UNIVERSITY OF LIVERPOOL

BBSRC bioscience for the future

Deciphering the role of surface lipoproteins in Treponemal Immunomodulation towards a vaccine for Bovine Digital Dermatitis

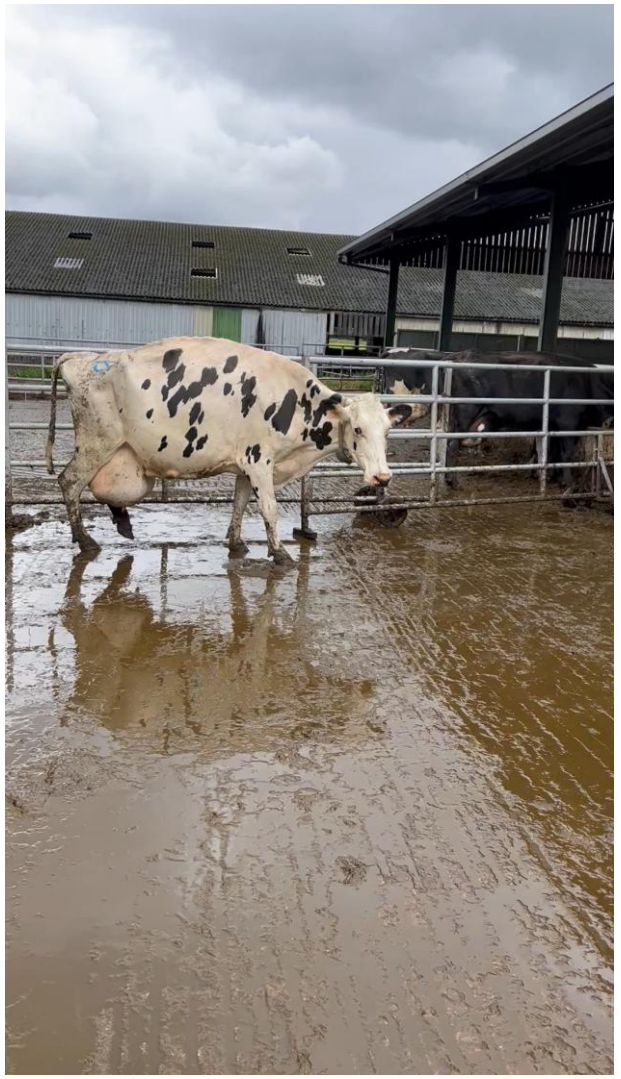


By
Ferdinand Ngale N.



Background – Bovine Digital Dermatitis







Estimated economic losses of around 80 million pounds annually in the UK.

Video Courtesy - Rachel Ridgway

Background - Aetiology

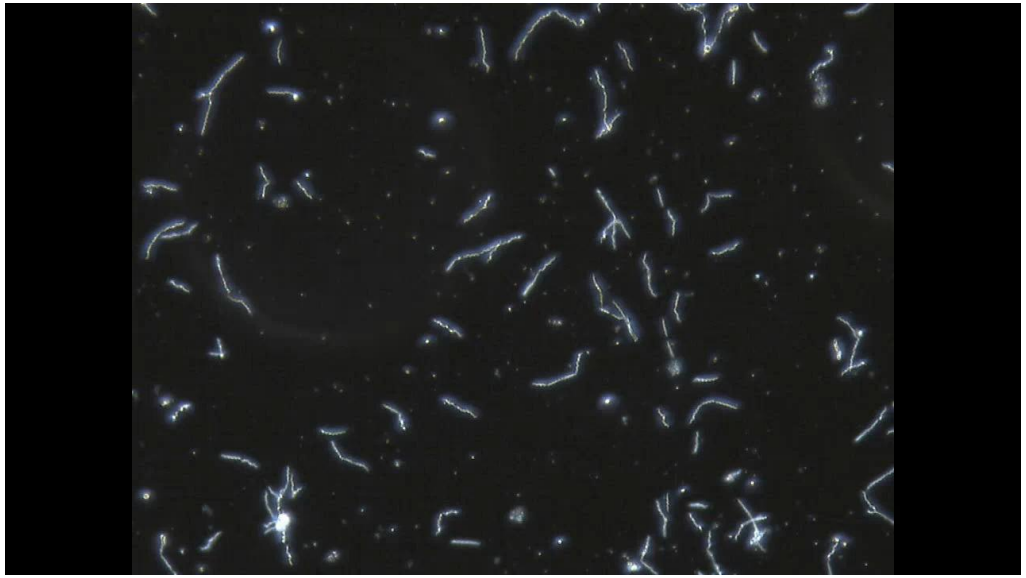




don whitley scientific
culturing innovation
50 YEARS OF CULTURING INNOVATION

Lesions are cultured under anaerobic conditions to isolate the fastidious beast responsible

Background - Aetiology



Three species associate with all DD positive farms-

Treponema phagedenis

Treponema medium

Treponema pedis

Representative strains at the University of Liverpool include T320A (***T. phagedenis***), T19 (***T. medium***) and T3552B (***T. pedis***).

Background – Disease Management



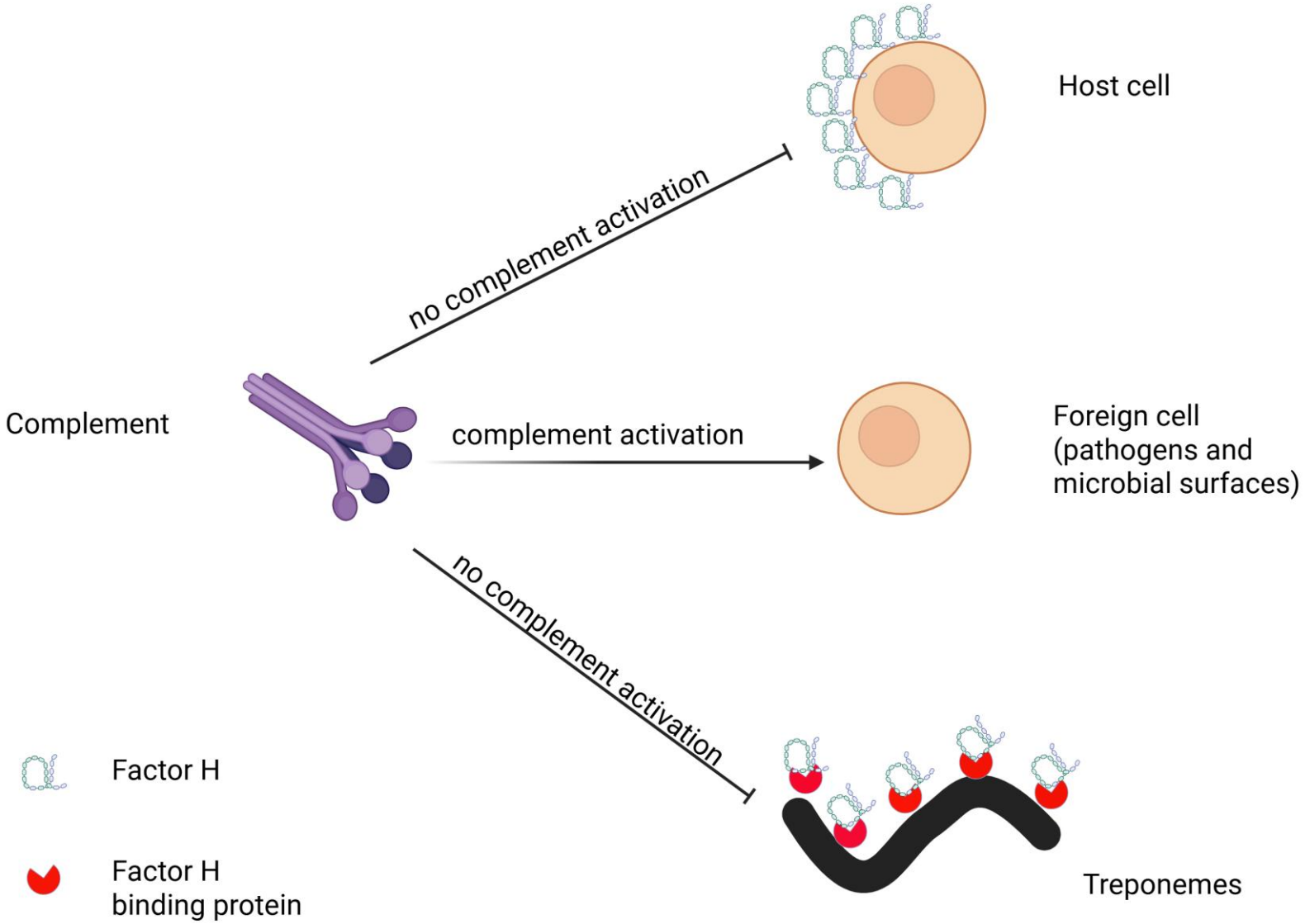
Treatment – Topical administration of antibiotics



Prevention - Foot baths in formalin or copper sulfate

A vaccine is therefore needed to ensure efficient management of the disease.

Background – Immune Evasion Strategy



*Plasminogen is also a regulator of the complement system - binds to C3 and cleaves C3b into fragments that lack effector function.

Project Goal

Overall goal is to screen conserved Surface lipoproteins in all three BDD treponeme species for Factor H and plasminogen binding and use the candidates to assess protection against BDD.

Objectives

Set out to identify potential Factor H and plasminogen binding proteins across DD Treponemes using AI

To screen selected *in-silico* candidates for binding efficacies to factor H (FH) and plasminogen *in-vitro*

Function restriction and thermostability assessment of confirmed FH and plasminogen binders and immunize mutated proteins in mice.

Investigate immune mechanisms of any observed protection

Experimental approach



Screen for Surface Lipoproteins

AlphaFold/Co-folding with BFH and plasminogen



Expression and purification of candidates.

In-vitro binding assays/function restriction and thermostability assays





Immunization of confirmed candidates

Assessment of protection

Results



Prof. Dan Ridgen



Dr. Emily Johnson

Screen proteomes of DD Treponemes using SignalP for surface lipoprotein

Generate a list orthologous surface lipoproteins (shared by all three DD Treponemes) – 35 proteins

Co-Fold all 35 candidates with **Human factor H** and **plasminogen** – co-folding was ranked using mpDOCKq and Iptm-ptm scores

Table of Results

No	Protein Candidate	Iptm_ptm (CFH)	Iptm_ptm (Plasminogen)	In-vitro Binding (CFH)	In-vitro Binding (Plasminogen)	Expression	Purification	Molecular weight (KDa)
1	C5O78_13570	N/A	N/A	ns	ns	Expressed	Soluble	53.2
2	C5O78_11575	N/A	N/A	ns	ns	Expressed	Soluble	48.4
3	C5O78_11520*	0.268	0.248	**	ns	Expressed	Soluble	70.6
4	C5O78_07615	N/A	N/A	**	***	Expressed	Soluble	45.4
5	C5O78_01830*	0.404	0.305	*	ns	Expressed	Soluble	42.4
6	C5O78_05920*	0.290	0.279	****	****	Expressed	Soluble	34.2
7	C5O78_00085*	0.301	0.317	****	ns	Expressed	Soluble	36
8	C5O78_09920	N/A	N/A	****	**	Expressed	Insoluble	28.9
9	C5O78_14240	0.458	0.254	**	*	Expressed	Soluble	55.2
10	C5O78_11735*	0.668	0.331	ns	ns	Expressed	Soluble	40.9
11	C5O78_04800*	0.265	0.257	ns	ns	Not observable	NA	63.3
12	C5O78_01335*	0.388	0.284	**	ns	Expressed	Soluble	78.2

Table of Results

No	Protein Candidate	Iptm_ptm (CFH)	Iptm_ptm (Plasminogen)	In-vitro Binding (CFH)	In-vitro Binding (Plasminogen)	Expression	Purification	Molecular weight (Kda)
13	C5O78_05090*	0.257	0.283	****	*	Expressed	Soluble	40.3
14	C5O78_06345*	0.306	0.692	*	**ns**	Expressed	Soluble	66.4
15	C5O78_06435*	0.231	0.245	**N/A**	**N/A**	N/A	N/A	58.7
16	C5O78_07725*	0.241	0.397	**ns**	**ns**	Expressed	Insoluble	62.8
17	C5O78_01490	0.674	0.280	**	**	Expressed	Soluble	34.9
18	C5O78_10660	0.327	0.308	**nd**	**nd**	Expressed	Insoluble	31.2
19	C5O78_01645	0.351	0.511	**ns**	**ns**	Expressed	Insoluble	23.9
20	C5O78_05085	0.224	0.329	**	****	Expressed	Soluble	38.1
21	C5O78_10445	0.264	0.772	**	****	Expressed	Soluble	26.4
22	C5O78_09835	0.273	N/A	**ns**	*	Expressed	Soluble	38.0
23	C5O78_02040	N/A	0.535	***	***	Expressed	Soluble	27.6
24	C5O78_04180	0.233	0.363	*	****	Expressed	Soluble	49.5

Table of Results

No	Protein Candidate	Iptm_ptm (CFH)	Iptm_ptm (Plasminogen)	In-vitro Binding (CFH)	In-vitro Binding (Plasminogen)	Expression	Purification	Molecular weight
25	C5O78_02730	0.423	0.354	**ns**	*	Expressed	Soluble	26.6
26	C5O78_10515	0.386	0.473	***	****	Expressed	Soluble	13.6
27	C5O78_01820	0.337	0.308	*	****	Expressed	Soluble	33.7
28	C5O78_04725	0.303	0.615	*	*	Expressed	Soluble	27.0
29	C5O78_09505	0.483	0.364	***	****	Expressed	Soluble	21.3
30	C5O78_12625	0.246	0.486	**ns**	**ns**	Expressed	Soluble	45.3
31	C5O78_05965	0.575	0.278	*	*	Expressed	Soluble	48.8

Results

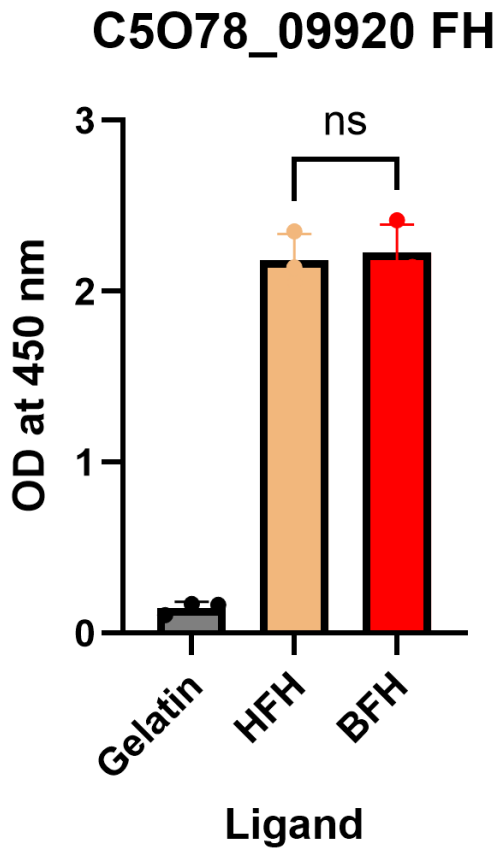
- Expression was attempted for **31** of the **35** shared lipoproteins with alphafold scores . **29** of those were successfully expressed and purified**. 20** were found to bind to either factor H and/or plasminogen.

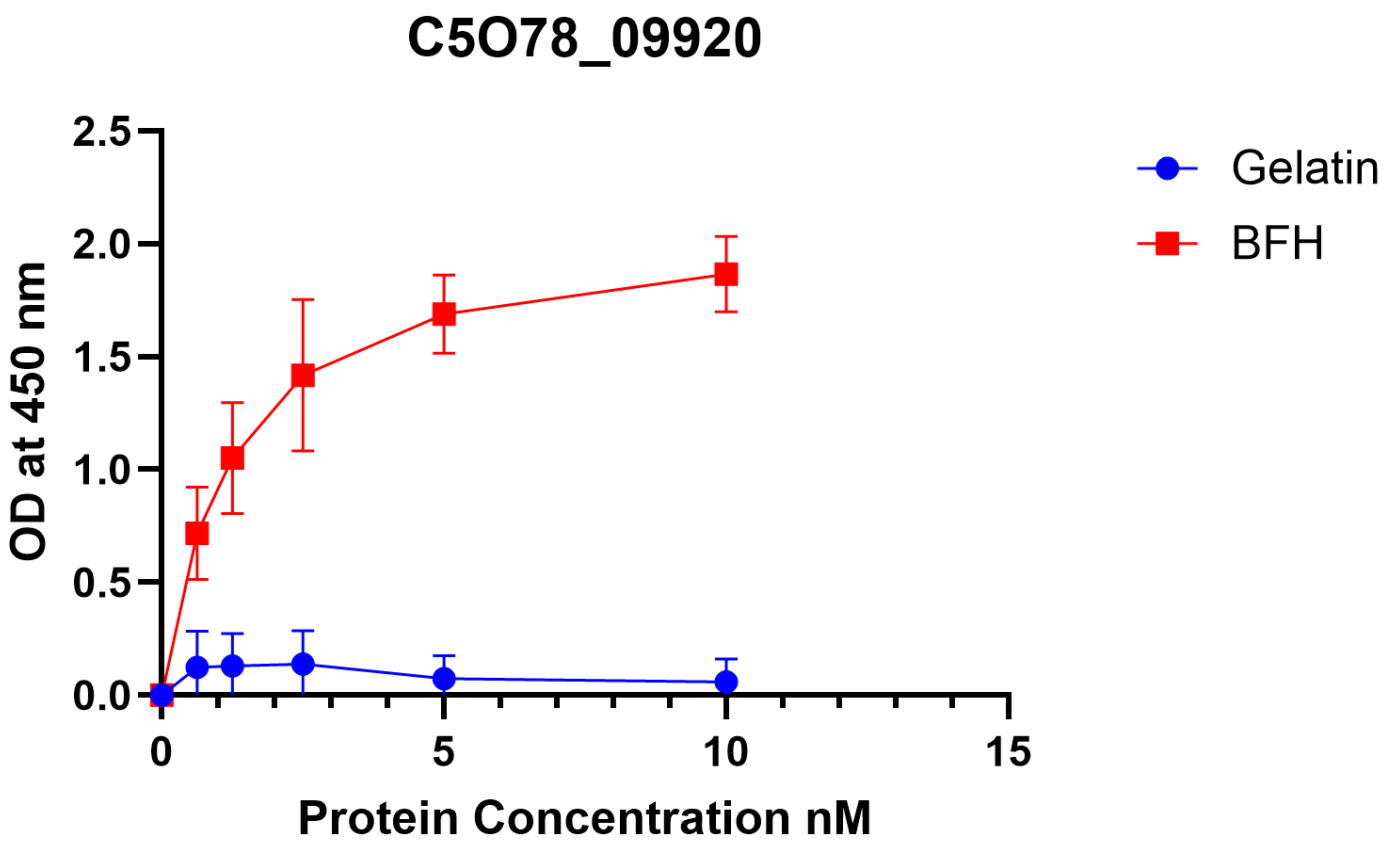
- Up **15** of the **20** candidates were found to bind to both plasminogen and factor H

- The iptm scores did not always agree with observed experimental binding data

- Saturation plots were obtained for **10** of the **20** candidates, suggesting the presence of a specific binding site and these 10 are being considered as the candidate set and advanced for further characterization and immunization

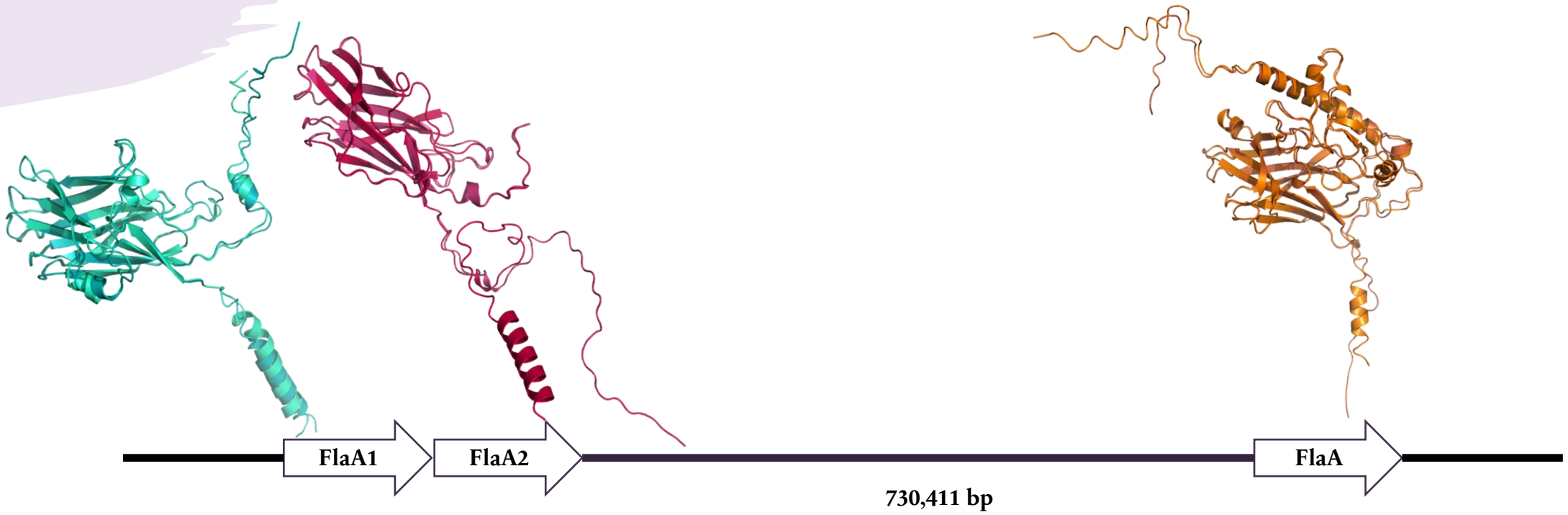
C5O78_09920 binds to BFH with low Kd value





Kd = 1.214±0.263 nM

C5O78_09920 is FlaA2



- C5O78_09920 is FlaA2
- All three paralogues are present in all Treponemes queried so far
- FlaA is Spirochetes-specific, indicating its role is crucial for spirochete motility and Biology in general

FlaA2 is a flagella sheath protein

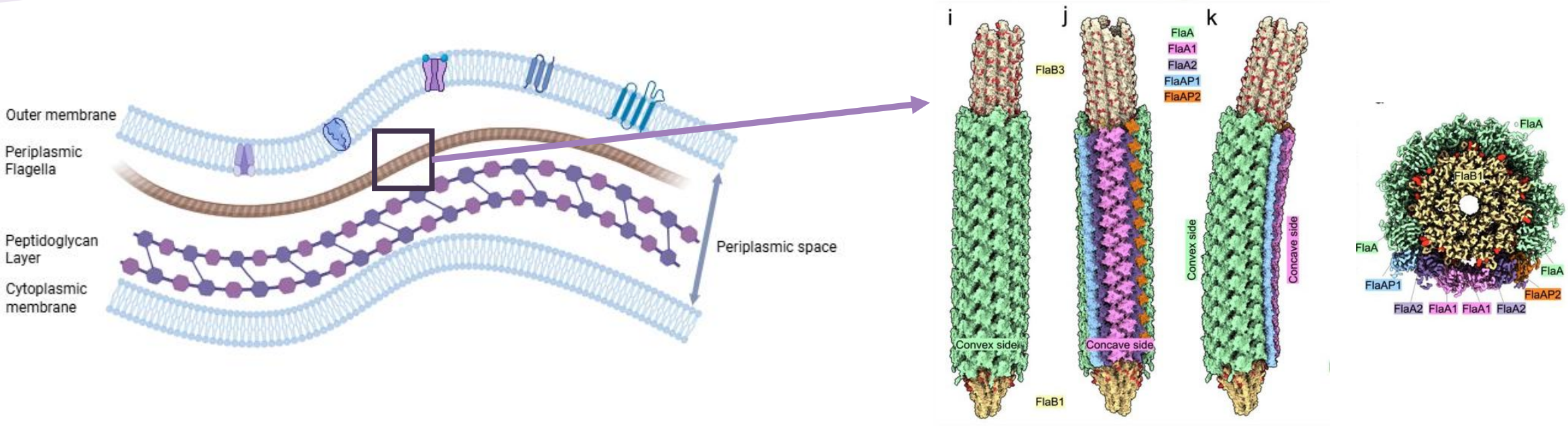
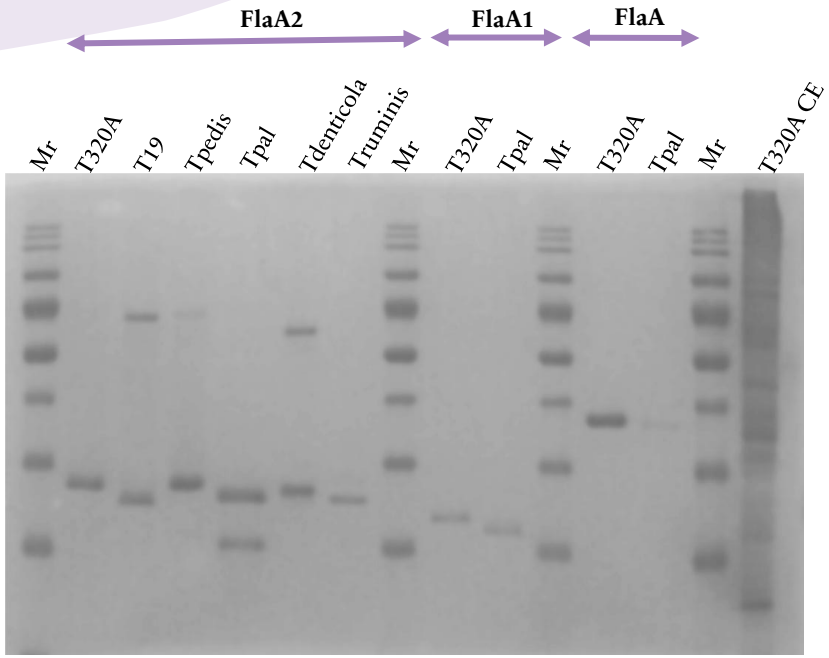
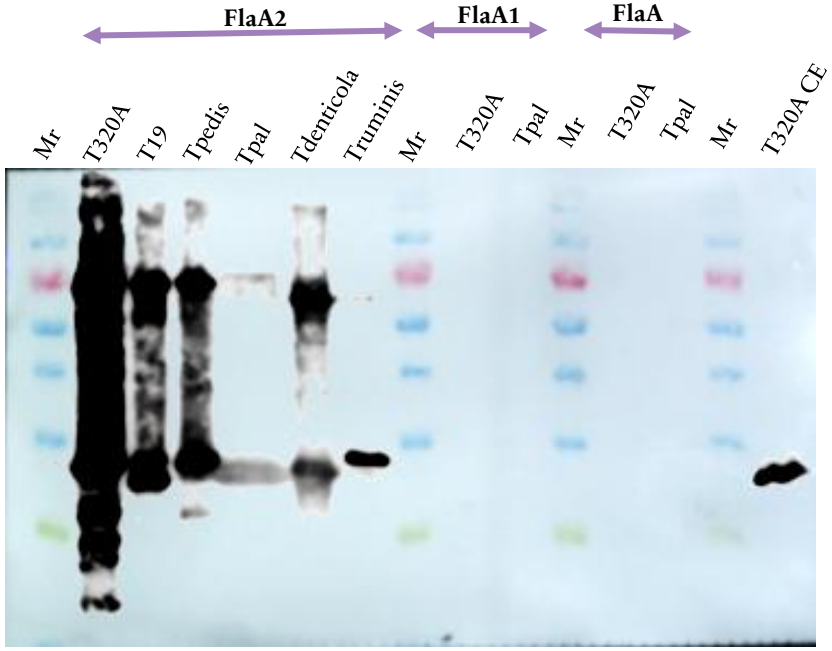


Image courtesy: Wang et al., 2026

Rabbit Anti-flaA2 antibodies are specific for flaA2

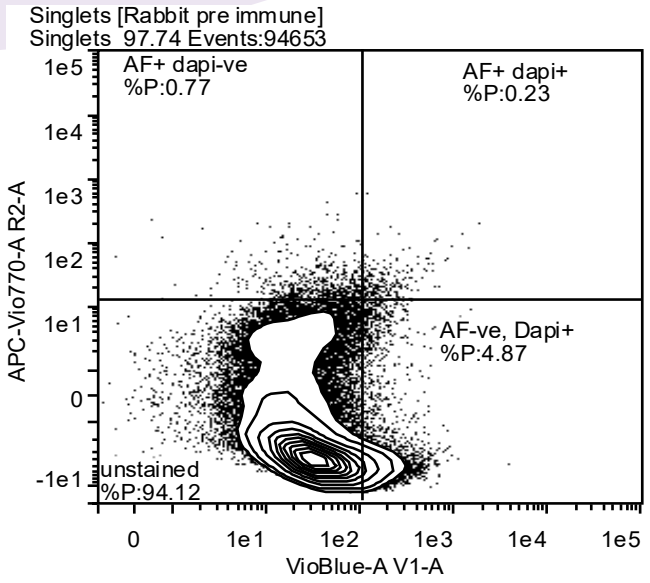


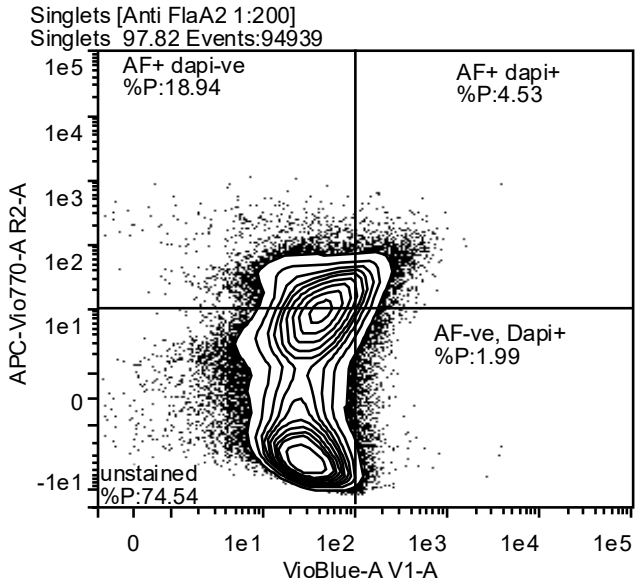
Coomassie staining

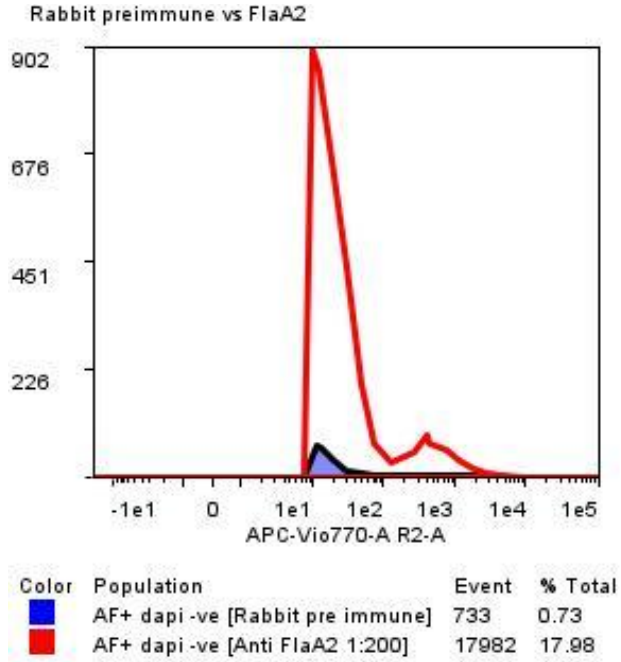


Rabbit anti-T32oA flaA2 polyclonal ab

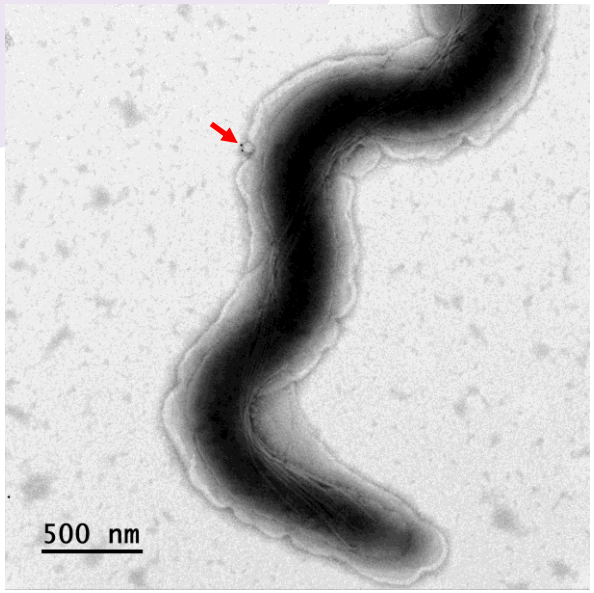
Flow cytometry suggests surface expression of FlaA2

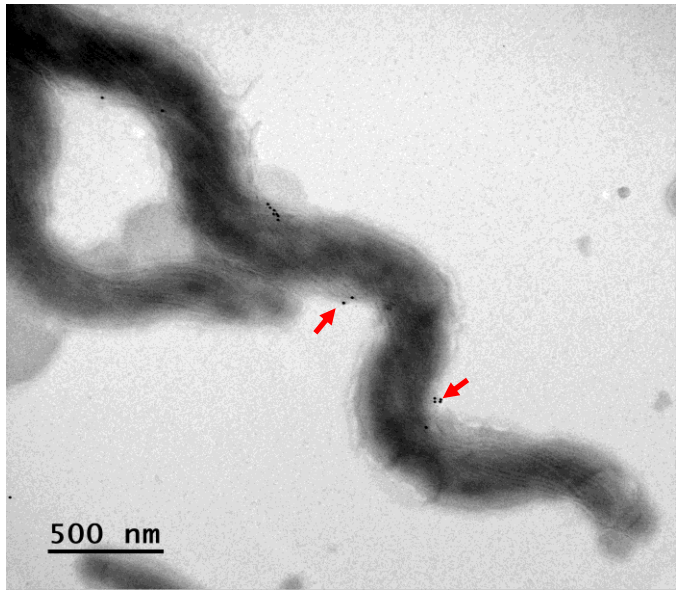


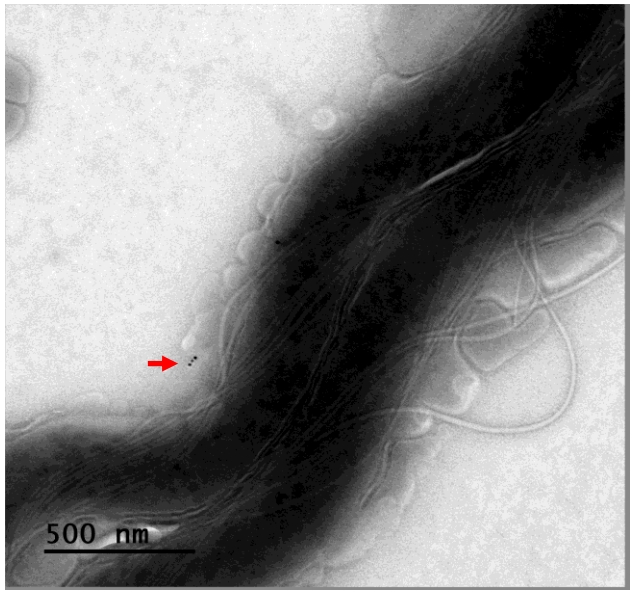


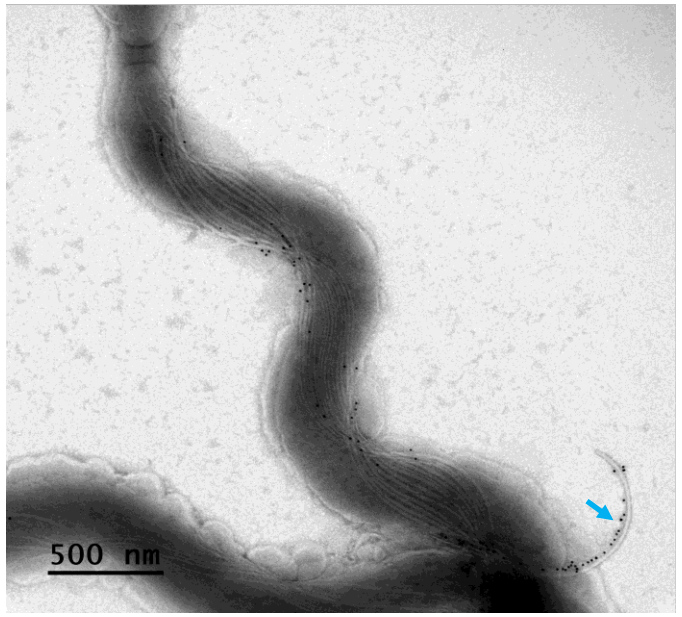


Immunogold EM identifies distinct flaA2 'puncta' on T320A surface

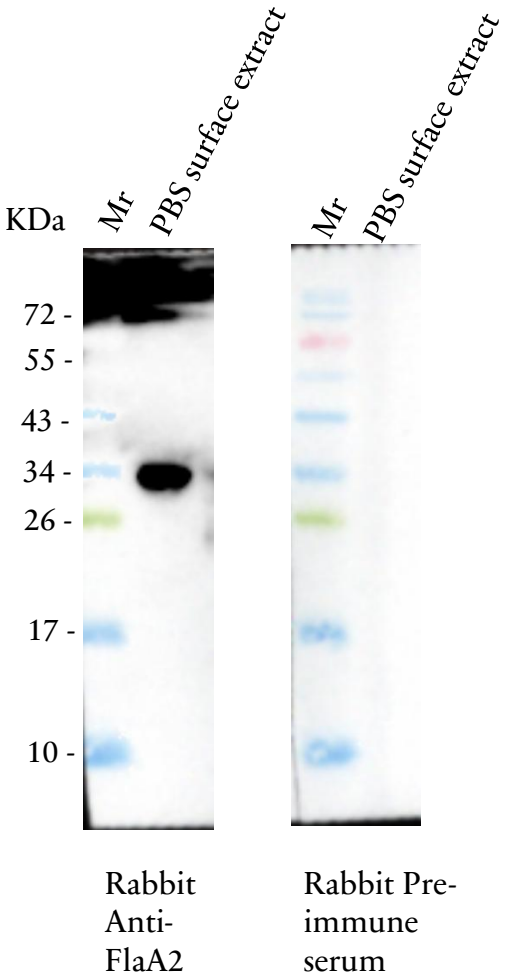




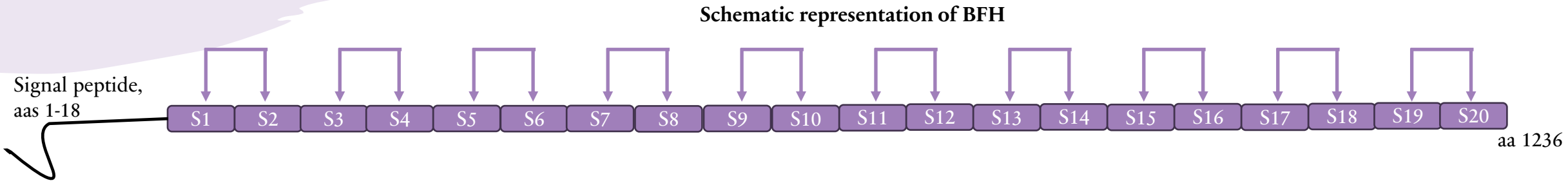


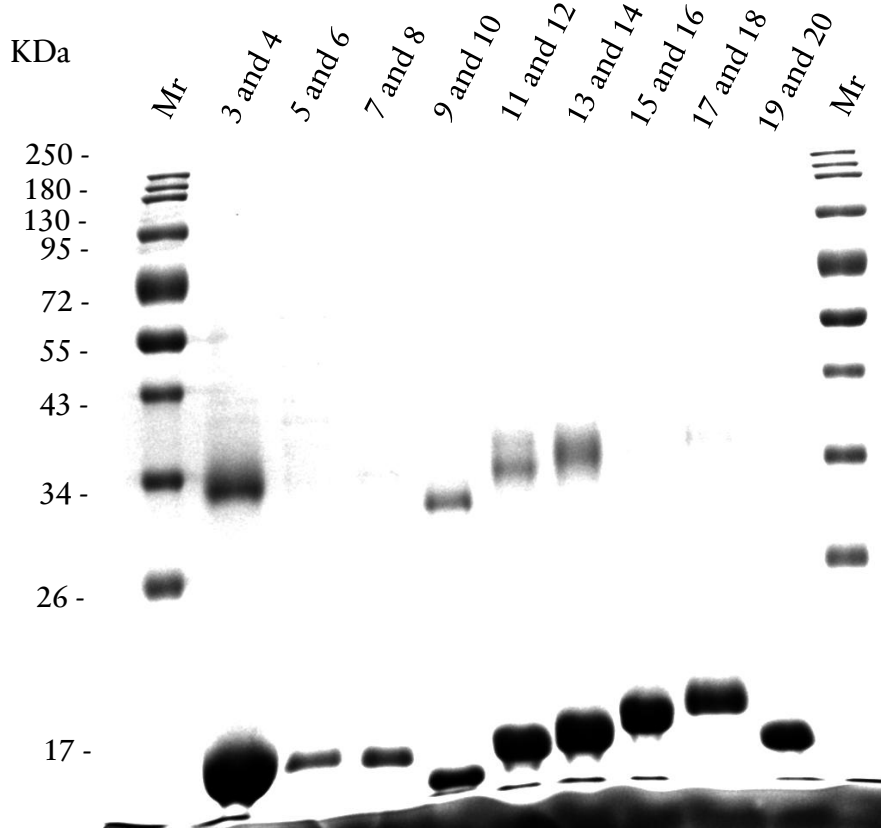


FlaA2 is present in PBS surface extract

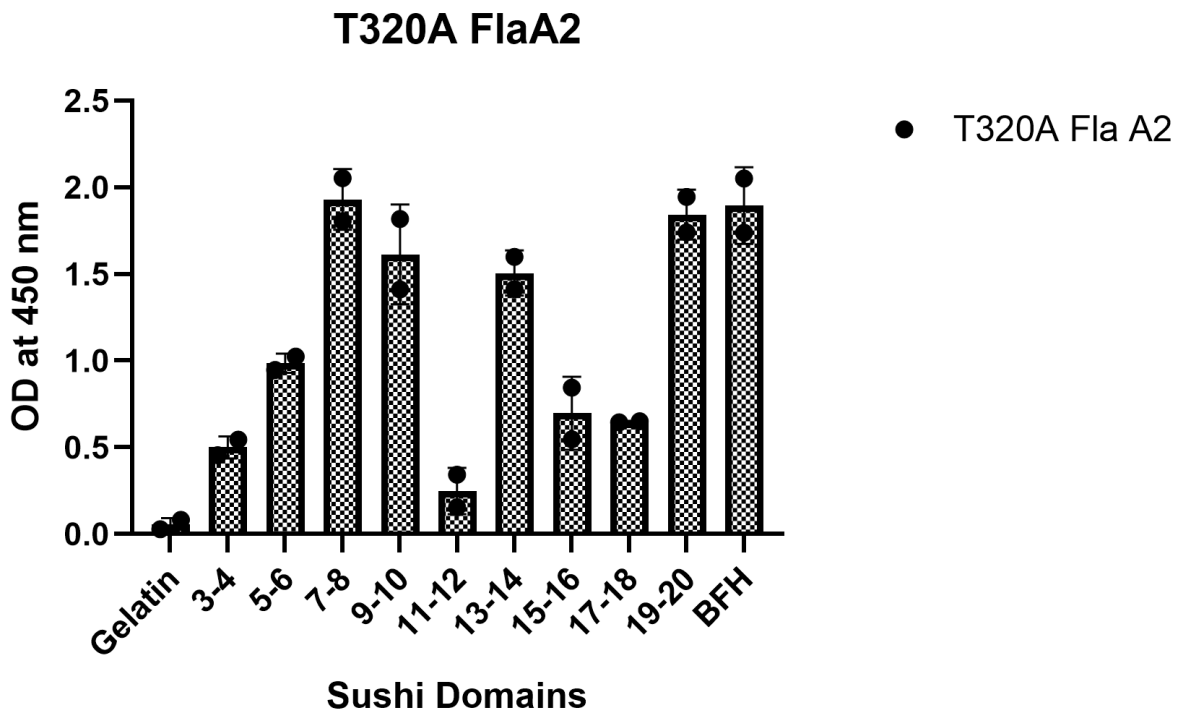


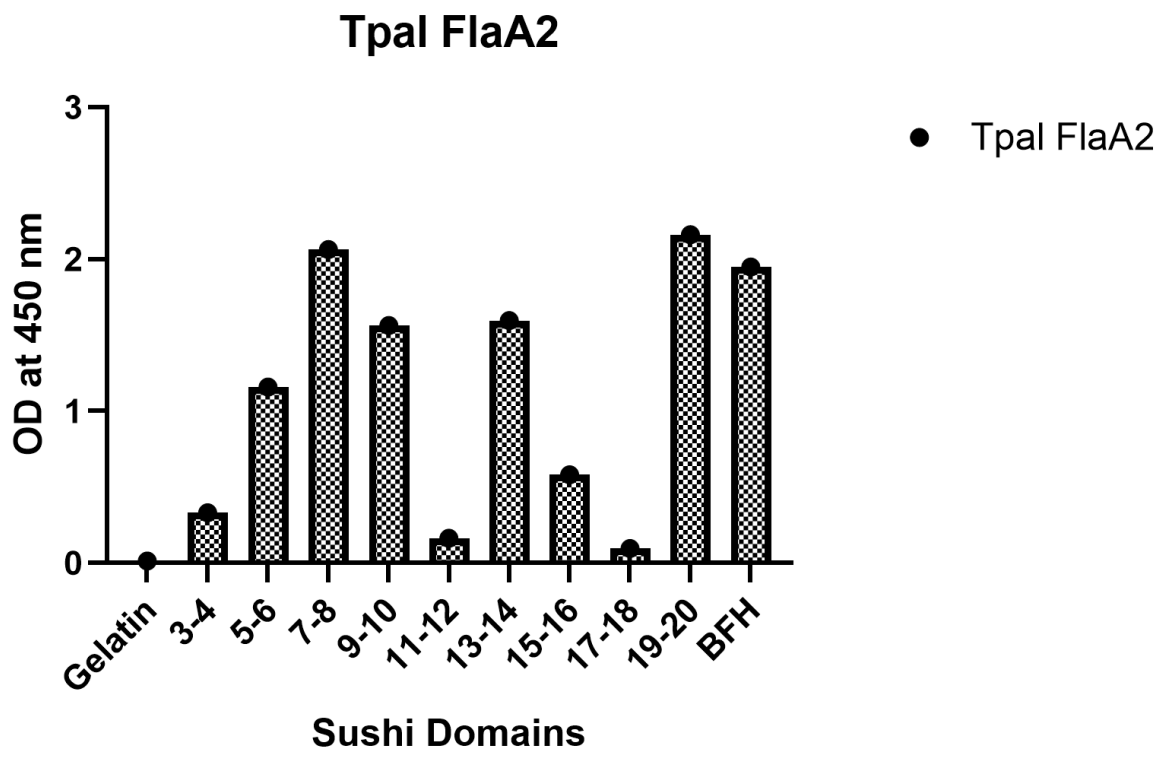
Purification of Bovine Factor H sushi domains





FlaA2 binds to multiple BFH sushi domains





Conclusions

- Plenty of Factor H and plasminogen binding proteins are present in DD Treponemes, suggesting functional redundancy

- FlaA2 is a BFH multi-sushi domain binding protein. It binds BFH with a very low Kd, indicating a very high affinity for BFH.

- FlaA2 is surface expressed in *Treponema phagedenis* (T320A)

- FlaA2 is therefore a key player in treponemal immunomodulation, and holds potential as a vaccine candidate for BDD

Future work

- Generation of functionally restricted mutants and thermostability assays for all the candidates

- Immunization and assessment of protection

Acknowledgements

Principal Investigator
Prof. Nick Evans

Prof. Emeritus Stuart Carter
Prof. Dan Ridgen
Dr. Emily Johnson
Dr. Hayley Crosby-Durrani

Lab Members
Postdoc – Dr. Abbie Hinds

PhDs
Rachel Ridgway
Lois Bent
Matty Lee
Kaixin Bao
Congriev Kumar
Hani Alnakhi

Leahurst Research Labs
Technical team

BBSRC
bioscience for the future

UNIVERSITY OF LIVERPOOL

Cheers