Purification, crystallization and functional analysis of the green algae (*Chlamydomonas reinhardtii*) ArsA1 enzyme

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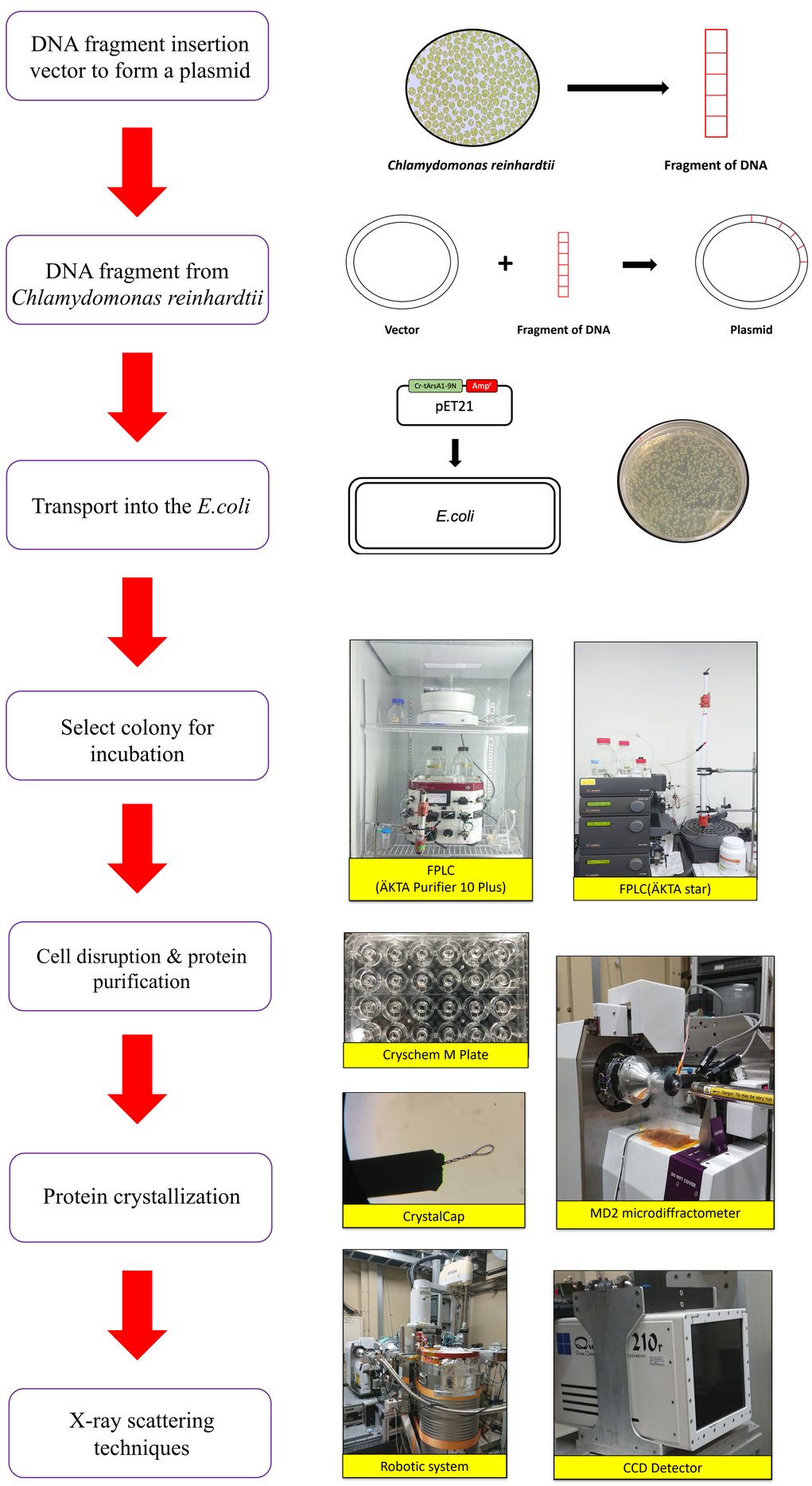
Abstract

In previous studies, the protein structure of *Chlamydomonas reinhardtii* Cr-tArsA1 has been successfully resolved. Interestingly, we discovered the unique interlocking hook structure of Cr-tArsA1. According to our Cr-tArsA1 crystal structure, the N-terminal α-helix 9 located near the top of the interlocking hook motif interaction interface is likely to act as a selective gate.

We mutated the N-terminal α-helix 9 of Cr-tArsA1 and obtained the protein Cr-tArsA1-9N by expression in *E. coli*. Finally, through purification, crystallization and functional analysis experiments. We obtained protein crystals of Cr-tArsA1-9N, and proved that Cr-tArsA1-9N obviously lost the selectivity of TA protein compared with Cr-tArsA1.

Method and Materials



Result

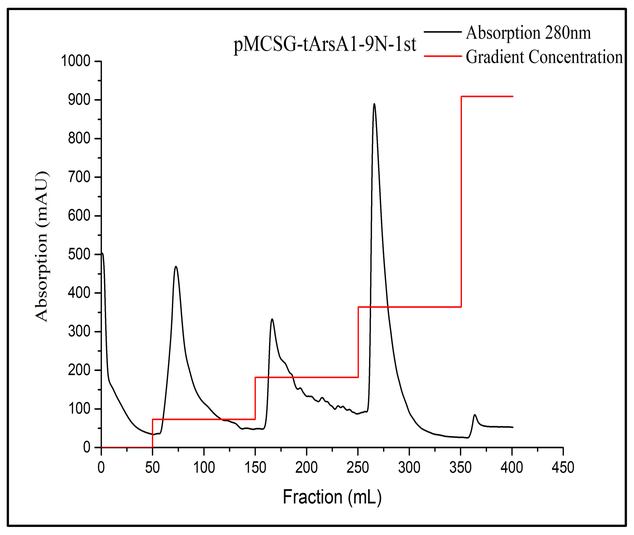


Figure 1. The first column purfifcation

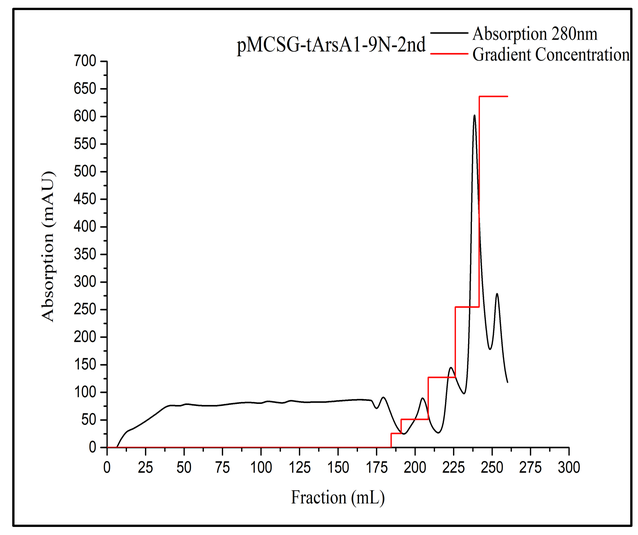


Figure 2. The second column purfifcation

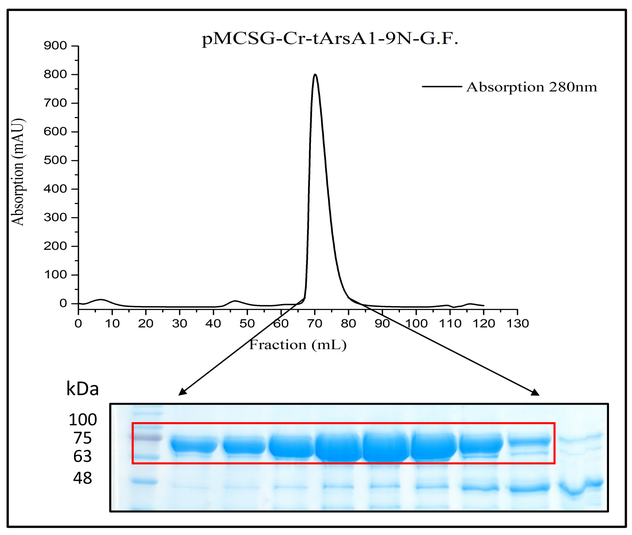


Figure 3. The Gel Filtration chromatography

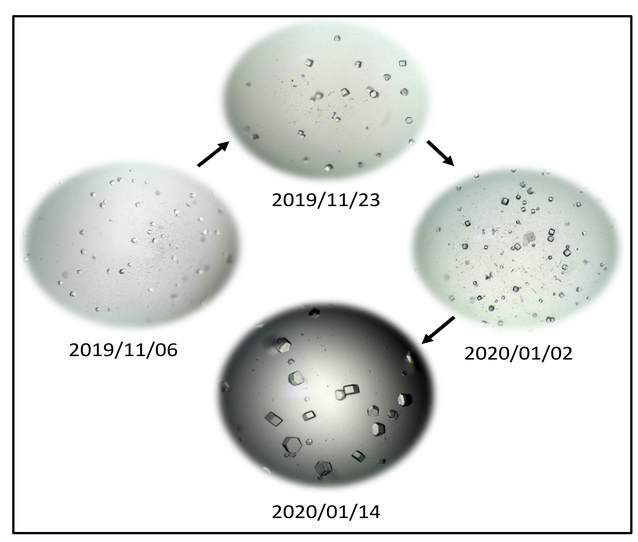
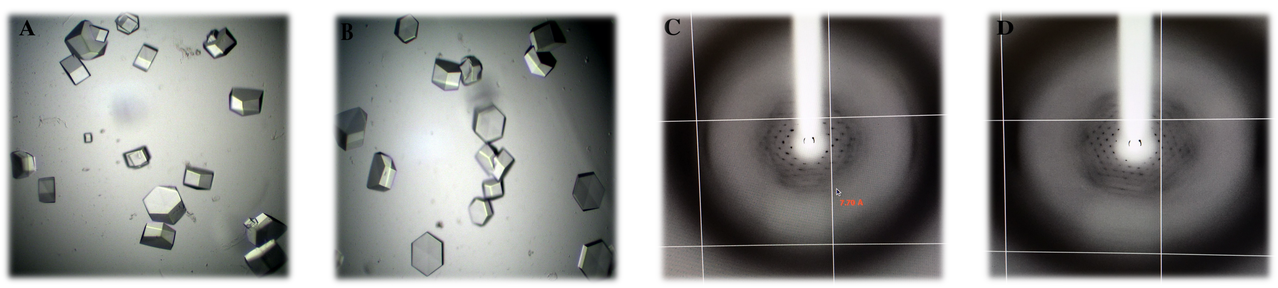


Figure 4. Process of crystallization



A & B : crystal of Cr-tArsA1-9N

C : X-ray diffraction pattern of Cr-tArsA1-9N （0˚）

D : X-ray diffraction pattern of Cr-tArsA1-9N （90˚）

Conclusions

1. The protein Cr-tArsA1-9N was successfully purified and Cr-tArsA1-9N crystals were obtained.
2. Previous studies suggest that Cr-ArsA1-9N lacks selectivity for different TA protein substrates compared to Cr-tArsA1, but our crystal structure has not been resolved, so the functional analysis of Cr-tArsA1-9N is unclear.

References

1. Chang HY, Hsiao CD, Chen CC et al. Structural analysis of chloroplast tail-anchored membrane protein recognition by ArsA1. *Plant J.* 2019 Jul;99(1):128-143.