Our research aims to **elucidate** the structure of the **HU-Junction** complex to clarify the **structural elements** that lead to HU **recognition** of the Holliday Junction.

# Elucidating the Structure of the HU-Junction Complex

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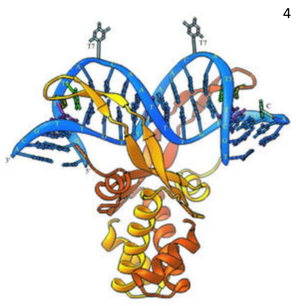
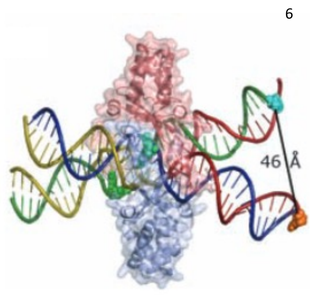
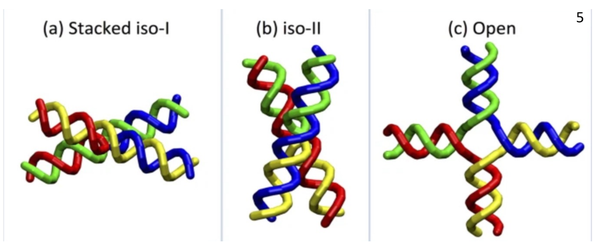
## Introduction



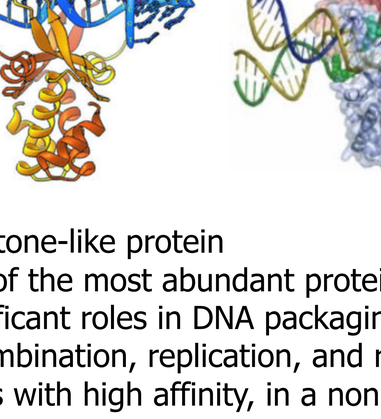



HU
- a histone-like protein
- one of the most abundant proteins in *E. coli*
- significant roles in DNA packaging, recombination, replication, and repair
- binds with high affinity, in a non-sequence specific manner, to Holliday or 4-Way Junction




5

Holliday (HJ) or 4-Way Junction (4WJ)
- important intermediate in recombination and repair
- J20: an immobile Holliday Junction with 20 base pairs per strand

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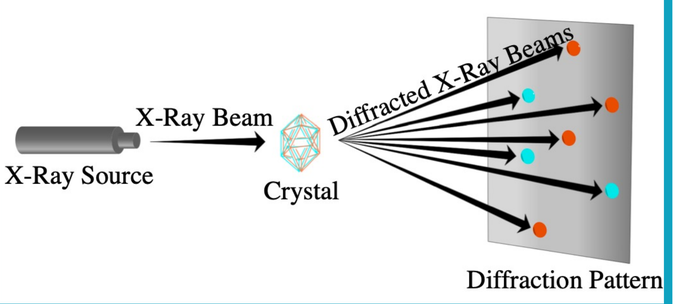
X-Ray Crystallography
- used to visualize structural features of biomolecules
- will provide atomic details of the HU-HJ interaction

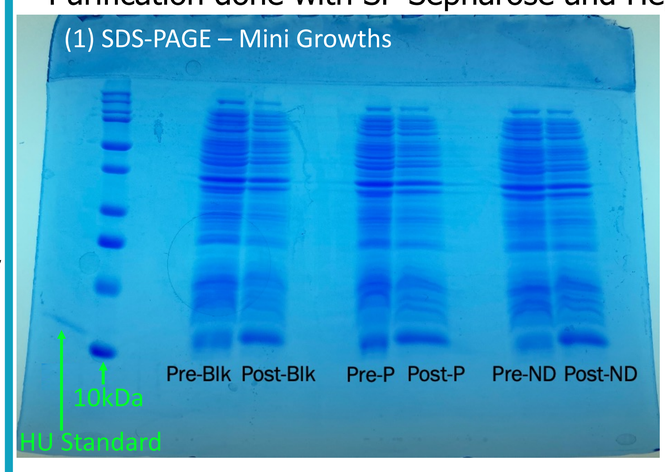


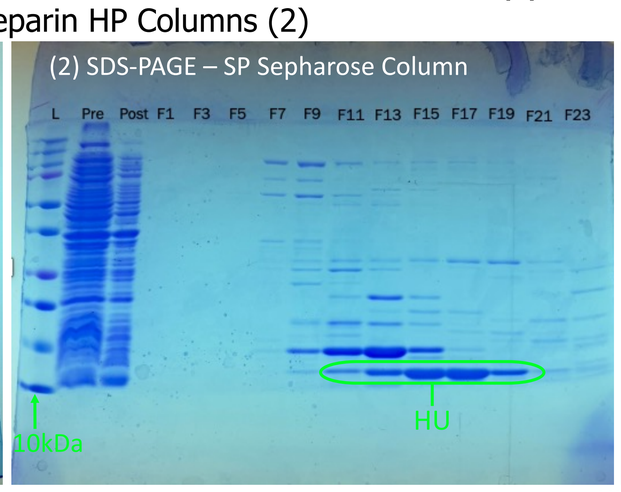

## Objectives

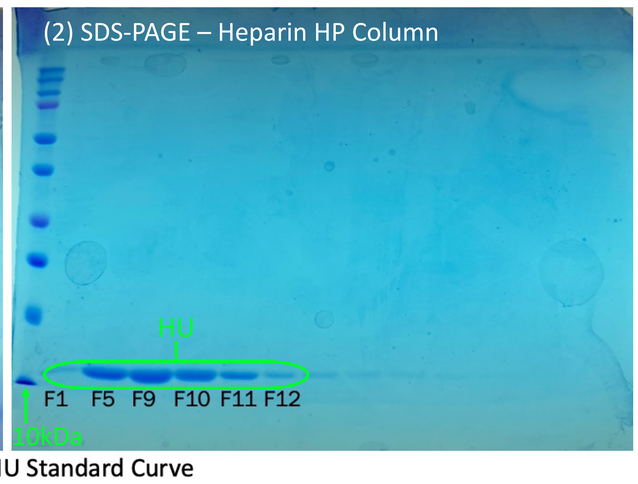
- Currently we are generating protein for crystal screens
- Assay protein to determine concentration for DNA binding activity
- Screen a wide range of conditions for trays
- Optimize screening conditions to generate diffraction-quality

## Methods & Results

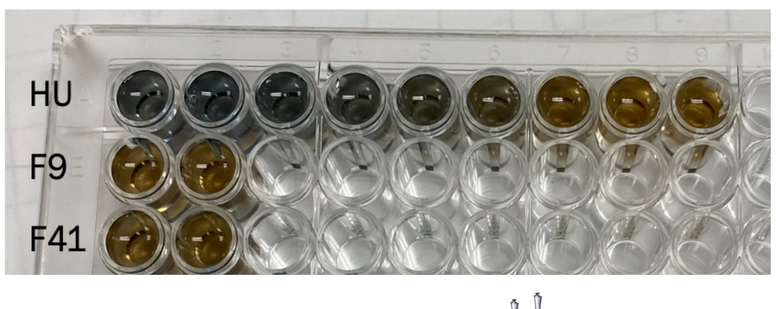
Purifying the HU Protein
- *E. coli* cells grown in Terrific Broth Media
- Pre-Induction, Post-Induction, and Fractions were run on 14% SDS-PAGE Gels (1)
- Purification done with SP Sepharose and Heparin HP Columns (2)



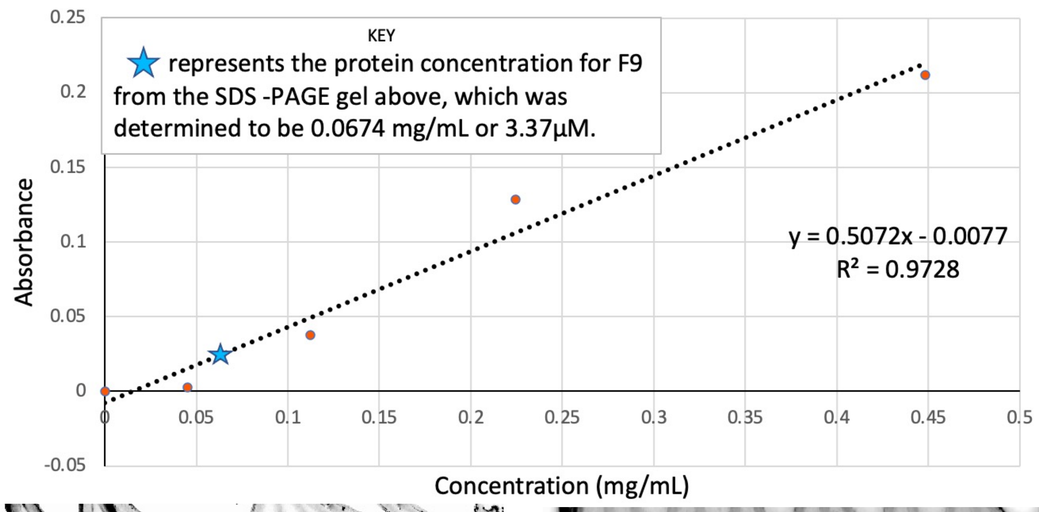



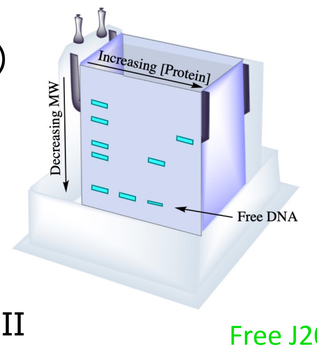

Bradford Assay
- used to determine protein concentration
- HU has no aromatic amino acids; absorption at 280nm cannot be used to determine protein concentration

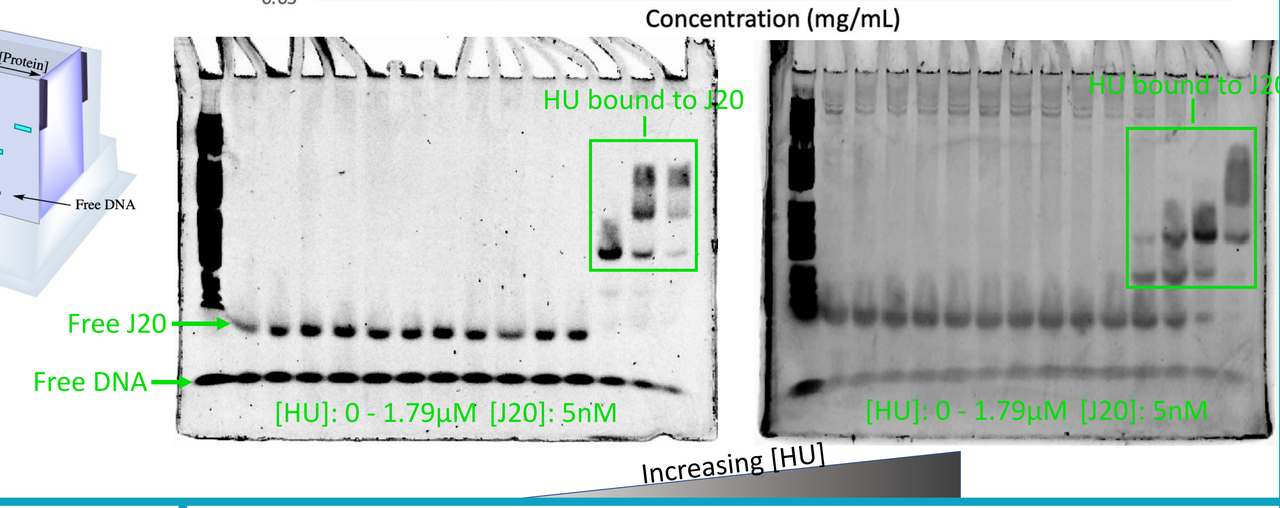



HU Standard Curve



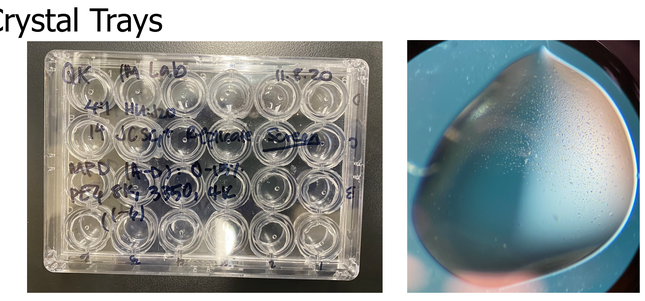

Gel Mobility Shift Assay (GMSA)
- used to determine protein binding activity
- HU-J20 complex has a higher MW and runs higher in gel
- HU binds to 4WJ with nanomolar affinity
- Lane 1: Ladder with pBRHaeIII digest
- 6.5% Native Gel



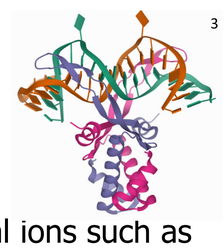




## Future Directions

Crystal Trays



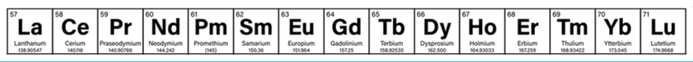
Determine stoichiometry of HU binding to J20
- Is HU binding to J20 2:1, 4:1, 1:1?



3

Try crystal screens with Anabaena HU
- Cyanobacteria
- Homodimer
- Crystal structure of AHU and DNA

Try crystal screens with different metal ions such as lanthanides
- Crystallophores, a part of the lanthanide family, have shown to promote crystal nucleation and solve the phase problem[1]




7

## Acknowledgements

I would first like to thank Dr. Mukerji for all her support, patience, and guidance throughout the school year and summer. I could not have asked for a better faculty research advisor. I would also like to thank my lab mates: Amy, Dan, Jack, and especially Zane for always answering my questions and being patient as I learned. Also, I would like to thank Ronnie and Dr. Taylor for all their support this summer and the endless hours they put into this program. I also want to thank the Ronald E. McNair Scholars Program for providing this opportunity and funding my project.

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7. Periodic Table Modified from Philip Harris

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