JMJD2DA c105 Protein expression, purification and crystallization

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Author: **Fairhead, Michael**
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Method

1. Protein expression, purification and crystallization (JMJD2DA-c105)

Vector: pNIC28-Bsa4 (50 μg/mL kanamycin)

Cell line: BL21[DE3]-pRARE2 (34 μg/mL chloramphenicol)

Tags and additions: N-terminal, TEV protease cleavable hexahistidine tag

Final protein sequence

MHHHHHHSSGVDLGTENLYFQ*SMETMKSKANCAQNPNCNIMIFHPTKEEFNDFDKYIAYMESQGAHRAGLAKIIPPKEWKARETYDNIS

(underlined sequence contains vector encoded His-tag and TEV protease cleavage site*)

Protein expression

BL21[DE3]-pRARE2 were transformed with the appropriate plasmid and selected on LB kanamycin/chloramphenicol plates.

A 6 x 10 mL overnight culture was grown in LB kanamycin/chloramphenicol and used to inoculate 6 x 1 L of AIM-TB (ForMedium) with 0.01 % Antifoam 204 and antibiotics in a 2.5 L baffled flask (Ultrayield, Thomson). Cells were grown for 4 h 37 °C 250 rpm shaking and then for a further 44 h 17 °C 250 rpm shaking. Cells were then harvested by centrifugation at 4,000 g for 30 minutes 4 °C and the pellet stored at -80 °C.

Cell Lysis

Lysis Buffer: 10 mM HEPES, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP, 20 mM imidazole pH 7.5

Cell pellet (180 g) was dissolved in 500 mL of lysis buffer and lysozyme and benzonase added to 0.5 mg/mL and 1 μg/mL respectively. After 30 minutes stirring in the cold room 30 mL of 10 % Triton X-100 was added and stirring continued for a further 30 minutes. Lysed cells were split across 24 x 50 mL Falcoln tubes and frozen at -20 °C overnight. Cells were

then thawed in a room temperature water bath and the volume of each tube brought to 50 mL with Lysis Buffer. Cells were centrifuged for 1 h at 5,000 g (4 °C).

Column 1: His GraviTrap columns (24 x 1 ml volume in a gravity-flow column)

Wash Buffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 20 mM imidazole, 0.5 mM TCEP

Elution Buffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 500 mM imidazole, 0.5 mM TCEP

The clarified cell extract was added to a 24 x 1 ml His GraviTrap columns pre-equilibrated with Wash Buffer. The columns were then washed with 2 x 10 ml Wash Buffer. The protein was eluted with 2.5 ml Elution Buffer.

Column 2: PD-10 desalting columns (24 x 8.3 ml volume in a gravity-flow column)

Wash Buffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 10 mM imidazole, 0.5 mM TCEP

Each 2.5 mL His GraviTrap fraction (24 x) was applied directly to a PD-10 column (24 x) for desalting and eluted with 3.5 mL of Wash Buffer

Tag cleavage

1 mg of TEV protease was added to every 10 mg of the eluted protein and the digestion was performed overnight at 4 °C.

Column 3: His GraviTrap columns (24 x 1 ml volume in a gravity-flow column)

Wash Buffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 20 mM imidazole, 0.5 mM TCEP

The TEV cleaved protein (3.5 mL) was applied to a His GraviTrap column pre-equilibrated in Wash Buffer for removal of His-tag, TEV and uncleaved protein. The column was washed with a further 2.5 mL of Wash Buffer for a final pool of 6 mL. The various desalted protein fractions were combined (24 x 6 mL). The protein was concentrated to 25 mg/mL using a 30 kDa MWCO concentrator.

Column 4: Yarra SEC 2000 300x21.2 mm column (300x21.2 mm column, 104 mL volume)

Gel Filtration Buffer: 10 mM HEPES pH 7, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP

The column was equilibrated with Gel Filtration buffer and the protein loaded, the peak corresponding to the target protein was taken and concentrated to 25 mg/ml using a 30 kDa MWCO concentrator, flash frozen in liquid nitrogen and stored at -80°C.

2. Protein crystallization

Protein was crystallized by mixing 75 nl of 25 mg/ml protein in 10 mM HEPES pH7.5, 500mM NaCl, 5% Glycerol, 0.5 mM TCEP with 75 nl of reservoir solution containing 31 % PEG3350, 150 mM ammonium sulfate, 0.1 M HEPES, pH 7. Crystals

appeared after 1-4 days from sitting drop plates at 20 °C. Crystals are good for a week and then a severe drop in diffraction quality is observed.

JMJD2DA crystallized in space group P 43 21 2 with typical unit cell dimensions of a = 71.5 Å, b = 71.5 Å, c = 150.6 Å, corresponding to one JMJD2DA molecule in the asymmetric unit. The crystals typically diffract between 1.3 and 1.6 Å.

Crystals