RNF168

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Tag:N-terminal tag: MGSSHHHHHHSSGLVPRGS
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMALPKDAIPSLSECQCGICMEILVEPVTLPCNHTLCKPCFQSTVEKASLCCPFCRRRVSSWTRYHTRR
NSLVNVELWTIIQKHYPRECKLRASGQESEEVADDYQPVRLLSKP

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure: Competent BL21 (DE3) cells (Invitrogen, C6000-03) were transformed and grown using the LEX system (HarbingerBiotech) at 37 °C in 2L bottles (VWR, 89000-242) containing 1800 ml of TB (Sigma, T0918) supplemented with 150 mM glycerol, 100 µM Kanamycin and 600 µl antifoam 204 (Sigma A-8311). When the OD(600) reached a value of about 6.0, the temperature was reduced to 15 °C, and one hour later the culture was induced with 100 µM IPTG (BioShop, IPT001) and incubated overnight (16 hours) at 15 °C. Cell pellets were collected by centrifugation (12,227 xg, 20 mins), frozen in liquid nitrogen, and stored at -80 °C.

Purification

Procedure
A volume of 2.0 mL settled Talon resin per 40 mL lysate (Clontech, 635504) was rocked with unclarified lysate for 60 minutes at 4 °C, batch-washed 4 times with 45 mL of cold Wash Buffer with 500 xg, 2 minute spins, and transferred to a column. After additional washing (50 column volumes), protein was eluted with 30 mL of Elution Buffer and dialyzed overnight at 4 degC against 50 volumes of Dialyses Buffer. The His6 tag was cut with thrombin (1unit/mg protein), and the resultant protein was further purified by gel-filtration and concentrated with a 3 KDa MW cut-off concentrator (Millipore, UFC900524) at 2850 xg to a final value of 0.75mM (~10 mg/mL). The protein yield was 8 mg per liter of bacterial culture. Coomassie-stained SDS-PAGE showed that the product was pure.

Extraction

Procedure
After resuspension in 30 mL per liter bacterial culture of Lysis Buffer, cells were lysed using sonication (Misonix Sonicator 3000) on ice for 10 minutes total sonication time (10 sec pulses at half-maximal frequency with 10 second rest).
Concentration:10 mg/mL.
Ligand
MassSpec:mass-spectroscopy by LCMS (Agilent 1100 Series) showed that the purified protein had the exact molecular weight.
Crystallization: Crystals were grown at 18 degC using the sitting drop method in Linbro plates by mixing equal volumes of protein (10 mg/ml) and Crystallization Buffer (1.5 NaMalonate pH7.3). Suitable crystals were cryoprotected by immersion in 2.4M NaMalonate pH7.3 supplemented with 10 TMAO % (v/v) prior to dunking and storage in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data was collected using a home source (Rigaku FRE SuperBright).
Data Processing: All data sets were integrated and scaled using the XDS package. The structure was solved by molecular replacement techniques using concomitantly the weak phases derived from a Sulfur-SAD data collected from a chromium source (Rigaku FRE) in the program MOLREP. The search model was PDB 3FL2. Automated model building using ARP/wARP, combined with iterative model building using the graphics program Coot and maximum-likelihood and TLS refinement with the program REFMAC5 using a higher resolution dataset collected from Copper source, were used to build the final model.