FXR1

PDB:3KUF

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC028983
Entry Clone Source:MGC: AT42-B4
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgrenlyfq*g.
Host:*E. coli* BL21(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgAELTVEVRGSNGAFYKGFIKDVHEDSLTVVFENNWQPERQVPFNEVRLPPPPDIKKEISEGDEVEVYSR
ANDQEPCGWWLAKVRMMKGEFYVIEYAACDATYNEIVTFERLRPVNQNKTVKKNTFFKCTVD

Vector:pET28-MHL

Growth

Medium:M9 SeMET growth media (Medicilon Inc).
Antibiotics:
Procedure:A fresh transformation was used to inoculate 20 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol . The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to innoculate 2L of M9 SeMET growth medium. The culture was grown in LEX at 37°C to OD600 of 2.3. Methionine biosynthesis inhibition and IPTG-based induction were carried out according to the manufacturer's protocol. The temperature was reduced to 14°C and the culture was incubated for a further 18 hours before harvesting the cells.

Purification

Procedure
Column 1: Affinity purification.
open Ni-NTA column Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads by rocking. After 1 hour incubation at 4ºC, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20mL EB.

Column 2: Size Exclusion, HiLoad 16/60 Superdex 75
Prep Grade Procedure: The eluent from from the NiNTA column was concentrated and loaded onto the size exclusion column in at 1 mL/min, fraction size 2mL. The fractions containing protein were identified on a SDS-PAGE gel.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes) and samples were centrifuged for 60 min at 70000 g.
Concentration:10 mg/ml.
Ligand
MassSpec:
Crystallization:1.4M (NH4)2SO4, 0.1 M HEPES pH 6.8, 0.25M NaCl, 10 mM DTT by sitting-drop vapour diffusion, using micro seading after equilibration for 3 hours. The drop was prepared by mixing 1 microL protein with 1 microL of reservoir solution. Protein concentration is 7.25mg/ml. Crystals appear after a period of 2 days.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using mother liquor containing 20% glycerol, and flash frozen in liquid nitrogen. Diffraction data were collected APS 19-ID to 2.75 Å.
Data Processing: