MS03-P24 | Preliminary Diffraction Experiments On The E.Coli Toxin Sat

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Background: Sat belongs to the serine protease autotransporter of *Enterobacteriaceae* (SPATEs). This family of proteins is normally associated with virulence of Gram-negative pathogens. Sat function has been studied specifically in Uropathogenic *Escherichia coli* strains (UPEC) responsible for urinary tract infections. **Results:** The Sat construct with the proteolytic activity abolished (S256I-S258A) from *E. coli* strain HB101 was used for overexpression in LB broth medium. The supernatant of the cell culture was incubated with ammonium sulfate (1.7 M) for protein precipitation (Overnight at 4 ^{0}C) and the pellet thus obtained was re-suspended in 20 mM Tris-HCL, 5 mM EDTA, pH 7.5, desalted and equilibrated using 6-8 KDa molecular-weight-cutoff (MWCO) dialysis membrane. Subsequently, 50 ml of this solution was loaded onto a preequilibrated Q-sepharose anionic exchange column equilibrated with 20 mM Tris-HCL, 5 mM EDTA, pH 7.5. A second chromatography purification was performed using size exclusion Superdex200 10/300. The process of the purification was monitored by SDS-PAGE (4-20 %). The crystals were obtained by sitting drop method and measured at beamline 19-ID at APS, Detector distance was 1000 mm, wavelength 0.9729 A. A data set was taken with 1 sec exposure for each image and 0.333 degree oscillation, in total 540 images. The unit cell has been determined to belong to the space group F432 with a=b=c=485 A and alpha=beta=gamma 90 degree. Currently the resolution is limited to 9.5 A.