PRKCBP1A (ZMYND8) PHD-BRD-PWWP module

PDB Code: 4COS

Material and Methods

Entry Clone Source: Synthetic

GI number: gi|34335262

Expressed sequence:

MHHHHHHSSGVDNKFNKERRRARREIRHLPNLNREQRRAFIRSLRDDPSQSANLLAEAKKLN
DAQPKGTENLYFQ^SMQDGRNDFYCWVCHREGQVLCCELCPRVYHAKCLRLTSEPEGDWFCP
ECEKITVAECIETQSKAMTMLTIEQLSYLLKFAIQKMKQPGTDAFQKPVPLEQHPDYAEYIF
HPMDLCTLEKNAKKKMYGCTEAFLADAKWILHNCIIYNGGNHKLTQIAKVVIKICEHEMNEI
EVCPECYLAACQKRDNWFCEPCSNPHPLVWAKLKGFPFWPAKALRDKDGQVDARFFGQHDRA
WVPINNCYLMSKEIPFSVKKTKSIFNSAMQEMEVYVENIRRKFGVFNYSPFRTPYTPNSQYQ
MLLDPTNPSAGTAKIDKQEKVKLNFDMTAS
^ TEV cleavage site

Construct DNA sequence:

ATGCACCATCATCATCATCATTCTTCTGGTGTGGATAACAAGTTCAACAAGGAGCGTCG
AAGAGCTCGCCGTGAAATTCGCCATCTGCCGAACCTGAACCGCGAACAGCGTCGCGCAT
TTATTCGCAGCCTGCGCGATGATCCGAGCCAGAGCGCGAACCTGCTGGCGGAAGCGAAG
AAGCTGAACGATGCGCAGCCGAAGGGTACCGAGAACCTGTACTTCCAATCCATGCAGGA
TGGACGGAATGATTTCTACTGCTGGGTTTGTCACCGGGAAGGCCAAGTCCTTTGCTGTG
AGCTCTGTCCCCGGGTTTATCACGCTAAGTGTCTGAGACTGACATCGGAACCAGAGGGG
GACTGGTTTTGTCCTGAATGTGAGAAAATTACAGTAGCAGAATGCATCGAGACCCAGAG
TAAAGCCATGACAATGCTCACCATTGAACAGTTATCCTACCTGCTCAAGTTTGCCATTC
AGAAAATGAAACAGCCAGGGACAGATGCATTCCAGAAGCCCGTTCCATTGGAACAGCAC
CCTGACTATGCGGAATACATCTTCCATCCAATGGACCTTTGTACATTGGAAAAGAATGC
GAAAAAGAAAATGTATGGCTGCACAGAAGCCTTCCTGGCTGATGCAAAGTGGATTTTGC
ACAACTGCATCATTTATAATGGGGGAAATCACAAATTGACGCAAATAGCGAAAGTAGTC
ATCAAAATCTGTGAACATGAGATGAATGAAATCGAAGTATGTCCAGAATGTTATCTAGC
TGCTTGCCAAAAACGAGATAACTGGTTTTGTGAGCCTTGTAGCAATCCACATCCTTTGG
TCTGGGCCAAACTGAAGGGGTTTCCATTCTGGCCTGCAAAAGCTCTAAGGGATAAAGAC
GGGCAGGTCGATGCCCGATTCTTTGGACAACATGACAGGGCCTGGGTTCCAATAAATAA
TTGCTACCTCATGTCTAAAGAAATTCCTTTTTCTGTGAAAAAGACTAAGAGCATCTTCA
ACAGTGCCATGCAAGAGATGGAGGTTTACGTGGAGAACATCCGCAGGAAGTTTGGGGTT
TTTAATTACTCTCCATTTAGGACACCCTACACACCCAACAGCCAGTATCAAATGCTGCT
CGATCCCACCAACCCCAGCGCCGGCACTGCCAAGATAGACAAGCAGGAGAAGGTCAAGC
TCAACTTTGACATGACGGCATCCTGA

Vector: pNIC-ZB

Tags and additions: Cleavable N-terminal His6-ZB tag.

Host: BL21 (DE3)R3-pRARE2 (Phage resistant strain)

Growth medium, induction protocol: 10 ml from a 50 ml overnight culture containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol were used to inoculate each of two 1 liter cultures of TB containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol with K/Na phosphates substituted with 5 g/l NaCl to prevent ZnCl2 precipitation. Cultures were grown at 37 oC until the OD600 reached ~2.5 then the temperature was adjusted to 18 oC. Expression was induced overnight using 100 Î¼M IPTG and 1 mM of ZnCl2 added at an OD600 of 3.0.

The cells were collected by centrifugation and the pellet re-suspended in binding buffer and frozen.

Binding buffer: 50 mM HEPES pH 7.5; 500 mM NaCl; 10 mM imidazole, 0.5 mM tris(2-carboxyethyl)phosphine (TCEP), 5% glycerol.

Extraction buffer, extraction method: Frozen pellets were thawed and fresh 0.5 mM TCEP, 1 mM PMSF added to the lysate. Cells were lysed using Avestin EmulsiFlex-C5 homogeniser. The lysate was centrifuged at 17,000 rpm for 60 minutes and the supernatant collected for purification.

Column 1: Ni-affinity. Ni-sepharose (Amersham), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Buffers:

Binding Buffer:50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 0.5 mM tris(2-carboxyethyl)phosphine (TCEP), 5% glycerol
Wash Buffer:50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 0.5 mM tris(2-carboxyethyl)phosphine (TCEP), 5% glycerol
Elution Buffer:50 mM HEPES pH 7.5, 500 mM NaCl, 0.5 mM tris(2-carboxyethyl)phosphine (TCEP), 5% glycerol, 60 to 300 mM Imidazole (step elution).

Procedure: The supernatant was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 30 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (60 mM, 90 and 300 mM); fractions were collected until essentially all protein was eluted.

Column 2 : Anion exchange. HP SP

Elution buffer:0.25 - 1 M NaCl

Procedure : Fractions containing recombinant protein were directly loaded onto an HP SP column on an Ã□KTA Purifier, were eluted with a 0.25 â□□ 1 M NaCl gradient and were combined

Enzymatic treatment : The Z-Basic (ZB) tag was removed by overnight incubation at 4 Ë□C with TEV protease (at 1:100 w/w).

Column 3 : Ni-affinity. Ni-sepharose (Amersham), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Procedure : The Z-Basic tag and other impurities were removed by binding to Ni-sepharose column. Flow through containing cleaved recombinant protein was collected for further purification.

Column 4 : Size Exclusion Chromatography. Superdex S75 16/60 HiLoad

Buffers : 10 mM HEPES, pH 7.5; 250 mM NaCl

Procedure : The protein was concentrated and applied to an S75 16/60 HiLoad gel filtration column equilibrated in 10 mM HEPES, pH 7.5; 250mM NaCl, using an Ã□KTAexpress system.

Mass spec characterization: LC- ESI -MS TOF gave a measured mass of 37785 for construct as predicted from the sequence of this protein.

Crystallisation Crystals were grown at 4 oC in 300 nl sitting drops from a 1:2 ratio of protein (11 mg/ml) to reservoir solution containing 1.8M (NH4)2SO4, 0.1 M MES pH 6.3 and 15 % (v/v) dioxane.

Data Collection: Crystals were cryo-protected using the well solution supplemented by 2M Li2SO4 and flash frozen in liquid nitrogen.
X-ray source: Diffraction Zn-SAD data were collected at Diamond Lightsource from one

crystal close to the Zn K-edge at 1.2829 Ã on beamline I03 and a second crystal was used to obtain higher resolution on beamline I04 at a wavelength of 0.9796 Ã. The final structure was refined to 1.67 Ã.
Phasing: The structure was solved by Zn-SAD.