JMJD2AA: Human jumonji domain containing 2A in complex with trimethylated H3K9 peptide

PDB:2OQ6

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC002558
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal TEV cleavable 6His tag- mhhhhhhssgvdlgtenlyfq*s(m), cleaves at *.
Host:E. coli BL21(DE3)-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMASESET LNPSARIMTFYPTMEEFRNFSRYIAYIES QGAHRAGLAKVVPPKEWKPRASYDDID
DL VIPAPIQQLVTGQSGLFTQYNIQKKAMTV REFRKIANSDKYCTPRYSEFEELERKYWK NLTFNPPIYGADVNGTLYEKHVDE
WNIGR LRTILDLVEKESGITIEGVNTPYLYFGMW KTSFAWHTEDMDLYSINYLHFGEPKSWYS VPPEHGKRLERLAKGFFPGSA
QSCEAFLR HKMTLISPLMLKKYGIPFDKVTQEAGEFM ITFPYGYHAGFNHGFNCAESTNFATRRWI EYGKQAVLCSCRKDMVKI
SMDVFVRKFQP ERYKLWKAGKDNTVIDHTLPTPEAAEFLK ESEL

Vector:pNIC28-Bsa4

Growth

Medium:TB + 50 µg/ml Kanamycin + 34 µg/ml chloramp.
Antibiotics:
Procedure:12 x 1 L TB in 2.5-L baffled flasks were inoculated with 5 ml overnight culture and grown at 37°C. The protein expression was induced with 0.2 mM IPTG at OD600 = 0.8 for 18 h at 18 degC. The cells were collected by centrifugation and frozen at -80 degC.

Purification

Procedure

Extraction

Procedure

Frozen cell pellets were thawed and resuspended in a total volume of 400 ml lysis buffer. The cells were disrupted by high pressure homogenisation (15 kpsi) followed by sonication. Cell debris were removed by centrifugation for 60 minutes at 40 000 x g.

Concentration: The protein was concentrated using an Amicon Ultracel centrifugal concentrator (10 kDa MWCO) to 11 mg/ml by A280.

Ligand

MassSpec: The mass determined for JMJD2AA-p020 was 44266 Da, in agreement with the predicted mass for the his-tagged protein.

Crystallization: Crystals were grown by vapor diffusion at 4°C. A sitting drop consisting of 100 nl protein (11 mg/ml) + 10 mM N-oxalylglycine and 50 nl well solution was equilibrated against well solution containing 0.1 M citrate pH 5.5, 20% PEG 3350, 4 mM NiCl 2 . Cryo-protection of the crystals used 0.1 M citrate pH 5.5, 20% PEG 3350, 4 mM NiCl 2 , 25% glycerol and 10 mM N-oxalylglycine.

NMR Spectroscopy:

Data Collection: Resolution: 2.0 Å; X-ray source: Synchrontron SLS-X10SA.

Data Processing: