Unwinding the Helicase Activity of *Thermus aquaticus* UvrD

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Introduction and Background

Background:
- DNA Mismatch Repair (MMR) is a repair system that contributes to genomic stability and the correction of mismatched base pairs during DNA replication.
- Errors within the MMR pathway are involved in the development of 93 types of human hereditary non-polypsis colon cancers.
- *Thermus Aquaticus* (Taq) is a thermophilic eukaryotic organism, which has a hybrid eukaryotic-prokaryotic MMR system.
- The protein UvrD from Taq may play a role in its MMR pathway and, from sequence analysis to be a helicase like UvrD of *E. Coli*, which has interactions with important MMR proteins.

Hypothesis: We theorize that if Taq UvrD is a thermophilic ATPase enzyme with a single-stranded binding site, then it will unwind DNA efficiently at high ATP concentrations and higher temperatures from the overhang side of a pre-assembled oligomer.

Significance: By better understanding of Taq UvrD’s abilities as a helicase in the context of MMR in a hybrid eukaryotic-prokaryotic context, we can further medical and pharmacological research in the treatment and detection of non-polypsis colon cancers.

![Figure 1. Overlayed Taq. UvrD (blue) and E. Coli. UvrD (green) with DNA (light blue) to show similarities in structures of the proteins.](image)

Conclusions + Future Directions

Conclusions
- **Our protein, Taq UvrD, cannot be characterized as a thermophilic helicase using our experimental conditions**
- In the helicase assays (figures 2 and 3) there was no significant unwinding difference when more protein or more incubation time was used.
- In the FRET assay (figure 4), we did not see a significant correlation between the decreasing emission and increased concentration of protein.
- In the ATPase assay, (figure 5) we did see more ATP hydrolysis with more protein which may indicate ATPase activity but not specific helicase activity.

In the future... more advanced purification techniques should be used to ensure that a functional protein can be used for experimentation.
- After purification, different substrates such as varying overhangs or G-quadruplexes should be tested to understand more about the mechanism of the protein in addition to different incubation temperatures.

Acknowledgements


*All graphics made in GraphPad Prism*  
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*Protein structures adapted from Pymol*