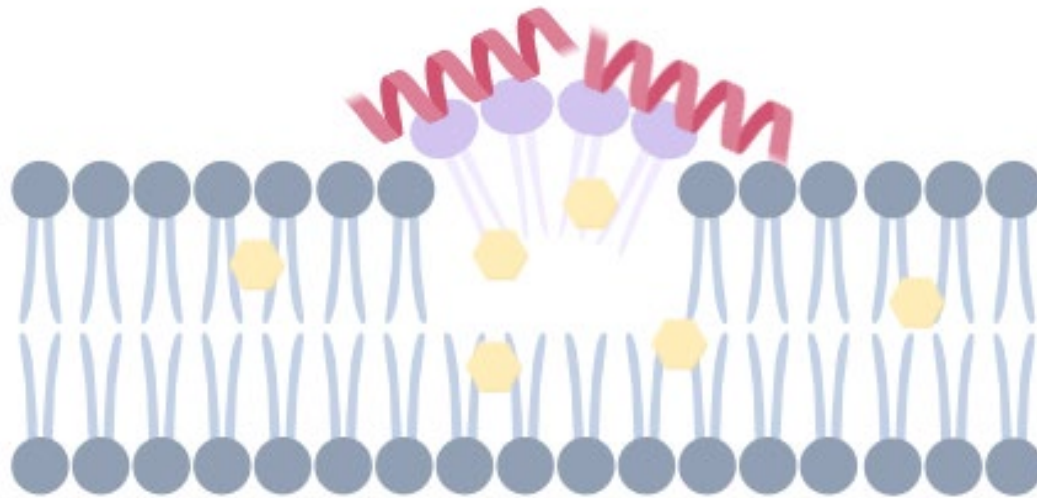


# Design and Evaluation of a Cell-Penetrating Peptide Library Based on SNARE Mimetics for Optimization of siRNA Delivery



By: Ryan Grosso  
Mentor: Emma Wezenberg  
PI: Professor Alexander Kros

## About Me

- ❖ Phoenix, Arizona
- ❖ Medical Microbiology at Arizona State University
- ❖ Fourth year of my Bachelor's
  - ❖ Currently applying to graduate programs and dream of working in academia and research
- ❖ Hobbies include reading fantasy novels/series, exploring new music (used to play cello and piano), hiking, swimming, and traveling

## My Research Journey

- ❖ At home, I am currently heading a research project where we use computational methodologies such as Molecular Dynamics simulations and AlphaFold to study the structure and function of Cas10 in CRISPR systems present in the *Deinococcus* genus.
- ❖ Here at Leiden University in the Netherlands, I am working on a project where we aim to create novel cell-penetrating peptides and test their efficacy in delivering siRNA into cells.

## Leiden, Netherlands



## Leiden's Ontzet



# Project Background

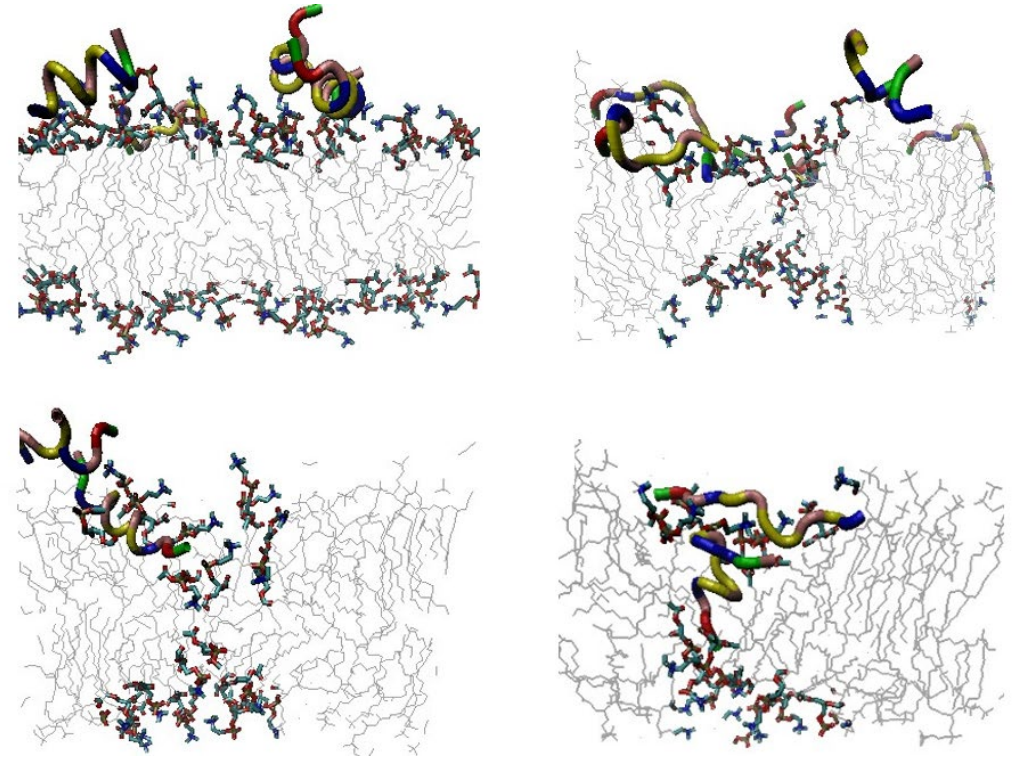
## What are Cell-Penetrating Peptides (CPPs)

### Cell-Penetrating Peptides Definition:

- Short peptides capable of crossing the cell membrane.
- Typically  $\alpha$ -helices, positively charged, and amphipathic.

### Applications:

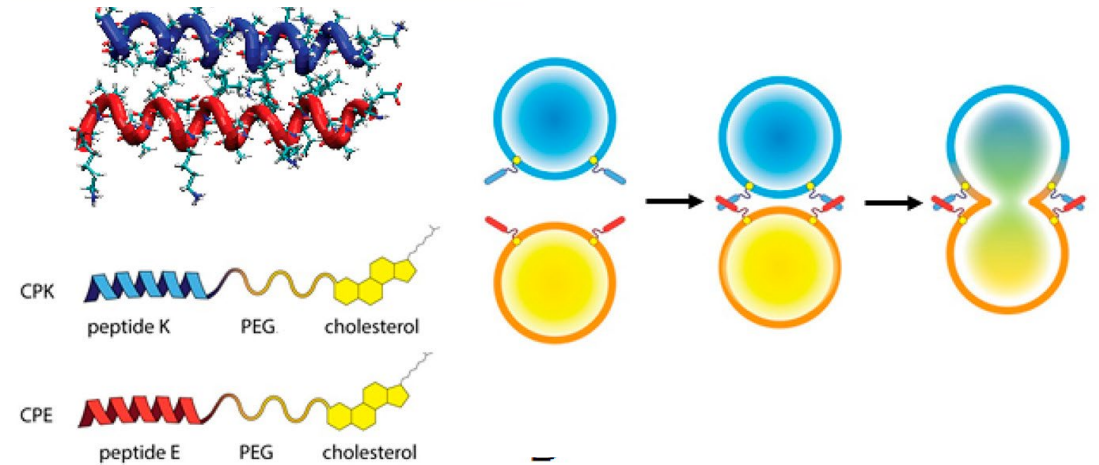
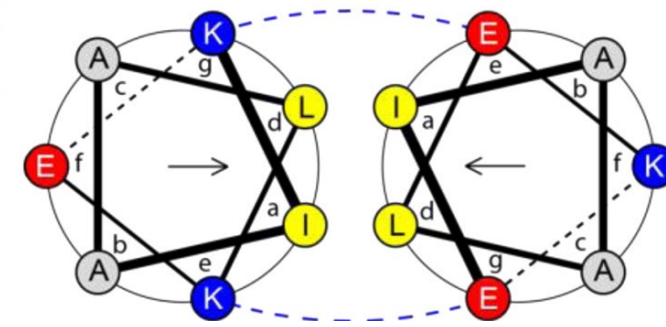
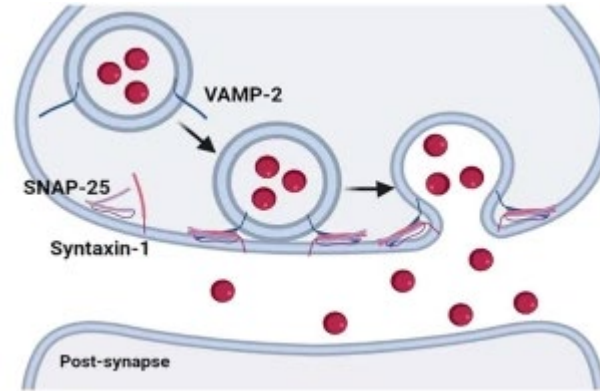
- Deliver cargo to the interior of cells.
  - Drug delivery
  - Gene therapy



Adjusted from Gao *et al.*, *Nanoscale* (2019)

## What is the SNARE mimetic system?

- SNARE proteins: Natural fusion proteins that mediate membrane fusion in cells
- SNARE Mimetics: Synthetic peptides designed to mimic SNARE function
  
- Peptides E and K (Developed by Prof. Kros's group, Leiden University):
  - E: (EIAALEK)<sub>4</sub>
  - K: (KIAALKE)<sub>4</sub>
  
- Potential Applications:
  - Drug delivery systems
  - Studying membrane fusion mechanisms
  - Developing synthetic cell-like systems



Adjusted from Jian Yang *et al.*, ACS Cent. Sci. (2016)

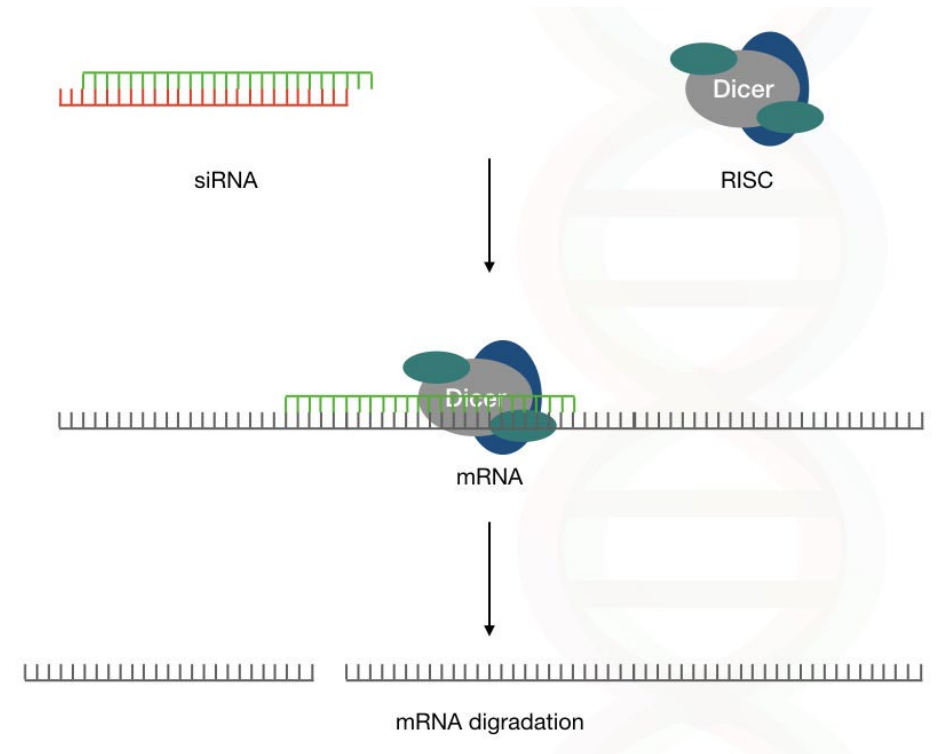
## What is Small Interfering RNA (siRNA)?

### Small Interfering RNA Definition:

- Short, double-stranded RNA molecules (20-25 base pairs).
- Interfere with specific gene expression by binding to complementary mRNA.

### Applications:

- Silences specific genes.
  - Target oncogenes (driving genes for cancer)
  - Suppress expression of mutated genes (in the case of inherited diseases)



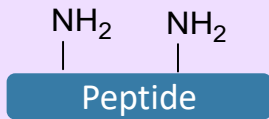
Adjusted from Tushar, Genetic Education (2019)

A detailed 3D molecular model of a cell membrane, showing a phospholipid bilayer with various proteins and DNA double helix structures. The colors are primarily purple, blue, and yellow.

# Current Progress and Future Work

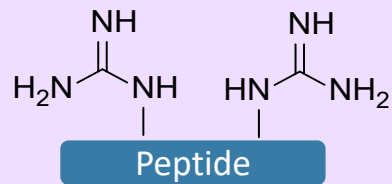
### Electrostatic interaction with lysine

- K3 and K4 are positively charged  $\alpha$ -helices that bind RNA.



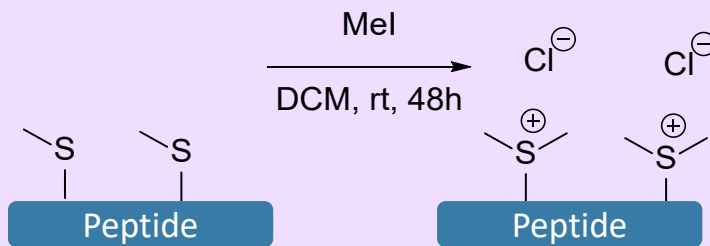
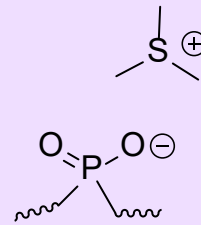
### Electrostatic interaction with arginine

- Arginine pKa: 13.8  $\rightarrow$  more positive
- Lysine pKa: 10.8  $\rightarrow$  less positive



### Permanent cationic interaction

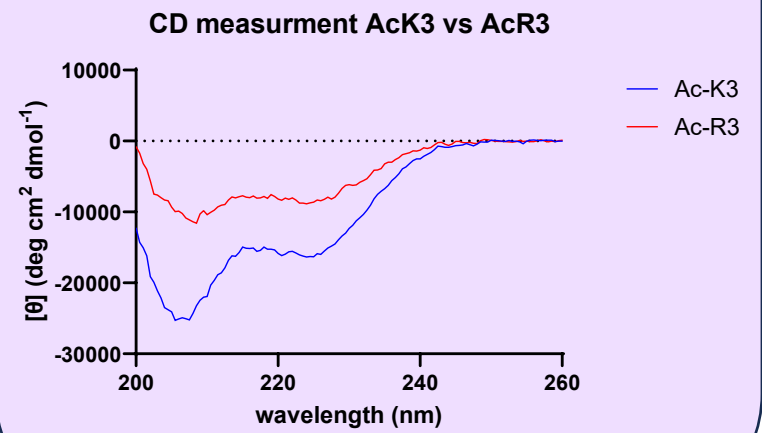
- Alkylated Methionine is permanently positively charged.





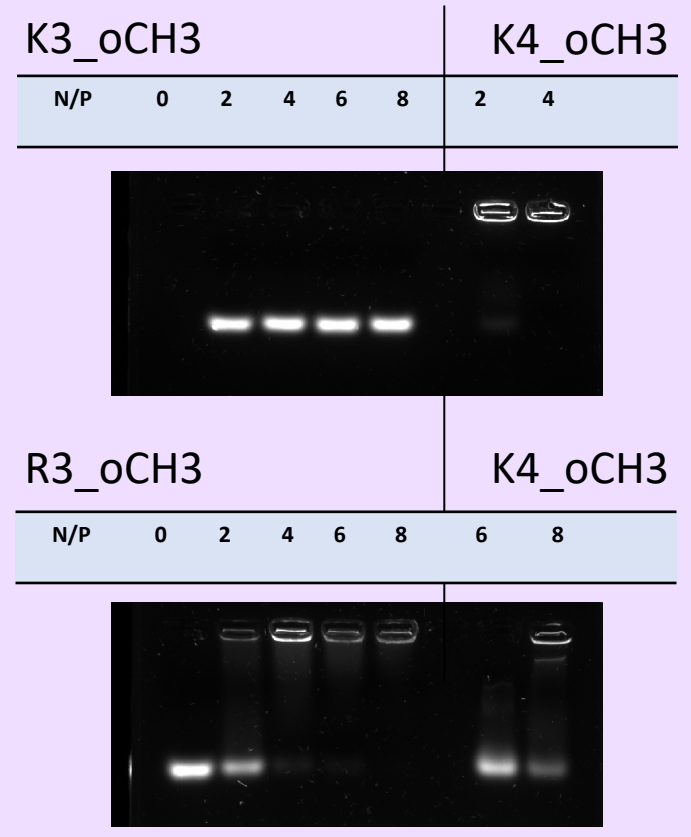
## Circular Dichroism (CD)

- Determination of Secondary Structure



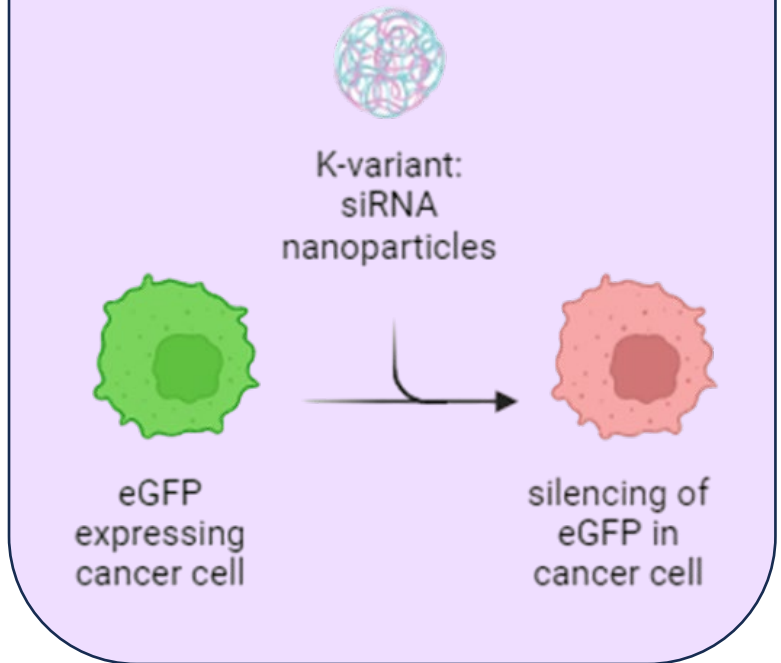
## Electromobility Shift Assay (EMSA)

- To test if the peptide can complex with siRNA at certain N/P ratios



## Flow Cytometry

- To measure effectiveness of silencing eGFP expression in cancer cell line



### **Acknowledgements:**

Thank you to Ms. Wezenberg and Professor Kros for teaching and mentoring me in the SBC lab at Leiden University.

Thank you to Ms. Usha Mohunlol for advising and making this wonderful opportunity possible.

### **References:**

Gao, X., Hong, S., Liu, Z., Yue, T., Dobnikar, J., & Zhang, X. (2019). Membrane potential drives direct translocation of cell-penetrating peptides. *Nanoscale*, 11(4), 1949-1958. <https://doi.org/10.1039/c8nr10447f>

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Yang, J., Bahreman, A., Daudey, G., Bussmann, J., Olsthoorn, R. C. L., & Kros, A. (2016). Drug Delivery via Cell Membrane Fusion Using Lipopeptide Modified Liposomes. *ACS Central Science*, 2(9), 621–630.  
<https://doi.org/10.1021/acscentsci.6b00172>



Any Questions?