Pv-ClpB-NBD: First nucleotide binding domain of chaperone ClpB (Pv089580) from Plasmodium vivax

PDB:2P65

Revision

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Entry Clone Accession: Pv089580
Entry Clone Source:
SGC Clone Accession: Pv089580:Y168-S353; plate MAC01Q:D10
Tag:mgsshhhhhhssgrenlyfq
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

gYQALEKYSRDLTALARAGKLDPVIGRDTEIRRAIQILSRRTKNNPILLGDPGVGKTAIVEGLAIKIVQGDVPDSLKGRKLVSLDLS
SLIAGAKYRGDFEERLKSILKEVQDAEGQVVMFIDEIHTVVGAGAVAEGALDAGNILKPMLARGELRCIGATTVSEYRQFIEKDKAL
ERRFQQILVEQPS

Vector:p15-tev-lic

Growth

Medium:TB
Antibiotics:100 microG/mL ampicillin and 34 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 2L bottle and cultured using the LEX system to an OD600 of ~5, 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 prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5M NaCl and equilibrated with Binding Buffer) and subsequently onto a 1.0-2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1-1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was

further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. EDTA was immediately added to the elution fraction to 1 mM; and TCEP was added to 1-5 mM after approximately 15 more minutes.

The His6-tag was cleaved with TEV overnight at 4degC in the presence of 1 mM TCEP and dialysed into Crystal Buffer. The cleaved sample was loaded onto a Sephadex S75 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak eluting at 164 mL corresponding to monomeric protein were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device from Millipore (15 kD cutoff). The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel. The concentrated protein was stored at 4degC.

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Bufferwith protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF). Resuspended pellets stored at -80 degC were thawed overnight at 4 degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5% CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged at ~75000 x g for 20 minutes at 10 degC.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a Linbro plate. The plate was set with 1.5 microL protein and 1.5 microL buffer in each drop, and 500 microL reservoir volume per well. Crystals grew in 1.4 M sodium citrate, 100 mM Tris at pH 8.5 and 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: