EPHA7

PDB:3NRU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:CM16-H1
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:epha7.0032.0204.173D03 (SDC173D03)
Tag:N-terminal tag: AA
C-terminal tag: EFVEHHHHHHHH
Host: Sf9 cells

Construct

Prelude: The ligand binding domain of ephrin receptor EphA7 (also known as HEK11 and EHK-3) was cloned from a Mammalian Gene Collection cDNA template (CM16-H1) into the pFHMSP-LIC-C vector (Alma Seitova, SGC) using the In-Fusion CF Dry-Down PCR Cloning Kit (Clontech, 639605) resulting in a plasmid called epha7.0032.0204.173D03 (SDC173D03) with the sequence shown below. This plasmid was transformed into DH10Bac E. coli cells (Invitrogen, 10361-012), and a mini-prep was performed to obtain recombinant viral DNA bacmid. SF9 cells (Invitrogen, 11496-015) were transfected with bacmid using Cellfectin reagent (Invitrogen, 10362-010) and recombinant baculovirus was generated. Viral stock was amplified from P1 to P3.
Sequence:

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EVLLLDSKAQQTELEWISSPPNGWEEISGLDENYTPIRTYQVCQVMEPNQNNWLRTNWISKGNAQRIFVELKFTLRDCNSLPGVLGT
CKETFNLYYYETDYDTGRNIRENLYVKIDTIAADESFTQGDLGERKMKLNTEVREIGPLSKKGFYLAFQDVGACIALVSVKVYYKK
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Vector:pFHMSP-LIC-C vector (Alma Seitova, SGC)

Growth

Medium: Serum Free Medium (HyClone SFX-Insect, SH3027802)
Antibiotics:
Procedure: Sf9 cells were grown in Serum Free Medium (HyClone SFX-Insect, SH3027802) at density of 3.5 million cells per milliliter of media and with viability not less then 97 % were infected with 10 mL of P3 viral stock for each 1 L of cell culture. Cell culture medium was collected after 3-4 days of incubation on a shaker at 100 rpm and 27 °C when cells viability dropped to 45-65 %.

Purification

Procedure
The cultured medium was centrifuged at 14,000 xg for 15 minutes, and the pH of the supernatant was adjusted to 7.5 at room temperature by adding Titrating Buffer. PMSF (Bioshop, PMS 123.50) and benzamidine (Sigma, B6506-100G) were added to final concentrations of 1 and 2 mM, respectively. A 3.2 L volume of medium was mixed with 30 ml pre-equilibrated NiNTA Superflow beads (Qiagen, 30450) and stirred (Talboys, 134-2) on ice for 1 hour. The resin was transferred to a 100 ml gravity column, washed with 300 ml of Wash Buffer and the protein was eluted with 30-40 ml of Elution Buffer. A second round of NiNTA batch absorption may have been performed for increased yield. The eluate was dialyzed against 50-100 X volume of buffer 50 mM Tris-pH8.0 and 0.15 M NaCl. Purified protein was concentrated using 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, Millipore) to a final value of 20 mg/mL. Average yield was about 8 mg/L. Coomassie-stained SDS-PAGE showed that the product was pure and mass-spectroscopy by LC/MS (Agilent 1100 Series) showed that the purified protein had a molecular weight slightly less (-3 Da) than expected.

Extraction

Procedure

Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown at 18 °C using the hanging drop method in greased VDX Plates (Hampton Research, HR3-306) by mixing equal volumes of protein (20 mg/ml) and Crystallization Buffer (0.3 M NH_4SO_4, 0.6 M $LiSO_4$, 0.1 M tri-NaCitrate pH 4.8. Prior to dunking and storage in liquid nitrogen, suitable crystals were immersed in 20% ethylenglycol.
NMR Spectroscopy:
Data Collection: Diffraction data was collected at beamline **xyz** at the **xyz** synchrotron. All data sets were integrated and scaled using either the HKL2000 program suite. The structure was solved by molecular replacement techniques using the program PHASER and search model PDB entry **xyz**. Automated model building using ARP/wARP, combined with iterative model building using the graphics program Coot and maximum-likelihood and TLS refinement with the program REFMAC5. Parameters for Translation/liberation/screw (TLS) refinement were generated using the TLSMD web server.
Data Processing: