WMS UPDATE

GROUP MEETING

27/3/2018

MUHAMMAD HASAN & ALICE FAYTER

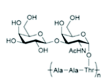
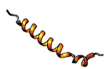
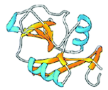
WARWICK

OVERVIEW

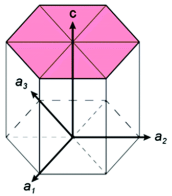
- Introduction
- AFPIII
- AFPI
- Cryopreservation of bacterial cells
- Future work

INTRODUCTION: AFPs

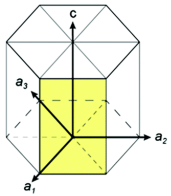
WARWICK

	AFGP	Type I AFP	Type II AFP	Type III AFP	Insect AFP
Mass (kg mol^{-1})	3-33	3-4.5	11-24	6.5	9
Representative structure					
PDB	-	1WFA	2PY2	1HG7	-
Binding plane					
Origin	Antarctic notothenioids, northern cods	Right-eyed flounders, sculpins	Herring, sea raven, smelt	Eel pout, ocean pout, wolfish,	Darkling beetle, spruce budworm moth, midge, fly

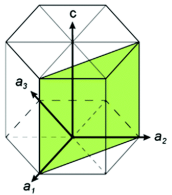
Crystal planes of hexagonal ice (Ih)



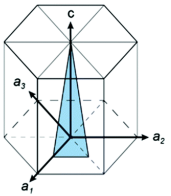
basal plane {0001}



primary prism plane $\{10\bar{1}0\}$



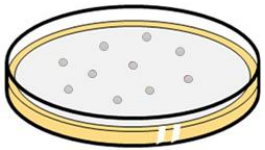
secondary prism plane $\{11\bar{2}0\}$



pyramidal plane $\{20\bar{2}1\}$

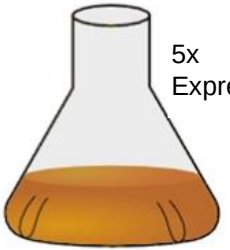
AFPIII: Expression and purification

Transformation



Inoculation

IPTG



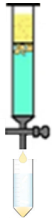
5x Expression

Pellet by centrifugation (4000 RPM)

Lysis

Centrifugation

Filter supernatant



Desalting

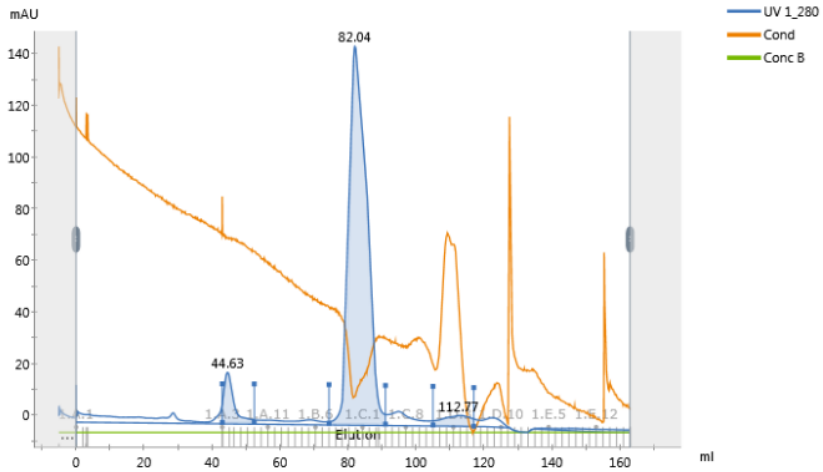
Concentrate sample

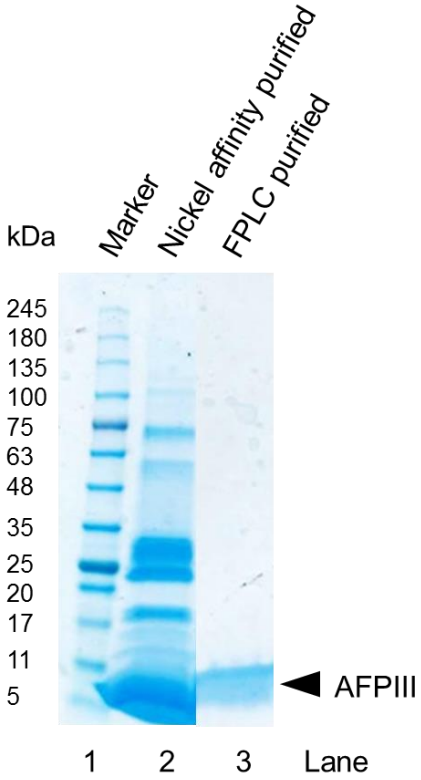
AFPIII

AFPIII: FPLC/Gels

Concentration recorded by:

- BCA Assay
- Nanodrop (A280 absorbance) - Tyr & Trp
- Difficulties with FPLC originally





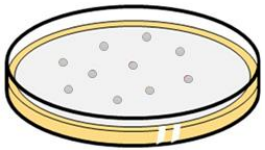
bpAFPI: Construct

TRX-tag – His-tag – Thrombin – S.tag – rEK – TEV – AFPI

- Goal: Separate TEV protease, tag and leftover impurities from the AFPI peptide.

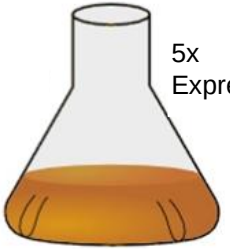
bpAFPI: Expression

Transformation



Inoculation

IPTG



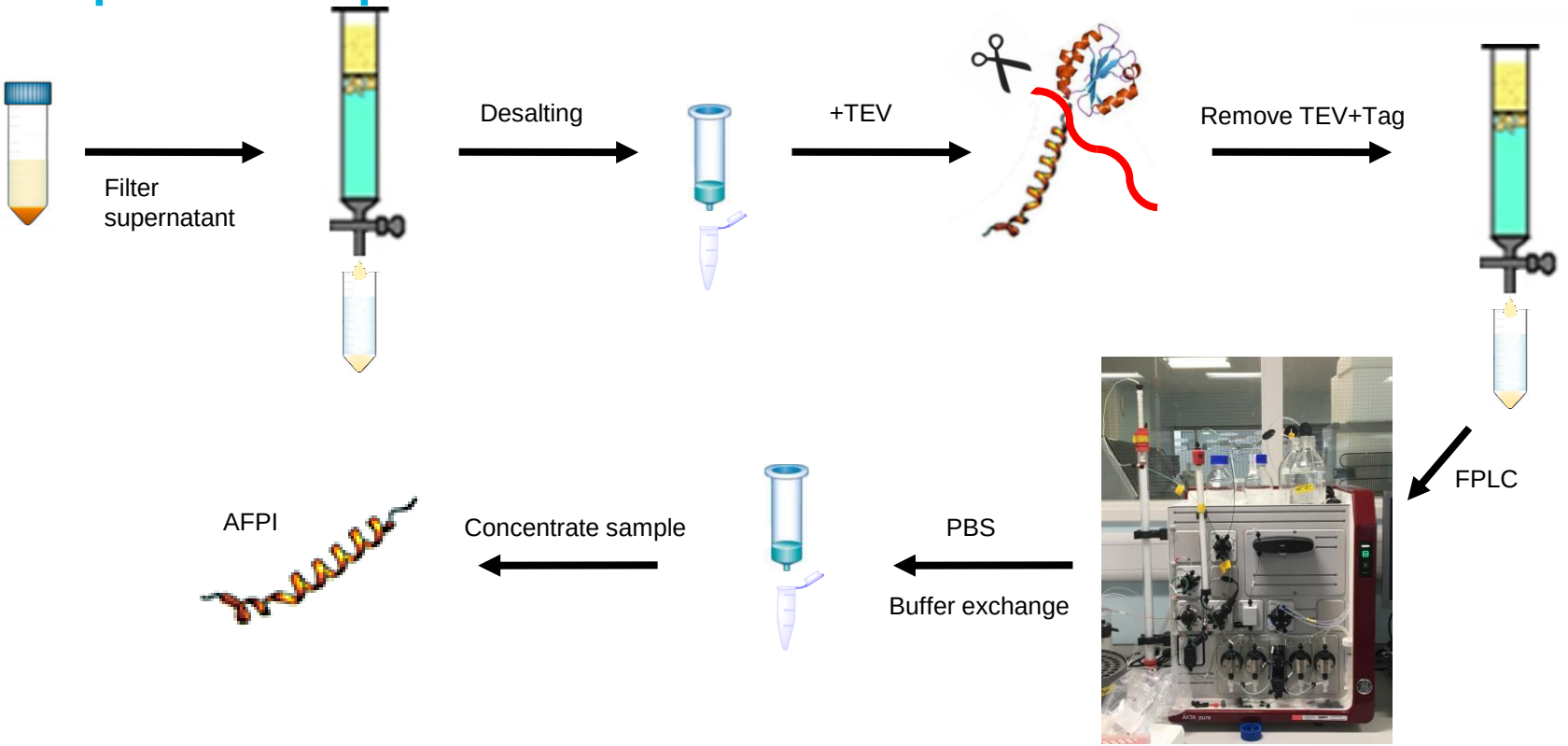
5x
Expression

Pellet by
centrifugation
(4000 RPM)

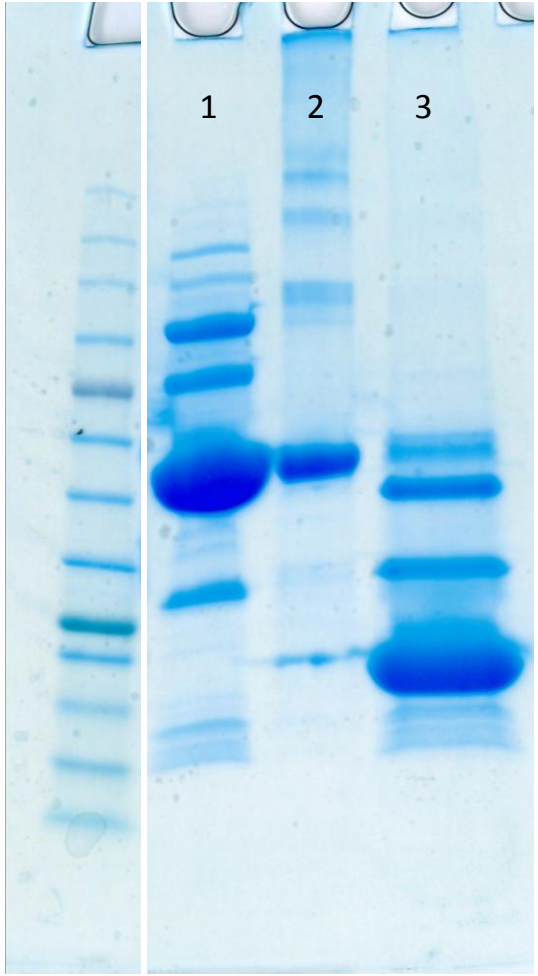
Lysis

Pellet

bpAFPI: purification



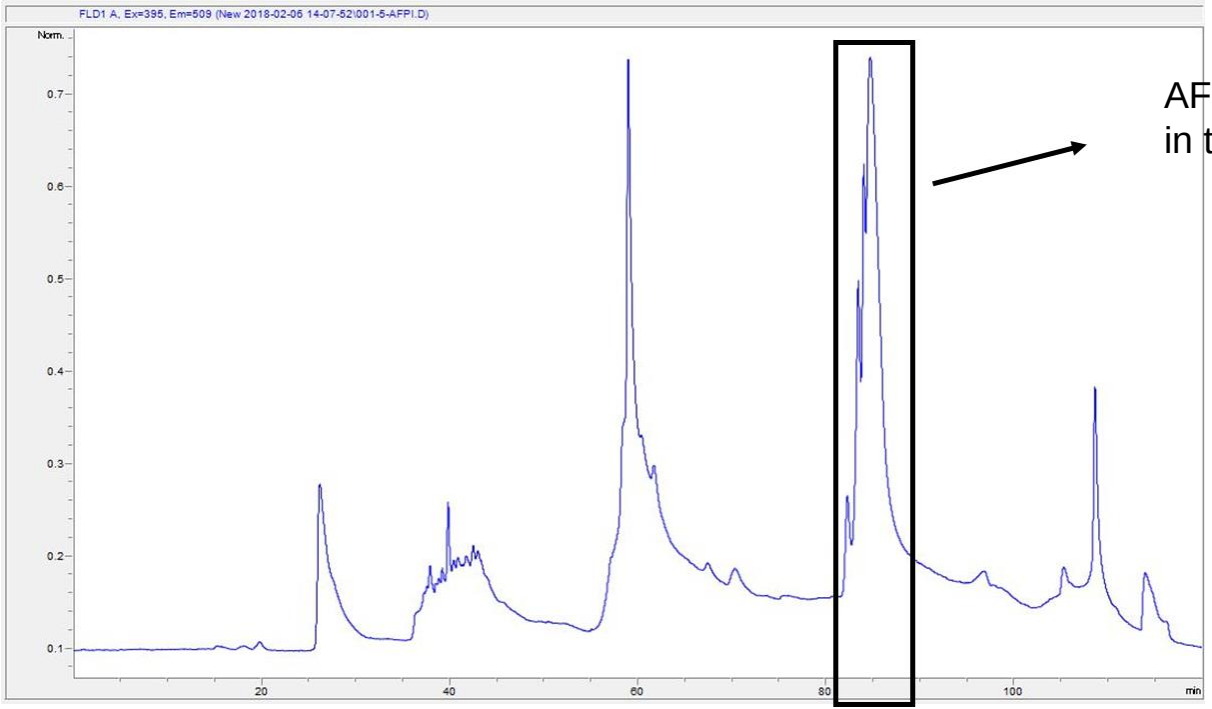
bpAFPI :Failed



1 – AFPI after IMAC
2 – TEV after IMAC
3 – overnight incubation of AFPI-TEV

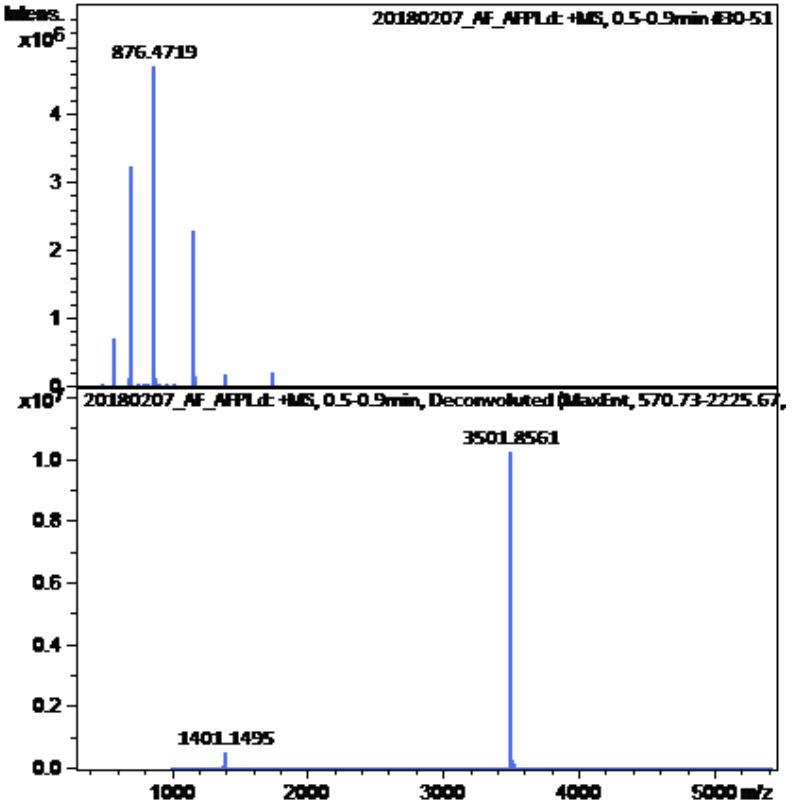
TRX-tag – His-tag – Thrombin – S.tag – rEK – TEV – AFPI

bpAFPI: HPLC



AFPI is contained in this peak

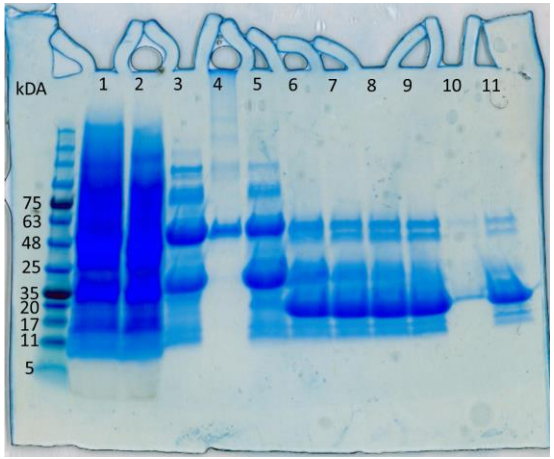
bpAFPI: Mass Spec QToF



bpAFPI: Success

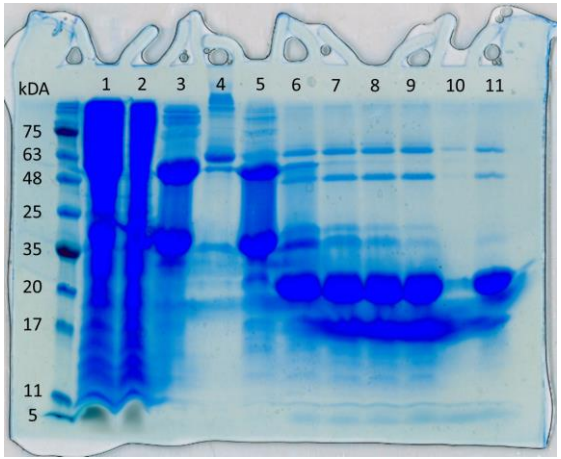
- The 40 AA peptide was expressed in fusion with a thioredoxin tag in pET32a vector (ref Davies paper).

Tris-glycine gel



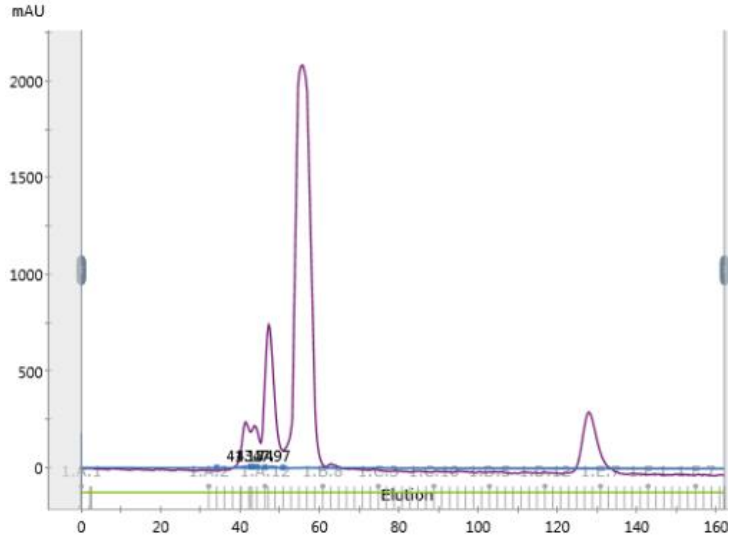
1 – Before IMAC
2 – Flow through
3 – Elution after PD10
4 – TEV
5 – 0 hrs after addition of TEV
6 – 1 hr
7 – 2 hrs
8 – 4 hrs
9 – Overnight
10 – FT from AFPI concentration
11 – left on the coluamn after second IMAC

Tris-tricine gel

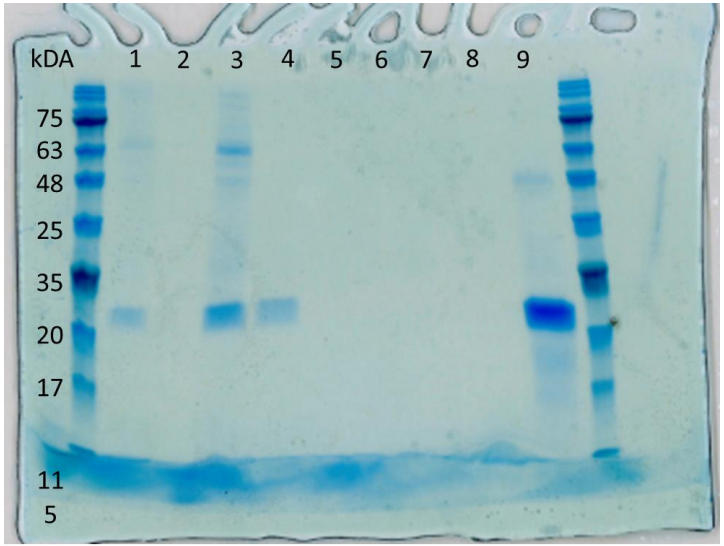


bpAFPI: FPLC & Gel

Superdex 75 column = didn't work
Superdex 30 column = worked



1 – AFPI after IMAC
2 – FT from concentrator
3 – Concentrated AFPI
4 – B1
5 – B4
6 – B5
7 – B6
8 – E5
9 – old peptide sample



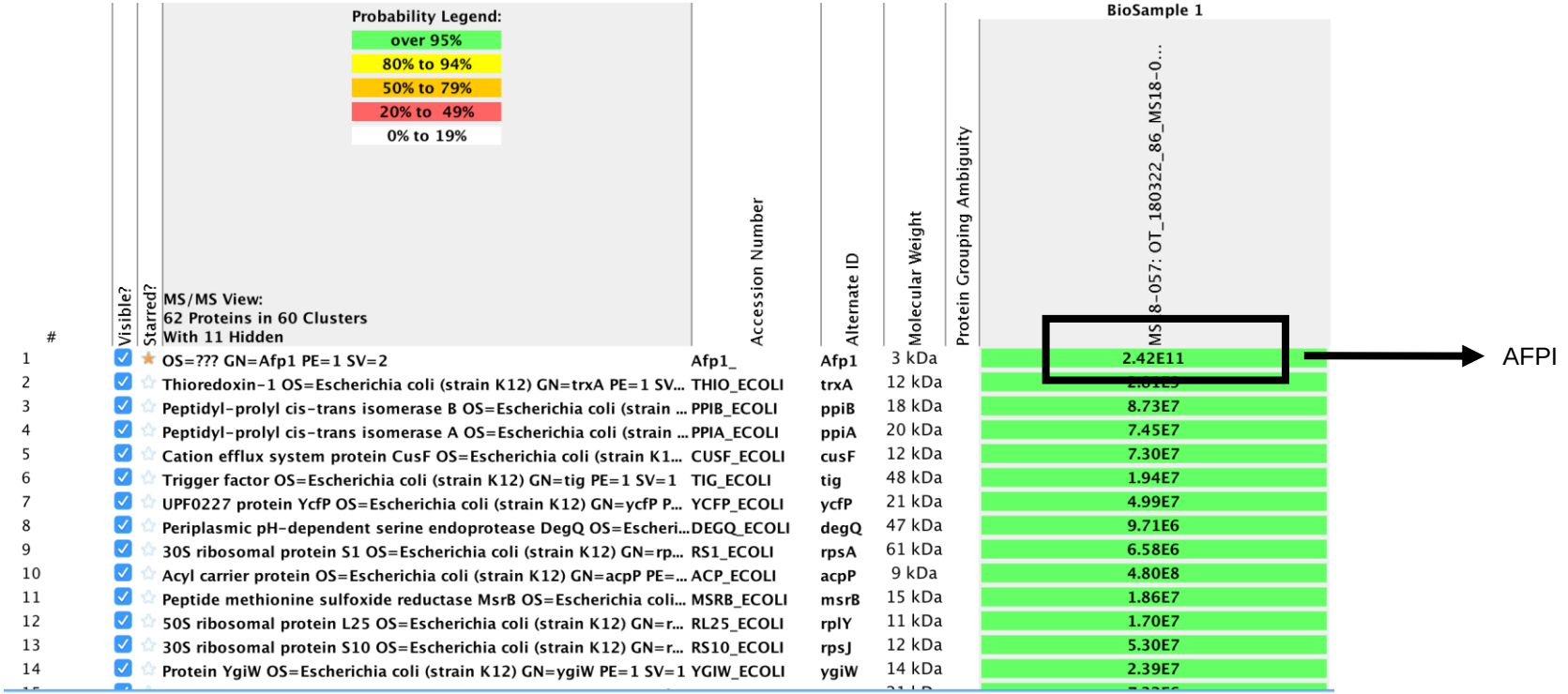
bpAFPI: Proteomics Results

WARWICK

Probability Legend:
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

#	Visible?	Starred?	MS/MS View: 62 Proteins in 60 Clusters With 11 Hidden	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	BioSample 1 MS18-057: OT_180322_86_MS18-0...
1	☑	★	OS=??? GN=Afp1 PE=1 SV=2	Afp1_	Afp1	3 kDa		2.42E11
2	☑	☆	Thioredoxin-1 OS=Escherichia coli (strain K12) GN=trxA PE=1 SV...	THIO_ECOLI	trxA	12 kDa		2.81E9
3	☑	☆	Peptidyl-prolyl cis-trans isomerase B OS=Escherichia coli (strain ...	PPIB_ECOLI	ppiB	18 kDa		8.73E7
4	☑	☆	Peptidyl-prolyl cis-trans isomerase A OS=Escherichia coli (strain ...	PPIA_ECOLI	ppiA	20 kDa		7.45E7
5	☑	☆	Cation efflux system protein CusF OS=Escherichia coli (strain K1...	CUSF_ECOLI	cusF	12 kDa		7.30E7
6	☑	☆	Trigger factor OS=Escherichia coli (strain K12) GN=tig PE=1 SV=1	TIG_ECOLI	tig	48 kDa		1.94E7
7	☑	☆	UPF0227 protein YcfP OS=Escherichia coli (strain K12) GN=ycfP P...	YCFP_ECOLI	ycfP	21 kDa		4.99E7
8	☑	☆	Periplasmic pH-dependent serine endoprotease DegQ OS=Escheri...	DEGQ_ECOLI	degQ	47 kDa		9.71E6
9	☑	☆	30S ribosomal protein S1 OS=Escherichia coli (strain K12) GN=rp...	RS1_ECOLI	rpsA	61 kDa		6.58E6
10	☑	☆	Acyl carrier protein OS=Escherichia coli (strain K12) GN=acpP PE=...	ACP_ECOLI	acpP	9 kDa		4.80E8
11	☑	☆	Peptide methionine sulfoxide reductase MsrB OS=Escherichia coli...	MSRB_ECOLI	msrB	15 kDa		1.86E7
12	☑	☆	50S ribosomal protein L25 OS=Escherichia coli (strain K12) GN=r...	RL25_ECOLI	rplY	11 kDa		1.70E7
13	☑	☆	30S ribosomal protein S10 OS=Escherichia coli (strain K12) GN=r...	RS10_ECOLI	rpsJ	12 kDa		5.30E7
14	☑	☆	Protein YgiW OS=Escherichia coli (strain K12) GN=ygiW PE=1 SV=1	YGIW_ECOLI	ygiW	14 kDa		2.39E7

bpAFPI: TADA!



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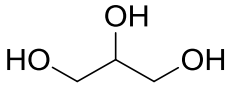
1	☑	★	OS=??? GN=Afp1 PE=1 SV=2	Afp1_	Afp1	3 kDa		2.42E11
2	☑	☆	Thioredoxin-1 OS=Escherichia coli (strain K12) GN=trxA PE=1 SV...	THIO_ECOLI	trxA	12 kDa		
3	☑	☆	Peptidyl-prolyl cis-trans isomerase B OS=Escherichia coli (strain ...	PPIB_ECOLI	ppiB	18 kDa		8.73E7
4	☑	☆	Peptidyl-prolyl cis-trans isomerase A OS=Escherichia coli (strain ...	PPIA_ECOLI	ppiA	20 kDa		7.45E7
5	☑	☆	Cation efflux system protein CusF OS=Escherichia coli (strain K1...	CUSF_ECOLI	cusF	12 kDa		7.30E7
6	☑	☆	Trigger factor OS=Escherichia coli (strain K12) GN=tig PE=1 SV=1	TIG_ECOLI	tig	48 kDa		1.94E7
7	☑	☆	UPF0227 protein YcfP OS=Escherichia coli (strain K12) GN=ycfP P...	YCFP_ECOLI	ycfP	21 kDa		4.99E7
8	☑	☆	Periplasmic pH-dependent serine endoprotease DegQ OS=Escheri...	DEGQ_ECOLI	degQ	47 kDa		9.71E6
9	☑	☆	30S ribosomal protein S1 OS=Escherichia coli (strain K12) GN=rp...	RS1_ECOLI	rpsA	61 kDa		6.58E6
10	☑	☆	Acyl carrier protein OS=Escherichia coli (strain K12) GN=acpP PE=...	ACP_ECOLI	acpP	9 kDa		4.80E8
11	☑	☆	Peptide methionine sulfoxide reductase MsrB OS=Escherichia coli...	MSRB_ECOLI	msrB	15 kDa		1.86E7
12	☑	☆	50S ribosomal protein L25 OS=Escherichia coli (strain K12) GN=r...	RL25_ECOLI	rplY	11 kDa		1.70E7
13	☑	☆	30S ribosomal protein S10 OS=Escherichia coli (strain K12) GN=r...	RS10_ECOLI	rpsJ	12 kDa		5.30E7
14	☑	☆	Protein YgiW OS=Escherichia coli (strain K12) GN=ygiW PE=1 SV=1	YGIW_ECOLI	ygiW	14 kDa		2.39E7

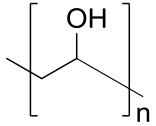
Cryopreservation of bacterial cells

- Storage of lab strains
- Structure-activity relationship
- Industrial application in light of microbiome research

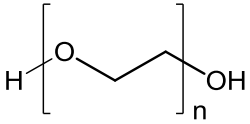
THE CANDIDATES



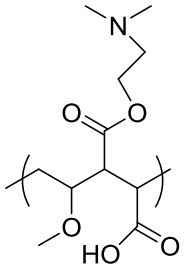
Glycerol



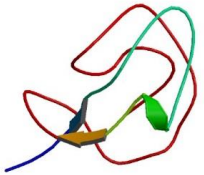
PVA



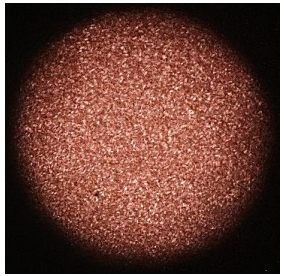
PEG



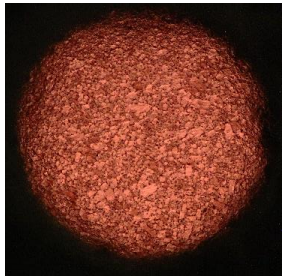
Poly(ampholyte)



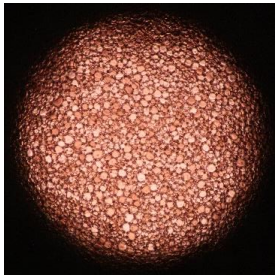
AFPIII



PEG/PVA

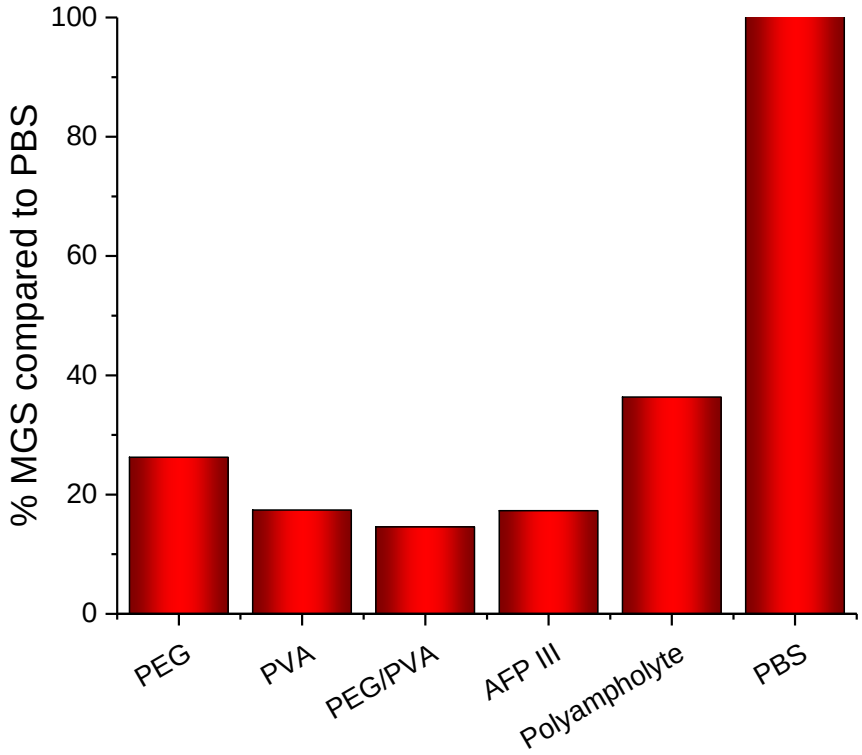


PVA

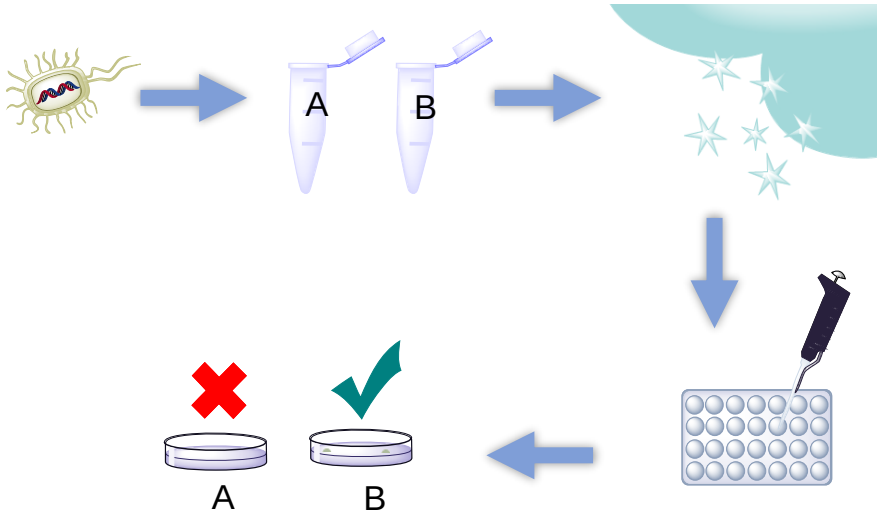


PEG

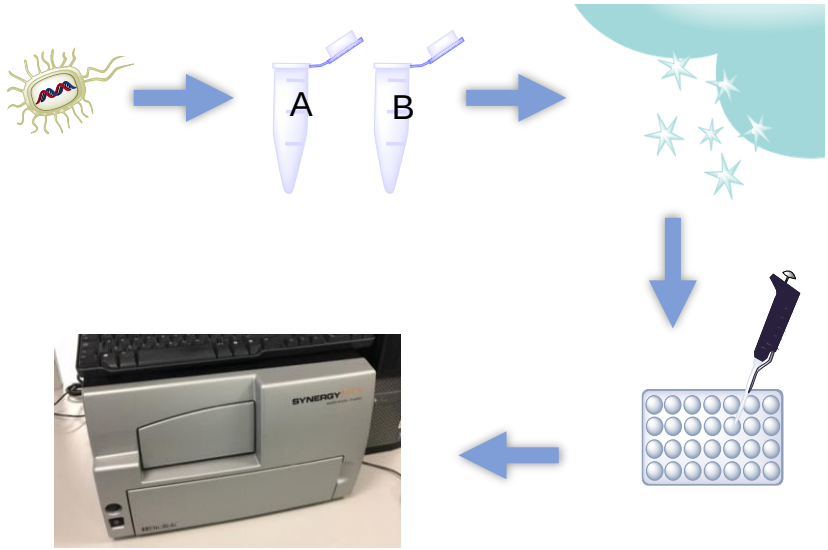
Ice-recrystallization inhibition: results



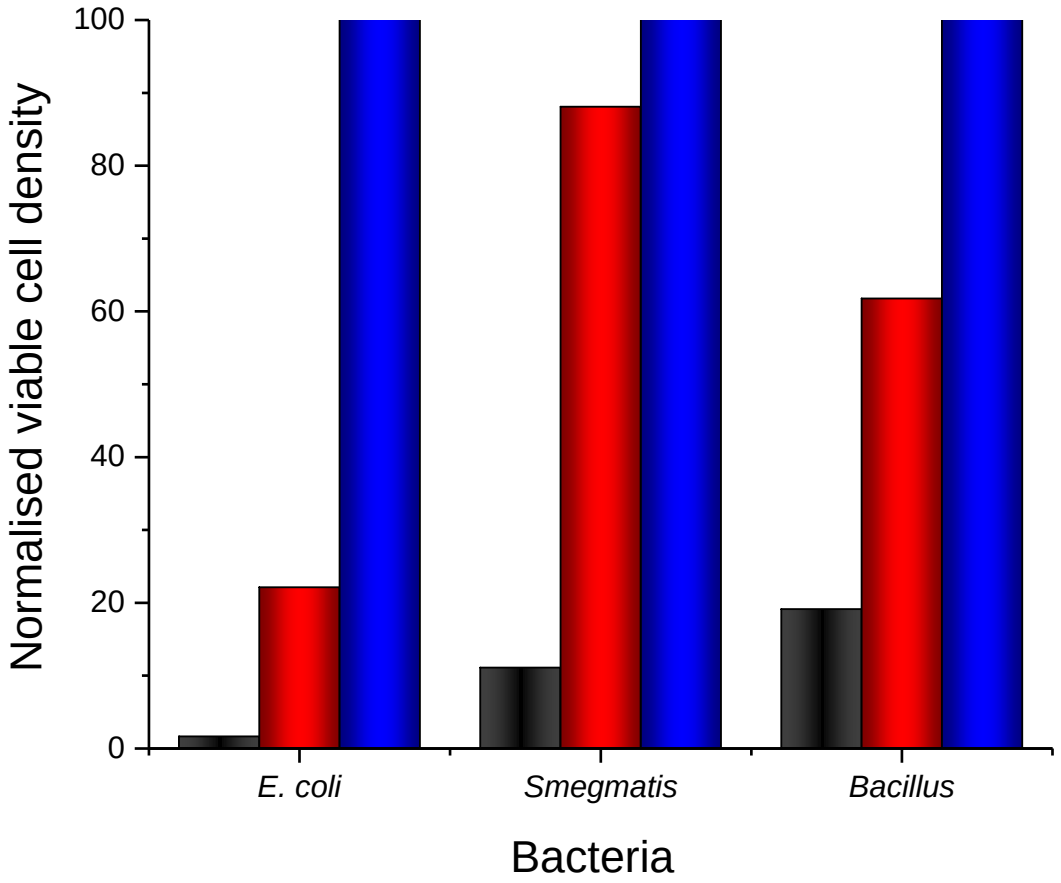
Methods: Freeze-tolerance assays



Counting colonies



Monitoring growth profiles at OD600

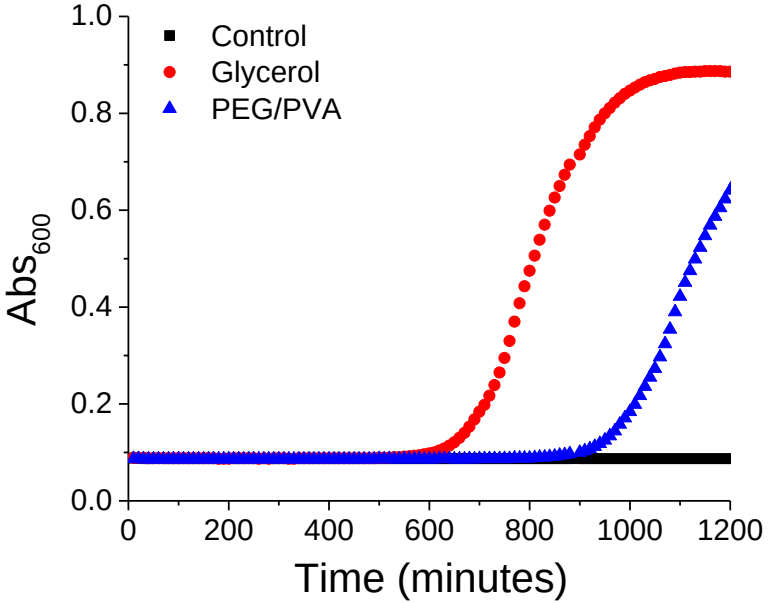


Control

Glycerol

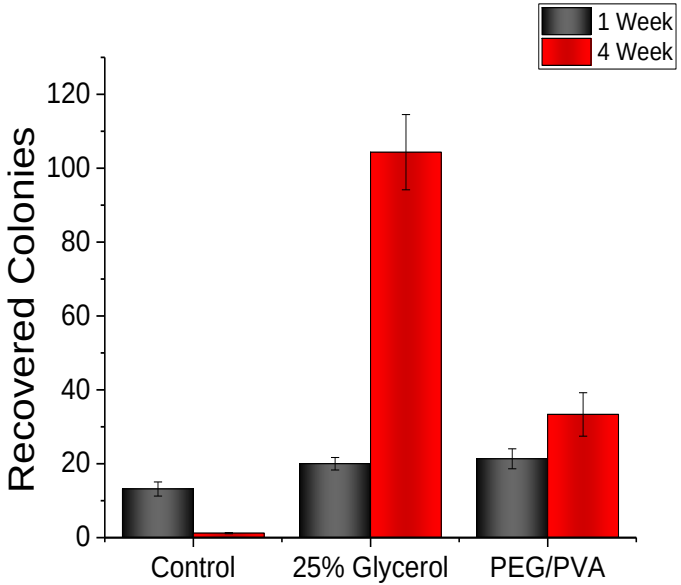
PEG/PVA

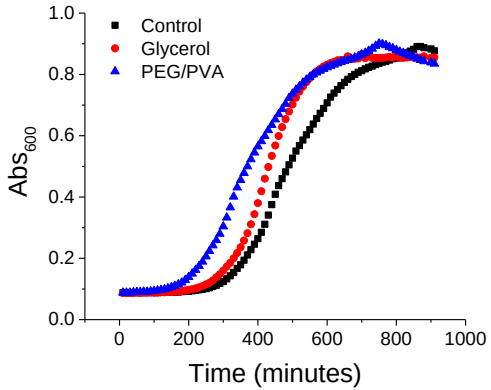
E. coli growth profile post 7xFT with storage at -20°C



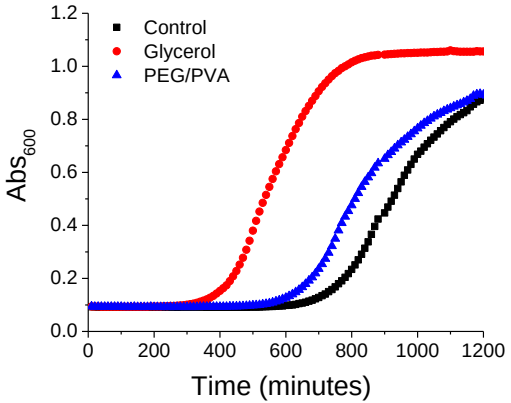
ALL

Results after storage at -20 °C



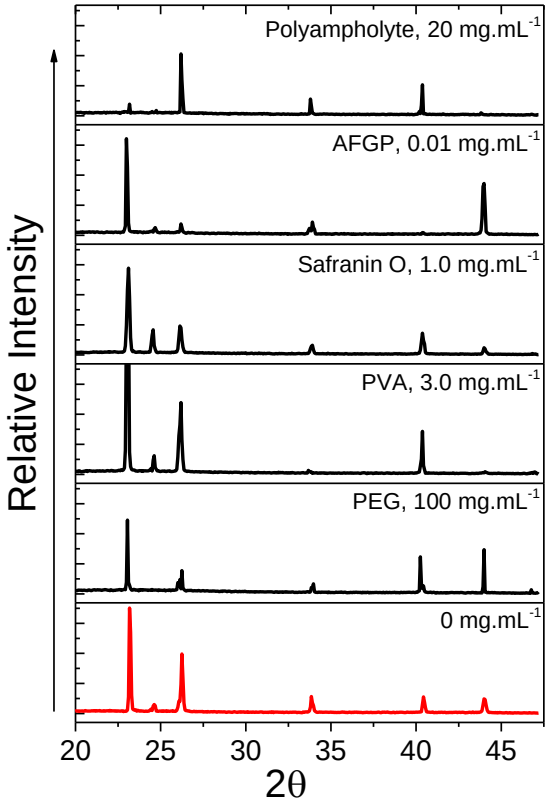


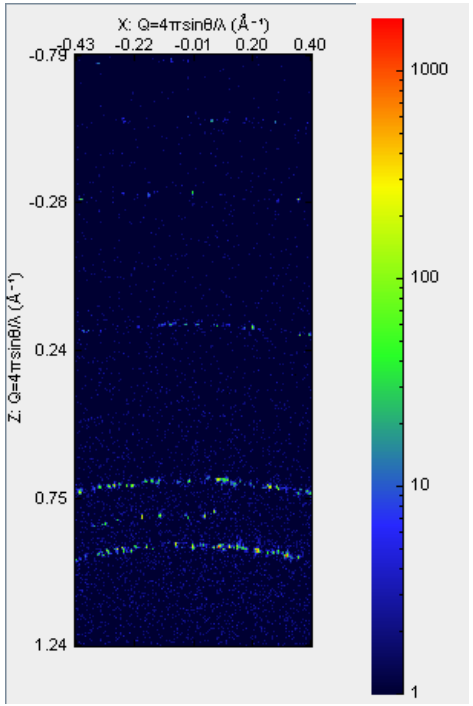
E. Coli after 1 freeze thaw cycle (Frozen in N_2 & left at -20 °C for 1 week)



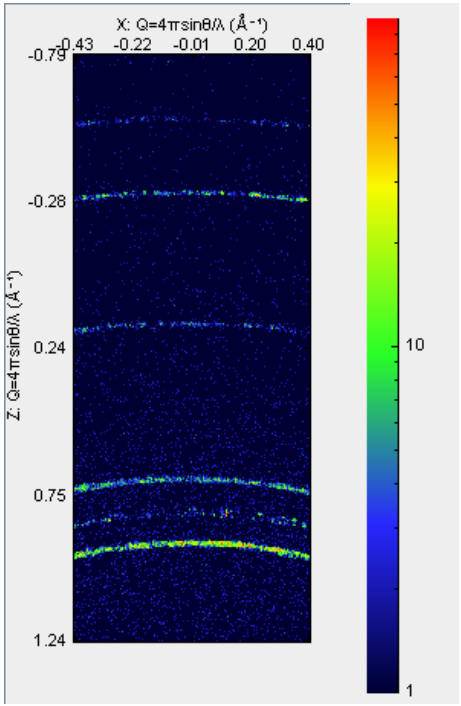
E. Coli after 1 freeze thaw cycle (Frozen in N_2 & left at -20 °C for 4 weeks)

WAXS: Results & Hypothesis





Scattering pattern for Safranin O (0.1 mg.mL^{-1})



Scattering pattern for Safranin O (1 mg.mL^{-1})

Future Work

- LEA
  - Intrinsically disordered
  - Known to potentially provide cryoprotection
  - Distinct to IRI
  - Stabilisation?
- INP
  - Good to have one
- CRP
  - Cryoprotective protein
  - 29 kDa
  - Have seen it is 1000x more active than BSA
  - Just arrived!
  - Plan to: get genome & identify sequence/structure of protein
  - SFR

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