Enzymatic Steroid Hormone Degradation: Purification of a Putative Estradiol-Degrading Protein for an Estrogen Biosensor

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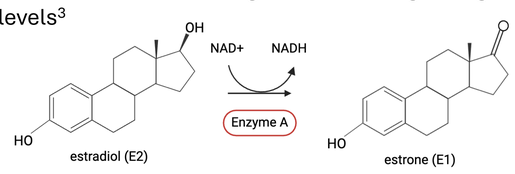
Introduction

- Estrogen fluctuation in both human and wildlife settings can directly correspond to a system's health
  - Breast cancer[1]
  - Fertility[1]
  - Wastewater steroid pollution[2]
- Awareness of estrogen levels in various environments would allow for the prevention and recognition of a multitude of illnesses
- Steroids are the second largest class of drugs, yet there are no commercially available wearable biosensors for measuring and monitoring estrogen levels[3]



Scheme 1 Diagram of Enzyme A Degradation of E2 to Form Product E1.

- We present Enzyme A (a censored name for an enzyme), a bacterial redox enzyme that putatively degrades estradiol
- Estradiol is responsible for the development of secondary sex characteristics in, predominantly, premenopausal women[1]

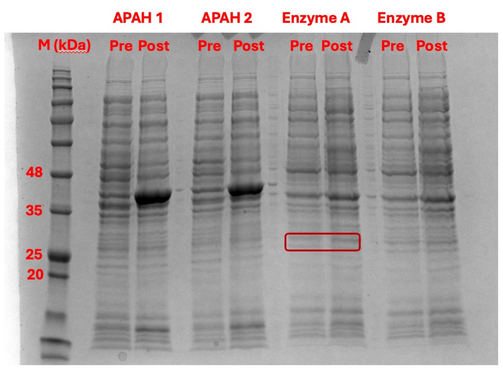


Fig. 1 SDS-PAGE with Enzyme A pre- and post-induction. Little expression at 27.7 kDa (molecular weight of Enzyme A).

Methods

Transformation into Chaperone Cell Line

- Grew seed cultures to perform plasmid miniprep for isolation of the plasmid encoding for Enzyme A
- Spread out transformed bacteria on agar plates

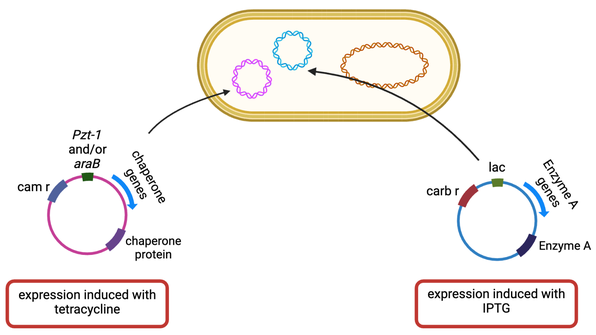


Fig. 2 Chaperone plasmid (from *E. coli* BL-21 (DE3)) insertion. Enzyme A plasmids isolated using the Monarch Miniprep Kit from New England Biolabs, chaperone cell lines from Takara Bio.

Test Expression

- Inoculated LB media with single colonies for test expressions
- Collected pre- and post-induction samples from five separate *E. coli* BL-21 (DE3) colonies each with a different chaperone plasmid: pG-Tf2 (Tf2), pGro7 (G7), pKJE7 (Kje7), pG-KJE8 (Kje8), pTf16 (Tf16)
- Highest expression of Enzyme A was seen with the pG-Tf2 chaperone plasmid

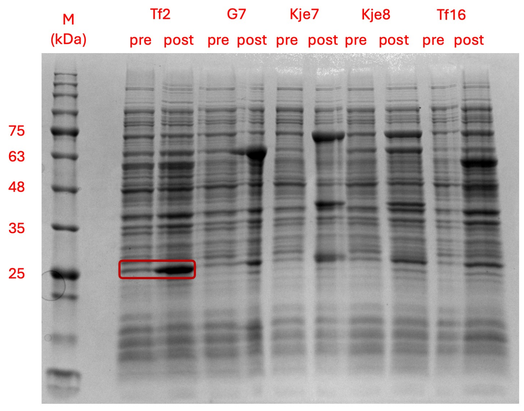


Fig. 3 Five chaperone plasmids with their pre- and post-induction samples.

Path A

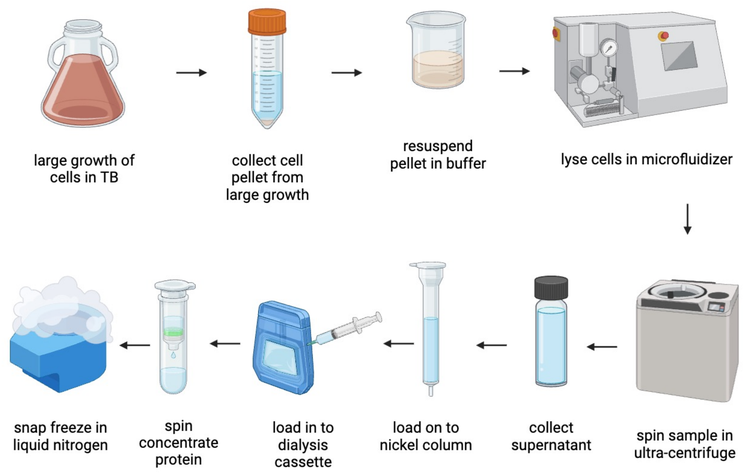
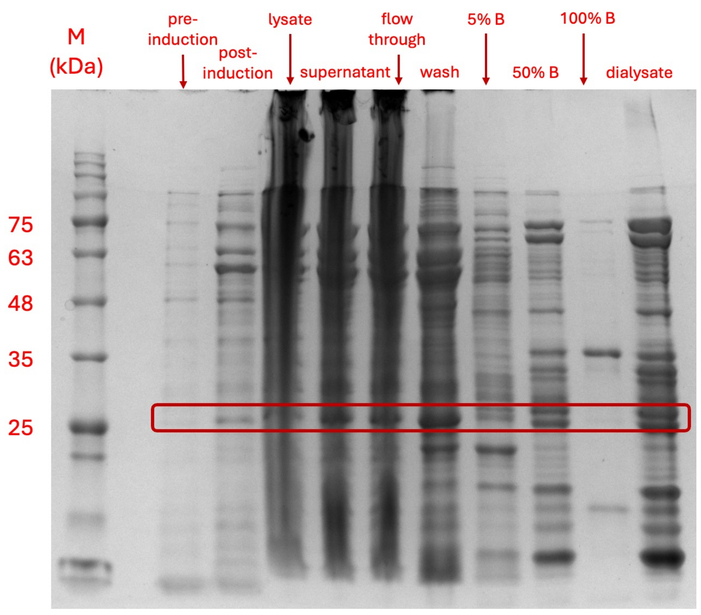
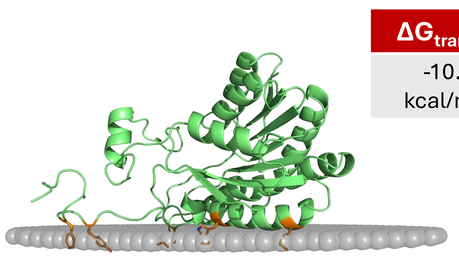


Fig. 4 Initial soluble protein purification protocol. Upon discovering the chaperone plasmid with the most expression, Tf2, a standard protein purification protocol was followed to isolate the transcribed Enzyme A genes. Samples from various steps were taken for an SDS-PAGE.

Fig. 5 Initial SDS-PAGE for Enzyme A purification. The red box at 27.7 kDa indicates where the band should be for Enzyme A. Although there are faint bands at Enzyme A's molecular weight (27.7 kDa), none of the bands are isolated. This demonstrates that the protein did not get adequately purified and further investigation would have to be performed to understand the lack of purity in our results.



Path B



$\Delta G_{transfer}$
-10.7 kcal/mol

Fig. 6 AlphaFold model of Enzyme A embedded in membrane with its $\Delta G_{transfer}$ from the Orientation of Proteins in Membranes (OPM) server[4]. OPM output suggests that the cause of purification challenges was due to Enzyme A being membrane-bound rather than soluble. The $\Delta G_{transfer}$ indicates a net drop in energy, suggesting that Enzyme A is more stable being bound to a membrane rather than in solution.

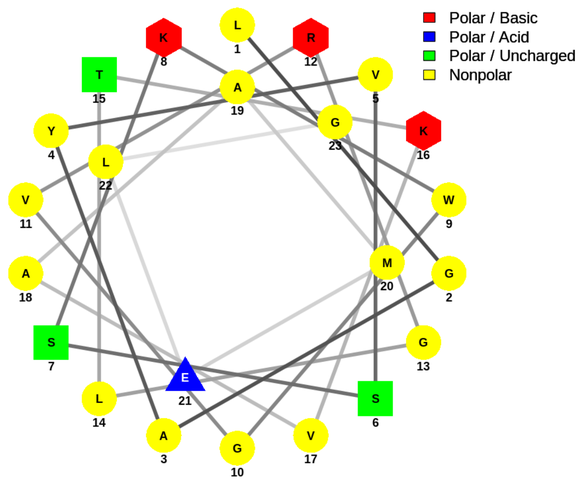


Fig. 7 Helical wheel diagram. This diagram shows how the amino acids of Enzyme A are arranged going around the alpha helix interacting with the membrane. All the yellow, non-polar, amino acids indicate that this helix is very hydrophobic, a characteristic of membrane-associated helices.

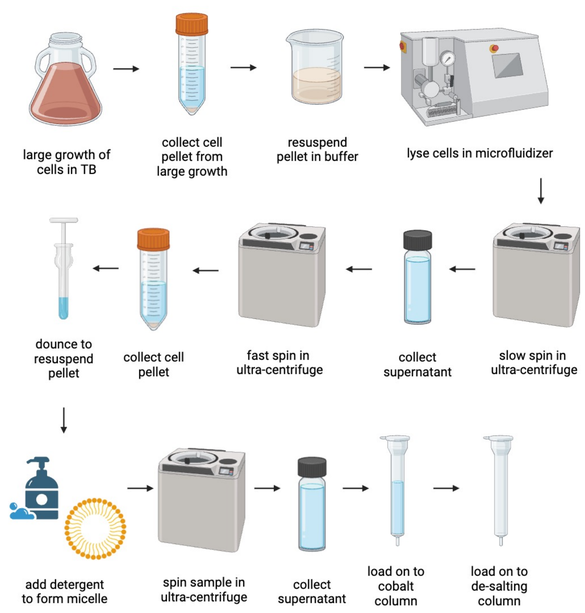


Fig. 8 Membrane-bound protein purification protocol. Following poor results from the soluble protein purification, a membrane-bound protocol was taken. The inclusion of the detergent Tween 20, in this process, disrupts the membrane and forms micelles around the protein. Samples from multiple steps were taken for an SDS-PAGE to assess the purity accomplished through this protocol.

Results

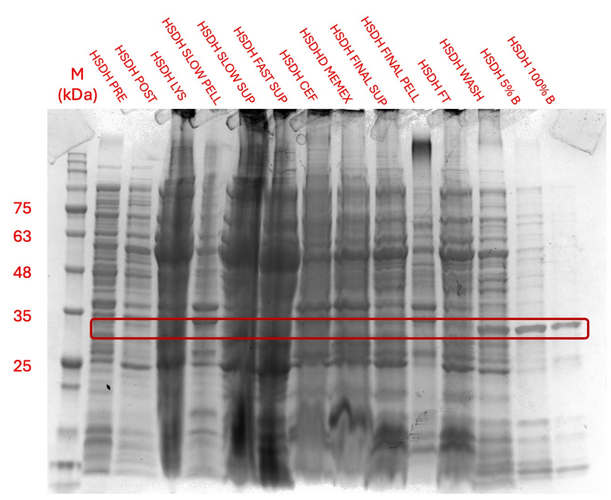


Fig. 9 Path B SDS-PAGE for Enzyme A purification. The red box at 27.7 kDa indicates where the band should be for the purification of Enzyme A. Through the implementation of a membrane-bound purification protocol (notably with the addition of a detergent and a cobalt and de-salting column), there is an isolated band at Enzyme A's molecular weight, 27.7 kDa. Though faint, the individual band indicates that our protein is likely membrane-bound.

Conclusion

- A combination of experiments in the lab coupled with computational models suggested that Enzyme A is membrane-bound
- Based on this insight, we developed an improved purification protocol that allowed for a much higher level of Enzyme A purity to be achieved

Future Directions

- Optimize the Path B membrane-bound protein purification protocol
  - Experiment with a different detergent
  - Optimize spin speeds and durations
- Perform a Western Blot test to confirm that the protein, apparently isolated in the SDS-PAGE, is truly Enzyme A
- Characterize the structure of Enzyme A through x-ray crystallography
- Determine steady-state kinetic parameters for Enzyme A
- In collaboration with the Galagan Lab, Enzyme A will hopefully be implemented into a multiplex sensor for real-time detection of multiple estrogens in various environments

References

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Acknowledgements

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