Pf-HSP: Plasmodium falciparum heat shock protein Pf-HSP PF14_0417 middle domain

PDB:1Y6Z

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PF14_0417
Entry Clone Source: Plasmodium falciparum 3D7 genomic DNA
SGC Clone Accession:PF14_0417:Q385-D645; plate MAC2004:C10
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGVDLGTENLYFQ*sm
Host:E.coli BL21 (Gold Magic)

Construct

Prelude:
Sequence:

smQLPIWKQDEKSLTENDYYSFYKNTFKAYDDPLAYVHFNVEGQISFNSILYIPGSLPWELSKNMFDEESRGIRLYVKRVFINDKFS
ESIPRWLTFLRGIVDSENLPLNVGREILQKSKMLSIINKRIVLKSISMMKGLKETGGDKWTKFLNTFGKYLKIGVVEDKENQEEIAS
LVEFYSINSGDKKTDLDSYIENMKEDQKCIYYISGENKKTAQNSPSLEKLKALNYDVLFSLEPIDEFCLSSLTVNKYKGYEVLDVNK
AD

Vector:pET21a-LIC

Growth

Medium:M9
Antibiotics:10 microG/mL ampicillin
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 Ni-NTA (nickel-nitrilotriacetic acid) column from Qiagen at 4ºC. The column was washed with Wash Buffer, and the protein was eluted with Elution Buffer. The protein was dialyzed overnight at 4 degC in the presence of TEV protease into the Binding

Buffer 2 with TCEP added to 1 mM.The product of dialysis was loaded onto a Ni-NTA column. The purified protein was dialyzed into Crystal Buffer and concentrated using Amicon Ultra centrifugal filter devices (Millipore).

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:10 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a 24-well Linbro plate. The plate was set with 1.5 microL cleaved protein (20 mg/mL) and 1.5 microL buffer in each drop, and 350 microL reservoir volume per well. Crystals grew to full size in 3 days in 25% PEG 3350, 0.1 M Tris, pH 8.2 at 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: