Assessing the Interaction Between Def1 and TFIIH Through X-Ray Crystallography

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Introduction

- Def1 is a protein that tags RNA Polymerase II with ubiquitin, marking it for degradation
- TFIIH is a transcription factor that crucial for transcription initiation
- New data reveals that DEF1 recruits TFIIH during transcription
- Alphafold predictions show an interaction between the Cue domain of DEF1 and the PH domain of TFIIH, which resembles ubiquitin
- The aim of our study is to experimentally prove and study this interaction by designing a protein crosslinking the Cue domain of DEF1 to the PH domain of TFIIH and imaging it through X-ray crystallography

Methods

Cloning

- We designed a vector expressing the Cue domain of DEF1 covalently linked to the PH domain of TFIIH
- We used the pET His6 TEV cloning vector for expression as it adds a HIS6 tag onto our protein for affinity binding during purification

Protein Purification

- We tested expression of our vector on a small scale (50mL of cells) to find the optimized conditions for large scale expression (6L of cells): Rosetta cells with a 1 hour IPTG induction.
- We used a nickel column to bind our protein, eluted the protein with a linear gradient, performed an overnight TEV cleavage to remove the HIS tag, used another nickel column to remove uncleaved proteins, and finally used a size exclusion column to further purify the protein before concentrating it.

Crystal Screening

- We tested two different protein concentrations: 10 mg/mL and 5 mg/mL
- With the help of the Van Duyne Lab, we set up five 96-well-plates for each concentration with different buffer conditions and refrigerated at 24°C
- We examined our plates under a microscope after one, two, and three weeks to screen for protein crystals

Acknowledgements

Thank you to Dr. Murakami and the Murakami Lab as well as Saira Montermoso and the Van Duyne Lab. Thank you the Center for Undergraduate Research Funding (CURF) and, specifically, the Penn Undergraduate Mentoring Program (PURM) for funding this project.

Results

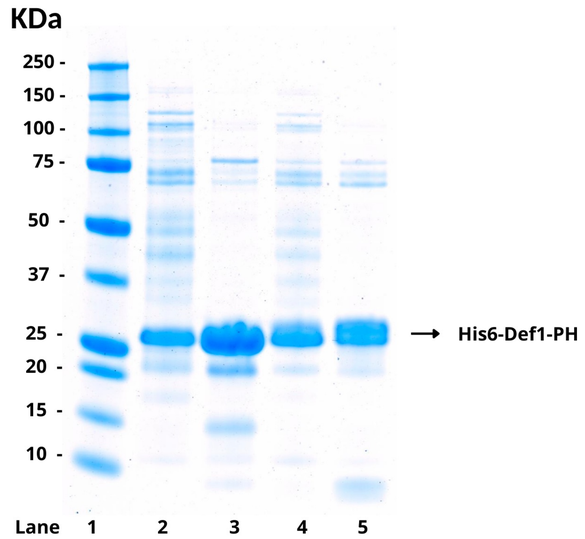

Figure 1. A SDS- page gel showing the protein bands following small scale protein expression. *Lane 1* shows the protein standard, *Lane 2* the protein expressed by BL21 cells after a 1 hour induction, *Lane 3* the protein expressed by BL21 cells after an 18 hour induction, *Lane 4* the protein expressed by Rosetta cells after a 1 hour induction, and *Lane 5* the protein expressed by Rosetta cells after an 18 hour induction. The His6-Def1-PH complex weighs about 25 kDa.

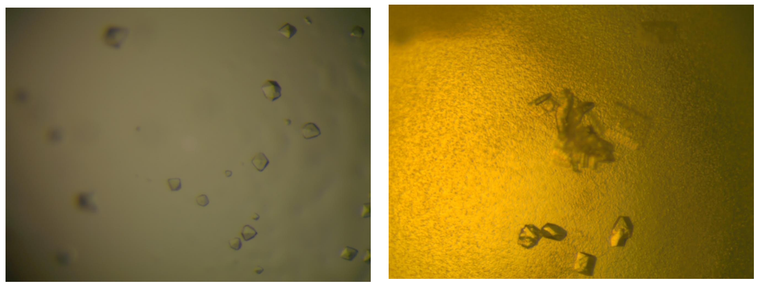

Figure 3. The two conditions that yielded protein crystals: 2100mM DL Malic acid pH 7.0 (*right*) and 40% PEG400, sodium citrate pH 5.5, and 200 mM $MgCl_2$ (*left*), viewed and photographed at 100x magnification.

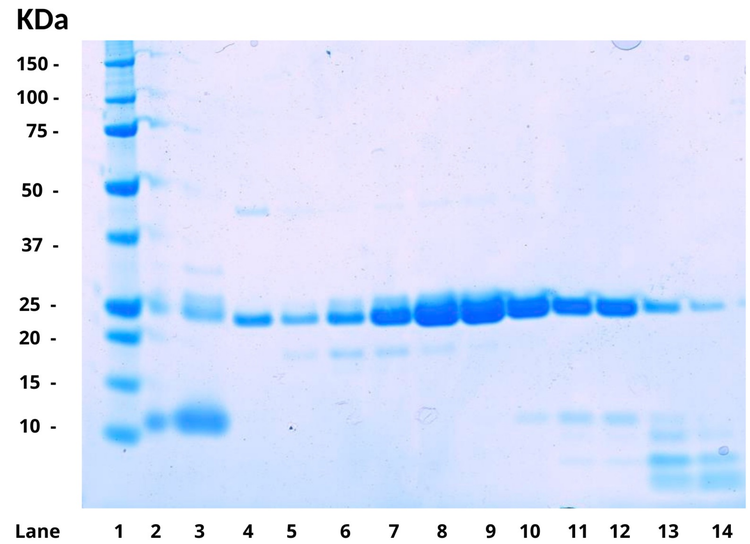

Figure 2. A SDS-page gel showing the protein fractions from large scale expression following the size exclusion column, the final step of purification. The protein in *lanes 7* through *12* were pooled together and concentrated to 17 mg/mL.

Discussion and Future Directions

The crystals we obtained thus far are too small to be imaged as they were from an initial screening. We set up another 24-plate well to further optimize conditions for the crystals pictured on the right in Figure 3. We created PEG400 glycerol concentrations from 30% to 40% in increments of 2% and varied the sodium citrate buffer pH from 5.3 to 5.6 in increments of 0.1. We chose to optimize conditions around this protein because the PEG400 glycerol in the buffer cryoprotects the protein. This will protect the protein during imaging, which takes place at freezing temperatures.

Once we obtain the structure , we can better analyze the residues involved in the interaction between Def1 and TFIIH and understand Def1's role in recruiting TFIIH during transcription.

References

1. Damodaren N, Van Eeuwen T, Zamel J, Lin-Shiao E, Kalisman N, Murakami K. Def1 interacts with TFIIH and modulates RNA polymerase II transcription. Proc Natl Acad Sci U S A. 2017 Dec 12;114(50):13230-13235. doi: 10.1073/pnas.1707955114. Epub 2017 Nov 27. PMID: 29180430; PMCID: PMC5740667.