Pf-DNCL1: Plasmodium falciparum Dynein Light Chain 1

PDB:1YO3

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Plasmodium falciparum 3D7 genomic DNA
SGC Clone Accession:PFL0660w:V10:G92; plate 2001:D4
Tag:N-terminal His-tag; MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (Gold)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsVVKNVDMTEEMQIDAIDCANQALQKYNVEKDIAAHIKKEFDRKYDPTWHCVVGRNFGSYVTHETKNFI
YFYIGQVAILLFKSG

Vector:pET28a-LIC

Growth

Medium: Terrific Broth (TB)
Antibiotics:50 microG/mL kanamycin and 25 microG/mL chloramphenicol
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 degC for 3 hours. The culture was then transferred into 1.8 L of above-specified growth medium with Antibiotics and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of ~1, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure
The cleared lysate was loaded onto a column of 3 mL Ni-NTA from Qiagen at 4 degC. The column was washed with 150 mL Wash Buffer and the protein was eluted with 15 mL Elution Buffer. About 18 mg of pure protein was obtained from 1L of cell culture. The purified protein was dialyzed overnight into Crystal Buffer at 4 degC and concentrated using a Amicon Ultra centrifugal filter device from Millipore (15 kD cutoff).

Extraction

Procedure
Cells were centrifuged and the cell pellets were resuspended in binding buffer with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication (1x 30 sec). Lysate was cleared by centrifugation at 75,000 x g.
Concentration:15 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a VDXm plate. The plate was set with 1.5 microL protein (20 mg/mL) and 1.5 microL buffer in each drop, and 350 microL reservoir volume per well. Crystals emerged in 18% PEG 3350, 0.2M di-ammonium citrate and pH 4.8 at 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: