DPYSL2

PDB:2GSE

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC067109
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host: Rosetta II pLysS

Construct

Prelude:
Sequence:

```
MHHHHHHSSGVDLGTENLYFQSMTSDRLLIKGGKIVNDDQSFYADIYMEDGLIKQIGENLIVPGGVKTIEAHSRMVIPGGIDVHTRF
QMPDQGMTSADDFFQGTKAALAGGTTMIIDHVVPEPGTSLLAAFDQWREWADSKSCCDYSLHVDISEWHKGIQEEMEALVKDHGVNS
FLVYMAFKDRFQLTDCQIYEVLSVIRDIGAIAQVHAENGDIIAEEQQRILDLGITGPEGHVLSRPEEVEAEAVNRAITIANQTNCPL
YITKVMSKSSAEVIAQARKKGTVVYGEPITASLGTDGSHYWSKNWAKAAAFVTSPPLSPDPTTPDFLNSLLSCGDLQVTGSAHCTFN
TAQKAVGKDNFTLIPEGTNGTEERMSVIWDKAVVTGKMDENQFVAVTSTNAAKVFNLYPRKGRIAVGSDADLVIWDPDSVKTISAKT
HNSSLEYNIFEGMECRGSPLVVISQGKIVLEDGTLHVTEGSGRYIPRKPFPDFVYKRIKARSRLAE
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Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:20 mL TB media was inoculated with Rosetta II pLysS cells, and the culture was grown overnight at 30 ºC in 100 mL shake flasks at 175 rpm. 1.5 L TB media supplemented with 8 g/L glycerol, 50 µg/mL kanamycin and approximately 20 µL BREOX (anti-foam solution) was inoculated with 20 mL of the over night culture. The large scale cultivations were grown in 2 L bottles in the LEX system water bath at 37 °C. The temperature of the bath is regulated by connection via a circulation (pumping) system to another tempered water bath, set to 41°C. Room tempered air is circulated and transfered to the bottles by an airline tubing and a sparger inside the bottle. The bottle was transferred to a neighbouring water bath of 18 °C when the OD600 reached 2.5. The protein expression was induced by adding IPTG to a final concentration of 0.5 mM when the OD600 reached 3.5. The protein expression was continued over night.

Purification

Procedure
Columns: HiTrap Chelating and HiLoad 16/60 Superdex 200

Purification was conducted automatically on an ÄKTA xpress system operated by UNICORN software. Prior to purification columns were equilibrated with IMAC Bind/Wash1 Buffer (HiTrap Chelating) and Gel filtration buffer (Superdex 200). The protein sample was loaded on the HiTrap Chelating column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with IMAC Elution Buffer and loaded onto the Gelfiltration column. The chromatogram from Gelfiltration showed one major protein peak that consisted of highly pure DRP2A-c011 confirmed by SDS-PAGE analysis. TCEP was added to the sample to a final concentration of 2 mM. The protein was concentrated to 33 mg/mL and stored at -80ºC.

Extraction

Procedure
A stock solution of Complete EDTA-free (protease inhibitor) was prepared by dissolving 24 tablets in 48 mL of IMAC binding and wash1 buffer. Cells were harvested by centrifugation for 10 min at 4000 g. The cell pellet was resuspended in 80 mL of IMAC lysis buffer complemented with 4 mL of Complete EDTA-free protease inhibitor stock and then frozen at -80˚C. The frozen cell pellet was briefly thawed by warm water. 4 µL (1000 U) benzonase was added to the sample prior to high-pressure homogenization with the HPH. The cells were run three times through the HPH before centrifugation for 20 min at 49 000 g in the Sorvall SS-34 rotor. The soluble fraction was decanted and filtered through 0.45mm prior to loading onto the ÄKTAxpress for furher purification.
Concentration:
Ligand
MassSpec:
Crystallization:0.2 M CaCl, 0.1 M Tris pH 8.5, 17% PEG 10k. Dilute the sample 1+1 with GF-buffer to 16.7 mg/ml. Set up 2 µl (protein) + 1 µl (well solution) hanging drops.
NMR Spectroscopy:
Data Collection:ESRF ID14.2 20060213
Data Processing: