Cp 14-3-3

PDB:2IJP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:cgd1_2980 (CryptoDB.org)
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal His6-tag with integrated TEC cleavage site (*): mgsshhhhhhssglvpr*gs
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqgITEKNMKLSEGAYRAKLADMVGNYKDVIKVLTESSDFRDNSLILLLAGSLRNRVTSIRNSLKSIKS
QEEKLRKEKSLNNEFIQVIEDIKRDFEESILLESEDVIRIIDDNLLMYSEEGARAFCIKLKGDLMRYKAEILKDEEKNQCIKQAVEF
YEDALQRERSFLEKYPSDPLYLATILNYTILKYDLLGNPEGAMKFANRAIQAAENSRSDSEQFSENTEKLLKILRDNVSQWEQGCSG
LLTSAFF

Vector:p15TV-L

Growth

Medium:TB
Antibiotics:100 microG/mL ampicillin and 34 microG/mL chloramphenicol
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 degC for 3 hours. The culture was then transferred into 1.8 L of above-specified growth medium with Antibiotics and 0.3 mL of antifoam (Sigma) in a 2L bottle and cultured using the LEX system to an OD600 of ~5, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

Purification

Procedure
The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and subsequently onto a 1.0 Â 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Â 1.5 mL/min. When all the lysate was loaded, both columns were washed with 15 mL Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 mL/min. After washing, the protein was eluted from the Ni-NTA column with 15 mL of Elution Buffer. EDTA was added immediately to 1 mM; and DTT was added to 1 mM 15 minutes later.
The eluted protein was applied to a Sephadex S200 26/60 gel filtration column (GE Healthcare) pre-equilibrated with Gel Filtration Buffer. The collected fractions corresponding to the eluted protein peak were concentrated to 20 mg/mL using a 15 mL Amicon Ultra centrifugal filter device (Millipore). Aliquots of the purified protein were stored at -80 °C.

Extraction

Procedure

Concentration:20 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized using the hanging drop vapour diffusion method in a 24-well Linbro plate. For the purpose of removing partially aggregated protein, the sample was first added to a solution of 14% PEG3350, 10% ethylene glycol, and 0.1 M HEPES, pH 7.0 in a ratio of 3:2 protein:buffer, and incubated at ~ 20 degC for 15 min. The solution was then centrifuged at 18,000 x g for 15 min. The supernatant was then applied to a coverslip in 3 microL hanging drops and placed over reservoir buffer containing 16% PEG3350, 10% ethylene glycol, and 0.1 M HEPES pH 7.0 and incubated at 20 °C. Large ~700 x 500 x 50 micrometer plate crystals grew to maximum size in two days.
NMR Spectroscopy:
Data Collection:
Data Processing: