NPAC

PDB:2UYY

Revision

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Entry Clone Accession:gi|40556376
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SGC Clone Accession:NPACA-c000
Tag:N-terminal, TEV cleavable hexahistidine tag. Tag sequence: mhhhhhhssgvdlgtenlyfq(*)sm
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmGSITPTDKKIGFLGLGLMGSGIVSNLLKMGHTVTVWNRTAEKCDLFIQEGARLGRTPAEVVSTC
DITFACVSDPKAAKDLVLGPSGVLQGIRPGKCYVDMSTVDADTVTELAQVIVSRGGRFLEAPVSGNQQLSNDGMLVILAAGDRGLYE
DCSSCFQAMGKTSFFLGEVGNAAKMMLIVNMVQGSFMATIAEGLTLAQVTGQSQQTLLDILNQGQLASIFLDQKCQNILQGNFKPDF
YLKYIQKDLRLAIALGDAVNHPTPMAAAANEVYKRAKALDQSDNDMSAVYRAYIH

Vector:pNIC28-Bsa4.

Growth

Medium:TB
Antibiotics:
Procedure:10µl of a BL21(DE3)-R3-pRARE2 glycerol stock carrying the expression plasmid were inoculated into 5ml of TB with 50µg/ml of kanamycin and 34µg/ml chloramphenicol and grown overnight at 37°C, 200rpm. 10ml of overnight culture were added to 3 x 1L of TB with 50µg/ml kanamycin and incubated at 37°C, 160rpm. After the OD600 reached 1.0, the temperature was dropped to 18°C and 500ul of 1M IPTG was added to the final concentration of ~0.5mM. The culture was then incubated with shaking overnight at 18°C, 160rpm. The following morning the 3L culture was harvested and centrifuged for 10min at 4000rpm. Supernatant was discarded and cell pellets were resuspended in 60ml of a lysis buffer and frozen at -80°C.

Purification

Procedure
The cell extract was loaded on the AKTA Express system The extinction at 280nm was monitored and fractions were collected and analyzed by SDS-PAGE. Positive fractions were pooled and concentrated.

Extraction

Procedure
The thawed cells were broken by 5 passes at 16.000 psi through a high pressure homogeniser followed by centrifugation for 45min at 15.000rpm.
Concentration: Using VivaSpin-15 concentrators with 30kDa cutoff, the sample was concentrated to 10mg/ml. Concentrations were determined from the absorbance at 280nm using NanoDrop.
Ligand
MassSpec: Calculated mass of the construct was 34053. The mass of 34052 was confirmed by mass spectrometry.
Crystallization: Crystals were grown by vapor diffusion at 20°C in 300nl sitting drops. Prior to the crystallisation 5mM NAD+ was added to the protein. The drops were prepared by mixing 200nl of protein solution and 100nl of precipitant consisting of 20% PEG 3350 and 0.2M KSCN. Crystals were transferred to a cryo-protectant consisting of 25% glycerol and 75% well solution with 5mM NAD+ before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection:Resolution: 2.6Å; X-ray source: SLS beam X10SA.
Data Processing: