Interactions of Insecticidal Proteins with Target Membranes

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Vegetative Insecticidal Proteins and Pests

- Several Lepidoptera are agricultural pests, eg the genus *Spodoptera*(1)
- These pests pose significant agricultural and economic risks: widespread in North and South America, and recently Sub-Saharan Africa (2)
- *Bacillus thuringiensis* (Bt) is a bacterium that produces insecticidal proteins including vegetative insecticidal proteins (Vips)(3)
- Vips kill some Lepidoptera, thought to be through pore formation in the midgut: exact mechanism poorly understood (1)
- This project aims to further understanding through a combination of biochemical and biophysical analysis, supported by molecular modelling approaches

Spodoptera frugiperda larva

Biochemical Characterisation

- Vips activated by proteinases – typically trypsin
- Gross conformational change (Fig. 1), and insertion into membranes (Fig. 2)

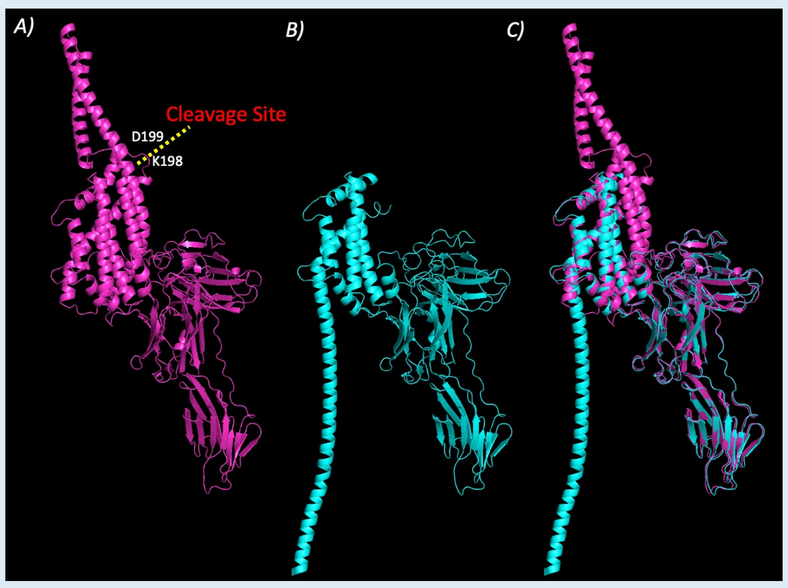

Figure 1: Vip3A activation. *A) Monomer of Vip3Aa16 protoxin. Cleavage site- yellow dashed line. B) Monomer of activated Vip3Aa16. C) Aligned protoxin and activated forms. Adapted from 6TFJ.pdb and 6TFK.pdb*(4,5)

Project will:

- Use brush border membrane vesicles and cell lines from *Spodoptera frugiperda* to:
  - Measure LD_{50} values
  - Quantify changes in electric potential across membranes
  - Examine impacts of mutations on toxicity
- Models will include susceptible and resistant insects
- Lipid/sugar interactions are poorly understood; dot blots and glycan arrays will be used to probe this
  - Compounds found in the insect midgut environment will be used as primary candidates e.g. chitotriose and N, N', N''-Triacetylchitotriose

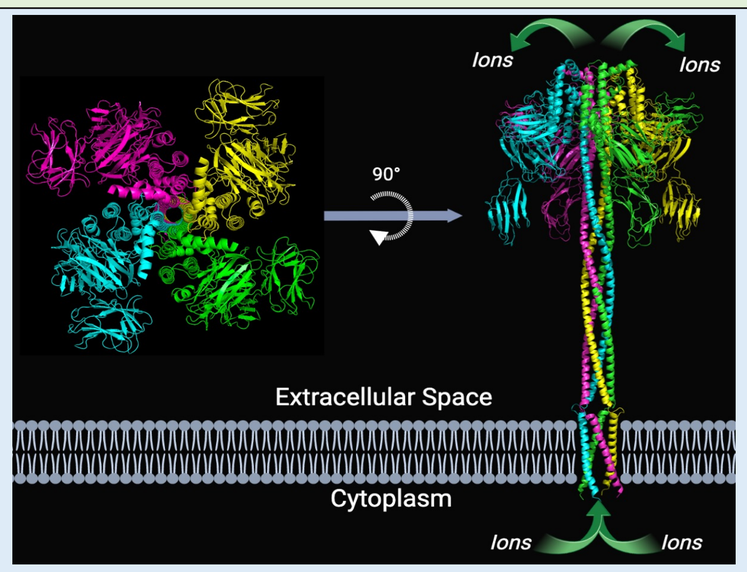

Figure 2: Predicted mode of Vip3A toxicity. *Left) Top-down view of Vip3A toxin, showing central pore. Right) Putative insertion of Vip3A toxin in cell membrane. Adapted from 6TFK.pdb.*(4,5)

Biophysical Analysis

- Giant unilamellar vesicles (GUVs) will act as artificial membranes to investigate protein insertion
- Quantitative and qualitative changes in membranes can be measured using DIC, iGOR and fluorescence microscopy (Fig. 3)
- GUVs can be created to reflect insect-like membranes

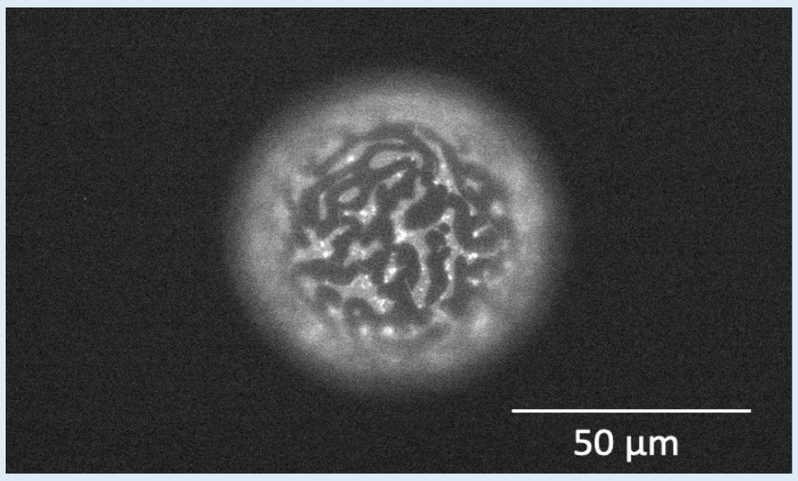

Figure 3: GUV image fluorescence microscopy.
GUV composed of 1:1, POPC:POPE with addition of 7 µM App6 pesticidal protein.

Molecular Modelling

- Molecular modelling used to model interactions of Vip3 with lipids/membranes and sugars
- AlphaFold to predict complete structures of activated Vips and other proteins
  - Extended α-helix (Domain 1) in coiled coil is too disordered in crystallography/Cryo-EM

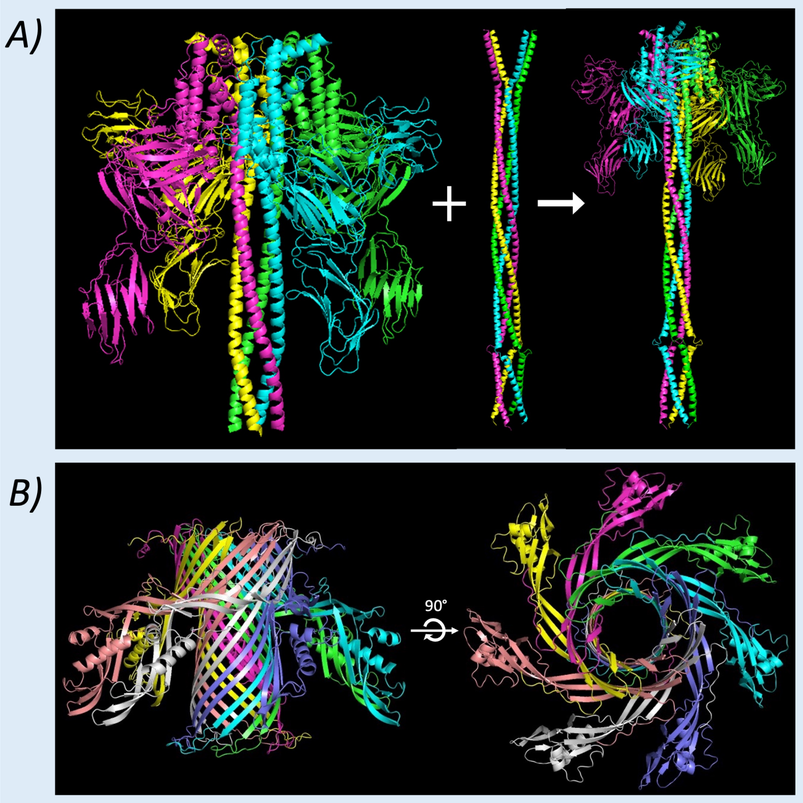

Figure 4: Predicted structures of insecticidal proteins. *A) Composite structure of Vip3Aa16; Left – Cryo-EM structure activated Vip3Aa16. Middle - AlphaFold prediction of tetrameric Domain 1. Right – Construction of complete Vip3Aa16. B) AlphaFold prediction of MppMp heptamer.*(4,5)

- Simulations of membranes carried out: GROMACS with CHARMM-GUI and PyLipID packages for membrane construction
  - Vip models to be inserted into these membranes
- Gross conformational change could be simulated through coarse-grain simulations

Summary

- An early stage project looking to establish Vip toxicity mechanisms
- Combinatorial approach with application of novel techniques
- Should mechanisms be elucidated, new generation pest-resistant crops could be developed, aiding food security globally
- Methods applicable to other Bt toxin families e.g. 3-Domain Cry proteins

References

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