First Bromodomain of BRDT in complex with the inhibitor (+)-JQ1 (4FLP) Materials & Methods

Entry clone source: Synthetic

SGC Construct ID: gi|46399198

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank].

DNA sequence:

```
CATATGCACCATCATCATCATCATTC
TTCTGGTGTAGATCTGGGTACCGAGA
ACCTGTACTTCCAATCCATGAATACT
AAGAAAAATGGGCGATTGACAAATCA
ACTTCAGTATCTACAAAAAGTTGTCC
TAAAGGATTTATGGAAGCATAGTTTT
TCATGGCCCTTTCAACGTCCTGTGGA
TGCTGTGAAACTACAGTTGCCTGATT
ATTATACCATTATAAAAAACCCAATG
GATTTAAATACAATTAAGAAGCGCTT
GGAGAATAAATATTATGCGAAGGCTT
CAGAATGTATAGAAGACTTCAATACA
ATGTTCTCAAATTGTTATTTATATAA
CAAGCCTGGAGATGACATTGTTCTTA
TGGCACAAGCTCTAGAGAAGCTGTTT
ATGCAGAAATTATCTCAGATGCCACA
AGAAGAGTGACAGTAAAGGTGGATAC
GGATCCGAA
```

Final protein sequence (Tag sequence in lowercase):

```
mhhhhhhssgvdlgtenlyfq^smNT
KKNGRLTNQLQYLQKVVLKDLWKHSF
SWPFQRPVDAVKLQLPDYYTIIKNPM
DLNTIKKRLENKYYAKASECIEDFNT
MFSNCYLYNKPGDDIVLMAQALEKLF
MQKLSQMPQEE

^ TEV protease recognition site
```

Tags and additions: Cleavable N-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°C until the OD_{600} reached ~2.5 then the temperature was adjusted to 18°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

Column 1 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).

Column 1 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 5ml 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 N-terminal His tag was cleaved by treatment with TEV protease, overnight.

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

Column 2 Buffers:
Gel Filtration buffer: 10 mM HEPES, pH 7.5; 500 mM NaCl, 5% glycerol

Column 2 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 14148.5 for the construct as predicted from the sequence of this protein

Concentration: The protein was concentrated to 23 mg/ml using an Amicon 3kDa cut-off concentrator.

Crystallization: Crystals were grown at 4°C in 150 nl sitting drops from a 1:2 ratio of protein (23 mg/ml + 1 mM (+)-JQ1) to reservoir solution 1.0 M bis-tris propane pH 8.0, 25 % PEG3350, 0.15 M KSCN and 10 % ethylene glycol.

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 at Diamond beamline I03 at a single wavelength of 0.9763Å and the structure was refined to 2.2Å.

Phasing: The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.