Freshwater Mussel Adhesive Protein Localization and Characterization

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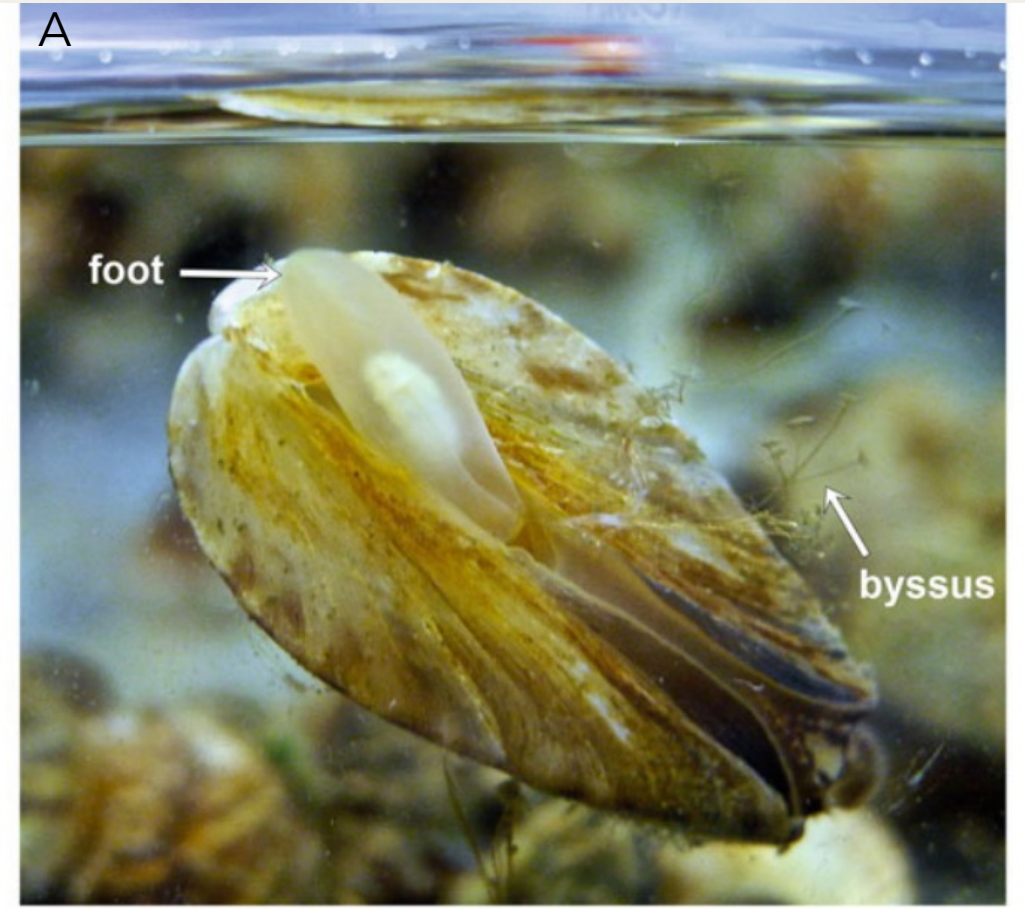
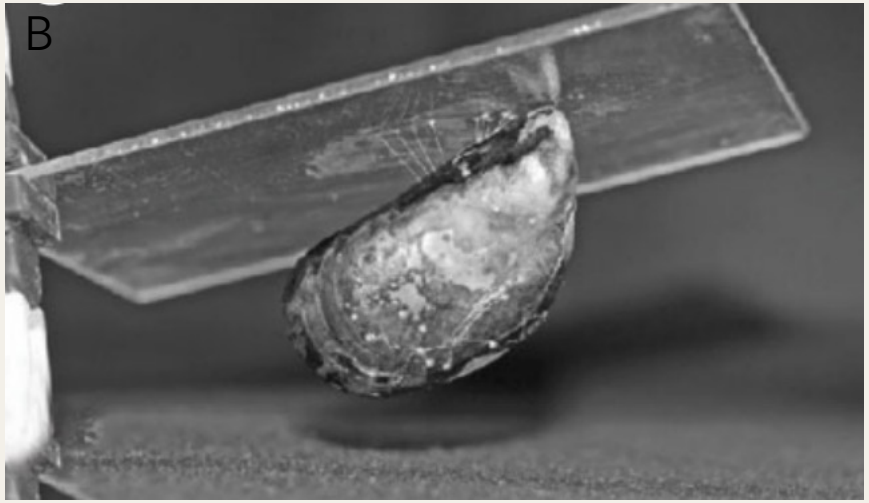
Background





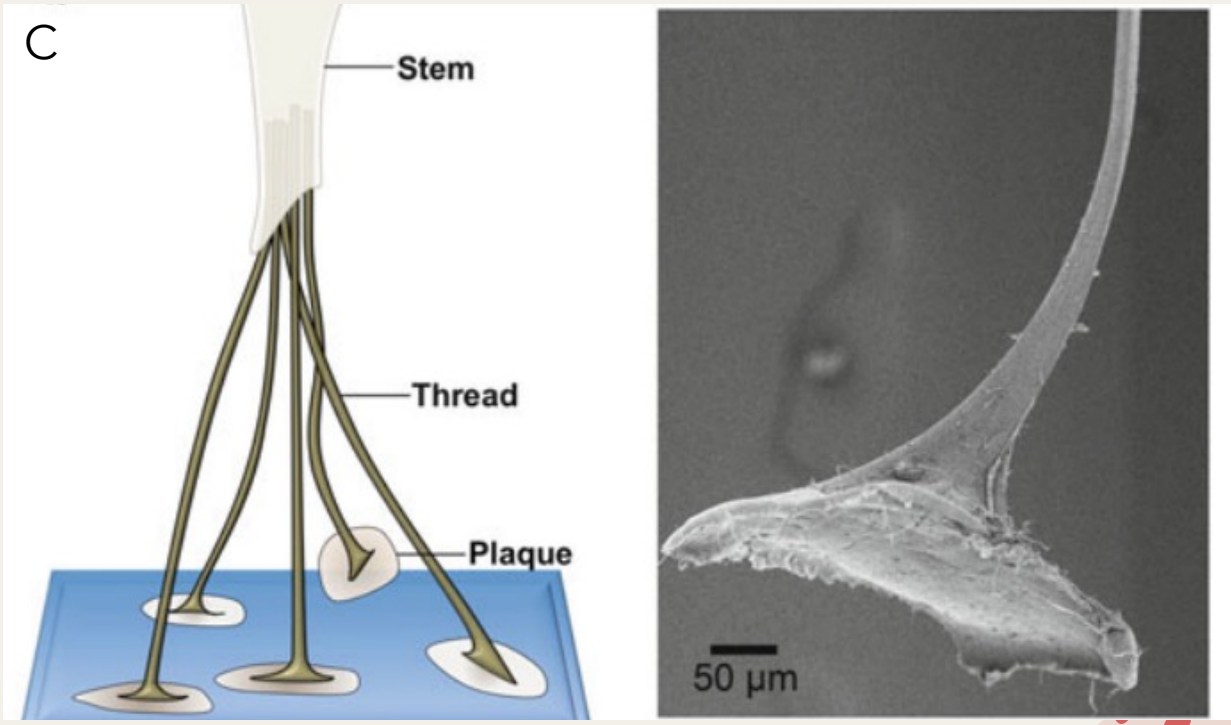


Figure 1. (A)(B)A freshwater quagga mussel is shown attaching to the side of a glass aquarium. The byssus is visible, as is the foot of the mussel, which produces the byssus. (C) The byssus, consisting of several threads and plaques attached to a substratum, is shown schematically (left) and as a SEM micrograph of a detached plaque and thread [1]

Objectives

Zebra mussel protein discovery [2]

Quagga mussel protein localization [3]

Zebra mussel adhesive proteins present extensive threonine/serine glycosylation [4]

Phosphorylation of serine residues has been detected in some marine mussel adhesive proteins [5]

Zebra Mussel Protein Localization

Analysis of Post-Translational (PTM) Modification of Quagga Mussel Proteins

Significance of the Study

This work provides a basis for the development of anti-fouling surfaces, which can help control biofouling of invasive species in freshwater habitats. In addition, this work can be further utilized to develop novel bioadhesive materials in medical and dental applications.

Zebra mussel protein Localization - Method

Determine the extraction buffer that provides the highest efficiency

Protein extraction with separating tubes collecting proteins from thread, plaque and footprint

SDS-PAGE following the extraction of proteins for protein identification

Collected protein samples will also be sent for nanodrop analysis to examine protein concentration

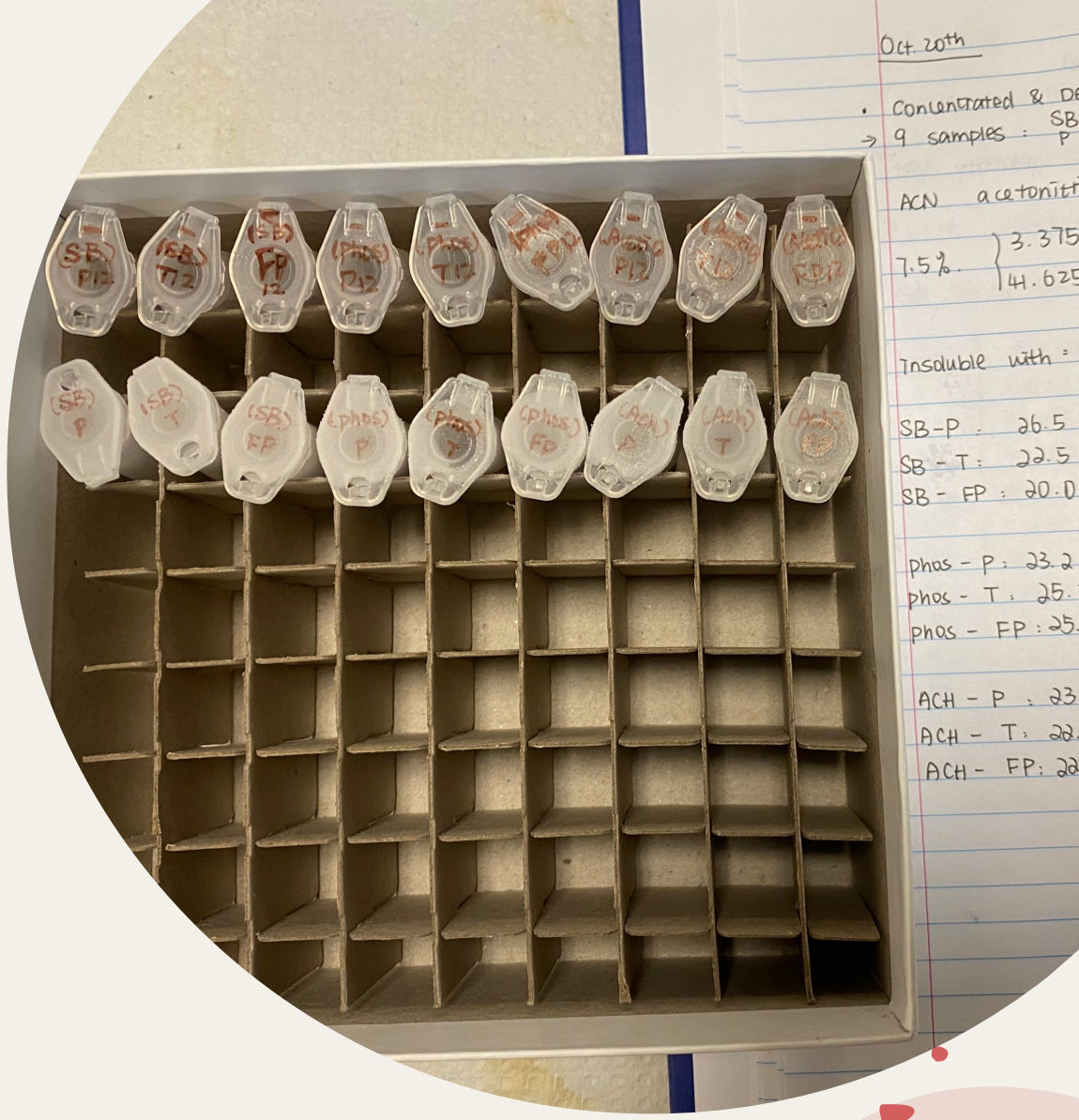
Zebra mussel protein Localization - Progress

- Three rounds of buffer experiments with

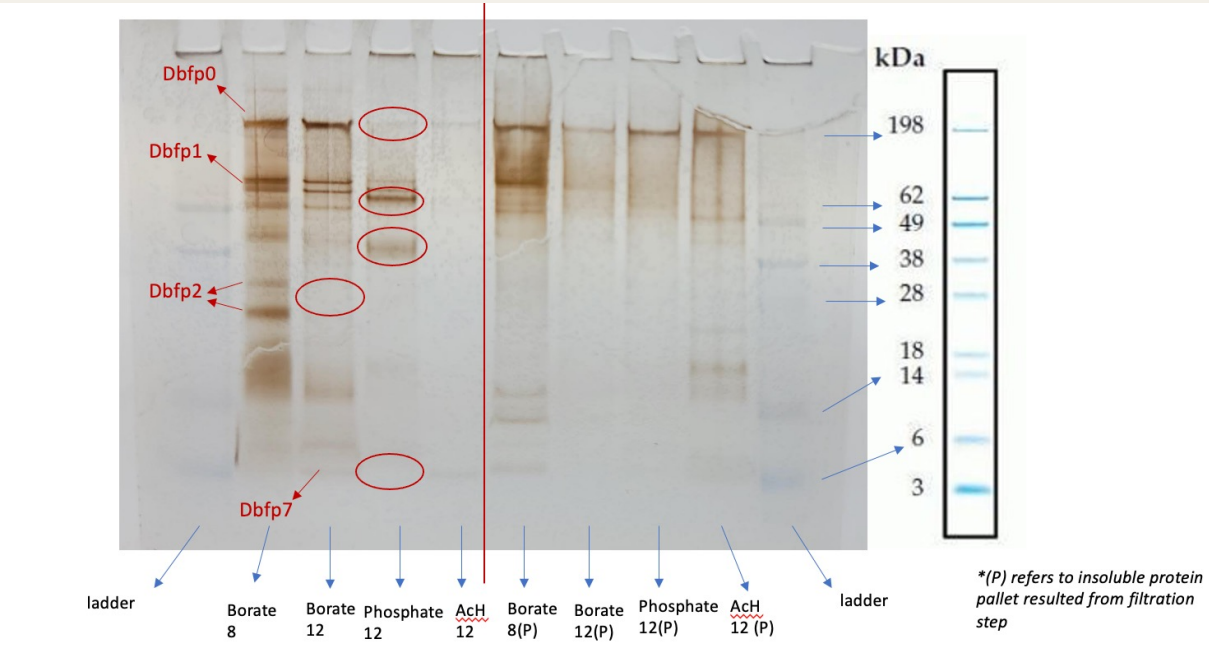
  Borate buffer (per Sam's protocol)

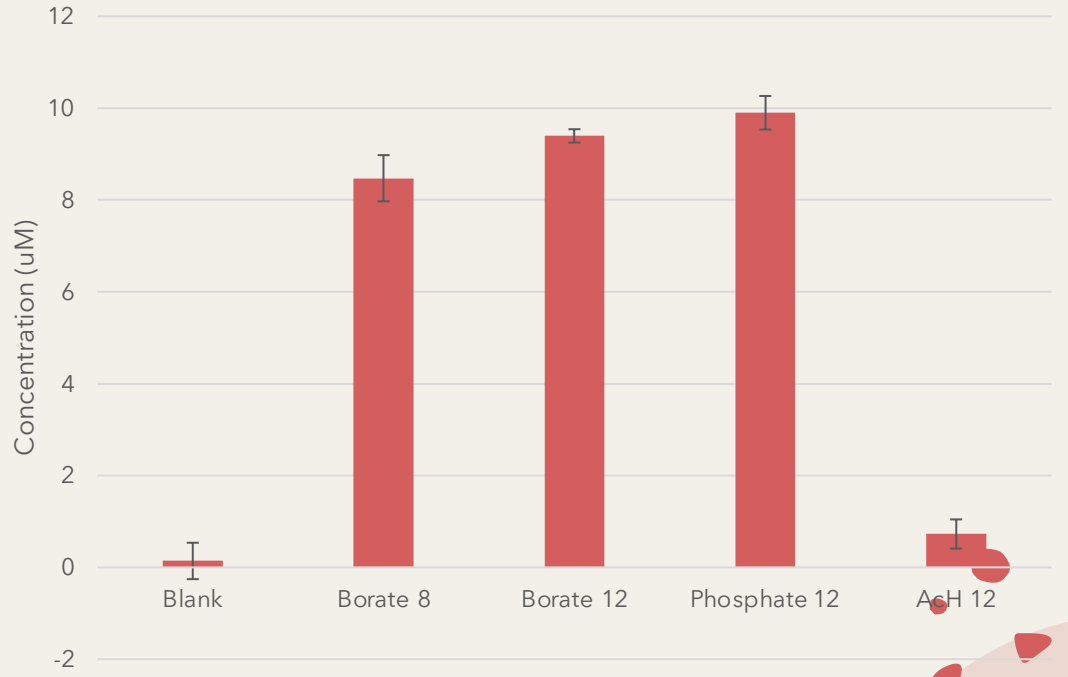
  Phosphate buffer (per Matt/Mimi protocol)

  5% acetic acid/8M urea (per Rzepecki 1993b [4])

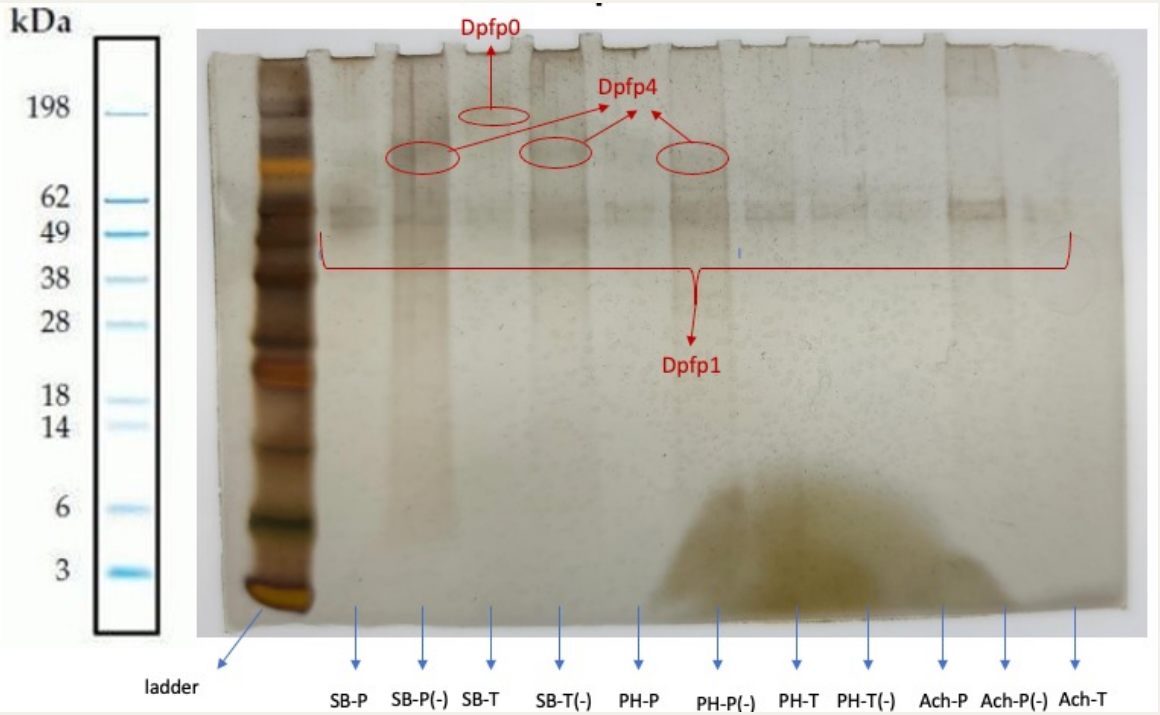


Results – Extraction Buffer Selection – Trial 1





Results – Extraction Buffer Selection – Trial 2



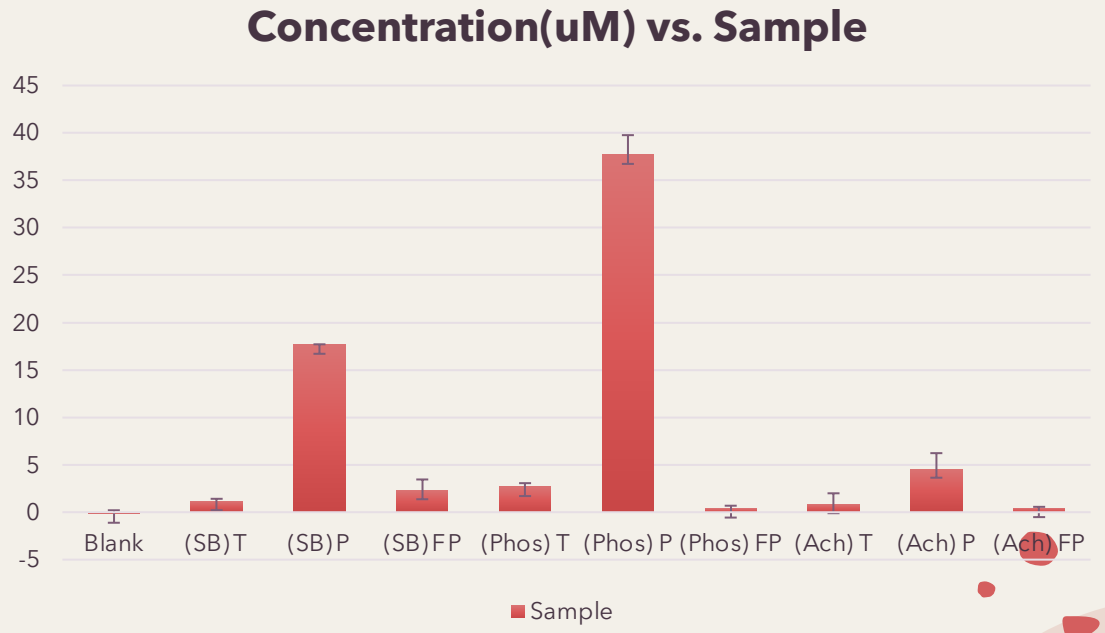
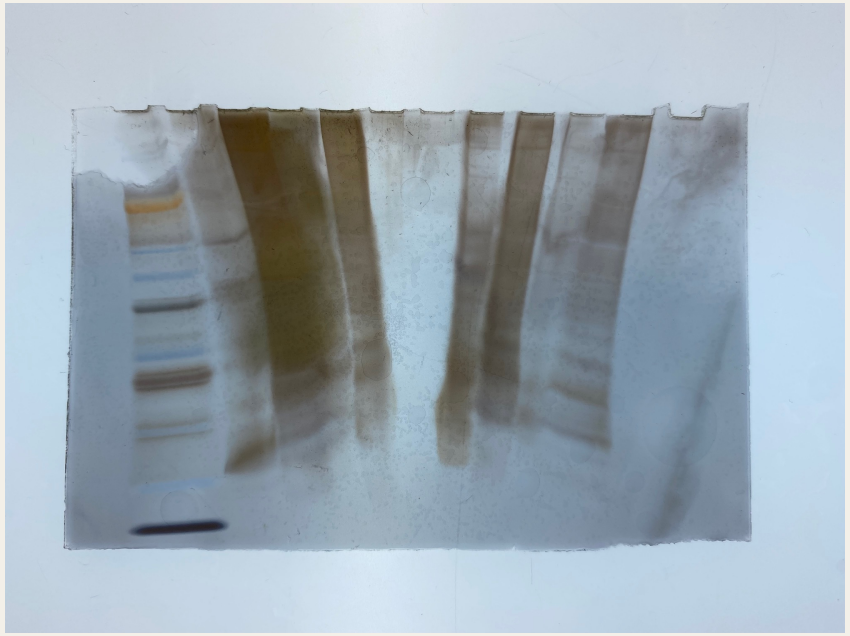
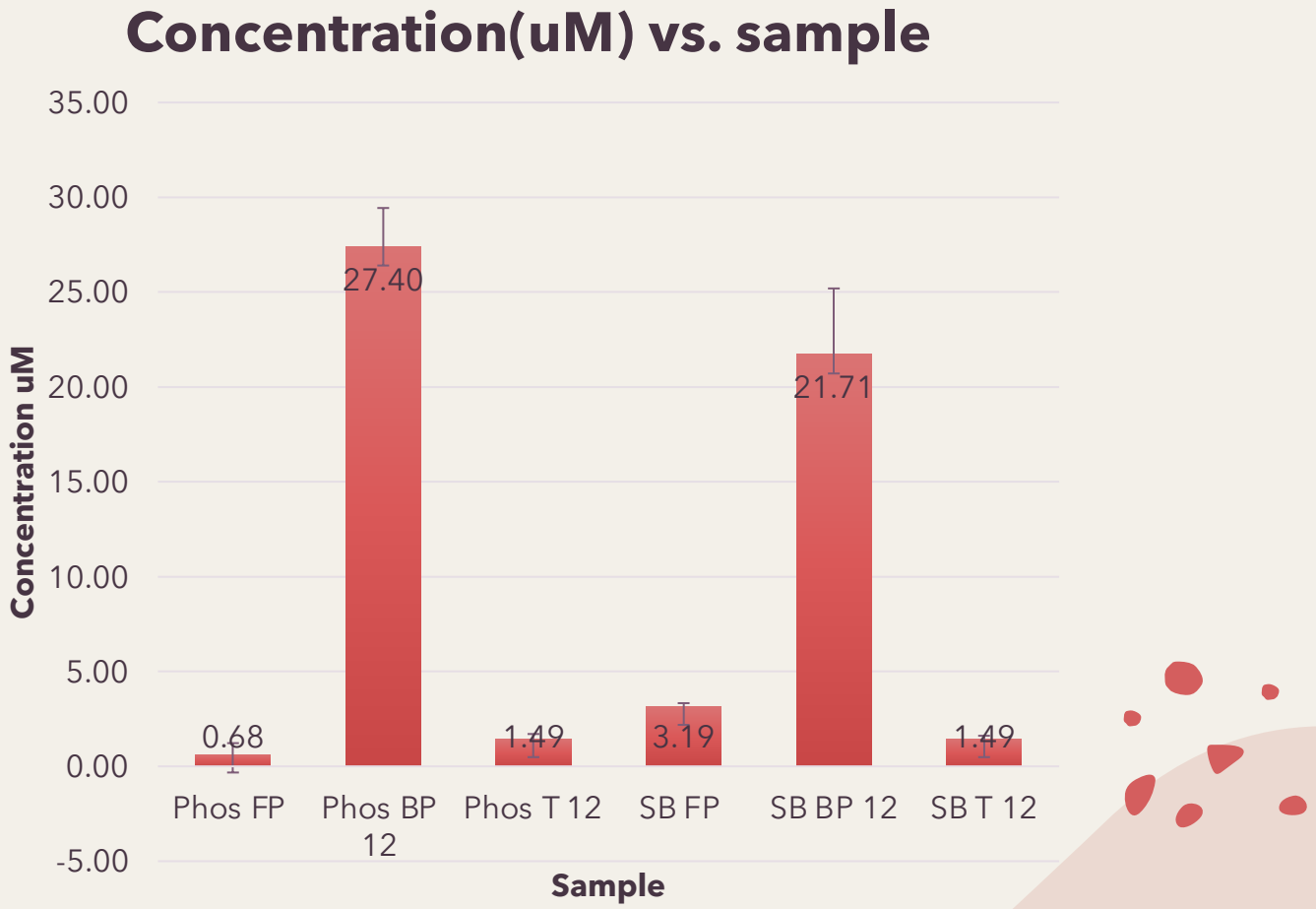


Figure 2. Results of zebra mussel proteins from week of Oct.19

Results – Extraction Buffer Selection – Trial 3 & 4





Zebra mussel protein localization – Discussion & Future plans

5% acetic acid/8M urea is not feasible according to the little amount of protein shown from nanodrop result. Phosphate buffer and borate buffer has similar results while phosphate buffer showed slightly higher efficiency

Pretreatment a few hours with 1% acetic/N-phenylthiourea before extraction on phosphate buffer and borate buffer

Generating the volcano graph for zebra mussel proteins based on the sequencing results

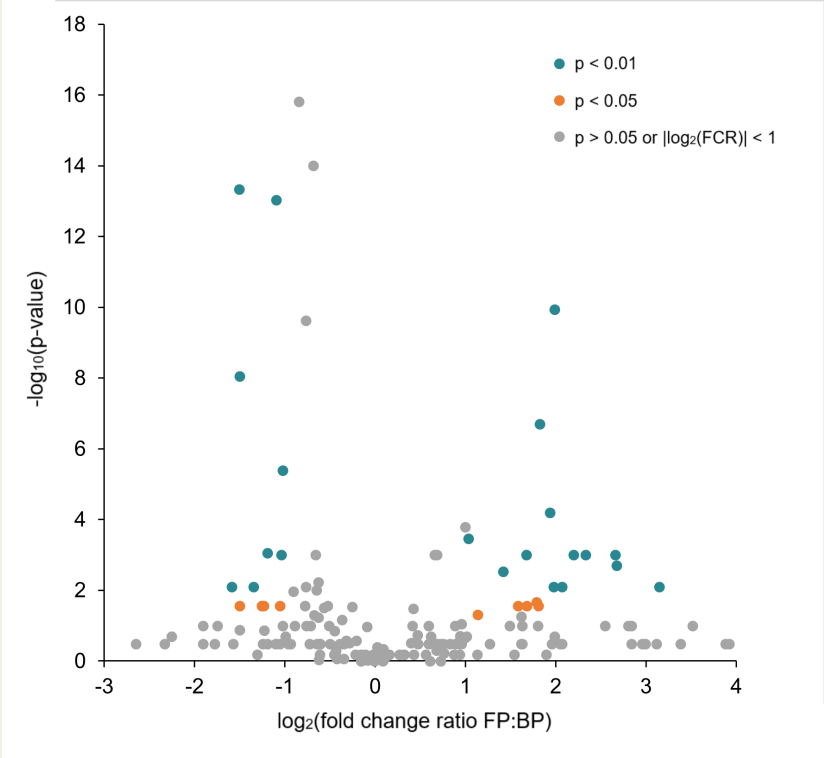


Figure 3. Volcano Graph of Quagga Mussel Proteins showing localizations. The log FCR <0 are bulk plaque proteins and the log FCR > 0 are footprint proteins [3].

Post-Translational Modification of Quagga Mussel Adhesive Protein - Method

- Stains-all – for both glycan & phosphate

Phosphorylation:

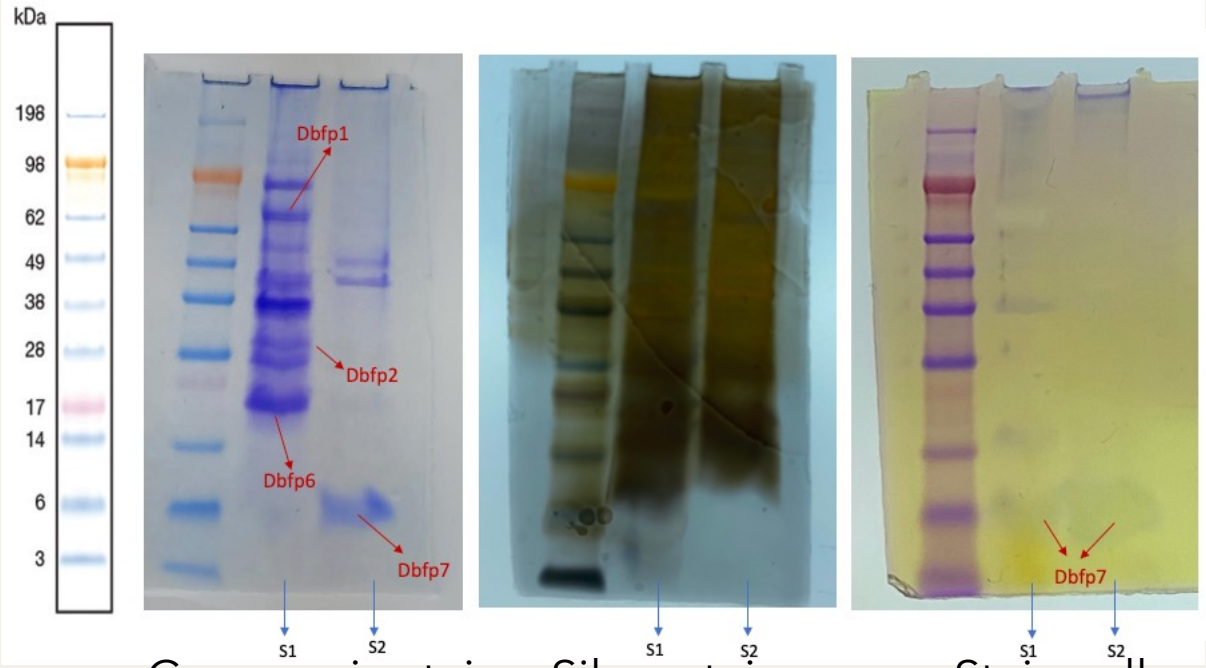
- Phosphorylation detection assay
- Pro-Q staining Phosphate Assay [9]

Glycosylation:

- Pro-Q staining Glycoprotein Assay [9]
- Periodic acid-Schiff (PAS)

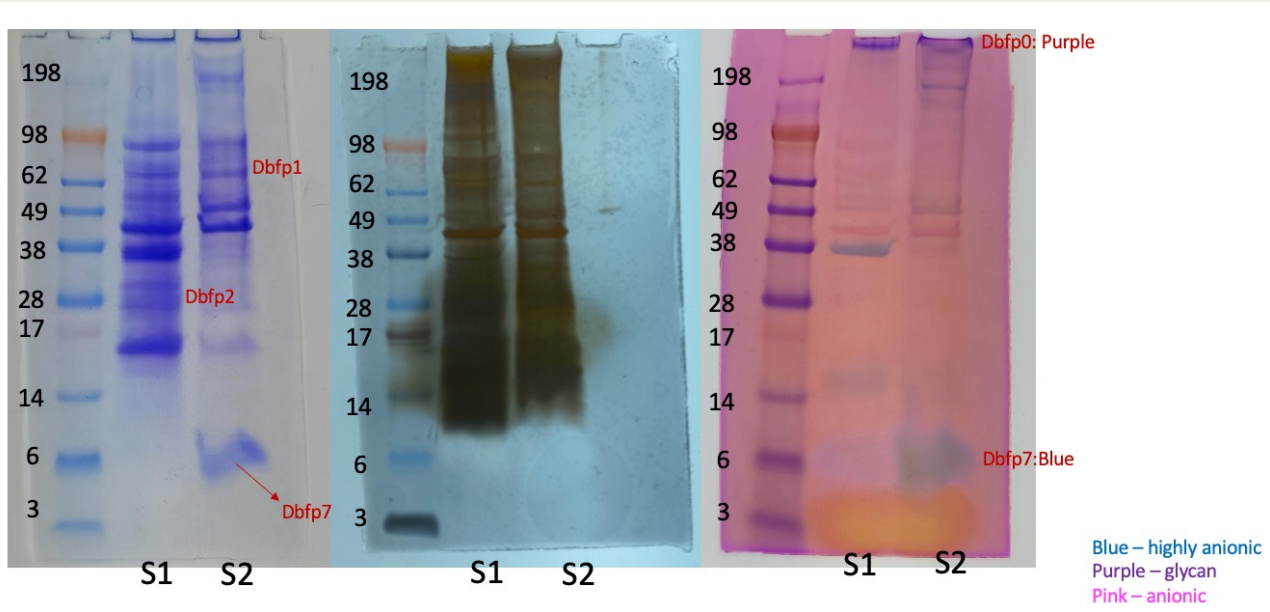
Results - Stainsall

Stains-all gel imaging result from week of Oct. 27



Coomassie stain Silver stain Stains-all

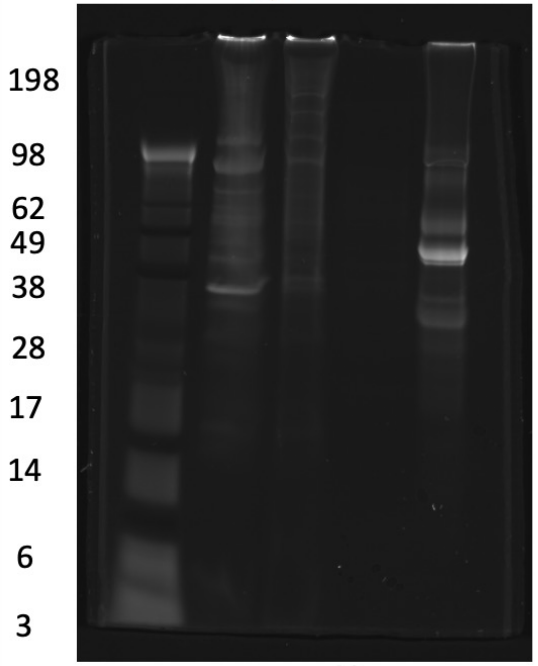
Stains-all gel imaging result from week of Nov. 1



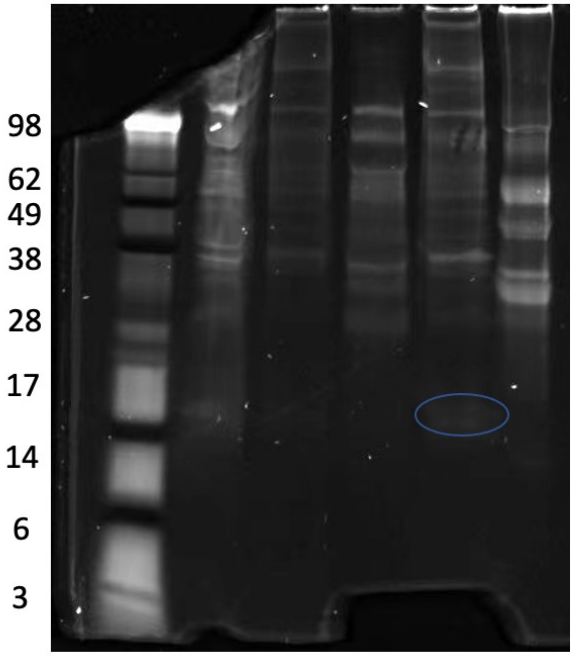
Coomassie stain Silver stain Stains-all

Results – Pro-Q Phosphate Kit

Pro-Q gel imaging result from week of Feb.7



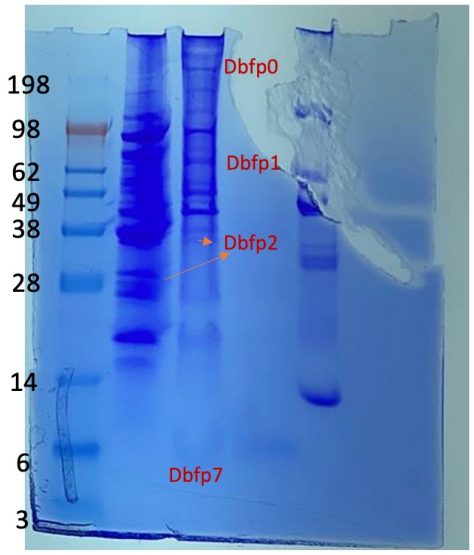
Pro-Q gel imaging result from week of Jan.17



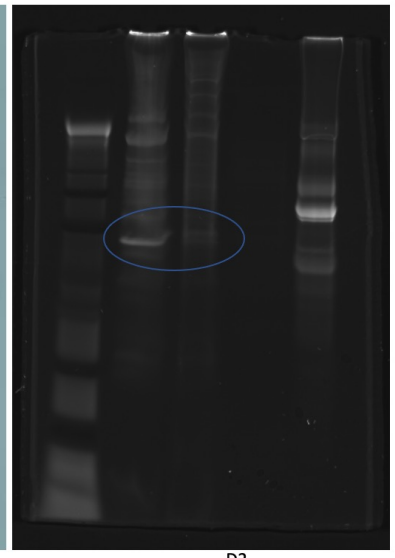
Section of S1& S2: ~38 kDa
Section of S1: ~98 kDa

Results – Pro-Q Phosphate (Continued…)

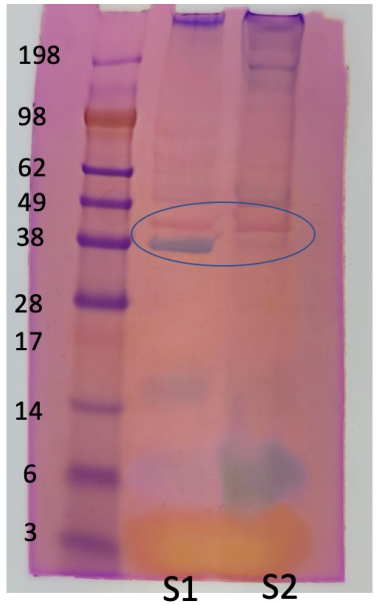
Coomassie control gel imaging from week of Feb.7



Pro-Q gel imaging from week of Feb.7



Stains-all gel imaging from week of Nov.1

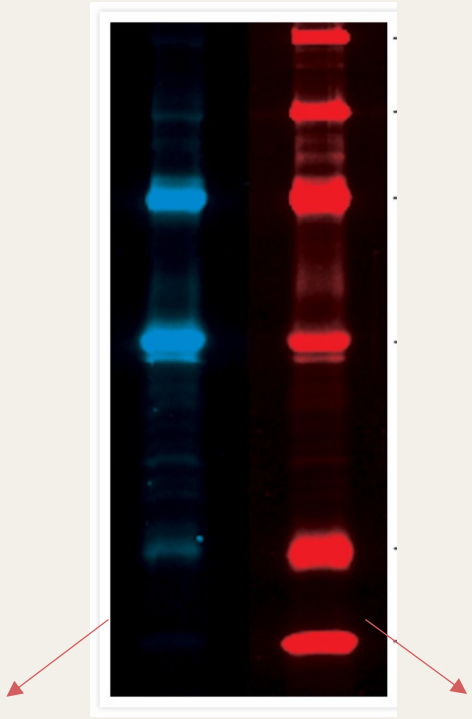


Blue – highly anionic
Pink – anionic
Purple – glycan

Section of S1:38 kDa blue – highly anionic
Section of S2: 38, 42, S1:42 kDa pink - anionic
Section S1: 49 kDa – 98 kDa pink color - anionic

Dbfp0: >200 kDa
Dbfp1: 69; 80 kDa
Dbfp2: 30 kDa
Dbfp7: 6-8 kDa

Results – SYRO Stain Comparison



Phosphate standard stained with pro-q stain

Phosphate standard stained with pro-q stain w/ SYRO stain

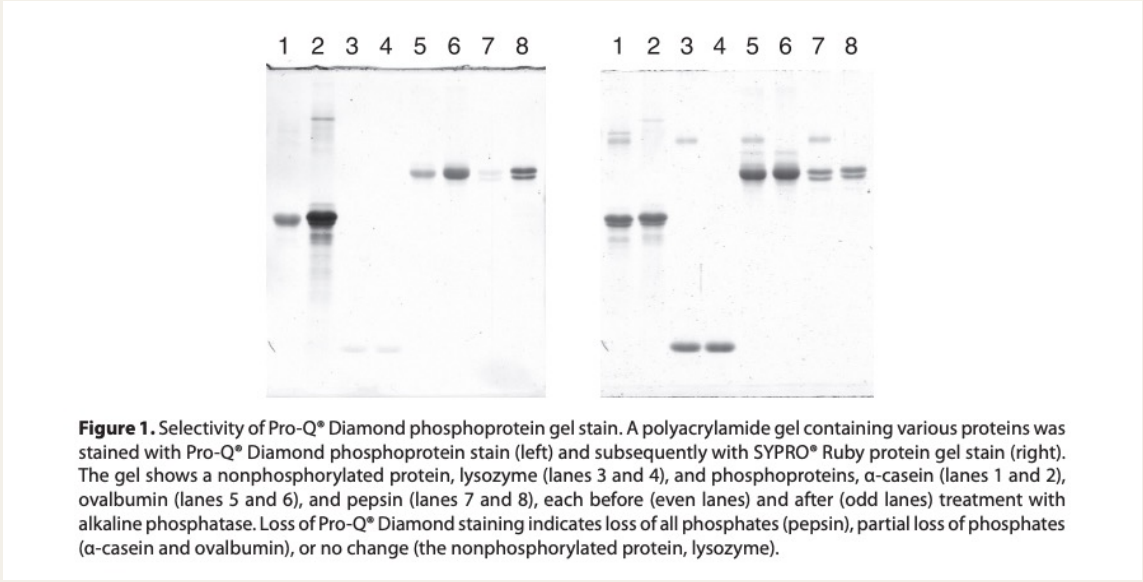
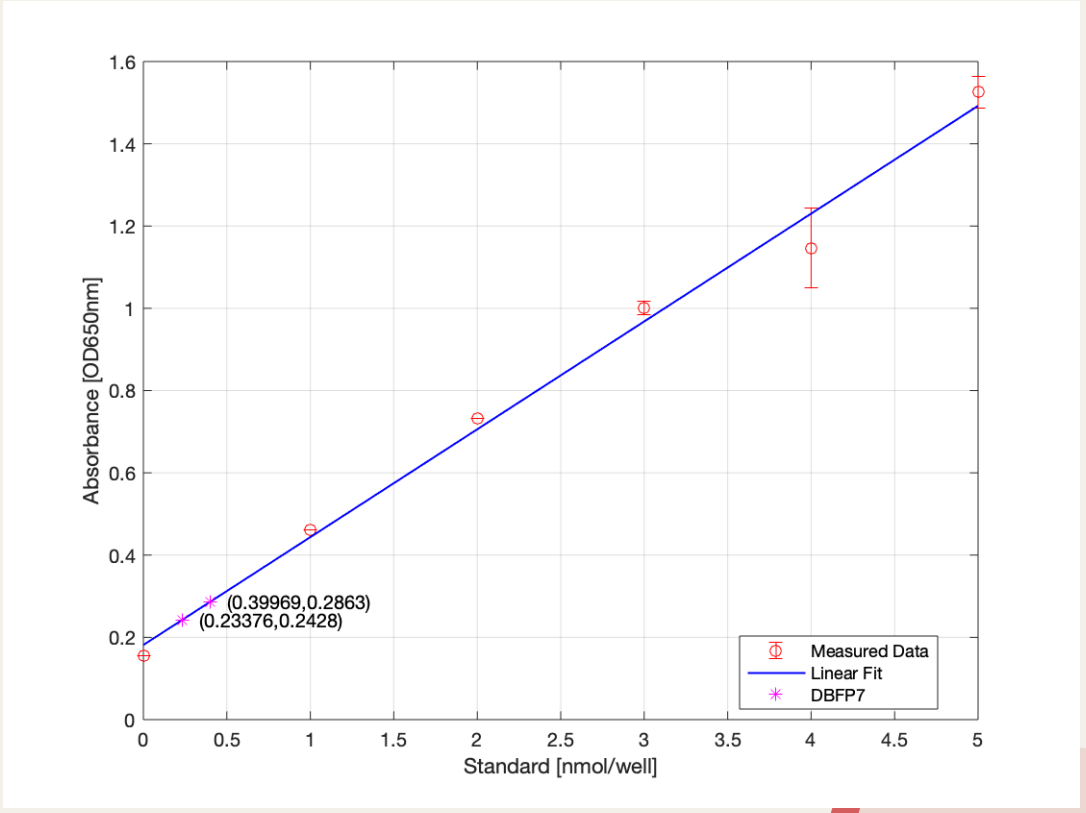


Figure 1. Selectivity of Pro-Q® Diamond phosphoprotein gel stain. A polyacrylamide gel containing various proteins was stained with Pro-Q® Diamond phosphoprotein stain (left) and subsequently with SYPRO® Ruby protein gel stain (right). The gel shows a nonphosphorylated protein, lysozyme (lanes 3 and 4), and phosphoproteins, α-casein (lanes 1 and 2), ovalbumin (lanes 5 and 6), and pepsin (lanes 7 and 8), each before (even lanes) and after (odd lanes) treatment with alkaline phosphatase. Loss of Pro-Q® Diamond staining indicates loss of all phosphates (pepsin), partial loss of phosphates (α-casein and ovalbumin), or no change (the nonphosphorylated protein, lysozyme).

Results – Phosphate Assay

	STD1	STD2	BSA2(AcH)	BSA5(AcH)	BSA10(AcH)	Dbfp7(2.5)	Dbfp7(5)
0	0.1557	0.1558	0.157	0.1649	0.1843	0.2384	0.2846
1	0.461	0.4611	0.1552	0.1605	0.1753	0.2472	0.288
2	0.7324	0.7314					
3	0.9886	1.0121				x	x
4	1.2154	1.0778				0.23376	0.39969
5	1.4982	1.5535					

Figure 1. A) Table showing data obtained from the plate reader under OD650 condition, value x is the nmol/well phosphate for Dbfp7 samples, generated from the line of best fit curve on the right B). Line of best fit of the standard curve, samples points are marked on the line



0. 23376 nmol/well & 0.39969 nmol/well
Dbfp7 concentration: 0.62 ug/ul
~85% phosphate per Dbfp7

Post-Translational Modification of Quagga Mussel Adhesive Protein - Plans

- Phosphorylation
  - Phosphate assay with more Dbfp7
  - Pro-Q staining with SYRO staining
- Glycosylation
  - Pro-q staining
  - Periodic acid-Schiff (PAS)

Thank you

Reference

[1] Sone, E.D. Interfacial Phenomena in Marine and Freshwater Mussel Adhesion, 2nd ed. [Smith, A. (ed.)] Biological Adhesives. 6, 129–151. (Springer, 2016).

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[9] "Interview with Ruixin" Nov. 17,2021.

[10] "Interview with Megda." Oct. 15,2021.