EPHA1 EPHA2

PDB:3KKA

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession: The SAM domain of human ephrin receptor EphA1: epha1.LIFESEQ2516475.OBS.2516475.pINCY
The SAM domain of EPH receptor A2: BC037166
Entry Clone Source: The SAM domain of human ephrin receptor EphA1: Open Biosystems
The SAM domain of EPH receptor A2: MGC
SGC Clone Accession:epha1.0911.0974.117A03 (SDC117A03);
epha2.904.970. 117B01 (SDC117B01)
Tag:N-terminal tag: MHHHHHHSSGRENLYFQ
Host:

Construct

Prelude:
Sequence:

The SAM domain of human ephrin receptor EphA1:

mhhhhhhssgrenlyfqGDGIPYRTVSEWLESIRMKRYILHFHSAGLDTMECVLELTAEDLTQMG
ITLPGHQKRILCSIQGF

The SAM domain of EPH receptor A2:
mhhhhhhssgrenlyfqGVPFRTVSEWLESIKMQQYTEHFMAAGYTAIEKVVQMTNDDIKRIGV
RLPGHQKRIAYSLLGLKDQVN
Vector:pET28-MHL

Growth

Medium:TB
Antibiotics:
Procedure: Competent BL21 (DE3) cells (Invitrogen C6000-03) were transformed and grown using the LEX system (HarbingerBiotech) at 37 oC in 2L bottles (VWR 89000-242) containing 1800 ml of TB (Sigma T0918) supplemented with 150 mM glycerol, 100 µM Kanamycin, and 600 µl antifoam 204 (Sigma A-8311). At OD600 = 6, temperature was reduced to 15 degC, and one hour later the culture was induced with 100 µM IPTG (BioShop IPT001). Cultures were grown overnight (16 hours) at 15 degC, and cell pellets were collected by centrifugation (12,227 xg, 20 mins) and frozen at -80 degC.

Purification

Procedure
Unclarified lysate was mixed with HisLink (Promega, V882A) (2.0 mL settled resin per 40 mL lysate) for 60 minutes at 4 °C. The resin was spun (500 xg for 2 minutes), batch-washed (4X45 mL of cold Wash Buffer, and transferred to a column. After additional washing (50 column volumes), protein was eluted with 50 mL of Elution Buffer and dialyzed against 50 volumes of Dialyses Buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 5 mM DTT) overnight at 4 degC. The protein sample was concentrated using a 3,000 molecular weight cut-off Amicon Ultra-15 (Millipore, UFC900524) at 4750 xg to a final concentration of 30 mg/mL. Proteins yield was 25-50 mg per liter of bacterial culture. Mass-spectroscopy by LCMS showed that the products were pure and of correct molecular weight

Extraction

Procedure
After resuspension with an Ultra-Turrax T18 homogenizer (IKA Works) in 40 mL per liter bacterial culture of Lysis Buffer, cells were lysed by sonication (Misonix, Sonicator 3000, 15-338-276) on ice for 10 minutes total sonication time (10 sec pulses at half-maximal frequency with 10 second rest).
Concentration:
Ligand
MassSpec:
Crystallization: Mixture of EphA1 SAM domain (58 g/L) and EphA2 SAM domain (27 g/L) in ratio 0.08 µM : 0.08 µM were set Crystals were grown at 20 degC using the hanging drop method with equal volumes of sample and Crystallization Buffer (2.1 M (NH4)2SO4, 2 % PEG400, 0.1 M Na HEPES pH 7.2). Immediately prior to setting-up crystallization plates, chymotrypsin was added to the protein sample to a final concentration of 5.7e-7 M (0.57 µM) and protein concentration of 2.5e-3 M (2.5 mM). Prior to dunking and storage in liquid nitrogen, suitable crystals were immersed in 1 uL CB and 1 uL cryoprotectant (20% (w/v) sucrose, 4% (w/v) glucose, 18% (v/v) glycerol and 18% (v/v) ethylene glycol).
NMR Spectroscopy:
Data Collection: Diffraction data from a crystal of the complex of sam domains of epha1 and epha2 was collected at beamline 19ID at the Argonne National Laboratory Advanced Photon Source. Data sets were integrated and scaled using the HKL3000 program suite.
Data Processing: The structure was solved by molecular replacement techniques using the program PHASER and search model PDB entry 3HIL. Iterative manual model building using the graphics program Coot and maximum-likelihood and TLS refinement with the program REFMAC5 led to a model with an R factor of 20.4% (Rfree 24.1%) for data between 30.44-2.40 Å. Parameters for Translation/liberation/screw (TLS) refinement were generated using the TLSMD web server.