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structure analysis of helices in biomembranes

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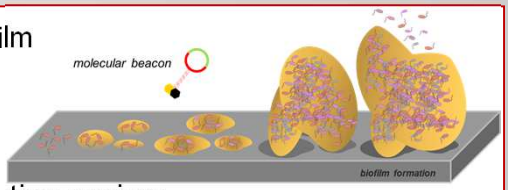
Aim of B2 topic: Find and lokalize different bacterial species in a complex biofilm

Approach: Develop specific *in vivo* reporter systems



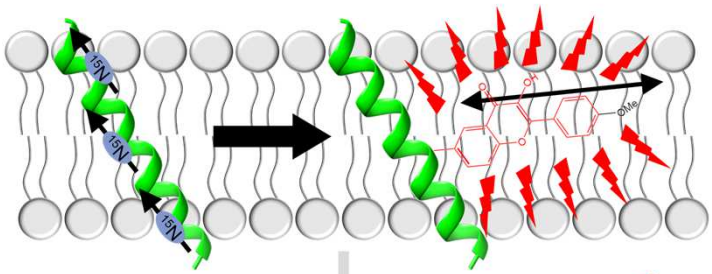
Challenge: Transport the molecular beacons into the bacteria with cell penetrating carriers

Our task: Structure analysis and application of helical cell penetrating peptides in membranes

Anisotropic-based methods for structure analysis

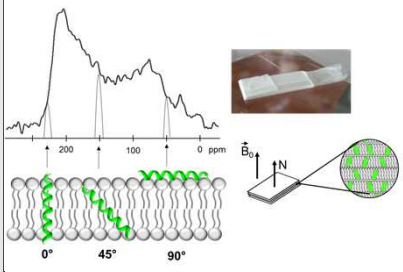
established: solid-state NMR

- accurate structures
- isotope labeling required
- need large amounts (10 - 15 mg)
- only in reconstituted systems
- unnatural conditions

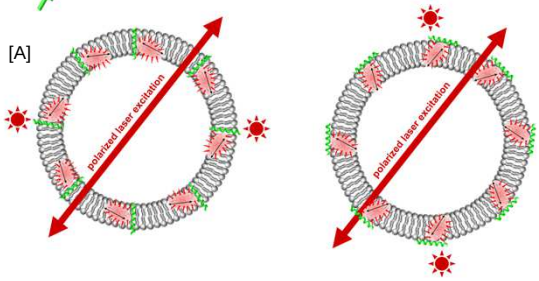


new approach: fluorescence

- highly sensitive (µg material)
- applicable *in vivo*
- novel side-chains have to be designed [B]
- new method has to be explored [3] [A]
- less accurate structures expected



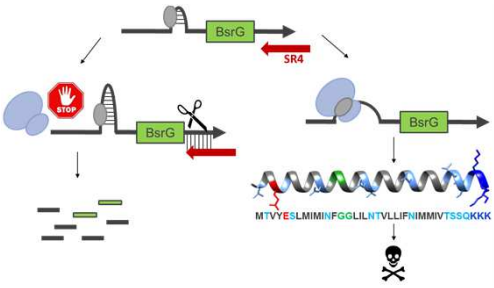
Solid-state NMR (ssNMR) sample of mechanically aligned lipid bilayers and reconstituted ^{15}N-labeled proteins on glass plates. The glass plate stack (wrapped in foil to prevent drying) is placed in the magnetic field so that the lipid bilayer normal is parallel to B_0. The anisotropy the ^{15}N chemical shift provides informations about the orientation of a helix in the model membranes.



For structure analysis by fluorescent imaging, peptides modified with a rigid fluorescent amino acid [B] [C] will be reconstituted in vesicles and exposed to polarized laser light [A]. Fluorophores with a transition dipolar moment parallel to the polarization plane of the polarized laser light are excited preferentially resulting in a detectable fluorescence signal, while fluorophores with dipol moments perpendicular to the polarization plane are not excited As the fluorophore is rigidly attached to the peptide backbone it is possible to determine the orientation of the whole peptide in the membrane.

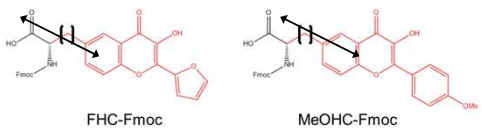
The toxin-antitoxin system type I BsrG/SR4 from *B.subtilis* [1][2]: an example how solid-state ^{15}N-NMR (and OCD) can be used to investigate the orientation of helices in membranes

Binding of SR4 (antitoxin mRNA, red) leads an RNA duplex formation what on the one hand stimulates the degradation of the duplex and on the other hand inhibits translation of the toxin BsrG due to resulting structural changes that prohibit a binding of the ribosome (blue) at the ribosome binding site (grey) what prevents toxin translation. BsrG leads to cell lysis in the absence of SR4.



Design of environment-sensitive fluorescent amino acids of 3HC family [B] with well defined dipolar transition moment (double headed arrow).

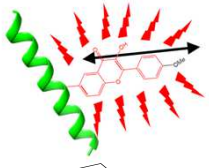
[B]



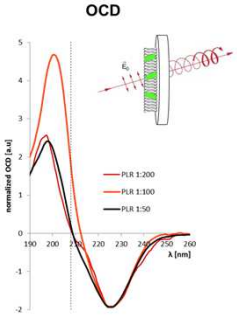
well-known test peptide PGLa: G M A S K A G A I A G K I A K V A L K A L-NH_2

Incorporation (positions 9, 10, 13, 14; shown as red arrow) into the well-known membrane-active peptide PGLa as testpeptide

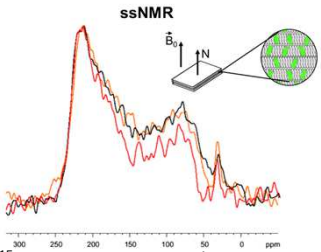
[C]



First investigations in vesicles [A] **Future investigations in living cells**



OCD measurements (used to screen and to optimize conditions for the high-resolution but more time consuming ssNMR) show a transmembrane alignment of BsrG in POPE/PG lipid membranes at different PLRs (positive ellipticity at the 208 nm fingerprint band).



1D ^{15}N ssNMR spectrums show resonances around 220 ppm chemical shift indicating a transmembrane orientation of BsrG, but also a substantial fraction of the protein was aggregated, as it can be seen at the powder lineshape at ~ 75 ppm chemical shift. The orientation of BsrG found by ssNMR results fits to the alignment of the protein observed by OCD. Interestingly, the alignment in the lipid bilayer is not affected by the PLR (protein-to-lipid ratio).

Acknowledgements
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Literature
[1] N. Jahn *et al., Molecular Microbiology*, **2012**, *83 (3)*, 579-598
[2]] S. Brantl *et al., RNA Biology*, **2012**, *9 (12)*, 1488-1490
[3] A.V. Strizhak *et al., Bioconjug. Chem.* **2012**, *23*, 2434-2443