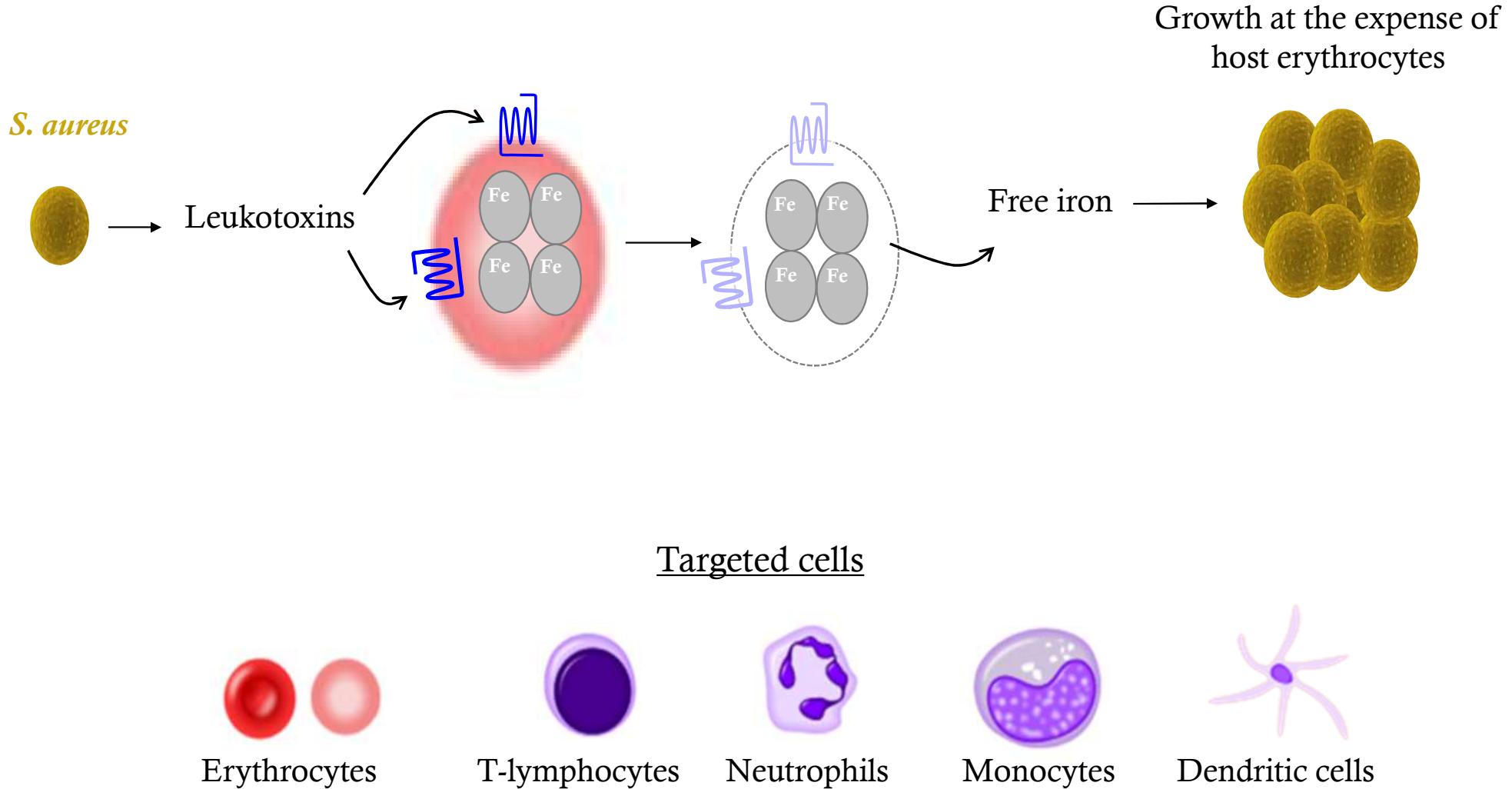


# Bacterial leukotoxins – Insights from Mass Spectrometry

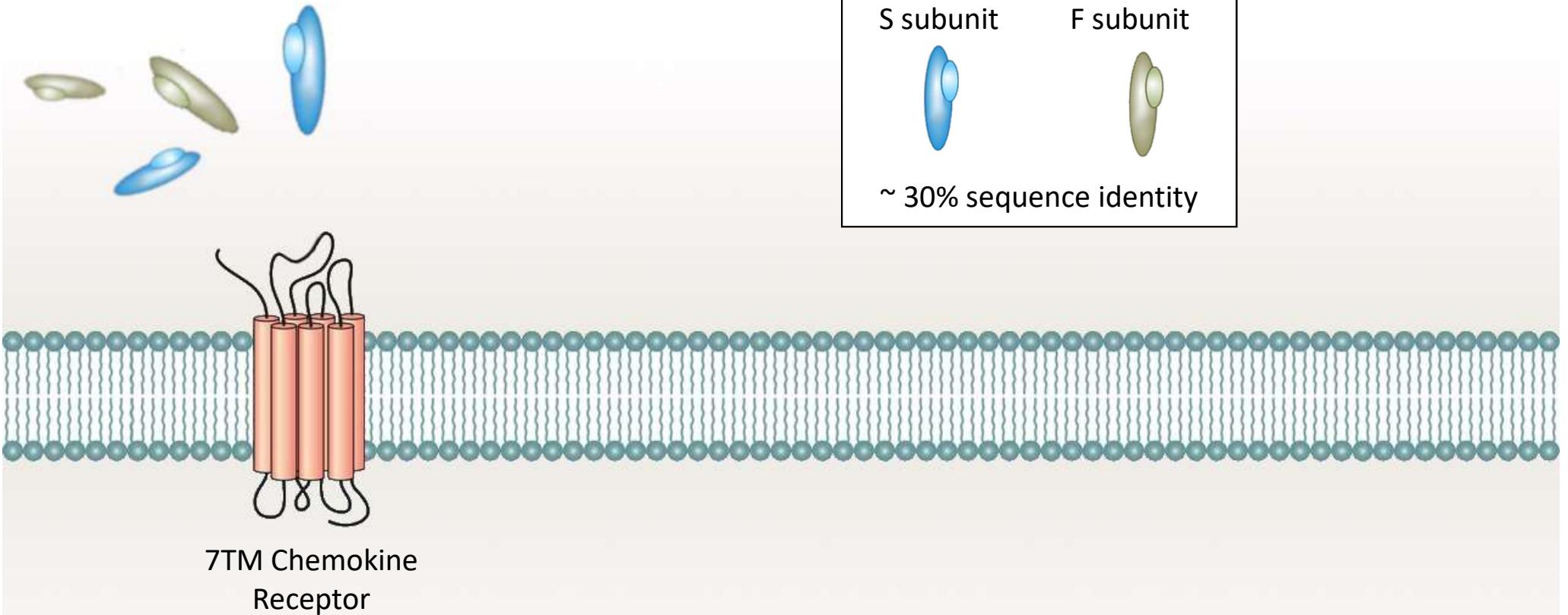
Internship presentation by Conor McKeon

Organisation: IGF  
Team: Granier-Mouillac  
Responsible: BECHARA Cherine  
Internship duration: Jan-July

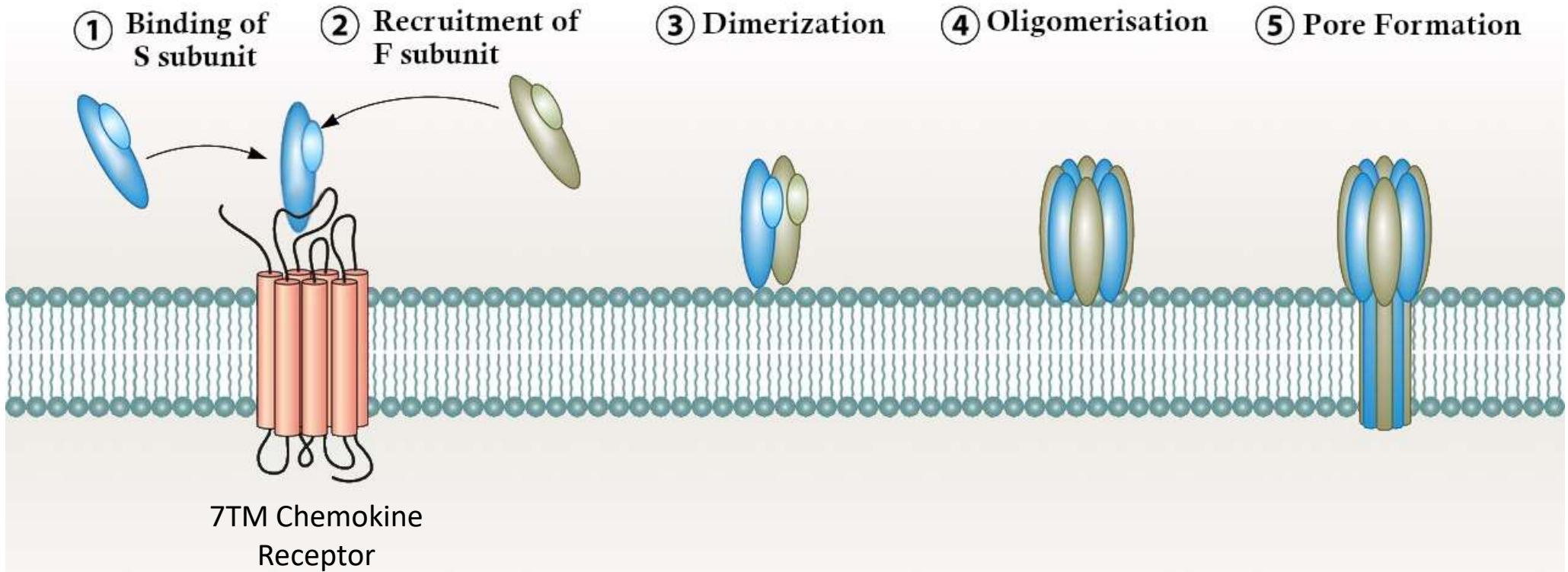
# *Staphylococcus aureus* – Protein weapons



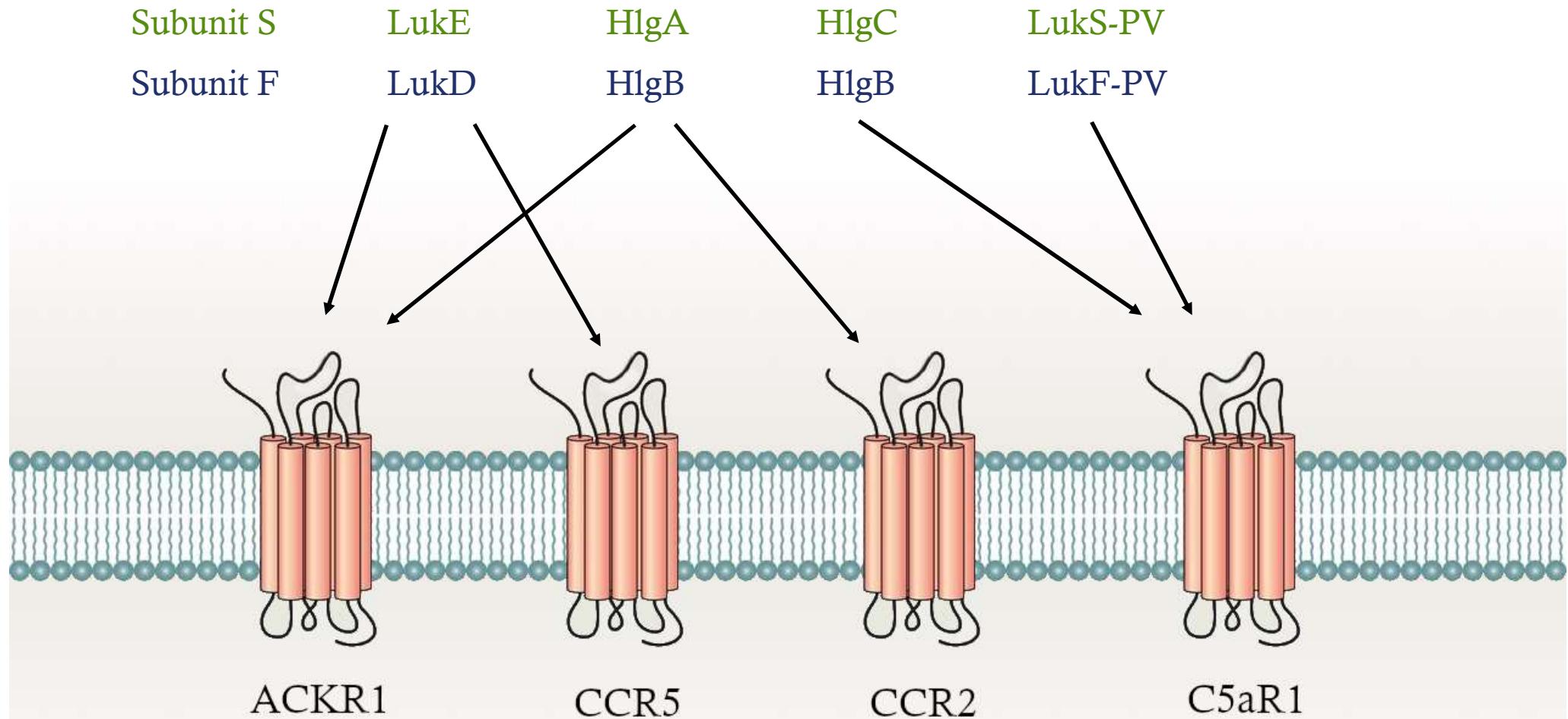
# *Staphylococcus aureus* – Pore forming toxins



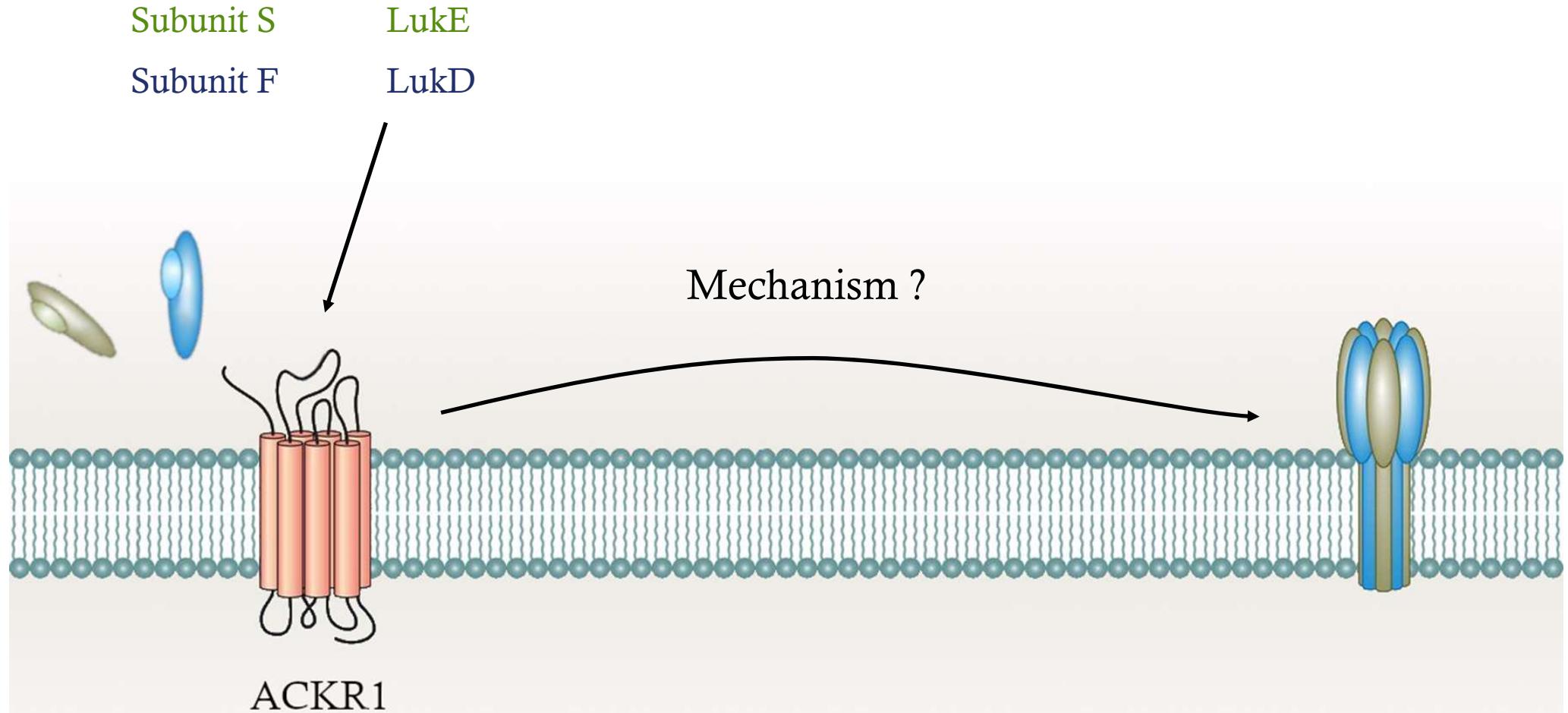
# *Staphylococcus aureus* – Pore forming toxins



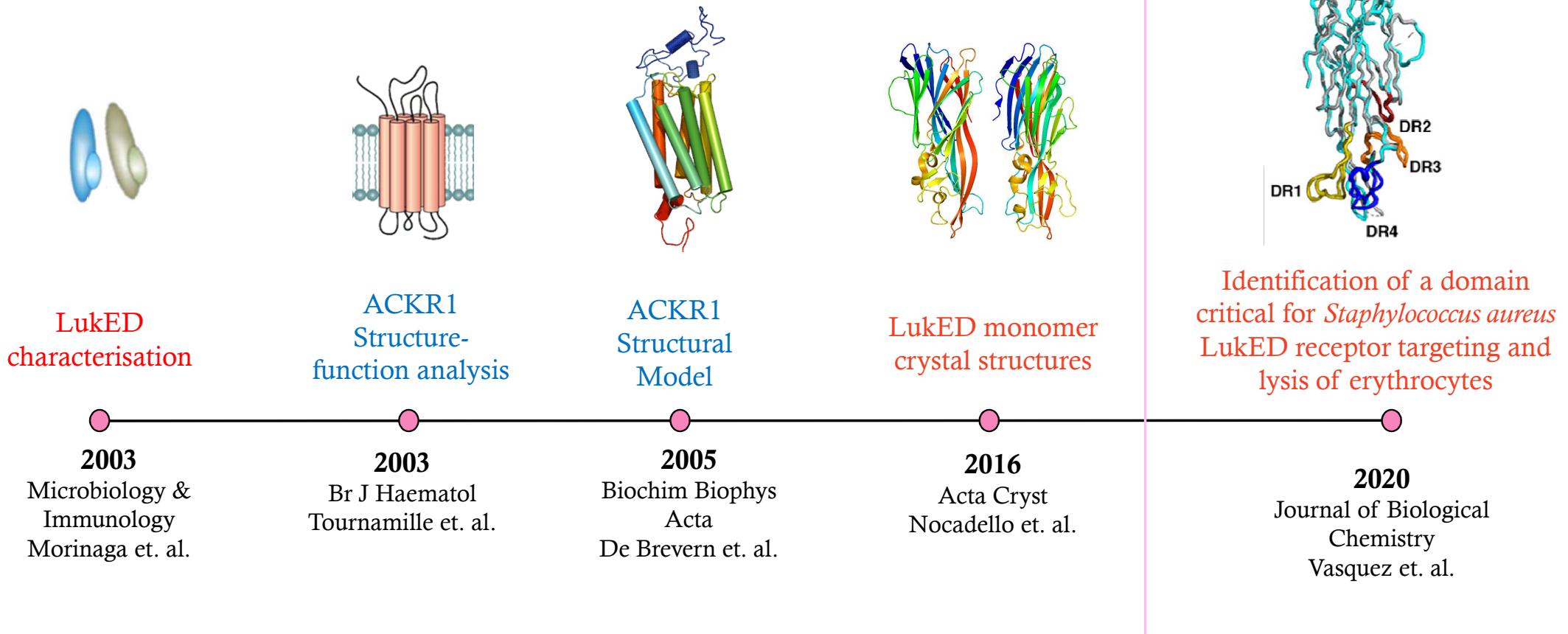
# Research overview



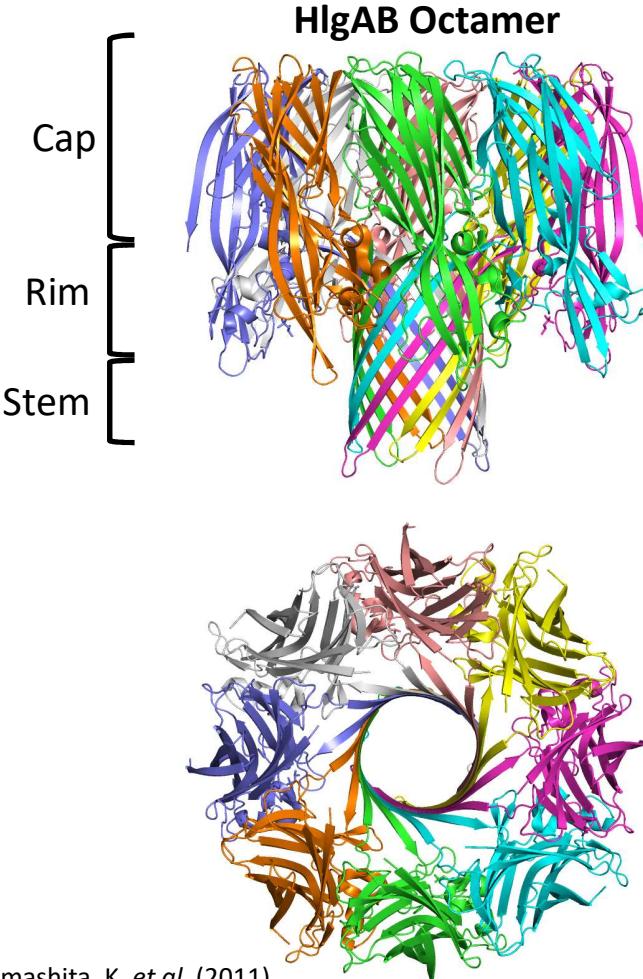
# Research overview



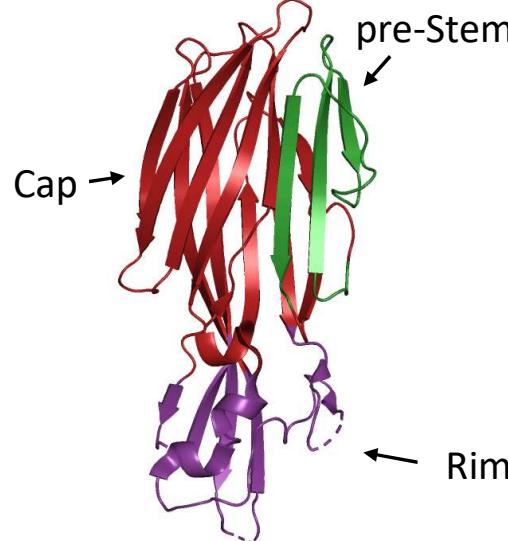
# Evolution of research in the field



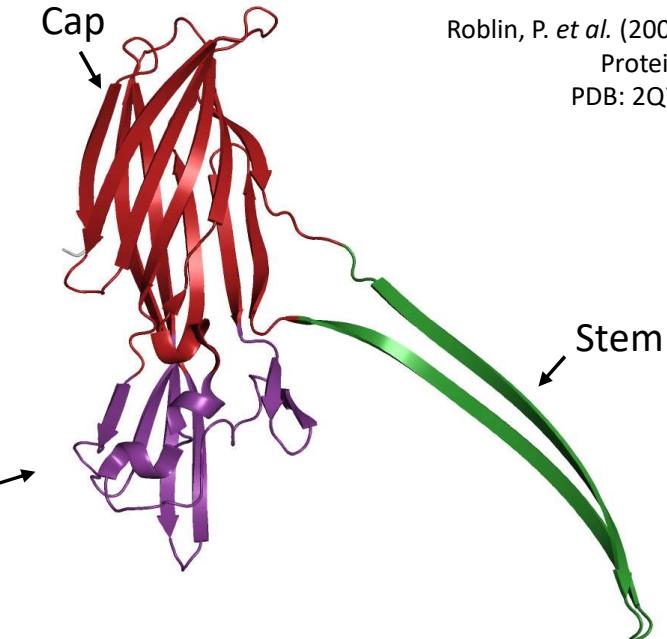
# Pore-Forming Toxins



**Monomeric HlgA**



**HlgA in mature pore**



- Toxin monomers must undergo a conformational change to form the octameric pore complex



## Identification of a domain critical for *Staphylococcus aureus* LukED receptor targeting and lysis of erythrocytes

Received for publication, August 23, 2020, and in revised form, October 7, 2020. Published, Papers in Press, October 13, 2020. DOI 10.1074/jbc.RA120.015757

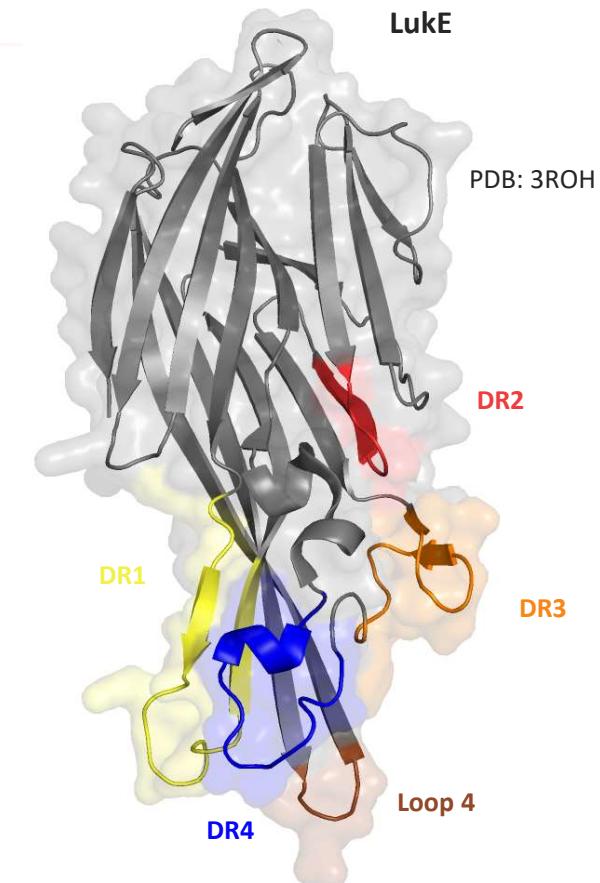
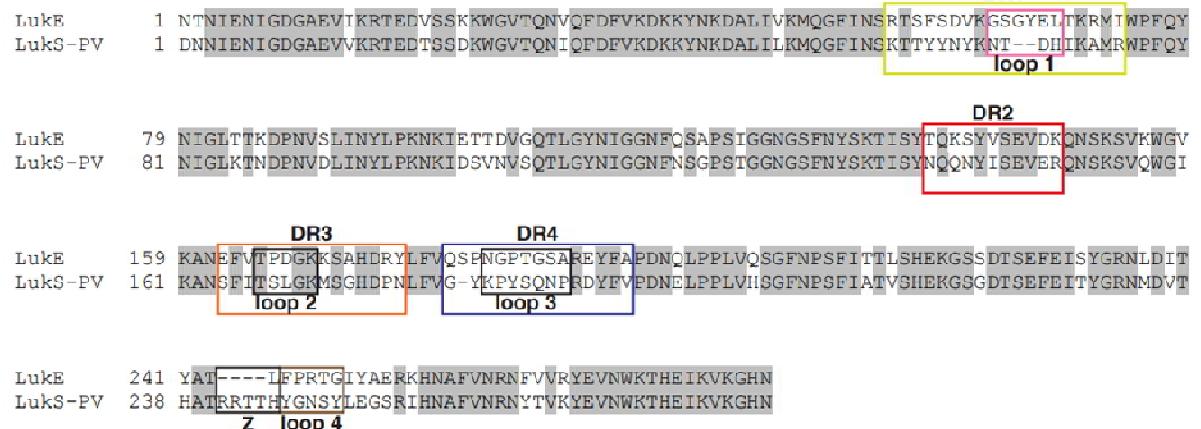
Marilyn T. Vasquez<sup>1,‡</sup>, Ashira Lubkin<sup>1,‡</sup>, Tamara Reyes-Robles<sup>1</sup>, Christopher J. Day<sup>2</sup>, Keenan A. Lacey<sup>1</sup>, Michael P. Jennings<sup>2</sup>, and Victor J. Torres<sup>1,\*</sup>

From the <sup>1</sup>Department of Microbiology, New York University Grossman School of Medicine, New York, New York, USA, and the

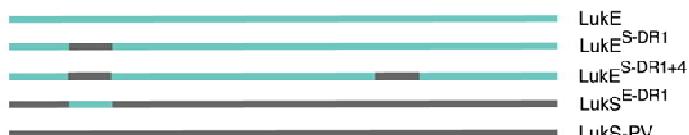
<sup>2</sup>Institute for Glycomics, Griffith University, Gold Coast, Queensland, Australia

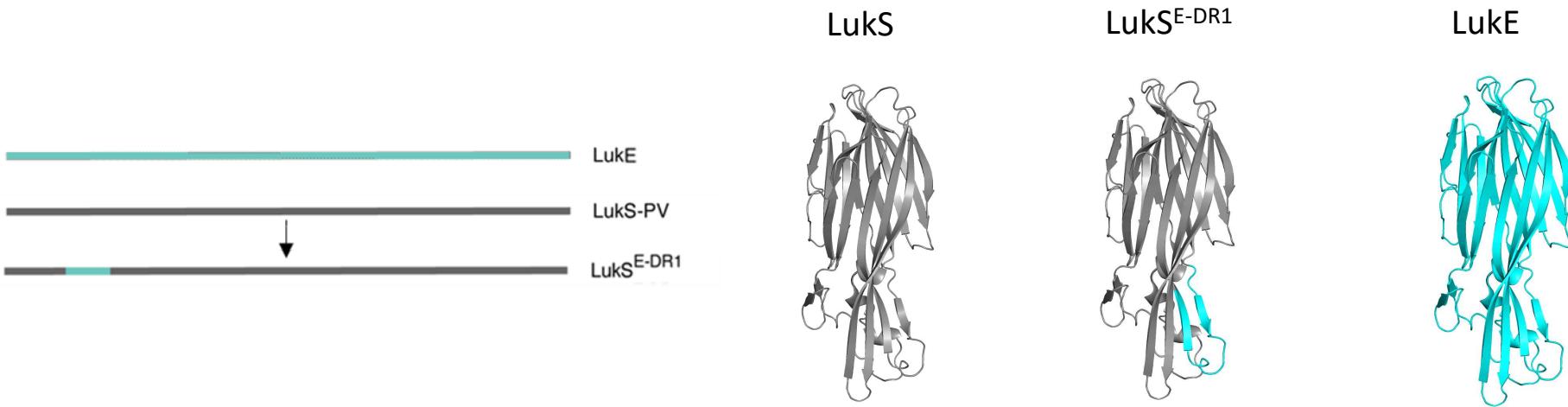
### LukE and LukS-PV sequence alignment

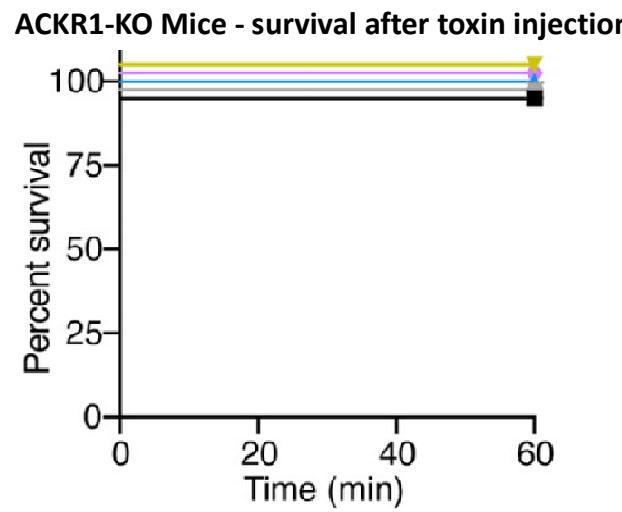
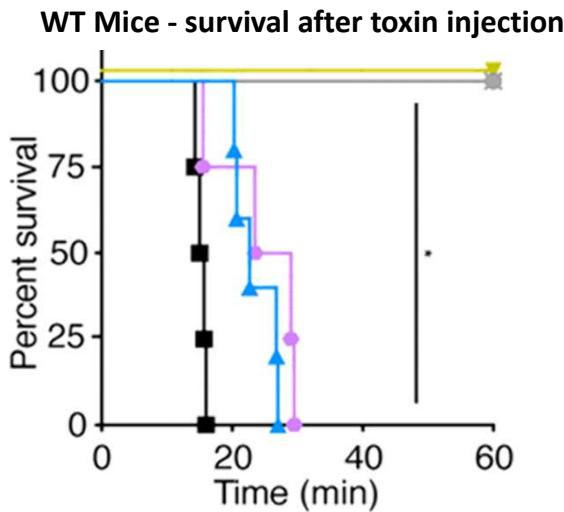
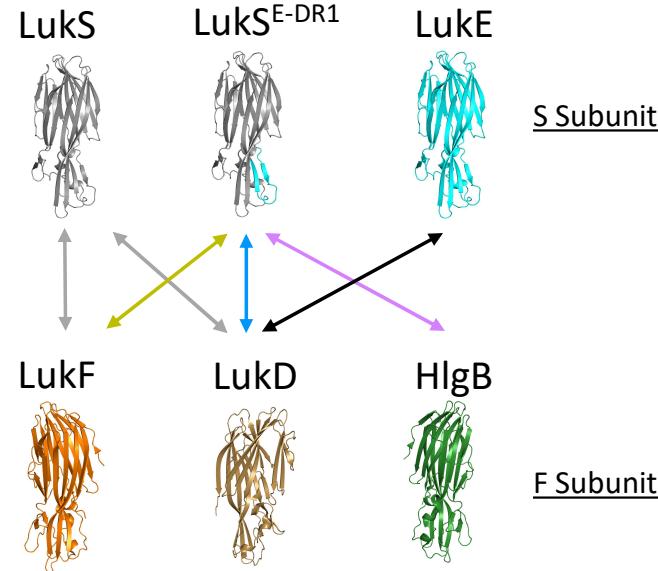
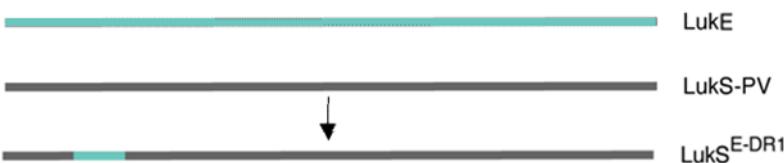
A



### Toxin mutagenesis schema







**Toxin pairs administered in mouse survival test**

- PBS
- LukS + LukF
- LukS + LukD
- ▼ LukS<sup>E-DR1</sup> + LukF
- LukD + LukE
- ▲ LukS<sup>E-DR1</sup> + LukD
- LukS<sup>E-DR1</sup> + HlgB

The DR1 region of LukE is able to render the inactive Luk-S - LukD toxin pair lethal in ACKR1-positive mice

# Aims - Characterising the interaction via Mass Spectrometry

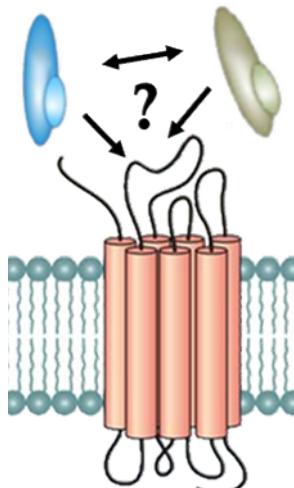
## Native Mass Spectrometry (nMS)

Identification of species by direct Mass analysis of intact protein complexes

## Hydrogen-Deuterium Exchange Mass Spectrometry (HDX)

Dynamic characterisation of the solvent accessibility by analysing the deuterium exchange at the peptide level

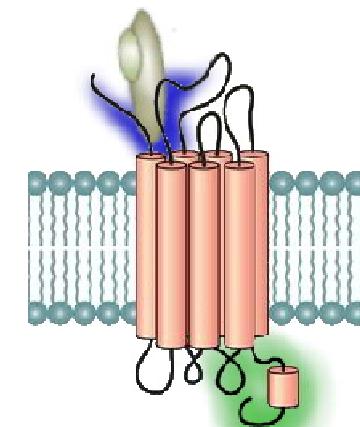
With these techniques we can learn about



