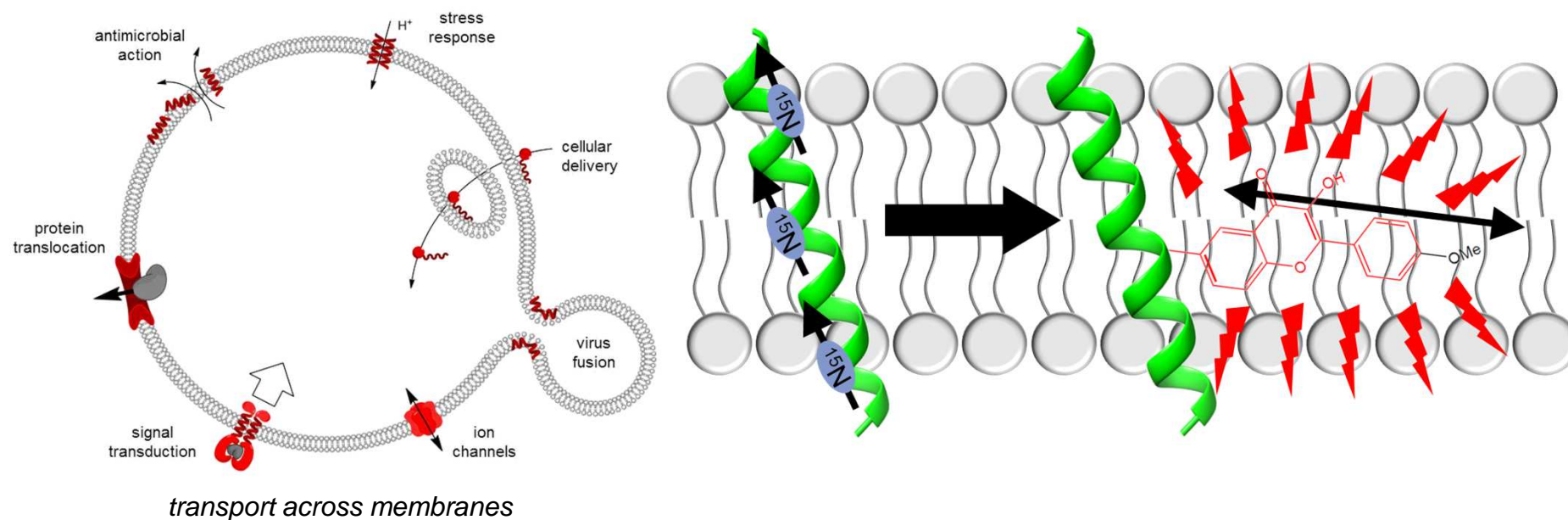


# Anisotropy-based methods for structure analysis of helices in biomembranes

Katharina Becker, AK Ulrich

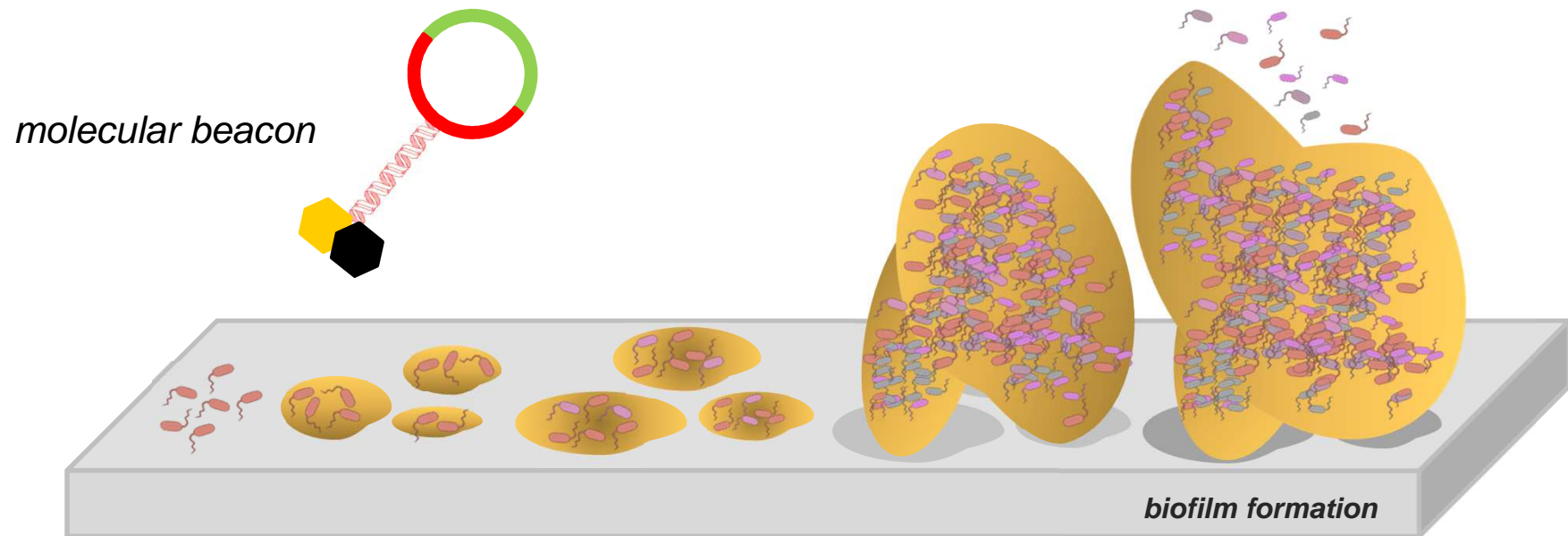


## B2: „Reporter systems for live cell imaging in bacterial communities“

---

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

**Approach:** Develop specific *in vivo* reporter systems

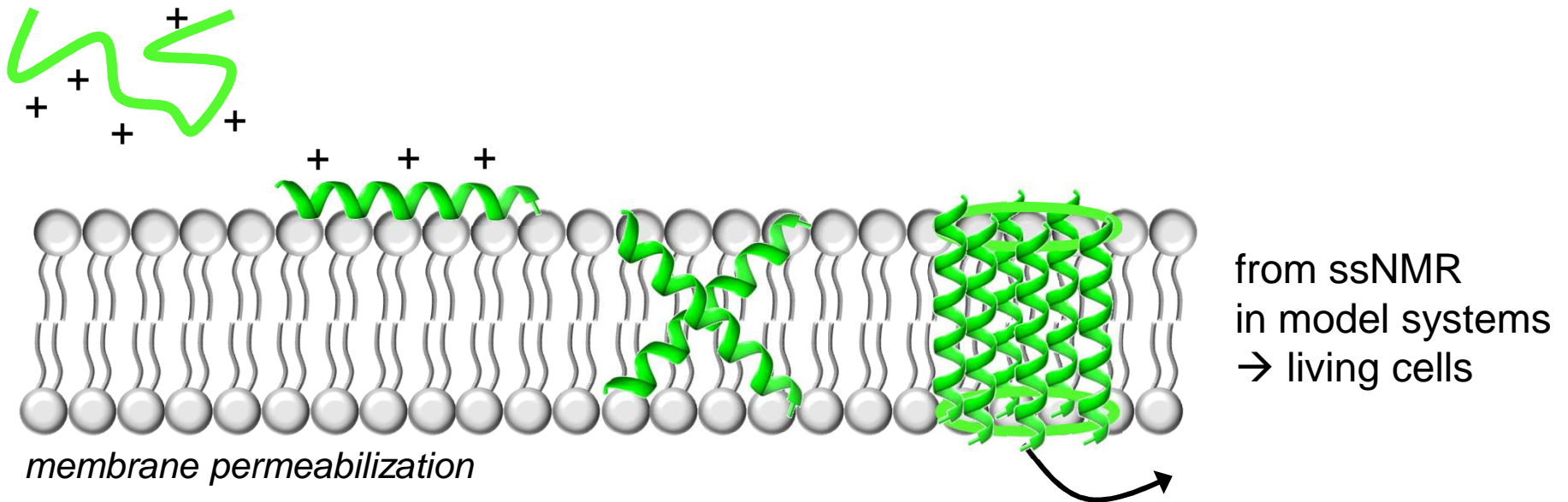


## B2: „Reporter systems for live cell imaging in bacterial communities“

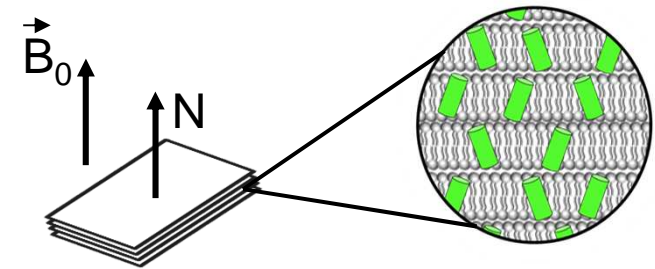
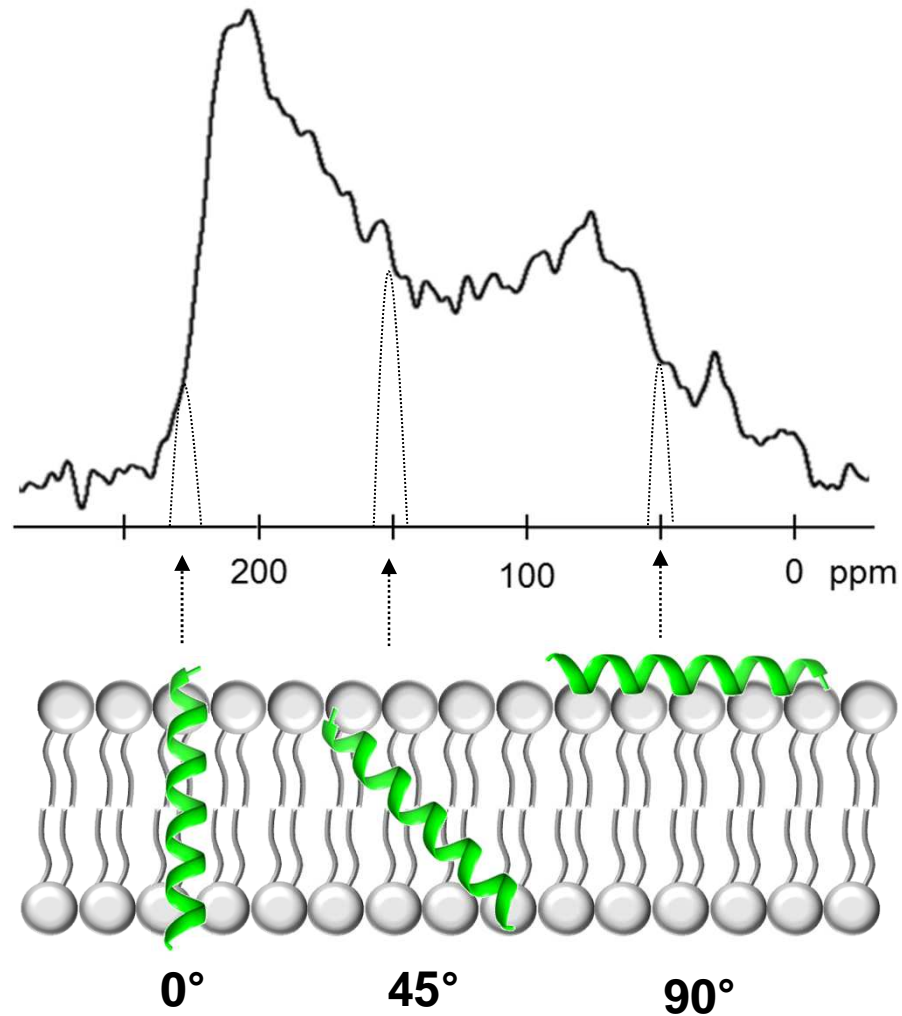
---

**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



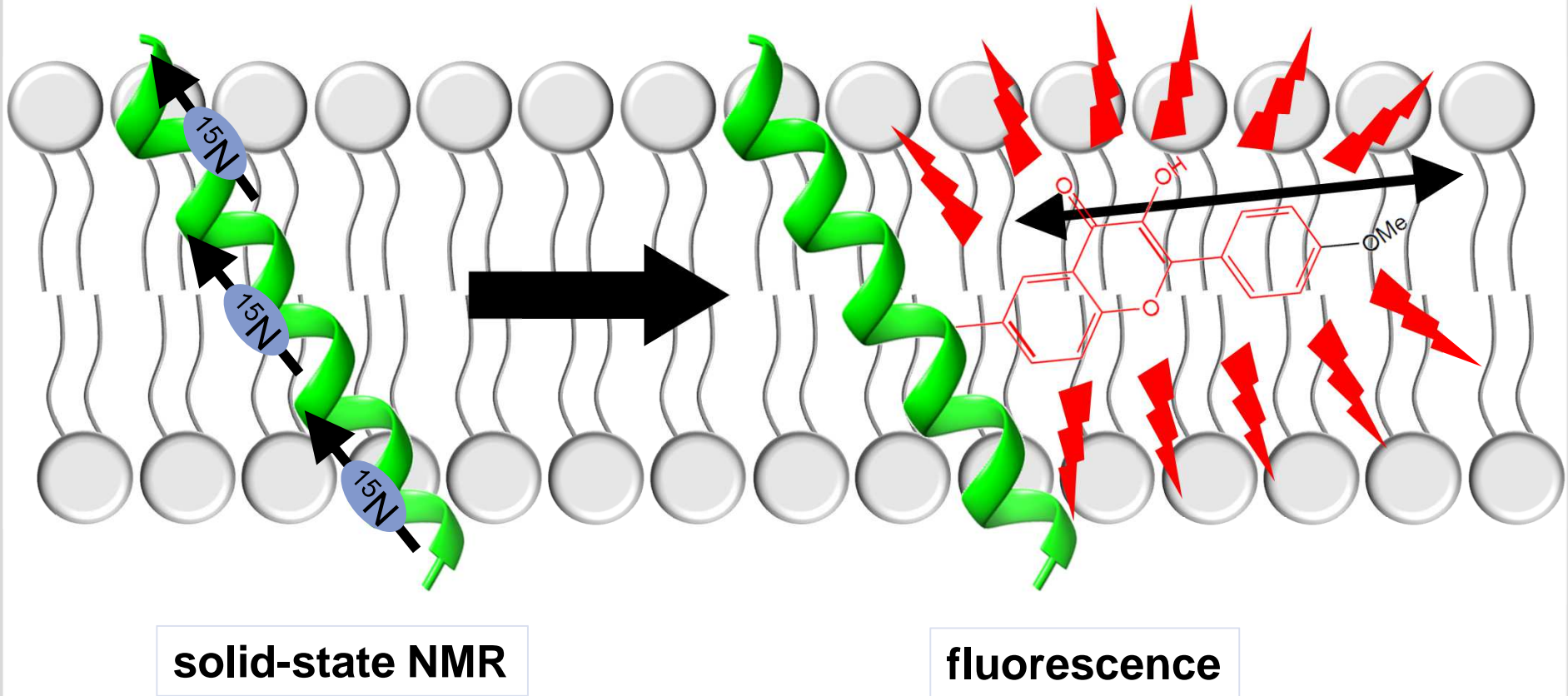
# 1D $^{15}\text{N}$ solid-state NMR



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

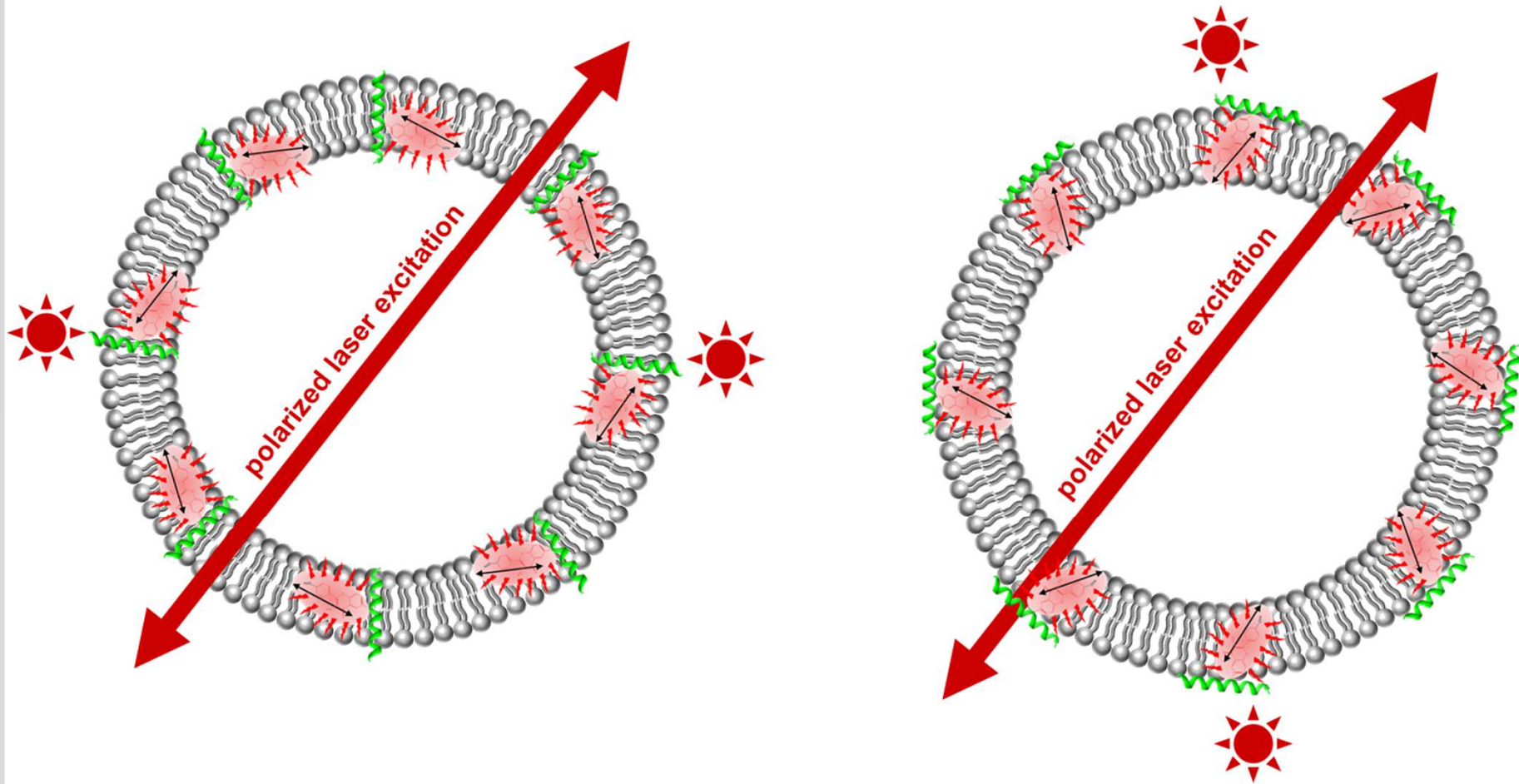
# My strategy

---



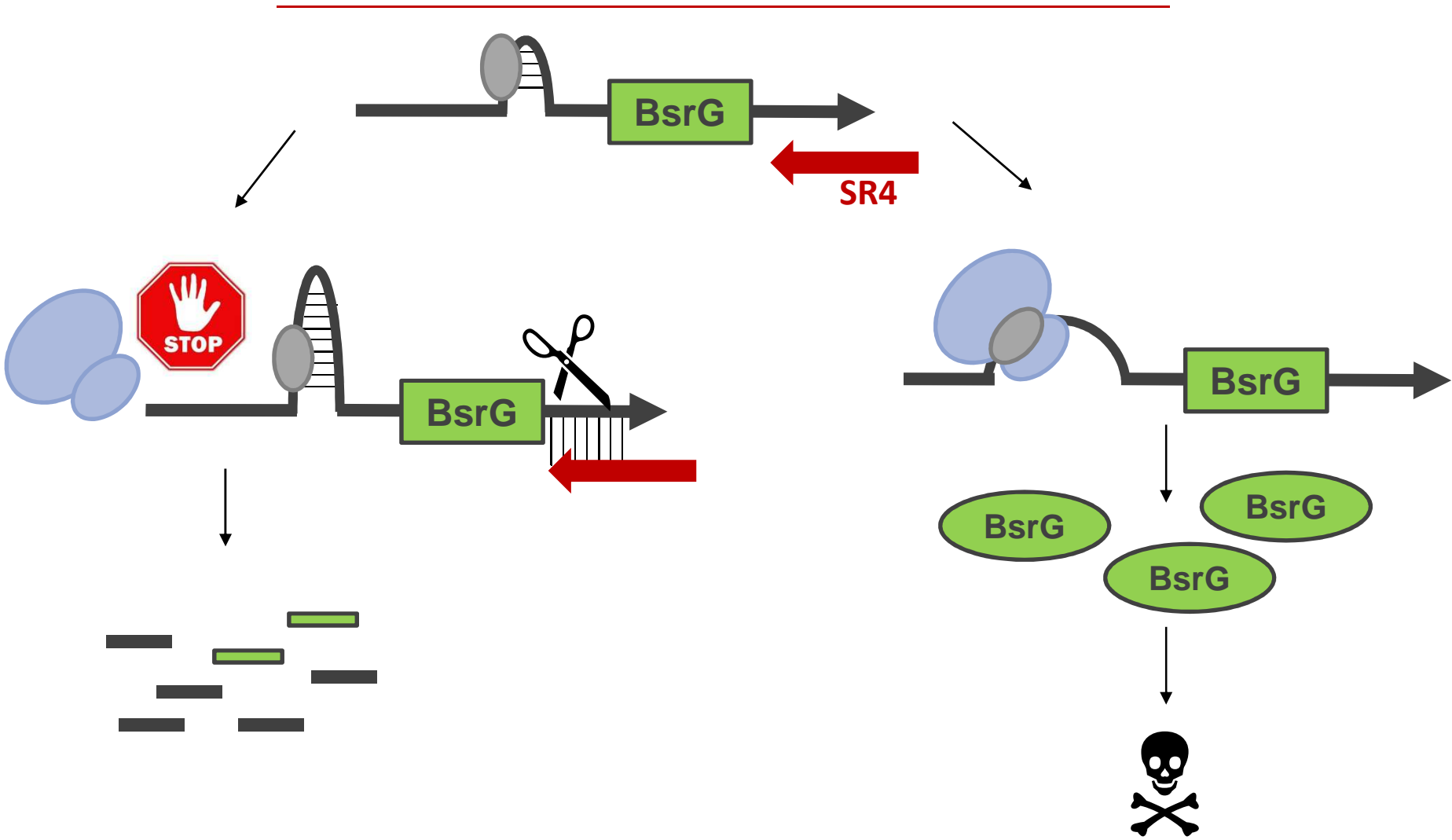
# Fluorescence anisotropy to determine peptide orientation in the membrane

