AKAP13

PDB:4D0O

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Collaborator.
SGC Clone Accession:
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMEAESWSRIIDSKFLKQQKKDVVKRQEVIYELMQTEFHHVRTLKIMSGVYSQGMMADLLFEQQMV
EKLFPCLDELISIHSQFFQRILERKKESLVDKSEKNFLIKRIGDVLVNQFSGENAERLKKTYGKFCGQHNQSVNYFKDLYAKDKRFQ
AFVKKKMSSSVVRRLGIPECILLVTQRITKYPVLFQRILQCTKDNEVEQEDLAQSLSLVKDVIGAVDSKVASYEKKVRLNEIYTK
Glu1976-Lys2211 The N-terminal residues, MHHHHHHSSGVDLGTENLYFQS, derive from the vecto
r.

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Cell Lysis: The resuspended cell pellet was thawed and lysed by sonication on ice. PEI (polyethyleneimine) was added to a final concentration of 0.15 %. The cell debris and precipitated DNA were spun down.Lysis Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% Glycerol, 0.5 mM TCEP. Purification: Column 1: 5 ml of Ni-Sepharose in a 2 cm diameter gravity flow column. Column 1 Buffers: Binding Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 20 mM Imidazole, 5% Glycerol, 0.5 mM TCEP. Wash Buffer 1: As Binding Buffer except 40 mM imidazole and 1M NaCl.Wash Buffer 2: As Binding Buffer except 60 mM imidazole.Elution Buffer: As Binding Buffer except 250 mM imidazole. Column 1 Procedure: The clarified supernatant was passed through the column. The column was washed with 50 mL of Binding Buffer, 50 mL each of Wash Buffer 1 and Wash Buffer 2, and 25 mL of Elute Buffer was

passed through to elute the protein. Column 2: S75 16/60 Gel Filtration (GE Healthcare) Column 2 Buffers: GF Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP Column 2 Procedure: The protein was concentrated to 5 ml volume and injected onto the column.

Extraction

Procedure
Expression strain: BL-21(DE3)-R3-pRARE2 (A homemade phage resistant version of BL21(DE3)). Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure. Expression: Colonies were used to inoculate 50 mL of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol in a 250 ml baffled shaker flask, which was placed in a 37°C shaker overnight. The next day 3x 10 mL of this starter culture was used to inoculate 3x 1L of LB media containing 40 µg/ml kanamycin in 2L baffled shaker flasks. When the OD600 was 0.4-0.5, the temperature was reduced to 20°C and the cells were induced by the addition of 0.5 mM IPTG. The expression was continued overnight. Cell harvest: Cells were harvested by centrifugation and the pellets resuspended in Lysis Buffer and then frozen at -20°C
Concentration: Fractions from gel filtration were pooled and passed through a gravity column of 5 mL Ni-Sepharose. The flow-through and an elution fraction with GF Buffer containing 10 mM imidazole were pooled and the complex was concentrated to 10.5 mg/ml (measured by 280 nm absorbance).
Ligand
MassSpec:
Crystallization: Crystallisation: Crystals grew from a mixture of 50 nL protein and 100 nL of a well solution containing 0.2 M ammonium sulphate, 0.1 M Bis-Tris pH 5.5, 25% (w/v) PEG 3350, using the vapour diffusion method.Crystals were equilibrated into reservoir solution plus 20% ethylene glycol before freezing in liquid nitrogen
NMR Spectroscopy:
Data Collection: Data was collected at Diamond synchrotron, beamline I03.
Data Processing: