DNMBP

PDB:4GLM

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-C10
Tag:N-terminal hexa-histidine tag , removed for crystallization
Host:BL21-V2R-pRARE2

Construct

Prelude:DNMBP:T246-P301
Sequence:

ggaaqpamaqgalTYGVALYRFQALEPNELDFEVGDKIRILATLEDGWLEGSLKGRTGIFPYRFVKLCPaaa

Vector:pHH0239, a modified pGEX vector, the GST tag is replaced by an 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 µg/mL Ampicillin and 34 µg/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 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 elute 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: Protein yield was 5 mg from per liter culture (20 mg in total). The stock concentration was 33 mg/ml.
Ligand
MassSpec:M.W. of the tag intact protein was measured to be 10381.0 Da, expected 10380.8 Da. Tag removed version not measured. Expected MW of the tag-removed protein was 7744.9 Da and the measured value was 7745.0 Da.
Crystallization:120uL of protein stock solution was mixed with 30uL 20mM (theoretically calculated concentration, the peptide is not well soluble) putative binding peptide DIPQFWFRGVFY solution before setting up crystallization.
Crystal was initially obtained from SGC-I screen condition B10 and G9.Crystal used for structure refinement was grown in SGC-II screen condition B10, i.e. in 25% PEG 8000, 0.2 M NaCl, and 0.1 M Tris 8.5, in sitting drop setup, using 0.5 uL protein plus 0.5 uL well solution against 90 uL reservoir buffer at room temperature.Crystals grow to a mountable size within three days. Cryoprotectant used mixture of 0.9V well solution plus 0.1V 100% EG.
NMR Spectroscopy:
Data Collection:
Data Processing: