Structural and Functional Characterisation of Chimeric Phage-like Attachment Proteins

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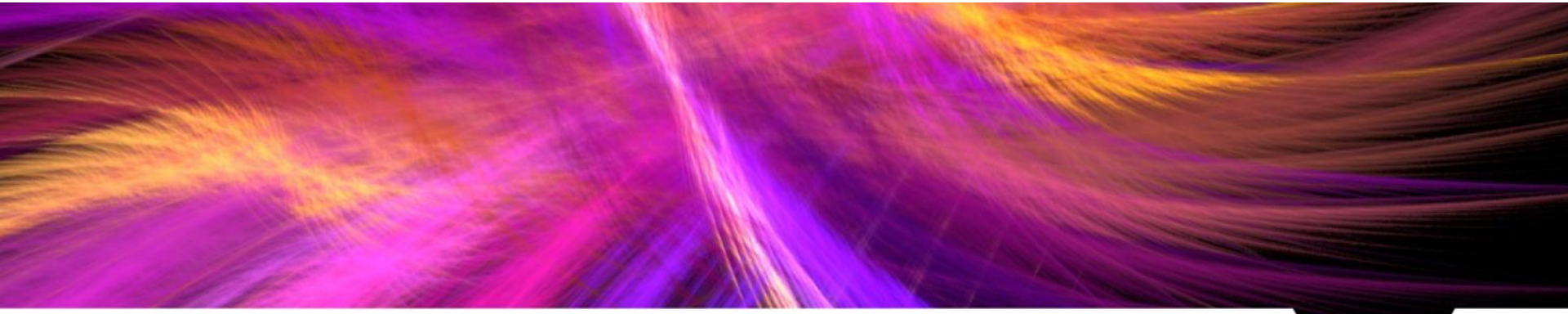

Chemistry PG Symposium 31/05/2017

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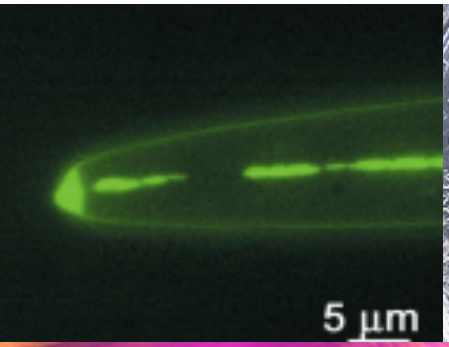
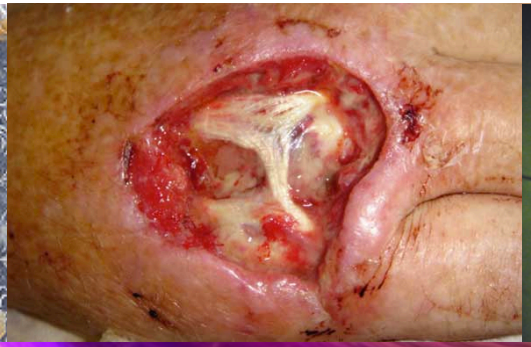
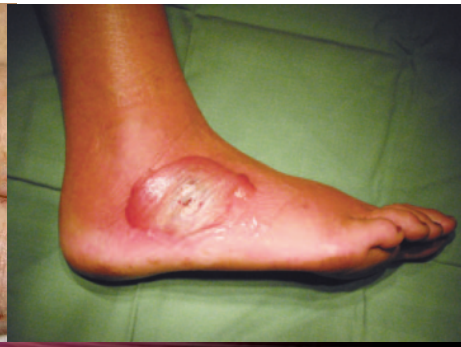
Weird and wonderful bacteria…

- *Photorhabdus* is an insect and occasionally human pathogen.
- Gram –ve, bioluminescent, rod-shaped bacterium
- Obligate symbiont of insecticidal nematodes
- Produces a **vast** number of antibiotics/secondary metabolites/toxins

- Particularly interested in a toxin delivery system called the

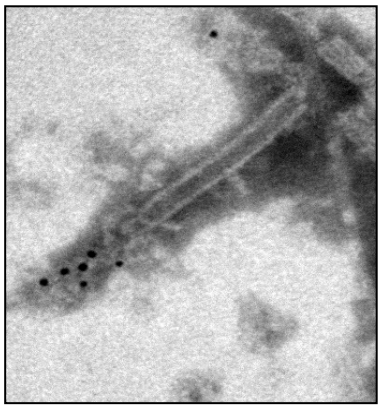
***Photorhabdus* Virulence Cassette (”PVCs”)**



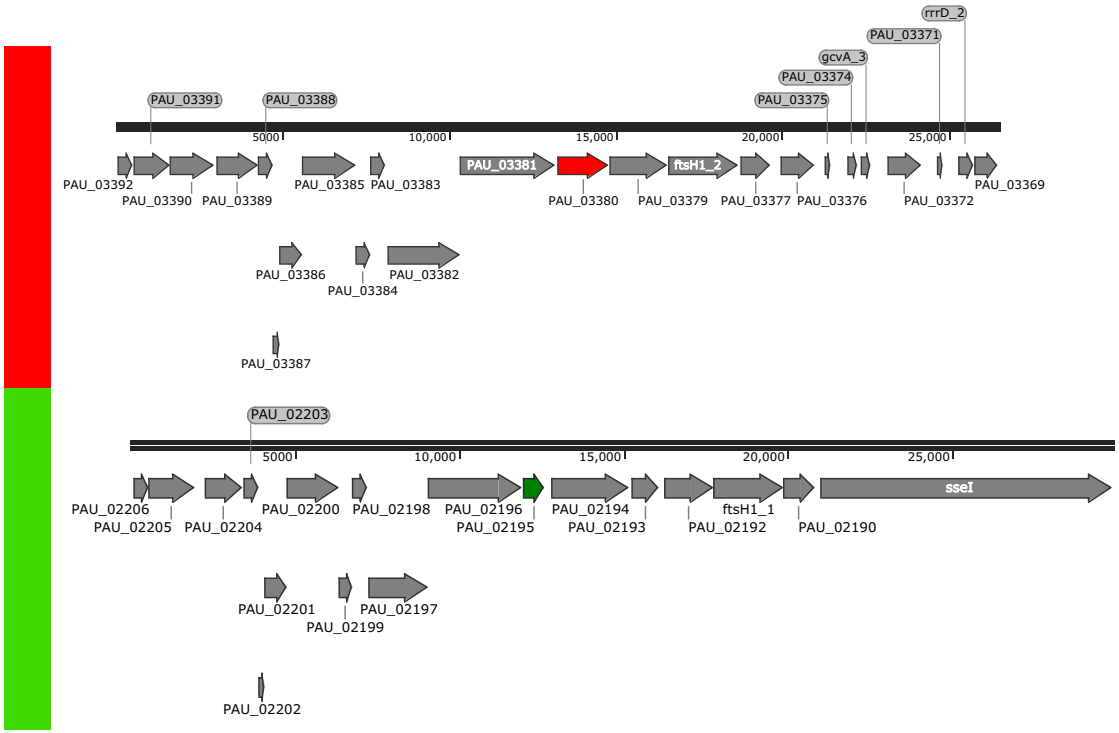
Forst, *et al. Annual Reviews in Microbiology* **51.1** (1997): 47-72.

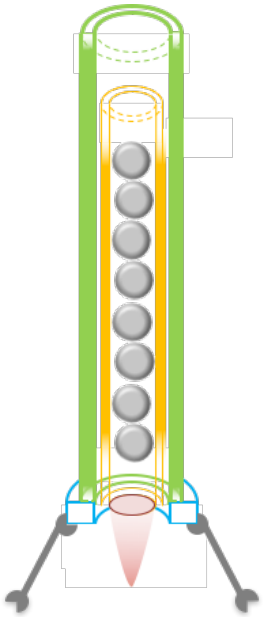
What's a PVC?

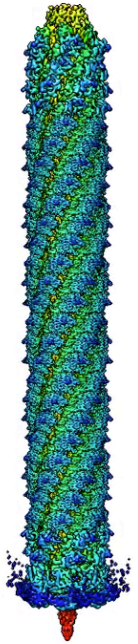


PVCs are 'nano-torpedos' which the bacterium uses to kill eukaryotic cells. They resemble phage tail tubes, loaded with toxins.

There are 5-6 gene clusters per genome, encoding variants – talk focuses on 2:

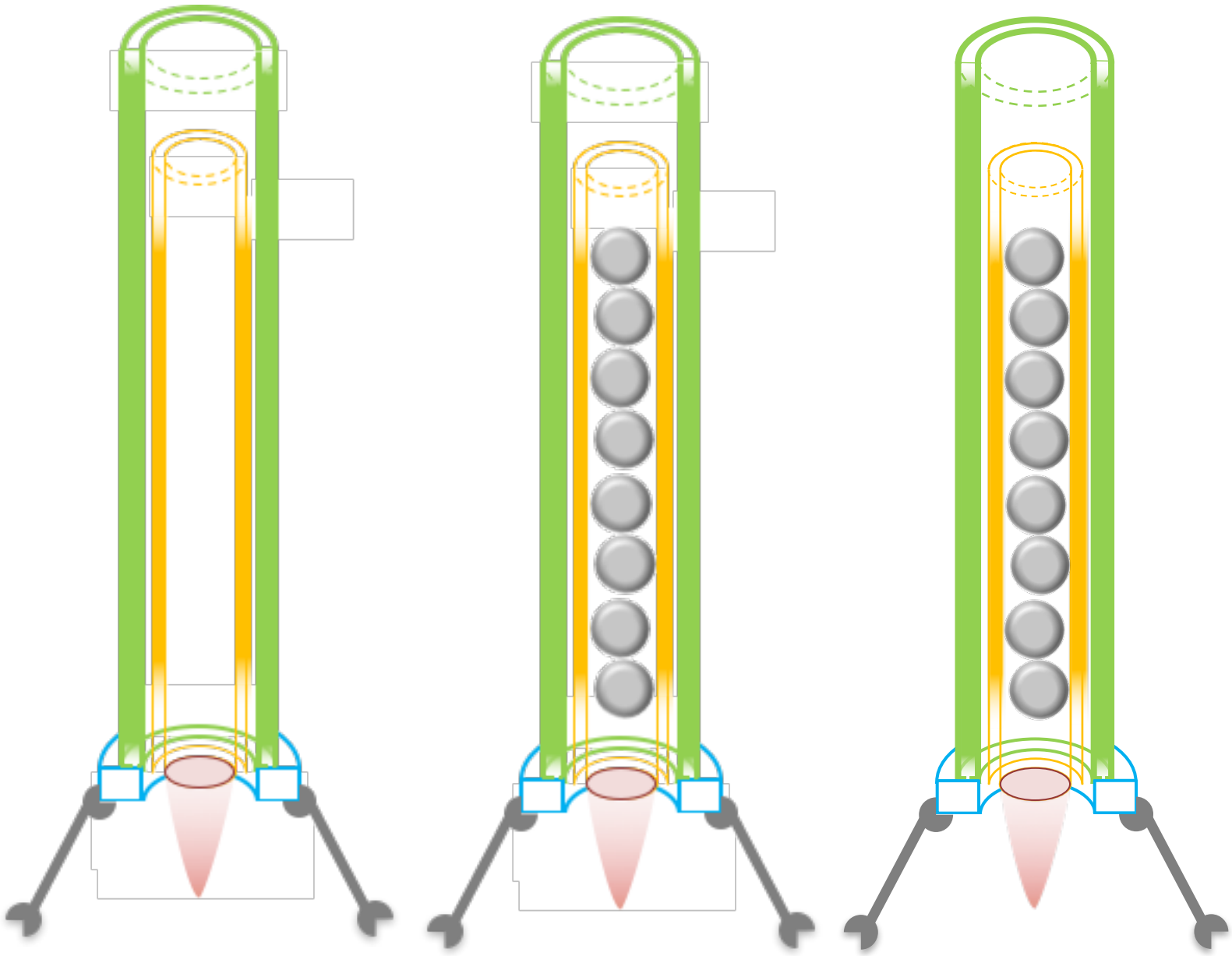






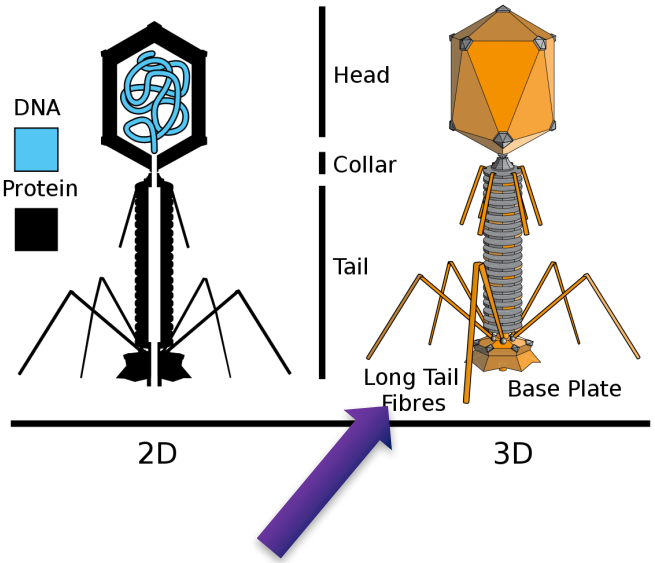
Ge, *et al. Nat struc & mol bio* **22.5** (2015)

Yang, G., *et al*. J Bacteriol, 2006. **188**(6): p. 2254-61



Tail Fibre Proteins: What are they?

- Tail fibre proteins (in phage etc) are responsible for target specificity/ attachment – always vs. prokaryotes.
- Bioinformatics studies suggests that these genes are the PVC equivalents.
- Seem very diverse/recombinant.



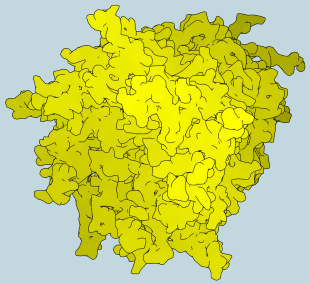
Most interestingly, they seem to be ***chimeric*** - this might account for eukaryotic activity.

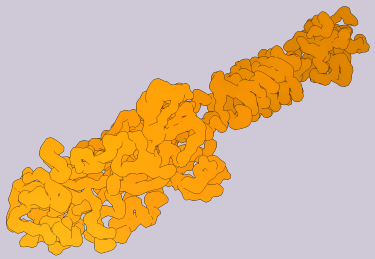
Tail Fibre Proteins: Weird chimeras?

DOMAINS:

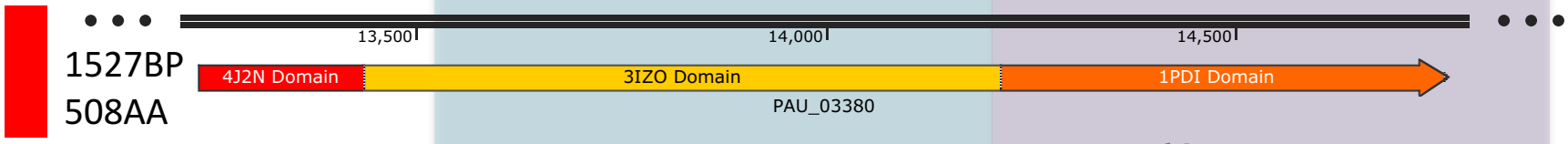
Adenovirus-like

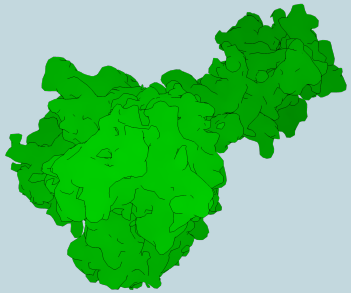
T4Phage-like

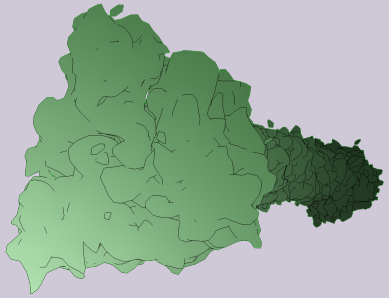




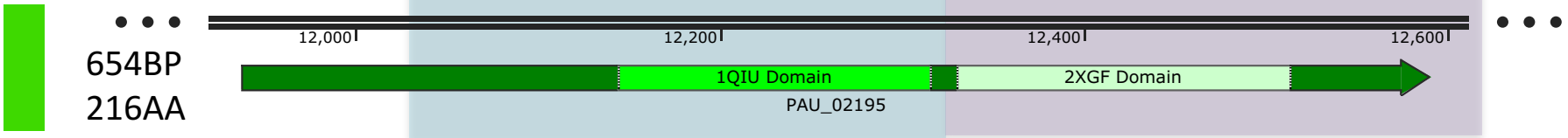
1527BP
508AA







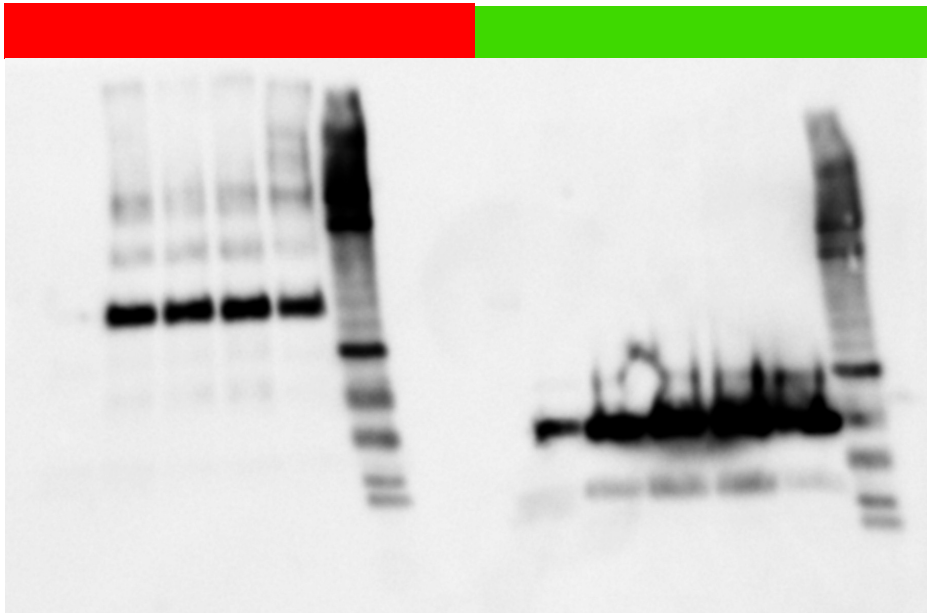
654BP
216AA



Tail Fibre Proteins: Cloning/Tagging

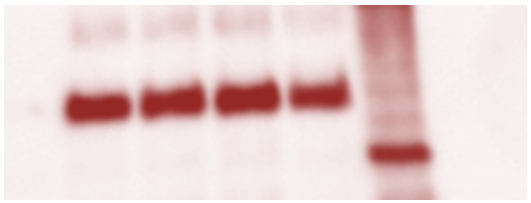
- 2 tail fibres cloned with terminal Histidine tags
- Cloned in to IPTG inducible pET vectors
- Induction/Expression performed in NEB NiCo21(DE3) IMAC optimised cells.

- Good expression seen after 4 hours & overnight
- Expression seemed better in LB+IPTG than in alternative media.



Tail Fibre Proteins: Expression/Purification

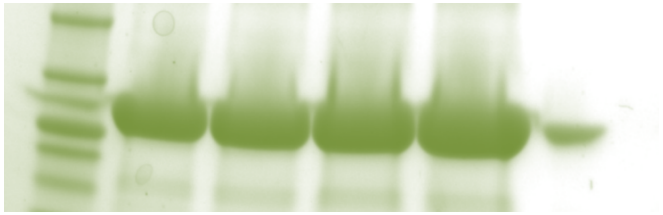
- First clue that we were on the right track – formation of trimers. Stable even with boiling and high concentration Urea+SDS.

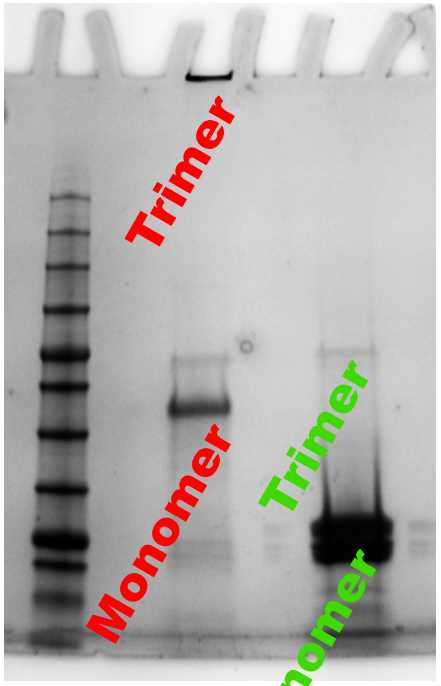


Monomer wt = 53.5 kDa

Ni-IMAC + Gel Filtration Purified

Monomer wt = 25.4 kDa





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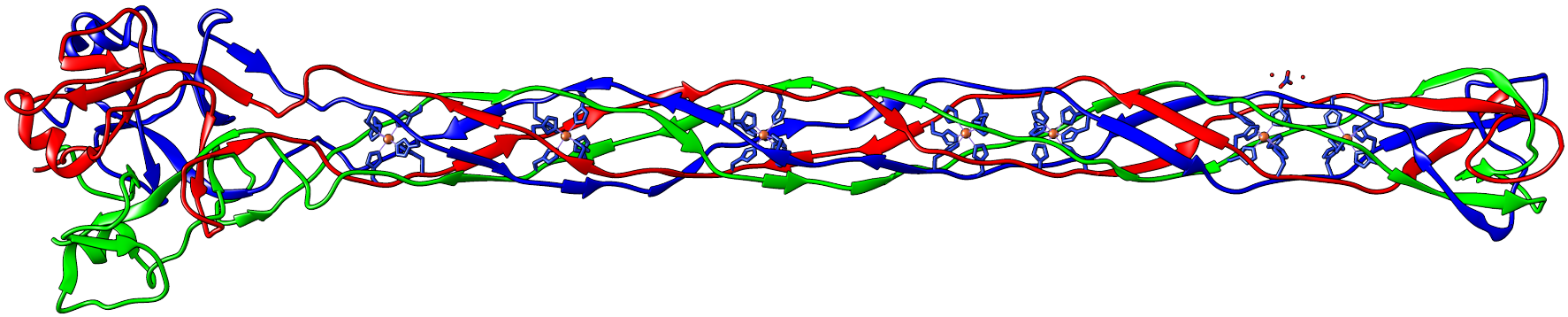
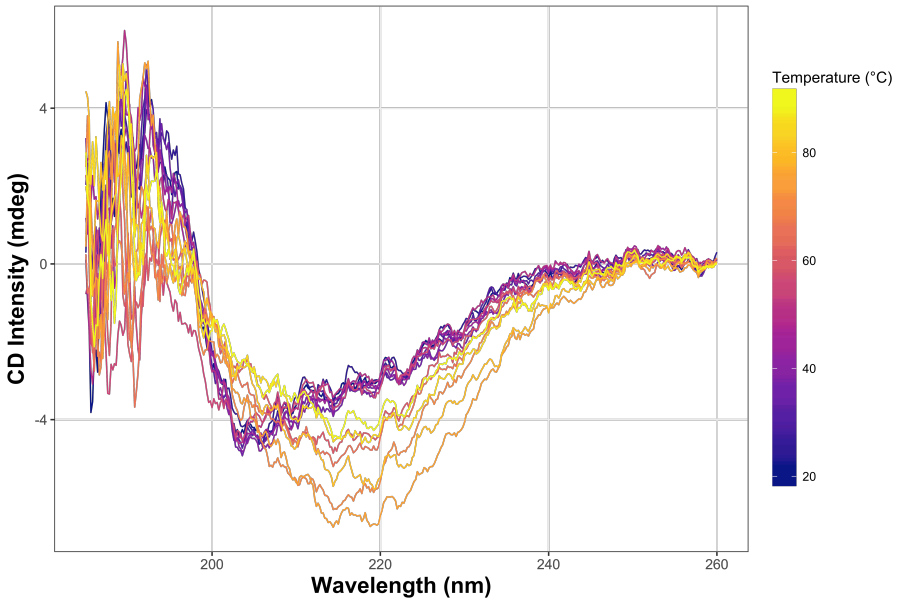


van Raaij, *et al*. *JMB* **314.5** (2001): 1137-1146.

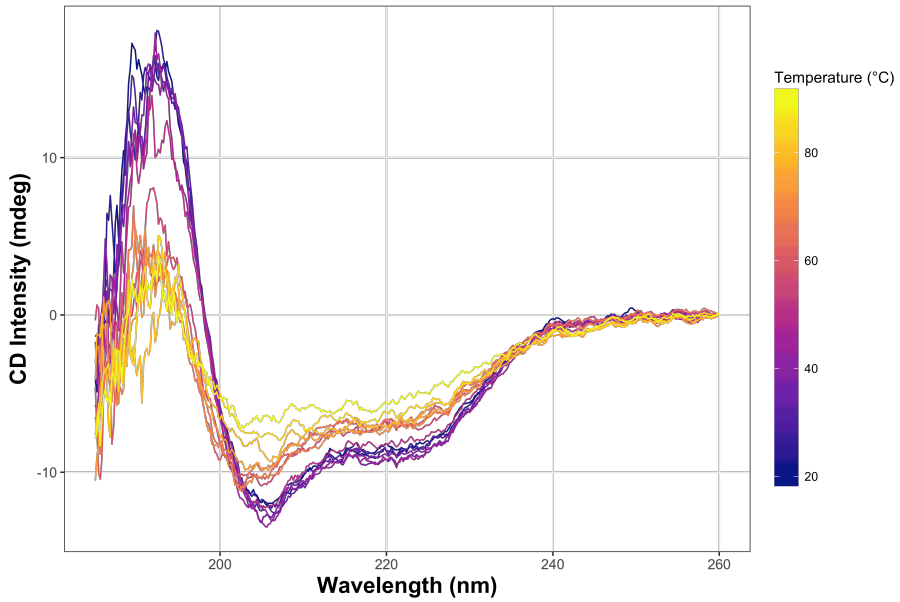
Tail Fibre Proteins: CD

- Stability seen on the gels made us curious to assess thermal stability more directly – CD melts.
- Also wanted to know whether the 2ndary structure is consistent with published fibres

PNF13

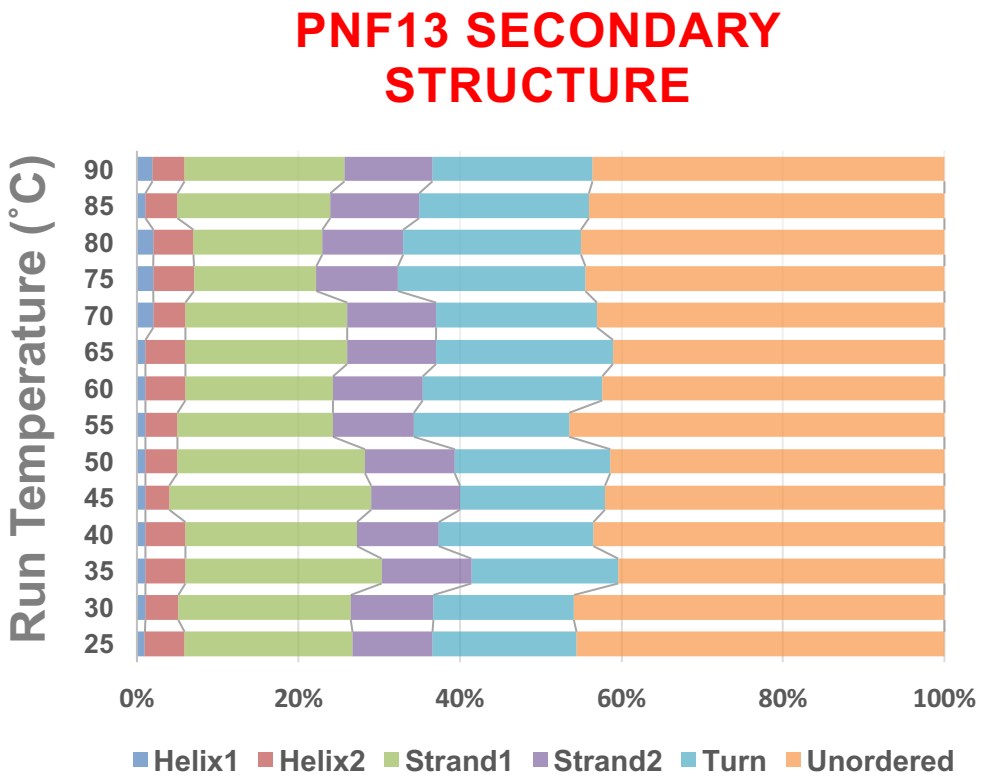


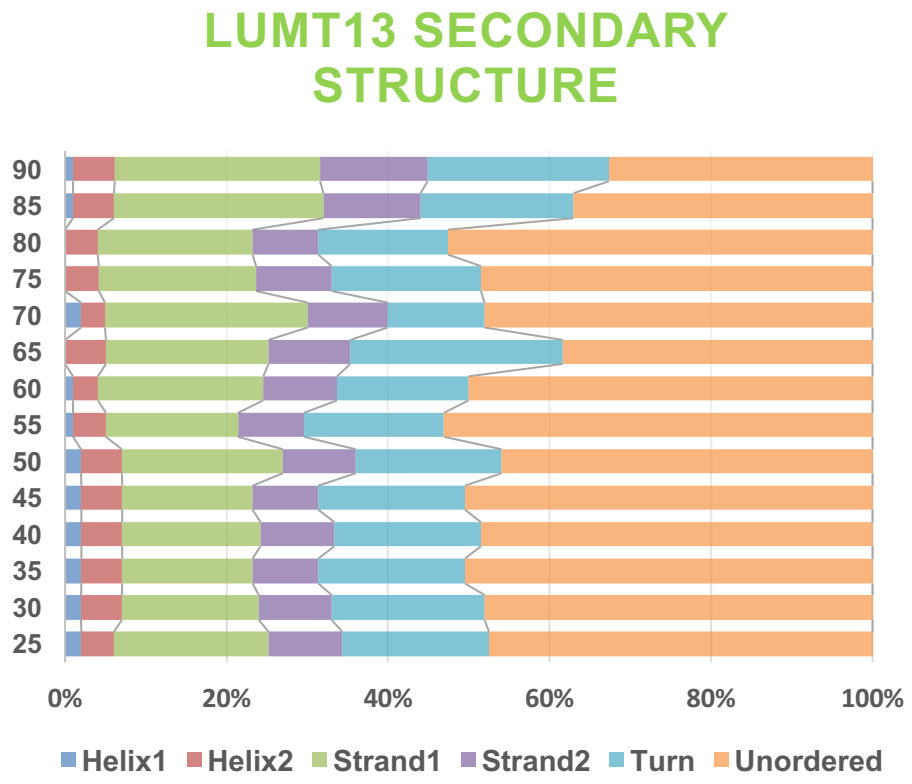
LUMT13



Tail Fibre Proteins: Secondary Structure Prediction

- Dichroweb gives good fits (CDSSTR).
- Consistent with published structures (dominated by turn/sheet/other)





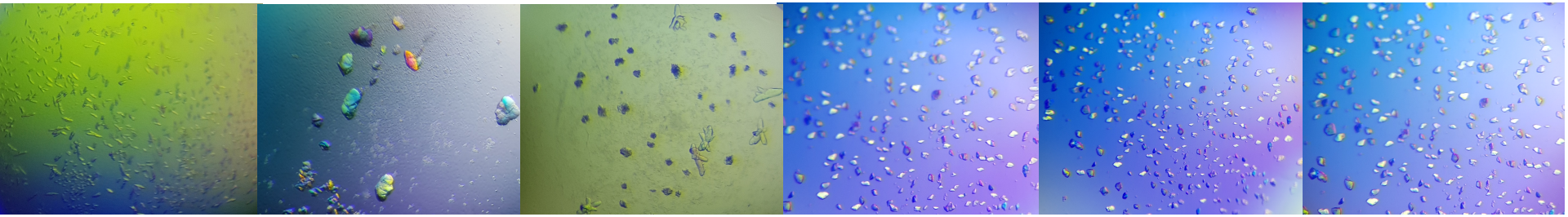
Tail Fibre Proteins: Crystallisation

- Working toward a structure.

- Had some initial success with crystallography:
  - First protein tested gave crystals in ~12 conditions in < 1 week.
    - No diffraction however

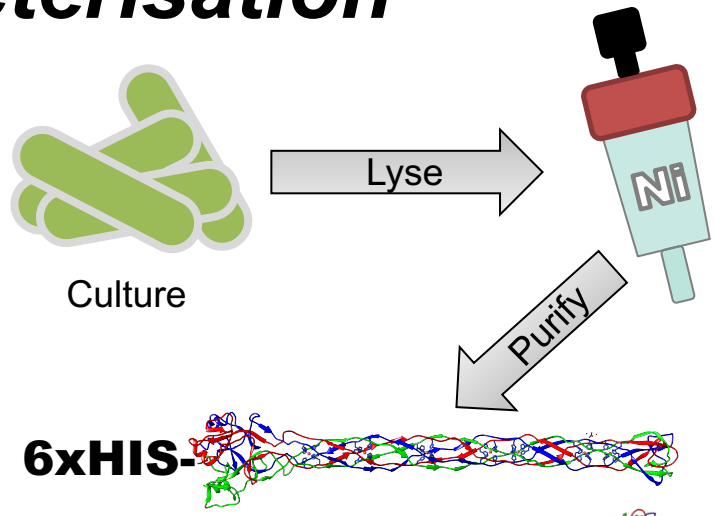
  - Testing with *in-situ* partial proteolysis gave crystals in another ~10 conditions overnight.

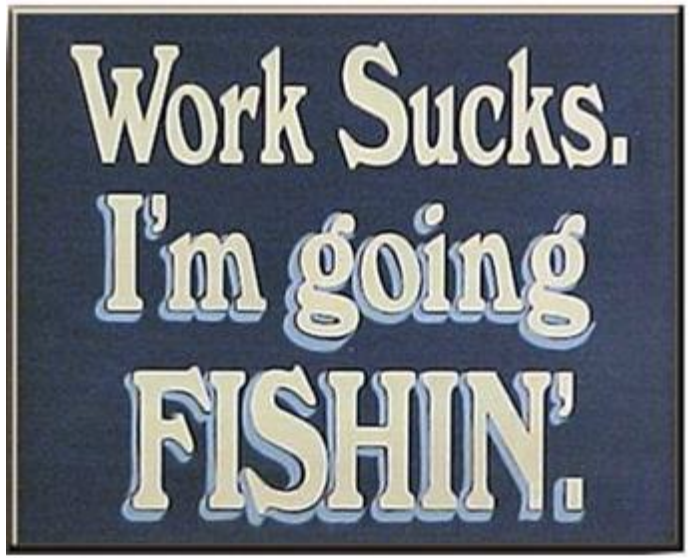
- Work is ongoing, and only tried with 1 protein so far.

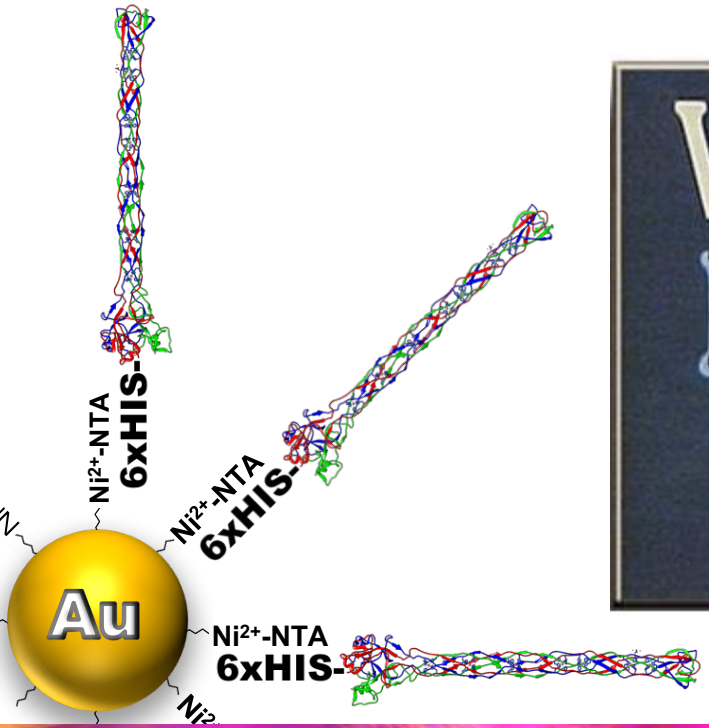


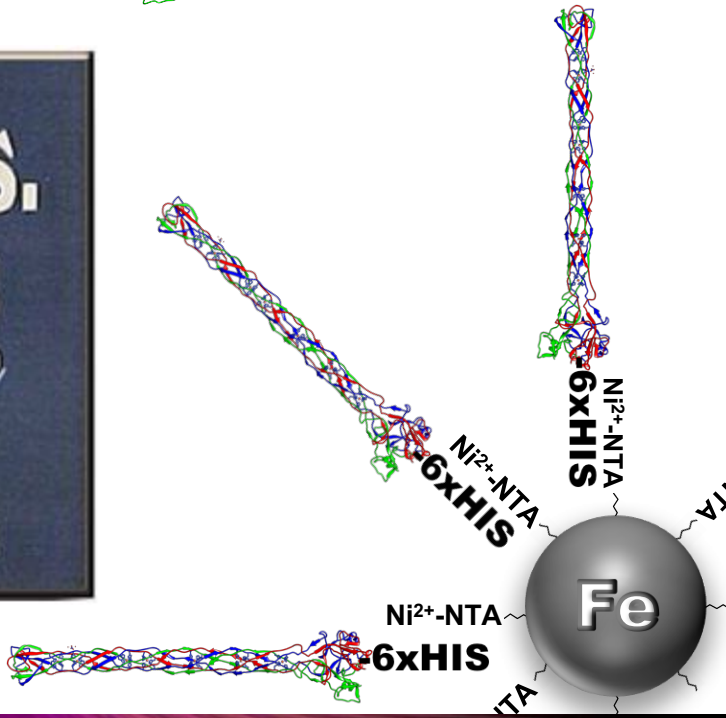
Functional Characterisation

- His-tag is a multifunctional 'handle':
  - Purification
  - Pulldowns
  - Quantification
- Binding to nanoparticles



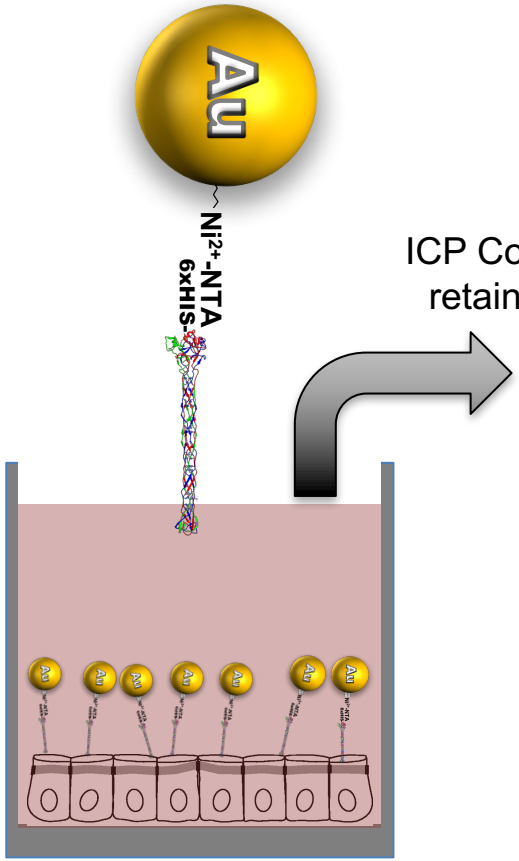




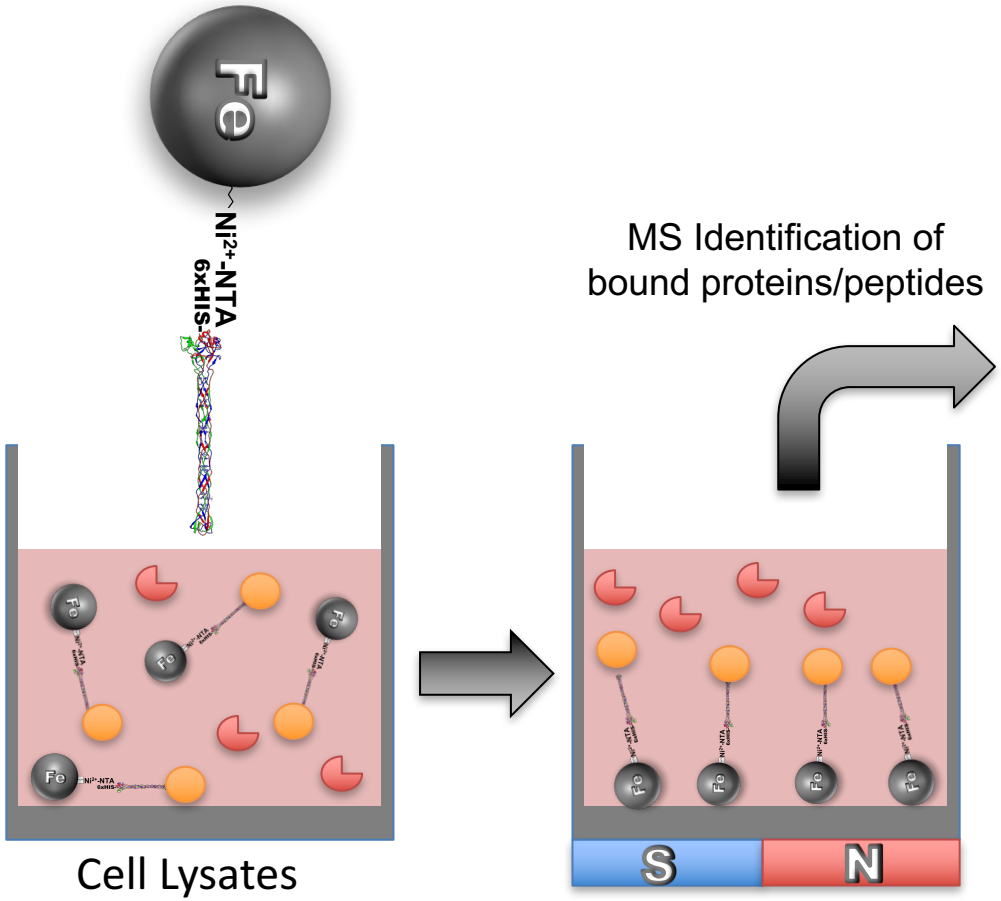


Functional Characterisation

TISSUE SPECIFICTY STUDIES

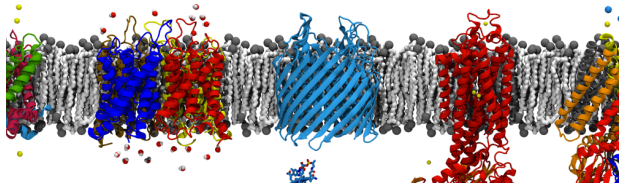


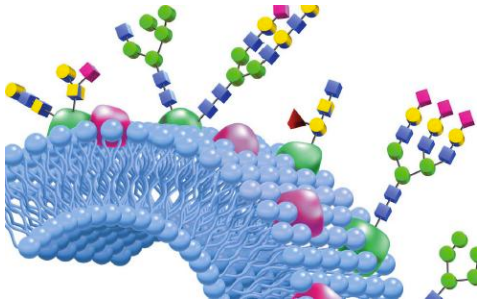
BINDING PARTNER MAG-PULLDOWNS

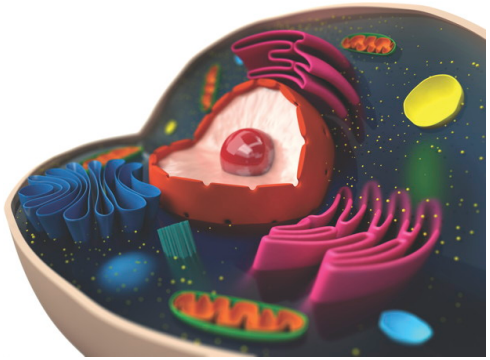


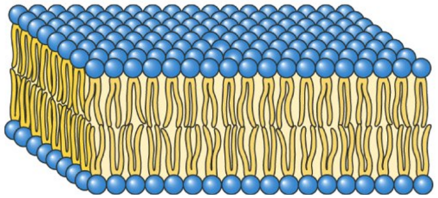
Functional Characterisation

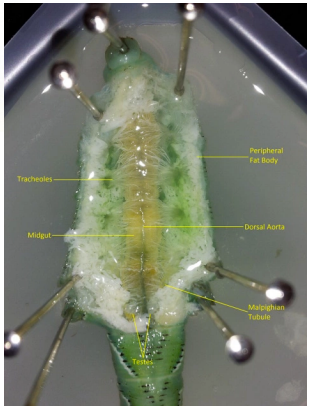
- Possibilities for binding partners:
  - Specific cell surface protein target -> **proteomics**
  - Internal cell component? -> **proteomics**
  - Sugar binding -> **glycan arrays**
  - Lipid interactions -> **fat blots**
  - Whole-animal tracing -> **ICP-AES**











Conclusions/Next Steps

Chimeric nature could be evolved against eukaryotic targets, but maintaining the ‘mounting hardware’.

Experimental data seems consistent with what we’d expect from known tail fibre proteins. Quite likely they are what we thought they were all along.

- Consistent secondary structure profile
- Consistent thermal stability
- Form homotrimers as expected.

Experiments planned:

- SAXS – attempt to model as rods for an envelope structure
- Further CD repetitions
- Continued crystallography optimisation/structure resolution attempts
- Continued pulldown assays etc to try and find binding partners for the tail fibres.

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Assay development for pulldowns/binding assays etc.

Acknowledgements

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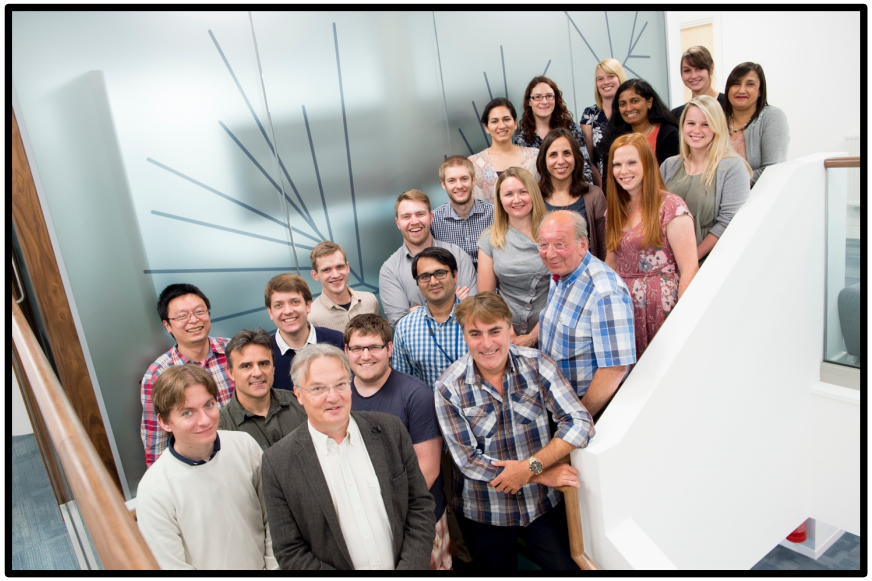

Microbiology and Infection Group

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- *Dr. Kathryn Styles*

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- *Tom Brooker*

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