Entry clone accession: BC134805

Entry clone source: DNA 09-F4

SGC clone accession: YTC047-E06

Expression Tag: N-terminal His6-tag

Construct comments: Sequence 100% identical

Construct sequence:

*mhhhhhhssgrenlyfqg*MGSGPIDPKELLKGLDSFLTRDGEVKSVDGIAKIFSLMKEARKMVSR
CTYLNIILQTRAPEVLVKFIDVGGYKLLNSWLTYSKTTNNIPLLQQILLTLQHLPLTVDHLK
QNNTAKLVKQLSKSSEDEELRKLASVLVSDWMAVIRSQS

The DNA sequence was verified by sequencing.

Vector: pET28-MHL

Expression host: BL21(DE3)V2R-pRARE2

Growth method:

Shaking

The target protein was over-expressed in *E. coli*. 12.5 mL of overnight culture, grown in Luria-Bertani media, was inoculated into 1 L of M9 medium containing ^{13}C-glucose and $^{15}NH_4Cl$, in the presence of 50 μg/mL kanamycin and 34 μg/mL chloramphenicol. When the OD600 of the culture reached ~ 0.8, the temperature was lowered to 16 °C and the culture was induced with 0.25 mM final IPTG concentration. The cells were allowed to grow overnight before harvesting by centrifugation (@7,000 rpm for 15 min using a Beckman JLA-8.1000 rotor). The cell pellets were flash frozen in liquid nitrogen and stored at -80 °C.

Lysis buffer: Phosphate-buffered saline pH7.4

Lysis procedure:

A Cell pellet corresponding to 1 L of growth culture was suspended in a total volume of 200 mL extraction buffer, with 1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 10 mins at 5" on 7" off duty cycle at 120W output power.

Purification buffers:

Washing Buffer: Phosphate-buffered saline, 30 mM imidazole

Elution Buffer: Phosphate-buffered saline, 250 mM imidazole

Gel Filtration Buffer: 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM DTT

Purification procedure:

The cell lysate was centrifuged at 16,000 rpm (25,800 *x g* RCF) for 60 minutes. The supernatant was supplemented with 6 mL Ni-NTA resin (50% flurry), and incubated on a rotary drum for 1 hour at 4 °C, then loaded onto a Bio-rad gravity column. The beads, deposited in an open column, were then washed with 50 mL lysis buffer, followed by 15 mL washing buffer. Bound proteins were eluted using 15 mL elution buffer. The protein was further purified using gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare) pre-equilibrated with gel filtration buffer. Fractions containing the target protein were pooled and concentrated by centrifugal filters. The yield of the protein was ~ 4 mg/L, and the purity above 95% judging from SDS-PAGE.

Functional multimerization: Monomer (as determined by Gel filtration chromatography)

Protein stock concentration:

Concentration used for NMR: ~ 8 - 10 mg/mL (400 - 500 mM)

NMR data acquisition:

Spectra were acquired at 30 °C on Bruker spectrometers operating at 600 or 800 MHz, and equipped with cryogenic probes. All 3D spectra were acquired with non-uniform sampling schemes in the indirect dimensions and were reconstructed by multi-dimensional decomposition software MDDNMR (1) or qMDD (2), interfaced with NMRPipe (3). Conventional backbone and NOESY spectra were acquired as described previously (4), and the automated program ABACUS (5) was used to aide in the assignment of backbone and sidechain resonances.

Structure calculation:

Initial automated NOE assignments and structure calculations were performed using the *noeassign* module in CYANA 3.0 (6). The best 20 of 100 CYANA structures from the final cycle were refined with CNSSOLVE (7) by performing a short restrained molecular dynamics simulation in explicit solvent (8). The NMR structural ensemble is comprised of the final 20 refined structures.

References

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4) Lemak, A., Gutmanas, A., Chitayat, S., Karra, M., Farès, C., Sunnerhagen, M., and Arrowsmith, C.H. (2011) A novel strategy for NMR resonance assignment and protein structure determination. *J. Biomol. NMR* **49**:27-38.

5) Lemak, A., Steren, C.A., Arrowsmith, C.H., and Llinás, M. (2008) Sequence specific resonance assignment via Multicanonical Monte Carlo search using an ABACUS approach. *J. Biomol. NMR* **41**:29-41.

6) Güntert, P. (2004) Automated NMR structure calculation with CYANA. *Methods Mol. Biol.* **278**: 353-378.

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