CTPS

PDB:2C5M

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC009408
Entry Clone Source:MGC (RZPD)
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:BL21(DE3)-Gold-tf2

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMKYILVTGGVISGIGKGIIASSVGTILKSCGLHVTSIKIDPYINIDAGTFSPYEHGEVFVLDDGG
EVDLDLGNYERFLDIRLTKDNNLTTGKIYQYVINKERKGDYLGKTVQVVPHITDAIQEWVMRQALIPVDEDGLEPQVCVIELGGTVG
DIESMPFIEAFRQFQFKVKRENFCNIHVSLVPQPSSTGEQKTKPTQNSVRELRGLGLSPDLVVCRCSNPLDTSVKEKISMFCHVEPE
QVICVHDVSSIYRVPLLLEEQGVVDYFLRRLDLPIERQPRKMLMKWKEMADRYDR

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:20 mL TB media was inoculated with BL21gold-DE3 cells, and all cultures were grown overnight at 30 ºC in 100 mL shake flasks at 175 rpm. 750 mL TB media supplemented with 8 g/L glycerol and 50 µg/mL kanamycin was inoculated with 10 mL of the over night culture. The large scale cultivations were grown in TunAir shake flasks at 37 °C, 225 RPM. The cells were harvested by centrifugation in the SLC-6000 rotor (6x1000mL) at 5000 rpm for 10 minutes.

Purification

Procedure
Purification was conducted automatically on an ÄKTA xpress system operated by UNICORN software at a flow of 0.8 mL/min. Prior to purification columns were equilibrated with IMAC Bind/Wash1 Buffer (HisTrap HP) and Gel filtration buffer (Superdex 200). The protein sample was loaded on the HisTrap HP column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with IMAC Elution Buffer and loaded onto the Gelfiltration column. The chromatogram from Gelfiltration showed one major protein peak that consisted of highly pure CTPSA-c007 as shown by SDS-PAGE analysis. TCEP was added to the sample to a final concentration of 2 mM. The protein was concentrated to 5.34 mg/mL and stored at -80ºC.

Extraction

Procedure
A stock solution of Complete EDTA-free (protease inhibitor) was prepared by dissolving 2x12 tablets in 2x30 mL of IMAC binding/washing buffer 1. Cells were harvested by centrifugation for 10 min at 4500 g. The cell pellet were resuspened in 90mL of IMAC lysis buffer complemented with 1mL of Complete EDTA-free protease inhibitor stock and then frozen at -80˚C. The frozen cell pellets were briefly thawed by warm water. 4 µL (1000 U) benzonase was added to the sample priorto high-pressure homogenization with the HPH. Cells were disrupted by high-pressure homogenization with the HPH before centrifugation for 30 min at 30 000 g in the Sorvall SS-34 rotor. The soluble fraction was decanted and filtered through 0.45mm prior to loading onto the ÄKTAxpress for furher purification.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained by the hanging drop vapour diffusion method using by mixing 1uL of the protein solution (5.3 mg/mL) with 1µL of the well solution consisting of 0.1 M Tris pH 8.8, 1.2 M AmSO4 and 0.1 M Malonic acid at room temperature. Crystals appeared after 3 days and were briefly transferred to cryosolution containing 0,1 M Tris pH 8.8, 1.4 M AmSO4, 50 mM Malonic acid, 25% glycerol, 0.2 M NaCl and 2 mM TCEP and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection:ESRF ID29, 20051001
Data Processing: The structure was initially solved by MR using MOLREP and then refined using COOT, REFMAC and CNS. Initially the structure was built and deposited (PDB code 2C5M) in space group P 41 with some additional symmetry erroneously interpreted as perfect twinning. Recent re-processing of the data suggests the space group to be P 41 21 2 with no signs of twinning judged from the output of XTRIAGE from the PHENIX suite. A ten amino-acid register error was corrected in 2C5M and the CTPS structure was re-deposited (PDB code 2VO1).