USP7

PDB:2KVR
Entry Clone Accession:AB1438_E01
Entry Clone Source: Origene
SGC Clone Accession:usp07.0537-0664.172B03 (SDC172B03)
Tag:N-terminal tag: MGSSHHHHHHSSGLVPRGS
Vector:pET28aLIC vector (GenBank, EF442785)

Sequence:PQQLVERLQEEKRIEAQKRKERQEAHLYMQVQIVAEDQFCGHQGNDMYDEEK
VKYTVFKVLKNSSLAEFVQSLSQTMGFPQDQIRLWPMQARSNGTKRPAMLDNEADGNK
TMIELSDNENPWTIFLET

Growth

Medium:M9 minimal media supplemented with 1 mM biotin and 1 mM thiamine; $^{15}NH_4Cl$ and ^{13}C-glucose were the sole nitrogen and carbon source

Procedure: Bacteria were grown in M9 minimal media supplemented with 1 mM biotin and 1 mM thiamine; $^{15}NH_4Cl$ and ^{13}C-glucose were the sole nitrogen and carbon source. Starter cultures (50 mL in a 250 mL flasks) were prepared with media supplemented with 100 µL of glycerol stock and shaken overnight (10 hours) at 220 rpm at 37°C. They were used to inoculate 1 L of minimal growth media in a modified LEX fermentation system at 37°C to an OD_{600} of 1.0. Cultures were induced with 1 mM IPTG and grown at 16°C overnight (10 hours). Cells were harvested by centrifugation and frozen in 50 mL Falcon tubes at -80°C.

Purification

Procedure: Lysate was clarified by centrifugation for 20 min at 4°C and the supernatant was mixed for 20 minutes at 4°C with 4 mL settled Ni^{2+} affinity beads. Beads were batch-washed twice with 10 mL of cold wash buffer (spun at 2000 rpm for 6 minutes), transferred to a column, and further washed with 5 mL of wash buffer. Protein was eluted with 5 mL of elution buffer. The His-tag was cut off using Thrombin. Protein was further purified using gel-filtration chromatography (Hi-Load Superdex 75 column). Purifies protein was buffer exchanged into NMR buffer and protein concentration was performed using VivaSpin concentrators with a 5,000 molecular weight cut-off at 3000 rpm, resulting in a final volume of 500 uL. Protein samples were transferred to a 5 mm NMR tube for data collection.

Extraction

Procedure: Frozen cell pellets from 1 L cultures were thawed, resuspended in 50 mL lysis buffer and lysed by sonication.

Structure Determination

NMR Spectroscopy:A series of spectra (^{13}C-edited aliphatic NOESY, ^{13}C-edited aromatic NOESY, ^{15}N-edited NOESY-HSQC, ^{13}C Constant Time HSQC, 3D HNCO, 3D HNCA, 3D CBCA(CO)NH, 3D HBHA(CO)NH, 3D (H)CCH-TOCSY, and 3D H(C)CH-TOCSY) were

collected at 25°C on Bruker Avance 600 MHz equipped with a z-shielded gradient triple resonance cryoprobe. Chemical shifts were referenced to external DSS. All spectra were non-uniformly sampled, and were processed using the NMRPipe, NMRDraw and SPARKY software. The resonance assignments were obtained by ABACUS approach using FMCGUI program[15]. Distance restraints for structure calculations were derived from cross-peaks in ^{15}N-edited NOESY-HSQC, ^{13}C-edited aliphatic and aromatic NOESY-HSQC spectra. The restraints for backbone phi and psi torsion angles were derived from chemical shifts of backbone atoms using TALOS+[16]. Automated NOE assignment and structure calculations were performed using CYANA[17] (version 2.1) using its standard protocol. The best 20 of 100 CYANA structures from final cycle were selected and subjected to molecular dynamics simulation in explicit water by the program CNS. The structures were inspected by PROCHECK and MolProbity using NESG validation software package PSVS.