EPHA7

PDB:3H8M

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:synthetic Codon Devices cDNA template (epha7.001.998.CDV.pOT50)
SGC Clone Accession:epha7.919.990.117D01 (SDC117D01)
Tag:N-terminal tag: MHHHHHHSSGRENLYFQG
Host:BL21(DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgTPDFTTFCSVGEWLQAIKMERYKDNFTAAGYNSLESVARMTIEDVMSLGITLVGHQKKIMSSIQTMRAQ
MLH

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:BL21 (DE3) V2R cells. A 2L bottle (VWR 89000-242) containing 1800 ml TB (Sigma T0918) supplemented with 1.5% glycerol, 50 µg/ml Kanamycin, and 600 µl antifoam 204 (Sigma A-8311) was inoculated with 50 ml overnight LB culture and aerated in a LEX system at 37 degC. The temperature of the media was reduced to 15 degC at OD600 = 6 and after one hour, 100 µM isopropyl-thio-β-D-galactopyranoside (BioShop Canada IPT 001) was added for induction. Cultures were aerated overnight (16 hours) at 15 degC and centrifuged to collect cells. Cells were frozen and stored at -80 degC.

Purification

Procedure
After additional washing (50 column volumes), protein was eluted with 30 mL of elution buffer and dialyzed against 50 volumes of dialyses buffer overnight at 4 degC. The protein sample was concentrated using a 3,000 molecular weight cut-off Amicon Ultra-15 (UFC900524, Millipore) at 3500 rpm to a final concentration of 40 mg/mL. Protein yield was 14 mg per liter of bacterial culture. Mass-spectroscopy by LCMS showed that the product was pure and of correct molecular weight.

Extraction

Procedure
Frozen cell pellets obtained from 2L cultures were thawed in warm water, resuspended in 80 mL lysis buffer, homogenized using an Ultra-Turrax T18 homogenizer (IKA Works) at maximal setting for 30-60 seconds per 2L pellet, and sonicated (Sonicator 3000 Misonix) on ice with 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 10 minutes total sonication time per pellet.Unclarified lysate was mixed with HisLink (Promega, V882A, 2.0 mL settled resin per 40 mL lysate) for at least 30 minutes at 4 degC. The resin was spun (500 x g for 2 minutes), batch-washed (4X45 mL of cold wash buffer), and transferred to a column.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals of the EphA7 SAM domain were grown at 20 degC using the sitting drop method by mixing equal volumes of protein (40 mg/ml) and crystallization buffer (30 % PEG 4000, 2.0 M NaOAc, 0.1 M Tris HCL pH8.5). Crystals were harvested without cryoprotection into liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: Diffraction data from a crystal of the x domain was collected at beamline x at the x synchrotron. All data sets were integrated and scaled using either the HKL2000 or HKL3000 program packages. The structure was solved by molecular replacement techniques using the program PHASER and search model PDB entry x. Automated model building using ARP/wARP3, combined with iterative model building using the graphics program O and maximum-likelihood and TLS refinement with the program REFMAC5 led to a model with an R factor of x% (Rfree x%) for data between x-x Å. Parameters for Translation/liberation/screw (TLS) refinement were generated using the TLSMD web server.