

# ATAD2

PDB:4QSW

## Revision

**Revision Type:**created

**Revised by:**created

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**Entry Clone Accession:**gi|24497618

**Entry Clone Source:**synthetic

**SGC Clone Accession:**

**Tag:**mhhhhhssgvdlgtenlyfq\*s(m) TEV-cleavable (\*) N-terminal his6 tag.

**Host:**BL21(DE3)-R3-pRARE2

## Construct

**Prelude:**

**Sequence:**

mhhhhhssgvdlgtenlyfq\*smQEEDTFRELRIFLRNVTHRLAIDKRFRVFTKPVDPDEVPDYVTVIKQPMDLSSVISKIDLHKY  
LTVKDYLRLDIDLICSNALYNPDRDPGDRLIRHRACALRDTAYAIIKEELDEDFEQLCEEIQESR

**Vector:**pNIC28-Bsa4

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**The expression plasmid was transformed into the host strain and plated on LB-agar containing 50 µg/mL kanamycin and 35 µg/mL chloramphenicol. Several colonies were combined to inoculate a 1mL culture in TB (+ 50 µg/mL kanamycin, 35 µg/mL chloramphenicol). The culture was grown overnight, glycerol was added to 15% v/v (from a 60% stock), and the resulting glycerol stock was frozen at -80 °C in 100 µL aliquots. When required a 50 mL overnight culture was started by inoculating a LB medium containing antibiotic as above. 5mL this overnight culture was used to inoculate similar 1 litre cultures (4L total) which were grown at 37°C until the OD600 reached ~0.5 at which point the temperature was adjusted to 18°C. Expression was induced overnight using 0.5 mM IPTG. The cells were collected by centrifugation and the pellet re-suspended in binding buffer and frozen. (Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol.)

## Purification

**Buffers**

**Procedure**

Column 1: Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatman), 10 g of

resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl, washed with 20 mL binding buffer prior to loading the sample. Buffers: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole. Procedure: Supernatant was applied by gravity flow, followed by a wash with 50 mL binding buffer. The column flow Column 2: Ni-affinity. Ni-NTA (Qiagen), 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, 50 to 250 mM Imidazole, 5% Glycerol. Procedure: The flowthrough from column 1 was loaded by gravity flow on the Ni-NTA column. The column was then washed with 100 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 and 250 mM); fractions were collected until essentially all protein was eluted. 10 mM DTT was added for overnight storage together with TEV protease for cleavage of the N-terminal hexahistidine tag. Column 3: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad Buffers: 25 mM HEPES, pH 7.5; 150 mM NaCl, 0.5 mM TCEP. Procedure: Protein was applied to a S200 gel filtration column using an ÄKTAexpress system at 1.0 mL/min. Eluted proteins were collected in 1.8-mL fractions and analyzed on SDS-PAGE. 10 mM DTT was added for storage. Column 4: Ni-affinity. Ni-NTA (Qiagen), 2 mL of 50% slurry in 1.5 x 10 cm column, washed with binding buffer. Procedure: The elution from column 3 (TEV cleaved) was loaded by gravity flow on a Ni-NTA column to remove Ni-binding contaminants. The column was washed with 10 mL wash buffer (as column 2) and proteins eluted as column 2. 10 mM DTT was added for overnight storage.

## Extraction

### Buffers

#### Procedure

Frozen pellets were thawed and fresh 1 mM PMSF and 0.5 mM TCEP were added. Cells were lysed by sonication. The lysate was centrifuged at 21,000 rpm for 60 minutes and the supernatant collected for purification.

**Concentration:** The cleaved purified protein was concentrated in a VivaSpin500 (5 K MWCO) to 12.2 mg/ml and stored at 4 °C. The protein concentration was determined spectrophotometrically

#### Ligand

**MassSpec:** Observed mass without histidine tag, 15430.6 (calculated mass without histidine tag, 15431).

**Crystallization:** Crystals were obtained using the vapor diffusion method. The protein was concentrated in gel filtration buffer to a protein concentration of 12.2 mg/mL. Sitting drops comprising 75 nl of the concentrated protein mixed with 75 nl of a well solution (2.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 0.1M BisTris pH 5.5) were equilibrated against well solution at 4°C. Crystals appeared within 2-3 days. For transfer/soaking experiments, the crystals were transferred into the stabilizing/soaking solution, containing either i) 45-50% MPD, 0.1 M bis-tris pH 5.5 and 0.1 M ammonium phosphate or ii) 28-32% PEG 3350, 50 mM bis-tris pH 5.5, 50 mM ammonium phosphate and 20% ethylene glycol. Fragments were supplemented in the soaking solution at 20-50 mM concentration (10% for NMP). Soaking time was varied from 4-9 hours. Peptide complexes: Soaking experiments were performed in a drop containing stabilizing solution (PEG 3,350, Bis-Tris, pH 5.5, ammonium phosphate) supplemented with 6 mM histone peptide and 25% ethylene glycol for 2 hours.

#### NMR Spectroscopy:

**Data Collection:** Crystals were cryo-protected using the well solution supplemented with 2M Li<sub>2</sub>SO<sub>4</sub> and flash frozen in liquid nitrogen (apo) or using ethylene glycol present in the soaking solution. Diffraction data were collected from a single crystal on a Rigaku FR-E SuperBright at a single wavelength of 1.5 Å. The structure was solved by molecular replacement and refined to

1.89 Å.

**Data Processing:**