Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5301753

SGC Construct ID: ACBD7A-c000

GenBank GI number: gi|89886362

Vector: pNIC28-Bsa4

Tags and additions: Tag sequence: N-terminal TEV-cleavable (at *) his-tag with the following sequence mhhhhhhssgvdlgtenlyfq*s

Final protein sequence:
mhhhhhhssgvdlgtenlyfq*sMALQAD
FDRAAEDVRKLKARPDDGELKELYGLYKQ
AIVGDINIACPGMLDLKGKAKWEAWNLKK
GLSTEDATSAYISKAKELIEKYGI

Host: BL21(DE3)-R3-pRARE2

Expression protocol: Competent BL-21 (DE3) phage resistant cells with were transformed with the plasmid DNA and plated out onto LB plate plus 50 µg/ml kanamycin. The next day colonies were picked into fresh deep well blocks containing 1 ml TB + 50 µg/ml kanamycin which were grown overnight. Glycerol stocks were prepared by adding 333 µl of 60 % glycerol to 1 ml of cell suspension, which were stored at -80°C to be used for future scale up preparations.
The glycerol stock was used to inoculate 10 ml of TB (terrific Broth) supplemented with 50 µg/ml kanamycin. This starter culture was grown overnight at 37°C and used to inoculate a 1 liter culture in the same medium. The culture was grown at 37°C until the OD_{600} reached ~3.0. After that the temperature was lowered to 18°C. Protein production was induced with 1mM IPTG and recombinant ACBD7A was expressed at that temperature over night. The next day cells were harvested by centrifugation at 4000 rpm for 15 minutes. The cell pellet was stored at -80°C.

Extraction buffer and extraction method:
2xExtraction buffer: 20mM imidazole, 1.0 M NaCl, 100mM NaH_2PO_4, pH8.0, 1 mM TCEP, 1x protease inhibitor cocktail (Complete PI EDTA free tablets); Benzonase Nuclease HC, (3 µl per 30ml).
Extraction buffer: 10mM imidazole, 500mM NaCl, 50mM NaH_2PO_4, pH8.0, 0.5mM TCEP, 1x protease inhibitor cocktail (Complete PI EDTA free tablets); Benzonase Nuclease HC, (3 µl per 60ml extraction buffer).
Procedure: The cell pellet (10 g) was re-suspended in one volume (10 ml) of 2x extraction buffer. The re-suspended cells were lysed two times through a Constant Systems cell breaker and subsequent sonication; the cell breaker was washed with 1x extraction buffer, bringing the total volume to 120 ml. DNA was precipitated by addition of PEI to a final concentration of 0.15 % during an incubation time of 30 min on ice, followed by a centrifugation at 17,000 rpm (4°C); The supernatant was further cleared by filtration through a 0.2 µm serum Acrodisc filter.

Column 1: Ni-affinity chromatography: HisTrap FF Crude, 5 ml (GE Healthcare).

Buffers: Binding Buffer: 10mM Imidazole, 500mM NaCl, 50mM NaH_2PO_4, pH 8.0, 5% glycerol, 0.5mM TCEP; **Wash Buffer**: 20mM Imidazole, 500mM NaCl, 50mM NaH_2PO_4, pH 8.0, 5% glycerol. 0.5mM TCEP; **Elution Buffer:** 300mM Imidazole, 500mM NaCl, 50mM NaH_2PO_4, pH8.0, 5% glycerol, 0.5mM TCEP.

Procedure: All purification steps were carried out using an AKTAexpress system (GE Healthcare) at 7ºC. The lysate was loaded on a pre-equilibrated His-trap column at 0.8 ml/min, using a standard purification method. After loading, the column was washed at 0.8 ml/min with 10 ml binding buffer, then 20 ml wash buffer, and protein was eluted with 5 ml of elution buffer. The peak fraction was collected automatically according to A280. The N-terminal his 6 -tag was cleaved by incubating the protein overnight with TEV protease.

Column 2: Size exclusion chromatography HiLoad 16/60 Superdex 75
SEC-Buffers: 10 mM Hepes, pH 7.4, 500 mM NaCl, 5% glycerol,10 mM DTT
Procedure: The ACBD7A containing fraction eluted of the Ni-affinity chromatography was automatically loaded on the SEC column at 1.2 ml/min. ACBD7A eluted at a retention time corresponding to the monomeric protein. Eluted fractions were 95% pure as judged by SDS-PAGE.
Mass spec characterization: ESI-MS revealed that the protein had the expected mass of 9878 Da.
Protein concentration: 18 mg/ml in SEC buffer using a centricon with a 10 kDa cut off.
Crystallization:. Palmitoyl-CoA was added to a final concentration of 10 µM. Crystals were grown by sitting drop vapour diffusion in nanolitre sitting drops at 20°C equilibrated against reservoir containing 25% PEG 3350; 0.20M $MgCl_2$; 0.1M BIS-TRIS pH 5.5. Crystals were cryoprotected in 30% glycerol and flash-frozen in liquid nitrogen.
Data Collection: Resolution: 2.05Å; **X-ray source:** Swiss Light source (SLS), beamline X-10.