Secondary structure and alignment analysis of membrane-active peptides in lipid bilayers by oriented circular dichroism

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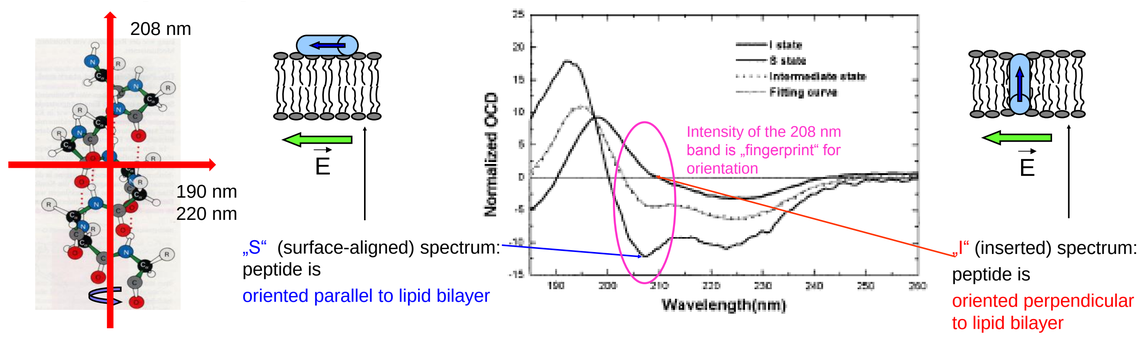
Oriented Circular Dichroism Spectroscopy (OCD)

OCD is a fast and sensitive spectroscopic method for analyzing the secondary structure and orientation of membrane-embedded peptides and proteins in lipid bilayers that are macroscopically aligned with respect to the light beam[1,2] . It helps, e.g., to understand the mechanisms during formation of transmembrane pores by anti-microbial peptides. The method is complementary to solid-state NMR structure analysis using the same oriented samples and exhibits characteristic features:

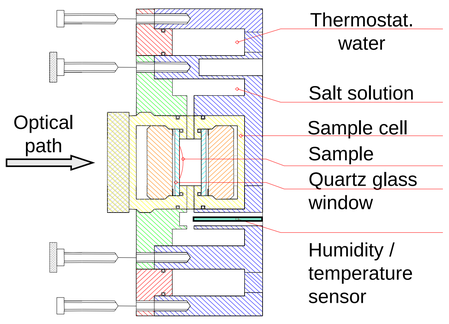
+ very high sensitivity, minimum amount of peptide required ~ 1 μg / sample
+ relative fast measurement (typically 3 hours / sample)
+ no isotope labeling required (wt peptide can be used)
+ simple sample preparation (similar to solid-state NMR)
+ exact control of temperature and humidity
- low resolution method: only global information on alignment and secondary structure of peptide
- at present theory is restricted to α-helical peptides

Basic principle



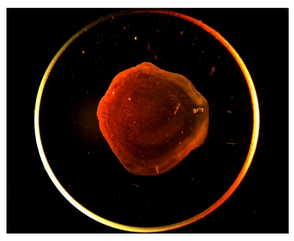
According to Moffit´s theory[3] the dipole moment of π-π electronic transitions of amide chromophores in a helix are polarized parallel or perpendicular to the helix axis. CD band intensity of α-helical peptides depends on their orientation. The OCD line shape of an α-helical peptide, which is oriented in macroscopically aligned lipid bilayers reveals its orientation with respect to the field vector E of the circularly polarized light. Different OCD spectra (S and I) are obtained for surface-aligned and inserted peptides in oriented membranes. Intermediate states can be fitted by a linear combination of the S- and I-state spectra.*

Experimental set-up OCD cell

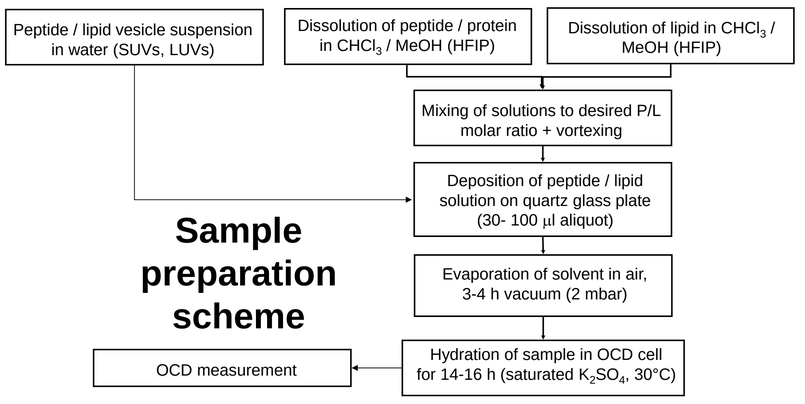


Schematic of the developed rotatable OCD cell, which was manufactured in-house to measure peptide alignment in lipid bilayers at constant temperature and humidity.

OCD cell mounted on rotation stage in JASCO J-810 spectropolarimeter; rotational averaging of spectra diminishes spectral artifacts caused by linear dichroism of the solid sample.

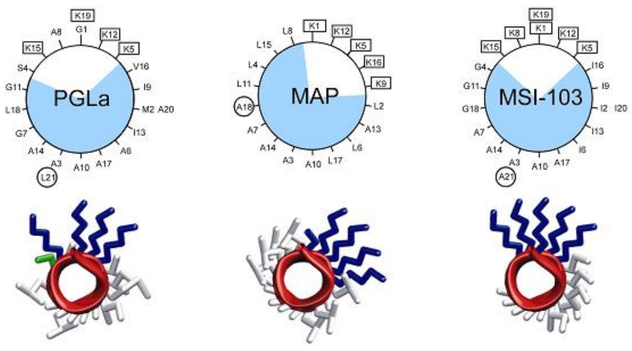


Photograph of OCD sample deposited on 20 mm Ø quartz glass window.



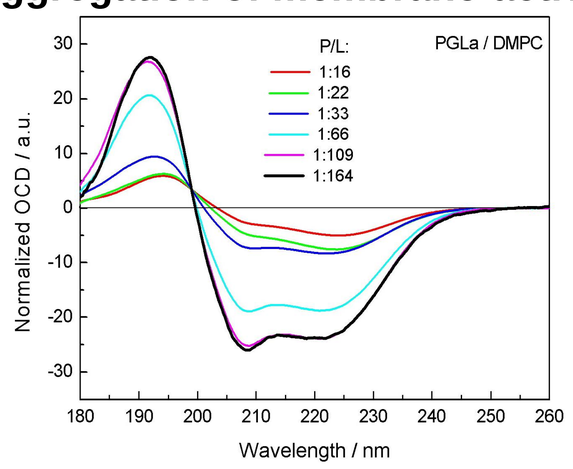
OCD reveals secondary structure, re-orientation and aggregation of membrane-active peptides in lipid bilayers

Most organism use antimicrobial peptides as a first line of defense against bacterial invasion. A peptide found in the skin of the African frog *Xenopus laevis*[4,5] is PGLa (GMASKAGAIAGKIAKVALKAL-NH_2). MSI-103 ([KIAGKIA]$_3$-NH_2), is a peptide designed based on the sequence of PGLa, and has a higher antimicrobial activity[6,7]. MAP (KLALKLALKALKAALKLA-NH_2) is also a designer-made peptide, which can penetrate cell membranes[8]. They all have amphipathic properties, bind to lipid bilayers and should form α-helices in membranes. We have used OCD to study their structure and orientation in DMPC bilayers for understanding structure / function relationships.

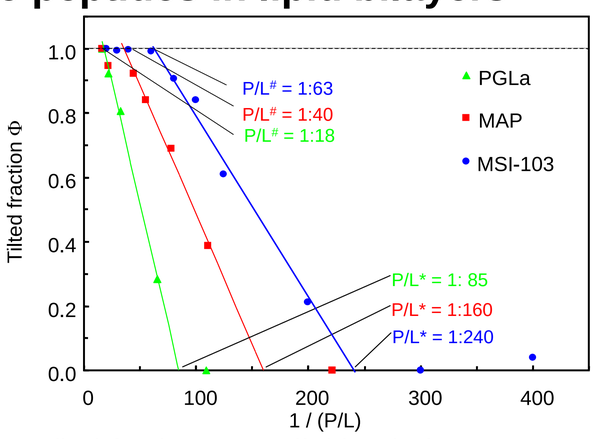


	PGLa	MAP	MSI-103
Charge:	+5	+6	+7
Hydrophobic moment μ_H[9]:	0.411	0.524	0.631

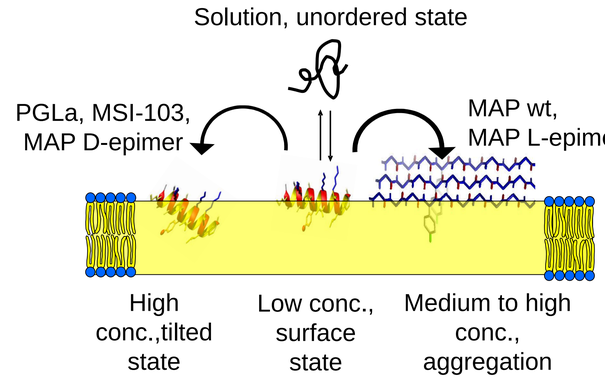
Helical wheel projections of the amphipathic α-helical peptides PGLa,, MAP and MSI-103. Charged residues are marked by rectangles, and the C-terminal amino acid by a circle. The hydrophobic sector is blue. In the panels below, an end-view of the helix is shown for each peptide with amino acids in stick representation. Here, blue marks positively and red negatively charged residues, polar residues are green and hydrophobic residues white. The peptide´s net charge and hydrophobic moment μ_H (norm. consensus scale[9]) are stated below.



OCD spectra of PGLa in DMPC bilayers, showing its re-alignment from a surface-bound α-helical S- to a tilted T-state induced by increasing the peptide/lipid ratio.



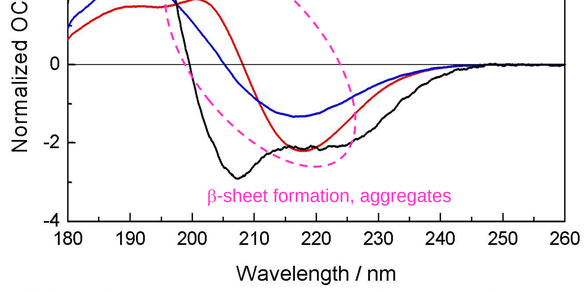
Tilted fraction Φ vs. 1/(P/L) ratio for PGLa, MAP and MSI-103 in DMPC bilayers; Φ was determined by fitting the intermediate spectra with a linear combination of the corresponding S- and T-state spectra.



OCD as an independent analytical method supports solid-state NMR results on behavior of the three peptides in DMPC lipid bilayers.

Results:

- PGLa, MSI-103 and the D-epimer of a MAP analogue exhibit mostly α-helical conformation and re-alignment in DMPC
- for low peptide concentration the S-state predominates, at threshold P/L* the T-state starts to appear, and above a higher threshold P/L# all peptides are in the T-state
- For all three peptides P/L* was about four times P/L#, but the value of P/L* varies strongly in the order MSI-103 < MAP < PGLa (same order found in NMR[10])
- the P/L* threshold is inversely correlated with the charge and hydrophobic moment of the peptides
- for MAP-wt and its L-epimer a change to β-pleated structure was found, thus OCD offers also a simple way to identify the formation of such aggregates



OCD of MAP mutant (L–epimer) in DMPC bilayers for varying P/L ratio showing the peptide in α-helical conformation and S-state at low and β-pleated structure at high P/L ratios.

Conclusion

OCD allows to screen and identify conditions where functionally relevant changes in peptide structure and orientation occur as a function of concentration, lipid environment, temperature, and humidity[11]. These conditions can then be used in high-resolution solid-state NMR structure and alignment analysis of such systems.

References

1. Wu, Y., Huang, H. W., and Olah, G. A., *Biophys. J.,* 1990, ***57***, 797–806. 2. Chen, F.-Y., Lee, M.-T., Huang, H. W., *Biophys. J.,* 2002, ***82***, 908–914. 3. Moffitt, W., *J. Chem. Phys.*, 1956, ***25***, 467. 4. Zasloff, M. , P*roc. Natl. Acad. Sci. USA,* 1987*,* ***84***, 5449-5453. 5. Soravia, E., Martini, G., and Zasloff, M. *FEBS Lett.,* 1988, ***228***, 337-340. 6. Maloy, W. L., and Kari, U. P., *Biopolymers,* 1995, ***37*****,** 105-122. 7. Blazyk, J., Wiegand, R., Klein, J., Hammer, J., Epand, R. M., Epand, R. F., Maloy, W. L., and Kari, U. P., *J. Biol. Chem.,* 2001, ***276***, 27899-27906. 8. Langel, U. *Cell-penetrating peptides: processes and applications*, CRC Press, Boca Raton, FL, 2002. 9. Eisenberg, D., Weiss, R. M., Terwilliger, T. C., and Willcox, W., *Faraday Symp. Chem. Soc.,* 1982, ***17***,109–120.10. Strandberg, E., Kanithasen, N., Tiltak, D., Bürck, J., Wadhwani, P., Zwernemann, O., and Ulrich, A.S., *Biochemistry*, 2008, **47**, 2601-2616. 11. Bürck, J., Roth, S., Wadhwani, P., Afonin, S., Kanithasen, N., Strandberg, E., and Ulrich, A. S., *Biophys. J.,* 2008, ***95***, 3872-3881.

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