---



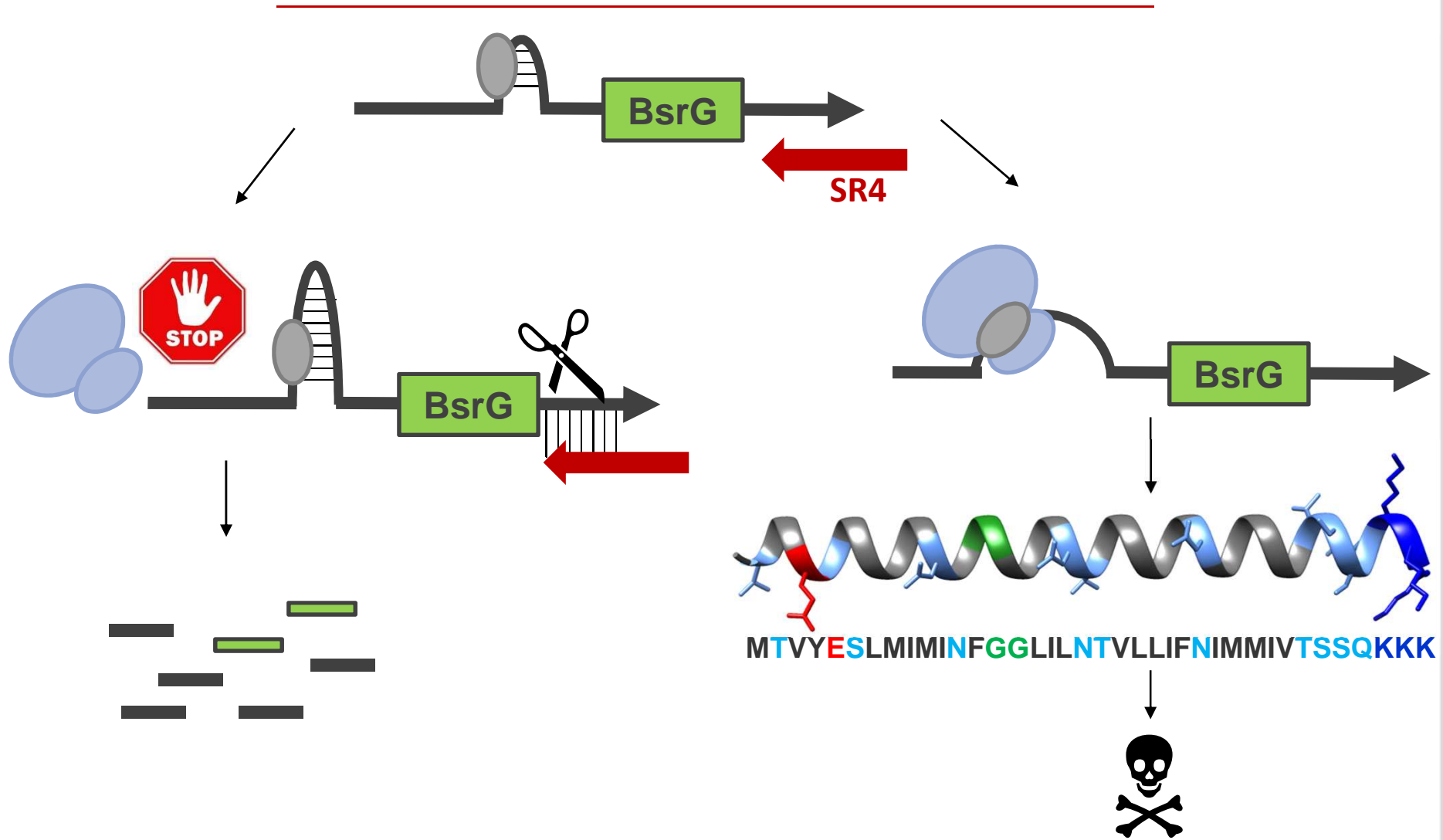


# Toxin-antitoxin system type I BsrG/SR4 (*B.subtilis*)



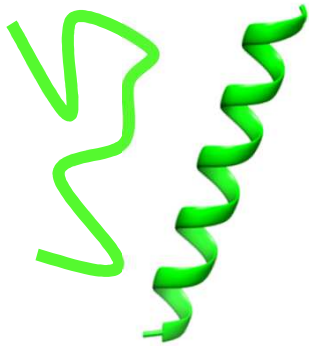


# Toxin-antitoxin system type I BsrG/SR4 (*B.subtilis*)



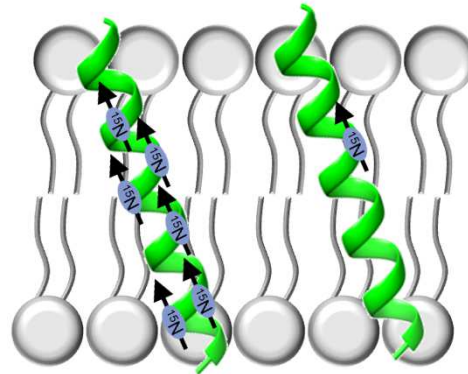
# Structure-function analysis of BsrG

secondary  
structure



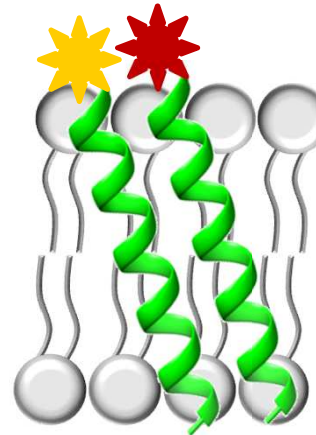
→ CD

orientation  
in membranes



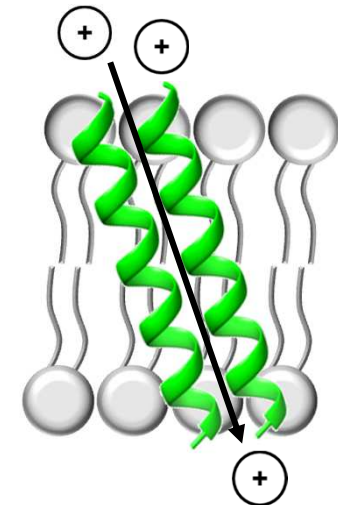
→ ssNMR (OCD)

oligomerization  
behaviour



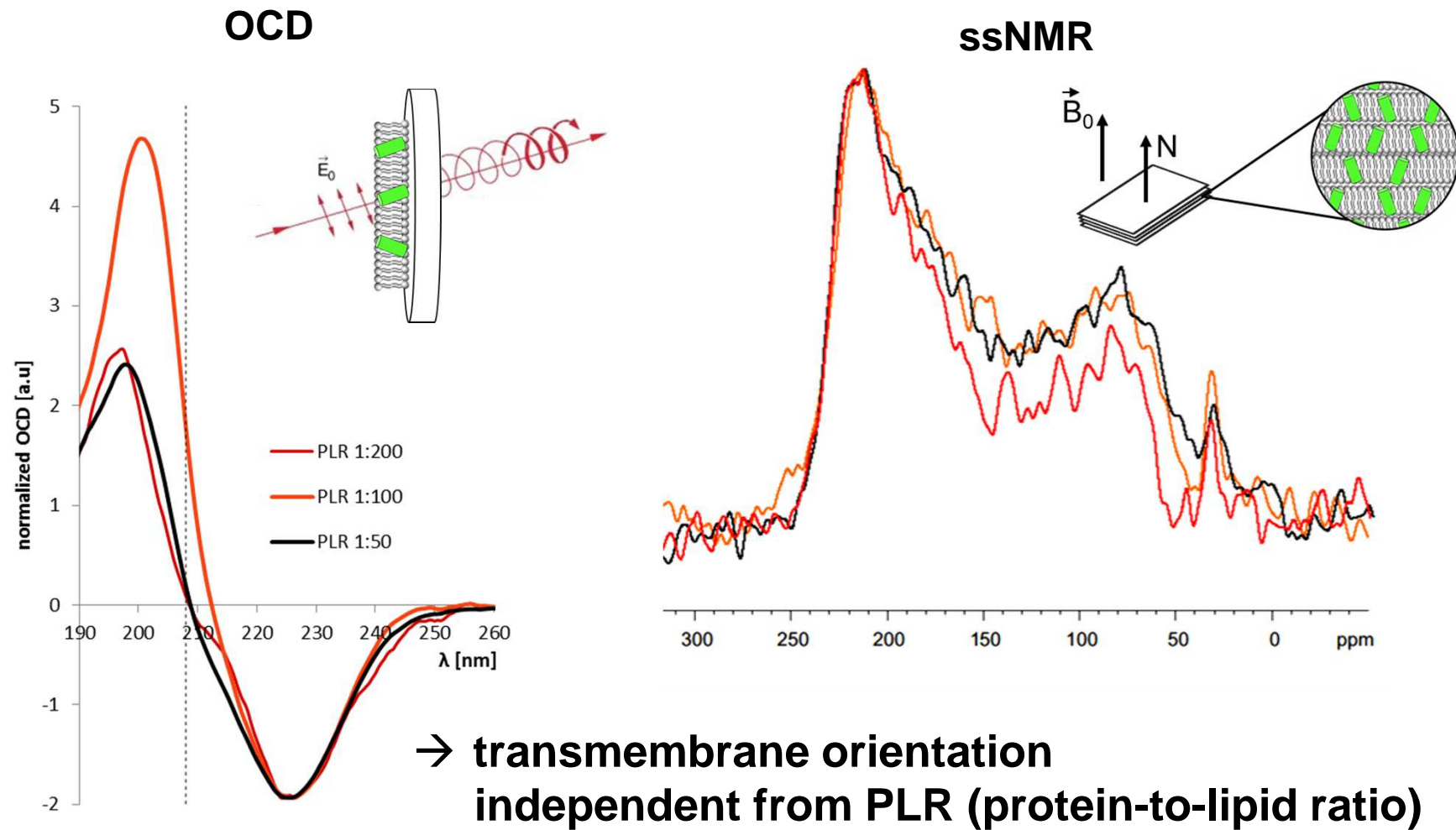
→ FRET

biofunctional  
assays



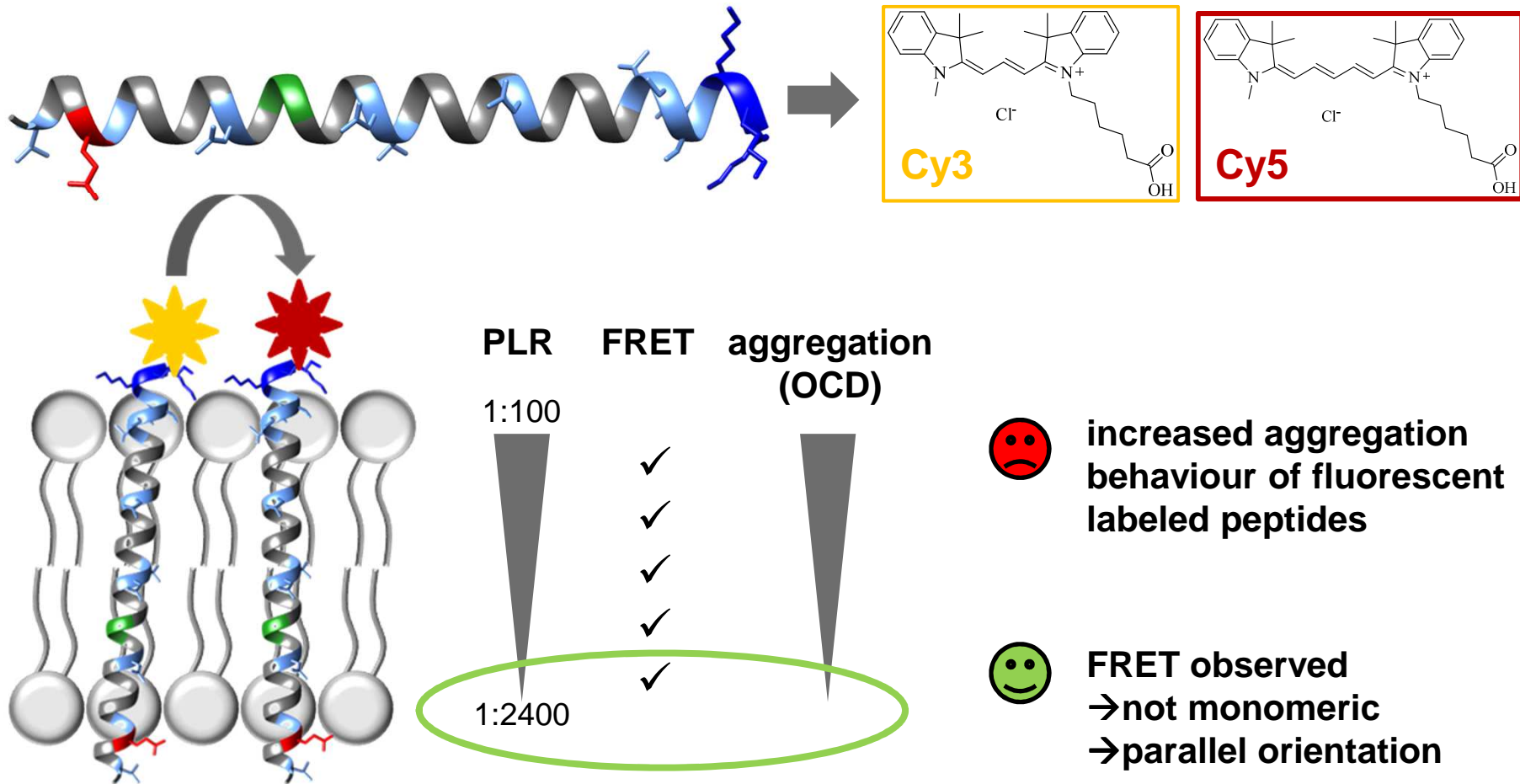
in different model membranes; wild type peptide and mutants

# Structure analysis of BsrG in oriented POPE/PG bilayers



# First FRET experiments






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









# 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 ( $\mu\text{g}$  material)
-  applicable *in vivo*
-  novel side-chain has to be designed
-  new method has to be explored
-  less accurate structures expected
-  increased peptide aggregation

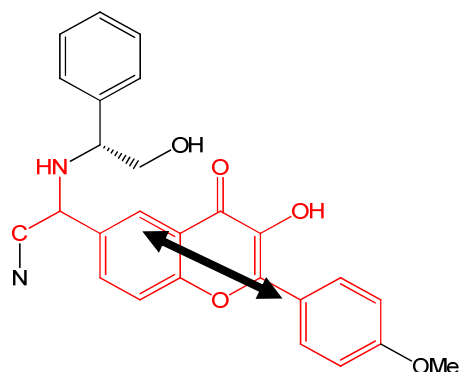
# Acknowledgements

---

Anne S. Ulrich

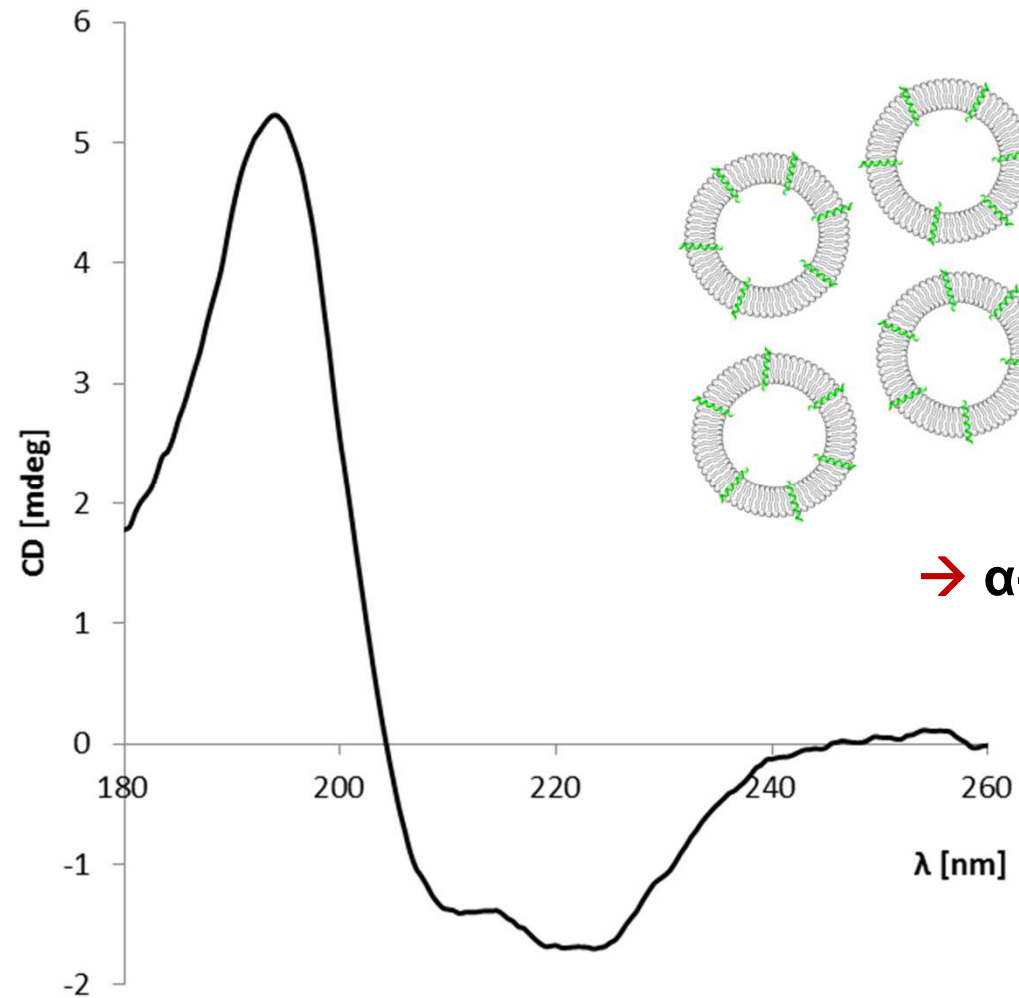
Sergii Afonin

Oleg Babii



partners from Kyiv: Prof. Igor V. Komarov

# CD spectrum of BsrG in POPE/PG lipid vesicles



→  $\alpha$ -helical secondary structure