PSIPS1

PDB:4FU6

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:BC044568AT98-b5
Entry Clone Source:MGC
SGC Clone Accession:JMC049-A09
Tag:MHHHHHHSSGRENLYFQG
Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude:PWWP domain of PSIP1
Tag not removed
Sequence:

mhhhhhhssgrenlyfqgMTRDFKPGDLIFAKMKGYPHWPARVDEVPDGAVKPPTNKLPIFFFGTHETAFLGPKDIFPYSENKEKYG
KPNKRKGFNEGLWEIDNNPKVKFSSQQAATKQSNASSDVEVEEKETSVSKEDTDHEEKASNEDVTK

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL ofovernight culture grown in Luria-Bertani medium into a 4L of Terrific Broth mediumin the presence of 50 mg/mL kanamycin and 30 mg/mL chloramphenicol at 37 degree.When OD600 reached ~3.0, the temperature of the medium was lowered to 16 degree andthe culture was induced with 0.4 mM IPTG. The cells were allowed to grow overnightbefore harvested and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure
The lysate was centrifuged at 16,000 rpm for 45 minutes and the supernatants weremixed with 8 mL 50% flurry of Ni-NTA beads and incubated at 4 degree on rotaryshaker for one hour. The mixture was then centrifuged at 2000 rpm for 5 min and thesupernatant discarded. The beads were then washed with 50 mL washing buffer, andfinally 25 mL the elution buffer. The protein further purified by a 5mL Hitrap Q HPcolumn and Superdex-75 gel filtration column. Fractions

containing the protein werecollected and concentrated with Amicon Ultra-15 centrifugal filter. The purity of thepreparation is tested by SDS-PAGE to be greater than 95%.

Extraction

Procedure
Frozen cells from 2L TB culture were thawed and resuspended in 200 mL extractionbuffer and 8 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed usingsonicator.
Concentration:25 mg/mL
Ligand
MassSpec:protein expected 17487.3, not measured
Crystallization: Crystallization was setup using in situ proteolysis method in sitting drops with RedWings and SGC-I screens initially. Diffracting crystals were from initial screen plate forRed Wings F01.Crystal used for structure determination was grown in 2.5M NH4SO4, 0.1M Tris buffer atpH 8.5.Crystals grow to a mountable size within 1 week
NMR Spectroscopy:
Data Collection:
Data Processing: