Solid-state NMR of membrane-active peptides

EURACT-NMR workshop,
Karlsruhe, 27.1.2010

Anne S. Ulrich
Karlsruhe Institute of Technology

Overview

1) Biological membranes and peptides as „magic bullets“

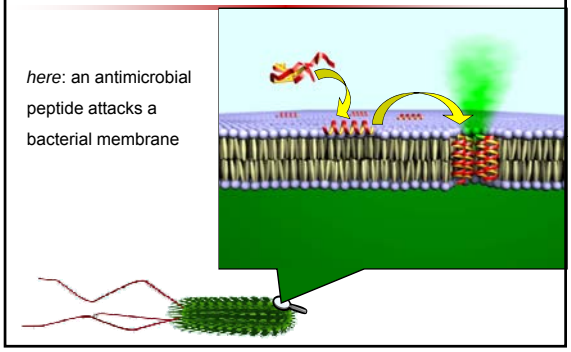
2) Structure analysis by ssNMR of lipids (^{31}P) and peptides (^{19}F, ^{2}H)

3) Antimicrobial peptide PGLa: structure, alignment, mobility

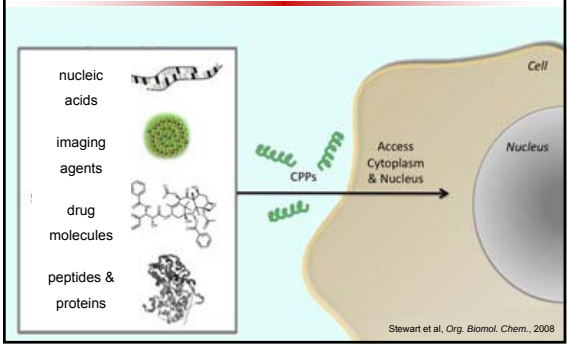
4) Comparison with other peptides and interactions with „real“ cells

Aim: explain molecular mechanism

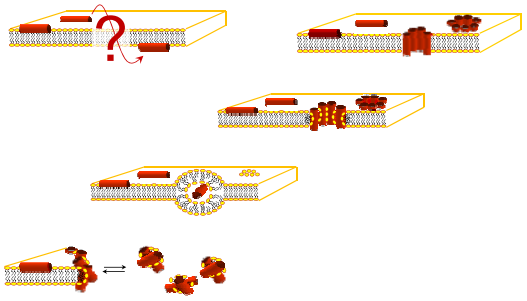
here: an antimicrobial peptide attacks a bacterial membrane



Cell penetrating peptides

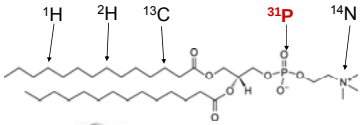


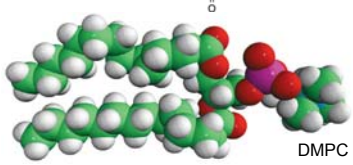
Cell penetrating mechanism ?



Solid state NMR of lipids

isotopes for studying lipids

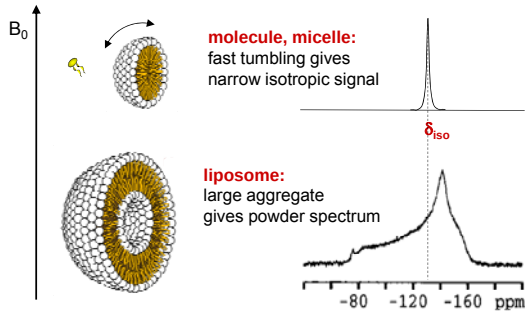




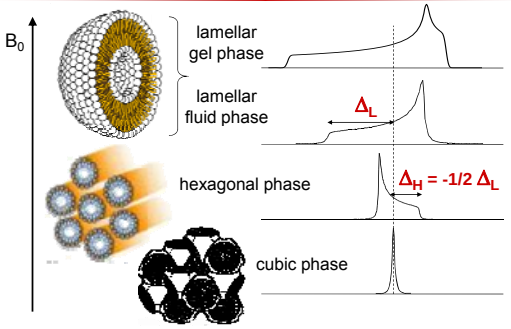
DMPC

- morphology
- dynamics
- structure
- influence of peptides/proteins

^{31}P-NMR of lipids (CSA)

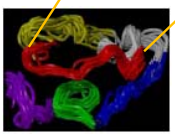


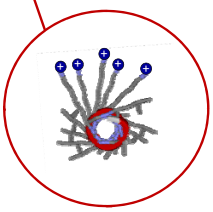
Different lipid phases



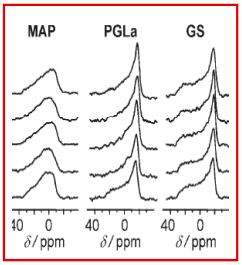
Different classes of CPPs

Transportan: AGYLLGKINLKALAALAKKIL
Penetratin: RQIKIWFQNRRMKWKK
MAP: KLALLKALKALKAALKLA
TAT(HIV): GRKKRRQRRRPPQ
Poly-arginine: RRRRRRRR

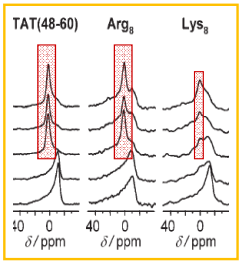




CPPs in membranes (^{31}P-NMR)

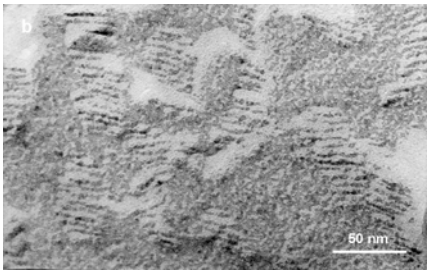


amphiphilic α-helices



high charge density,
short, disordered

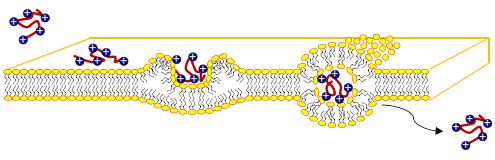
Electron microscopy of TAT



→ TAT induces short rod-like bundles in DMPC membranes

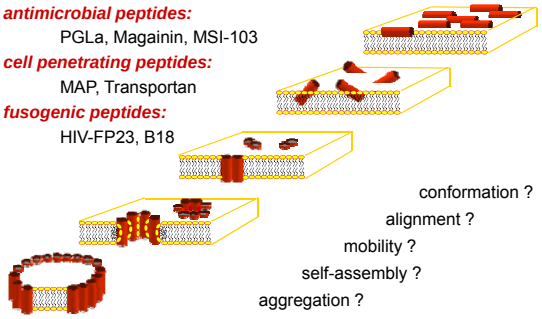
Cell penetrating peptide TAT

- the **Arginine-rich TAT** has no well-folded structure
- it induces isotropic phase (rod-like micelles) in DMPC
- complex formation between arginine and phospholipids
- **translocation via inverted lipid micelles**



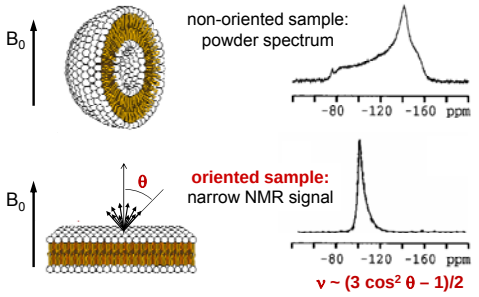
Amphiphilic peptides

antimicrobial peptides:
PGLa, Magainin, MSI-103
cell penetrating peptides:
MAP, Transportan
fusogenic peptides:
HIV-FP23, B18



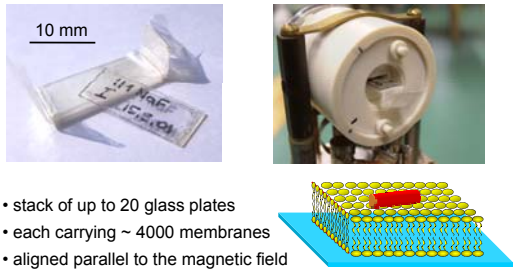
conformation ?
alignment ?
mobility ?
self-assembly ?
aggregation ?

Anisotropy in oriented samples



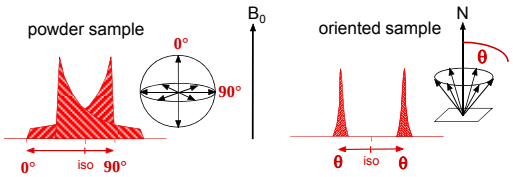
$\nu \sim (3 \cos^2 \theta - 1)/2$

Oriented NMR sample



- stack of up to 20 glass plates
- each carrying ~ 4000 membranes
- aligned parallel to the magnetic field

Orientational constraints

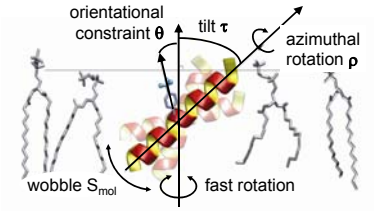


chemical shift anisotropy: $\nu = \Delta_{CSA} (3\cos^2\theta - 1)/2$

dipole (quadrupole) splitting: $\Delta\nu_D = \left(\frac{3\gamma^2\hbar}{2\pi r^3}\right) \frac{(3\cos^2\theta - 1)}{2}$

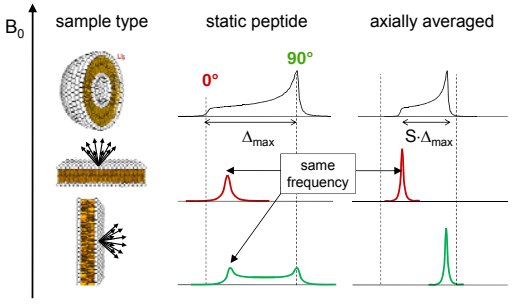
Solid state NMR of peptides

Place single isotope labels (^{2}H, ^{15}N, ^{19}F) into the peptide frame

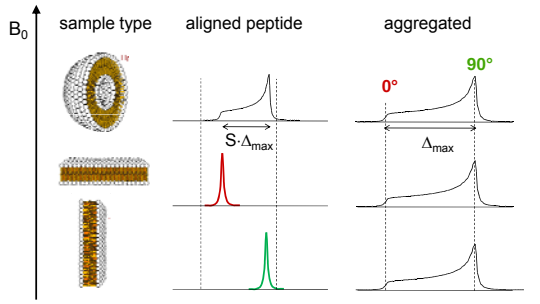


Several labels (**θ**) reveal peptide conformation (**α**-helix, **β**-strand), its alignment (**τ, ρ**) and dynamics (S_{mol}, rotation in the membrane)

Detect fast peptide diffusion



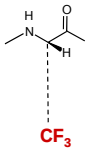
Detect order vs. aggregation



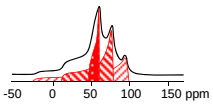
Labeling of synthetic peptides

^{15}N-amino acid
☺ non-perturbing
☹ low sensitivity

$^{2}H_3$-alanine
☺ suitable for Ala
☺ medium sensitivity

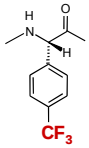


CF_3-label
☺ high sensitivity
☺ no background
☹ perturbation?



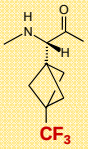
dipolar splitting with sign (+/-) from CSA

^{19}F-labeled amino acids



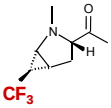
CF_3-Phg
(racemization)

use *D*-epimer as steric probe in functional studies



CF_3-Bpg
(custom design)

F_3-Aib
(long coupling)

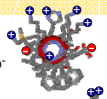


CF_3-Pro
(stereochemistry)

Cationic amphiphilic α-helices

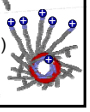
PGLa (antibiotic from frog skin)
GMASKAGAIAGKIAKVALKAL-NH_2

Magainin (synergistic antibiotic)
GIGKFLHSAKKFGKAFVGEIMNS-COO^-



MSI-103 (designer-made antibiotic)
KIAGKIAKIAGKIAKIAGKIA-NH_2

MAP (designed cell-penetrating peptide)
KLALKLALKALKAALKLA-NH_2



Antimicrobial defence

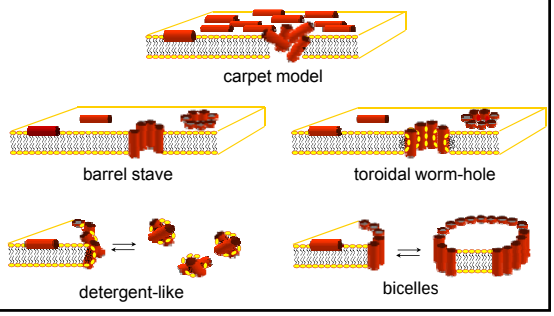
Antimicrobial peptides were discovered in the 1980s in the skin of amphiphibians, hundreds were found since.

Selectivity of PGLa

Bacterial membranes	**Eukaryotic membranes**
▪ negatively charged lipids ▪ no cholesterol ▪ 200 mV potential	▪ zwitterionic lipids ▪ up to 50% cholesterol ▪ 20 mV potential
electrostatic attraction	*hydrophobic interactions*

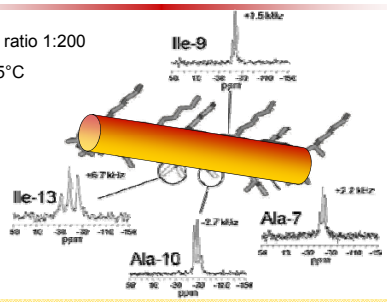
Cationic **antibiotic** peptides preferentially bind bacterial membranes, but **hemolytic** side effects can occur

Membrane disruption ?



^{19}F-NMR of PGLa

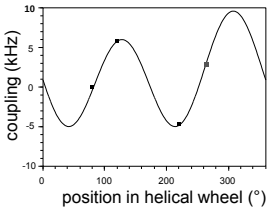
peptide:lipid ratio 1:200
in DMPC, 35°C



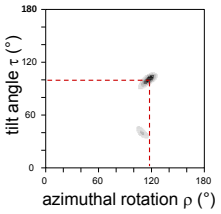
PGLa: GMASKAGAIAGKIAKVALKAL-NH_2 CF_3

^{19}F-NMR structure calculation

Dipolar wave plot:



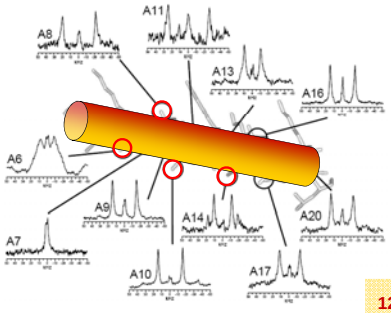
RMSD analysis:



→ τ = 90 ±10°, ρ = 115 ±10°, S_{mol} = 0.7

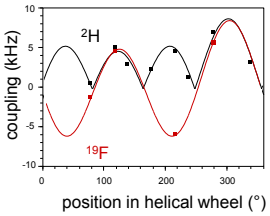
PGLa: GMASKAGAIAGKIAKVALKAL-NH_2 CF_3

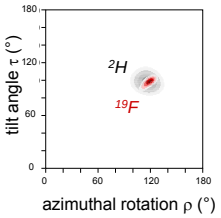
^{2}H-NMR analysis of PGLa



12x CD_3

Accurate ^{2}H-NMR analysis

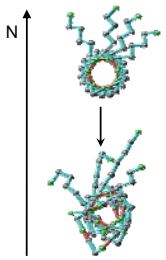




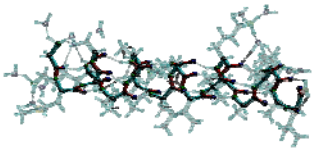
→ τ = 98 ±5°, ρ = 115 ±5°, S_{mol} = 0.7

PGLa: GMASKAGAIAGKIAKVALKAL-NH_2 CD_3

All-atom MD simulation of PGLa

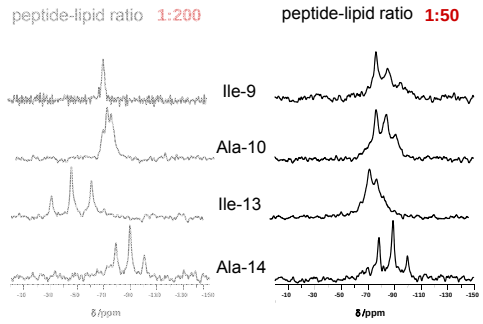


orientational constraints act as pseudo-forces
to align peptide with respect to bilayer normal:

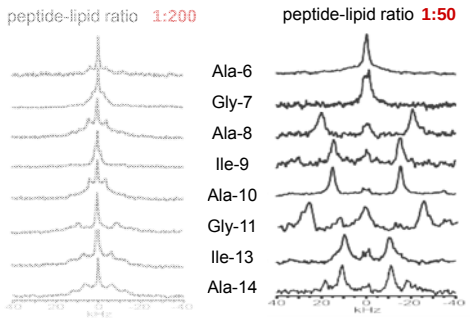


PGLa: GMASKAGAIAGKIAKVALKAL-NH_2 CD_3

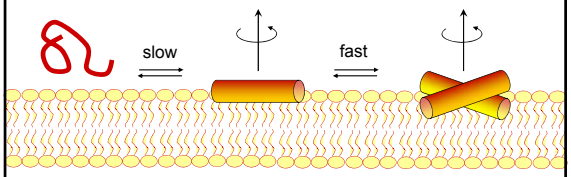
^{19}F-NMR at higher concentration



^{2}H-NMR at higher concentration



Binding and re-alignment



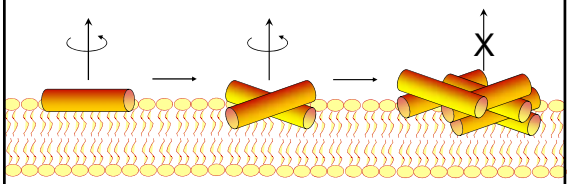
free peptide

surface bound: S-state (τ ≈ 98°)

tilted state T-state (τ ≈ 126°)

⟶ monitor exchange (ms time-scale) and threshold (~1:100)

Concentration dependence



1:200

S-state: rotating monomer

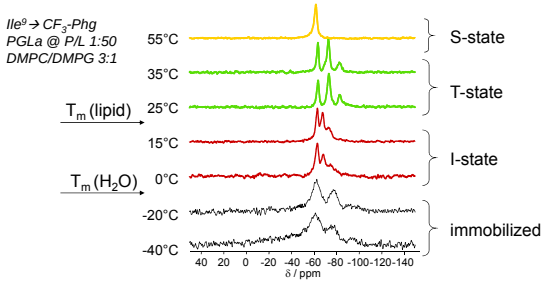
1:50

T-state: rotating dimer

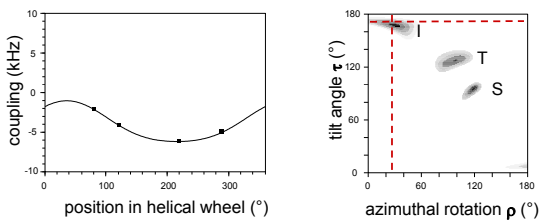
1:20

immobilized: laterally aggregated

^{19}F-NMR temperature dependence

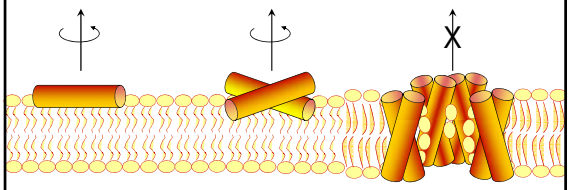


PGLa in lipid gel phase



⟶ τ = 168 ±10°, ρ = 92 ±10°, S_{mol} = 0.83, **inserted I-state**

Dependence on lipid phase



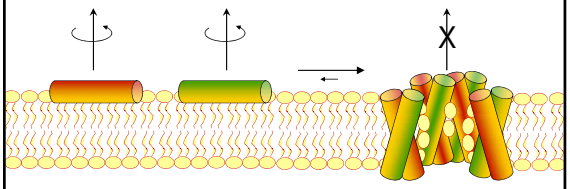
T ≥ 45°C (***fluid***)
S-state (τ ≈ 82°)

35 - 25°C (***fluid***)
T-state (τ ≈ 54°)

15°C ≥ T (***gel***)
I-state (τ ≈ 23°)

⟶ discrete states, pore can be trapped in lipid gel phase

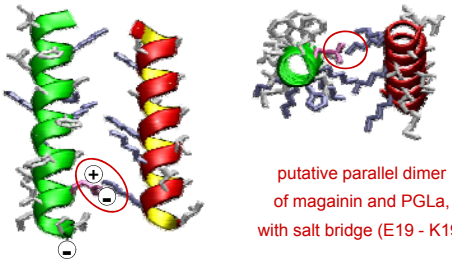
Synergy of PGLa + Magainin



monomeric PGLa + monomeric Magainin → heterodimeric I-state

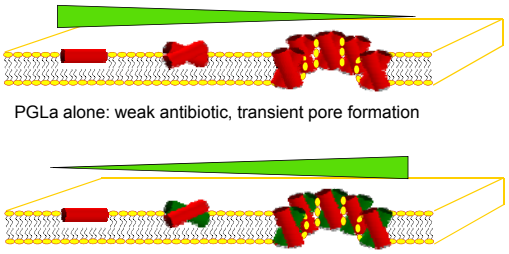
⟶ pore of heterodimers is stable (low conc., fluid phase)

Model of heterodimer



putative parallel dimer
of magainin and PGLa,
with salt bridge (E19 - K19)

Position of equilibrium



PGLa alone: weak antibiotic, transient pore formation

PGLa + magainin: synergistic effect, stabilizes pore

Teamwork …

Happy couples …

Choreographic success !

Comparison with other peptides

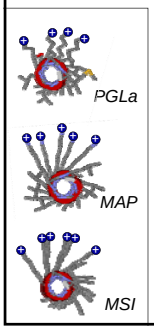
PGLa (antibiotic from frog skin)
GMASKAGAIAGKIAKVALKAL-NH_2

Magainin (synergistic antibiotic)
GIGKFLHSAKKFGKAFVGEIMNS-COO^-

MSI-103 (designer-made antibiotic)
KIAGKIAKIAGKIAKIAGKIA-NH_2

MAP (cell-penetrating peptide)
KLALKLALKALKAALKLA-NH_2

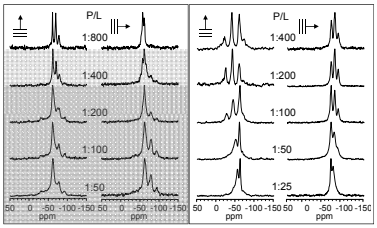
Different thresholds



Binding to DMPC:	PGLa > MAP > MSI
to DMPG:	MSI > MAP > PGLa
Tilting:	MSI > *D*-MAP > PGLa
Immobilization:	MAP > MSI ≈ PGLa
Antibiotic activity:	MSI > *D*-MAP > PGLa
Hemolytic activity:	MAP > MSI ≈ PGLa

Unexpected behavior of MAP

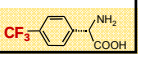
L-CF_3-Phg



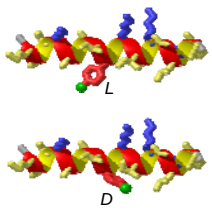
D-CF_3-Phg

⟶ aggregation of MAP at low concentration (1:400)

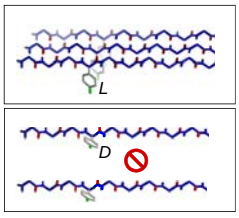
MAP: KLALKLALKALKAALKLA-NH_2



Steric effect of *D*-amino acid

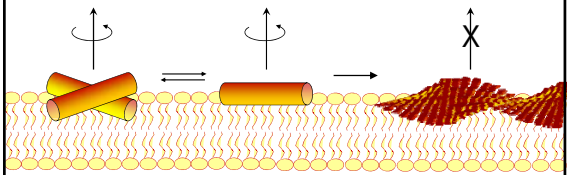


both *L*- and *D*-CF_3-Phg can be accommodated in α-helix



L-form is compatible with β-sheet but *D*-form prevents aggregation

Aggregation tendency of MAP



analyzed from ***D*-CF_3-Phg**

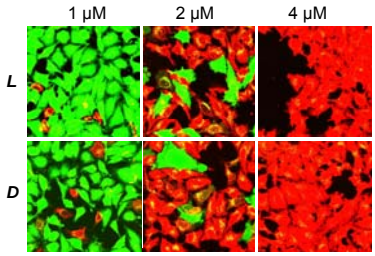
monomeric only at low concentration

all-*L* aggregates as β-amyloid

⟶ aggregation behaviour of MAP can be controlled

Functional state ?

Cell-penetration of MAP epimers



HeLa cells: incubated 30 min with CPP at 37°C

red: fluorescent-labeled CPP

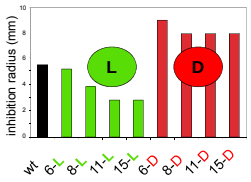
green: live cells

yellow: successful endocytosis and/or cell penetration

⟶ effect of aggregation cannot be examined, as MAP is toxic

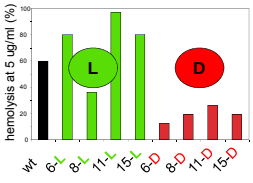
Any other use of MAP epimers ?

Antimicrobial activity



better antibiotic when aggregation is prevented

Hemolytic activity



less hemolysis when aggregation is prevented

Summary of NMR strategy

1. Check lipid alignment by ^{31}P-NMR, detect any perturbation
2. Screen conditions with sensitive ^{19}F-NMR (P/L=1:3000 to 1:8)
3. Rough peptide alignment (τ, ρ, S_{mol}) from CF_3-Phg labels
4. Confirm accurate peptide structure from D_3-Ala labels
5. Observe peptide *in vivo* by background-free ^{19}F-NMR
6. Do functional studies with analogues (e.g. with *D*-CF_3-Phg)
7. Derive rules to design new analogues with improved activity

Acknowledgements

Erik Strandberg, Parvesh Wadhwani, Sergii Afonin, Jochen Bürck, Marina Berditsch, Stephan Grage, Ulrich Sternberg, Christian Mink, Ralf Glaser, Pierre Tremouilhac, Deniz Tiltak, Marco Ieronimo.

Igor Komarov	University of Kiev
Jesus Salgado, Santi Esteban	University of Valencia
Irene Wacker-Schröder	KIT, IBG-1
Sevil Weinkauf, Sybille Bayerl	TU München

€€ KIT, CFN, DFG, FCI, AvH, DAAD €€