NR1D2

PDB:3CQV

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_005117
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal His tag with integrated TEV protease site:
MHHHHHHSSGRENLYFQG
Host:*E. Coli* BL21-Gold(DE3)pLysS (Stratagen)

Construct

Prelude:
Sequence:

SSPPSSDFAKEEVIGMVTRAHKDTFMYNQEQQENVPIDGFSQNENKNSYLCNTGGRMHLVCPMSKSPYVDPHKSGHEIWEEFSMSFT
PAVKEVVEFAKRIPGFRDLSQHDQVNLLKAGTFEVLMVRFASLFDAKERTVTFLSGKKYSVDDLHSMGAGDLLNSMFEFSEKLNALQ
LSDEEMSLFTAVVLVSADRSGIENVNSVEALQETLIRALRTLIMKNHPNEASIFTKLLLKLPDLRSLNNMHSEELLAFKVHP

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure: Rev-erbβ was expressed in E. Coli BL21-Gold(DE3) in selenomethionine medium in the presence of 50 µg/ml kanamycin, 50 µg/mL chloramphenicol and 12.5 µM hemin (Sigma). Cell were grown at 37 degC to an OD600 of 1.2 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 25 °C.

Purification

Procedure
Following centrifugation of sonicated cell lysate, protein was purified from clarified supernatant using Ni-NTA affinity chromatography. Once loaded, the column was washed with 300ml of buffer containing 30 mM imidazole, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP and 50 mM Hepes pH 7.5. Elution of purified Rev-erbβ from the column was done using an equivalent buffer containing 250 mM imidazole. Protein was dialysed overnight into a buffer containing 500 mM NaCl, 0.5 mM TCEP and 50 mM Hepes pH 7.5.

Extraction

Procedure
Cells were harvested by centrifugation at 8,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 °C. In preparation for purification, the cell paste was thawed, resuspended in lysis buffer (5 mM imidazole, 500 mM NaCl, 0.5 mM TCEP, 5% glycerol 50 mM Hepes pH 7.5) and sonicated on ice (3 second intervals) for 5 min.
Concentration:17 mg/ml.
Ligand
MassSpec:
Crystallization: Rev-erbβ was crystallized using the hanging drop vapor diffusion method at 18 °C by mixing 2 µl of the protein solution with 2 µl of the reservoir solution containing 1.6 M Ammonium sulfate, 0.1 M Na Hepes pH 7.6, 4% Jeffamine M-600. Partial proteolysis of Rev-erbβ was performed in the crystallization drop by adding a 1:2000 ratio (v/v) of trypsin (1.5 mg/ml) to the protein.
NMR Spectroscopy:
Data Collection:
Data Processing: