Molecular Biology

Entry Clone Accession: NM_017545
Entry Clone Source: Origene
SGC Construct ID: HAO1A-c002
Protein Region: M1-S368
Vector: pNIC28-Bsa4
Tag: N-6HIS; TEV cleavage site
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MHHHHHHSSGVDLGTENLYFQSMLPRLICINDYEQHAKSVLPKSIYDYYRSGANDEETL
ADNIAAFSRWKLYPRMLRNVAETDLSTSVLGQRVSMPICVGATAMQRMAHVDGELATV
RACQSLGTGMMLSSWATSSIEEVAEAGPEALRWLQLYIYKDREVTKKLVRQAEKMGYK
AIFVTVDTPYLGNRLDDVRNRFKLPPQLRMKNFETSTLSFSPEENFGDDSGLAAYVAKAI
DPSISWEDIKWLRRLTSLPIVAKGILRGDDAREAVKHGLNGILVSNHGARQLDGVPATIDV
LPEIVEAVEGKVEVFLDGGVRKGTDVLKALALGAKAVFVGRPIVWGLAFQGEKGVQDV
LEILKEEFRLAMALSGCQNVKVIDKTLVRKNPLAVS
Sequence after tag cleavage:
SMLPRLICINDYEQHAKSVLPKSIYDYYRSGANDEETLADNIAAFSRWKLYPRMLRNVAE
TDLSTSVLGQRVSMPICVGATAMQRMAHVDGELATVRACQSLGTGMMLSSWATSSIEEV
AEAGPEALRWLQLYIYKDREVTKKLVRQAEKMGYKAIFVTVDTPYLGNRLDDVRNRFK
LPPQLRMKNFETSTLSFSPEENFGDDSGLAAYVAKAIDPSISWEDIKWLRRLTSLPIVAKGI
LRGDDAREAVKHGLNGILVSNHGARQLDGVPATIDVLPEIVEAVEGKVEVFLDGGVRKG
TDVLKALALGAKAVFVGRPIVWGLAFQGEKGVQDVLEILKEEFRLAMALSGCQNVKVI
DKTLVRKNPLAVS
DNA sequence:
CATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGCTCCCCCGGCTAATTTGTATCAATGATTATGAACAACATGCTAAATC
AGTACTTCCAAAGTCTATATATGACTATTACAGGTCTGGGGCAAATGATGAAGAAACTT
TGGCTGATAATATTGCAGCATTTTCCAGATGGAAGCTGTATCCAAGGATGCTCCGGAAT
GTTGCTGAAACAGATCTGTCGACTTCTGTTTTAGGACAGAGGGTCAGCATGCCAATAT
GTGTGGGGGCTACGGCCATGCAGCGCATGGCTCATGTGGACGGCGAGCTTGCCACTG
TGAGAGCCTGTCAGTCCCTGGGAACGGGCATGATGTTGAGTTCCTGGGCCACCTCCTC
AATTGAAGAAGTGGCGGAAGCTGGTCCTGAGGCACTTCGTTGGCTGCAACTGTATATC
TACAAGGACCGAGAAGTCACCAAGAAGCTAGTGCGGCAGGCAGAGAAGATGGGCTA
CAAGGCCATATTTGTGACAGTGGACACACCTTACCTGGGCAACCGTCTGGATGATGTG
CGTAACAGATTCAAACTGCCGCCACAACTCAGGATGAAAAATTTTGAAACCAGTACTT
TATCATTTTCTCCTGAGGAAAATTTTGGAGACGACAGTGGACTTGCTGCATATGTGGCT
AAAGCAATAGACCCATCTATCAGCTGGGAAGATATCAAATGGCTGAGAAGACTGACAT
CATTGCCAATTGTTGCAAAGGGCATTTTGAGAGGTGATGATGCCAGGGAGGCTGTTAA
ACATGGCTTGAATGGGATCTTGGTGTCGAATCATGGGGCTCGACAACTCGATGGGGTG
CCAGCCACTATTGATGTTCTGCCAGAAATTGTGGAGGCTGTGGAAGGGAAGGTGGAA
GTCTTCCTGGACGGGGGTGTGCGGAAAGGCACTGATGTTCTGAAAGCTCTGGCTCTT
GGCGCCAAGGCTGTGTTTGTGGGGAGACCAATCGTTTGGGGCTTAGCTTTCCAGGGG
GAGAAAGGTGTTCAAGATGTCCTCGAGATACTAAAGGAAGAATTCCGGTTGGCCATG
GCTCTGAGTGGGTGCCAGAATGTGAAAGTCATCGACAAGACATTGGTGAGGAAAAAT
CCTTTGGCCGTTTCCTGACAGTAAAGGTGGATACGGATCCGAA

Protein Expression

Medium: Terrific Broth
Antibiotics: Kanamycin
Procedure: An overnight culture (20 mL LB) was used to inoculate 2L auto-induction TB containing 50 µg/ml each of kanamycin and chloramphenicol. Cells were cultured at 37°C for 6 hours followed by 48 h incubation at 18°C.

Protein Purification

Procedure: Cell pellet from 2 L of culture was re-suspended in 150 mL Extraction Buffer, lysed by sonication for 15 minutes, and centrifuged at 37000 x g for 1 hour at 4°C. The clarified cell extract was incubated with 5 mL of Ni-NTA resin pre-equilibrated with lysis buffer before applying to 1.5 x 10 cm column by gravity flow. The column was washed with 10 column volumes of Binding Buffer, 10 column volumes of Wash Buffer, and eluted with 5 x 5 mL Elution Buffer. Fractions containing hHAO1 were loaded onto gel filtration column (Superdex 200 Hiload 16/60) pre-equilibrated with GF buffer. Fractions containing hHAO1 were pooled.

Extraction Buffer: 500 mM NaCl, 50 mM HEPES pH 7.5, 20 mM imidazole, 0.5 mM TCEP, 5% glycerol, 1:1000 of Merck Protease Cocktail II, 0.5 mg/mL lysozyme and 0.2 µg/mL benzonase.
Binding Buffer: 500 mM NaCl, 50 mM HEPES pH 7.5, 20 mM imidazole, 0.5 mM TCEP, 5% glycerol.
Washing Buffer: 500 mM NaCl, 50 mM HEPES pH 7.5, 40 mM imidazole, 0.5 mM TCEP, 5% glycerol.
Elution Buffer: 500 mM NaCl, 50 mM HEPES pH 7.5, 250 mM imidazole, 0.5 mM TCEP, 5% glycerol.
GF buffer: 500 mM NaCl, 550 mM HEPES pH 7.5, 0.5 mM TCEP, 5% glycerol.

Concentration: 13.7 mg/ml
Mass-spec Verification: Yes

Structure Determination

Crystallization: Crystals were grown by vapour diffusion at 4°C in sitting drops of 13.7 mg/mL protein equilibrated against well solution containing 25-35% PEG1000, sodium malonate-imidazole-boric acid pH 8.0. Approximately 50 crystals of suitable size were identified per 96-well crystallisation plate, and a total of 10 plates generated sufficient crystals for the entire fragment campaign.

Data Collection: Crystals were soaked for two hours with fragments from the Diamond-SGC Poised Library before being harvested using XChem SHIFTER technology, cryo-cooled in liquid nitrogen, and data sets collected at the beamline I04-1 in “automated unattended” mode.
Data Processing: The XChemXplorer pipeline was used for structure solution with parallel molecular replacement using DIMPLE (template 2NZL), followed by map averaging and statistical modelling to identify weak electron densities generated from low occupancy fragments using Pandda software.