PDB: 6NFT

Entry Clone Accession:

Entry Clone Source:

SGC Clone Accession: SDC132-E06

Tag: N-terminal tag: MHHHHHHSSGRENLYFQG

Host: BL21 Codon Plus RIL

Vector: pet28-MHL

Sequence:
gGEVRQVSKHAFSLKQLDNPARIPPCGWKCSKCDMRENLWLNLTDGSILCGRRYFDGSG
GNNHAVEHYRETGYPLAVKLGTITPDGADVYSYDEDDMVLDP
SLAEHLSHFGIDMLKMQKTD

Growth:

Medium: M9

Antibiotic: 50 µg/mL Kanamycin, 34 µg/mL chloramphenicol

Shaker: Cultures were grown in M9 media supplemented with 50 µM $ZnCl_2$, and grown at 37ºC until OD_{600} of 0.6 and induced with 0.5 mM IPTG at 15ºC for overnight growth. Cell pellets were collected by centrifugation: 6000 RPM for 10 min (JLA8.1000), frozen and stored at -80ºC.

Purification:

Buffers: Buffer A: 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP; Buffer B: 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP, 15 mM imidazole; Buffer C: 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP, 300 mM imidazole

Procedure: Clarified lysate rocked with 5 mL Ni-NTA resin (Qiagen) for 1 hour at 4˚C. Beads washed with 100 mL Buffer A, then 200 mL Buffer B before elution with 2x15 mL Buffer C. Eluent supplemented with TEV and dialysed with snakeskin MWCO 3500 against 1 L Buffer A for 2 hours then fresh 2 L Buffer A overnight. Cleavage of protein was verified with SDS-PAGE analysis. Cleaved protein incubated with 5 mL Ni resin and rocked for 1 hour at 4˚C. The beads were eluted with Buffer C, concentrated to 5 mL and run on S75 16/60. The protein was concentrated (Amicon MWCO 10,000) to 8 mg/mL. The yield of the protein was approximately 2 mg per litre of bacterial culture.

Extraction:

Cells were re-suspended in 400 mL of Buffer A and supplemented with benzonase and 1x protease inhibitors and lysed by sonication for 10 mins at 5” on 7” off at 85W output power. Lysates were clarified by centrifugation: 15,000 RPM for 1 hour (JLA 16.250).

Mass Spec: 13517.29 g/mol

Structure Determination:

Ligand: (4-oxoquinazolin-3(4H)-yl)acetic acid

Concentration: protein: 12 mg/mL

Crystallization: Crystals of the complex were grown at 291 K using vapor diffusion sitting drop by mixing equal volumes of 1:2.5 protein (12 mg/mL, 1.1% DMSO (v/v)): ligand and crystallization buffer (2 M sodium/potassium phosphate pH 7.0). The crystals were cryoprotected in 25% ethylene glycol (v/v) and cryo cooled in liquid nitrogen.

Data Collection & Processing: X-ray diffraction data was collected at 100 K at a Rigaku FR-E Superbright copper source. Diffraction data were processed with the XDS and AIMLESS, using the Xia2 interfaces and were solved by direct refinement of protein chains A and B of the isomorphous PDB entry 2G43.