SUV420H2

PDB:3RQ4

Revision

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Entry Clone Accession:GI:31543169
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SGC Clone Accession:APC065C12
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:E.coli BL21 (DE3) V2R-pRARE

Construct

Prelude:
Sequence:

gGPDRVTARELCENDDLATSLVLDPYLGFRTHKMNVSPVPPLRRQQHLRSALETFLRQRDLEAAYRALTLGGWTARYFQSRGPRQEA
ALKTHVYRYLRAFLPESGFTILPCTRYSMETNGAKIVSTRAWKKNEKLELLVGCIAELREADEGLLRAGENDFSIMYSTRKRSAQLW
LGPAAFINHDCKPNCKFVPADGNAACVKVLRDIEPGDEVTCFYGEGFFGEKNEHCECHTCERKGEGAFRTRPRE

Vector:pET28-MHL

Growth

Medium:M9 minimal medium
Antibiotics:50 µg/ml of kanamycin
Procedure:SUV420H2 was expressed in E.coli BL21 (DE3) V2RpRARE in M9 minimal medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37°C to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15°C

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES, pH 7.4, containing 250 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES pH 7.4, 250 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was then loaded on to a Superdex200 (26X60, Amersham Biosciences) column equilibrated in 20 mM PIPES, pH 6.5 buffer containing 250 mM NaCl. TEV protease was added to combined fractions containing SUV420H1. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES,

pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 24 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For the purification the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES, pH 7.4, 500 mM NaCl, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:15 mg/ml
Ligand
MassSpec: Enzymatic treatment: TEV - Expected MW is 28137.0 Da, measured mass is 28137.3 Da.
Crystallization: Purified SUV420H2 (10 mg/mL) was complexed with S-adenosyl-L-methionine (SAM) (Sigma) at 1:10 molar ratio of protein:SAM and crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 12% PEG8K, 0.1 M HEPES pH 7.2, 12% Ethylene glycol.
NMR Spectroscopy:
Data Collection:
Data Processing: