RNF2

PDB:3H8H
Entry Clone Accession:BC012583
Entry Clone Source: Mammalian Gene Collection (rnf2.BC012583.MGC.AU54-E8.pDNR-LIB)
SGC Clone Accession:
Tag:N-terminal tag: MHHHHHHSSGRENLYFQG
Host:BL21
Vector:pET28-MHL

Sequence:
mhhhhhhssgrenlyfqgMDGASEIELVFRPHPTLMEKDDSAQTRYIKTSGNATVDHLSKYLAVR
LALEELRSKGESNQMNLDTASEKQYTIYIATASGQFTVLNGSFSLELVSEKYWKVNKPME
LYYA

Growth

Procedure: Competent E.coli BL21 CodonPlus cells were grown in 2 L of LB media at 37 degC to OD600=1.0, then cells were induced with 1mM IPTG for 10 hrs.

Purification

Procedure: Cleared cell lysate in Lysis Buffer was loaded onto a 5 mL TALON metal-affinity resin column equilibrated in Wash Buffer at room temperature. The column was washed with 100 mL of Wash Buffer and the protein was eluted with 30 mL of Elution Buffer. Upon addition of 1 mg of TEV protease, 2 mM DTT and 2 mM EDTA, the eluant was incubated for 10 hrs at 4 °C. The protein was further purified by gel filtration on a HighLoad Superdex 75 column equilibrated with Gel-filtration Buffer. Fractions containing protein were pooled and concentrated using Amicon Ultra centrifugal filter with 3kD cutoff membrane to a final concentration of 8 mg/ml.

Structure Determination

Crystallization: Crystals were grown in hanging drops by mixing 1.5 µl protein solution (7 mg/ml) with 1.5 µl Crystallization Buffer (2.0 M ammonium sulfate, 2% PEG 400, 0.1M Hepes, pH 7.5) at 18 °C. For cryoprotection, the crystals were soaked in 2 M ammonium sulfate, 2% PEG 400, 25% Glycerol and 0.1 M Hepes, pH 7.5.

Data Processing: Data from crystals of a selenomethionine derivative of the Ring1B (res. 220-330) were collected on beamline 19-ID of the Advanced Photon Source at the selenium peak wavelength, and processed using the HKL-2000 program suite. Solve and Resolve were used to locate the selenium substructure and to build the initial model. A native data set was collected on a home-source Rigaku FR-E and used for the final refinement of the structure. Automatic model building using ARP/wARP, was followed by iterative manual model building aided by the graphics program Coot, and TLS and restrained refinement using REFMAC, leading to a final model with an Rwork of 0.20 and an Rfree of 0.24, for data from 28.27-2.00 angstroms. Initial parameters for TLS refinement were obtained from the TLSMD server.