DNAJC2

PDB:2M2E

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:SGC cDNA collection 36-A2
Entry Clone Source:
SGC Clone Accession:YTC004H10
Tag:N-terminal His6-tag, removed
Host: Competent BL21 (DE3) cells (Invitrogen, C6000-03)

Construct

Prelude:ZRF1:F551-K621
Sequence:

gFTPWTTEEQKLLEQALKTYPVNTPERWKKIAEAVPGRTKKDCMKRYKELVEMVKAKKAAQEQVLNASRAKK

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure: The plasmid is transformed into competent cell strain BL21(DE3) (Invitrogen, C6000-03) and the cells were inoculated in a 125 ml flask containing 50 ml of M9 minimal media (100 uM ZnSO4, 8.55 mM NaCl, 47.6 mM Na2HPO4, 22 mM KH2PO4, 100 mM MgSO4, 2 mM biotin, 1.5 mM thiamine.HCl, and 0.1 M CaCl2) supplemented with 15NH4Cl, 13C6-D-glucose, and 50 µg/ml kanamycin at 37 °C shaking at 220 rpm. The overnight starter culture was transferred to a 2 L flask containing 0.5 L of 2X M9 minimal media supplemented with 15NH4Cl, 13C6-D-glucose, and 50 µg/ml kanamycin, and incubated at 37 °C. When the OD600 reached 1.0, protein over-expression was induced with 1mM isopropyl-thio-beta-D-galactopyranoside (IPTG) and the cells were further grown overnight (15.5 hours) at 15 °C with shaking at 220 rpm. Cell pellets were collected by centrifugation (7000 rpm, 20 mins) and frozen in 50 mL Falcon tubes at -80 °C for storage.

Purification

Procedure
The frozen cell pellet stored in a 50 ml Falcon tube obtained from 1L of culture was thawed and resuspended in 25 mL lysis buffer. The cell pellet was lysed by sonication (Branson Sonicator) on ice for 5 minutes and 30 secs total sonication time (30 sec pulses at amplitude of 72% frequency

with 30 second rest). The lysate was clarified by centrifugation for 20 min at 4 °C. The supernatant was mixed with 2 mL of Ni-NTA affinity beads per 25 mL lysate. The mixture was incubated on a rotary drum for 20 minutes at 4 °C . The lysate was spun at 2000 rpm for 6 minutes, and the supernatant was decanted. The remaining resin was resuspended and washed twice with lysis buffer, followed by two washes with 5 mL of cold washing buffer. The resin was then transferred to a gravity filter column and further washed with 2 mL of wash buffer. Samples were eluted from the resin by 5mL elution buffer.

The N-terminal His-tag was removed by overnight incubation with TEV protease (1:30 w/w) at 4 degree. Uncut proteins and TEV protease were removed by passing the solution through Ni-NTA beads.

The buffer of the purified protein was exchanged from elution buffer into Tris-based NMR buffer by ultracentrifugation using 5 mL concentrators with a 5,000 molecular weight cut-off (VivaSpin 2 MES) at 3000 rpm, resulting in a final volume of 300ul (final protein concentration of 0.5 mM). The concentrated protein was transferred to a 5mm Shigemi NMR tube.

Extraction

Procedure

Concentration:
Ligand
MassSpec:
Crystallization: Initial hits were seen from conditions Red Wings G07 and SGC-II A10. After optimization the pH of the buffer, different PEG (PEG1500, PEG3350, PEG8000) and the ratio of protein to buffer, the third RLD domain of HERC1 was crystallized by vapor diffusion at 18°C from a hanging drop consisting of 2.0ul protein (15.0 mg/ml) and 1.0ul well solution containing 20% PEG1500, 0.2M MgCl2, 0.1M HEPES pH 7.5. The crystal was transferred to a cryo protectant containing 15% ethylene glycerol in the well solution before flash-frozen in liquid nitrogen.
NMR Spectroscopy:A series of spectra (3D 1H-13C NOESY, 3D 1H-15N NOESY, 2D 1H-13C Constant Time HSQC, 3D HNCO, 3D HNCA, 3D CBCA(CO)NH, 3D HBHA(CO)NH, 3D 1H-13C Aromatic NOESY, 3D (H)CCH-TOCSY, and 3D H(C)CH-TOCSY) were generated using a 600MHz Bruker AVANCE spectrometer and a 800MHz Bruker AVANCE spectrometer. NMR data was processed and analyzed using Topspin, NMRPipe, NMRDraw, Sparky, FMC-GUI, CYANA, CNS, TALOS, and PSVS.
Data Collection:
Data Processing: