Tag:N-terminal hexahistidine tag
Host: *BL21-CodonPlus (DE3)-RIL*

Sequence:
MHHHHHHSSGFSWGNYINSNSFIAAPVTCFKHAPMGTCWGDISENVRVEVPNTDCSLPT
KVFWIAGIVKLAGYNALLRYEGFENDSGLDFWCNICGSDIHPVGWCAASGKPLVPPRTIQ
HKYTNWKAFLVKRLTGAKTLPPDFSQKVSESMQYPFKPCMRVEVVDKRHLCRTRVAVV
ESVIGGRLRLVYEESEDRTDDFWCHMHSPLIHHIGWSRSIGHRFKRSDITKKQDGHFDTPP
HLFAKVKEVDQSGEWFKEGMKLEAIDPLNLSTICVATIRKVLADGFLMIGIDGSEAADGS
DWFCYHATSPSIFPVGFCEINMIELTPPRGYTKLPFKWFDYLRETGSIAAPVKLFNKDVPN
HGFRVGMKLEAVDLMEPRLICVATVTRIIHRLLRIHFDGWEEEYDQWVDCESPDLYPVG
WCQLTGYQLQPPASGSAGSAGSAGSAGSAGSAQGFVSKTLDSASAQFAASALVTSEQLM
G
Vector:p28a-MHL

Growth

A 250 mL flask containing LB supplemented with 50 µg/mL kanamycin was inoculated from a glycerol stock of the bacteria. The flask was shaken overnight (16 hours) at 250 rpm at 37 °C. Using the Lex system, a 2L bottle containing 1800 mL of TB supplemented with 1.5% glycerol, 50 ug/ mL kanamycin and 600 µl antifoam 204 was inoculated with 50 mL overnight LB culture, and incubated at 37 °C. The temperature of the media was reduced to 16 °C one hour prior to induction and induced at OD600 = 1.2 with 300 µM isopropyl-thio-ß-D-galactopyranoside. Cultures were aerated overnight at 16 °C, and cell pellets collected by centrifugation and frozen at -80 °C.

Purification
Procedure
Column 1: Affinity purification: open Ni-NTA Superflow column.
Column 2: Prep Ion exchange: HiTrapQ 5ml.
Column 3: Gel filtration: Superdex 200

The supernatant was incubated with 6ml of 50% slurry Ni-NTA beads on ice. After 1 hour incubation the beads were washed with 100 ml of lysis buffer. The protein was eluted using 16 mL of EB. The eluent from Ni column was loaded onto the gel filtration column in GF buffer at 1ml/min, fraction size 2mL. The proteins were further purified by anion-exchange HiTrapQ chromatography and subsequent gel-filtration chromatography in a buffer containing 25 mM Tris-HCl pH 8.0, 150 mM NaCl. Peak fractions were concentrated to 16 mg/mL.The fractions containing protein were identified on a SDS-PAGE gel.

Extraction
Procedure
cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. The cells were homogenised and sonicated on ice with 10second pulses and 30 second rest intervals. The output level was 8.5 for 6 mins. The sample was centrifuge at 16,000rpm for 1 hour. The supernatant was purified by affinity, size exclusion and ion exchange.
Concentration:16mg/ml.
Crystallization: Crystals of MBTD1-EPC1 were obtained using a reservoir solution composed of 0.1 M sodium citrate tribasic dihydrate pH 5.0, 10% w/v polyethylene glycol 6,000. The crystals were soaked in a cryoprotectant of crystallization solution supplemented with 20% glycerol before being flash-frozen