OTUD5

PDB:3PFY
Entry Clone Accession:otud5.BC028225.OBS.MHS1010-7507944.pCMV-SPORT6
Entry Clone Source: Open BioSystems
SGC Clone Accession:otud5.0173-0344.201F09 (SDC201F9)
Tag:N-terminal: MHHHHHHSSG
Host: Competent BL21 (DE3) cells
Vector:pET28-MHL vector (GenBank, EF456735)

Sequence:

MHHHHHHSSGRENLYFQGAGYNSEDEYEAAAARIEAMDPATVEQQEHWFEKALRDKKGFIIKQMKEDGACLFRAVADQVYGDQDMHE
VVRKHCMDYLMKNADYFSNYVTEDFTTYINRKRKNNCHGNHIEMQAMAEMYNRPVEVYQYSTEPINTFHGIHQNEDEPIRVSYHRNI
HYNSVVNPNKA

Growth

Medium: TB (Sigma, T0918) supplemented with 150 mM glycerol, 50 mg/ml Kanamycin and 600 µl antifoam 204 (Sigma A-8311)

Procedure: Competent BL21 (DE3) cells (Invitrogen, C6000-03) were transformed and grown using the LEX system (HarbingerBiotech) at 37 °C in 1L bottles (VWR, 16157-191) containing 900 ml of growth medium. When the OD_{600} reached a value of about 6.0, the temperature was reduced to 15 °C, and one hour later the culture was induced with 1 mM IPTG (BioShop, IPT001) and incubated overnight (16 hours) at 15 °C. Cell pellets were collected by centrifugation (12,227 xg, 30 mins), frozen in liquid nitrogen, and stored at -80 °C. To produce the SeMet derivative, the same bacterial cells were grown using M9 SeMet High-Yield growth media kit package (Medicilon, MD045003) according to manufacturer's instructions.

Purification

Procedure: A volume of 1.0 mL settled TALON resin per 60 mL lysate (Clontech) was rocked with unclarified lysate for 60 minutes at 4 °C, washed with 45 mL of cold Wash Buffer, spun at 1000 g for 5 minutes, and transferred to a column. After additional washing (50 column volumes), protein was eluted with 5 mL (per mL of settled TALON resin) of Elution Buffer and dialyzed overnight at 4 oC against 200 volumes of Dialyses Buffer. The protein sample was concentrated using a 10 KDa MW cut-off concentrator (Millipore, UFC900524) at 3500 xg to a final value of 1mM (~40 mg/mL). Protein yield was 10 mg per liter of bacterial culture.

Extraction

Procedure: After resuspension in 30 mL per liter bacterial culture of Lysis Buffer, cells were lysed using a microfluidizer (Microfluidics, M110-EH) at 18,000 psi.
Concentration:40 mg/mL

Structure Determination

MassSpec:MW = 21 858.20 g/mol
Crystallization: Crystals were grown at 18 oC using the sitting drop method in intelliplate (ARI, 102-0011-00) by mixing equal volumes of protein (32 mg/ml) containing 1 uM Dispase (Sigma, D4818-2mg) and Crystallization Buffer (2 M NH_4SO_4, 2% PEG400, 0.1 M Sodium HEPES pH 7.5). Suitable crystals were cryoprotected by immersion in well solution supplemented with 25 % (v/v) glycerol prior to dunking and storage in liquid nitrogen.