# PFE0790c

**PDB:**2KDN

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**PFE0790c:NMR
**Entry Clone Source:**
**SGC Clone Accession:**
**Tag:**N-terminal tag: mgsshhhhhhsgrenlyfaghm
C-terminal tag: gs
**Host:**BL21 (DE3)

## Construct

**Prelude:**
**Sequence:**

mgsshhhhhhsgrenlyfaghmCIQKVIEDKLSSALKPTFLELVDKSCGCGTSFDAVIVSNNFEDKKLLDRHRLVNTILKEELQNIH
AFSMKCHTPLEYDKLKSKgs

**Vector:**

## Growth

**Medium:**M9
**Antibiotics:**
**Procedure:**Bacteria were grown in M9 minimal media supplemented with biotin, thiamine, and10 µM ZnSO4; $^{15}$NH4Cl and $^{13}$C-glucose were the sole nitrogen and carbon source. Starter cultures (50 mL in a 250 mL flasks) were prepared with media inoculated with 100 µL of glycerol stock and shaken overnight (18 hours) at 220 rpm at 37°C. They were used to inoculate 500 mL of growth media in a modified LEX fermentation system at 37°C to an OD600 of 1.0. Cultures wereinduced with 1 mM IPTG and grown at room temperature overnight (15.5 hours). Cells were harvested by centrifugation and frozen in 50 mL Falcon tubes at -80°C.

## Purification

**Procedure**
Frozen cell pellets from 500 ml cultures were thawed, resuspended in 25 mL lysisbuffer, and lysed by sonication. Lysate was clarified by centrifugation for 20 min at 4°C and the supernatant was mixed for 20 minutes at 4°C with 2 mL settled$Ni^{2+}$ affinity beads. Beads were batch-washed twice with 5 mL of cold wash buffer (spun at 2000 rpm for 6 minutes), transferred to a column, and further washed with 2 mL of wash buffer. Protein was eluted with 5 mL of elution buffer.

Buffer exchange into NMR buffer and protein concentration was performed using VivaSpin concentrators with a 5,000 molecular weight cut-off at 3000 rpm, resulting in a final volume of 300 µL. Protein samples were transferred to a 5 mm shigemi NMR tube for data collection.

## Extraction

**Procedure**

**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**
**NMR Spectroscopy:**All NMR spectra were collected at 25°C on Varian Inova 600, 750, and 800 MHz spectrometer. Chemical shifts were referenced to external DSS. All spectra were processed using the program Felix 2007 software. The backbone assignments were obtained using HNCO, HNCACB, CBCA(CO)NH, HBHA(CO)NH, HNCA and $^{15}$N-edited NOESY, $^{1}$H-$^{13}$C NOESY, C(CO)NH, H(CCO)NH, $^{1}$H-$^{13}$N HSQC, $^{1}$H-$^{13}$C HSQC, HCCH-TOCSY spectra. The software SPARKY 3.110 was used for peak picking and data analysis.

Distance restraints for structure calculations were derived from cross-peaks in $^{15}$N-edited NOESY, $^{13}$C-edited NOESY. Structure calculations were performed using the program CYANA 3.0. Structure refinement used CNS 1.1. The quality of the NMR structure quality was assessed using NESG validation software package PSVS 1.3.
**Data Collection:**
**Data Processing:**