ITSN2

PDB:4IIO

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:N/A
Entry Clone Source: Chemically synthesized, originally from Sachdev Sidhu Lab at University of Toronto
SGC Clone Accession:YTC002-C07
Tag:N-terminal hexa-histidine tag , removed for crystallization
Host:BL21-V2R-pRARE2

Construct

Prelude:
Sequence:

ggmaqgalLKAQALCSWTAKKDNHLNFSKHDIITVLEQQENWWFGEVHGGRGWFPKSYVKIIPaaa

Vector:pHH0239, a modified pGEX vector, the GST tag is replaced by a His6 tag.

Growth

Medium:
Antibiotics:
Procedure: The target protein was expressed in E. coli by inoculating 50 mL of overnight culture (grown in Luria Bertani medium) into 2 L of Terrific Broth medium containing 100 ug/mL Ampicillin and 34 ug/mL Chloramphenicol. The culture was grown in the LEX system (Harbinger BEC) at 37 °C. When the OD600 reached ~2.0, the temperature of the medium was lowered to 18 °C and the culture was induced with 1 mM IPTG. The culture was grown overnight and cells were harvested by centrifugation, then flash frozen in liquid nitrogen, and stored at -80 °C.

Purification

Procedure
The clarified lysate was mixed with 5 mL of 50% slurry of Ni-NTA beads (Qiagen) and incubated at 4 ºC on a rotary shaker for 1 hour. The mixture was then centrifuged at ~700 x g (2000 rpms) for 5 min and the supernatant was discarded. The beads were washed with 50 ml Binding Buffer and 50 ml of Washing Buffer. The protein was eluted with 10 ml of Elution Buffer. The eluted protein was mixed with TEV (home source) at 1:1 molar ratio and the mixture was subjected to dialysis (1:400 v/v) with Dialysis Buffer overnight at 4 ºC. The dialyzed sample was mixed with

1 ml of 50% slurry of Ni-NTA beads (Qiagen) and incubated at 4 ºC on rotary shaker for 1 hour. The sample was then centrifuged at ~700 x g (2000 rpms) for 5 min and only the supernatant was collected. It was further purified on a home-packed 26/60 Superdex-75 gel filtration column that was pre-equilibrated with Gel Filtration Buffer. The flow rate was 2.5 ml/min. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter (3 kDa molecular weight cut off). The purity of the protein preparation was greater than 95% as judged by SDS-PAGE.

Extraction

Procedure
Frozen cells from 4L TB culture were thawed and re-suspended in 250 ml Extraction Buffer. Cells were lysed by 10 minutes ten second pulses sonication on ice at 120W with ten second break between each pulse. The lysate was clarified by centrifugation at ~38000 x g (16,000 rpms) for 1 hour.
Concentration: The stock concentration was 30 mg/ml.
Ligand
Peptide ligand (chemically synthesized) WRGSLSYLKGPL**MassSpec:**M.W. of the tag intact protein was measured to be 10381.0 Da, expected 7352.4 Da. Measured value for the tag removed version was 7352.6 Da.
Crystallization: Peptide was disolved in gel filtration buffer to a 10 mM concentration.Protein was incubated with 5x peptide (molarity ratio) overnight at 4 degC, and the mixture was used to set up crystallization trials.

Crystal was initially obtained from Redwing screen condition D02.Crystal used for structure refinement was grown in Redwing screen condition D02, i.e. in 25 % PEG3350, 0.1 M (NH4)2SO4, and 0.1 M Tris, pH 8.5, in sitting drop setup, using 0.5 uL protein mixture plus 0.5 uL well solution against 90 uL reservoir buffer at 18 degC. Crystals grow to a mountable size within three days. Cryo: 0.86V well solution + 0.14V 100% EG.
NMR Spectroscopy:
Data Collection:
Data Processing: