**Entry Clone Source:** MGC

**Entry Clone Accession:** IMAGE:7262237

**SGC Construct ID:** CRYBB3A-c008

**GenBank GI number:** gi|4758074

**Vector:** pNIC28-Bsa4. Details [PDF ]; Sequence [ FASTA ] or [ GenBank ]

**Amplified construct sequence:**

```
CATATGCACCATCATCATCATCATTC
TTCTGGTGTAGATCTGGGTACCGAGA
ACCTGTACTTCCAATCCATGGGGGGC
AGCTACAAGGTGATCTTGTACGAACT
AGAGAACTTCCAAGGCAAACGCTGCG
AGCTCTCGGCCGAGTGCCCCAGCCTG
ACCGACAGCCTGCTGGAGAAGGTGGG
CTCCATCCAAGTGGAGTCCGGGCCGT
GGCTGGCATTTGAGTCCAGGGCCTTC
CGCGGGGAGCAGTTTGTTCTGGAGAA
GGGGGATTATCCTCGCTGGGATGCCT
GGTCCAACAGCCGTGATAGTGACAGC
CTTCTGTCCCTCCGGCCTCTGAATAT
TGATAGTCCACATCACAAGCTGCATC
TGTTTGAGAACCCAGCTTTCAGTGGC
CGCAAGATGGAGATAGTGGATGATGA
CGTGCCCAGCCTGTGGGCTCATGGCT
TCCAGGACCGTGTGGCGAGTGTCCGT
GCCATCAACGGGACGTGGGTTGGCTA
TGAGTTCCCCGGCTACCGTGGGCGCC
AGTACGTGTTTGAGCGGGGCGAGTAC
CGCCACTGGAATGAGTGGGACGCCAG
CCAGCCGCAGCTGCAGTCTGTGCGCC
GCATCCGTGACTGACAGTAAAGGTGG
ATACGGATCCGAA
```

**Final protein sequence (Tag sequence in lowercase):**

```
smGGSYKVILYELENFQGKRCELSAE
CPSLTDSLLEKVGSIQVESGPWLAFE
SRAFRGEQFVLEKGDYPRWDAWSNSR
DSDSLLSLRPLNIDSPHHKLHLFENP
AFSGRKMEIVDDDVPSLWAHGFQDRV
ASVRAINGTWVGYEFPGYRGRQYVFE
RGEYRHWNEWDASQPQLQSVRRIRD
```

The N-terminal residues, sm, derive from the vector following TEV protease digestion to remove the expression tag.

**Tags and additions:** N-terminal, TEV cleavable hexahistidine tag.

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

**Growth medium, induction protocol:** The construct DNA was transformed into competent cells of the expression strain by a standard heat shock procedure. A number of colonies were

used to inoculate 1ml of LB media containing 50µg/ml kanamycin and 34µg/ml chloramphenicol, which was incubated in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture. 5µl of a glycerol stock was used to inoculate 50ml of LB containing 50µg/ml kanamycin and 34µg/ml chloramphenicol, which was incubated at 37°C overnight. 15ml starter culture were used per litre TB, containing 50µg/ml kanamycin. The culture was incubated at 37°C until $OD_{600}$ reach ~1.2, when the temperature of the incubator was reduced to 18°C. Expression was induced with 0.1 mM IPTG and the culture continued overnight. Cells were pelleted at 6238g for 15min at 4°C, and stored at -80°C. The yield was 7.9g cells/litre culture.
**Lysis buffer**: 50 mM HEPES, pH 7.5; 500 mM NaCl; 10 mM Imidazole; 5% Glycerol; 1 mM PMSF; 0.5 mM TCEP.
**Extraction buffer, extraction method:** The pellets were resuspended in lysis buffer. They were passed 4 times through an Emulsiflex C5 high-pressure homogeniser, collecting a final volume of approximately 35ml/L culture. cell debris and DNA were spun down at 45,000g for 60 minutes (Beckman JA18 17500rpm). The supernatant was collected to which Benzonase was added to the supernatant, with a 60 minute incubation on ice.

**Column 1:** Ni-sepharose column.

**Column 1 Buffers**:
**Wash Buffer:** 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 30 mM Imidazole; 0.5 mM TCEP.
**Elution Buffer:** 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 250 mM Imidazole; 0.5 mM TCEP.

**Column 1 Procedure:** The supernatant was loaded onto an equilibrated Ni-sepharose column (1ml resin/L culture). The flow through was collected. The column was first washed with 18CV of Lysis/Binding Buffer, followed by 10CV of Wash Buffer and finally eluted with 6x1CV elution buffer. Each fraction was collected and analyzed on SDS-PAGE.

**Column 2:** Gel filtration. GiLoad S200 16/60 - 120ml volume.

**Column 2 Buffer**: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5 mM TCEP.

**Column 2 Procedure:** The gel filtration column was pre-equilibrated with Gel Filtration Buffer. The pooled Ni-sepharose eluants were loaded on the gel filtration column at a flow rate of 1.2ml/min. Eluted proteins were collected in 1.8ml fractions. The fractions containing protein were analyzed by SDS-PAGE.

**Enzymatic treatment:** Peak fractions from the gel filtration containing CRYBB3 were pooled and TEV protease was added at a molar ratio of 1:15. The digestion was left overnight at 4°C. SDS-PAGE and Mass Spec confirmed TEV digestion. His-TEV and contaminating proteins were removed by binding to Ni resin, pre-equilibrated in GF buffer.

**Mass spectrometry characterization:**
Measured: 20988.4Da
Expected: 20987.4Da

**Protein concentration:** The flow through containing TEV-cleaved protein, was collected and concentrated using an Amicon centrifugal filter with a 10kDa MW cut off. To remove any precipitation, the concentrated protein was centrifuged at 14000rpm for 20 min at 4°C and the supernatant was collected. The final concentration of protein was 46mg/ml and yield 15 mg/L culture. The protein was flash frozen and stored at -80°C in 70µl aliquots.

**Crystallisation:** Crystals grown at 4°C by vapour diffusion in sitting drops mixing protein (46mg/ml) and precipitant solution (0.1 M HEPES pH 7.5, 1.5 M $Li_2SO_4$) in a 2:1 ratio.

Crystals were cryo-protected in 20% ethylene glycol before being flash-frozen in liquid nitrogen.

**Data collection:** Data was collected to a resolution of 1.8Å at Diamond Light source beamline I02.