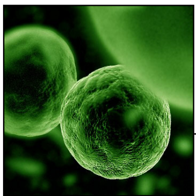
Self-organized peptide lipid complexes:

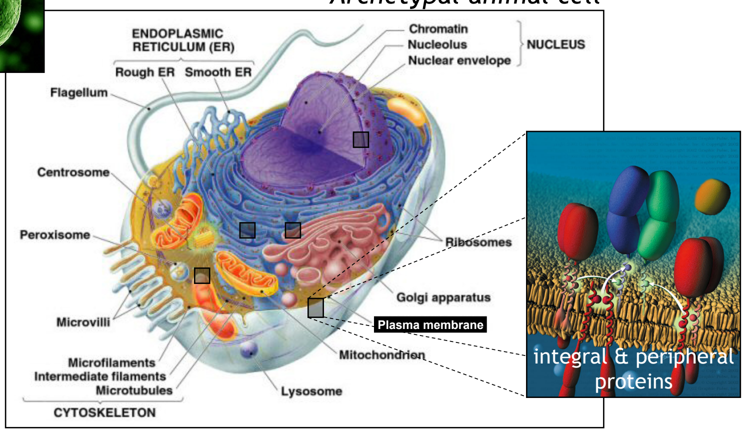
Peptide induced transmembrane water pores.

Cell Membranes

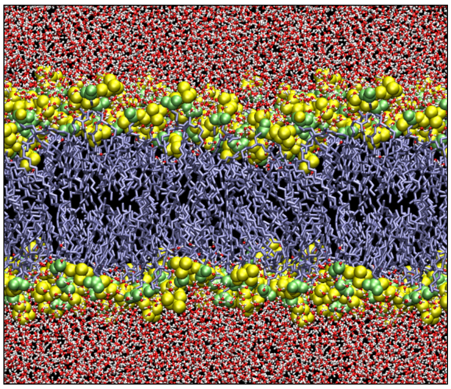


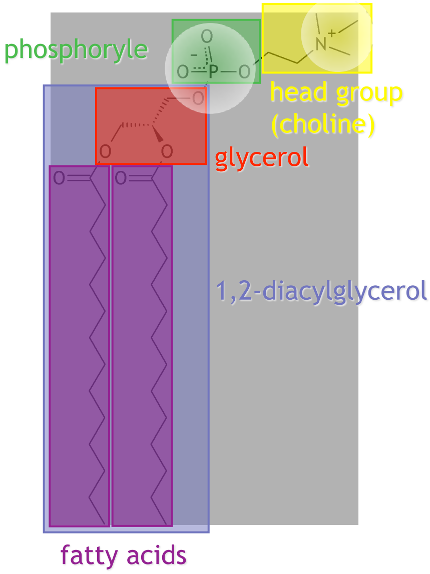
- Membrane structures highly dynamic.
- Transformations primarily driven by lipid physics
- Modulated by proteins

Archetypal animal cell



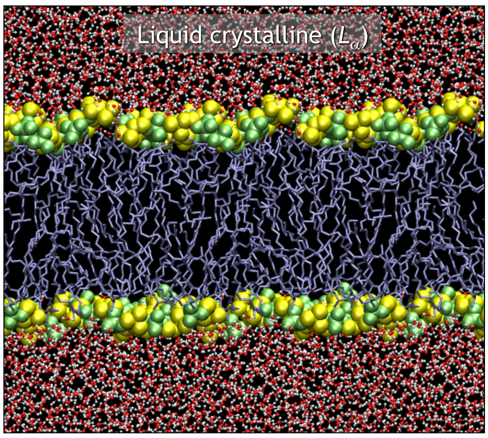
Cell Membranes





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

Cell Membranes



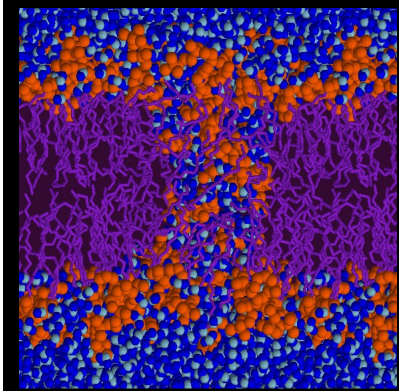
Continuous lipid matrix

However partial breakdown required for:

- transport of ions,
- transport small molecules
- membrane fusion etc.

Pore formation in lipid bilayers.

water pore

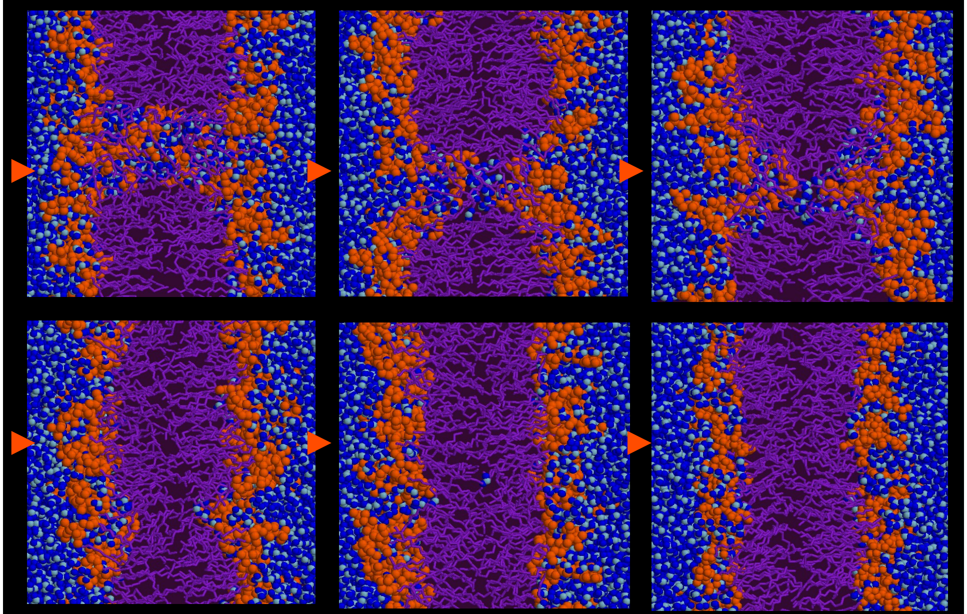


Pores formed:
- spontaneously
(how primitive cells could take up ions)
- during aggregation
- after application of lateral tension
- electroporation

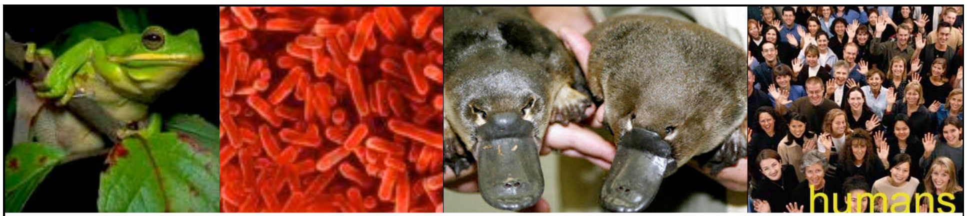
- Membrane curved at the openings
- Local perturbation of lipids.

- Hour-glass shape or toroidal pore.

Pores Metastable : Spontaneous Closure



S.J. Marrink

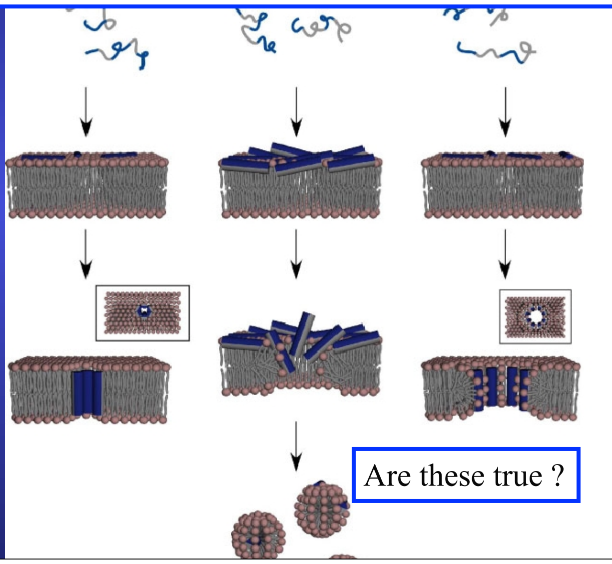


Pore Forming Peptide Toxins:
Models of Membrane Protein Assembly

- 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

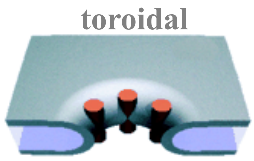


Pore formation by antimicrobial peptides: Case 1 Magainins

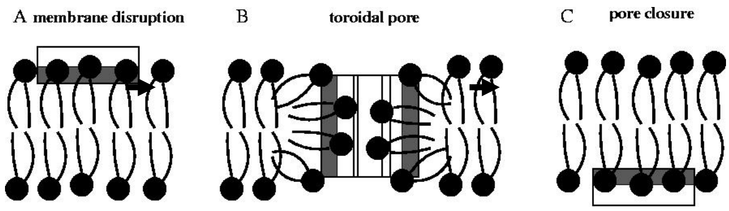
- Short cationic peptides from the skin of *Xenopus Leavis (*African clawed frog).
- Permeabilize the lipid matrix -> cell death.
- Bind preferentially to anionic lipids i.e. outer membrane of the bacterial cells.
- Broad antibacterial and anticancer activity (not hemolytic).
- Suggest formation of a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

What is known:

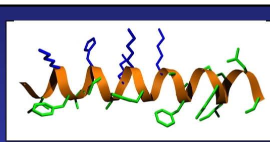
1. bind to membrane.
2. aggregate.
3. form pore (release ions).



Proposed General mechanism



Magainin H2 (MG-H2)



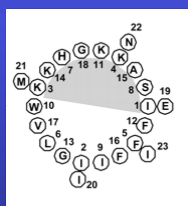
sequence:

ILE ILE LYS(+q) LYS(+q) PHE LEU HIS SER ILE TPR LYS(+q)

PHE GLY LYS(+q) ALA PHE VAL GLY GLU(-q) ILE MET

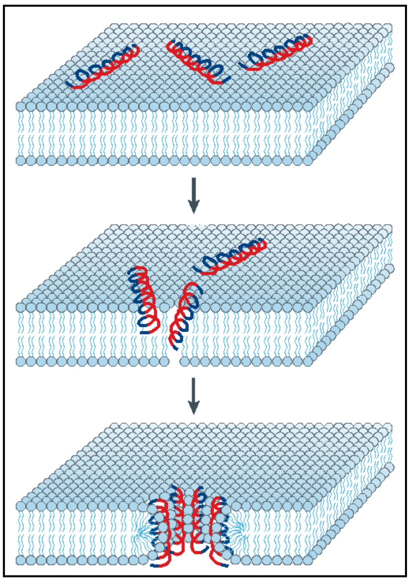
ASN ILE

23 amino acids
total charge 3+



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

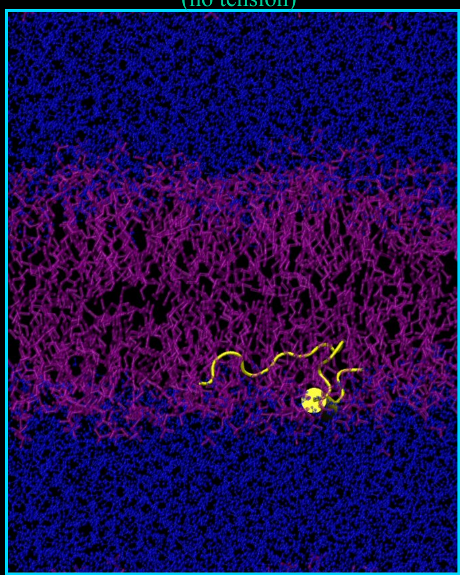
Proposed Mechanism of Toroidal Pore Formation



Brogden, Nat. Rev. Micr. 3,238-250(2005)

Magainin H2 (MG-H2)

1 copy placed randomly in solution (no tension)



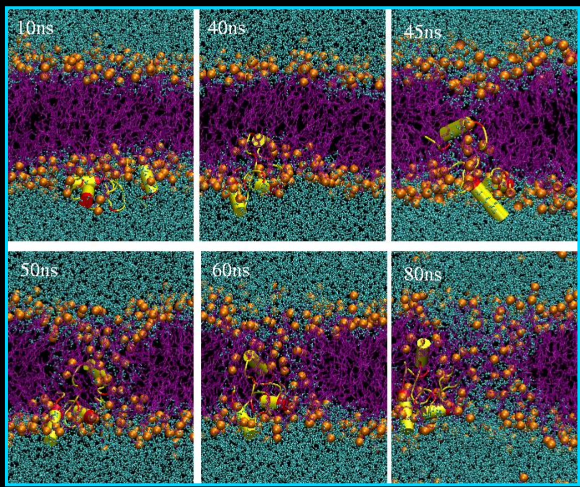
Hari Leontiadou

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

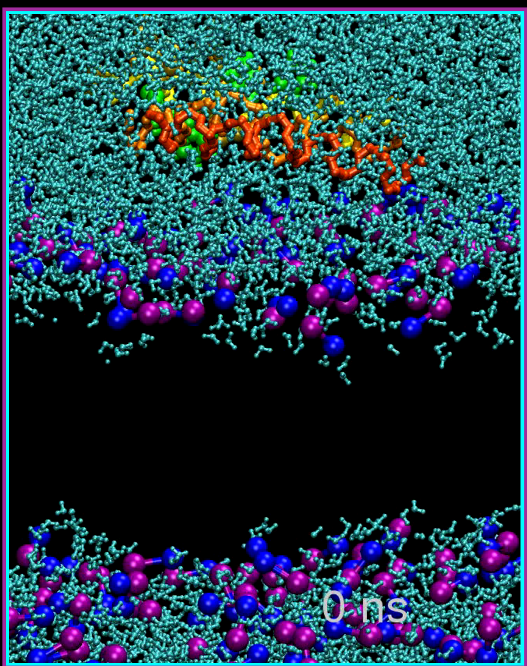
Magainin H2 (MG-H2)

- 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



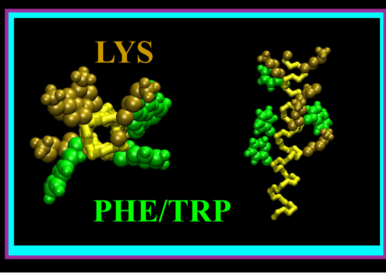
One peptide,

nothing happens …

Two peptides,

still nothing happens.

Four peptides, however …

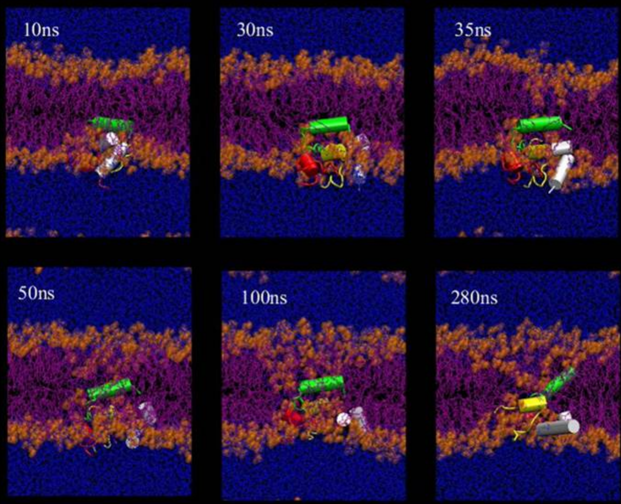


Magainin H2 (MG-H2)

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

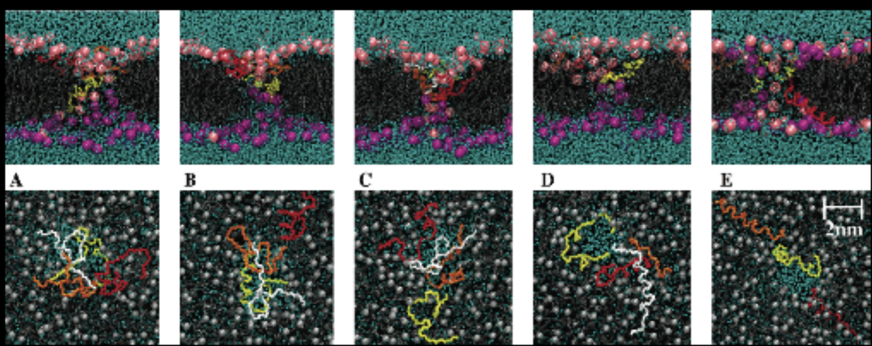
- Aggregates
- Migrates to interface
- Embeds

<u>Spontaneous PORE FORMATION</u>



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

The final structures of five independent simulations



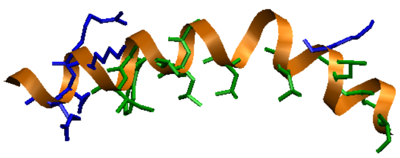
Highly disordered yet still compatible with most of the available experimental data.

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

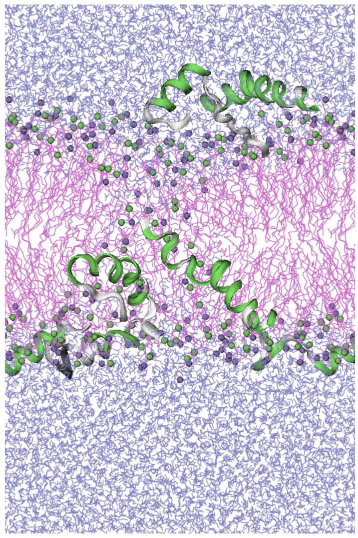
Antimicrobial Peptides: Case 2 Melittin

Principle component of Bee venom
26 amino acids.
+6 charge at pH 7.0
Acts by forming toriodal 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



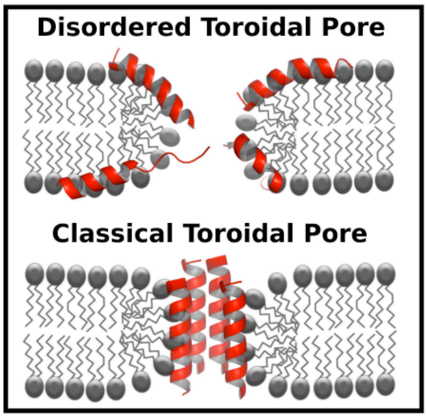
Melittin

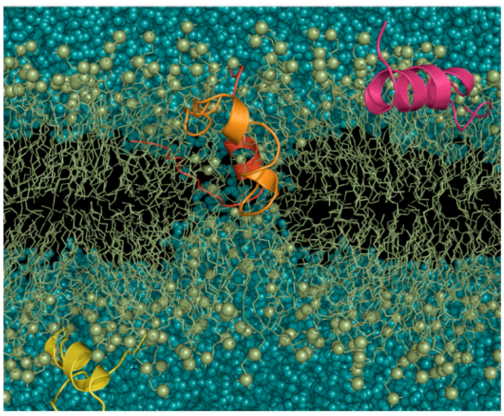


6 melittin, GROMOS 43a2 FF
128 DPPC *(Berger Parameters)*
$NP_{||}P_zT$ ensemble; Reaction Field; PBC
Temp. 323K ; SPC water

Durba Sengupta

Effect of Melittin on Lipid Bilayers



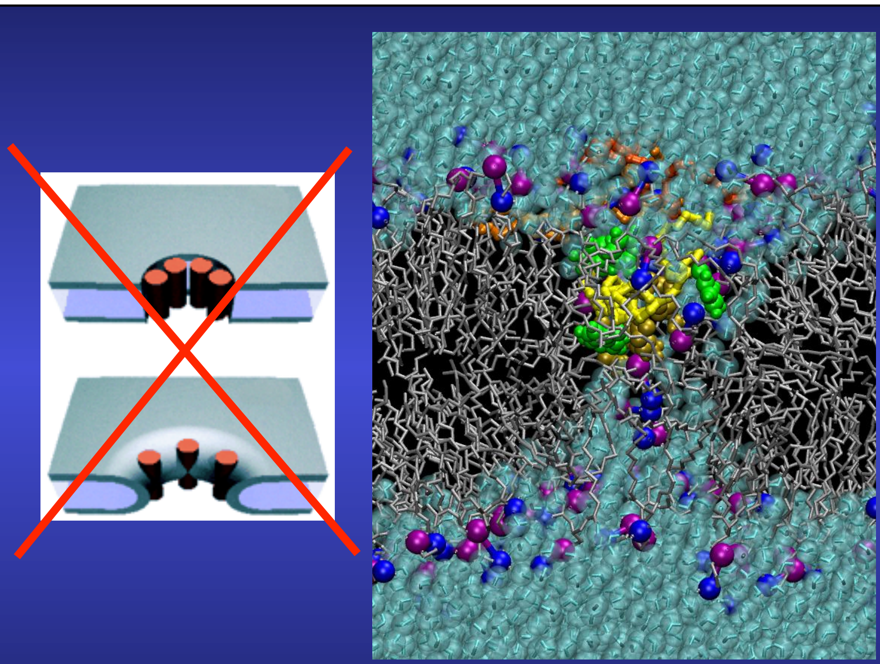


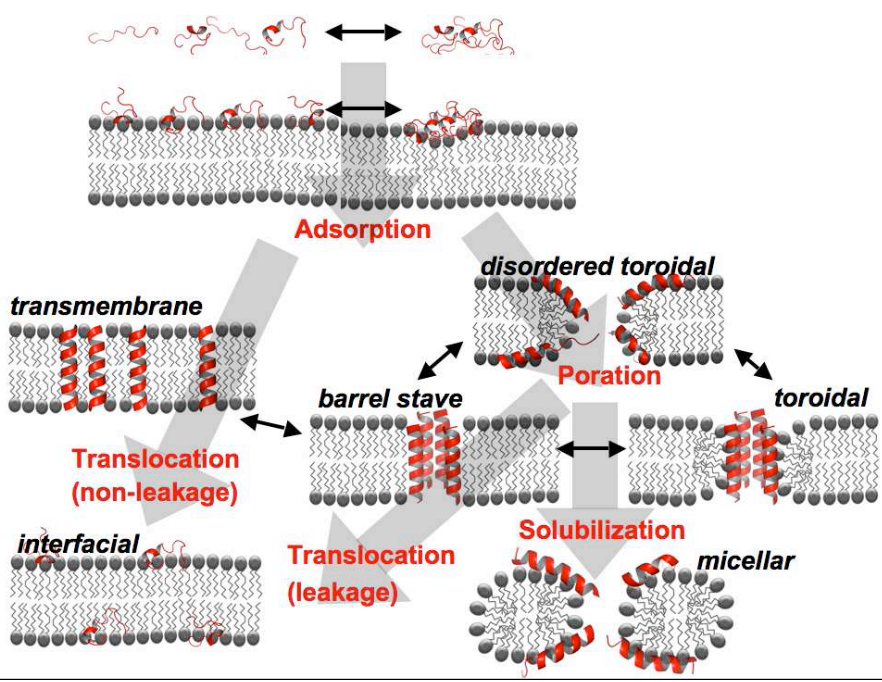
Sengupta et al. *Biochim. Biophys. Acta - Biomembranes.* 2008, **1778,** 2308-2317.

Effect of Melittin on Lipid Bilayers

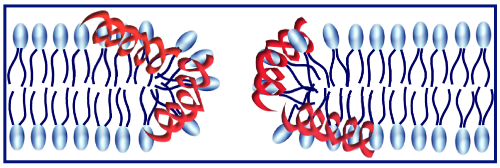
A. Below a critical concentration pores did not form.
B. Pores did not form if the peptides did not cluster.
C. Pore formation required at least 3 peptides.
D. Screening the charge interactions slows pore formation.
E. Removing positively charged amino acids blocks pore formation.

Durba Sengupta





Disordered Model For a Toroidal Pore



Peptides not fully Inserted
Partially Unfolded

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?).*

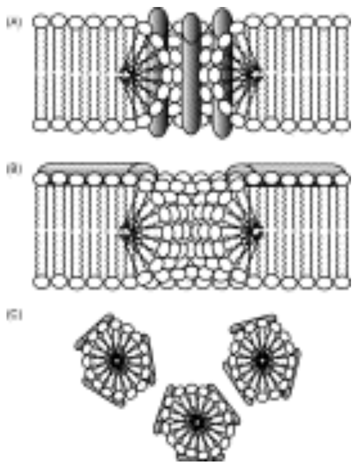
Antimicrobial peptides from Australian frogs

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

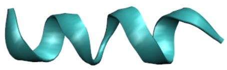


- Peptides lining channel walls
- Peptide binding to surface
- Cause formation of highly curved micelles after breaking the bilayer

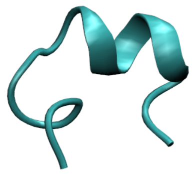
Sequence

Peptide	Sequence	AA	Net charge
Aurein 1.2	GLFDIIKKIAESF-NH_2	13	+1
Citropin 1.1	GLFDVIKKVASVIGGL-NH_2	16	+2
Maculatin 1.1	GLFGVLAKVAAHVVPAIAEHF-NH_2	21	+3
Caerin 1.1	GLLSVLGSVAKHVLPHVVPVIAEHL-NH_2	25	+4

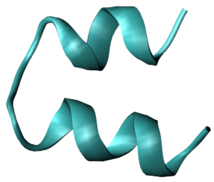
Structure



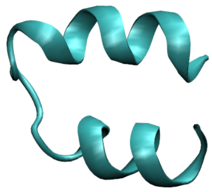
Aurein 1.2



Citropin 1.1

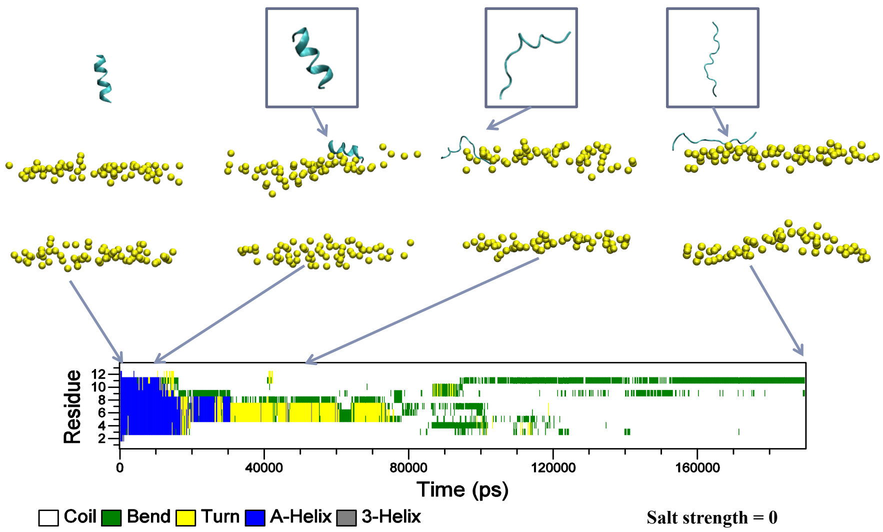


Maculatin 1.1



Caerin 1.1

Structure of Aurein 1.2 on DMPC bilayers

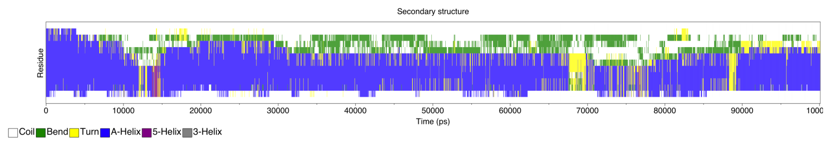


Salt strength = 0

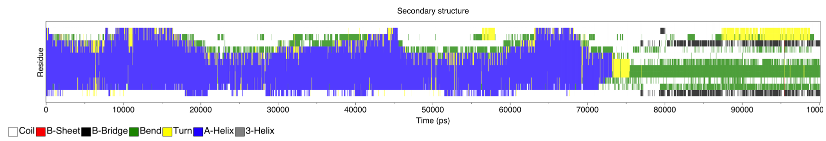
- T=303 K, anisotropic pressure coupling (Corrected GROMOS 53a6)

Aurein: Water 300K

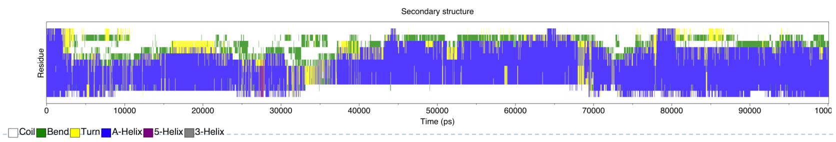
Sim 1



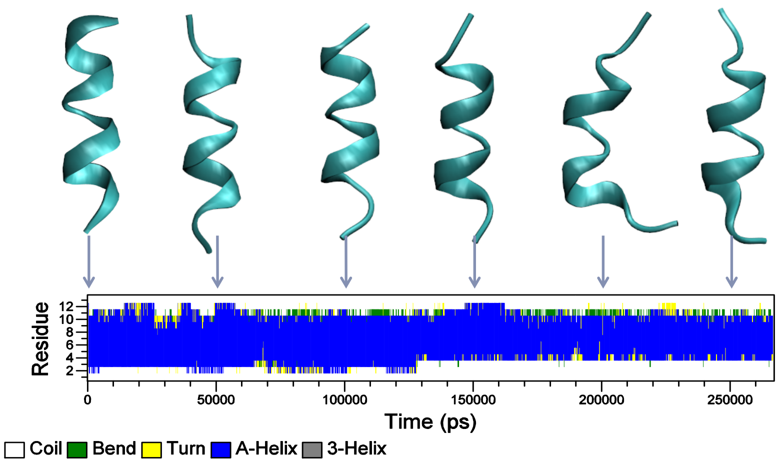
Sim 2



Sim 3

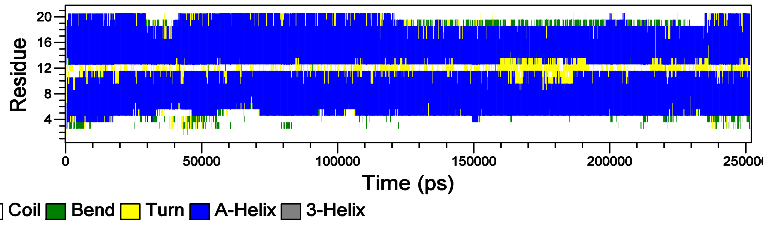


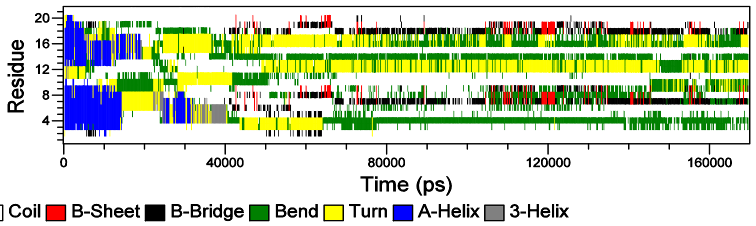
Structure of Aurein 1.2 in 20 mol % TFE (50 volume %)



T=303 K, isotropic pressure coupling

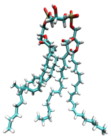
Structure of Maculatin 1.1 in TFE (top) and on DMPC bilayers (bottom)



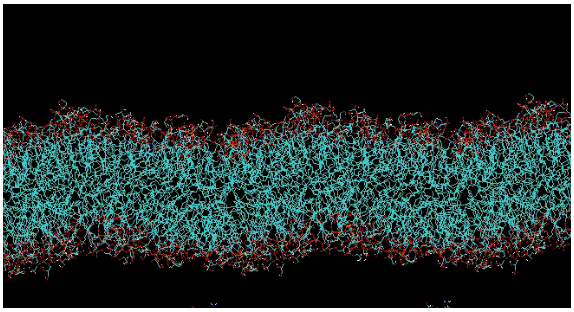


Aurein with PG-Cardiolipin membrane

60/40% PG/CL (tail ratio)

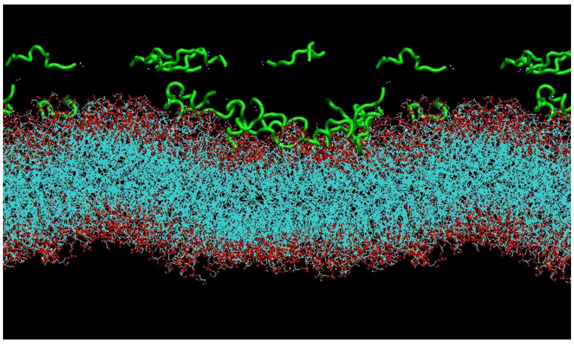


Cardiolipin



Aurein with PG-Cardiolipin membrane

60/40% PG/CL (tail ratio)



8/25/10

The interaction of Kalata B1 within membranes (binding and self-assembly)

Rong Chen

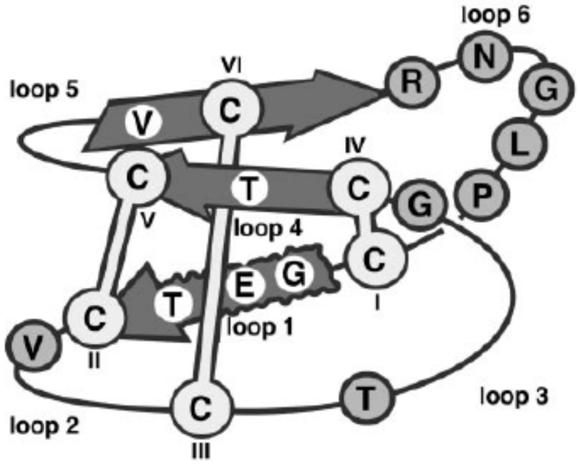
August 25, 2010

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Introduction

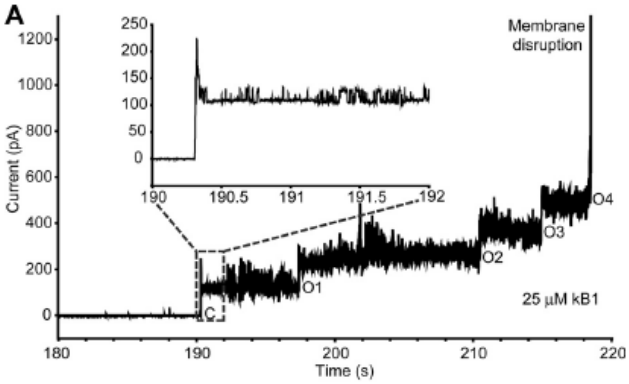
- Kalata B1 (KB1)
  - A 29-residue cyclic peptide isolated from plants
  - Remarkably stable structure
    - Head-to-tail cyclic backbone
    - Three disulfide bonds between 6 Cysteines
  - Biological activities include
    - Antivirus, antimicrobial, antifouling, etc.
  - Mechanism of action
    - Membrane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

Structural representation of KB1



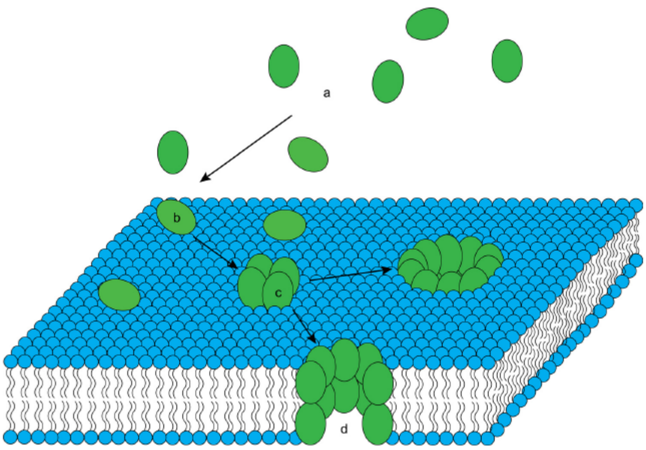
Rosengren et al. 2003. J. Biol. Chem. 278:8606

Recordings of asolectin patchs when 25 μM KB1 was added



Huang et al. 2009. J. Biol. Chem. 284:20699

A proposed model for pore formation by KB1



Huang et al. 2009. J. Biol. Chem. 284:20699

Aims

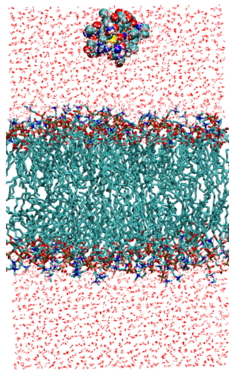
- To investigate
  - How KB1 binds to membranes
  - The association of KB1 into oligomers
  - How KB1 may form trans-membrane water-filled pores

Methods

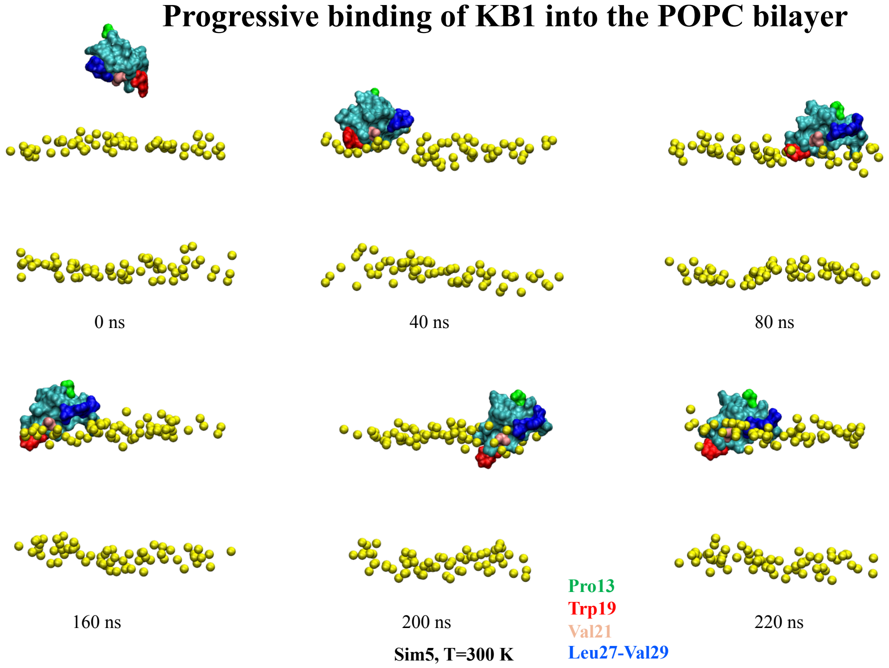
- Molecular dynamics simulations
  - GROMOS 53a6 force field
  - GROMACS 3.3.3 simulation engine
  - 4 fs time step
  - Twin-range cutoff (0.8 nm, 1.4 nm)
  - Reaction field
  - Berendsen weak-coupling method (NpT)

Simulation one

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

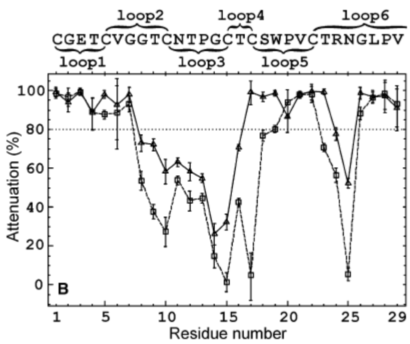


Progressive binding of KB1 into the POPC bilayer

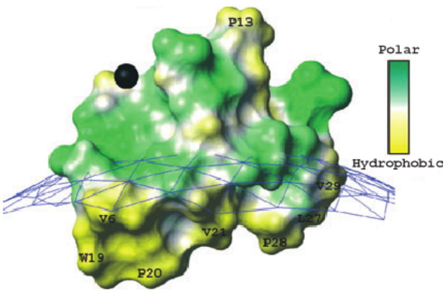


Sim5, T=300 K

Spatial structure of KB1 in DPC micelles derived from experiment



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

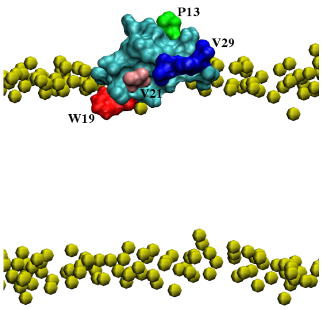


Proposed model for KB1 in DPC (dodecylphosphocholine) micelles

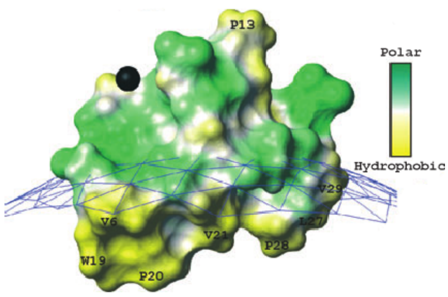
Shenkarev et al. 2006. FEBS J. 273:2658

Compare simulation to experiment

Simulation E (5)



Sim5, 220 ns



Experiment

Shenkarev et al. 2006. FEBS J. 273:2658

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

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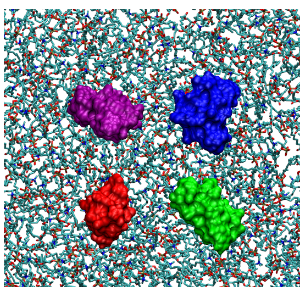
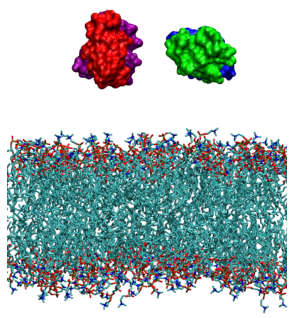
Initial structure (4 KB1, water and ions not shown)





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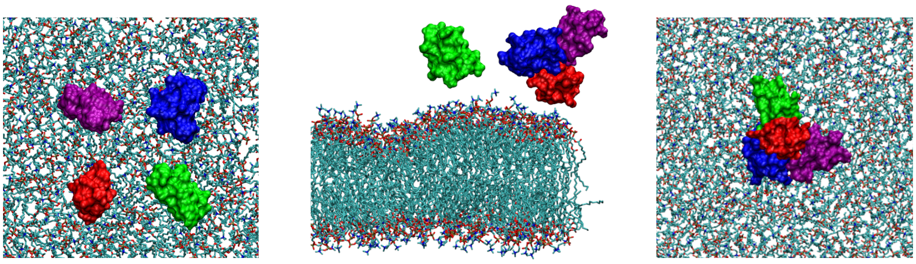
KB1 readily forms oligomers in solution



0 ns 20 ns 180 ns

T=300 K

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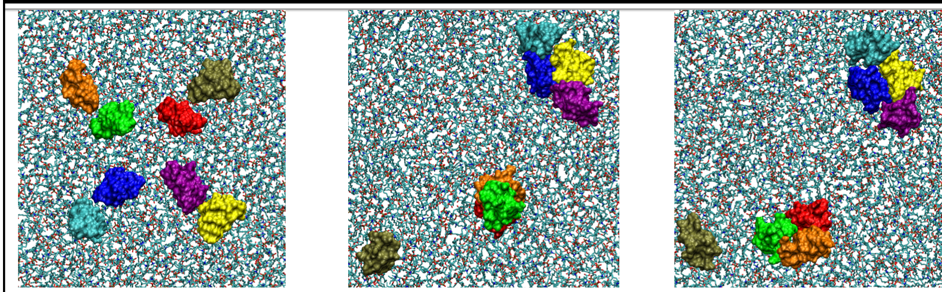
No spontaneous formation of pores



0 ns 20 ns 110 ns

T=300 K

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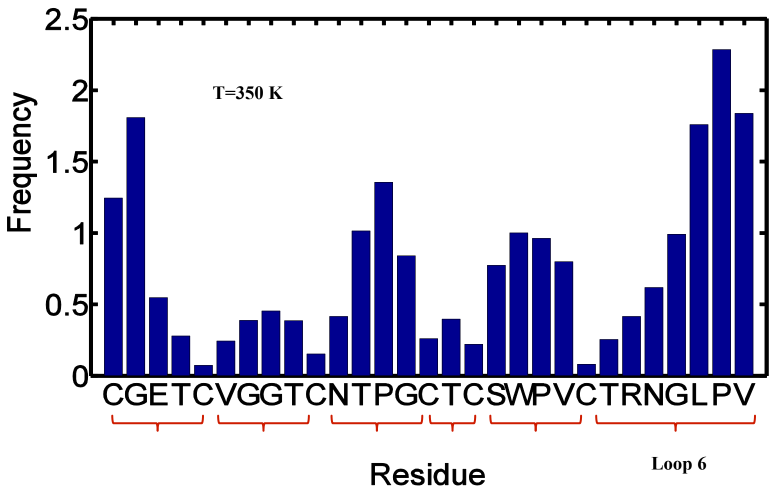
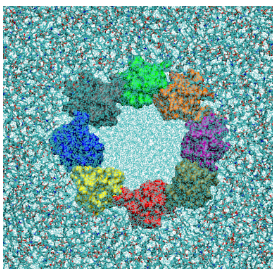
Self association via loops 6 and 1

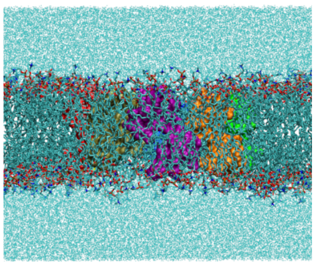


Stabilization of pre-formed pores

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

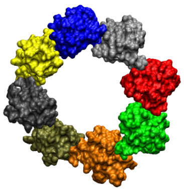


Top view

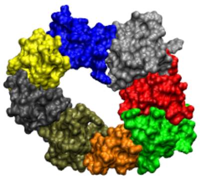


Side view

The pore diameter shrank from 4.5 nm to 2.0 nm in 90 ns



0 ns, D ≈ 4.5 nm

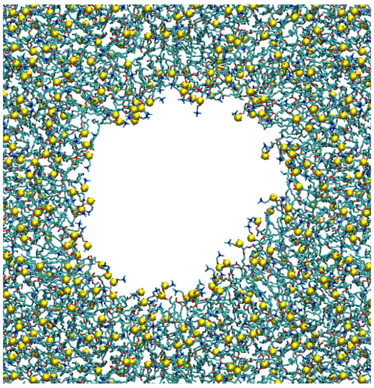


90 ns, D ≈ 2.0 nm

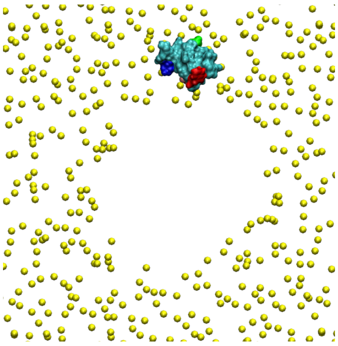
T=400 K, NVT

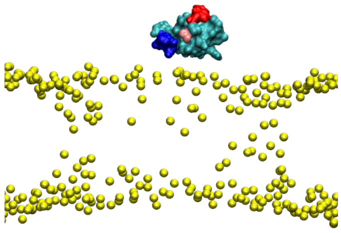
Stabilization of pre-formed pores

- A equilibrated membrane pore

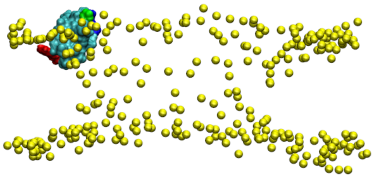


One KB1 placed above the pore

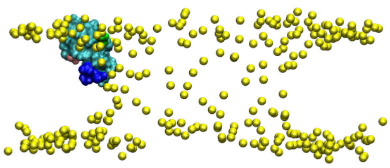




Rapidly inserts deeply into the rim of the pore (< 10 ns)



Sim1, 10 ns

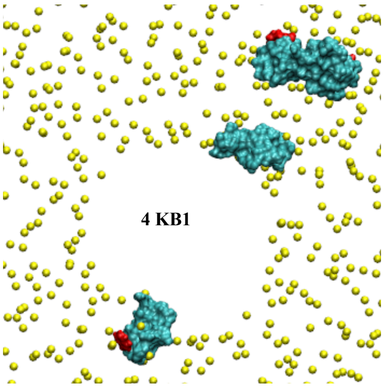


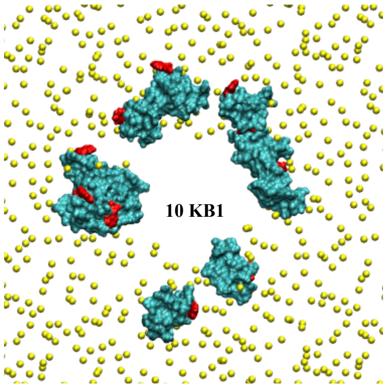
Sim2, 10 ns

T=350 K

Fixed area in the bilayer plane

More peptides added





T=350 K

Fixed area in the bilayer plane

Conclusions

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