DDX2A/eIF4A

PDB:2G9N

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:
Tag:
Host:BL21(DE3)

Construct

Prelude:
Sequence:

SMEGVIESNWNEIVDSFDDMNLSESLLRGIYAYGFEKPSAIQQRAILPCIKGYDVIAQAQSGTGKTATFAISILQQIELDLKATQAL
VLAPTRELAQQIQKVVMALGDYMGASCHACIGGTNVRAEVQKLQMEAPHIIVGTPGRVFDMLNRRYLSPKYIKMFVLDEADEMLSRG
FKDQIYDIFQKLNSNTQVVLLSATMPSDVLEVTKKFMRDPIRILVKK

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:30 µl competent BL-21 (DE3) cells were transformed with 2 µl plasmid miniprep for 30 min on ice followed by heatshock at 42?C for 45 sec. SOC, 125 µl, was added to the cellsuspension which was then incubated for 1 hour at 37? and plated on LB-plates containing kanamycin (50 µg/ml). 20 ml TB with 100 µg kanamycin/ml was inoculated with cells and grown overnight (ON) at 30?C. The inoculation culture was added to 1.5 L TB (supplemented with 50 µg kanamycin/ml) in 2 L bottles. The flask was incubated in the LEX system-water bath at 37?C until OD600 reached 1. At this time the flask was transferred to an 18?C water bath in the LEX-system. Expression of protein was induced by addition of 0.5 mM IPTG and continued for approximately 18 hours.

Purification

Procedure
The protein was purified on a Hi-Trap chelating column followed by a Superdex 75 gel filtration column on the Äkta Express. The fractions containing protein were pooled, the TCEP-

concentration adjusted to 2 mM, and concentrated to 16.2 mg/ml. The protein was aliquoted and quick-frozen in liquid nitrogen.

TEV-cleavage: TEV-cleavage was performed by adding 400 µl His-tagged TEV (30 µM) to 10 mg protein in a total volume of 0.6 ml Gel filtration buffer with 2 mM TCEP for approximately 18 hours at 4?C. Cleaved protein was loaded onto a His-trap crude column, detected in the flow-through and concentrated in AmiconUltra MWCO 10000 concentrators to a concentration of 19.2 mg/ml.

Reductive methylation:Reductive methylation of 5 mg TEV-cleaved DEAD-domain eIF4A protein was performed according to the manufacturer´s instructions (JBS Methylation Kit, JenaBioscience) in a final volume of 1 ml Gel filtration buffer containing 2 mM TCEP. The methylated protein was concentrated to 18.1 mg/ml before setting up drops.

Extraction

Procedure
Cells were harvested by centrifugation in a SLC-6000 rotor for 10 minutes at 5000 rpm (OD 600 8.2; WCV 24.7 g). Pellets were suspended in 90 ml 50 mM Nafosfate pH 7.5, 10 % glycerole, 0.5 mM TCEP and 500 mM NaCl, 10mM imidazole and Complete EDTA-free protease inhibitor (Roche Biosciences). Suspended cells were stored at -80?C until further use.Before lysis, 50 µl 200 mM ADP and 4 µl of 250U/µl benzonase (Novagen) was added to the suspended cells which were passed 2 times through a high pressure homogenizer (Stansted, at 5000 Psi) The sample was spun for 30 min at 20500 rpm in a Sorvall SA-800 rotor. The soluble fraction was decanted and filtered through a 0.45 µm syringe filter.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized by the sitting drop vapor diffusion method, 200 nl of the methylated protein was mixed with 100 nl of unbuffered reservoir solution consisting of 200 mM ammonium nitrate and 20 % (w/v) PEG3350. Crystal clusters grew within three days. For data collection a single crystal was separated from the cluster and soaked in a cryo solution consisting of 20 mM HEPES pH 7.5, 500 mM NaCl, 200 mM ammonium nitrate, 20 % (w/v) PEG3350 and 20 % Glycerol before flash cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: