RNGTT

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Revision

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Entry Clone Accession:gi|4506563
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SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:E. coli BL21(DE3).

Construct

Prelude:
Sequence:

Vector:pLIC-SGC1

Growth

Medium:
Antibiotics:
Procedure: Two litre of TB + 50 µg/mL Kanamycin in two 2.5-L baffled flasks were inoculated with 10 mL overnight culture. The culture was grown at 37°C and transfered to a 18°C shaker at an OD of 3.3. Protein expression was induced with 1 mM IPTG for 12 h. The cells were then collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-affinity chromatography.
Buffers: Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP. Wash buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP. Elution buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP.
Procedure : The cell extract was loaded on the column at 0.8 mL/minute on an AKTA-express system (GE/Amersham). The column was washed with 10 volumes of lysis buffer and 10 volumes of wash buffer. The his-tagged protein was eluted with a liner gradient of elution buffer at 0.8 mL/min. The eluted peak recorded at 280nm was automatically collected.
Column 2: Size exclusion chromatography Hiload 16/60 Superdex 75 prep grade 120 mL, Code no. 17-1069-01 Amersham Biosciences.
Buffers : SEC- buffer : 10 mM HEPES, pH 7.5, 500 mM NaCl, 0.5 mM TCEP.

Procedure: The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 1.20 mL/min. Eluted proteins were collected in 2 mL fractions. Eluted fractions were 95% pure as judged by SDS-PAGE.

Extraction

Procedure
Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, Complete protease inhibitors (1 tablet/50 mL). Frozen cell pellets were thawed on ice over night and re-suspended in a total volume of 50 mL lysis buffer. The cells were disrupted by a high pressure cell disrupter followed by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI from a 5% (w/v) stock. After an incubation time of 15 minutes the lysate was centrifuged clear at 40 000xg for 30 minutes. The supernatant was then further clarified by filtration (0.45µm).
Concentration: Centricon with a 10kDa cut off in SEC-buffer.
Ligand
MassSpec:
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 10 mg/ml by mixing 100 nl of the concentrated protein with 100nl of a well solution containing 2M Potassium citrate. Plate like crystals appeared after 3 days at 4°C.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution and 30% sucrose and flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line X10 at a single wavelength (0.99 nm).
Data Processing: