https://doi.org/10.17952/37EPS.2024.P2293

MOLECULAR INSIGHTS INTO THE LIPID BILAYER INTERACTIONS OF THE CYCLIC LIPODEPSIPEPTIDE TOLAASIN

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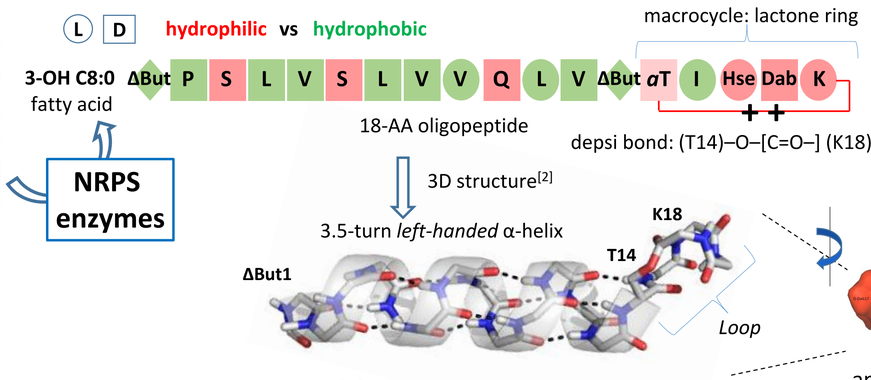
Tolaasin – not your average AMP

- Bacterial secondary metabolite of rhizosphere-dwelling *Pseudomonas tolaasii*
- Cyclic lipodepsipeptide with *non-proteinogenic* amino acids[1]

P. tolaasii



biosurfactant

- Double-edged antifungal activity[3]:

Crop Protecting Agent[4] ✔ *vs* Toxin of Brown Blotch Disease[5] ✘

Few (left) vs many (right) tolaasin producers in soil of cocoyam crop fields in Cameroon

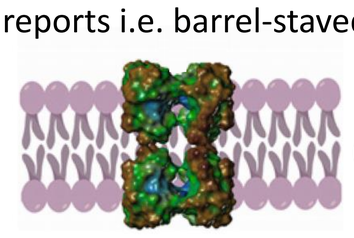
Decreases production in edible mushroom farms of *Agaricus bisporus* by 8–15% in Europe

Figure adapted from [5]

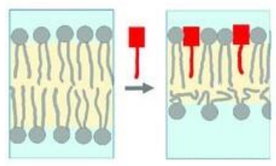
- Antifungal activity via *cell membrane* interactions. Main mode of action?

→ Mixed reports i.e. barrel-staved pore formation[2,6] *vs* asymmetry stress[7]

Adapted from [6d]



Oligomer vs Monomer (high *P/L*) (low *P/L*)



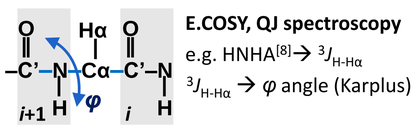
Adapted from [7b]

Via Ion Channel Measurements + ATR-IR *Via Fluorescence Leakage*

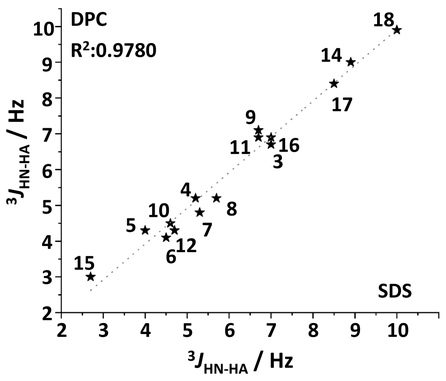
→ Objective: molecular insights into the interaction of tolaasin and the lipid bilayer using novel biophysical and computational approaches.

I) Revisiting the tolaasin conformation using *J*-coupling based NMR spectroscopy

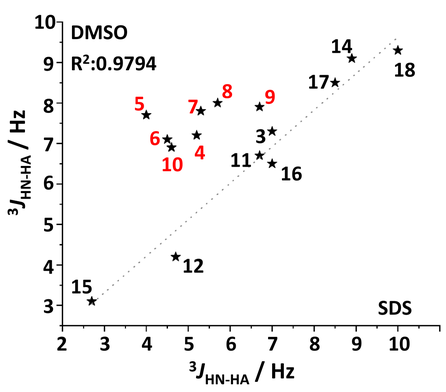
1) $^3J[\phi]$-couplings



E.COSY, QJ spectroscopy
e.g. HNHA[8] → $^3J_{H\text{-}H\alpha}$
$^3J_{H\text{-}H\alpha}$ → φ angle (Karplus)

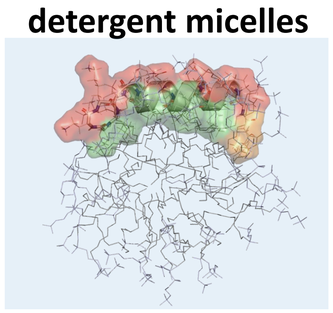


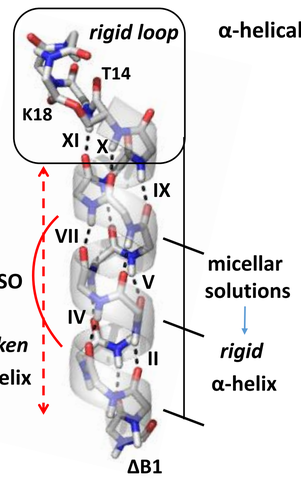
Quasi-identical backbone conformations with SDS(–) vs DPC (+/–) micelles



Last helical turn (11-14) + loop (14-18) preserved but α-helix disrupted in DMSO

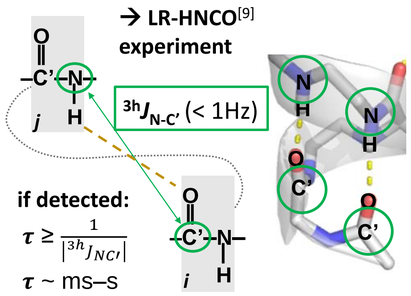
with membrane mimic detergent micelles





2) H-bond coupling

donor ^{15}N–H ↔ acceptor ^{13}C'=O
[^{15}N–H]-^{13}C' frequencies correlated



α-helical (*i*+4;*i*) hydrogen bonds detected:

Bond	SDS	DPC	DMSO
I	✔	✔	✘
II	✘	✘	✘
III	✔	✔	✘
IV	✔	*overlap*	✘
V	*overlap*	✔	✘
VI	✔	✔	✘
VII	✔	✔	✘
VIII	✔	✔	✘
IX	✘	✘	✘
X	✘	✘	✘
XI	✔	✔	✔

Results I

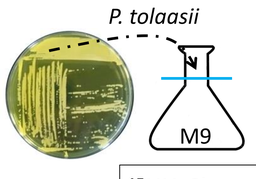
- The backbone conformation of tolaasin as determined with SDS micelles is fully maintained with DPC despite the charge difference.
- Long-lived hydrogen bond network detected @water:lipid interface.
- Loop + last helix turn most rigid, maintained in organic solvents too.

tolaasin ^{13}C,^{15}N isotope labeling : $^{12}C_{94}H_{163}O_{25}{}^{14}N_{21}$ → ~100% $^{13}C_{94}H_{163}O_{25}{}^{15}N_{21}$ → **C and N spins for NMR!**

Nucleus	%	NMR
^{1}H	99.97	✔
^{12}C	98.90	✘
^{14}N	99.60	✘
^{13}C	1.10	✔
^{15}N	0.40	✔

Recipe:

bacterial growth in M9 salt medium[10]

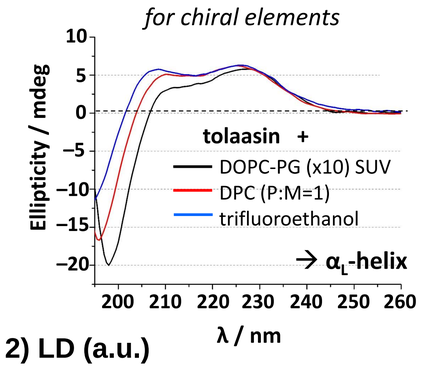


$^{15}NH_4Cl$
U-^{13}C-glucose

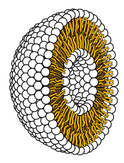
II) Tolaasin membrane interaction characterized by polarized light spectroscopy

1) Circular Dichroism (CD)

for chiral elements



with membrane mimic liposome SUVs



~100 nm

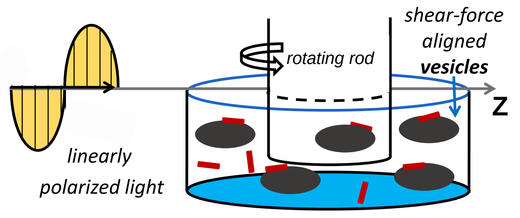
DOPC (+/–) or DOPC-DOPG (–)

↓ effect of charge

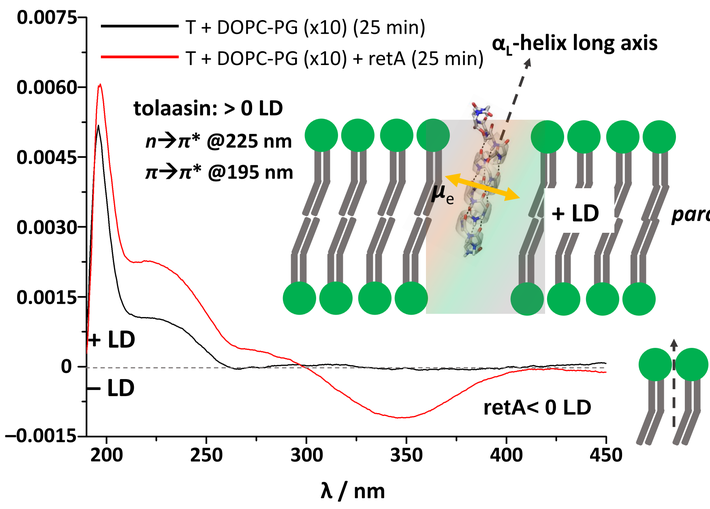
2) Linear Dichroism (LD)

for orientation[11]

LD: $A_{\parallel(Z)} - A_{\perp(Z)}$ → orientation of μ_e → orientation of molecule



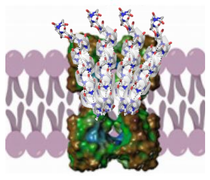
2) LD (a.u.)



inserted α_L-helix

↓

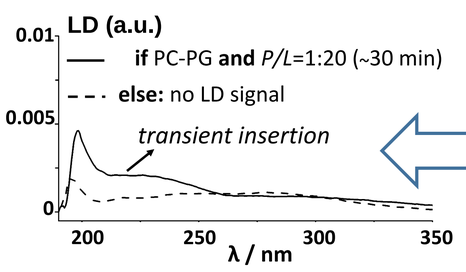
suitable orientaton for oligomerization / pore formation ?



illustration

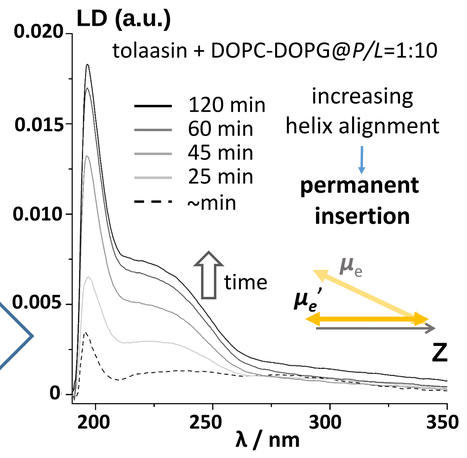
The system and time dependence of the tolaasin LD signal

- liposomes: pure DOPC (+/–)or mixed (4:1) DOPC-DOPG (–) SUVs
- peptide:lipid ratios (*P/L*): 1:10–50 (with [Tol]= 75 µM in PBS)



tolaasin + *liposome* mixed with a particular *P/L*

if PC-PG **and** *P/L*=1:10



Results II

- Tolaasin maintains its α_L-helical conformation upon interaction with lipid bilayers regardless of their charge.
- With negatively charged, (4:1) mixed DOPC-DOPG vesicles the α-helix of tolaasin is inserted into the lipid bilayer more parallel with its surface norm. Using *P/L*=1:10 LD signal of tolaasin shows gradual increase that plateaus on the timescale of hours and remains constant.
- Conditions are crucial! Using zwitterionic liposomes or lower *P/L* ratio tolaasin remains weakly aligned suggesting more random orientation within the lipid bilayer or transient alignment.

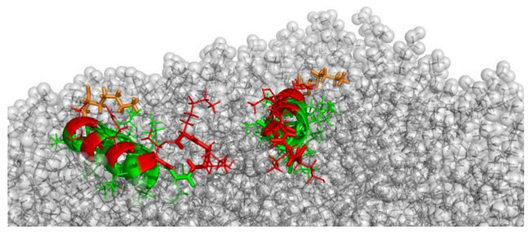
Discussion and Outlook

- Isotopically labeled NMR methods allowed to quantify backbone torsion angles and directly detect long-lived backbone hydrogen bonds for the cyclic lipodepsipeptide tolaasin. These data can now be implemented as novel restraints to improve the current model of tolaasin oligomerization and pore formation processes.
- Polarized light spectroscopy confirmed that the tolaasin helix is able to insert itself into the lipid bilayer parallel with the surface norm which agrees with ATR-IR results[6c]. The full insertion process takes place gradually on the timescale of *hours* and shows irreversible provided that, in our setup, 20% negatively charged SUVs and high peptide-to-lipid ratio (1:10) are used. To demonstrate potential oligomerization and pore formation processes under these conditions imaging techniques are planned to be involved. However, in general, at low *P/L* values or min timescale insertion of the tolaasin helix into the lipid bilayer is yet inefficient and no pore formation is expected.[7b]

III) Initial molecular dynamics simulations of the lipid bilayer interactions of tolaasin

- AMBER ff14SB force field[13], TIP3P water solvent, lipid bilayer builder: CHARMM-GUI Membrane Builder[14]

→ setup: 2 tolaasin +128 POPC molecules (*P/L*=64), 100 ns



Observations

- The tolaasin molecules remain parallel with the surface.
- They interact with the lipid headgroup region mostly.
- No dimerization takes place in the applied setup.

Abbreviations

ΔBut – dehydroaminobutyril, AMP – antimicrobial peptide, ATR-IR – attenuation total reflection infrared spectroscopy DAB – 2,4 diaminobutyril, DMSO – dimethyl sulfoxide, DOPC – 1,2-dioleoyl-sn-glycero-3-phosphocholine or (18:1) PC, DOPG – 1,2-dioleoyl-sn-glycero-3-phosphoglycerol or (18:1) PG, DPC – dodecyl phosphocholine, HNHA – experiment [8], Hse – homoseryl, LD – linear dichroism, LR-HNCO – long-range HNCO experiment [9], MOA – mode of action, NRPS – non-ribosomal peptide synthetase, NMR – nuclear magnetic resonance, PBS – phosphate buffer saline solution, *P/L* – molar peptide to lipid ratio, retA – retinoic acid, SDS – sodium dodecyl sulfate, SUV – small unilamellar vesicle

References

[1] Nutkins, J.C. *et al.*, *JACS*, 1991, **113**, 2621–2627. [2] Jourdan, F. *et al.*, *Proteins: Struct., Funct., Genet.*, 2003, **52**, 534–543 [3] Geudens, N., Martins., J.C. *Front. Mirobiol.* 2018, **9**, 1–18 C. [4] Olorunleke, F.E. *et al.* unpublished data [5] Soler-Rivas, S. *et al., FEMS Microbiol. Rev.*, 1999, **23**, 591–614. [6] a) Cho, K.H. and Kim, Y. K., *FEMS Microbiol. Lett.*, 2003, **221**, 221–226. b) Brody, C.L. *et al., Mol. Plant Microb. Interact.* 1991, **4**, 407–411. c) Coraiola, M. *et al.*, *BBA*, 2006, **1758**, 1713–1722. d) Jo, G. *et al.*, *J. Microbiol. Biotechnol.*, 2011, **21**, 1097–1100. [7] a) Andolfi, A. *et al.*, *Perspect. Med. Chem.*, 2008, **2**, 1177391X0800200–112. b) Steigenberger, J. *et al.*, *Front., Mol., Biosci.*, 2022, **9**, 2296-889X. [8] Vuister, G.W. and Bax, A., *JACS*, 1993, **115**, 7772–7777. [9] Cordier, F. *et al.*, *Nat. Protoc.*, 2008, **3**, 235–241. [10] Geudens, N. *et al. Molecules,* 2019, **24**, 2257. [11] Nordén, B. *et al.,* Linear Dichroism and Circular Dichroism: A Textbook on polarized-light spectroscopy, *RSC Books*, Cambridge, UK, 2010. [12] Svensson, F.R. *et al., J. Phys. Chem. B, 2007,* **111**, 10839–48. [13] Maier, J.A. *et al., J. Chem. Theory Comput.*, 2015, **11**, 3696–3713. [14] a) Jo, S. *et al., PLoS ONE,* 2007, **2**, e880. b) Jo, S. *et al., Biophys J.*, 2009, **97**, 50–58.

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