Pf-FtsJ: Plasmodium falciparum ribosomal RNA methyltransferase + SAM

PDB:2PLW

Revision

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SGC Clone Accession:PF13_0052:N70-K270; plate MAC02A:A8
Tag:N-terminal His6-tag with integrated TEV cleavage site (*): mhhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

gNYRSRAAYKLIELDNKYLFLKKNKIILDIGCYPGSWCQVILERTKNYKNKIIGIDKKIMDPIPNVYFIQGEIGKDNMNNIKNINYI
DNMNNNSVDYKLKEILQDKKIDIILSDAAVPCIGNKIDDHLNSCELTLSITHFMEQYINIGGTYIVKMYLGSQTNNLKTYLKGMFQL
VHTTKPKASRNESREIYLVCKNFLGRKK

Vector:pET15-MHL

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 1.0-2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 2.5-3.0 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 Ni-NTA was further washed with 5 mL of Binding Buffer. The Ni-NTA column was then washed with 150 mL of Wash Buffer. After washing, the protein was eluted with

Elution Buffer. EDTA was immediately added to the elution fraction to 1 mM; and DDT was added to 5 mM after approximately 15 more minutes. SAM was added to eluted protein to 2 mM.

The protein from Ni-NTA column was applied to a Superdex S75 26x70 Gel Filtration column which is pre-equiibrated with Gel Filtration Buffer. The fractions from the peak corresponding to a monomeric protein were collected.

The eluted protein was treated overnight at 4 degC with TEV protease and DTT added to 1 mM,with the protease:protein molar ratiodetermined based on measured activity of the available TEV. The cleaved protein was separated from the uncleaved protein by passage through another 0.5 mL Ni-NTA column pre-equilibrated with Binging Buffer. The flow-through was collected; and the column was rinsed with an additional 5 mL of Binding Buffer. The cut protein was concentrated using a 15 mL Amicon Ultra centrifugal filter device from Millpore (10 kDa cutoff). Protein was aliquoted, flash frozen and stored at -80degC.

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with 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 sonicated for 6 minutes and the cell lysate was centrifuged using a Beckman JLA-16.250 rotor at ~38,400 x g (16,000 rpms) for 45 minutes at 4 degC.
Concentration:
Ligand
2 mM S-(5'-Adenosyl)-L-methionine chloride (SAM) was added to the protein after Ni-NTA column and during the concentration.**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 uncleaved protein (20 mg/mL) and 1.5 microL buffer in each drop, and 350 microL reservoir volume per well. Crystals emerged in 24% PEG3350, 0.1M (NH4)2SO4, 0.1M Bis-Tris at pH 5.9 and 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: