TTK

PDB:3CEK

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:5270012
Entry Clone Source:MGC
SGC Clone Accession:
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3) phage resistant Rosetta strain

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfq*smSVKGRIYSILKQIGSGGSSKVFQVLNEKKQIYAIKYVNLEEADNQTLDSYRNEIAYLNKLQQH
SDKIIRLYDYEITDQYIYMVMECGNIDLNSWLKKKKSIDPWERKSYWKNMLEAVHTIHQHGIVHSDLKPANFLIVDGMLKLIDFGIA
NQMQPDTTSVVKDSQVGTVNYMPPEAIKDMSSSRENGKSKSKISPKSDVWSLGCILYYMTYGKTPFQQIINQISKLHAIIDPNHEIE
FPDIPEKDLQDVLKCCLKRDPKQRISIPELLAHPYVQIQTH[L]VNQMAKGTTEEThe construct contains a mutatio
n P797L highlighted in square brackets.
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Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin and 35 µg/ml chloramphenicol was used to inoculate 1 liter of LB media containing the same concentration of antibiotics. Cultures were grown at 37°C until the OD600 reached ~0.3. After that the temperature was adjusted to 20°C. Expression was induced for 4 hours using 1mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5% glycerol, 10 mM imidazole.

Purification

Procedure
Column 1: Ni-affinity chromatography.Buffers: Binding buffer: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 5% Glycero, 10mM Imidazole. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 30 mM Imidazole , 5% glycerol. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 50 to 250 mM imidazole, 5% Glycerol.Procedure: 5 ml of 50% Ni-NTA slurry (Qiagen)

was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 50 ml binding buffer. The lysate was applied to the column which was subsequently washed with 50 ml wash buffer 1 and 2. The protein was eluted by gravity flow by applying 5 ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150mM, 250 mM); fractions were collected until essentially all protein was eluted. The eluted protein was analyzed by SDS-PAGE. DTT was added to the protein sample to a final concentration of 5mM.Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)SEC-Buffers: 50 mM Hepes, pH 7.5, 150 mM NaCl, 5 mM DTT.Procedure: The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 ml using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 0.8 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

Extraction

Procedure
Cell pellets were lysed by C5 high pressure homogenizer (Avestin). The lysate was centrifuged at 21,000 rpm for 60 minutes and the supernatant collected for purification.
Concentration: Centricon with a 30 kDa cut off in SEC-buffer.
Ligand
MassSpec: The mass of the protein was calculated to be 36064 Da and experimentally determined mass was 36785 Da for the His tag containing protein. The identity of the protein was reconfirmed to be correct by DNA sequencing both DNA strands of this expression construct. It is therefore likely that the difference in Mass is due to posttranslational modifications of the protein in regions not visible in the electron density.
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 11 mg/ml by mixing 150nl of the concentrated protein with 150 nl of a well solution containing: 55w/v PEG 300; 0.25M NaCl; 6.4 pH Na/K phosphate.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution supplemented with an additional 25% ethylene glycol and flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line SAX10. Resolution: 2.4 Å.
Data Processing: