Molecular Biology

Entry Clone Accession: NM_017415.2
Entry Clone Source: MGC
SGC Construct ID: KLHL3A-c003
Protein Region: S298-L587
Vector: pNIC28-Bsa4
Tag: N-6HIS;N-TEV
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MHHHHHHSSGVDLGTENLYFQSMSLPKVMIVVGGQAPKAIRSVECYDFEEDRWDQIAE
LPSRRCRAGVVFMAGHVYAVGGFNGSLRVRTVDVYDGVKDQWTSIASMQERRSTLGAA
VLNDLLYAVGGFDGSTGLASVEAYSYKTNEWFFVAPMNTRRSSVGVGVVEGKLYAVGG
YDGASRQCLSTVEQYNPATNEWIYVADMSTRRSGAGVGVLSGQLYATGGHDGPLVRKSV
EVYDPGTNTWKQVADMNMCRRNAGVCAVNGLLYVVGGDDGSCNLASVEYYNPVTDK
WTLLPTNMSTGRSYAGVAVIHKSL
Sequence after tag cleavage:
SMSLPKVMIVVGGQAPKAIRSVECYDFEEDRWDQIAELPSRRCRAGVVFMAGHVYAVG
GFNGSLRVRTVDVYDGVKDQWTSIASMQERRSTLGAAVLNDLLYAVGGFDGSTGLASVE
AYSYKTNEWFFVAPMNTRRSSVGVGVVEGKLYAVGGYDGASRQCLSTVEQYNPATNEW
IYVADMSTRRSGAGVGVLSGQLYATGGHDGPLVRKSVEVYDPGTNTWKQVADMNMCR
RNAGVCAVNGLLYVVGGDDGSCNLASVEYYNPVTDKWTLLPTNMSTGRSYAGVAVIHK
SL
DNA Sequence:
CATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGAGCCTTCCCAAGGTCATGATTGTGGTTGGCGGCCAGGCACCCAAG
GCAATCCGCAGTGTGGAGTGCTATGATTTCGAGGAGGACCGGTGGGATCAGATTGCTG
AGCTTCCTTCCAGAAGATGCAGAGCAGGTGTGGTGTTCATGGCTGGCCACGTGTATGC
CGTGGGAGGGTTTAATGGCTCACTGCGGGTGCGGACAGTGGATGTGTATGACGGCGT
GAAGGACCAGTGGACGTCCATTGCCAGCATGCAGGAGCGCCGGAGCACACTGGGCG
CAGCGGTGCTCAATGACTTGCTCTACGCAGTGGGAGGCTTTGATGGCAGTACTGGCCT
AGCATCGGTGGAAGCCTACAGCTACAAGACCAACGAGTGGTTCTTTGTGGCCCCGAT
GAACACGCGGCGGAGCAGTGTGGGTGTGGGCGTTGTGGAGGGGAAGCTATATGCTGT
TGGGGGTTATGATGGAGCTTCCCGCCAGTGTCTGAGCACTGTGGAGCAGTACAACCC
AGCGACCAATGAATGGATATACGTGGCGGACATGAGCACCCGCCGCAGTGGCGCAGG
GGTTGGAGTGCTTAGCGGACAGCTGTACGCCACAGGTGGGCATGATGGGCCTTTGGT
GAGGAAGAGCGTTGAGGTTTACGATCCTGGAACAAATACCTGGAAGCAAGTGGCAGA
CATGAACATGTGCCGGCGCAACGCAGGGGTCTGTGCAGTAAATGGGCTCCTGTATGTG
GTTGGAGGGGATGATGGATCCTGCAACTTGGCTTCGGTGGAGTACTACAATCCTGTCA
CTGACAAATGGACGCTGCTTCCAACGAACATGAGCACGGGGCGGAGCTATGCAGGTG
TTGCCGTGATTCACAAGTCCTTGTGACAGTAAAGGTGGATACGGATCCGAA

Protein Expression

Medium: Lysogeny broth
Antibiotics: Kanamycin
Procedure: KLHL3A-c003 was transformed into BL21(DE3)-R3-pRARE2. 10mL E.coli overnight culture was inoculated into 2L autoclaved LB medium with 50ug/mL of each Kanamycin and Chloramphenicol. E.coli cells were grown in 37^{o}C with 160RPM shaking until

the OD_{600} reached 0.6. Expression was induced with 0.4mM Isopropyl β-D-1-thiogalactopyranoside and cultured overnight in 18^{o}C with 160RPM shaking

Protein Purification

Procedure: After cell lysis with 15min sonication, His-KLHL3 was purified by IMAC and eluted out by imidazole. Eluted fractions were pooled and then gel filtrated. His tag was cleaved by TEV protease. Untagged KLHL3 was purified by reverse IMAC. Purified KLHL3 was concentrated to 10mg/mL using 10kD MWCO concentrator. KLHL3 final stock was buffered in 50mM HEPES pH7.5, 300mM NaCl and 0.5mM TCEP.

Columns: Column 1: 5mL Ni column; Column 2: Gel F 16/60 S75; Column 3: 0.5 mL Ni rebind

Concentration: 10 mg/ml
Mass-spec Verification: Intact mass confirmed. (expected - 31499.6. observed – 31500.4)

Structure Determination

Crystallization: KLHL3 protein was co-crystalized with 4mM WNK3 degron peptide (ECEETEVDQHV). Crystals were yielded after micro-seeding. Seeds were obtained from 8% PEG4000 -- 0.1M acetate pH 4.5 at 4^{o}C in coarse screen. Those early crystals were transferred into an eppendorf tube containing 50 µL reservoir solution and a seed bead (Hampton Research), then vortexed for 2 min. Seed-stocks were diluted in 500 fold when in use. The drops were spiked with 20 nL of diluted seed-stock solution. The best-diffracting crystals of the KLHL3 complex were obtained at 4°C by mixing 75 nL of protein with 75 nL of a reservoir solution containing 6% PEG4K -- 0.1M acetate pH 5.1. Prior to vitrification in liquid nitrogen, crystals were cryoprotected by direct addition of reservoir solution supplemented with 25 % ethylene glycol.

Data Collection: *Beamline:* Dmnd I03; *Resolution:* 2.8 Å; *Wavelength*: 0.9763Å;
Data Processing: The data for KLHL3-WNK3 crystal were processed in software PHENIX version1.9 (Adams, Afonine et al. 2010). Molecular replacement was performed with PHENIX.Phaser-MR using PDB code 4CH9 chain A (Kelch domain of KLHL3) as the model.