CAMK2B + inhibitor

PDB:3BHH

Revision

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Revised by:created
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Entry Clone Accession:gi|26051204
Entry Clone Source:MGC
SGC Clone Accession:IMAGE:5014712
Tag: Cleavable N-terminal His6 tag
Host:BL21(DE3)-R3-pRARE2-Phage resistant

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*SMTDEYQLYEDIGKGAFSVVRRCVKLCTGHEYAAKIINTKKLSARDHQKLEREARICRLLKHSNI
VRLHDSISEEGFHYLVFDLVTGGELFEDIVAREYYSEADASHCIQQILEAVLHCHQMGVVHRDLKPENLLLASKCKGAAVKLADFGL
AIEVQGDQQAWFGFAGTPGYLSPEVLRKEAYGKPVDIWACGVILYILLVGYPPFWDEDQHKLYQQIKAGAYDFPSPEWDTVTPEAKN
LINQMLTINPAKRITAHEALKHPWVCQRSTVASMMHRQETVECLKKFNARRKLKGA

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Transformed 50 µl competent BL-21 (DE3) phage resistant cells with 10 µl of the plasmid DNA and plated out onto LB plate plus 50 µg/ml kanamycin and 35 µg/ml chloramphenicol. The next day colonies were picked into fresh deep well blocks containing 1 ml LB + 50 µg/ml kanamycin and 35 µg/ml chloramphenicol. Cultures were grown overnight and glycerol stocks were prepared by adding 333 ml of 60 % glycerol to 1 ml of cell suspension, which were stored at -80°C and used for future scale up preparations.The glycerol stock was used to inoculate 10 ml of LB supplemented with 50 µg/ml kanamycin and 35 µg/ml chloramphenicol. 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 OD600 reached ~0.5. After that the temperature was lowered to 18°C. Protein production was induced with 1mM IPTG and recombinant CAMK2B 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 degrees.

Purification

Procedure
Column 1: Ni-affinity chromatographyThe cell pellet (38 g) was re-suspended in one volume (38 ml) of binding buffer. The re-suspended cells were lysed by sonication. The lysate was cleared DNA was by a centrifugation at 17,000 rpm (4°C).5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 100 ml binding buffer. The lysate was applied to the column and was subsequently washed with 50 ml wash buffer 1 and 2. CAMK2B was eluted with 25 mls of elution buffer. The eluted protein was collected and analyzed by SDS-PAGE. DTT was added to the protein sample to a final concentration of 10mM. The N-terminal his6-tag was cleaved by incubating the protein overnight with TEV protease.Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 mls using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 0.8 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

Extraction

Procedure

Concentration:10.6 mg/ml in SEC buffer using a centricon with a 10kDa cut off
Ligand
{4-[4-(5-Cyclopropyl-1H-pyrazol-3-ylamino_-6-methylamino-pyrimidin-2-ylamino]-phenyl}-acetonotrile**MassSpec:**ESI-MS revealed that the protein had the expected mass of 33515 Da for the TEV cleaved protein
Crystallization:CAMK2B was crystallized at 4°C using the sitting-drop vapor diffusion method at 10.6 mg/ml in the presence of 1 mM {4-[4-(5-Cyclopropyl-1H-pyrazol-3-ylamino_-6-methylamino-pyrimidin-2-ylamino]-phenyl}-acetonotrile. Diffraction quality crystals were obtained by mixing 150 nl of protein solution with 50 nl of 20% PEG 3350; 0.20M (NH4)Cl.
NMR Spectroscopy:
Data Collection: Crystals were flash frozen in liquid nitrogen. Diffraction data were collected to 2.1 Å at the Swiss light source beam-line X10SA at a single wavelength of 0.9999 Å.
Data Processing: