RNF31

PDB:4JUY
Entry Clone Accession:BC012077
Entry Clone Source:MGC
SGC Clone Accession:YTC010-F07
Tag:N-terminal His6-tag, not removed. Cleavage motif visible in crystal structure
Host:BL21-V2R-pRARE2

Construct

Prelude:RNF31:M1-D180
Sequence:

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mhhhhhhssgrenlyfqgMPGEEEERAFLVAREELASALRRDSGQAFSLEQLRPLLASSLPLAARYLQLDAARLVRCNAHGEPRNYL
NTLSTALNILEKYGRNLLSPQRPRYWRGVKFNNPVFRSTVDAVQGGRDVLRLYGYTEEQPDGLSFPEGQEEPDEHQVATVTLEVLLL
RTELSLLLQNTHPRQQALEQLLED
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Vector:pET28-MHL

Growth

Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 30 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 ug/mL kanamycin at 37 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 16 degree and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (7,000 rpm 15min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure
The lysate was centrifuged at 16,000 rpm for 60 minutes and the supernatants were loaded onto 5 mL Talon metal-affinity resin column (ClonTech). The column was then washed 3 times each with 25 mL washing buffer. Bound proteins were eluted using 25 mL elution buffer. Pooled fractions giving a total approximate volume of 25ml were then diluted to 100 ml using Q Buffer A and injected into Q column. Protein was eluted using a linear gradient of 0-100% Q Buffer B over 20 column volumes, and eluted at ~40%~50% Buffer B. Fractions containing the target protein were pooled and concentrated using Amicon Ultra-15 centrifugal filter (mwco 10 kDa). The purity of the protein is >95% which was confirmed by SDS-PAGE.

Extraction

Procedure
Pellet from 4L cell culture was resuspended in a total volume of 200 ml lysis buffer and the cells disrupted by sonication.
Concentration:27.0 mg/mL measured by nanodrop, using absorbance coefficiency: Abs(280)=0.703
Ligand
MassSpec:uncut version native protein expected 22645.4, measured 22645.9.

Crystallization:RNF31 was crystallised by vapor diffusion at 18°C from a hanging drop consisting of 1.5ul protein (27.0 mg/ml) and 1.5ul well solution containing 0.8M Potassium sodium tartrate tetrahydrate, 0.1M Tris pH8.5, 0.5% W/V Polyethylene glycol monomethyl ether 5,000. The crystal was transferred to a cryo protectant containing the mother liquor and 20% glycol before flash-cooling in liquid nitrogen.