Py-CyP: Plasmodium yoelii cyclophilin (PY00382)

PDB:1Z81

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PY00382
Entry Clone Source: Plasmodium yoelii 17NXL genomic DNA
SGC Clone Accession:PY00382:; plate 2001:E10
Tag: His-tag with integrated thrombin protease site: mgsshhhhhhssglvprgs
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMKSNSKDSENKKVENLVLDDNDENTIIPYYLSNLLTNPSNPVVFMDINLGNNFLGKFKFELFQNIVPK
TSENFRQFCTGEYKVNNLPVGYKNTIFHRVIKEFMIQGGDFINHNGSGSLSIYGEKFDDENFDIKHDKEGLLSMANSGPNTNGCQFF
ITTKKCEWLDGKNVVFGRIIDNDSLLLLKKIENVSVTPYIYKPKIPINVVECGEL

Vector:p28a-LIC

Growth

Medium: Terrific broth (TB)
Antibiotics:50 microG/mL kanamycin
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 above-specified medium 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 in a 2L baffled flask and grown at 37 degC overnight in an Innova shaker from New Brunswick Scientific (150 rpm).

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 10 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 35,000 x g and passed through DE52 from Whatman in 0.5 M NaCl.
Concentration:15 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 uncleaved protein (15 mg/mL) and 1.5 microL buffer in each drop, and 500 microL reservoir volume per well. Crystals emerged in 1.15 M ammonium sulfate, 200 mM lithium chloride, 100 mM Tris at pH 8.5 and 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: