HKR3

PDB:3B84

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|4885419
Entry Clone Source:MGC
SGC Clone Accession:IMAGE:3906038
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfq *s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*sMSFVQHSVRVLQELNKQREKGQYCDATLDVGGLVFKAHWSVLACCSHFFQSLYGDGSGGSVVLP
AGFAEIFGLLLDFFYTGHLALTSGNRDQVLLAARELRVPEAVELCQSFKPKTSV

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Starter cultures (10 ml LB, 50 µg/ml kanamycin) were inoculated from a glycerol stock and grown overnight. LB media (1L) was inoculated with 5 ml of culture and grown at 37°C, 170 rpm until OD600 was 0.5. The flask was then transferred to an 18°C incubator and protein expression induced with 1mM IPTG (final concentration) at an OD600 of approximately 0.5. Cells were harvested the following morning by centrifugation (10min, 6500rp, 4°C). The pellet was resuspended in 25 ml binding buffer and frozen at -20°C.

Purification

Procedure
Column 1a & 1b : IMAC Sepharose 6 Fast Flow resin, placed in series.Nickel charged IMAC sepharose beads were prepared by packing 4 mls of gel slurry (approximately 50% beads in 30% EtOH) in a small column and equilibrating in approximately 10 mls of Binding Buffer. Both columns were then placed in series and the protein containing supernatant passed through under gravity. This was followed by washing using 50 mls of Binding Buffer followed by 50 mls of Wash Buffer. Elution of the protein proceeded using a stepwise gradient of elution buffer.Enzymatic treatment: Protein containing fractions were pooled and the His-tag cleaved using TEV protease (reaction proceeded overnight at 4°C).Column 2: Gel Filtration, HiLoad 16/60 Superdex 200 prep grade column run on an AKTA-express system.The protein solution was concentrated using a Vivaspin 20 ultrafiltration device with a 5 kDa MWCO to about 10 ml. A pre-equilibrated gel filtration column was injected with 2 ml of the solution and two runs undertaken at a flow rate of 0.8 ml/min.

Extraction

Procedure
The cell pellet was defrosted and cells were lysed by sonication, 10 s on, 2 s off at 40% amplitude for 8 min. PEI was added to a final concentration of 0.1 % and the cell debris and precipitated DNA were spun down (18000 rpm, JA18 rotor, 90 min).
Concentration: Using Vivaspin 5 ultrafiltration spin columns with a 5 kDa MWCO the protein was dialysed against 50 mM HEPES, pH 7.5, 150 mM NaCl, 0.5 mM TCEP and concentrated to 19.3 mg/ml approximated using UV absorbance at 280 nm.
Ligand
MassSpec: The calculated mass for this construct without histidine tag was 13,042Da. This correlated well with the mass spec data (13,041.6) following TEV treatment of the protein.
Crystallization: Crystals were obtained using the vapor diffusion method. The protein was concentrated in gelfitration buffer to a protein concentration of 12 mg/ml by mixing 50nl of the concentrated protein with 150nl of a well solution containing the following components: 0.2M MgCl2; 0.1M BIS-TRIS pH 6.5; 25% PEG 3350.Crystallization experiments were setup at 4°C.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution supplemented with an additional 20% ethylene glycol and flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line SAX10 to 1.7 Å
Data Processing: