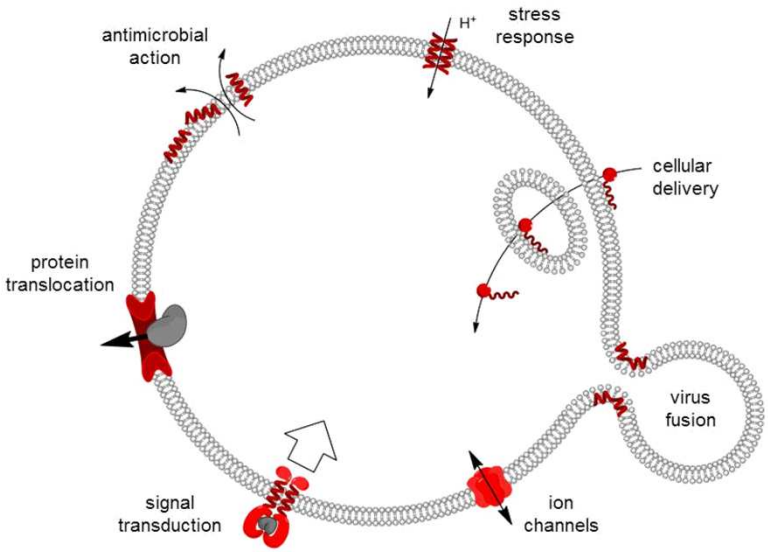
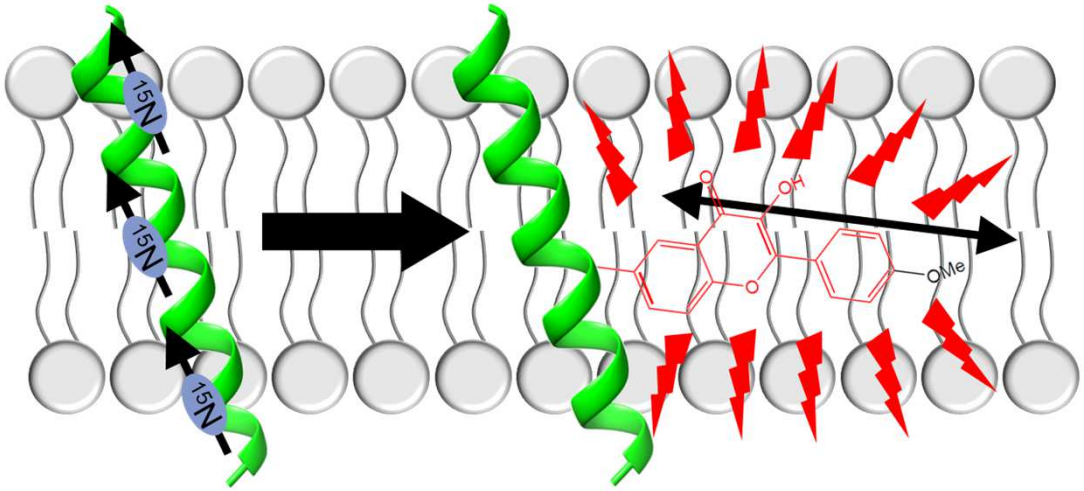
Anisotropy-based methods for structure analysis of helices in biomembranes

Katharina Becker, AK Ulrich



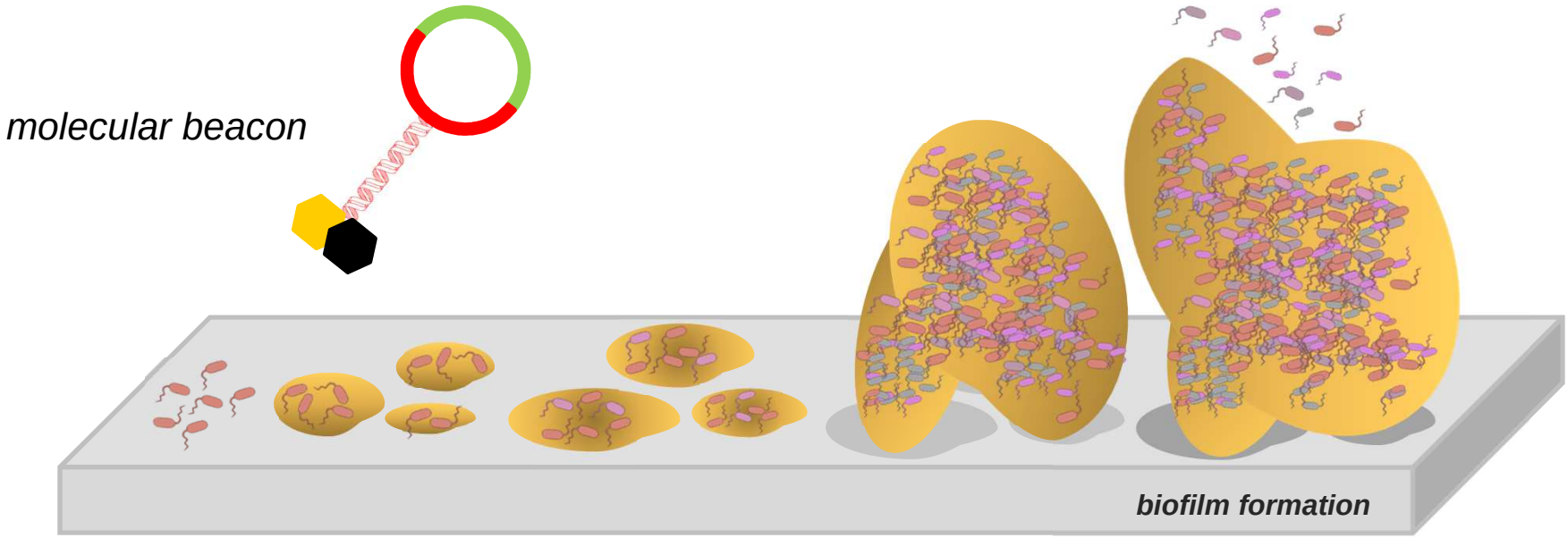
transport across membranes



B2: „Reporter systems for live cell imaging in bacterial communities“

Aim: Identify and localize different bacterial species in a complex biofilm

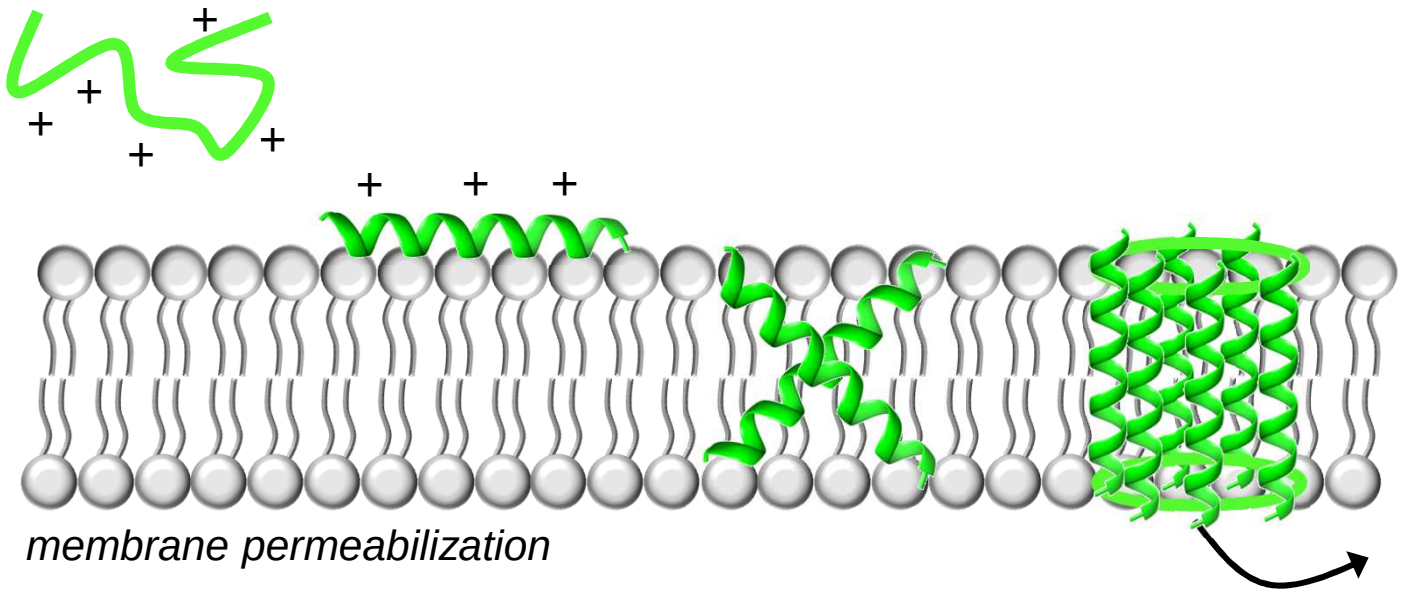
Approach: Develop specific *in vivo* reporter systems



B2: „Reporter systems for live cell imaging in bacterial communities“

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

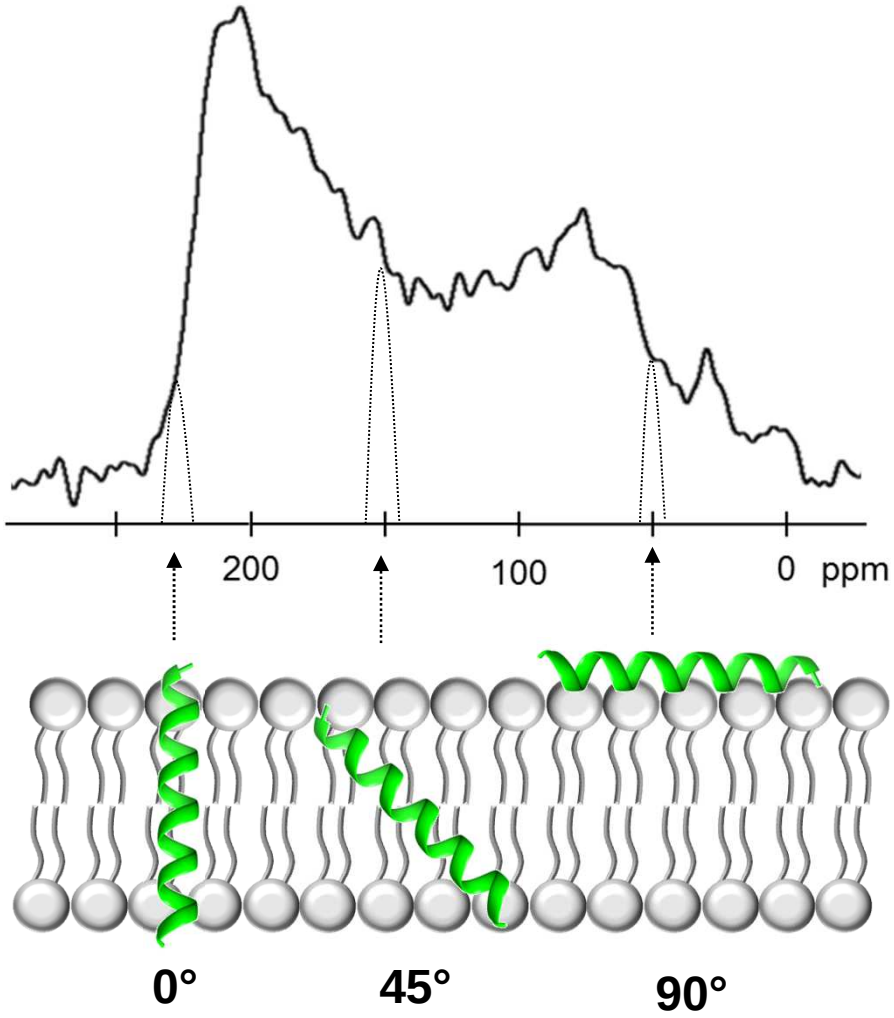
My task: Structure analysis and application of helical cell penetrating peptides in membranes

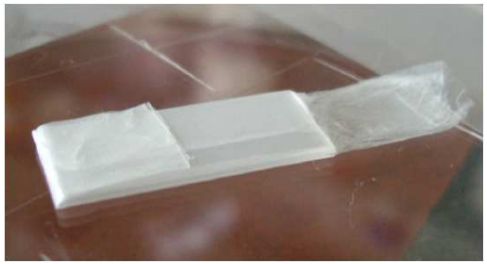


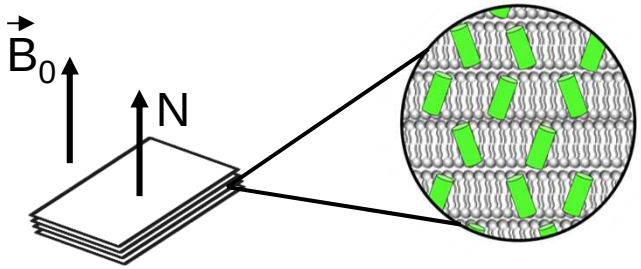
membrane permeabilization

from ssNMR
in model systems
→ living cells

1D ^{15}N solid-state NMR

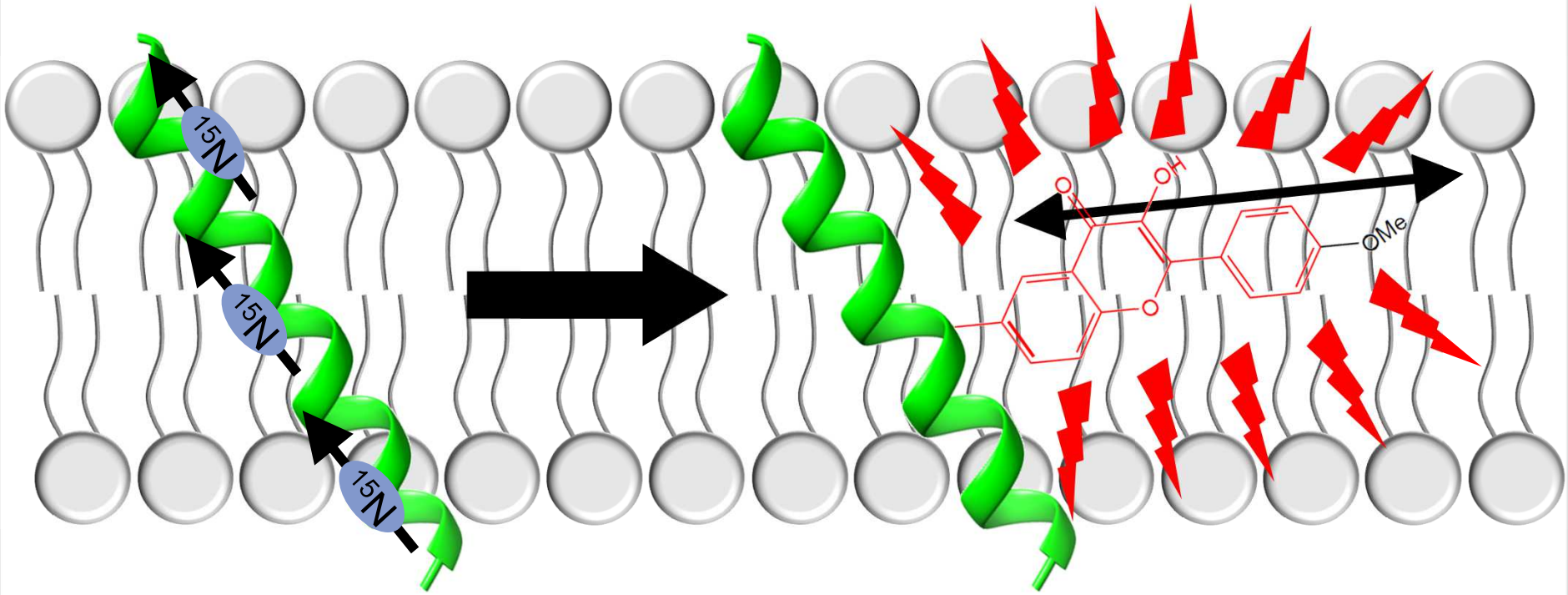






10-15 mg ^{15}N labelled protein
24 hours NMR time

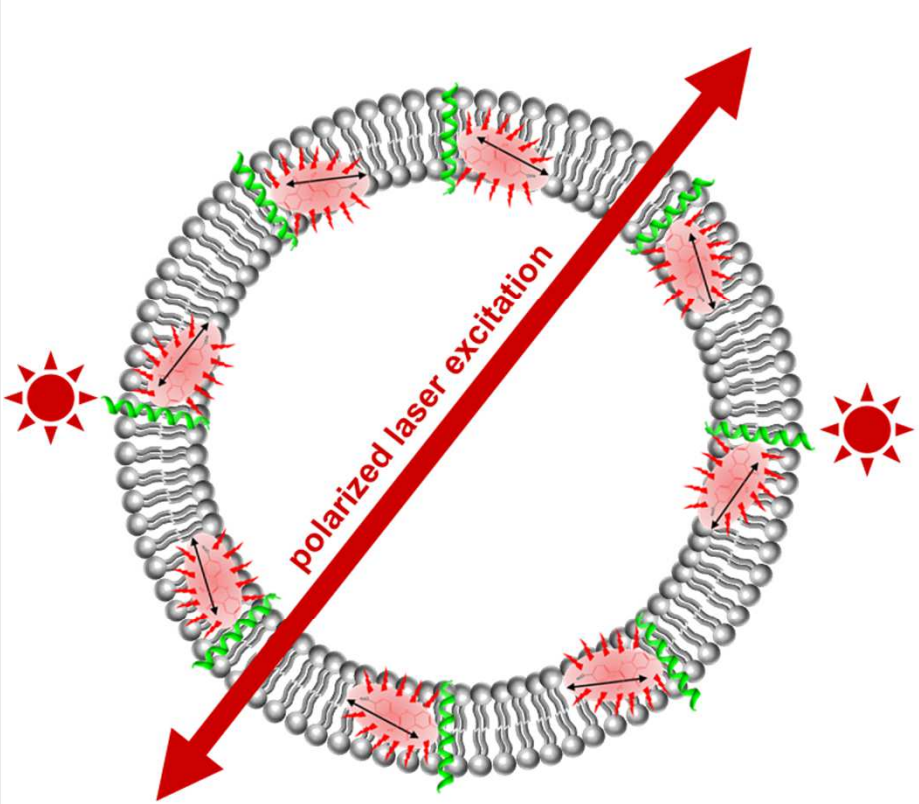
My strategy

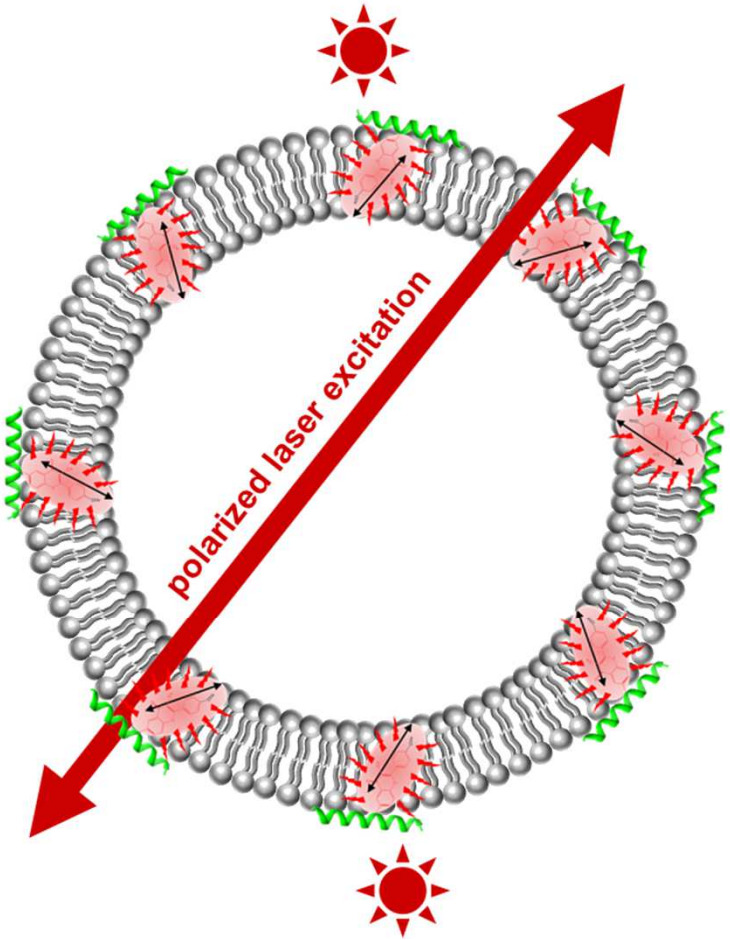


solid-state NMR

fluorescence

Fluorescence anisotropy to determine peptide orientation in the membrane

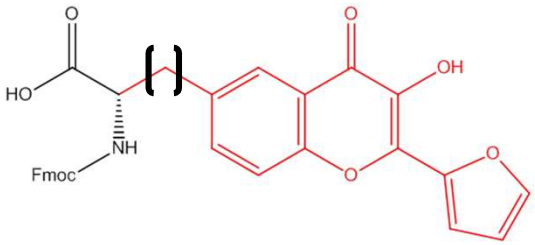




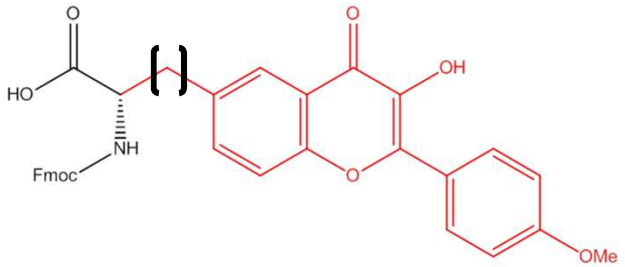
Synthesis of fluorescent amino acids and modified PGLa

Colaboration partners from University of Kiev (Prof. Igor Komarov) and Dr. Sergii Afonin (Ulrich group)

fluorescent amino acids:



FHC-Fmoc

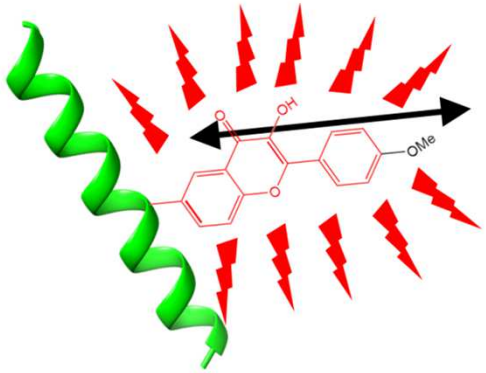


MeOHC-Fmoc

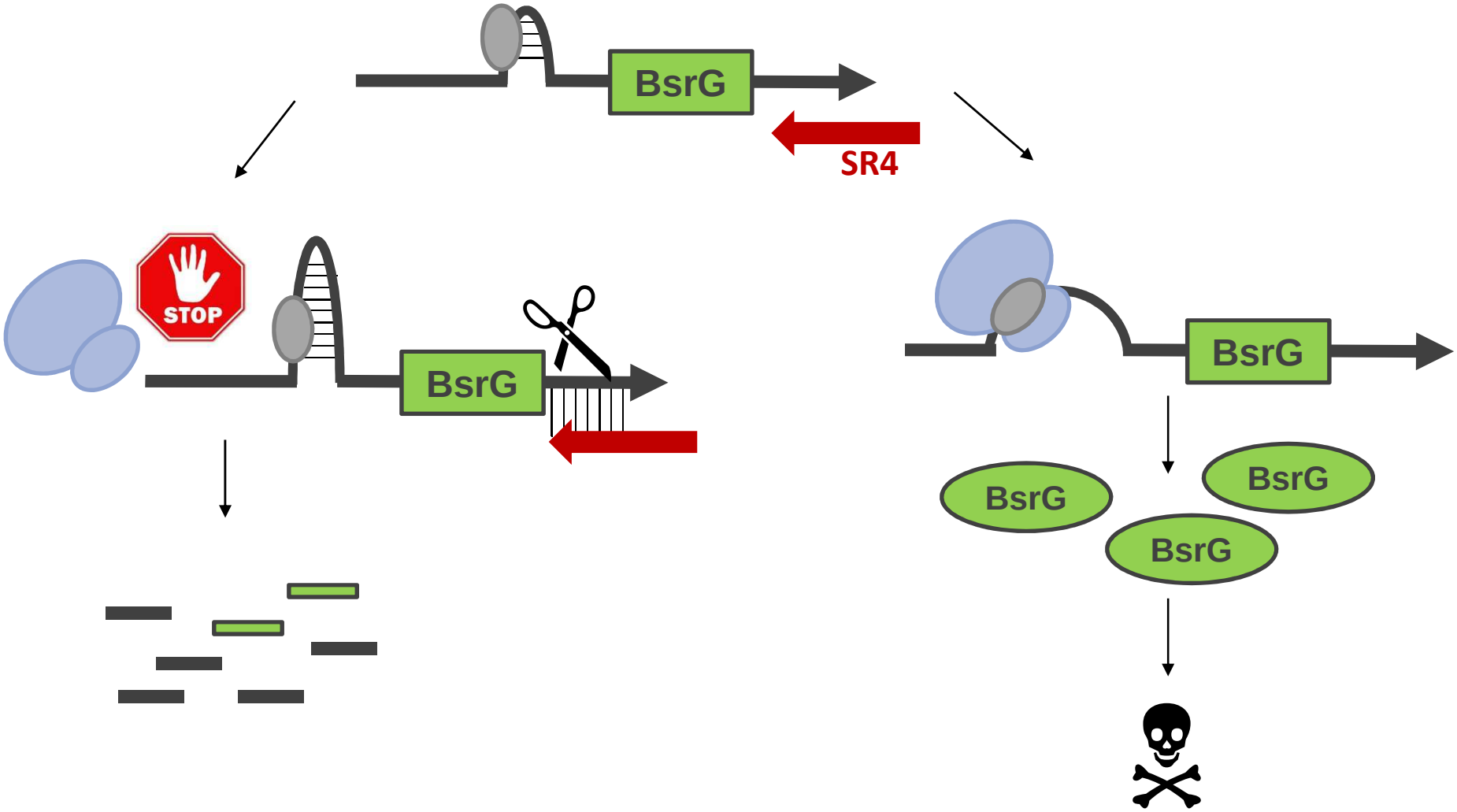
well-known test peptide PGLa: **G M A S K A G A I A G K I A K V A L K A L-NH$_2$**

Current status:

- **flexible** amino acids successfully synthesized
- PGLa analogues successfully synthesized
- synthesis of **rigid** amino acids in progress



Toxin-antitoxin system type I BsrG/SR4 (*B.subtilis*)



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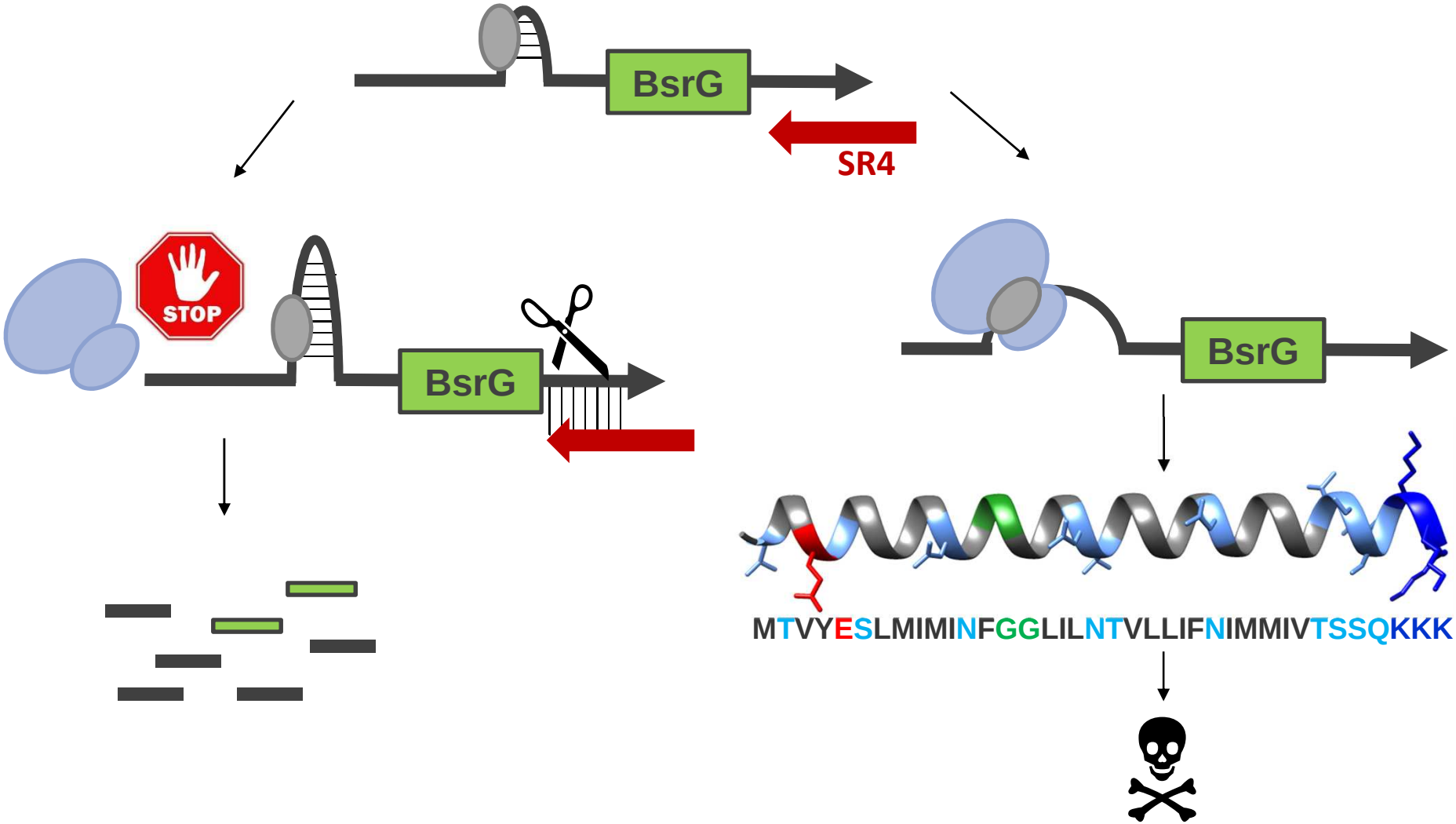
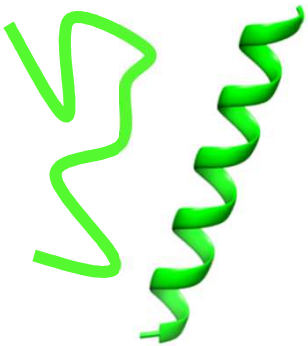


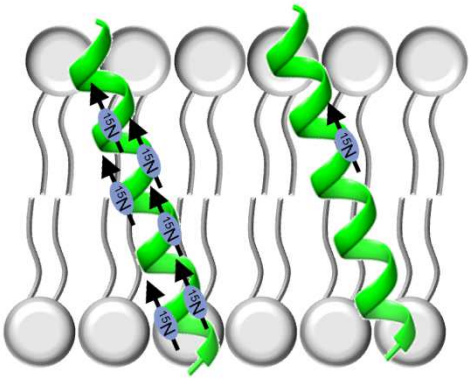
Structure-function analysis of BsrG

secondary structure



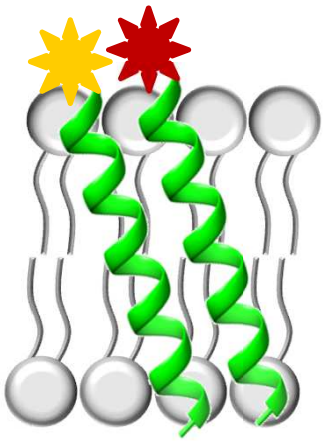
→ CD

orientation in membranes



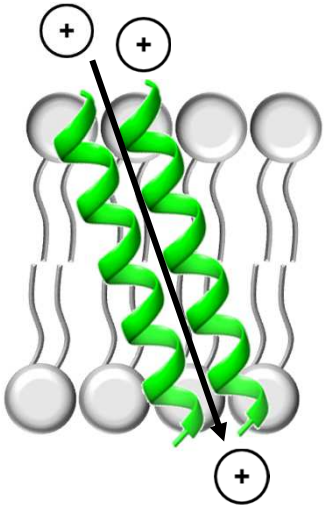
→ ssNMR (OCD)

