# Enzyme Kinematics of UvrD Helicase from *Thermoanaerobacter tengcongensis*

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

John Albright, Kopal Gupta, Isha Hawkins, Andrew Withrow, Sarina Jones, Noah Schomburg, Thomas C. Freeman, Jr.

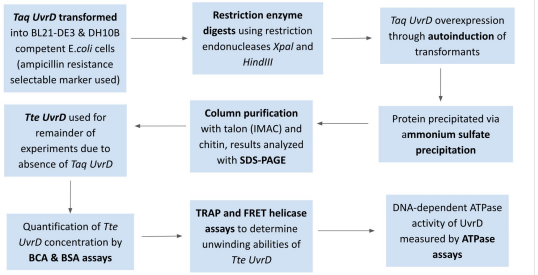
Department of Chemistry, College of Arts and Sciences, University of North Carolina, Chapel Hill, NC 27599

## Motivations

DNA mismatch repair (MMR) is a conserved cellular mechanism across bacteria and eukaryotes that serves as a safeguard to the genome by correcting base substitution and insertion-deletion mismatches. In eukaryotes, defective MMR proteins are the cause of many sporadic cancers such as colorectal, endometrial, and other types of gastrointestinal cancers (1), and understanding how these proteins function may lead to insights to the development of these cancers. While the methyl-directed MMR pathway in E. *coli* has been extensively studied, this pathway shows less homology to eukaryotic MMR as in other bacteria (2). For instance, there has not yet been identified a eukaryotic homolog to the E. *coli* UvrD, an ATP-dependent helicase enzyme, which serves to unwind DNA during MMR. Interestingly, other bacteria such as *Thermus aquaticus (Taq)* and *Thermoanaerobacter tengongenis (Tte)* share homology in their MMR mechanistic pathway to eukaryotes and have a homolog to E. *coli* UvrD, making this protein a prime candidate for studying potential differences in these pathways.

The UvrD helicase is a type of ATP-dependent enzyme that uses the energy of ATP hydrolysis to drive conformational changes associated with DNA unwinding. **Because the energy barrier to hydrolysis of ATP is lowered at higher temperatures, both the DNA-dependant ATPase activity and catalytic DNA unwinding activity of thermophilic *Taq* and *Tte* UvrD are postulated to be higher at their temperature optima than E. *coli* UvrD.**

## Methods




## Results

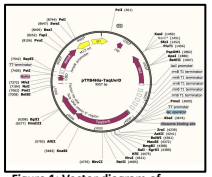


**Figure 1: Vector diagram of pTYB4His-TaqUvrD plasmid** containing *Taq* UvrD sequence, alongside HindIII and XbaI restriction sites.

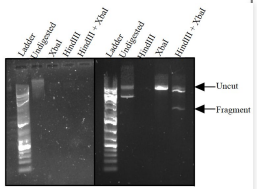



**Figure 2: Restriction enzyme digest** on agarose gel of amplified pTYB4His-TaqUvrD extracted from DH10β transformants.

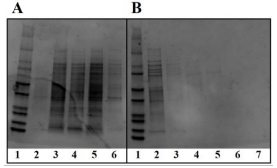



**Figure 3: SDS-PAGE of aliquots taken during ammonium sulfate precipitation (A) and column purification (B)**. Lane 1 corresponds to the protein ladder. (A) Lanes 2-6 correspond to the ammonium sulfate supernatant, induced insoluble, induced soluble, induced, and uninduced aliquots, respectively. (B) Lanes 2-7 correspond to the talon flow-through, talon wash, talon eluate, chitin flow-through, chitin wash, and concentrated chitin eluate aliquots, respectively.

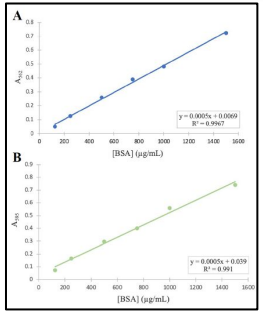



**Figure 4: Standard curves for the BCA assay (A) and Bradford assay (B)** used for *Tte* UvrD quantification. Absorbance values of standards were obtained at 562 nm and 595 nm respectively and plotted against known concentrations of BSA. Curves were used to calculate [*Tte* UvrD] which was found to be 60.2 μg/mL via the BCA assay standard curve and 88.5 μg/mL via from the Bradford assay standard curve.

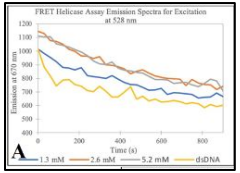



**Figure 5: Acceptor emission spectra at 670 nm for FRET helicase assay** reactions overlain with dsDNA control emission spectrum corresponding to excitation at 528 nm. Varying ATP concentrations used in the reaction are shown in figure legend.

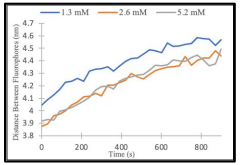



**Figure 6: Relative distance between fluorophores** Cy3 (donor) and Cy5 (acceptor) on oligos Cy3-3PA57-Atto488 and Cy5_3PA110 over time for FRET helicase assay reaction, as calculated via FRET efficiency and $R_0$ between fluorophores. Varying ATP concentrations used in the reactions are shown in figure legend

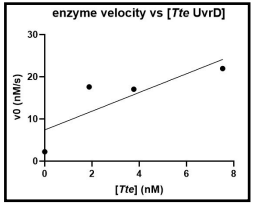



**Figure 7: Linear plot of enzyme velocity ($v_0$) vs. concentration of *Tte* UvrD helicase.** The slope of 2.20 $s^{-1}$ is equal to $K_{cat}$, a measure of enzymatic efficiency at a saturating level of substrate. Calculation of $K_{cat}/K_m$ (catalytic efficiency) yields 0.007 $s^{-1}uM^{-1}$, which indicates poor relative catalytic efficiency of *Tte* to *E. coli* (1.6 $s^{-1}uM^{-1}$).

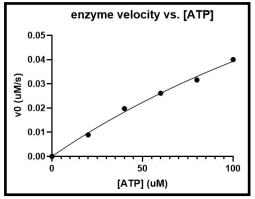



**Figure 8: Michaelis-Menten plot of enzyme velocity vs. concentration of ATP substrate.** $V_{max}$ = 0.1627 uM/s was estimated via extrapolation of the curve to its horizontal asymptote. The $K_m$ of 314.0 uM indicates poor affinity of *Tte* UvrD to its substrate compared to *E. coli* UvrD (46.7 uM)

## Conclusions

- *Tte* UvrD has dsDNA unwinding capabilities as shown through the FRET assay: acceptor emission decreased (Fig. 6) and fluorophore distance increased (Fig. 7) upon enzymatic incubation.
- *Tte* UvrD has ATPase activity at 65°C but has poorer substrate binding ($K_m$) and catalytic efficiency ($K_{cat}/K_m$) than *E. coli* UvrD at 37°C.
- Low catalytic efficiency may be due to ATPase assays not formed at optimal growth temperature of *Tte* (75°C) or conformational rigidity to withstand high temperatures
- Helped establish baseline helicase activity for thermophilic bacteria
- *Taq* UvrD was unable to be investigated due to procedural failures

## Future Directions

*Repeat FRET assay at lower ATP concentrations to establish activity-substrate relationship
*Repeat ATPase assays at the optimal growth temperature of *Tte* (75°C) with improved temperature control to obtain more accurate data
*Perform cross-linking experiments with DNA to determine crystal structure of bound *Tte* UvrD to uncover conformational changes
*Repeat experiments with *Thermus aquaticus* UvrD after controlling for procedural errors (i.e. plasmid verification after transformation)

## References

(1) Fukui, K. DNA Mismatch Repair in Eukaryotes and Bacteria. *Journal of Nucleic Acids*, **2010**, 2010, 260512.
(2) Hsieh et. al. DNA mismatch repair: Molecular mechanism, cancer, and ageing. *Mech. Ag. and Dev.* **2008**, 129, 391-407.
(3) Robert et. al. Mutations in Motif II of Escherichia coli DNA Helicase II Render the Enzyme Nonfunctional in Both Mismatch Repair and Excision Repair with Differential Effects on the Unwinding Reaction. *Journal of Bacteriology*, **1995**, 177, 5612-5621.

## Acknowledgements

We would like to thank Thomas Freeman, Sarina Jones, Noah Schomburg, and the UNC Chemistry Department for their guidance, contributions, and delivery of equipment and resources for this project.