Structural Investigation of Conjugative Protein Complexes

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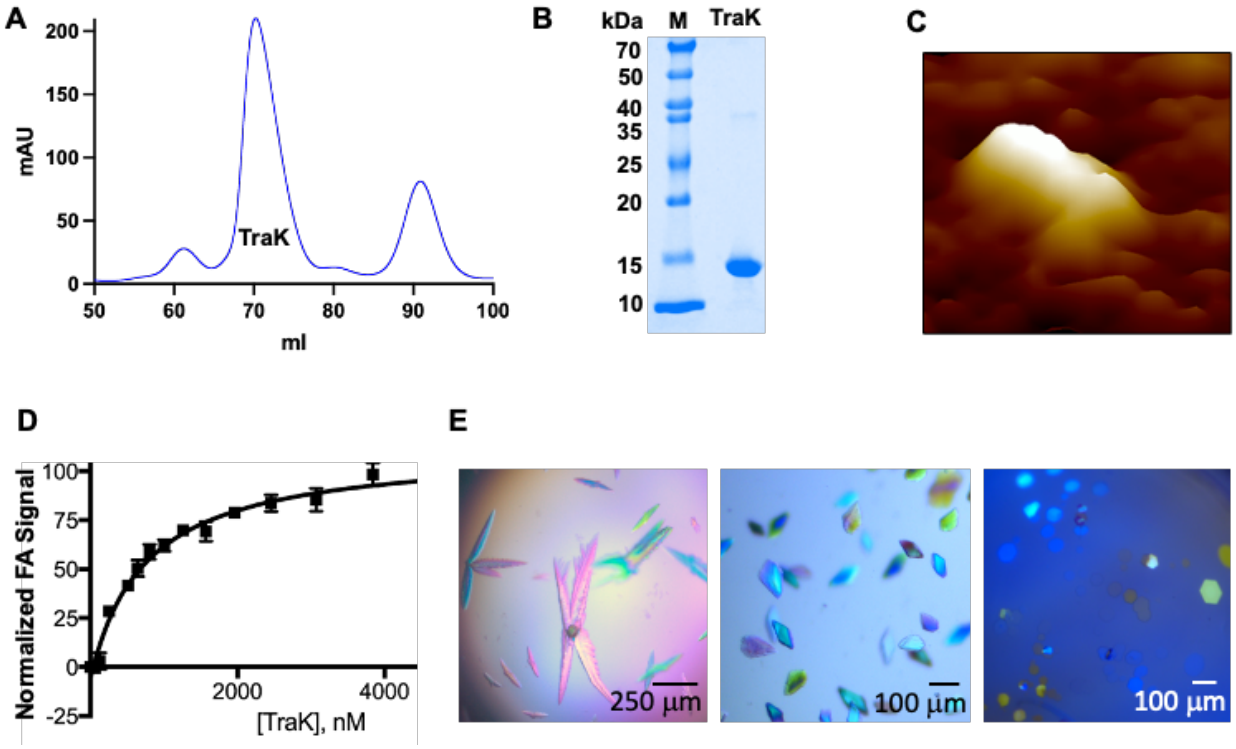
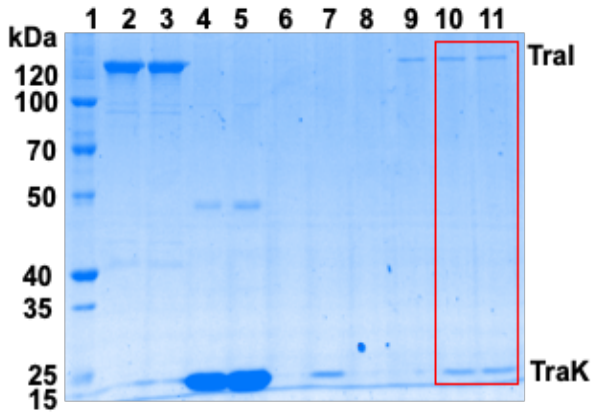


Fig. 1 pCU1 TraK Preliminary Data. (A) TraK purification gel filtration data with peak labeled and (B) SDS-PAGE showing purity. (C) AFM image of TraK depicting example of one TraK molecule corresponding to a tetramer by volume analysis. (D) Fluorescence anisotropy (FA) data showing TraK binding to region of pCU1 *oriT* with nanomolar affinity (726 nm). (E) Three examples of crystals grown with TraK bound to DNA.



Lane	Contents
1	Ladder
2	TraI Application (Bait Control)
3	TraI Application Wash
4	TraK Application Wash (Sample 1)
5	TraK Application Wash (Sample 2)
6	2nd Wash (Control)
7	2nd Wash (Sample)
8	*empty*
9	Bait Control Elution
10	Sample 1 Elution
11	Sample 2 Elution

Fig. 2. TraK and TraI Bind in Pulldown Assay. SDS-PAGE showing results of a pulldown assay. Purified His-tagged TraI was applied to Ni^{2+} affinity resin (Lanes 2-3). The TraI bound resin was separated into three: control where no TraK was added, and two samples where TraK was added individually at two different concentrations (Lanes 4-5). Representative washes for the control and a sample are shown in Lans 6 & 7. Elution with imidazole from the control shows only TraI (Lane 9) while TraK and TraI elute together in the samples (Lanes 10-11, red box). TraK did not have any affinity tags attached.