores did not form if the peptides did not cluster.
C. Pore formation required at least 3 peptides.
D. Screening the charge interactions slows pore formation.
E. Removing positively charged amino acids blocks pore formation.
Disordered Model For a Toroidal Pore

Mechanism
• Asymmetric binding of peptides induces stress in second layer.
• Spontaneous formation of pore.
• Pore stabilized by binding primarily to the entrance of the pore.

Advantages:
• Simple
• Does not require insertion of peptides into lipid matrix.
• Pore metastable (Collapse as peptides migrate through the pore?).
Antimicrobial peptides from Australian frogs

Structure in membrane mimic environments

Mode of Action

- **Positive** curvature linked to formation of toroidal pores and micelles, compared to \( H_i \)

- **Negative** curvature explained by the aggregate model, non-bilayer intermediate resembles \( H_{II} \)

- **Cubic** phases can lead to porous membrane structure or fragmentation into micelle like structures
Peptides inducing positive curvature

- Peptides lining channel walls
- Peptide binding to surface
- Cause formation of highly curved micelles after breaking the bilayer

Sequence

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>AA</th>
<th>Net charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aurein 1.2</td>
<td>GLFDIJKIAESF-NH₂</td>
<td>13</td>
<td>+1</td>
</tr>
<tr>
<td>Citropin 1.1</td>
<td>GLFDVIKKVASVIGGL-NH₂</td>
<td>16</td>
<td>+2</td>
</tr>
<tr>
<td>Maculatin 1.1</td>
<td>GLFGVLAKVAAHVVPAIAEHL-NH₂</td>
<td>21</td>
<td>+3</td>
</tr>
<tr>
<td>Caerin 1.1</td>
<td>GLLGSVAKHVLPHVVPVIAEHL-NH₂</td>
<td>25</td>
<td>+4</td>
</tr>
</tbody>
</table>
Structure

Aurein 1.2

Citropin 1.1

Maculatin 1.1

Caerin 1.1

Structure of Aurein 1.2 on DMPC bilayers

T=303 K, anisotropic pressure coupling (Corrected GROMOS 53a6)
Aurein: Water 300K

Structure of Aurein 1.2 in 20 mol % TFE (50 volume %)

T=303 K, isotropic pressure coupling
Structure of Maculatin 1.1 in TFE (top) and on DMPC bilayers (bottom)

Aurein with PG-Cardiolipin membrane

60/40% PG/CL (tail ratio)
Aurein with PG-Cardiolipin membrane
60/40% PG/CL (tail ratio)

The interaction of Kalata B1 within membranes
(binding and self-assembly)
Rong Chen
August 25, 2010
Introduction

- Kalata B1 (KB1)
  - A 29-residue cyclic peptide isolated from plants
  - Remarkably stable structure
    - Head-to-tail cyclic backbone
    - Three disulfide bonds between 6 Cysteines
  - Biological activities include
    - Antivirus, antimicrobial, antifouling, etc.
  - Mechanism of action
    - Membrane mediated

August 25, 2010

Structural representation of KB1

Recordings of asolectin patches when 25 µM KB1 was added

A


A proposed model for pore formation by KB1

Aims

- To investigate
  - How KB1 binds to membranes
  - The association of KB1 into oligomers
  - How KB1 may form trans-membrane water-filled pores

Methods

- Molecular dynamics simulations
  - GROMOS 53a6 force field
  - GROMACS 3.3.3 simulation engine
  - 4 fs time step
  - Twin-range cutoff (0.8 nm, 1.4 nm)
  - Reaction field
  - Berendsen weak-coupling method (NpT)
Simulation one

- Initial structures
  - 1 copy of KB1
  - POPC bilayer
    - 64 lipids/leaflet
  - Explicit water
  - Na⁺ and Cl⁻ (0.1 mol/L)
  - 300K→350K

Progressive binding of KB1 into the POPC bilayer

0 ns
40 ns
80 ns
160 ns
200 ns
220 ns
Sim5, T=300 K

Pro13
Trp19
Val21
Leu27-Val29
Spatial structure of KB1 in DPC micelles derived from experiment

Attenuation in a 100-ms NOESY spectra of KB1-DPC micelle complex when 5-doxystearate (Δ) or 16-doxystearate (○) was incorporated.

Proposed model for KB1 in DPC (dodecylphosphocholine) micelles.

Compare simulation to experiment

Simulation E (5)

Sim5, 220 ns

Experiment
Aggregation Studies

- Initial structures
  - 4 or 8 copies of KB1 (~6mM/L)
  - POPC bilayer
    - 128 lipids/leaflet for 4 KB1
    - 256 lipids/leaflet for 8 KB1
  - Explicit water
    - 300 K (150 - 300 ns) → 350 K (30 ns)
  - Two simulations for each system

Initial structure (4 KB1, water and ions not shown)
KB1 readily forms oligomers in solution

No spontaneous formation of pores
Self association via loops 6 and 1

![Bar chart showing frequency of residues in Loop 6 at T=350 K]

Stabilization of pre-formed pores

- Initial structure
  - A pore was preformed by 8 dimers of KB1

![Images showing top and side views of the initial structure]

August 25, 2010
The pore diameter shrank from 4.5 nm to 2.0 nm in 90 ns

Stabilization of pre-formed pores

- A equilibrated membrane pore
One KB1 placed above the pore

Sim1, 10 ns

Sim2, 10 ns

T=350 K

Fixed area in the bilayer plane

Rapidly inserts deeply into the rim of the pore (< 10 ns)
More peptides added

T=350 K
Fixed area in the bilayer plane

Conclusions

- Can reproduce the structure of KB1 bound to a membrane suggested by experiment
- KB1 can form tetramers or higher
- KB1 shows a strong preference for binding to regions of positive curvature