Binding strengths & stoichiometries



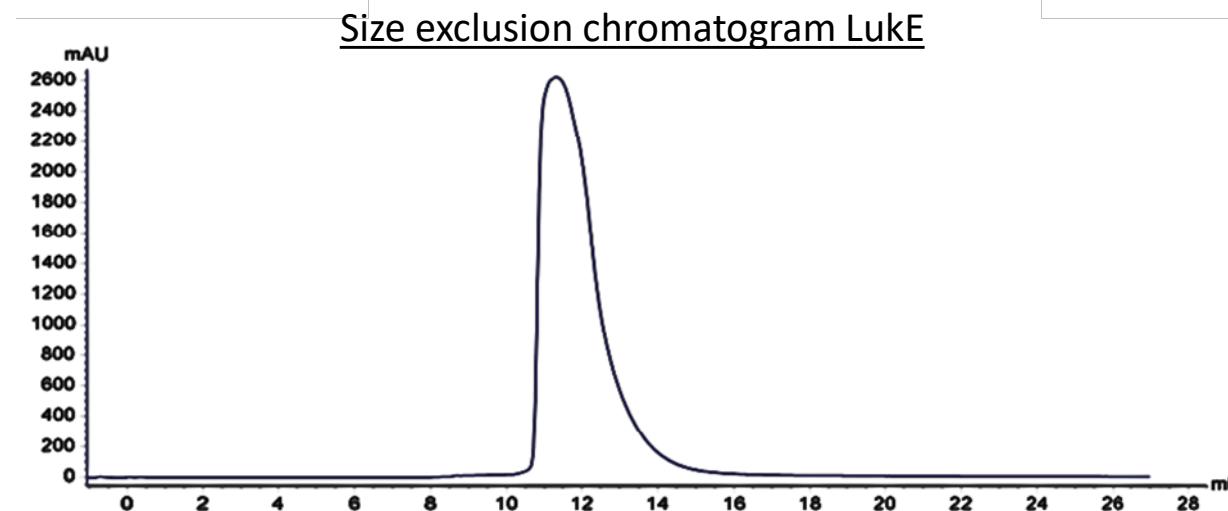
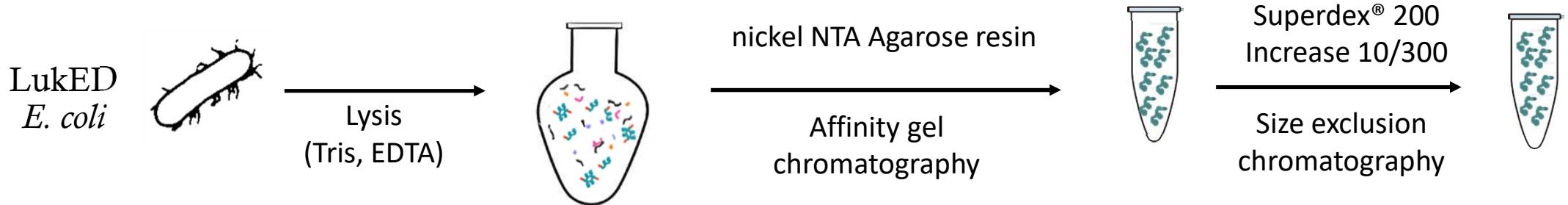
Interaction interfaces



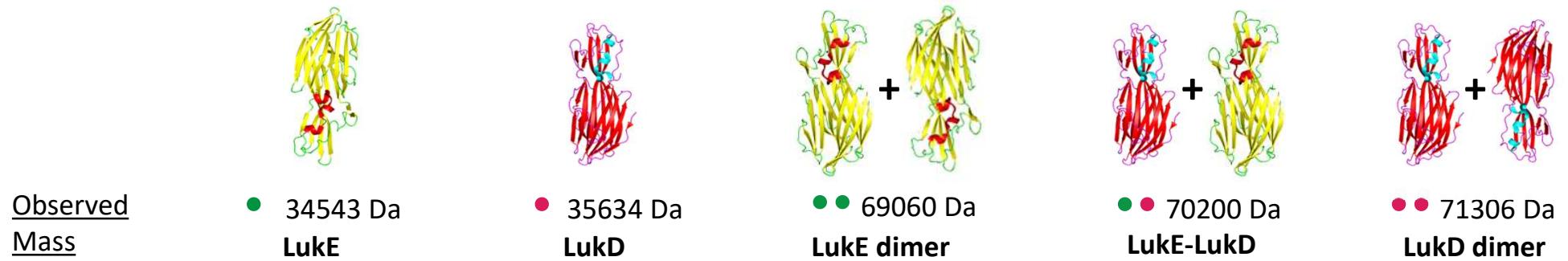
Conformational changes

# LukED - Purification

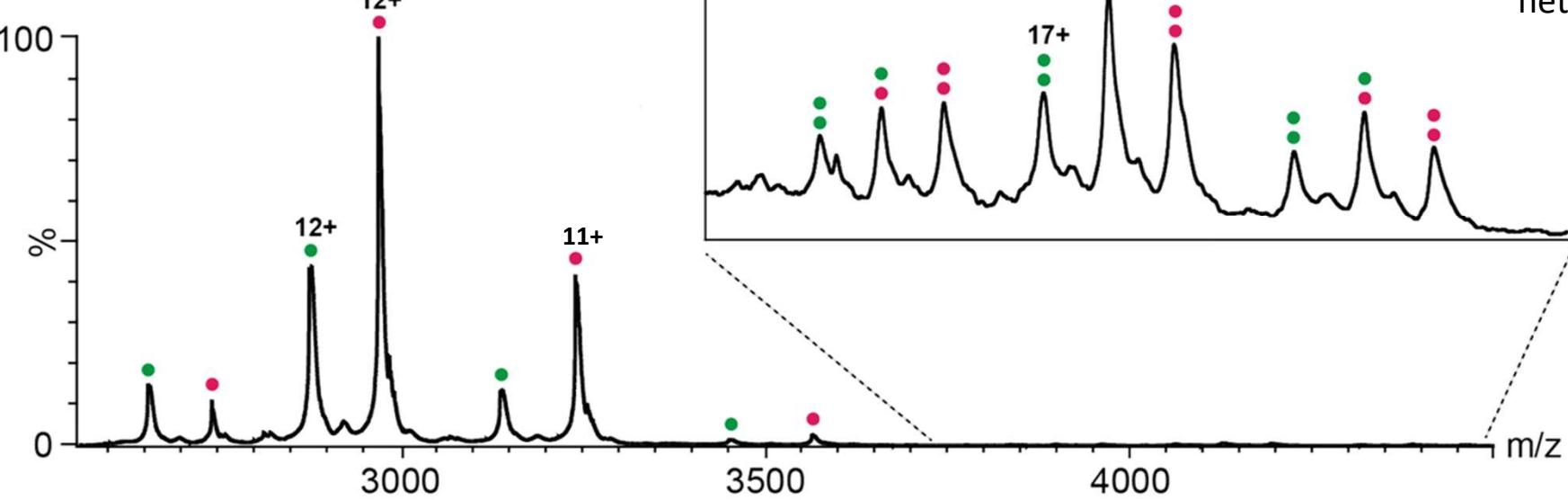
- Pure LukE was obtained after affinity gel chromatography



# LukED Interaction - Native MS

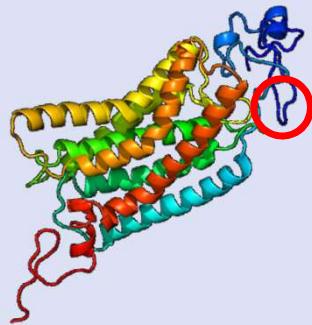


LukE + LukD  
Mass spectrum

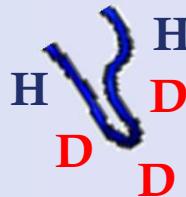


# Analysis Method - Hydrogen-Deuterium Exchange

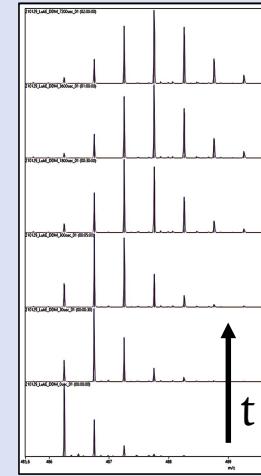
Apo



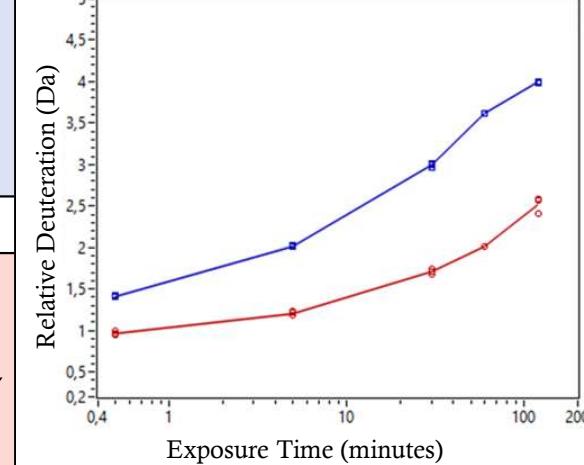
$M/Z = x + 3$



LCMS analysis

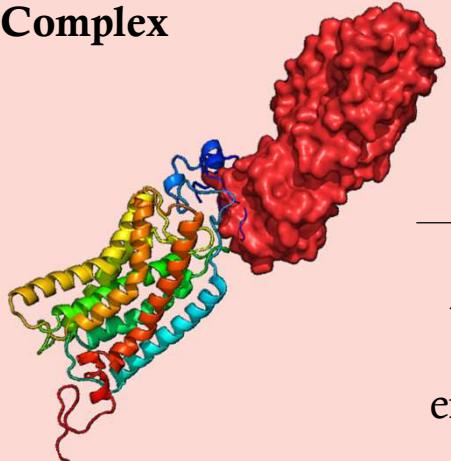


Comparative analysis of deuterium uptake

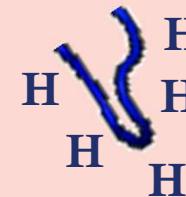


VS

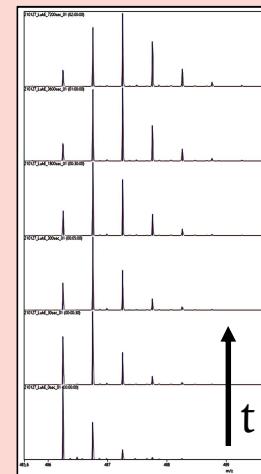
Complex



$M/Z = x$

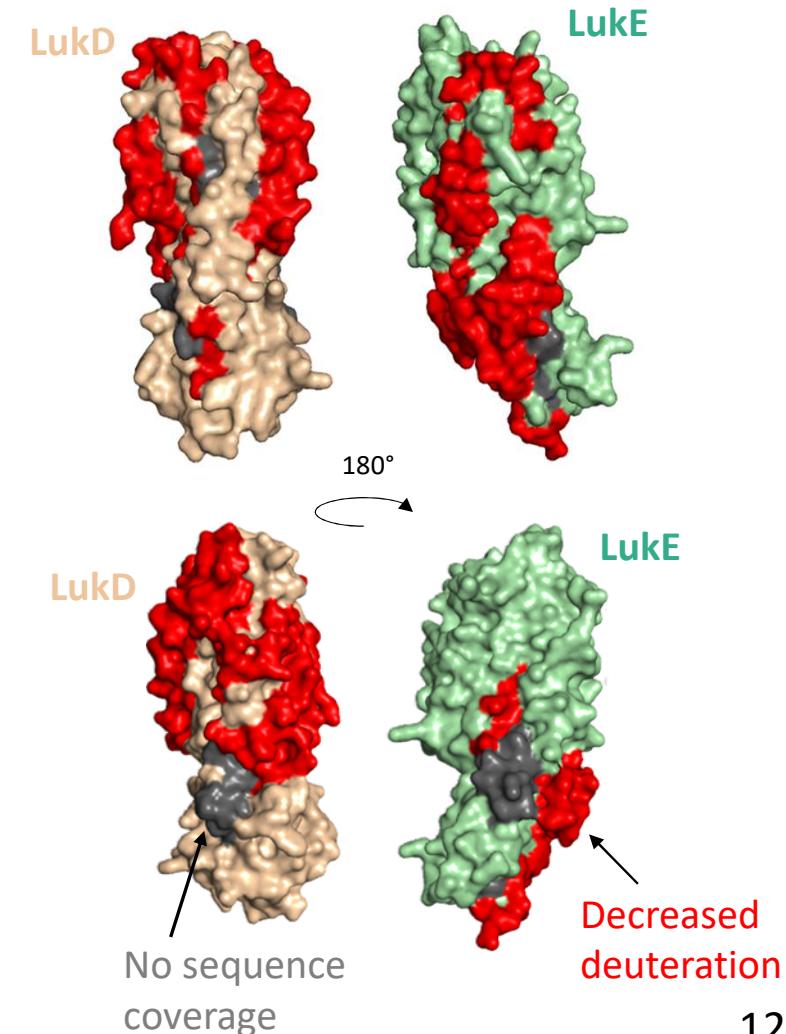
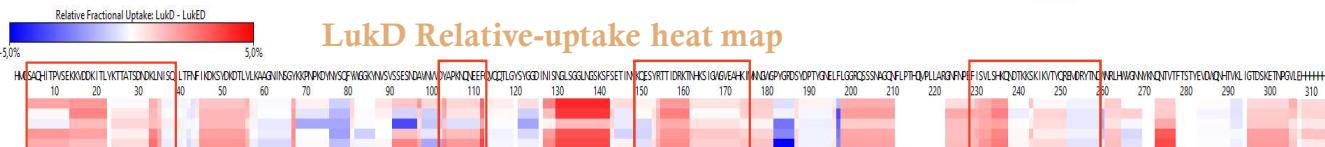
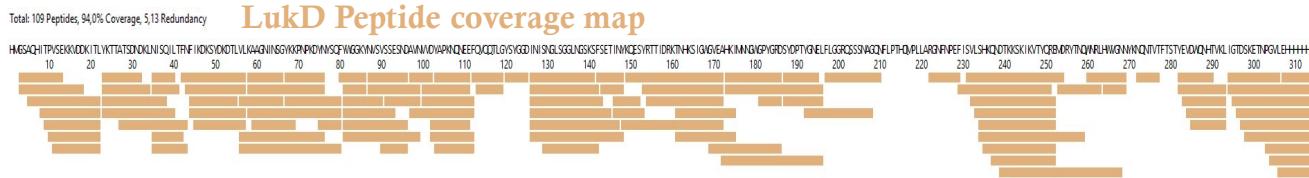
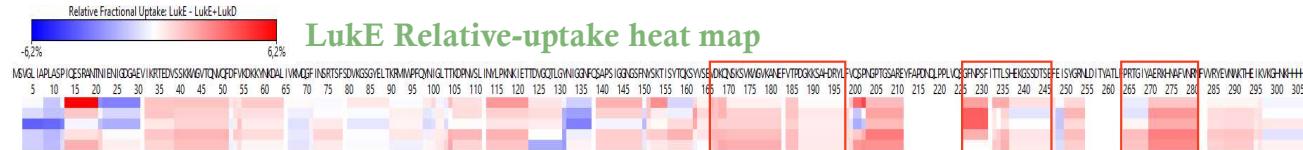
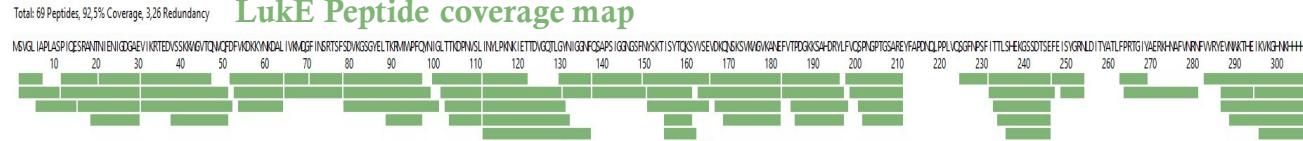


LCMS analysis



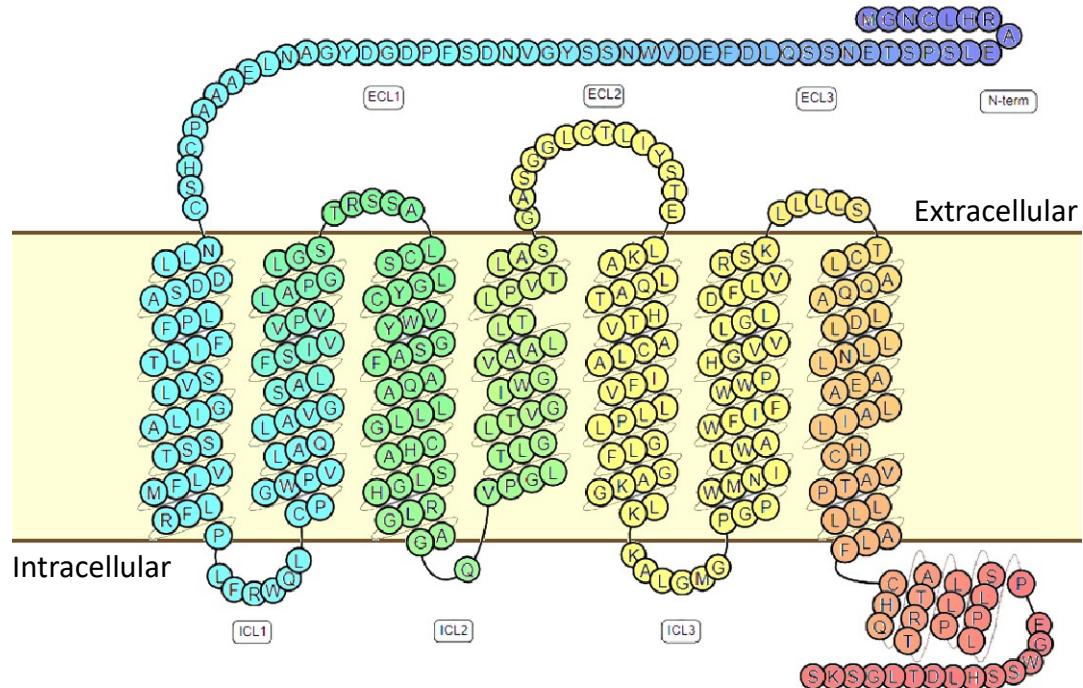
# LukED Interaction - HDX

- Several regions of LukE and LukD show less solvent accessibility upon complexation

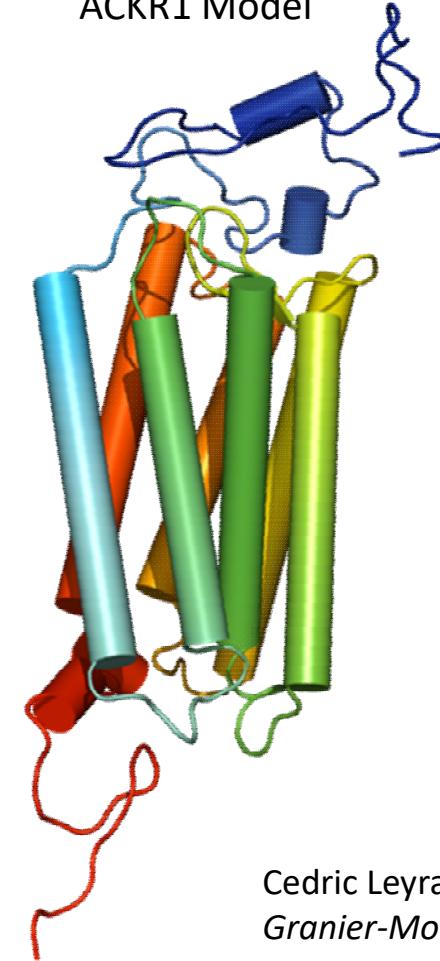


# ACKR1 Receptors

ACKR1 Snake Plot



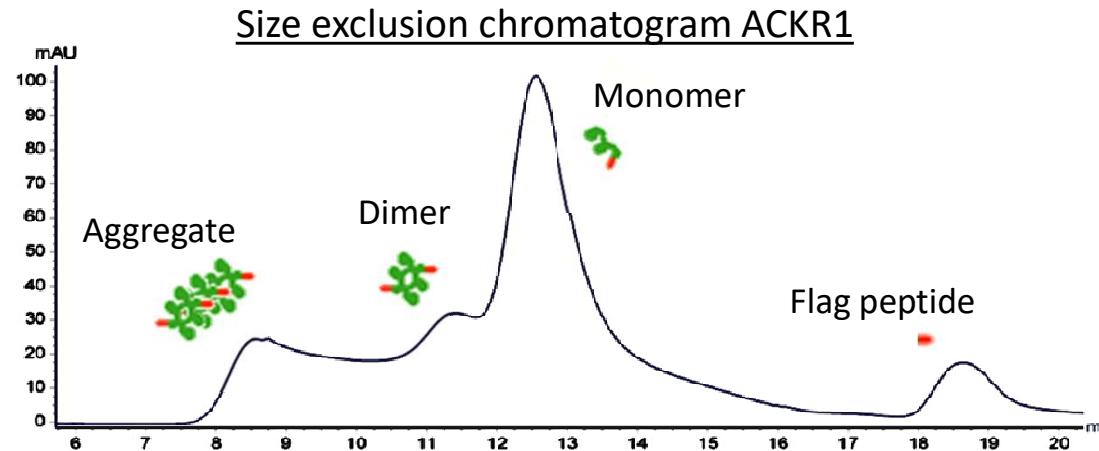
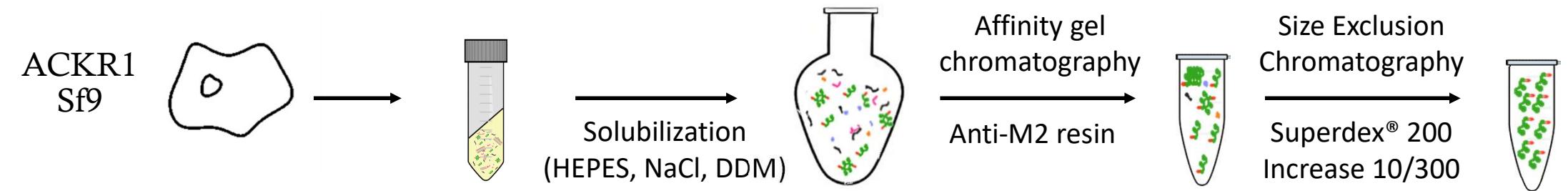
ACKR1 Model



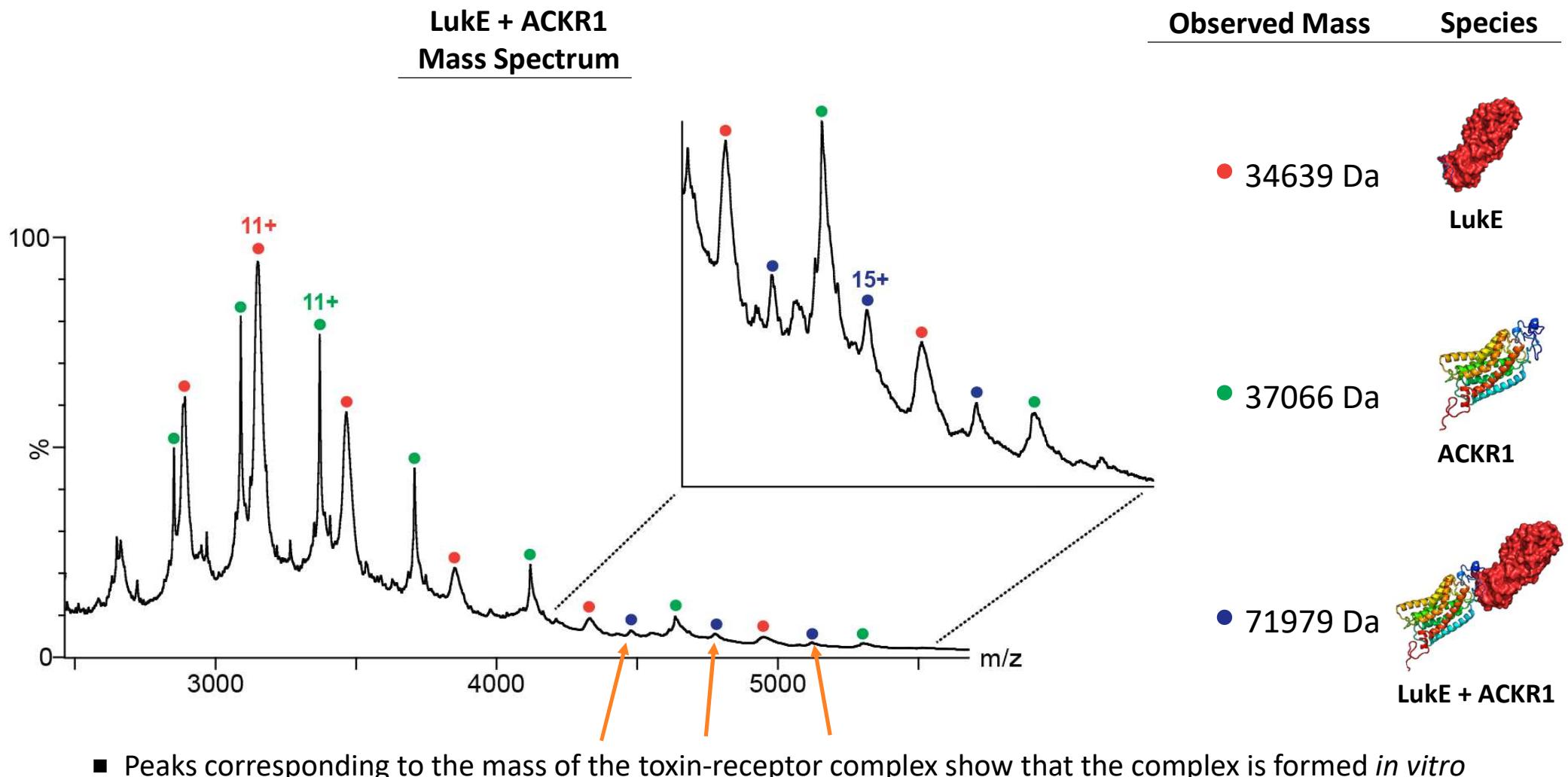
Cedric Leyrat  
Granier-Mouillac lab

# ACKR1 - Purification

- Pure monomeric ACKR1 was obtained after Size Exclusion Chromatography

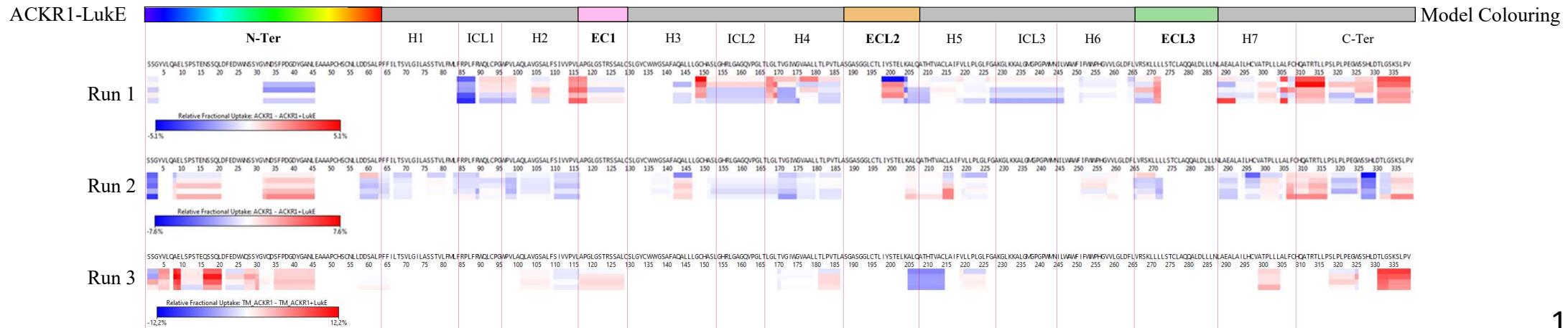
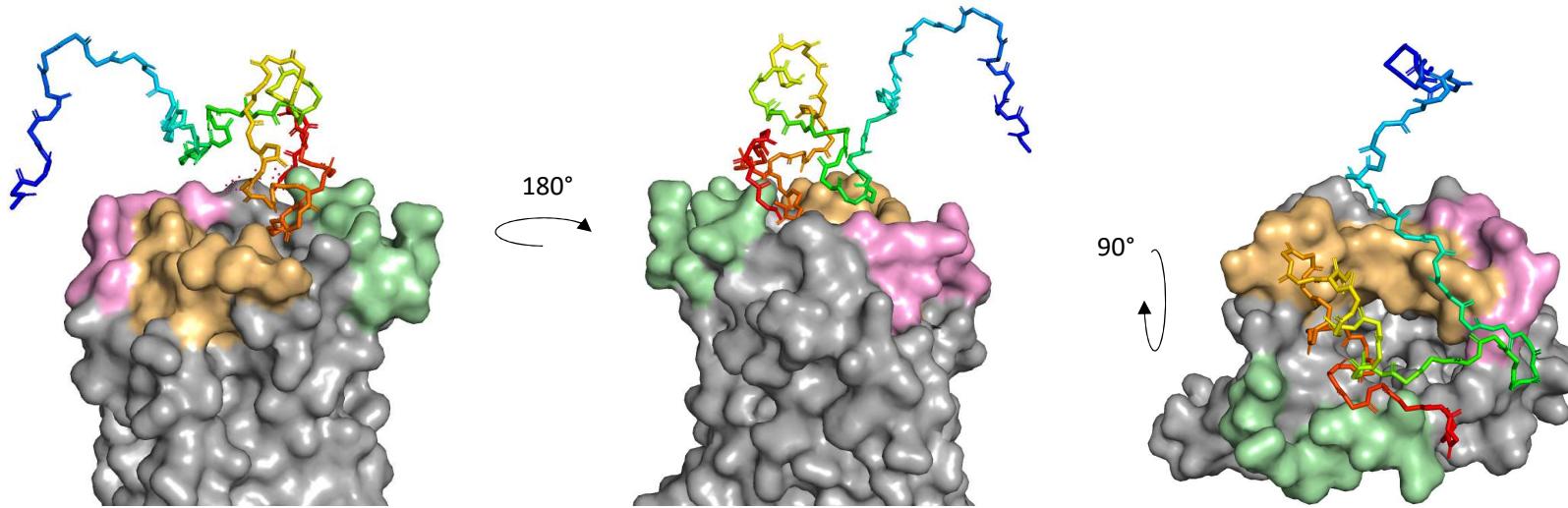


# Toxin-Receptor Interaction - Native MS



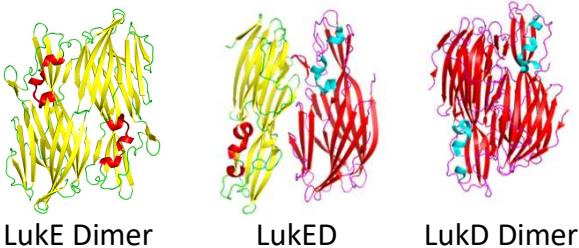
# Toxin-Receptor Interaction - HDX

- HDX results for ACKR1-LukE were inconclusive under the experimental conditions

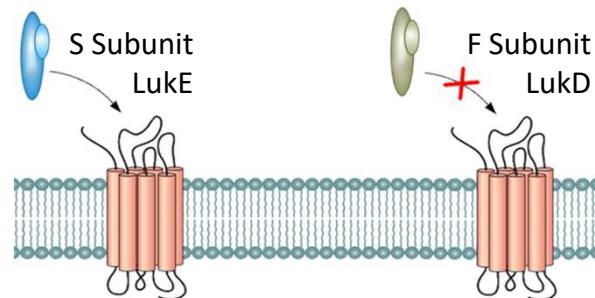


# Conclusions

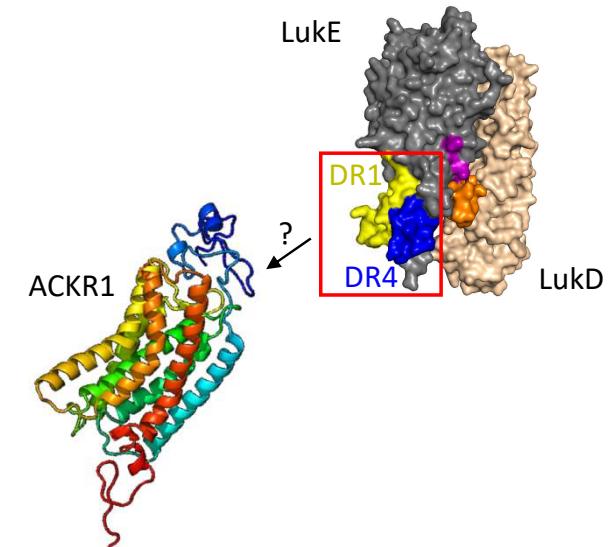
- LukE and LukD show homodimerisation and heterodimerisation in solution



- ACKR1-LukE complexation was observed. No binding was observed between ACKR1 and LukD



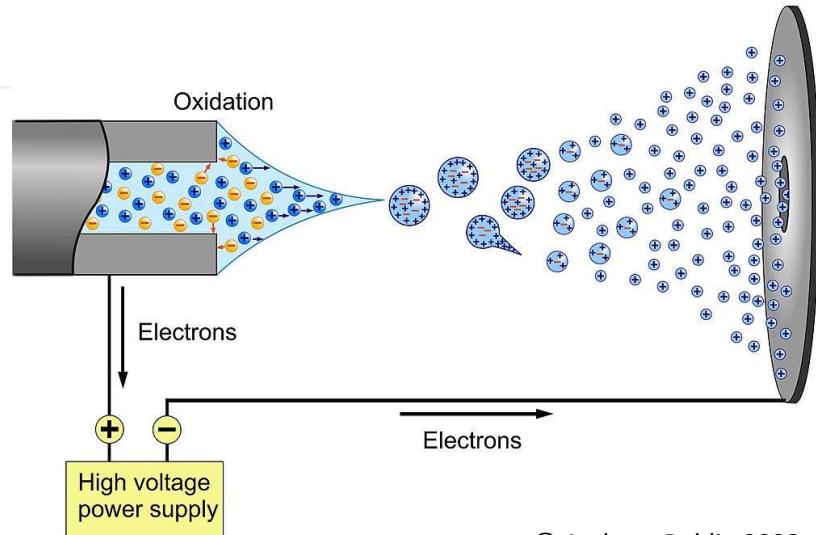
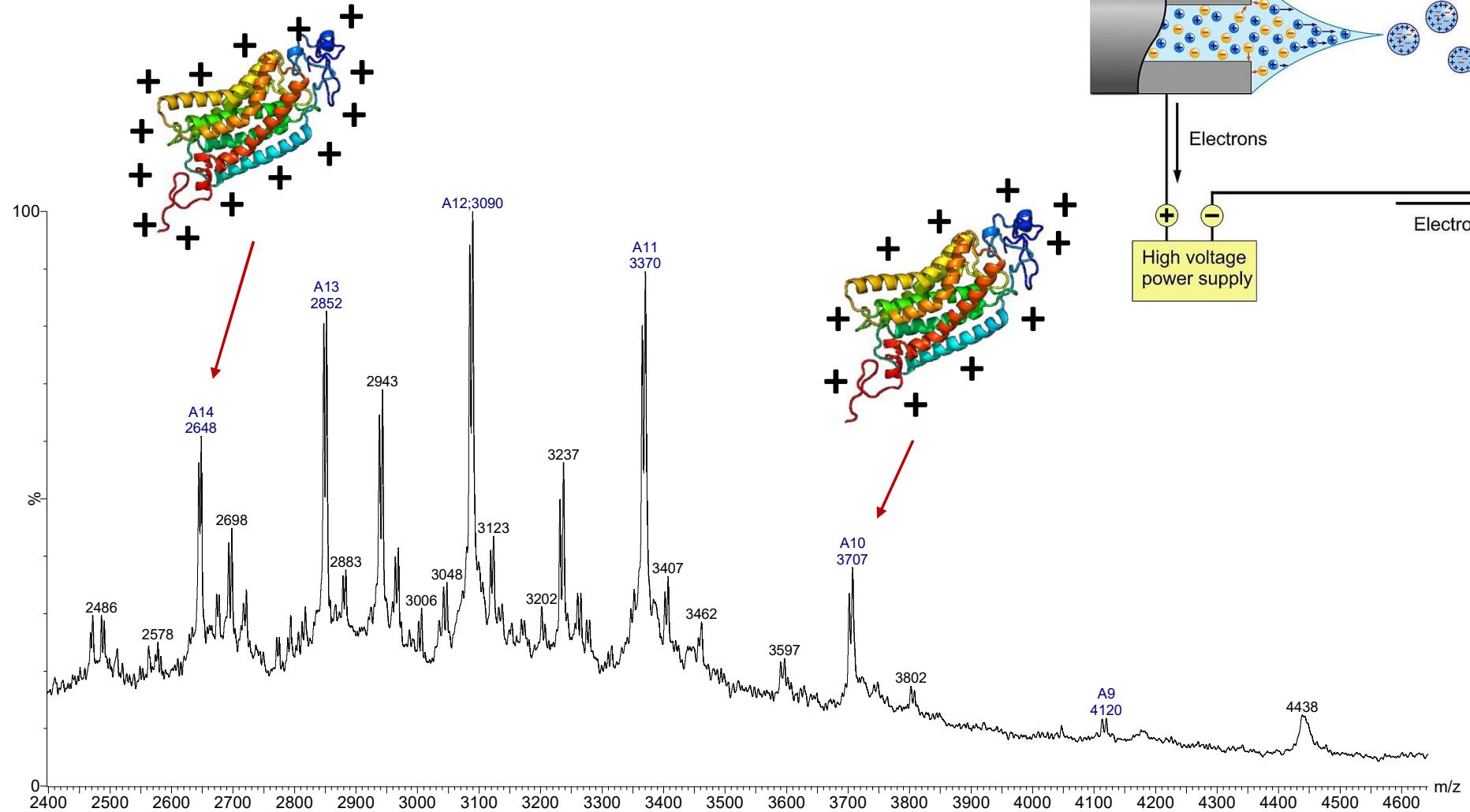
- Receptor targeting may be possible for toxin heterodimers as necessary binding regions remain exposed





Thank you for your attention

# Analysis Method - Native MS



© Andreas Dahlin 2008

# Optimisations

## Effective Application of Bicelles for Conformational Analysis of G Protein-Coupled Receptors by Hydrogen/deuterium Exchange Mass Spectrometry

Duc NM, Du Y, Zhang C, et al.

*J Am Soc Mass Spectrom.*

2015;26(5):808-817.

