ITCH

PDB: 5C7M
Entry Clone Accession: ITCH: BC011571
Entry Clone Source: MGC:AU66-H3
SGC Clone Accession: ITCH: SDC094-D06

Ubv.it.02: YTC006-E08
Tag: N-terminal His6-tag, TEV cleavable
Host: BL21(DE3)-V2R
Vector: ITCH: pET28-MHL; Ubv.it.02: pNIC-CH
Prelude: itch.483.862, in reference to sequence NP_113671.3

Sequence: ITCH:
mhhhhhhssgrenlyfqgYVRDFKAKVQYFRFWCQQLAMPQHIKITVTRKTLFEDSFQQIMSFSP
QDLRRRLWVIFPGEEGLDYGGVAREWFFLLSHEVSNPMYCLFEYAGKDNYCLQINPASYI
NPDHLKYFRFIGRFIAMALFHGKFIDTGFSLPFYKRILNKPVGLKDLESIDPEFYNSLIWVK
ENNIEECDLEMYFSVDKEILGEIKSHDLKPNGGNILVTEENKEEYIRMVAEWRLSRGVEE
QTQAFFEGFNEILPQQYLQYFDAKELEVLLCGMQEIDLNDWQRHAIYRRYARTSKQIMW
FWQFVKEIDNEKRMRLLQFVTGTCRLPVGGFADLMGSNGPQKFCIEKVGKENWLPRSH
TCFNRLDLPPYKSYEQLKEKLLFAIEETEGFGQE

Ubiquitin Variant, Ubv.it.02:
MHILVKTLRGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLFGGNKLEDGRTLSDYNI
QKESNLYLLLRRLGSKFhhhhhh

Growth

Procedure:LEX Bubbling. The target proteins were expressed in E. coli by inoculating 50 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 ug/mL kanamycin, 600 uL anti-foam at 37 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 18 degree and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (12,227g 10min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure: 4L cell pellet was resuspended in a total volume of 200 ml lysis extractionbuffer and the cells disrupted by sonication (10min, 10 sec on, 10 sec off,output 100-120 W). The lysate was centrifuged at 16,000 rpm (25,800xgRCF(average) for 60 minutes and the supernatant was loaded onto 5 mL opencobalt metal-affinity resin column. The column was then washed 3 times with 15mL washing buffer each. Bound proteins were eluted using 15 mL elution bufferin total. Pooled fractions were diluted to ~100mL using Q column buffer A andloaded on a HiTrap Q column (GE Healthcare) and eluted with 1M NaCl gradient(20CV). The elutants containing the target protein was pooled and concentrated.The final protein concentration is 30 mg/mL measured by nano-drop based on UV-absorbance at 280nM. The purity of the preparation is tested by SDS-PAGE to be >95%.

Structure Determination

Ubiquitin variant Ubv.IT.02 MassSpec: ITCH, uncut version native protein expected 47429.3, measured 47429.9

Ubv.it.02, measured 9748.3 Da, as expected

Crystallization: The ITCH and ubiquitin variant ubv.it.02 were mixed at molarity ratio 1:2, and then concentrated to 17mg/ml.The protein sample was mixed with 1mg/mL chymotrypsin at a 1:1000 (W/W) chymotrypsin:protein ratio right before set up crystallization.Crystal was initially obtained from SGC-I screen condition A05.Crystal used for structure refinement was grown in 1.6M NH_4SO_4, 0.2M NaAc, 0.1M HEPES pH 7.5, 5% Ethylene Glycol in hanging drop setup, using 1.2uL protein, 1.2uL well solution over 0.5 mL reservoir buffer at 20 °C. Crystals grow to moutable size for ~1 weeks.Harvested crystal was flash-frozen in liquid nitrogen. A well solution containing 20% glycerol was used as the cryo-protectant.