Expression and Purification of the Fatty Acid Synthase Thioesterase Domain

Kelly M. Dellovo, Emma L. Welsh, Ian B. Lehner, Maxwell G. Foisey,
Maddison E. Ouellette, and Paul W. Baures
Keene State College, Department of Chemistry, Keene, NH 03435

Abstract

The overall objective of this research project has been to further develop the expression and purification of the fatty acid synthase thioesterase (FASNN-TE) domain in support of research developing FASN-TE inhibitors for breast cancer. The goal of this specific research project was to design a streamlined protocol for which our lab to follow from the expression of protein in *E. coli* to enzymatic assay and crystallization plates. The new protocols have resulted in protein that is functionally active and soluble at higher concentrations, indicating proper folding and function

Introduction

Fatty acid synthase (FASN) is an enzyme that is expressed at high levels in many tumor types. Its responsibility includes producing palmitic acid which aids in the growth of many cancers. Recently, FASN was defined as a potential therapeutic target. Previous research recognized the inhibition of FASN with active site-modifying agents that can block tumor cell proliferation, elicit tumor cell death, and prevent tumor growth in animal models. An increase in FASN expression correlates with the prognosis of multiple cancers, including breast cancer, leading to our general interest in inhibiting FASN as part of cancer chemotherapy.

In previous research, inhibitors of the thioesterase domain have been discovered. These inhibitors block the action of FASN through competitive inhibition of the thioesterase domain and elicit FASN-dependent tumor cell death. The development a new FASN-TE inhibitor in our laboratory provides an important lead for investigating FASN-TE inhibition in treating breast cancer.

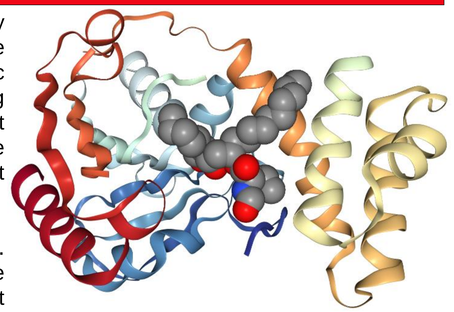

Figure 1 The fatty acid synthase thioesterase domain. Image generated from the Protein Data Bank, 2PX6

Purification Pipeline

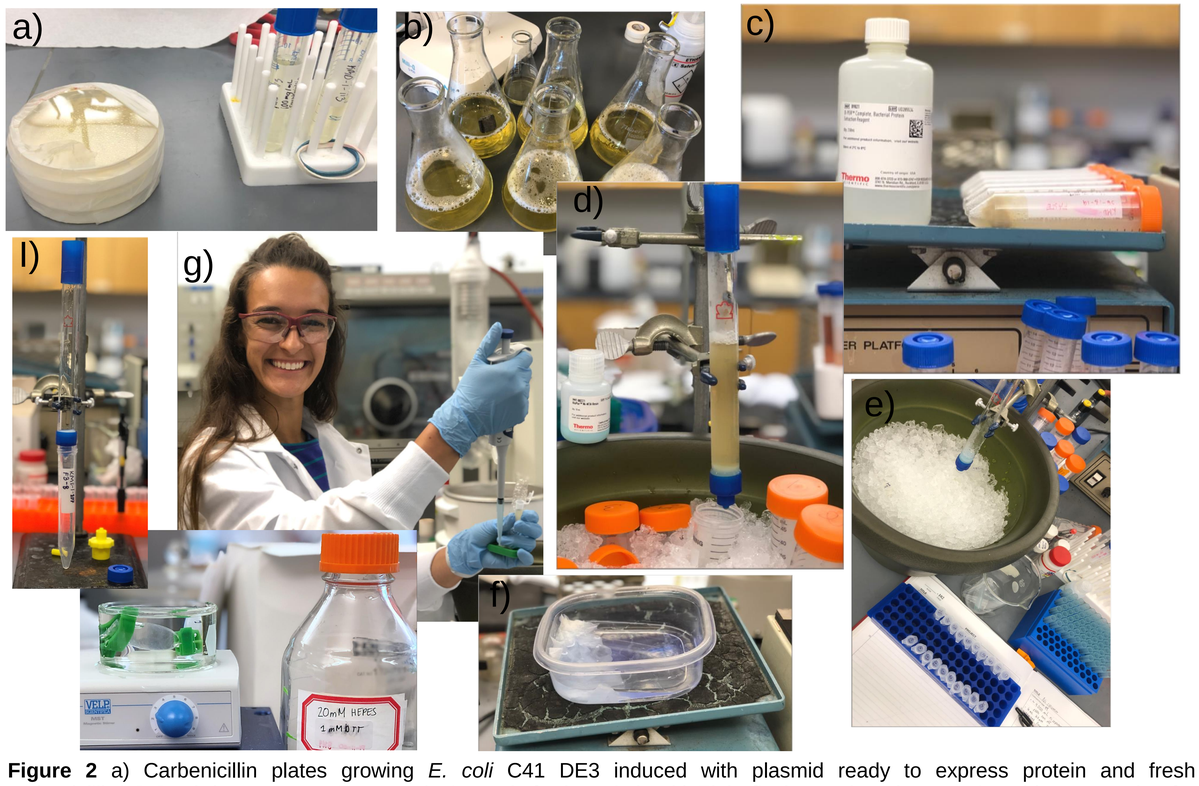

Figure 2 a) Carbenicillin plates growing *E. coli* C41 DE3 induced with plasmid ready to express protein and fresh carbenicillin/LB Broth in separate 15 mL conicals ready for inoculation b) Eight flasks ready to be autoclaved in preparation for cell culture c) Lysing of cells with B-PER in PBS with 20 mM imidazole d) All protein passing through nickel sepharose on the column in which the FASN His-tag will bind to nickel molecules e) Fractions created with an imidazole gradient in PBS to 'wash away' or 'collect' the bound protein from nickel column and eluted into DTT on ice to protect the FASN-TE from oxidation degradation f) SDS-PAGE on destain after the nickel column g) Kelly Dellovo combines the most 'pure' fractions (determined by SDS PAGE) for over night dialysis into low salt DTT/HEPES buffer h) snakeskin dialysis tubing filled with pure FASN-TE dialyzing in 20 mM HEPES/1mM DTT I) DEAE column and 15 mL collection tube to house pure FASN-TE, ready to be concentrated.

Analysis during Purification: SDS-PAGE

FASN-TE purification is evaluated by SDS-PAGE. A gel is run after each stage in the purification process to determine the location of FASN-TE and purify in the collected fractions. The figures below demonstrate a single experiment.

Crude cell extraction → (Ni2+) → Crude FASN-TE → (1.) Dialysis 2.) DEAE) → Pure FASN-TE → (Concentration) → FASN-TE stock for crystallization and assay use

Figure 3 (above) Flowchart demonstrating the purification process

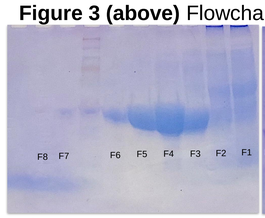

Figure 4 After the nickel sepharose column, fractions 3-8 were combined for dialysis before a DEAE column

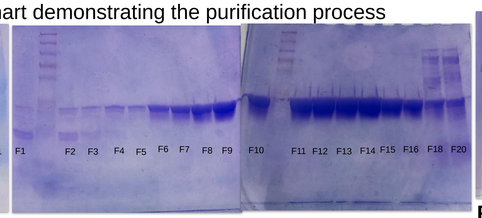

Figure 5 Two gels, placed side-by-side after the DEAE column, fractions 5-14 were collected for further purification

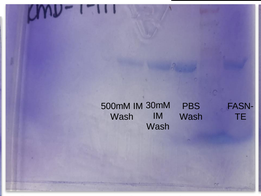

Figure 6 After the low affinity nickel column, PBS wash and FASN-TE were combined and the 500mM/30 mM Imidazole (IM) washes were stored

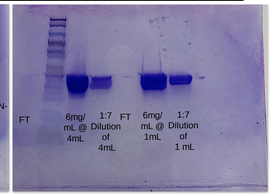

Figure 7 After concentrating to determine both the presence and absence of FASN-TE in solution and flow through (FT) respectively

Crystallization and Enzymatic Assay

- The Nanodrop 2000 was used during protein concentration to quantify the amount of protein in solution
  - It is necessary to concentrate protein at 6mg/mL for crystallization plates
- AMICON 8200 ultrafiltration concentrator was used effectively for large batch protein
- We aim to crystallize our pure protein to determine the structure and ultimately bound to inhibitors determines if the protein is functional and support that the protein is folded properly
- We utilize plate assay to determine if our protein has activity and is folded correctly

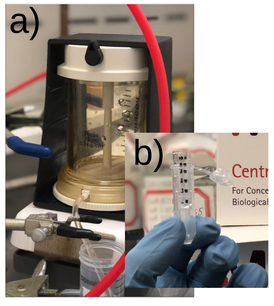

Figure 8 a) The AMICON8200 concentrator with flow through passing into the 50 mL conical b) a minicentrifuge spin column

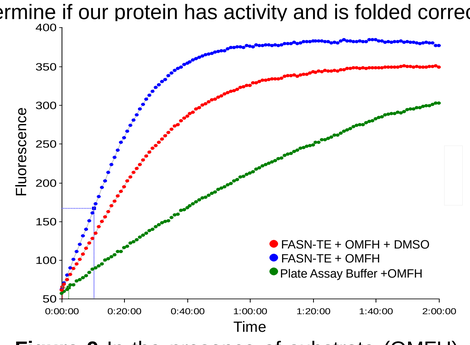

Figure 9 In the presence of substrate (OMFH) alone, FASN-TE demonstrates the highest amount of fluorescence. Goal: Analysis of enzymatic assay data at 10% or less of the maximum fluorescence.

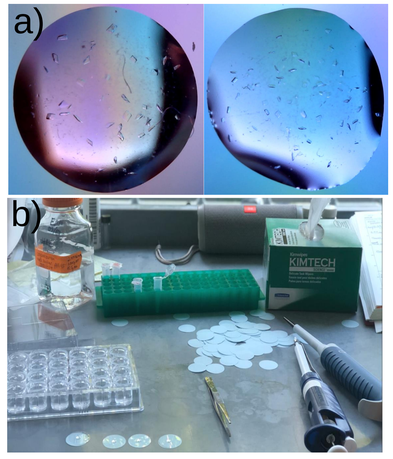

Figure 10 Crystal formation from most recent batch of protein (b) Setting up crystallization plates using pure FASN, and FASN + inhibitors. Crystallization is accomplished when pure protein is mixed with super saturated well buffer.

Challenges, Conclusions, and Future Directions

- Over the course of the summer we experienced some challenges with concentrating our protein, several of those challenges include:
  - Protein binding or aggregation on the cellulose membrane of the minicentrifuge spin columns
  - Precipitation due to contaminations and/or poor isolation/purification techniques
  - Instrumentation reliability i.e. Nanodrop 2000 utilizing various trials with a standard, bovine serum albumin (BSA)
- Overall, this project has effectively and thoroughly improved the purification pipeline start to finish
- Ultimately, I was able to effectively produce a large quantity of pure FASN to be used for crystallization, enzymatic assay, and future experiments
- Future direction for my project is to have produced viable crystals that will be sent for X-ray crystallography

Acknowledgements

Research reported on this poster was supported by New Hampshire-INBRE is greatly appreciated. The authors thank the NSF (1337206) for support of the KSC NMR facility, as well as infrastructure support through an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103506. The support of Dean Gordon Leversee and Brian Moore is also appreciated.