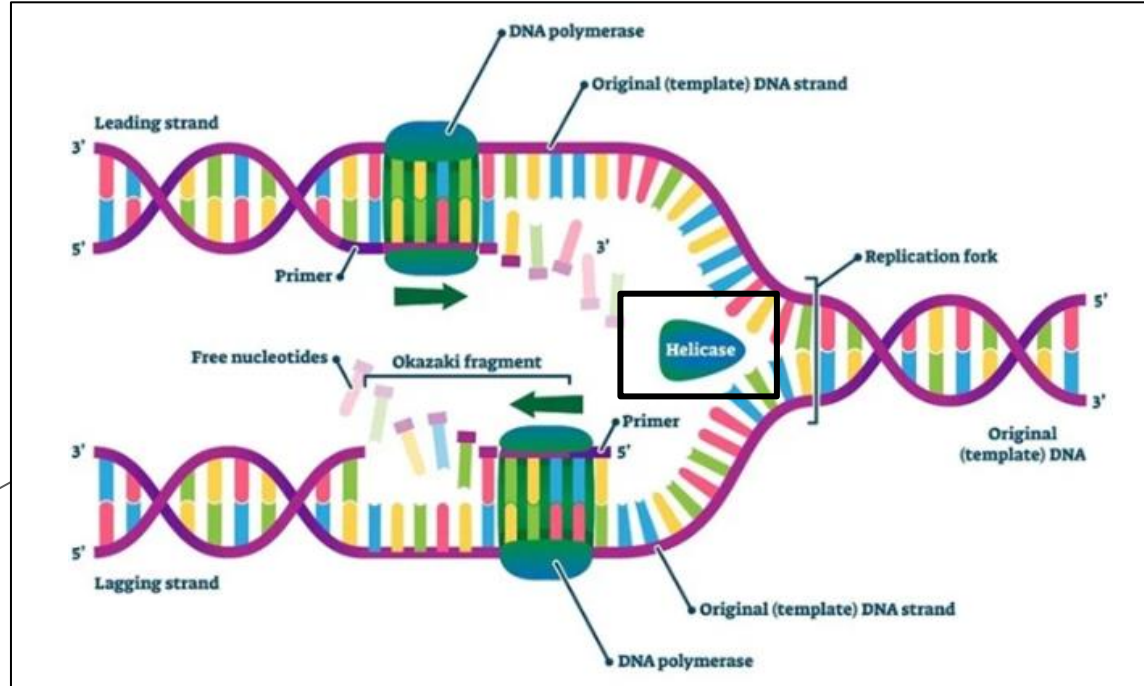
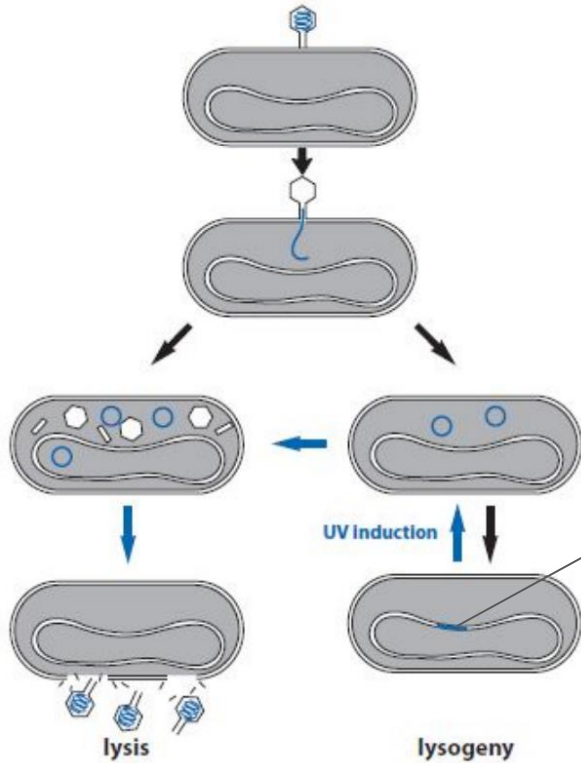


DnaJ and DnaK interactions with DnaB Helicase + λ P (BP) in *Bacteriophage* λ

By: Aya Sara Elwassif & Jefferson Estrada
B3 REU Summer 2023
Mentor: David Jeruzalmi

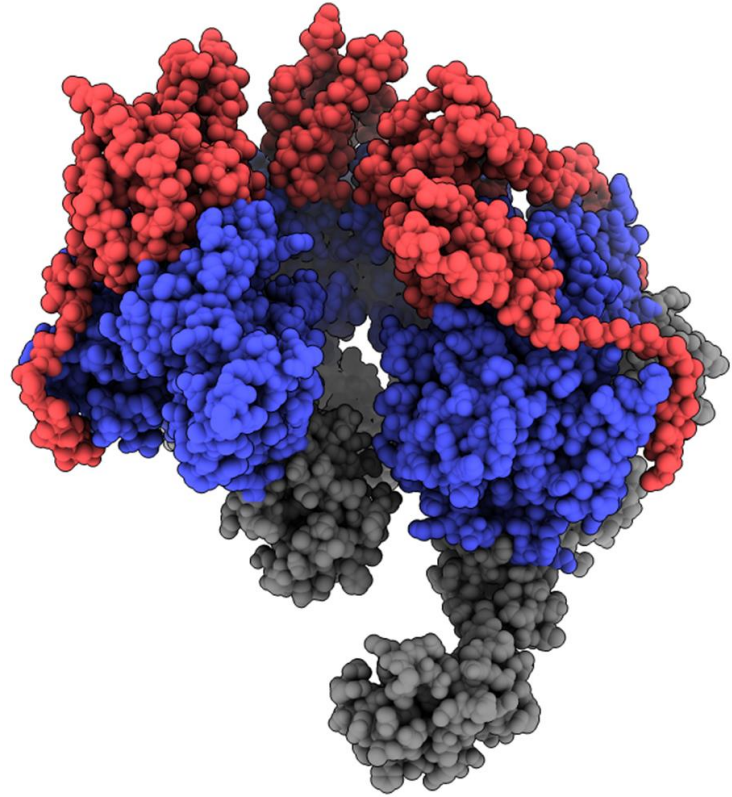
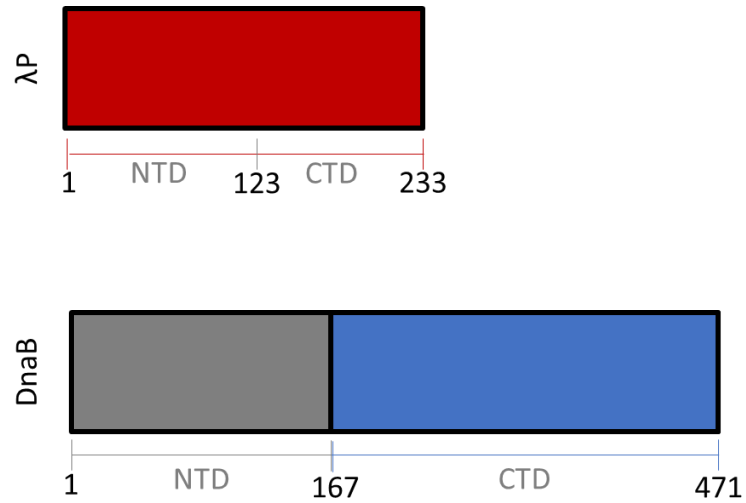
Bacteriophage λ - Two Pathways



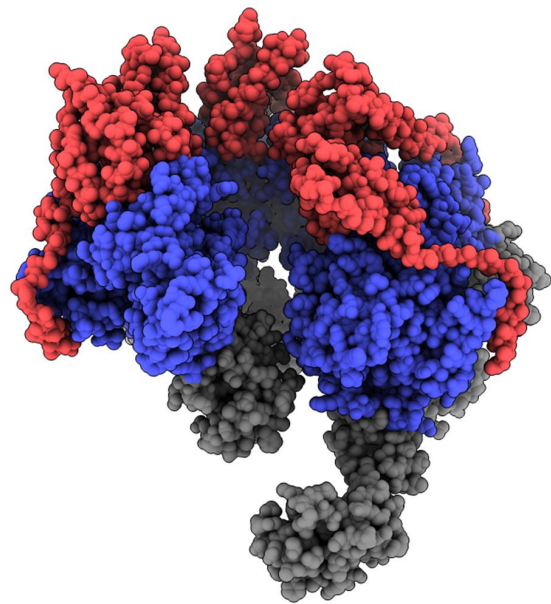
[2] (Mandal, 2022).

[1] (Ptashne, 2004)

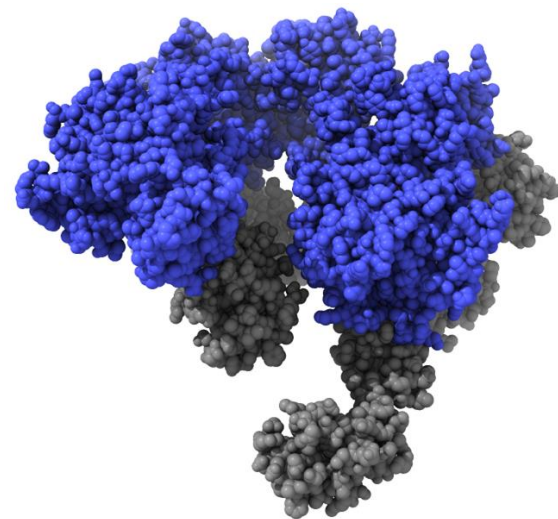
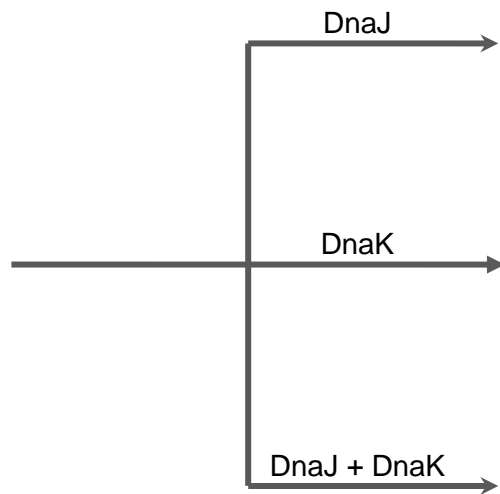
DnaB Helicase + λ P Complex



Experiment

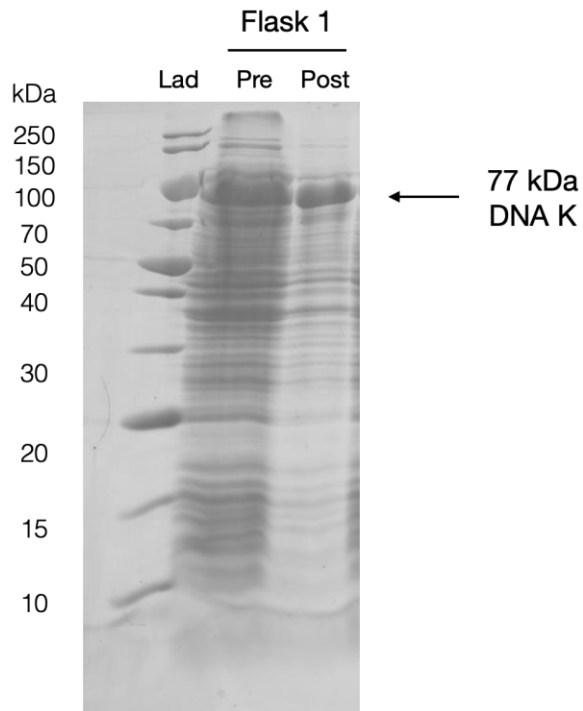


Open Helicase

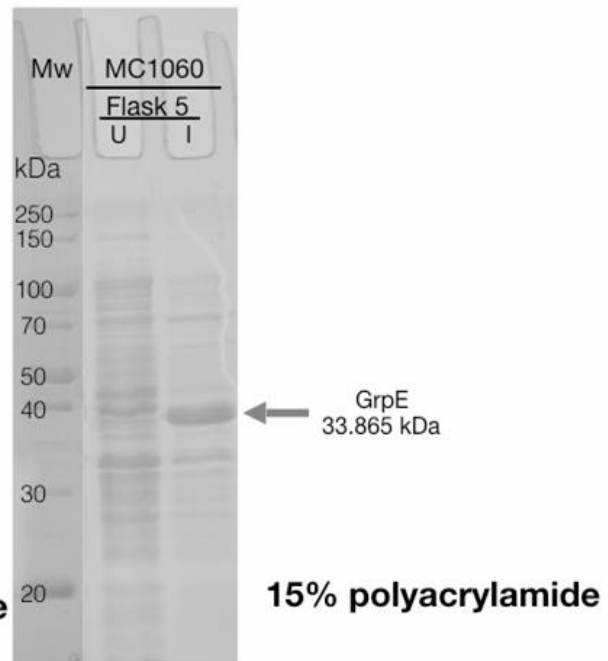
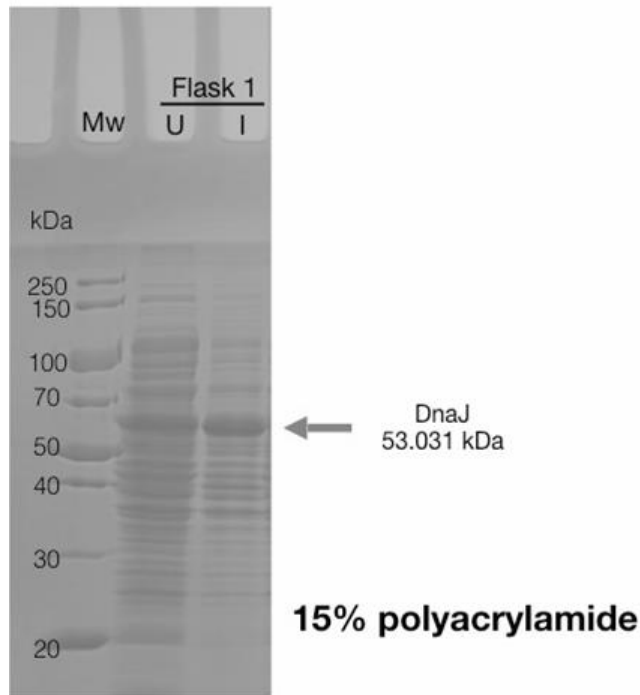


Closed Helicase

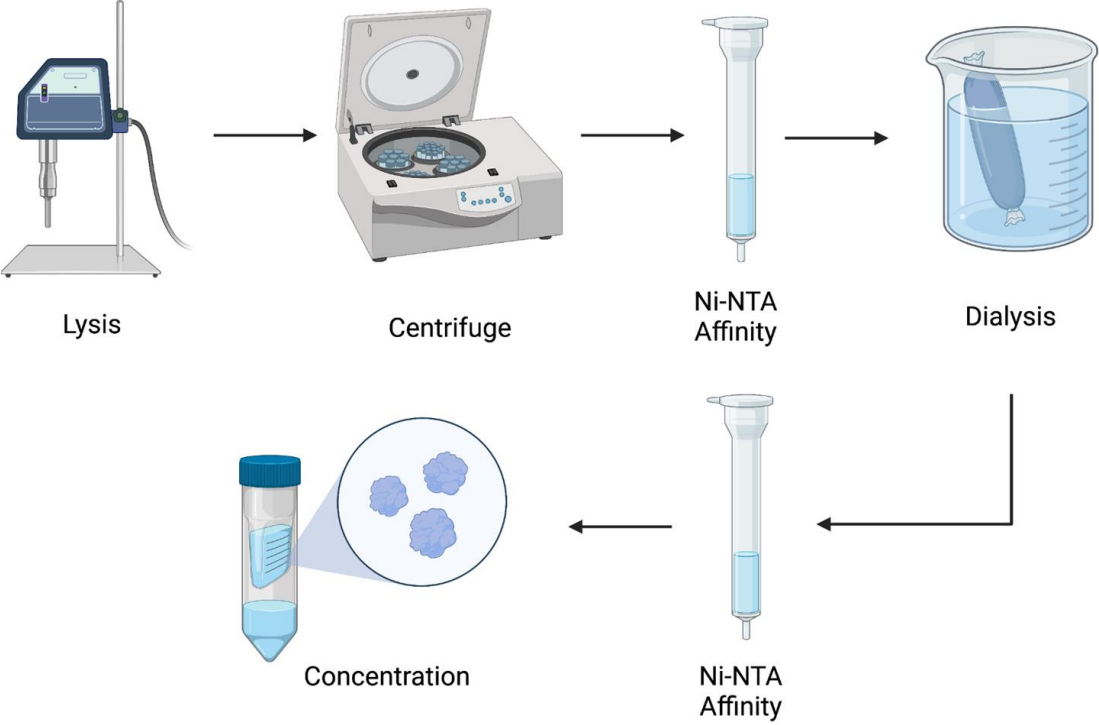
Growth/Induction



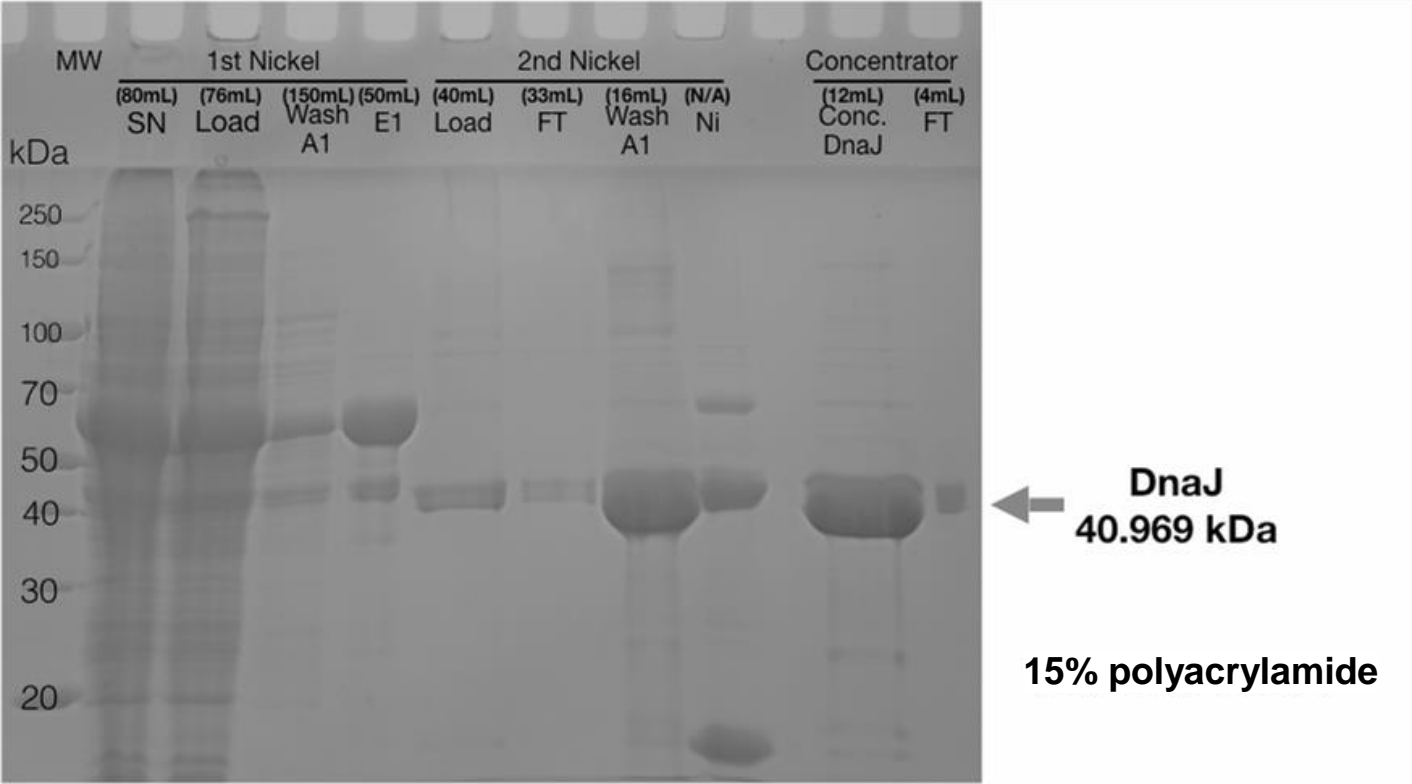
15% polyacrylamide



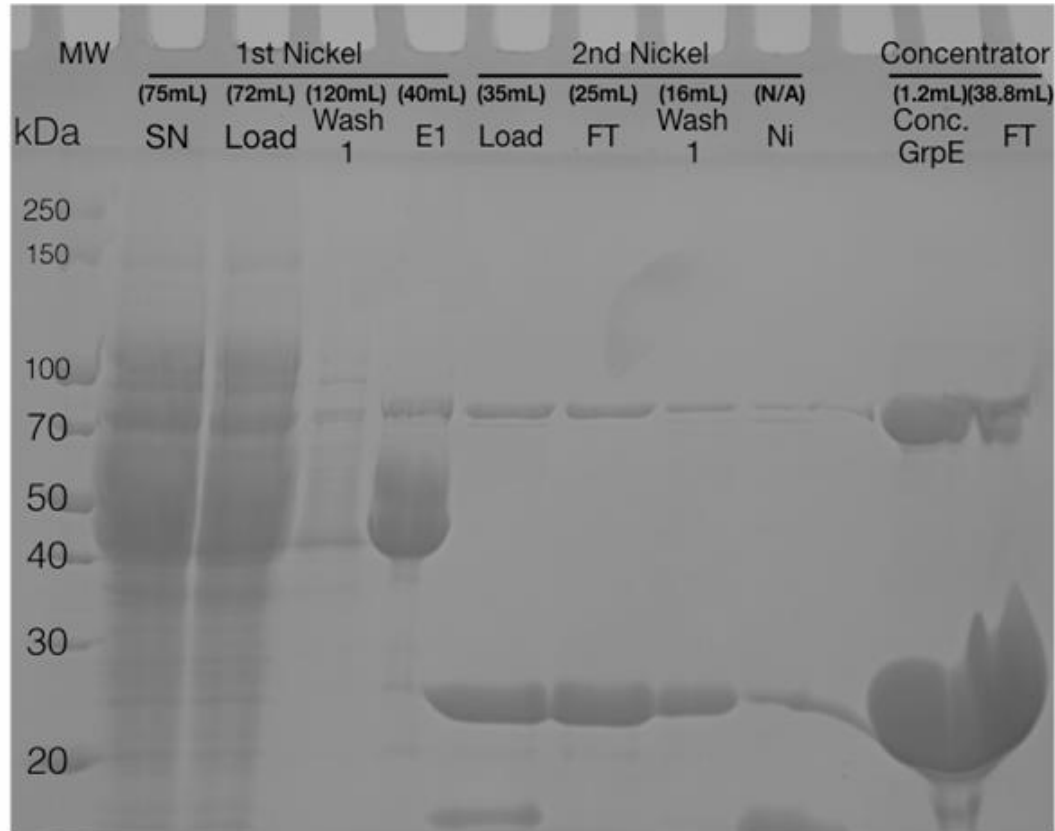
Protein Purification- DNA J & GrpE



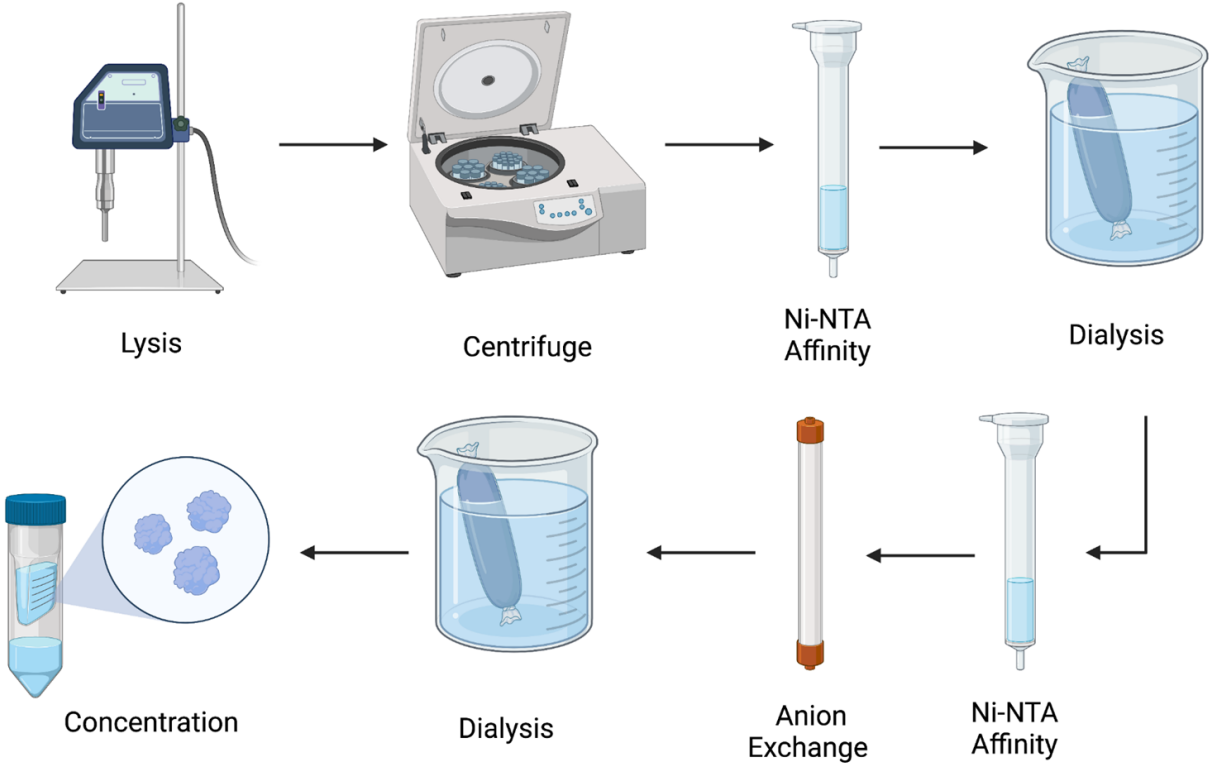
DNA J Summary Gel



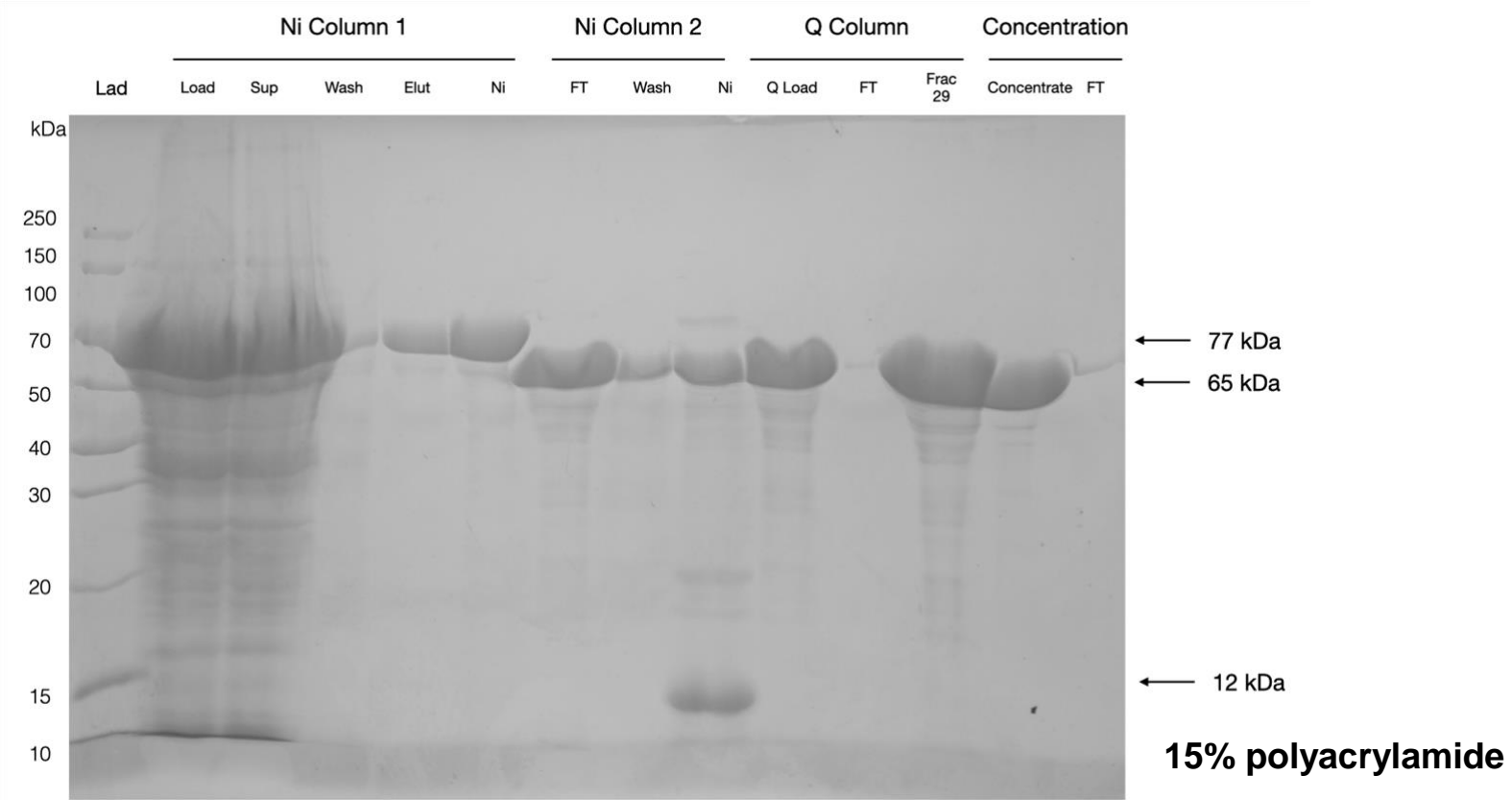
GrpE Summary Gel



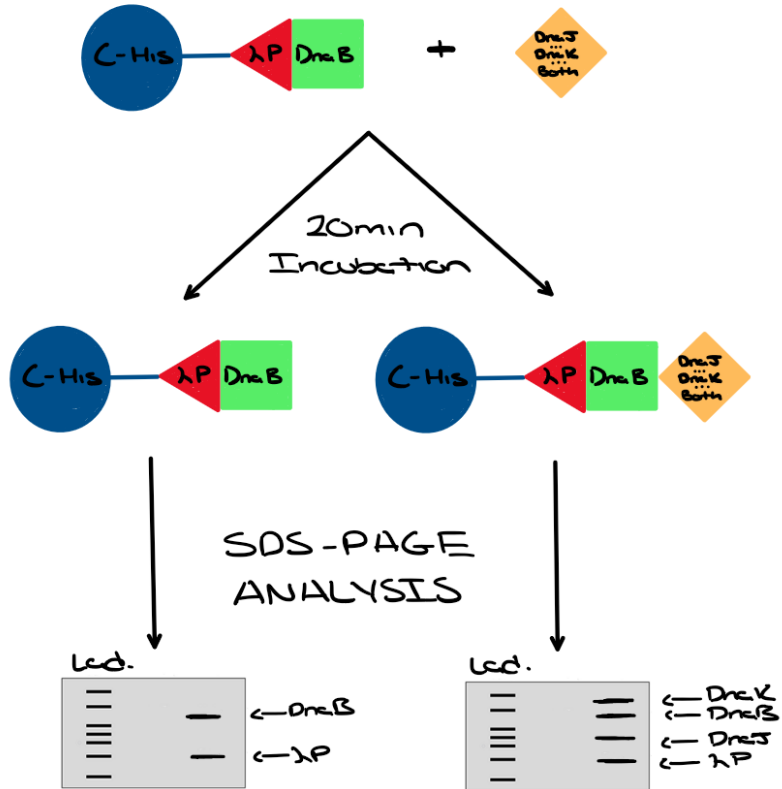
Protein Purification- DNA K



DNA K Summary Gel



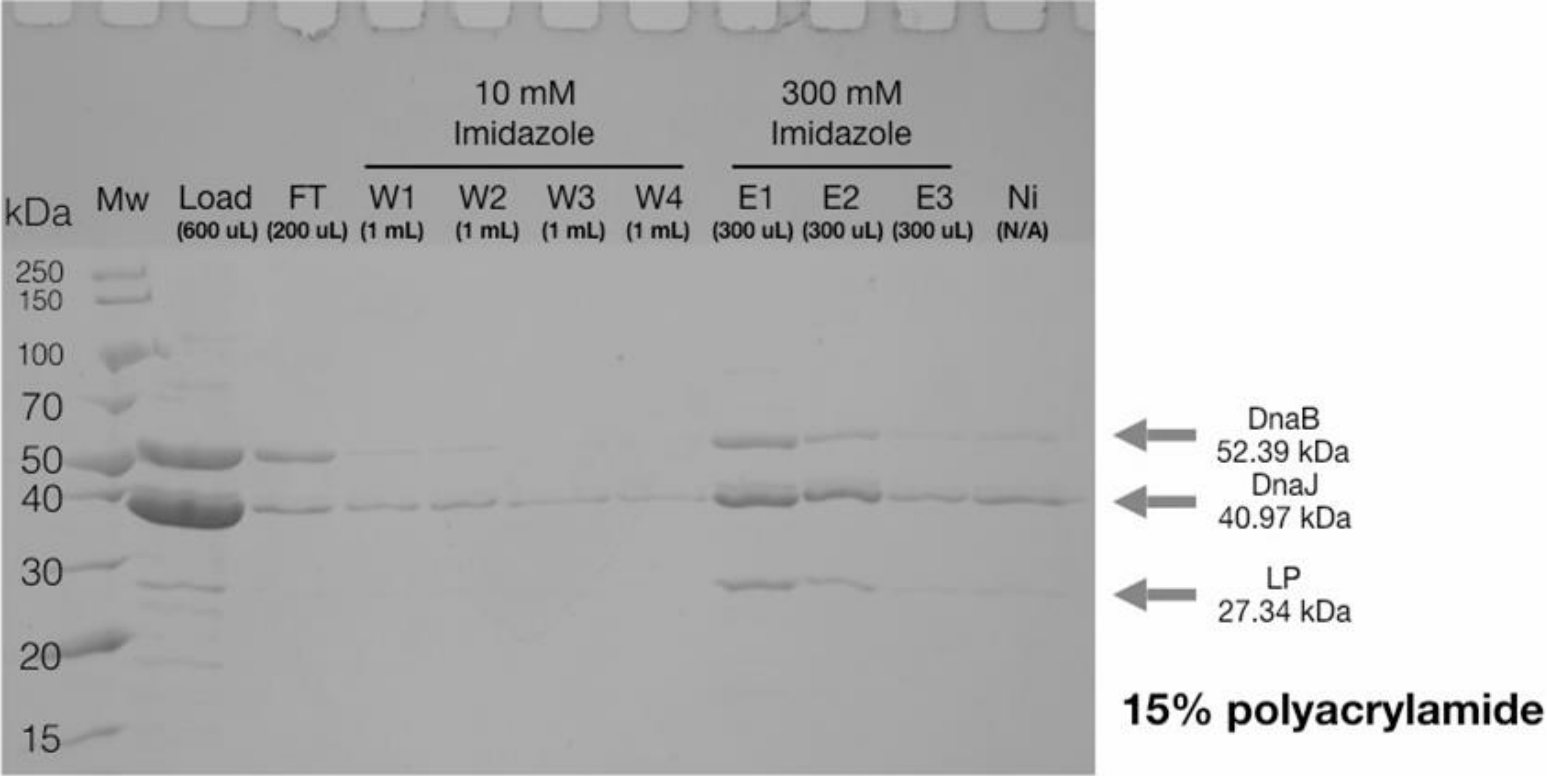
Pull-Down Explained



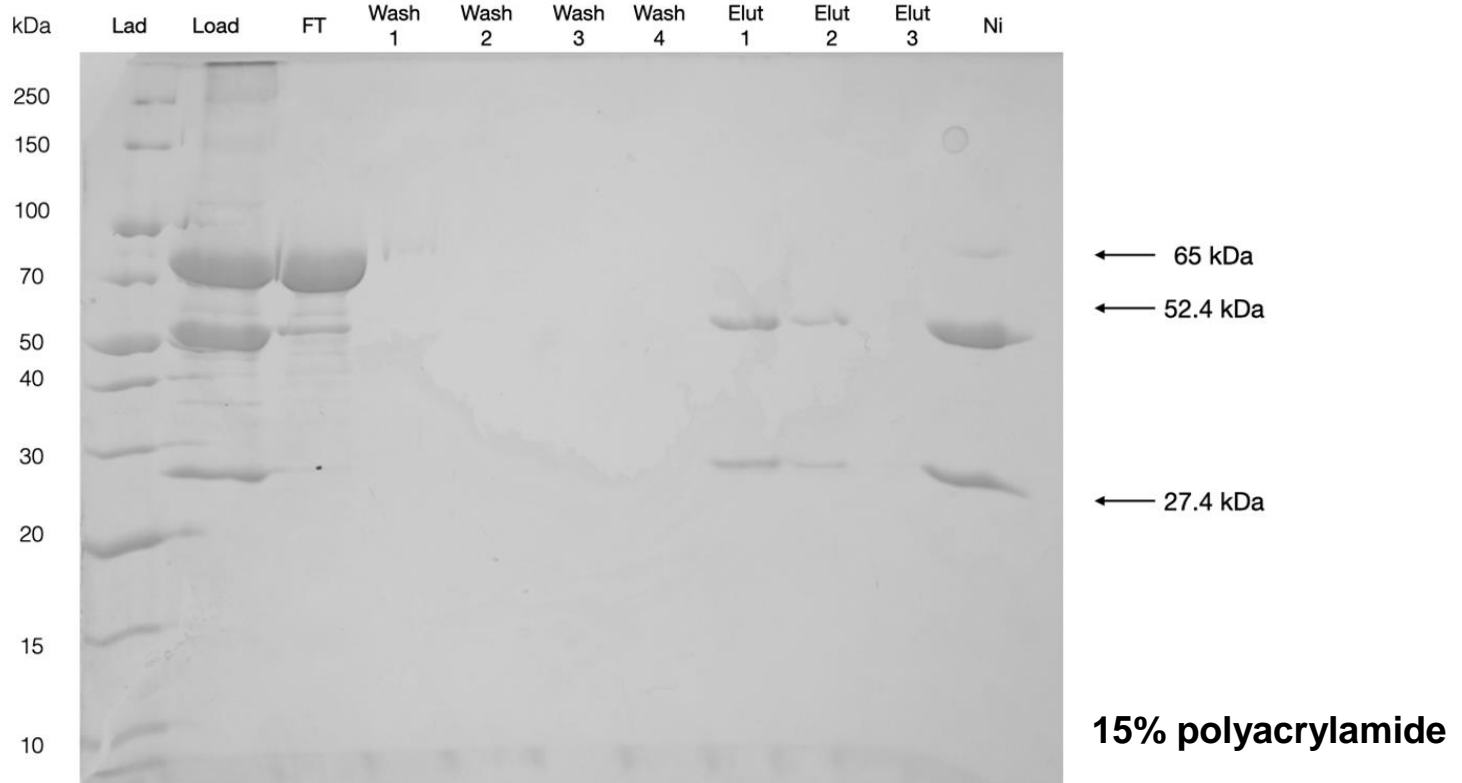
4 Washes (10 mM Imidazole)

3 Elutions (300 mM Imidazole)

BP CHis Tagged+ DNAJ Pull-Down



BP CHis Tagged + DNA K Pull-Down



BP CHis Tagged+ DNA K + DNA J Pull-Down



15% polyacrylamide

What's Next?

- Native mass spectrometry of BP-DnaJ to understand the stoichiometry of the complex
- Further pull downs with BP-DnaK, BP-DnaK-DNAJ and BP-DnaK-DNA followed by native mass spectrometry to understand the stoichiometry of the complexes
- Cryo-EM studies of BP-DnaK, BP-DnaJ, BP-DnaJ-DnaK-DNA to understand the three dimensional structure of the complex.

References

1. Ptashne, M. (April 2004). *A Genetic Switch*. Cold Spring Harbor Laboratory Press.
2. Mandal, Ananya. (2022, December 30). *What is DNA Polymerase?* www.news-medical.net/life-sciences/What-is-DNA-Polymerase.aspx.
3. Jillian Chase, Andrew Catalano, Alex J Noble, Edward T Eng, Paul DB Olinares, Kelly Molloy, Danaya Pakotiprapha, Martin Samuels, Brian Chait, Amedee des Georges, David Jeruzalmi (2018). Mechanisms of opening and closing of the bacterial replicative helicase. *eLife* 7:e41140.