oligomerization behaviour



→FRET

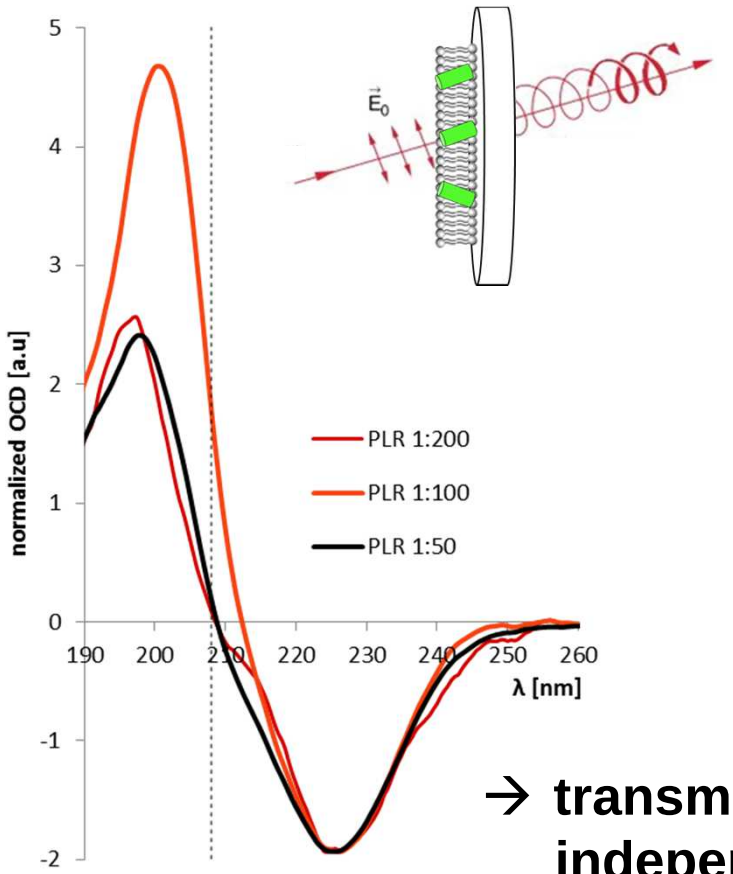
biofunctional assays



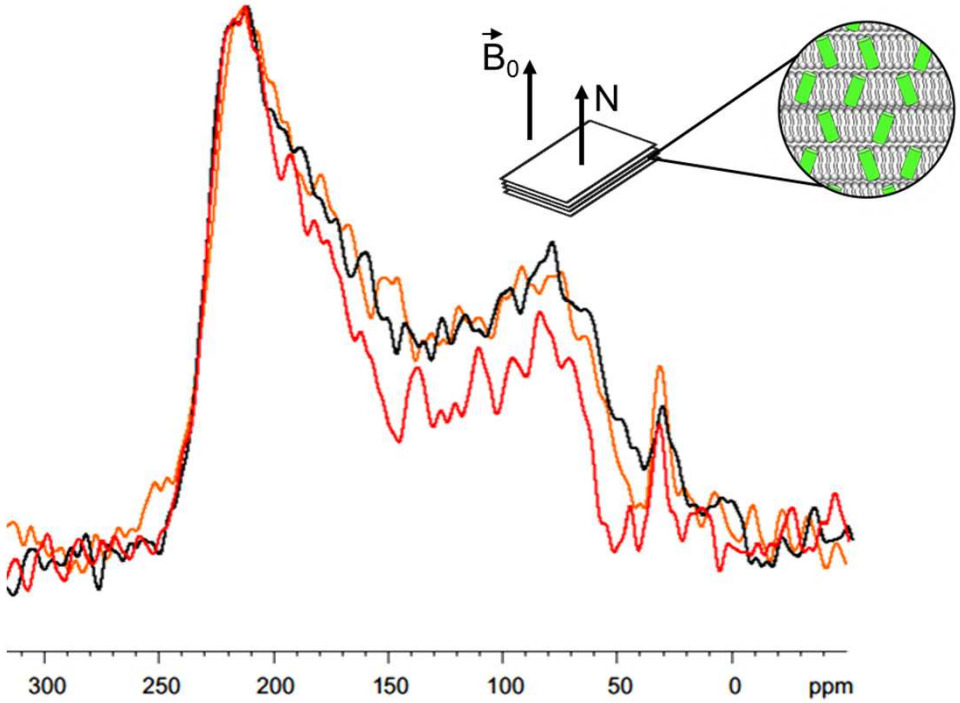
in different model membranes; wild type peptide and mutants

Structure analysis of BsrG in oriented POPE/PG bilayers

OCD



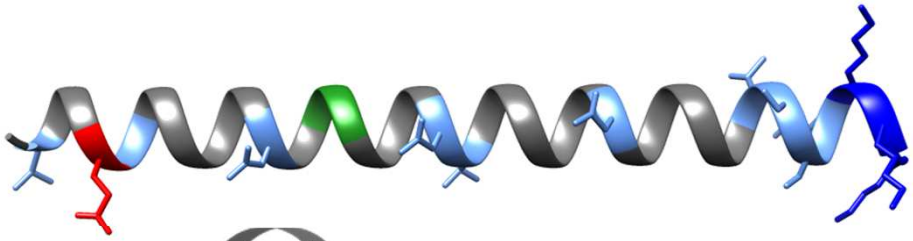
ssNMR

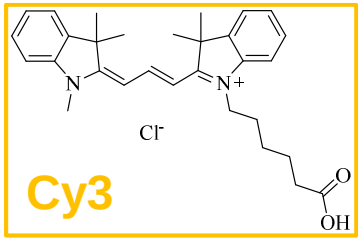


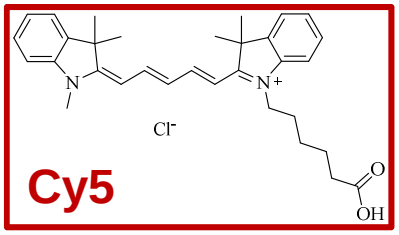
→ **transmembrane orientation independent from PLR (protein-to-lipid ratio)**

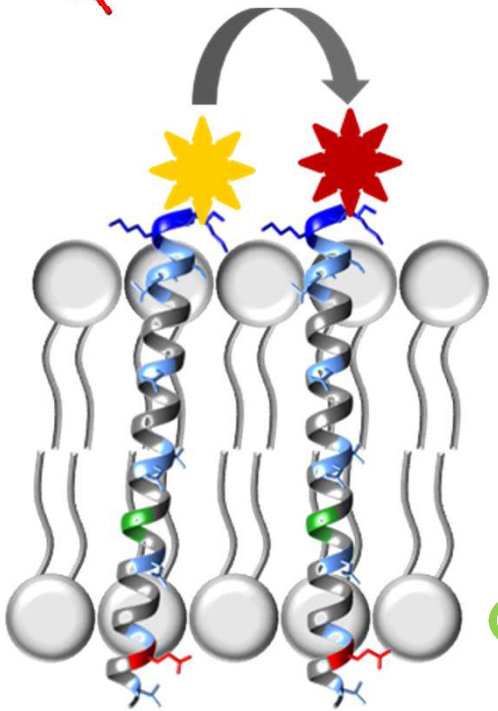
First FRET experiments

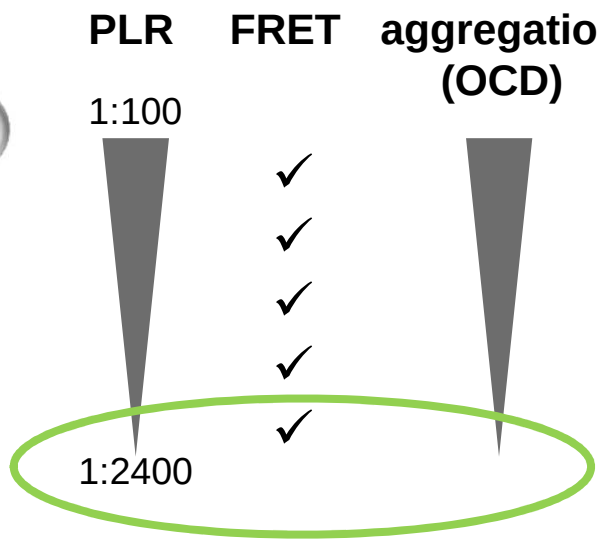
Work in progress, Carolin Pykta, Master‘s Thesis (Ulrich group)











increased aggregation behaviour of fluorescent labeled peptides

FRET observed
→not monomeric
→parallel orientation

Structure analysis of cell penetrating peptides

solid-state NMR

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

fluorescence

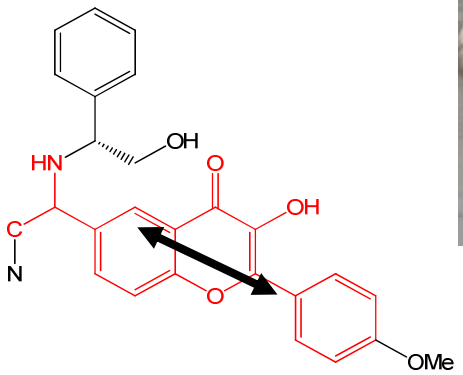
- highly sensitive (µg material)
- applicable *in vivo*
- novel side-chain has to be designed
- new method has to be explored
- less accurate structures expected
- increased peptide aggregation

Acknowledgements

Anne S. Ulrich

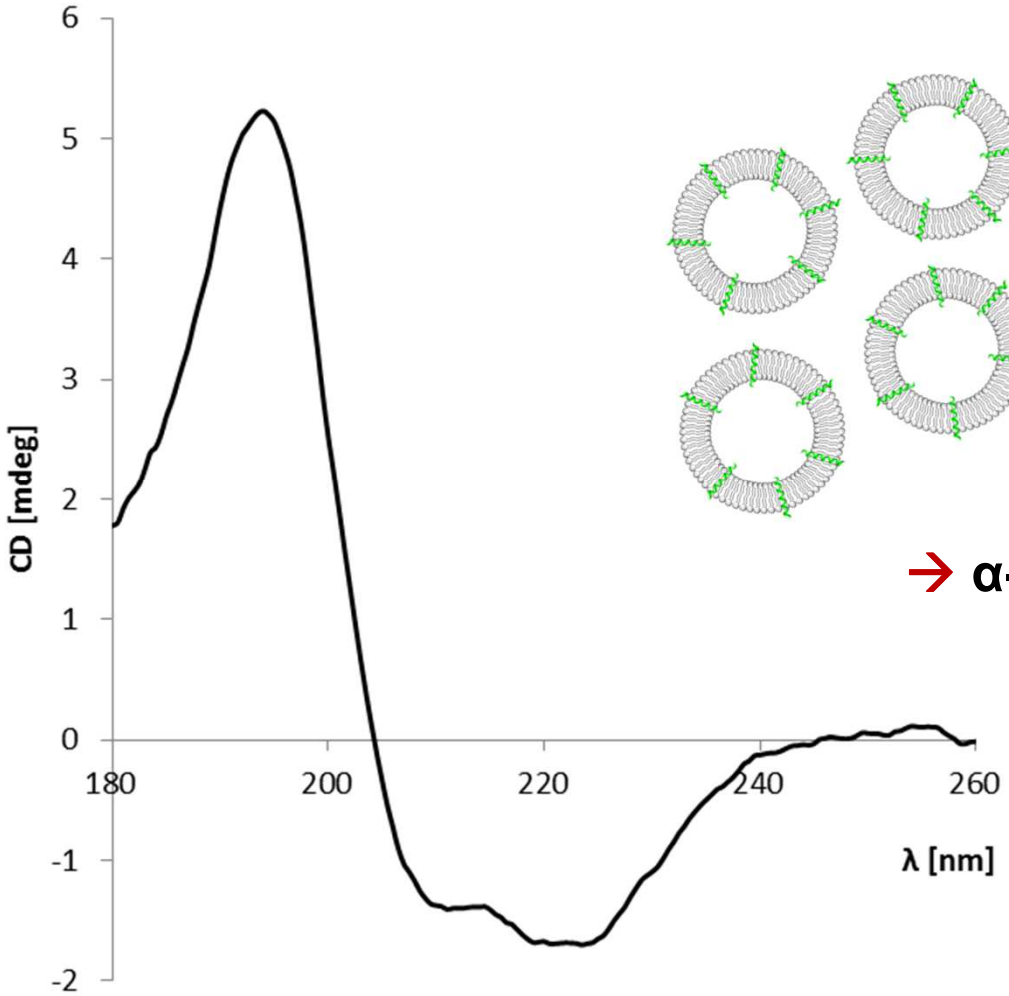
Sergii Afonin

Oleg Babii



partners from Kyiv: Prof. Igor V. Komarov

CD spectrum of BsrG in POPE/PG lipid vesicles



→ **α-helical secondary structure**