Entry clone source: Synthetic

GI number: gi|30794372

Final protein sequence:

MGDSMISSATSDTGSAKRKSKKNIRKQRMKILFNVVLEAREPGSGRRLCDLFMVKPSKKDYPDYYKIILEPM
DLKIIEHNIRNDKYAGE
EGMIEDMKLMFRNARHYNEEGSQVYNDAHILEKLLKEKRKELGPLPDDDDMASPAENLYFQ^SHHHHHHDYK
DDDDK

^ TEV cleave site

Amplified construct sequence:

Vector: pNIC-CTHF

Tags and additions: Cleavable C-terminal His6 tag.

Host: BL21 (DE3)R3-pRARE2 (Phage resistant strain)

Growth medium, induction protocol: 10 ml from a 50 ml overnight culture containing 50 μg/ml kanamycin and 34 μg/ml chloramphenicol were used to inoculate each of two 1 liter cultures of TB containing 50 μg/ml kanamycin and 34 μg/ml chloramphenicol. Cultures were grown at 37 ^{o}C until the OD_{600} reached ~2.5 then the temperature was adjusted to 18 ^{o}C. Expression was induced overnight using 0.1 mM IPTG at an OD_{600} of 3.0. The cells were collected by centrifugation and the pellet re-suspended in binding buffer and frozen.

Binding buffer: 50 mM HEPES pH 7.5; 500 mM NaCl; 10 mM imidazole, 5% glycerol.

Extraction buffer, extraction method: Frozen pellets were thawed and fresh 0.5 mM TCEP, 1 mM PMSF added to the lysate. Cells were lysed using sonication. The lysate was centrifuged at 17,000 rpm for 60 minutes and the supernatant collected for purification.

Column 1: Ni-affinity. Ni-sepharose (Amersham), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Buffers:

Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 5% glycerol

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, 5% glycerol, 50 to 250 mM Imidazole (step elution).

Procedure: The supernatant was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 30 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 mM, 200 and 250 mM); fractions were collected until essentially all protein was eluted.

Enzymatic treatment: The C-terminal His tag was cleaved by treatment with TEV protease, overnight.

Column 2: Size Exclusion Chromatography. Superdex S75 16/60 HiLoad

Buffers: 10 mM HEPES, pH 7.5; 500 mM NaCl, 5% glycerol
Procedure: The protein was concentrated and applied to an S75 16/60 HiLoad gel filtration column equilibrated in 10 mM HEPES, pH 7.5; 500mM NaCl, 5% glycerol using an ÄKTAexpress system.
Mass spec characterization: LC- ESI -MS TOF gave a measured mass of 17434 for this construct as predicted from the sequence of this protein.
Protein concentration: Protein was concentrated to 12.0 mg/ml using an Amicon 3 kDa cut-off concentrator.
Crystallization: Protein buffer was exchanged to 10 mM HEPES pH7.5, 150 mM NaCl and 2% glycerol prior concentration to 81 mg/ml. The protein was crystallized employing the sitting drop evaporating method: crystals were grown at 4 ^{O}C in 300 nl sitting drops from a 1:2 ratio of protein to reservoir solution containing 25 % PEG MME2000, 0.015 M NiCl and 0.1 M Tris pH 8.5.
Data Collection: Crystals were cryo-protected using the well solution supplemented by 20 % ethylene glycol and flash frozen in liquid nitrogen. **X-ray source:** Diffraction data were collected from a single crystal on Diamond beamline I03 at a single wavelength of 1.00 Å and the structure was refined to 2.13 Å. **Phasing:** The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.