SELS

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Revision

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Tag:MHHHHHHSSGRENLYFQ*G
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfq*gSARLRALRQRQLDRAAAAVEPDVVVKRQEALAAARLKMQEELNAQVEKHKEKLKQLEEEKRRQKIEMW
DSM

Vector:pET28-MHL

Growth

Medium:LB
Antibiotics:
Procedure: Overnight cultures were grown from glycerol stocks in LB media. Large-scale inoculations were performed by adding 50 mLs of overnight culture into 2 L of TB media supplemented with 1.5% glycerol, 50 µg/ ml kanamycin and 300 microL antifoam 204 (Sigma A-8311). Cells were grown at 37 degC using the LEX bubbling system until OD reaches ~5, at which time the temperature of the cultures were reduced to 15 degC. Cultures were induced when the cell density was between 6-8 with a final concentration of 100 µM IPTG and left overnight. Cells were harvested by centrifugation at 12,000xg for 15 minutes.

Purification

Procedure
Lysed cells from 2 L cultures were diluted 1:1 in lysis buffer; imidazole was added to a final concentration of 30 mM, and 1.5 mL of his-Link resin (Promega) was added. This mixture was then rocked at 4 degC for 1 hour, centrifuged at 500 xg, and decanted carefully. The resin was washed four times with IMAC buffer, resuspended, and loaded into a column. The resin was washed with 5 column volumes of low imidazole buffer and eluted with 10 mL of elution buffer. A volume of 10 microL TEV (1.6 mg/mL) per milligram of protein was added and incubated

overnight at 4 degC to cleave the His-tag. The sample was loaded onto an XK 16x65 column packed with highLoad Superdex 200 resin (GE Healthcare). Peak fractions were pooled and concentrated using Amicon concentrators with 10,000 MWCO (Millipore) to 22 mg/mL.

Extraction

Procedure
Frozen cell pellets from 2 L cultures were thawed on ice, resuspended in 25 mL lysis buffer, homogenized, and lysed using sonication
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained in hanging drop plates at 18 degC using the follow crystallization conditions: 13% PEG2000 MME, 0.1M Sodium acetate pH 4.6, 0.1M KSCN. Crystals were harvested into cryoprotectant containing 30% glycerol and flash frozen in liquid nitrogen
NMR Spectroscopy:
Data Collection:
Data Processing: