Structural Investigation of the Conjugative Protein TraK

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Introduction

Increasing prevalence of antibiotic resistant bacterial infections contributes to financial and global public health issues. Antibiotic resistance spreads predominantly through conjugative plasmid transfer (CPT), the process in which bacteria transfer segments of their genetic material on plasmids to other bacteria. CPT is mediated by three main components: the relaxosome, which processes the DNA substrate; a Type IV Coupling Protein, which transports the DNA to the Type IV Secretion system (T4SS); and the T4SS, where the DNA is transferred from donor to recipient cell. The relaxosome contains a DNA binding integrative host factor to hold the DNA in place, a DNA processing relaxase to unwind the DNA, and various accessory proteins. The relaxosome of pCU1, a plasmid isolated from *Salmonella* Typhimurium, contains an accessory protein known as TraK. The structure, function, and activity of TraK are relatively unknown, but it may interact with other components of the relaxosome and components of the T4SS.

Methods

TraK, containing a CPD His tag, transformed into *E. coli* cells, were grown in LB media.

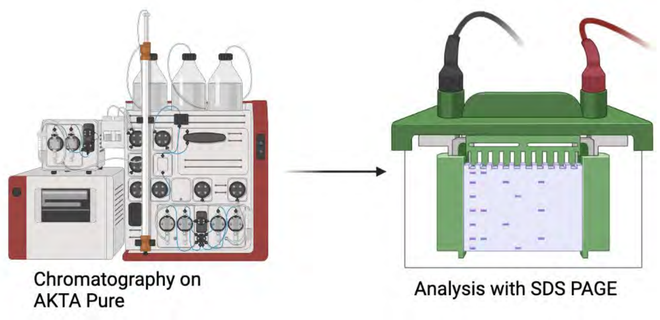

Figure 1: TraK Purification and Purity Analysis. After induction, clarified lysate containing TraK was run on the AKTA Pure for nickel affinity and size exclusion chromatography. SDS PAGE was used to analyze purification results.

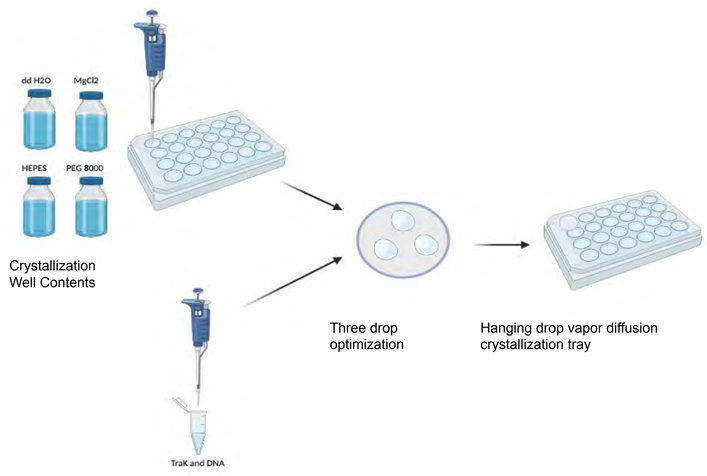

Figure 2: TraK Crystal Trays. Purified TraK with oriT DNA was used in hanging drop crystal trays.

Results

Purification: TraK was successfully purified to ~89% purity

Figure 3A.

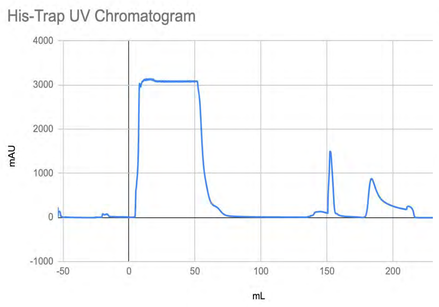

Figure 3B.

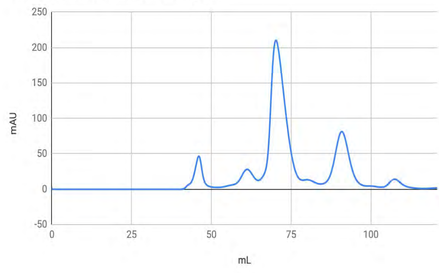

Figure 3A: His-Trap UV Chromatogram. This is a UV chromatogram from the AKTA Pure His Trap column used for Nickel Affinity Chromatography. The TraK peak is around 155 mL and the CPD peak is around 185 mL.

Figure 3B: Gel Filtration UV Chromatogram. This is a UV chromatogram from the AKTA Pure S200 Gel Filtration used for Size Exclusion Chromatography. The TraK peak is around 70 mL (corresponds to lane 10 in Figure 4) and the CPD peak is around 90 mL (corresponds to lane 3 in figure 4).

Figure 4.

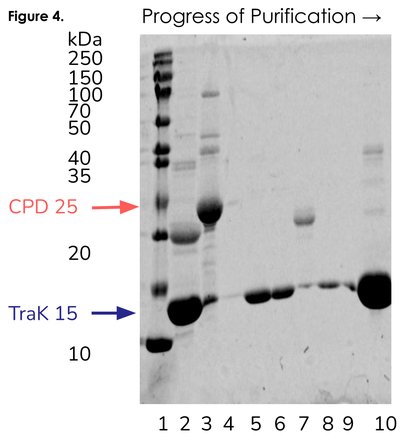

Figure 4: TraK Purification SDS Gel. Lane 1 contains a DNA ladder (left). **Lane 3** corresponds to the last step of nickel affinity chromatography.The band at 25 kDa indicates that the CPD his tag was cleaved from TraK. **Lane 10** contains the final sample of TraK, around its monomer molecular weight of 15 kDa. This, in addition to the presence of few other bands, indicates a high level of purity, a successful purification.

Crystallization: TraK was successfully crystallized bound to DNA

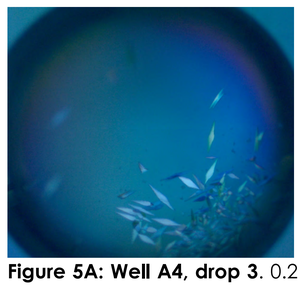

Figure 5A: Well A4, drop 3. 0.2 mm crystals.

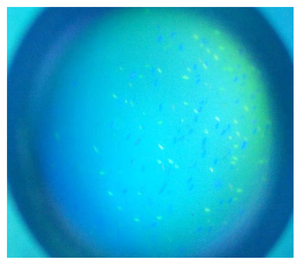

Figure 5B: Well C1, drop 2. 0.1 mm crystals.

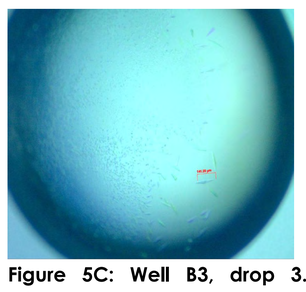

Figure 5C: Well B3, drop 3. Microcrystals.

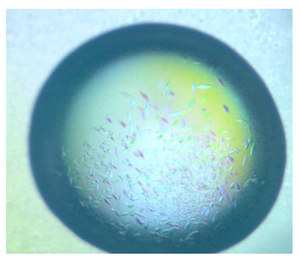

Figure 5D: Well A6, drop 3. 0.2 mm crystals.

Figure	PEG 8000	HEPES	$MgCl_2$	Observation time
5A	1.75%	0.08M	0.03M	24 hours
5B	4%	0.18M	0.03M	24 hours
5C	3%	0.10M	0.02M	24 hours
5D	3%	0.09M	0.03M.	24 hours

Future Directions

- Analyze TraK footprinting data to find where TraK binds to the pCU1 oriT plasmid DNA
- Crystallize TraK with selenomethionine substituted for methionine to solve the phase problem of crystallography
- Collect x-ray data for both the native and selenomethionine crystals in order to elucidate the structure of TraK

References

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