Prkch C2 domain

PDB:2FK9

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC037268
Entry Clone Source:MGC AT24-D3 pBluescriptR
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated thrombin protease cleavage site: mgsshhhhhhssglvpr*gs(M)
Host:

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprlgsMSSGTMKFNGYLRVRIGEAVGLQPTRWSLRHSLFKKGHQLLDPYLTVSVDQVRVGQTSTKQKTNKPT
YNEEFCANVTDGGHLELAVFHETPLGYDHFVANCTLQFQELLRTTGASDTFEGWVDLEPEGKVFVVITLTG

Vector:p28-LIC-Thrombin

Growth

Medium:
Antibiotics:
Procedure: The Prkch C2 domain was expressed in E. coli (DE3) in Terrific Broth (TB) in the presence of kanamycin (50 µg/mL). A stab culture was taken from glycerol stocks and inoculated into 100mL of LB with 50ug/mL kanamycin in a 250mL flask and incubated with shaking at 250rpm overnight at 37 ºC. The culture was transfered into 1.8L TB with 50ug/mL kanamycin and 0.6mL of antiforam (Sigma) in 2L bottles and cultured using the LEX system to an OD600 of 4.0-5.0 before induction. The culture was colled to 15ºC and isopropyl-1-thio-D-galactopyranoside (IPTG) was added to 100ug/mL then incubated overnight at 15ºC before harvesting by centrifugation and storage at -80ºC.

Purification

Procedure
4M imidazole was added to the cleared cell lysate to bring it to a final concentration of 5mM imidazole before loading onto 4 mL bed-volume of Ni-NTA agarose affinity resin (Qiagen #30250). The column was then washed with 50mL of binding buffer and 50mL of Wash buffer (50mM Tris, 500mM NaCl, 20mM Imidazole, 0.5mM TCEP) before eluting into 7mL of Elution

buffer (50mM Tris, 500mM NaCl, 250mM imidazole, 0.5mM TCEP). Using an AKTAxpress (GE Healthcare) this eluate was loaded onto an An XK 16x65 column packed with HighLoad Superdex 200 resin that was pre-equilibrated in Binding buffer. This gel filatration column was run at a flow rate of 1.5 mL/min and 2mL fractions collected. The Prkch C2 domain eluted with a single major peak, the fractions from this peak were pooled and concentrated to 1.4mg/mL using an Amicon YM10 centrifugal filter device and set into crystal trays.

Extraction

Procedure
The cell-pellet was thawed, then resuspended in 50mL of binding buffer (50mM Tris, 0.5M NaCl, 0.5mM TCEP pH 8.0) containing Sigma's protease inhibitor cocktail (P2714-1BTL). The thawed cells were homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds. The cells were then lysed by sonication (Virtis408912, Virsonic) on ice with a 10 seconds pulse at half-maximal frequency then 10 seconds rest for a total of 5 minutes sonication time. The resulting lysate was then centrifuged at 63 000 xg for 30 minutes at 10 ºC.
Concentration:
Ligand
MassSpec:
Crystallization: The purified Prkch C2 domain was crystallized using the hanging drop vapour diffusion method. On glass slides 3uL of protein at 1.1 mg/mL was mixed with an equal volume of resevoir solution that consisted of 22% w/v polyethylene glycol 3350 and 0.2M CaCl2. The drop was sealed against 1mL of reseveroir and allowed to reach equilibrium at 18 ºC, crystals grew overnight. After harvesting crystal were soaked in a cryo-solution consisting of reservoir plus 300mg/mL D-glucose before freezing in liquid nitrogen and mounting for X-ray diffraction studies.
NMR Spectroscopy:
Data Collection:
Data Processing: