Danielle Bennetsen
VDS Summer 2019

Week 1
Date: 6-7-2019
Brief Summary of What I did this time period: Made SDS page gels for characterization of expressed protein for next week, expressed target protein using IPTG induction (old process) and autoinduction (new process) [results are yet to be analyzed], transformed BL21 and DH5 alpha cells (DH5 alpha failed first time, second time worked successfully), made LB media and Kan plates (51 in total) for future needs

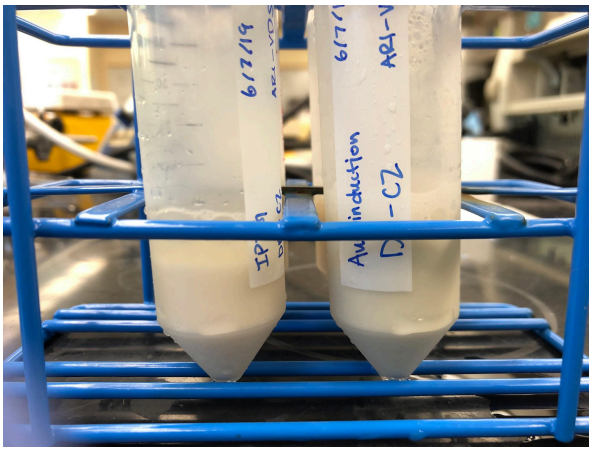

Figure 1

Figure 1: Mine and Cara's pellets from IPTG induction and autoinduction after being spun down once and resuspended (notice difference in color between the two; IPTG is a milky color, autoinduction is slightly green)
Analysis: After spin down, autoinduction and IPTG expression did yield fairly big pellets (autoinduction pellets were giant). The results of the IPTG and autoinduction expression processes have yet to be analyzed since the suspended pellets still need to be spun down again and filtered for purification next week, but will most likely need to redo autoinduction expression just to tweak the process for better efficiency.

Week 2
Date: 6-14-2019
Brief Summary of What I did this time period: Resuspended, spun down, and purified the pellets from autoinduction and IPTG expression, nanodropped Elution 1 & 2 samples of autoinduction and IPTG from purification, ran Elution 1 & 2 samples in an SDS page gel, conducted a DSF run with master mixes of the autoinduction and IPTG samples, made buffers and other solutions for purification, characterization, and DSF as needed

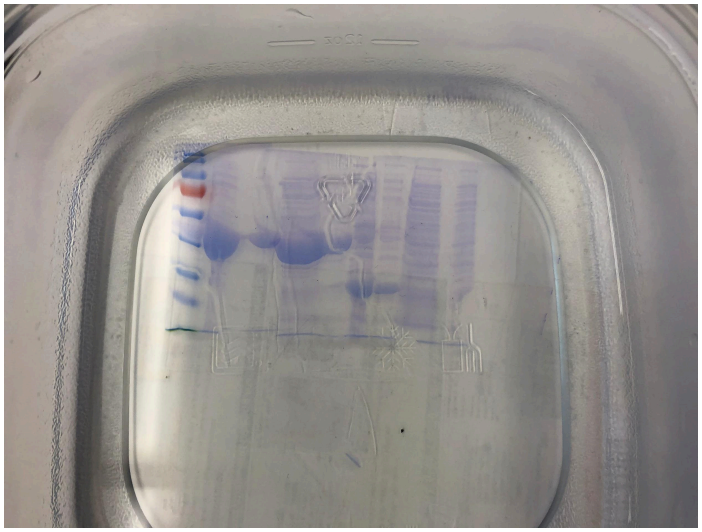

Figure 1 SDS Page Gel of Autoinduction and IPTG Induction Samples of Target Protein MtDala
Lanes (left to right): Protein Ladder, IPTG Elution 1, IPTG Elution 2, Autoinduction Elution 1, Autoinduction Elution 2

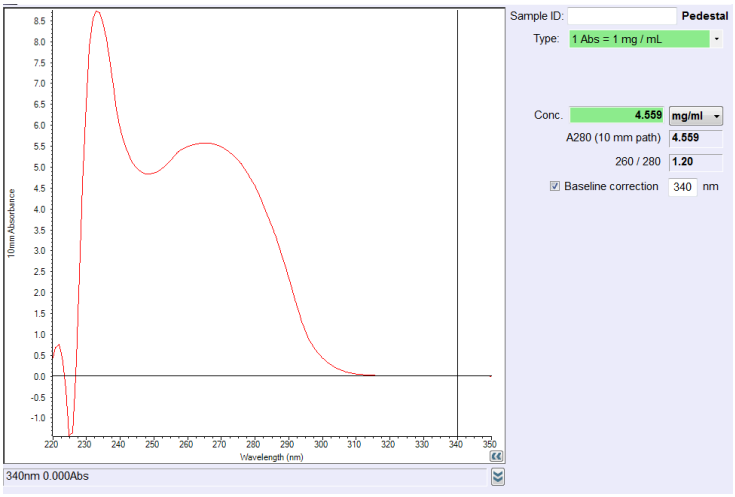

Figure 2 Nanodrop Graph of IPTG Induction Elution 1 Sample from MtDala

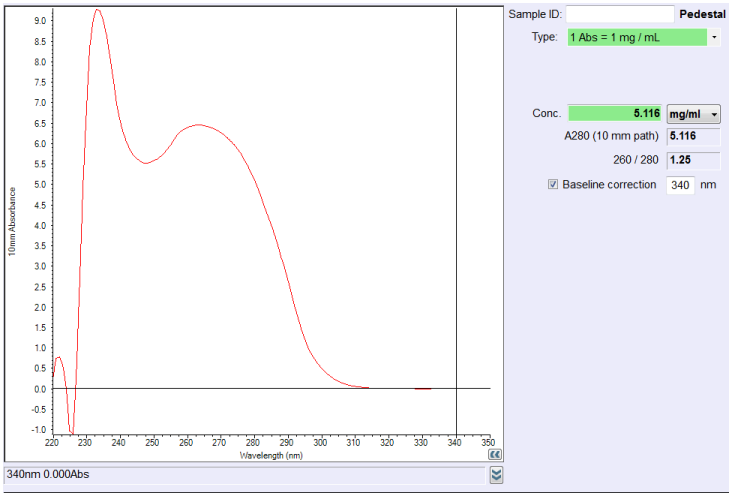

Figure 3 Nanodrop Graph of Autoinduction Elution 1 Sample from MtDala

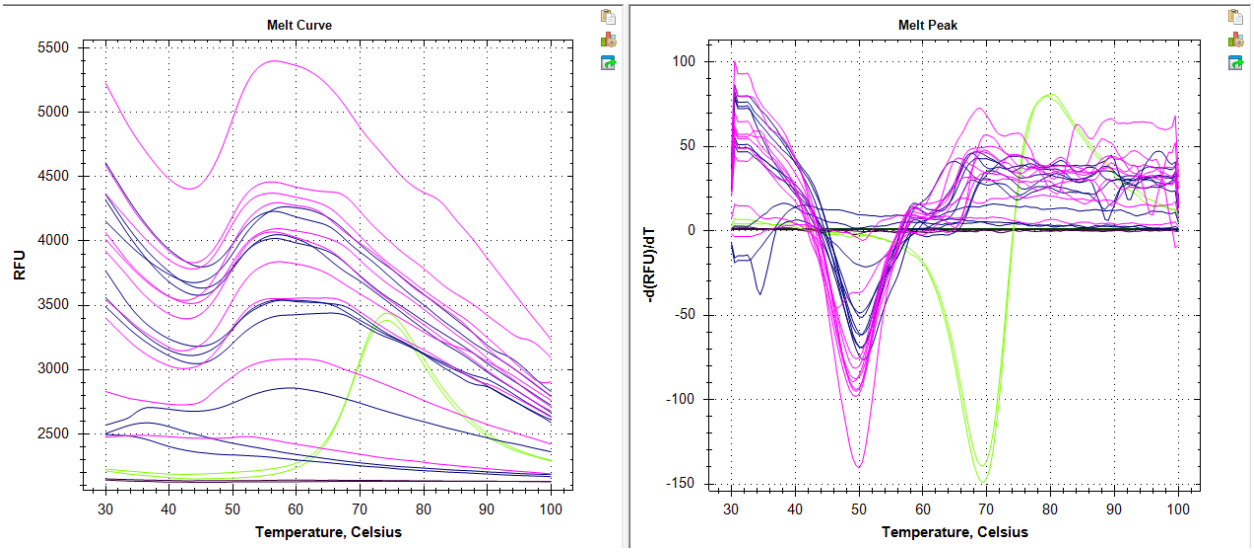

Figure 4 DSF Graph & First Derivative Curve of IPTG Induction and Autoinduction from MtDala
Key: Pink = Autoinduction, Purple = IPTG Induction, Green = Lysozyme Control, Magenta = Water Control

Analysis: The results of the purification, characterization, and DSF processes show that autoinduction did in fact yield a large amount of the target protein. As compared to the results of IPTG induction, more protein was expressed with the autoinduction process. More trials of autoinduction will be conducted in the later weeks of research, and the process as a whole will be tweaked to be more efficient in general as well as working well for all target proteins.

Week 3
Date: 6-21-2019
Name: Danielle Bennetsen
Brief Summary of What I did this time period: analyzed DNA sequence in computer lab, performed Midi Prep of target plasmid DNA and ran on an agarose gel, autoinduced target protein (expression) for the second time and adjusted some variables for the sugar mix/phosphate buffer needed, and purified the target protein for the second time

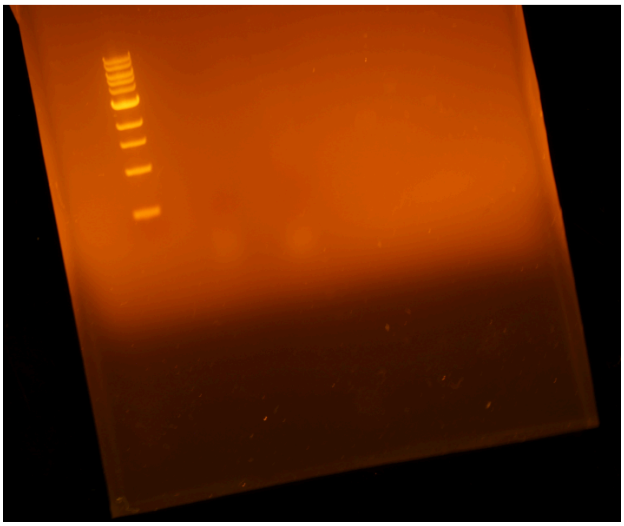

Figure 1 Gel Check of MtDala Plasmid DNA
Lanes: 1 = 1 kb DNA ladder, 2 = MtDala Plasmid DNA, 3 = NDM-1 Plasmid DNA

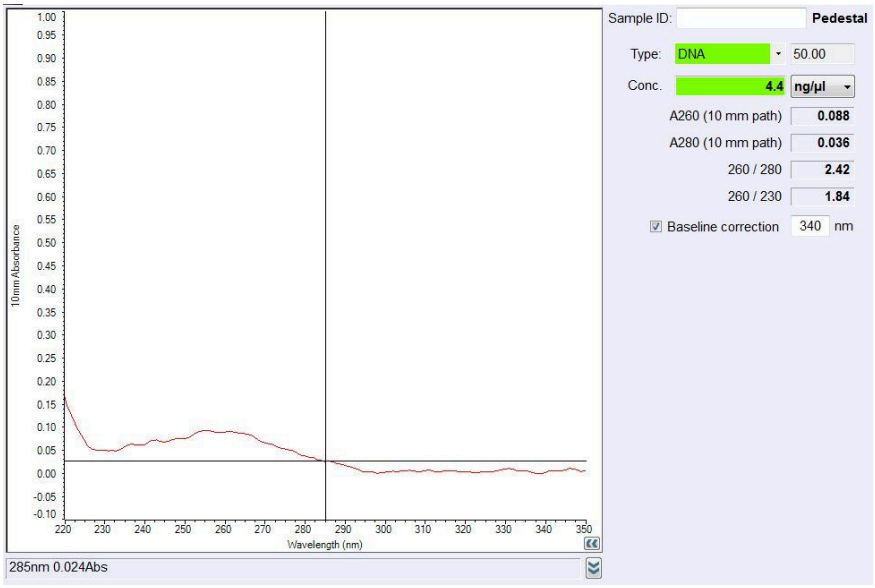

Figure 2 Nanodrop Graph of MtDala Plasmid DNA & Concentration

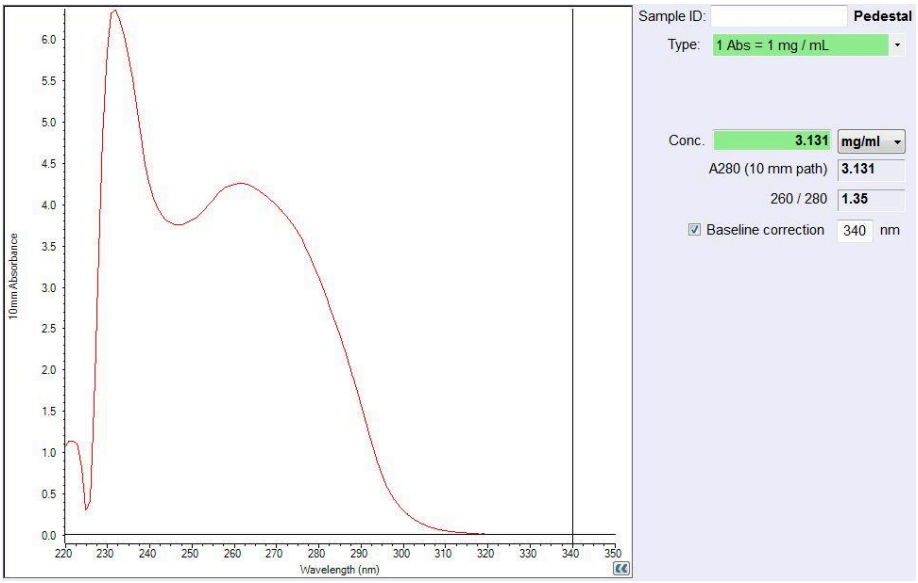

Figure 3 Nanodrop Graph of Purified Elution 1 from MtDala Protein & Concentration

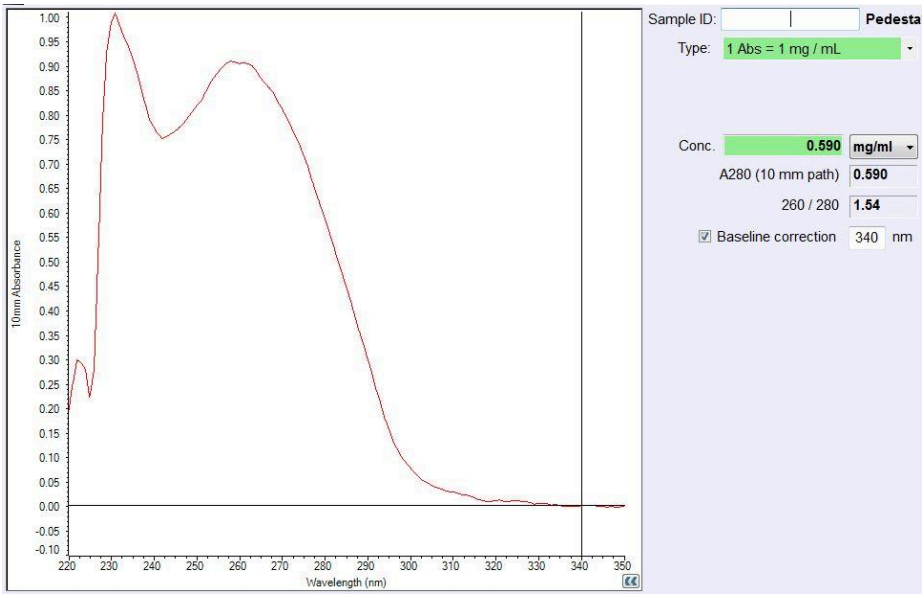

Figure 4 Nanodrop Graph of Purified Elution 2 from MtDala Protein & Concentration

Analysis: Midi Prep did not work out very well at all due to the nonexistent amount of MtDala plasmid DNA present, which is shown in Figures 1 & 2. A second trial of autoinducing MtDala protein showed that the yield of protein was less than the first trial. The pellet weight from the second trial was reduced by around half as compared to the first trial. Purification showed that a smaller amount of protein was produced by the bacterial colonies, as modeled in Figure 3 & 4. Whether or not the target protein is present for sure cannot be concluded until characterization, which will be done sometime next week.

Week 4

Date: 6-27-19

Brief Summary of What I did this time period: Protein samples from elution 1 and 2 of autoinduction trial 2 were characterized through an SDS-Page gel, snap frozen and glycerol stored versions of the protein samples were analyzed through a DSF

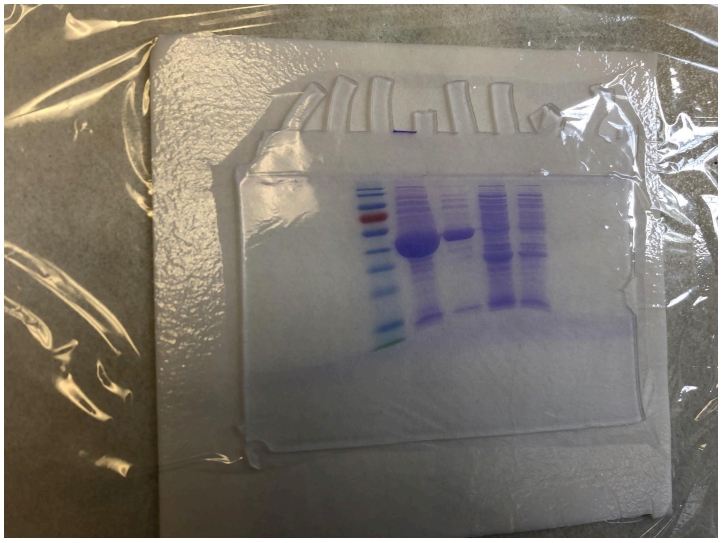

Figure 1 Gel Containing MtDala Elution 1 & 2 Samples from Trial 2 of Autoinduction Expression

Lanes: 1= PageRuler Protein Ladder, 2= MtDala Elution 1, 3= MtDala Elution 2, 4= NDM-1 Elution 1, 5= NDM-1 ELution 2

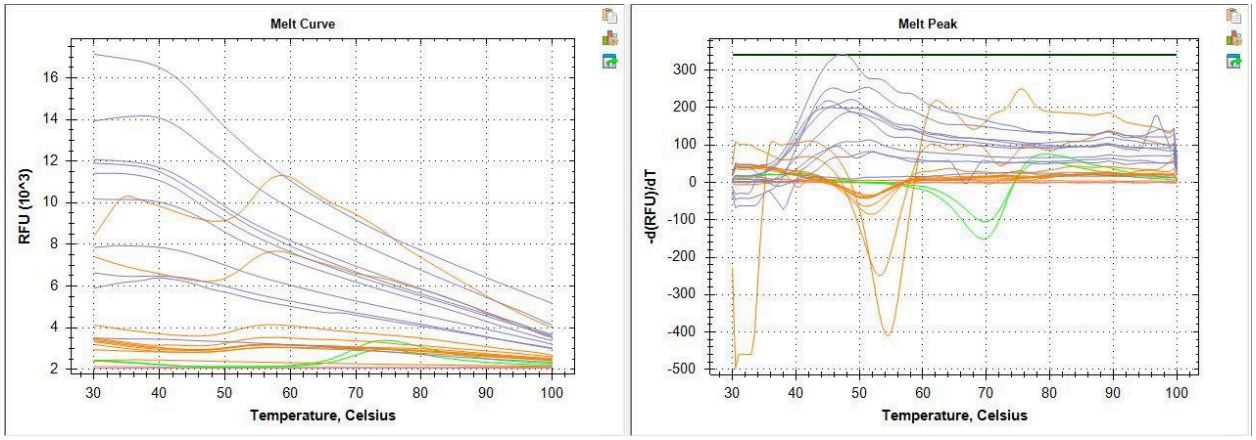

Figure 2 DSF Curve of Snap Frozen and Glycerol Stored MtDala Elution 1 Protein Samples; First Derivative Curve of Snap Frozen and Glycerol Stored MtDala Elution 1 Protein Samples
Key: Purple= Snap Frozen Sample, Orange= Glycerol Stored Sample, Green= Lysozyme Control, Pink= Water Control

Well	Fluor	Target	Content	Sample	Melt Temp
D03	HEX		Unkn		32.50
D04	HEX		Unkn		None
D05	HEX		Unkn		38.00
D05	HEX		Unkn		33.50
D06	HEX		Unkn		None
D07	HEX		Unkn		None
D08	HEX		Unkn		37.50
D09	HEX		Unkn		None
D10	HEX		Unkn		32.50
D11	HEX		Unkn		None
E02	HEX		Unkn		33.00
E02	HEX		Unkn		54.50
E03	HEX		Unkn		53.00
E04	HEX		Unkn		52.00
E05	HEX		Unkn		51.00
E05	HEX		Unkn		40.50
E06	HEX		Unkn		50.50
E07	HEX		Unkn		51.00
E08	HEX		Unkn		51.00
E09	HEX		Unkn		51.50
E10	HEX		Unkn		51.50
E10	HEX		Unkn		70.50
E11	HEX		Unkn		32.50
H02	HEX		Unkn		69.50
H03	HEX		Unkn		70.00
H04	HEX		Unkn		None
H05	HEX		Unkn		99.50

Figure 3 Table of Calculated Melting Temperatures for MtDala Snap Frozen and Glycerol Stored Protein Samples

Analysis: The second trial of autoinduction using lactose did in fact produce the target protein, but the amount produced wasn't as much as the first trial in which arabinose was used. As shown in Figure 2, the Snap Frozen Protein samples were mostly denatured, whereas the

Glycerol Stored samples were still viable. For MtDala, snap freezing may not be a good method and storing the samples via glycerol seems to be a better option for optimal performance.

Week 5
Date: 7-5-19
Brief Summary of What I did this time period: Made and tested BL21 competent cells, performed autoinduction process on EhPTP, transformed cells and started autoinduction process on Yop-H

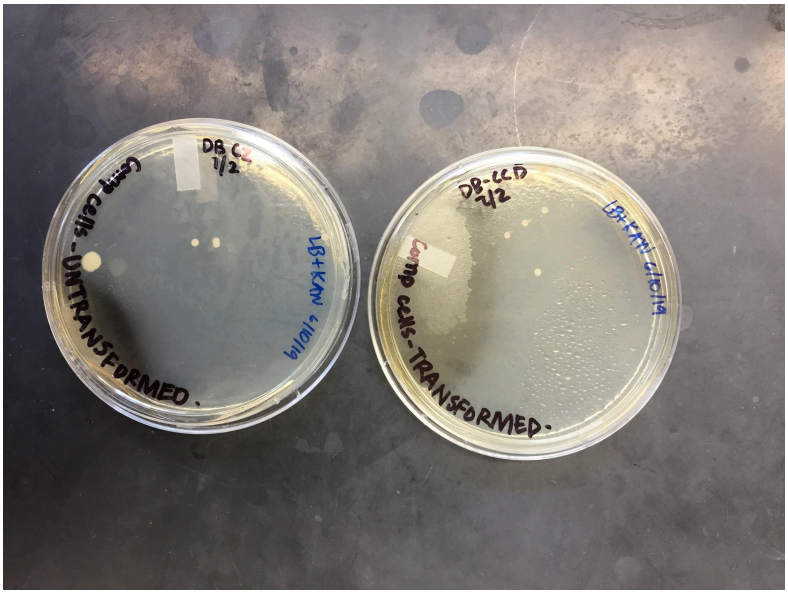

Figure 1 BL21 Competent Cells Efficiency Test (Transformed with NDM-1)

Analysis: The homemade BL21 competent cells did not work successfully when transformed NDM-1, so the cells could be tested for again using a different target to be sure of their efficiency. If they still do not work properly, the homemade competent cell process will have to be redone, or a different competent cell process will have to be used by trial and error. The autoinduction process did work on EhPTP, as an average amount of protein was produced. Purification and characterization will be done to ensure that the EhPTP protein was actually produced. Yop-H will be autoinduced next week.

Week 6
Date: 7-12-19
Brief Summary of What I did this time period: Purified and characterized EhPTP from autoinduction; expressed, purified, and characterized YopH from autoinduction; Ran a DSF using MtDala with random ligands; Re-transformed using YopH for BL21 competent cell efficiency test

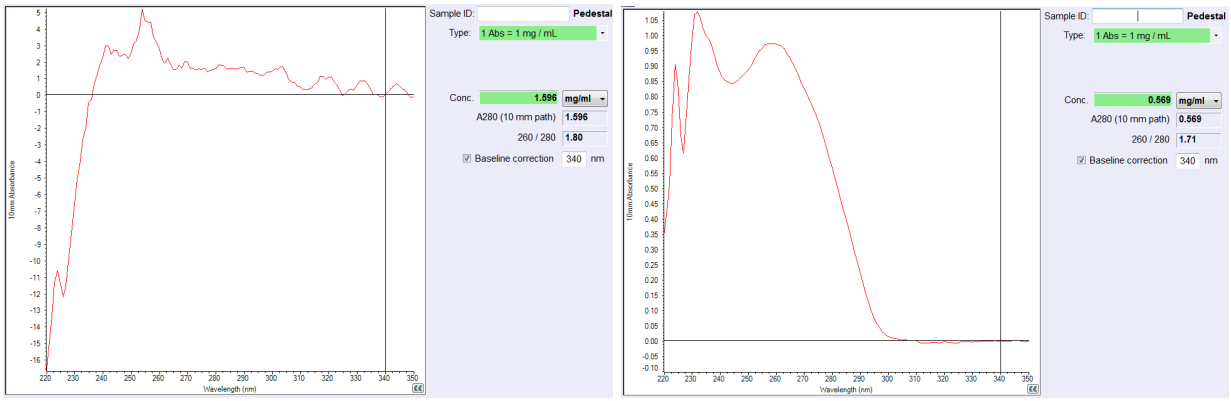

Figure 1 Nanodrop Graphs of Autoinduced EhPTP Elution 1 & 2 Protein Samples

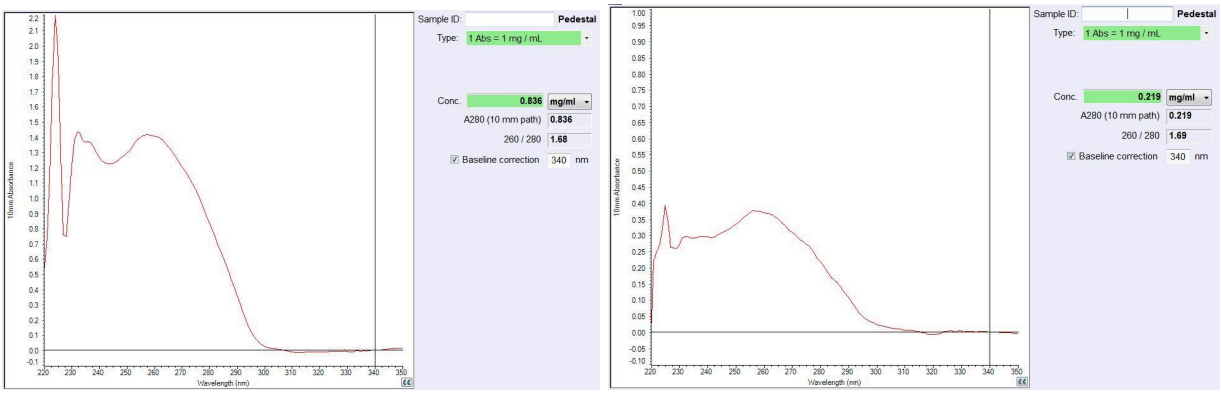

Figure 2 Nanodrop Graphs of Autoinduced YopH Elution 1 & 2 Protein Samples

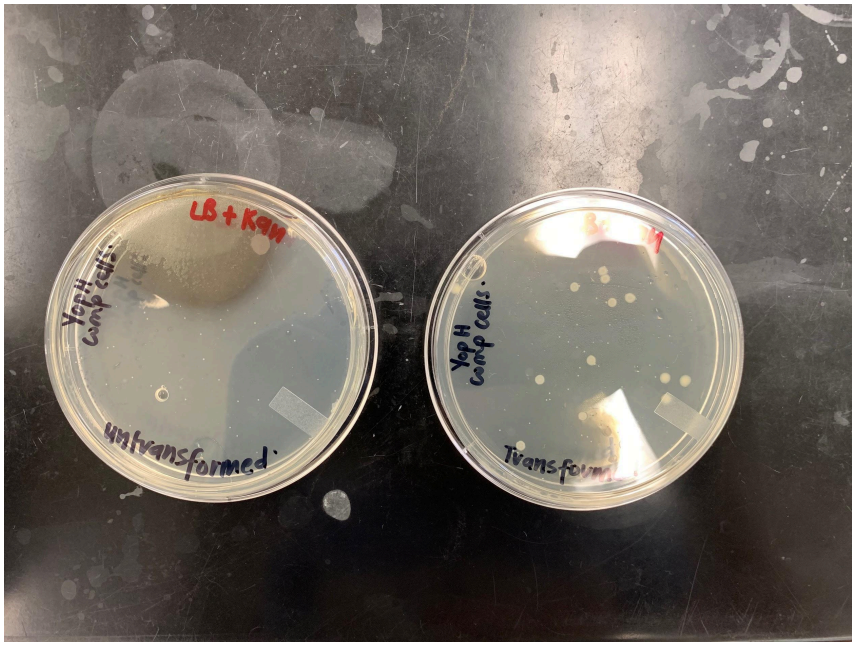

Figure 3 Homemade BL21 Competent Cell Efficiency Test Plates using YopH Plasmids for Transformation

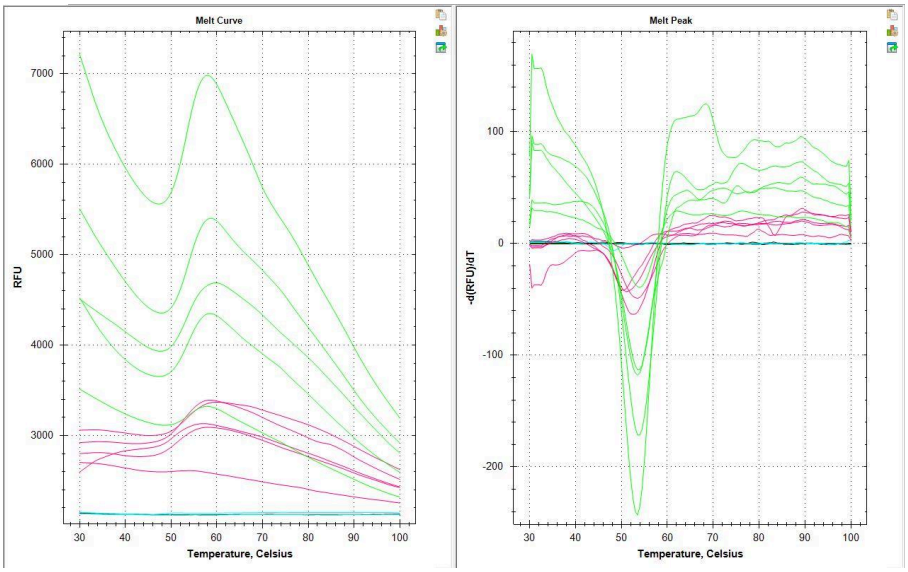

Figure 4 DSF Graph & First Derivative Curve of Glycerol-Stored MtDala Elution 1 Protein Sample with 2 Random Ligands

Key: Green = Ligand #1, Pink = Ligand #2, Blue = Water Control, Black = Lysozyme Control

Well	Fluor	Target	Content	Sample	Melt Temp
D02	HEX		Unkn		54.00
D03	HEX		Unkn		53.50
D04	HEX		Unkn		54.00
D05	HEX		Unkn		53.50
D06	HEX		Unkn		53.50
D07	HEX		Unkn		53.50
D07	HEX		Unkn		32.50
D08	HEX		Unkn		52.50
D08	HEX		Unkn		32.50
D09	HEX		Unkn		50.50
D10	HEX		Unkn		32.50
D10	HEX		Unkn		51.00
D11	HEX		Unkn		50.50
D11	HEX		Unkn		32.50
F02	HEX		Unkn		65.00
F02	HEX		Unkn		96.50
F03	HEX		Unkn		48.50
F03	HEX		Unkn		69.00
F04	HEX		Unkn		60.50
F04	HEX		Unkn		95.00
F05	HEX		Unkn		85.50
F05	HEX		Unkn		61.00

Figure 5 Table of Calculated Melting Temperatures of Glycerol-Stored MtDala Elution 1 Protein Sample with 2 Random Ligands

Analysis: The purification of autoinduced EhPTP showed that a small amount of protein was present in the sample, but there was quite a bit of contamination. Characterization of the sample showed that the target protein, EhPTP, was present in the sample but confirmed that there was contamination. The expression of autoinduced YopH yielded a fairly good-sized pellet, while the purification of the YopH sample showed that there was a very small amount of protein present. Characterization of autoinduced YopH has yet to be completed until the next week. The homemade BL21 competent cell efficiency test was redone using the YopH plasmid, and the plates confirmed that the homemade cells are in fact successfully competent. The DSF of MtDala using random ligands showed that Ligand #1 may be a good contender for binding to the protein, and the ligand will be further tested by DSF.

Week 7

Date: 7-19-19

Brief Summary of What I did this time period: Ran a DSF on MtDala with random ligands, ran DSF on YopH, dried and characterized gel of YopH, did an enzyme assay on YopH, screened and analyzed MtDala against two novel ligand libraries through GOLD

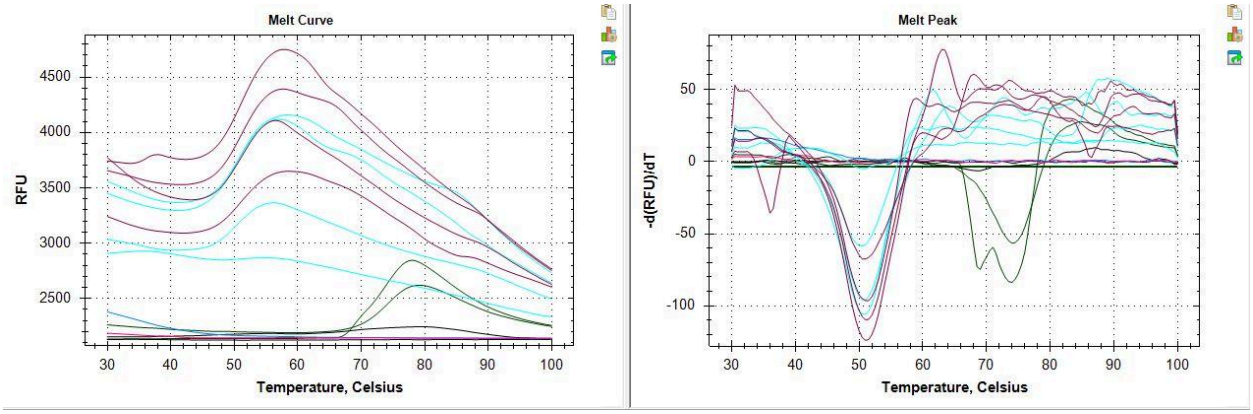

Figure 1 DSF Curve and First Derivative Curve of Autoinduced MtDala Elution 1 Protein Sample with Random Ligands #1 and #2

Key: Purple = Ligand #1, Teal = Ligand #2, Green = Lysozyme Control, Pink = Water Control, Black = Ligand Control

Well	Fluor	Target	Content	Sample	Melt Temp
D02	HEX		Unkn		50.50
D03	HEX		Unkn		51.50
D04	HEX		Unkn		51.00
D05	HEX		Unkn		51.00
D05	HEX		Unkn		36.00
D06	HEX		Unkn		51.00
D07	HEX		Unkn		50.50
D08	HEX		Unkn		51.00
D09	HEX		Unkn		50.50
D09	HEX		Unkn		32.50
D10	HEX		Unkn		None
D11	HEX		Unkn		None
F08	HEX		Unkn		68.50
F08	HEX		Unkn		72.00
F09	HEX		Unkn		None
F10	HEX		Unkn		74.00
F10	HEX		Unkn		69.00
F11	HEX		Unkn		74.00

Figure 2 Table of Melting Points for Autoinduced MtDala Elution 1 Protein Sample with Random Ligands #1 and #2

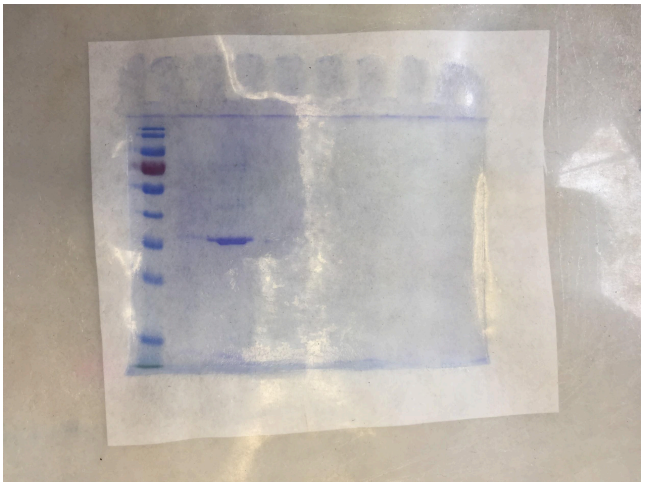

Figure 3 Gel of Autoinduced YopH Elution 1 and 2 Protein Samples with SDS PageRuler Protein Ladder
Key: Lane 1 = SDS PageRuler Protein Ladder, Lane 2 = YopH Elution 2 Sample, Lane 3 = YopH Elution 1 Sample

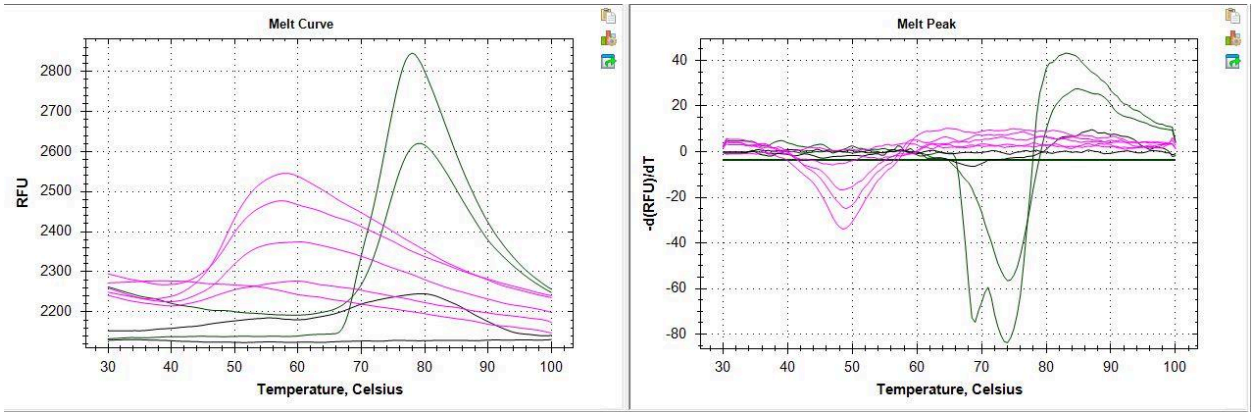

Figure 4 DSF Curve and First Derivative Curve of Autoinduced YopH Elution 1 Protein Sample
Key: Pink = YopH Elution 1 Sample, Green = Lysozyme Control, Black = Water Control

Well	Fluor	Target	Content	Sample	Melt Temp
F02	HEX		Unkn		48.50
F03	HEX		Unkn		48.50
F04	HEX		Unkn		49.00
F05	HEX		Unkn		47.00
F06	HEX		Unkn		None
F08	HEX		Unkn		68.50
F08	HEX		Unkn		72.00
F09	HEX		Unkn		None
F10	HEX		Unkn		74.00
F10	HEX		Unkn		69.00
F11	HEX		Unkn		74.00

Figure 5 Table of Melting Points for Autoinduced YopH ELution 1 Protein Sample

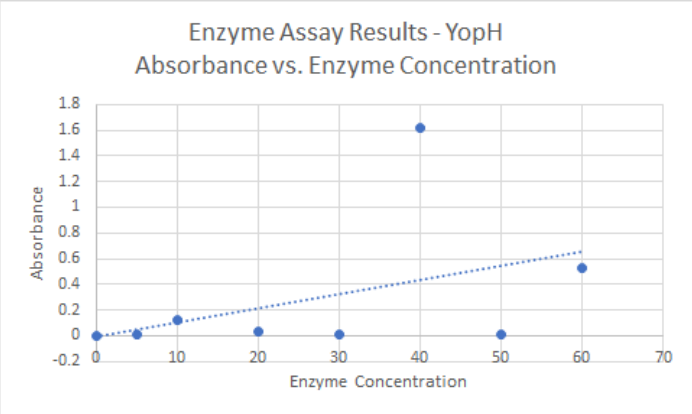

Figure 6 Graph of Sample Absorbance vs. YopH Concentration with a Linear 'Line of Best Fit'

Rank	Score	S(PLP	)	S(hbon	S(cho)	S(metal)	DE(clash)	DE(tors)	intcor	time	File name	Ligand Name
1	102.33	-95.8	0.94	1.99	0	0	1.62	0.97	19.2	'output_27469_36624/gold_soln_MicroFormats3D_m32640_3.sdf'	'm'	
2	100.27	-98.31	0.69	1	0	0	2.25	1.4	57.338	'output_64093_73248/gold_soln_MicroFormats3D_m68394_6.sdf'	'm'	
3	99.59	-102.71	0	0	0	0.01	3.64	4.17	65.241	'output_1_9156/gold_soln_MicroFormats3D_m5153_7.sdf'	'm'	
4	98.35	-100.57	0	0	0	0.18	2.49	1.55	66.852	'output_9157_18312/gold_soln_MicroFormats3D_m13685_4.sdf'	'm'	
5	97.8	-101.79	0	0	0	0	2.8	1.61	63.036	'output_9157_18312/gold_soln_MicroFormats3D_m13751_7.sdf'	'm'	
6	97.13	-92.56	1.34	1	0	0	1.58	0.72	63.399	'output_18313_27468/gold_soln_MicroFormats3D_m26376_9.sdf'	'm'	
7	97.11	-92.67	1.98	0	0	0.03	3.78	6.09	20.655	'output_1_9156/gold_soln_MicroFormats3D_m2790_2.sdf'	'm'	
8	97.03	-95.85	1.85	0	0	0	3.49	2.58	54.045	'output_82405_91560/gold_soln_MicroFormats3D_m84013_1.sdf'	'm'	
9	96.15	-99.5	0	0	0	0	2.57	1.37	68.768	'output_1_9156/gold_soln_MicroFormats3D_m303_6.sdf'	'm'	
10	96.12	-96.6	0.95	0	0	0.01	3.01	2.69	72.504	'output_18313_27468/gold_soln_MicroFormats3D_m24795_6.sdf'	'm'	
11	96	-99.4	0	0	0	0	3.57	3.23	76.193	'output_1_9156/gold_soln_MicroFormats3D_m548_2.sdf'	'm'	
12	95.85	-98.71	0.45	0	0	2.56	2.08	1.69	70.526	'output_1_9156/gold_soln_MicroFormats3D_m458_2.sdf'	'm'	
13	95.55	-95.69	0.78	0	0	0	2.39	2.3	33.392	'output_18313_27468/gold_soln_MicroFormats3D_m22882_2.sdf'	'm'	
14	95.17	-97.26	0	0	0	0.01	2.1	2.04	35.229	'output_9157_18312/gold_soln_MicroFormats3D_m18201_4.sdf'	'm'	
15	94.7	-92.75	1.65	0	0	0	2.71	2.43	13.202	'output_45781_54936/gold_soln_MicroFormats3D_m46301_3.sdf'	'm'	

Figure 7 GOLD Scores of Ligands Screened Against MtDala from MicroFormats Library

Rank	Score	S(PLP)	S(hbond)	S(cho)	S(metal)	DE(clash)	DE(tors)	intcor	time	File name	Ligand name
1	58.93	-51.45	2.63	0	0	6.66	0.58	7.41	8.758	'output_1_3963/gold_soln_Fragment-set_3D_m1074_7.sdf	'5154566'
2	58.53	-52.88	2	0	0	22.15	0.18	22.15	11.128	'output_1_3963/gold_soln_Fragment-set_3D_m2548_6.sdf	'5920505'
3	57.92	-59.14	0	0	0	0.58	0.35	0.05	3.576	'output_1_3963/gold_soln_Fragment-set_3D_m3718_2.sdf	'9044636'
4	57.8	-52.22	1	0.99	0	0	0.2	0	5.374	'output_1_3963/gold_soln_Fragment-set_3D_m3428_5.sdf	'7948268'
5	57.78	-52.73	0.91	0.99	0	0	0.33	0	5.006	'output_1_3963/gold_soln_Fragment-set_3D_m3434_4.sdf	'7948692'
6	57.74	-58.15	0	0	0	0	0.23	0.05	15.892	'output_1_3963/gold_soln_Fragment-set_3D_m1455_2.sdf	'5262725'
7	57.48	-52.61	1	0.95	0	0.03	0.49	0	4.48	'output_1_3963/gold_soln_Fragment-set_3D_m1156_1.sdf	'5185392'
8	57.4	-55.25	1	0.03	0	0	0.5	0.06	4.566	'output_1_3963/gold_soln_Fragment-set_3D_m3939_3.sdf	'9073118'
9	57.25	-48.47	1	2	0	0	0.11	0	3.467	'output_1_3963/gold_soln_Fragment-set_3D_m2005_2.sdf	'5457871'
10	56.72	-50.5	1.73	0.67	0	2.73	0.78	3.34	11.499	'output_1_3963/gold_soln_Fragment-set_3D_m3260_5.sdf	'7795927'
11	56.41	-52.79	1.34	0	0	0	0.2	0.01	3.292	'output_1_3963/gold_soln_Fragment-set_3D_m1468_1.sdf	'5263402'
12	56.05	-53.22	1	0	0	0	1.12	2.07	3.63	'output_1_3963/gold_soln_Fragment-set_3D_m3088_1.sdf	'7291926'
13	56.04	-50.21	1	1.34	0	0.48	0.76	0.8	11.204	'output_1_3963/gold_soln_Fragment-set_3D_m2205_8.sdf	'5548057'
14	56.04	-51.71	1.47	0	0	2.8	0.37	3.45	6.15	'output_1_3963/gold_soln_Fragment-set_3D_m2685_2.sdf	'6167670'
15	55.94	-54.87	0.8	0	0	0	1.27	1.19	11.205	'output_1_3963/gold_soln_Fragment-set_3D_m3537_2.sdf	'7995607'

Figure 8 GOLD Scores of Screened Ligands Against MtDala from Fragments Library

Analysis: The DSF done on MtDala with random ligands did not exhibit any good ligands to further test, as the melting point of the target protein did not shift according to Figures 1 and 2. According to the thick band compared to the ladder on the gel in Figure 3, YopH was the protein produced. In doing a DSF of YopH, Figures 4 and 5 show that the melting point of the target protein could not be determined. Therefore, the quality of the protein sample was not very good. In Figure 6, the graph shows that low amounts of substrate was produced, indicating that the YopH enzyme did not work efficiently. Overall, the GOLD scores received from screening the

two novel ligand libraries MicroFormat and Fragments are very good. The top 15 ligands from each library screened are shown in Figures 7 and 8.