

EATING THE POISON

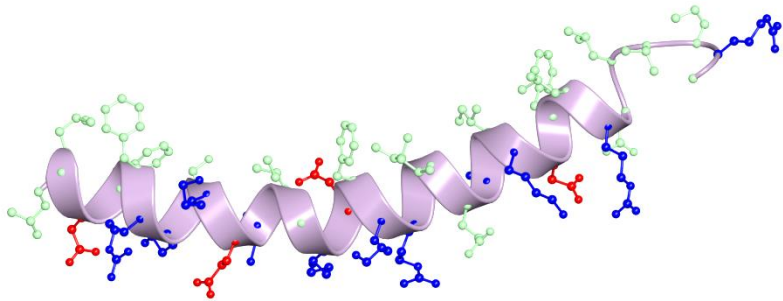
Bryony Ackroyd
University of York
CBMNet ECR Event
September 2016

Overview

- CAMPs
- Sap project so far
- Introduction to Yej and peptidases project

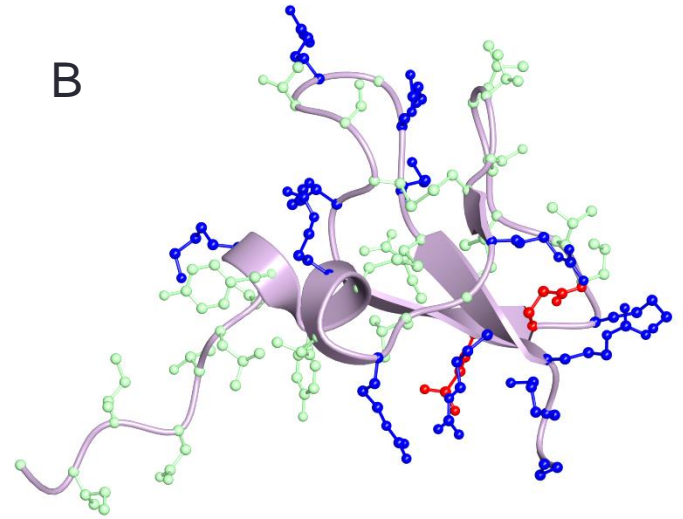
Antimicrobial Peptide Structures

A



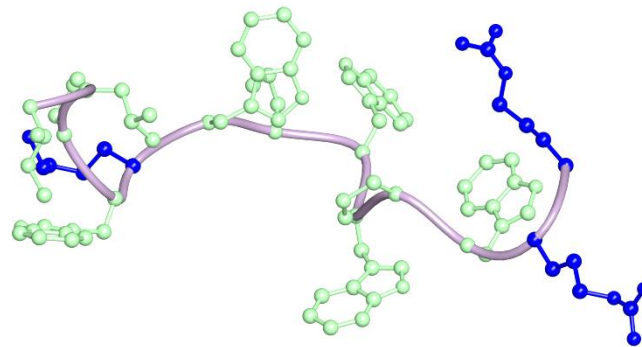
LL-37

B



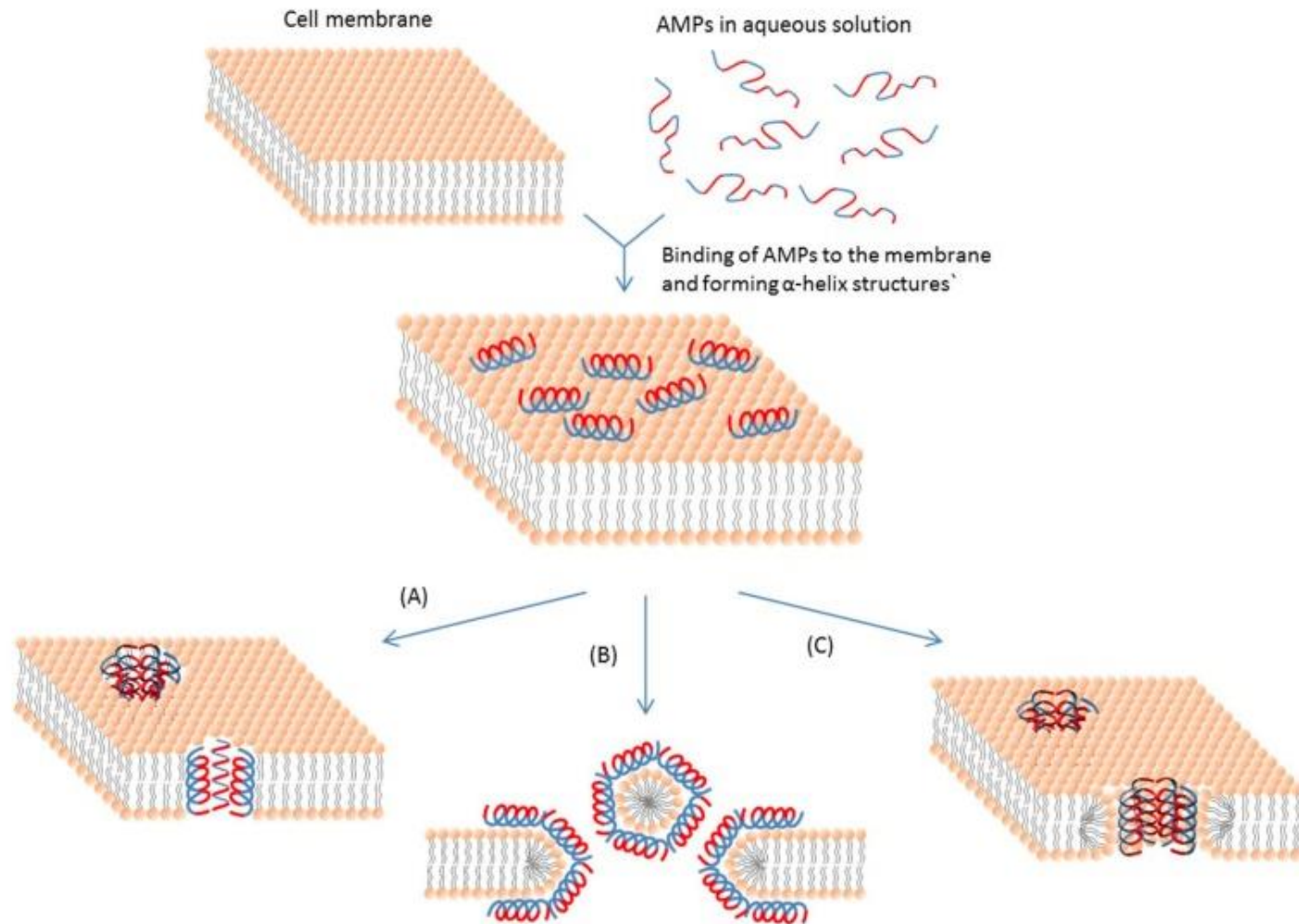
Human β -defensin 3

C

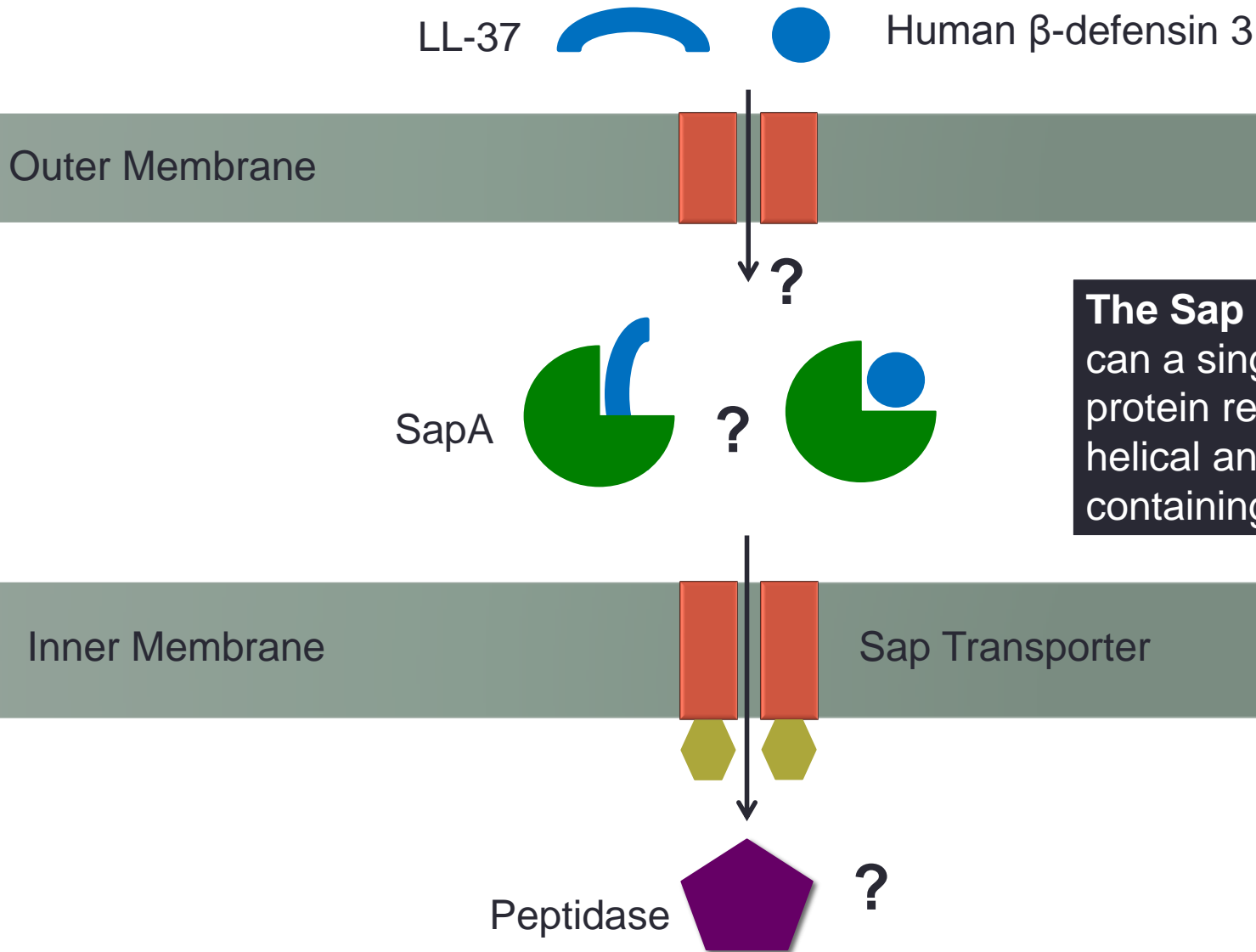


Indolicidin

Cationic Antimicrobial Peptides

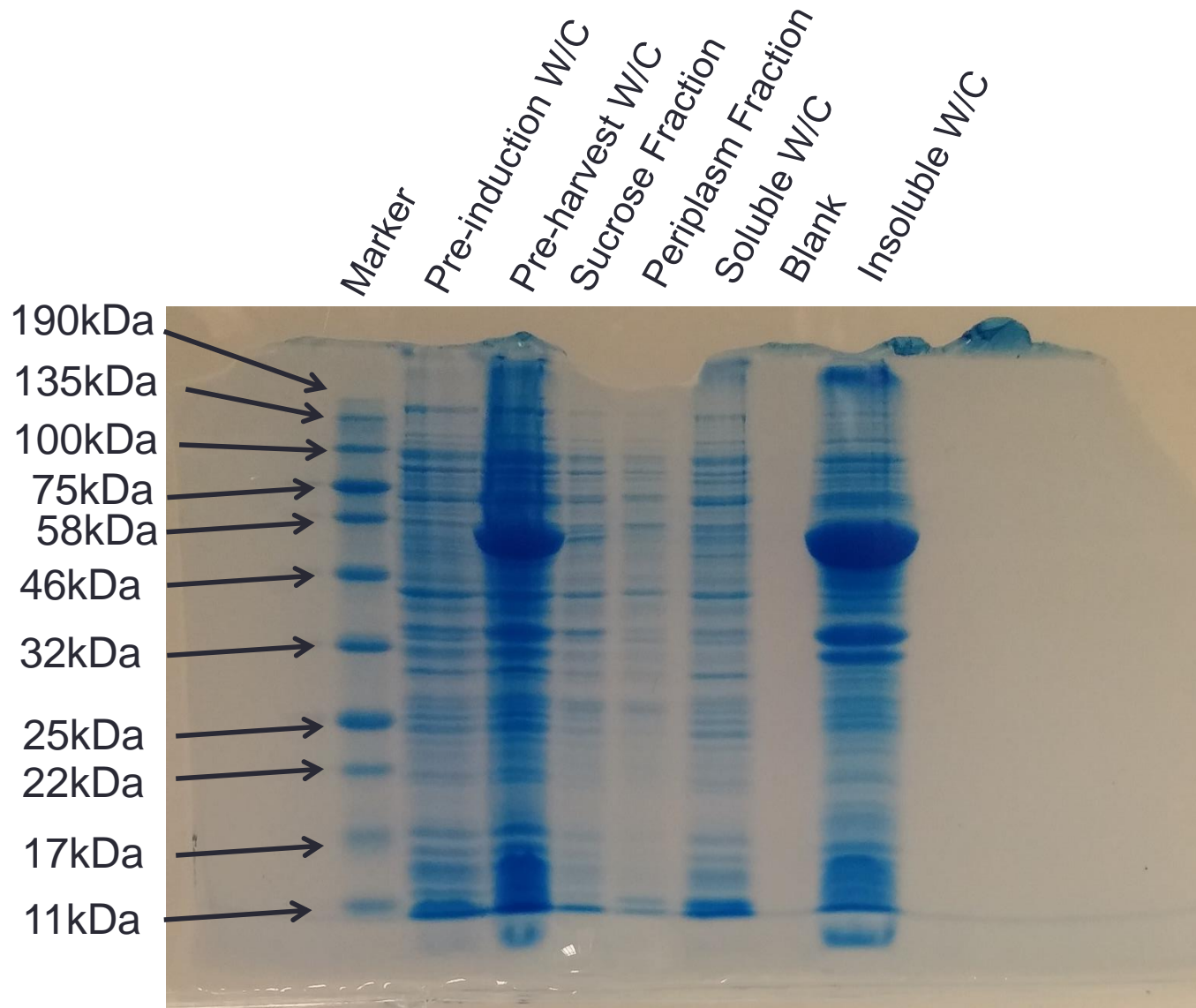


How does the Sap System Work?



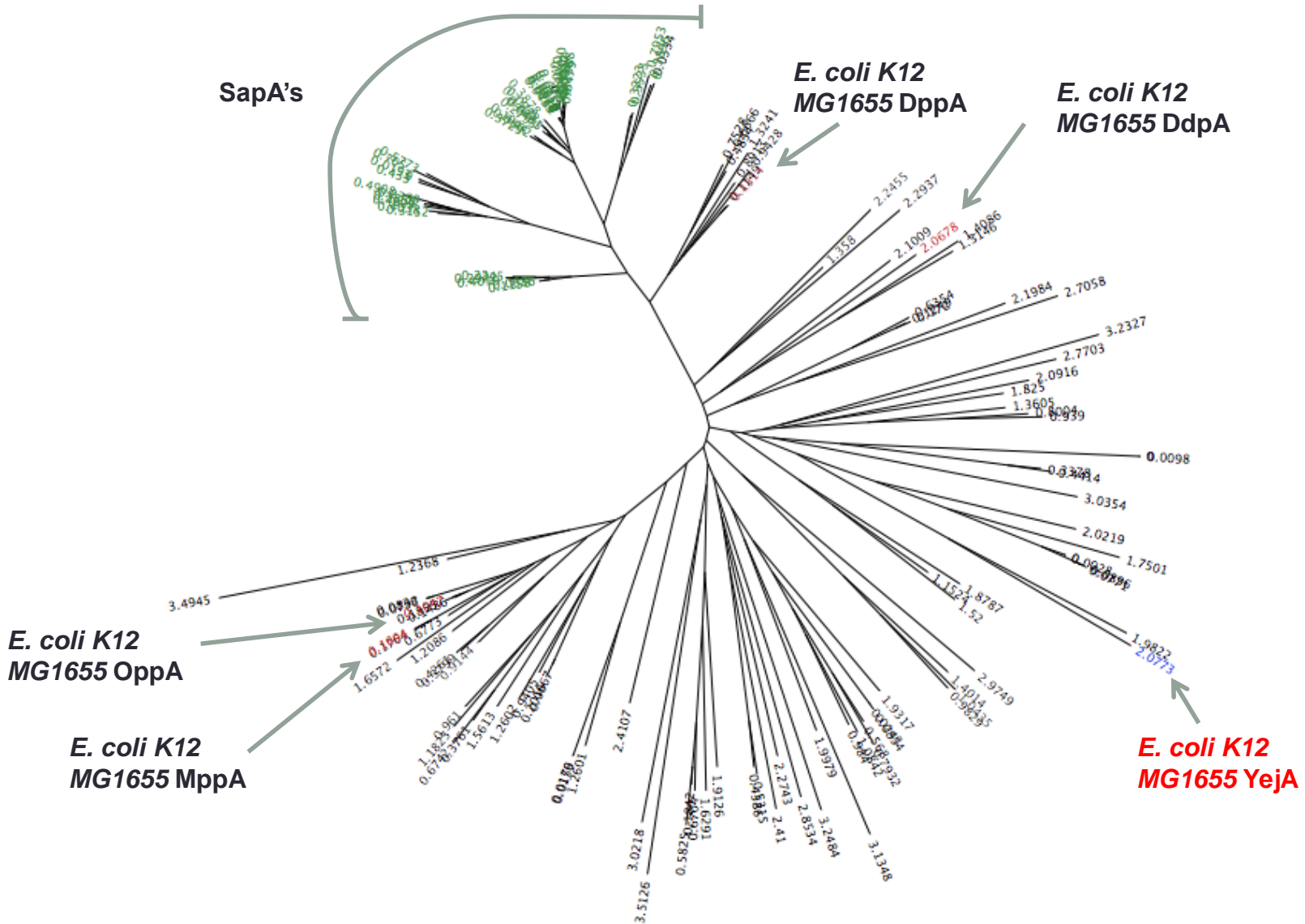
The Sap paradox – how can a single binding protein recognise **both** α -helical and β -sheet containing peptides?

Solubility of *S. Typhimurium* SapA

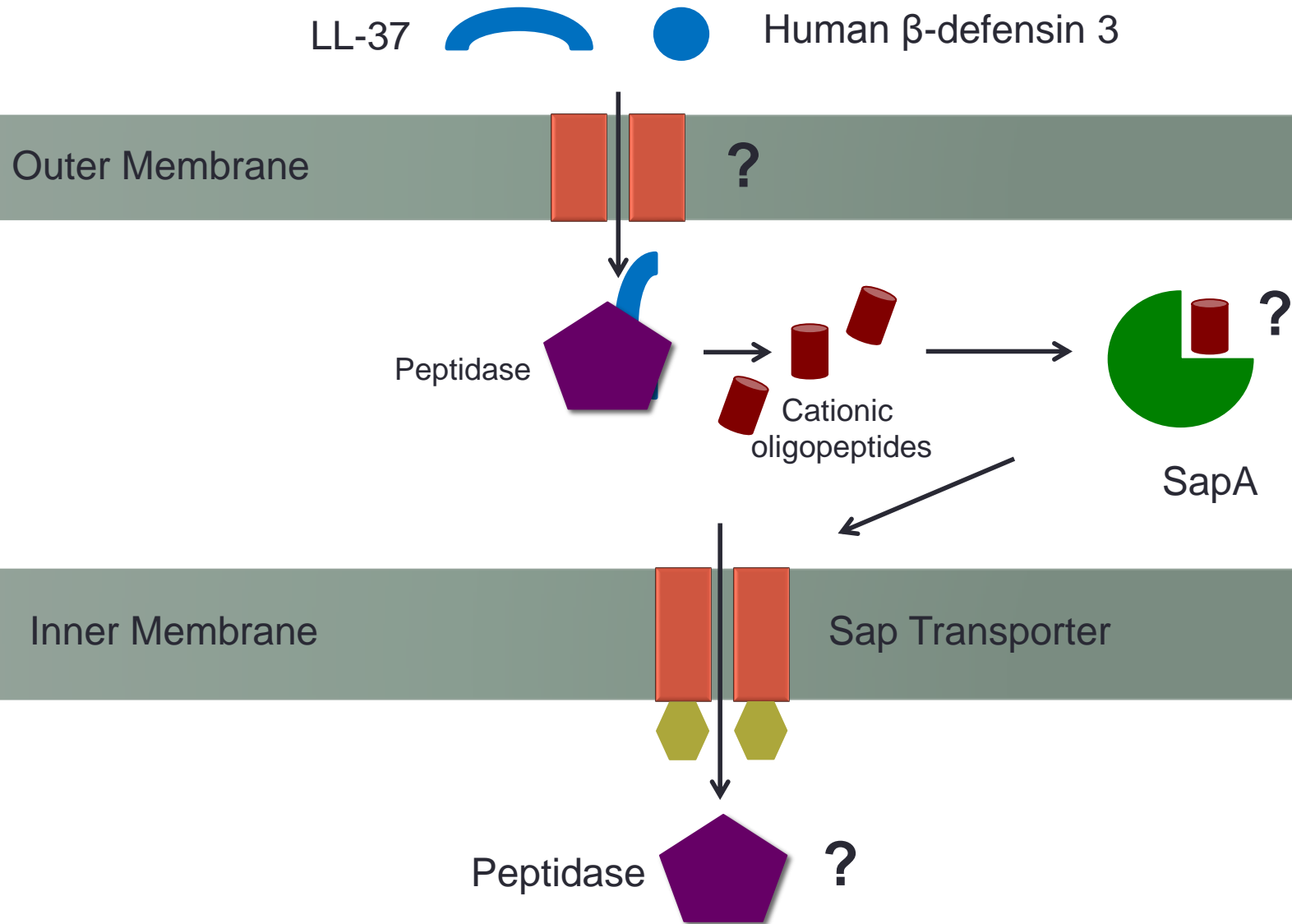


W/C = Whole Cell

SapA is Closely Related to DppA



How does the Sap System Work?

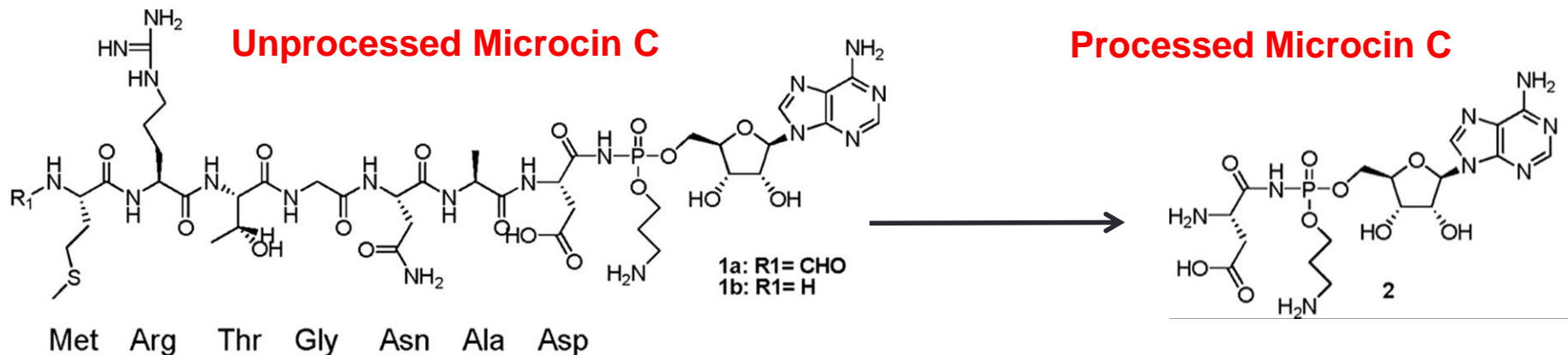


Introduction to YejABEF ABC Transporter

- *S. Typhimurium* *yejF* mutants more susceptible to protamine, melittin and polymyxin B than the WT. *yejB* and *yejE* mutants also more susceptible to protamine and polymyxin B (*Eswarappa et al., 2008*)

Introduction to YejABEF ABC Transporter

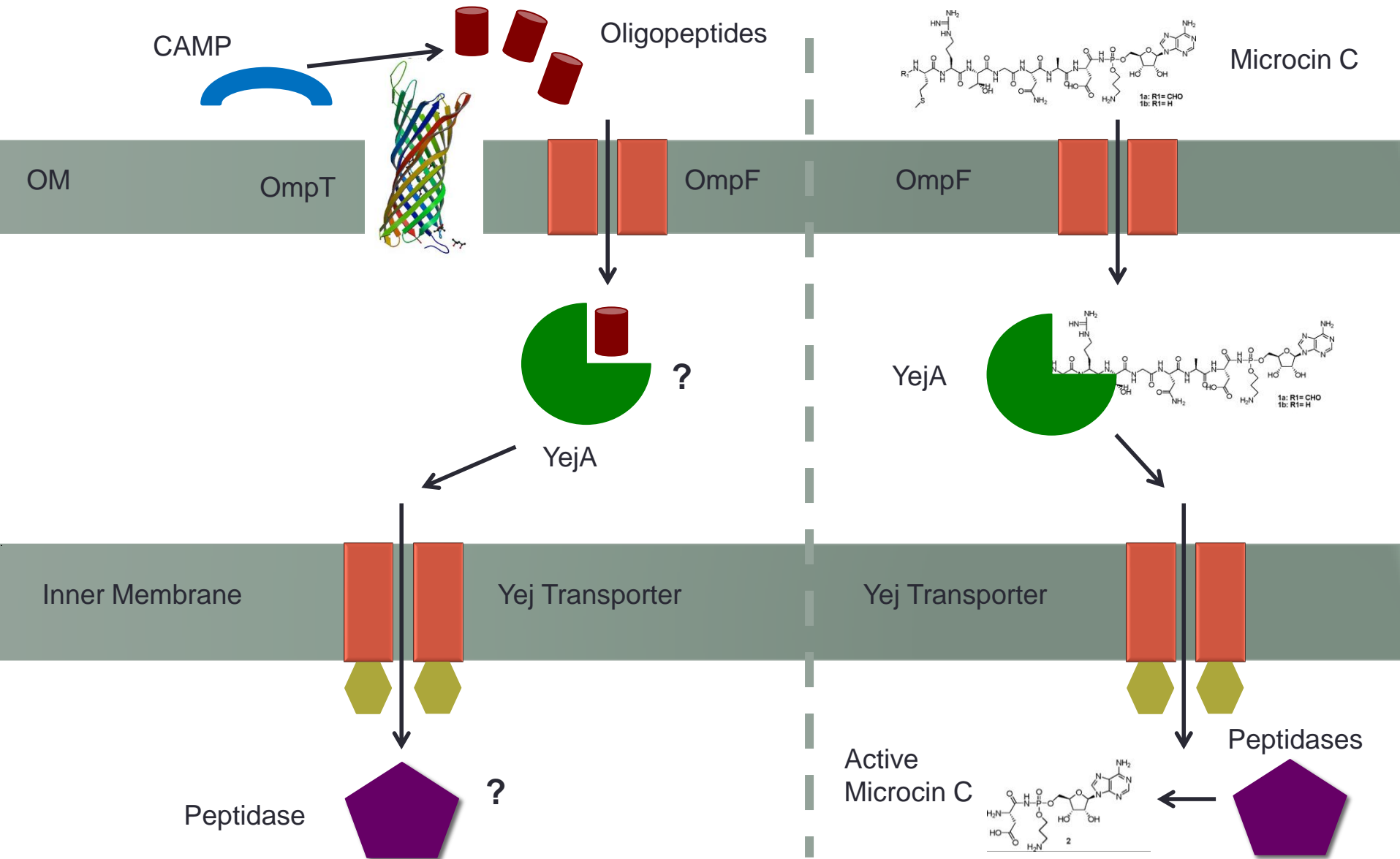
- Discovered via transposon mutagenesis *E. coli* Yej transports Microcin C (*Severinov et al., 2007*)
- Made Microcin C analogues to discover *E. coli* Yej transports peptides between 7-13 amino acids (*Vondenhoff et al., 2011*)



Introduction to Proteases

- A strain of *E. coli* that lacks the extracytoplasmic proteases DegP, Protease III and OmpT are more susceptible to protamine than the control strain (*Bakker et al., 1997*)
- Only deletion of *ompT* in *E. coli* leads to hypersusceptibility to protamine (*Bakker et al., 1998*)
- HPLC data used to show degradation of protamine by OmpT (*Bakker et al., 1998*)

How does the Yej System Work?



Future Work

Short Term Aim

Overexpression and purification of soluble YejA

Long Term Aims

1. To fully understand the resistance mechanism employed by bacteria to counteract CAMPs
2. Use this knowledge to design new antibiotics

Acknowledgements

Supervisors

Prof. Tony Wilkinson

Dr. Gavin Thomas



TAP Members

Dr. Marjan Van Der Woude

Dr. Martin Fascione



Wilkinson and Thomas lab members

welcometrust