ITCH

PDB:4ROH
Entry Clone Accession:NP_001244066.1.
Entry Clone Source: Open Biosystems
SGC Clone Accession:ITCH (ITCH:YTC002-B06): M139-E298
Tag:N-terminal tag:
MKIEEHHHHHHSSGKLMSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRN
KKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRY
GVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVV
LYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLE
VLFQGPLSSGLVPRGSGTAAQPAENLYFQGRRASVEL
Host: Escherichiacoli BL21 (DE3)
Vector:pET28GST-lic (modified)

Construct Sequence:
grrasvelEFDPLGPLPPGWEKRTDSNGRVYFVNHNTRITQWEDPRSQGQLNEKPLPEGWEM
RFTVDGIPYFVDHNRRTTTYIDPRTGKSALDNGPQ

Growth

Procedure: Lex system, 37 Â°C 3-4h, 15 Â°C 18-24h

Purification

Procedure: IMAC: Unclarified lysate was mixed with 3-4 mL of Ni-NTA superflow Resin (Qiagen) per 200 mL lysate. The mixture was incubated with mixing for at least 30 minutes at 4°C. The mixture was then loaded onto an empty comLum (BioRad) and washed with 40 mL wash buffer. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280

Gel filtration chromatography: An XK 16x60 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1.0 mL/min. The concentrate sample (approx. 3 mL, concentrated by using 15 mL concentrators with a 3,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm) from the IMAC step was loaded onto the column at 1 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols. Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

Extraction

Procedure: Frozen cell pellet contained in bags (Beckman 369256) obtained from 2L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 200 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 seconds pulse at 80% of maximal frequency (8.0), 10 seconds rest, for 10 minutes total sonication time per pellet.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 10 mg/mL.

Structure Determination

Crystallization: Recombinant human ITCH ww3-ww4 domain was mixed with PPCY peptide of TXNIP at molar ratio of 1:3 and co-crystallized using the sitting drop vapour diffusion method at 18 °C. The crystals were obtained in a buffer containing 30% PEG 4K, 0.2 M MgCl2, 0.1 M Tris HCI, pH 8.5. Crystals were soaked in a cryoprotectant consisting of 100% reservoir solution and 15% glycerol.