GRM3

PDB:3SM9

Revision

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Tag:N-terminal tag: APEHHHHHHDYDIPTTENLYFQGAMD
Host: Sf9 insect cells

Construct

Prelude:
Sequence:

gamdHNFLRREIKIEGDLVLGGLFPINEKGTGTEECGRINEDRGIQRLEAMLFAIDEINKDDYLLPGVKLGVHILDTCSRDTYALEQ
SLEFVRASLTKVDEAEYMCPDGSYAIQENIPLLIAGVIGGSYSSVSIQVANLLRLFQIPQISYASTSAKLSDKSRYDYFARTVPPDF
YQAKAMAEILRFFNWTYVSTVASEGDYGETGIEAFEQEARLRNIsIATAEKVGRSNIRKSYDSVIRELLQKPNARVVVLFMRSDDSR
ELIAAASRANASFTWVASDGWGAQESIIKGSEHVAYGAITLELASQPVRQFDRYFQSLNPYNNHRNPWFRDFWEQKFQCSLQNKRNH
RRVCDKHLAIDSSNYEQESKIMFVVNAVYAMAHALHKMQRTLCPNTTKLCDAMKILDGKKLYKDYLLKINFTAPFNPNKDADSIVKF
DTFGDGMGRYNVFNFQNVGGKYSYLKVGHWAETLSLDVNSIHWSRNSV

Vector:pFHMSP-LIC-N

Growth

Medium:
Antibiotics:
Procedure: Plasmid transfer vector pFHMSP-LIC-C containing the gene was transformed intoDH10Bac E.coli cells (Invitrogen) to obtain recombinant viral DNA. SF9 cells weretransfected with Bacmid DNA using Cellfectin reagent (Invitrogen), and recombinantbaculovirus was generated. Viral stock was amplified from P1 to P3.

Sf9 cells grown in HyQ® SFX Insect Serum Free Medium (Cat.# SH3027802) atdensity of 3 million cells per milliliter of media and with viability not less then 97 %were infected with 7 mL of P3 viral stock for each 1 L of cell culture. Cell culturemedium was collected after 4 days of incubation on a shaker at 100 RPM and 27 °C when cells viability dropped to 25-45 %.

Purification

Procedure
IMAC purification: A 4.8 L volume of medium was mixed with 45 mL pre-equilibratedNiNTA

Superflow beads and stirred (Talboys/Troemner) for 1 hour. The resin wastransferred to a 50 mL gravity column, washed with 600 mL of Washing Buffer1,240mL of Washing Buffer 2 and the protein was eluted with 30 mL of Elution Buffer.A second round of NiNTA batch absorption has been performed for increased proteinyield. The protein was then TEV cleaved to remove the poly histidine tag. TEV wasadded in the ration of 50:1 GRM3:TEV. The reaction was incubated at 4°C for ~2 days then loaded onto the Gelfiltration (GF) column. The chromatogram from gel filtrationshowed one major protein peak that consisted of GRM7 confirmed by SDS-PAGEanalysis.

Extraction

Procedure
The cultured medium was centrifuged at 14,000 xg for 15 minutes, and the pH ofthe supernatant was adjusted to 7.5 at room temperature by adding 10x Buffer_A.Protease inhibitors were added to final concentrations of 1 mM phenylmethanesulfonylfluoride (PMSF, Bioshop) and 2 mM benzamidine hydrochloride (Sigma).
Concentration: Purified protein was concentrated using 15 mL concentrators with an appropriatemolecular weight cut-off (Amicon Ultra-15 50,000 MWCO, Millipore) to a final value of5 mg/mL. Average yield was about 3.5 mg/L.
Ligand
LY341495**MassSpec:**
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screensinitially at 293K.

Crystal used for structure determination were grown in: 1.5 M Ammonium Sulfate0.1 M BisTris Propane pH 7.0 at protein concentration 3.5-5mg/mL with theprecence of 2mM LY341495

Cryoprotectant used: 2 M Ammonium Sulfate 0.1 M BisTris Propane pH 7.010% Glycerol
NMR Spectroscopy:
Data Collection:
Data Processing: