EIF4E

PDB:3SMU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: The plasmid containing the full-length cDNA for human eIF4E was a gift from Dr N. Shimma, Chugai Pharmaceutical Co., Japan.
SGC Clone Accession:
Tag:
Host:*E.coli* BL21 (DE3)

Construct

Prelude:
Sequence:

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EIF4E:MATVEPETTPTPNPPTTEEEKTESNQEVANPEHYIKHPLQNRWALWFFKNDKSKTWQANLRLISKFDTVEDFWALYNHIQL
SSNLMPGCDYSLFKDGIEPMWEDEKNKRGGRWLITLNKQQRRSDLDRFWLETLLCLIGESFDDYSDDVCGAVVNVRAKGDKIAIWTT
ECENREAVTHIGRVYKERLGLPPKIVIGYQSHADTATKSGSTTKNRFVV

4EBP1:PGGTRIIYDRKFLMECRNSP
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Vector:pT7 (gift from Dr N. Shimma, Chugai Pharmaceutical Co., Japan)

Growth

Medium:M9 minimal medium
Antibiotics:ampicillin (100µg/L)
Procedure: Luria Bertani (LB) or M9 minimal medium, containing ampicillin (100µg/L), were inoculated and grown at 37 °C until an OD_{600} = 0.6-0.8. For protein expression, cells were induced at 30 °C for 18 h by addition of 0.5 mM isopropyl-beta-D-thiogalactopyranoside (IPTG). Cultures were harvested by centrifugation (7,000 rpm, 15 min., 4 °C) and frozen at -20 °C.

Purification

Procedure
The lysate was cleared by centrifugation (18,000 rpm, 30 min., 4 °C) and the supernatant was clarified by filtration on a 0.45 mum membrane and loaded onto m7GDP-sepharose (cap column). After four washes (30 ml each; 1. lysis buffer, 2. wash buffer, 3. wash buffer containing 0.1 mM GTP and 4. wash buffer), protein was eluted with ~ 60 ml of elution buffer. The eluate was concentrated using an Amicon ultra centrifugal filter device (10 K MWCO, Millipore) to ~4 mL, and then applied to a size-exclusion column (Superdex 75, Amersham) using gel filtration buffer. FPLC fractions were pooled for further analysis and the purity of eIF4E was greater than 95% judging from SDS-PAGE gel. The protein concentration was measured at 280 nm using an extinction coefficient of 53440 M-1 cm-1. The final yield of soluble protein was 6-10mg grown in either LB medium or M9 minimal medium.

Extraction

Procedure
The cell pellet was then resuspended in 30 ml of lysis buffer and cells were disrupted by sonication on ice.
Concentration:eIF4E protein samples for crystallization trials were exchanged into 20mM HEPES, 100mM KCl, 1mM TCEP, pH 7.3 using an Amicon ultra centrifugal filter device (10 K MWCO, Millipore) and concentrated to 8-9mg/mL.
Ligand
MassSpec:
Crystallization:eif4e stock solution in the presence of 0.001 M 4ebp1 peptide and 0.02 M ribavirin was mixed in a 1:1 ratio with 2 M Ammonium Sulfate, 2% PEG400, 0.1 M Na HEPES, pH 7.5. Crystals grew to mountable size within 10-14 days.
NMR Spectroscopy:
Data Collection:
Data Processing: