UMPS

PDB:2JGY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC000364
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m)
Host:BL21(DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmELSFGARAELPRIHPVASKLLRLMQKKETNLCLSADVSLARELLQLADALGPSICMLKTHVDIL
NDFTLDVMKELITLAKCHEFLIFEDRKFADIGNTVKKQYEGGIFKIASWADLVNAHVVPGSGVVKGLQEVGLPLHRGCLLIAEMSST
GSLATGDYTRAAVRMAEEHSEFVVGFISGSRVSMKPEFLHLTPGVQLEAGGDNLGQQYNSPQEVIGKRGSDIIIVGRGIISAADRLE
AAEMYRKAAWEAYLSRLG

Vector:PNIC-BSA4

Growth

Medium:TB
Antibiotics:
Procedure:MB4933:
750 ml TB media supplemented with 50 µg/mL kanamycin was inoculated with 20 ml of the overnight cultures. The large scale cultivations were grown in tunair flasks 37°C until OD600 reaches approximately 1.8-2. The flasks were then moved to 18°C and induced with 0,5 mM IPTG after 1 hour. Cultures were allowed to grow overnight at 18°C.

Purification

Procedure
Columns:Columns:
Ni column: HiTrap IMAC HP
GF column:HiLoadÂ 16/60 Superdex 200 Prep Grade

Purification was performed on an ÄKTAprime. Prior to purification, columns were equilibrated with IMAC Bind/Wash1 Buffer (HiTrap Chelating) and Gel filtration buffer (Superdex 200). The

protein sample was loaded on the HiTrap Chelating column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with IMAC Elution Buffer and loaded in the Gel filtration column.

Extraction

Procedure
Harvest by centrifugation, rotor F8S-4X 1000y (=SLC-6000) at 5000 rpm for 10 minutes in 4°C and freeze the pellet at -80°C. Prior to purification the cell pellet was resuspended in Lysis Buffer. Cells were disrupted by sonication (4s on 4 off 3 min 80% amplitude) and samples were centrifuged for 20 min at 20500 rpm. The soluble fraction was filtered through 0.2 µm and subjected to further purification on the ÄKTAprime.
Concentration:23 mg/mL
Ligand
MassSpec:
Crystallization: The 23 g/L stock solution was diluted 1:1 to 11.5 g/L using the gel filtration buffer and mixed 0.1+0.1 µL with well solution consisting of 30% (w/v) PEG 3350 and 0.1 M HEPES pH 7.5 using a Phoenix crystallization robot from Art Robbins Instruments at 20ºC and then left in 4ºC for equilibration. A single crystal appeared somewhere in between day 3 and day 12 and was frozen in cryo solution consisting of 20% PEG3350, 20% glycerol and 0.1M Hepes pH 8.4.The product containing crystal was obtained in a drop of 0.2 µL protein+0.1 µL well solution consisting of 16% (w/v) PEG 3350 and 0.1 M HEPES pH 7.6. The crystal was soaked 15 minutes in a solution of 22% PEG3350, 0.1M HEPES pH 7.5, 20% Glycerol, 10mM uridine 5Â□-monophosphate (UMP) before being flash cooled in liquid nitrogen.
NMR Spectroscopy:
Data Collection: For the apo-crystal X-ray data in space group P212121 (59.8, 77.8, 152.6, $\alpha = \beta = \gamma = 90$) were collected at MaxLab (i911-2) and processed using XDS and XSCALE. The crystal soaked in UMP had the same space group and very similar cell dimensions; these data were collected at ESRF (id29) and processed using Mosflm and Scala of the CCP4 package to a resolution of 2 Å.
Data Processing: The apo structure was solved by molecular replacement using MOLREP with the Escherichia coli structure (1DQW) as our search model. The initial solution was then auto(re)built in ARP/WARP, which was possible due to the high quality of the 1.95 Å data. For both the apo and the UMP complex structure the final cycles of model building were performed in COOT with REFMAC5 as the refinement program.