

# LMSAH

**PDB:**3G1U

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**Q4Q124

**Entry Clone Source:**in-house cloning

**SGC Clone Accession:**LMSAHA-k006

**Tag:**N-terminal hexahistidine tag with integrated TEV protease cleavage site:  
mhhhhhhsgvd1gtenlyfq\*sm

**Host:***E.coli* BL21(DE3) R3 pRARE, where R3 denotes a derivative of BL21(DE3) resistant to a strain of T1 bacteriophage (SGC Oxford) and the pRARE plasmid originating from the Rosetta strain (Novagen) supplies tRNAs for rare codons.

## Construct

**Prelude:**

**Sequence:**

mhhhhhhsgvd1gtenlyfq\*smADYKVKD1S1AEWGRKAI1E1AENEMPGLM1RREYGPSQPLKGAK1AGCLHMTVQTA1LIETL  
KALGAE1LRWSSCN1F1STQDNAAAIAKTGV1F1WKG1ETDEEYEW1C1AQTVKG1F1SGDGLPNM1LDDGGDLTN1VIDR1P1E1V1PK1F1G  
1SE1ETT1GV1K1N1Y1K1R1L1S1K1G1N1P1S1A1N1V1N1D1S1V1T1K1S1F1D1N1L1Y1G1C1R1E1S1V1D1G1K1R1A1T1D1V1M1A1G1K1T1C1V1C1G1Y1G1D1V1G1K1G1C1A1A1L1R1A1F1G1A1R1V1  
VV1TE1V1D1P1N1A1L1Q1A1S1M1E1G1Y1Q1V1A1L1V1E1D1V1M1A1D1A1H1F1V1T1T1G1N1D1I1T1S1D1H1F1P1H1M1R1D1A1I1V1C1N1I1G1F1D1T1E1I1Q1V1G1W1L1E1A1N1A1K1E1H1V1E1K1P1Q1D1  
R1Y1T1M1E1N1R1H1I1L1A1K1G1R1L1V1N1G1C1A1S1G1H1P1S1F1V1M1S1N1F1T1N1Q1V1L1A1Q1E1L1W1S1N1R1D1N1G1K1Y1P1R1G1D1K1A1G1V1F1L1P1K1A1L1D1E1K1V1A1A1L1H1A1H1V1G1A1K1L1T1  
K1L1T1P1Q1A1E1Y1I1N1C1P1V1N1G1P1F1K1

**Vector:**pNIC-Bsa4

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**Cells from a glycerol stock were grown in 20 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (20 ml) was used to inoculate 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 0.75 ml 204 Antifoam A6426 (Sigma). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD600 reached ~2. The bottle was down-tempered to 18 °C over a period of 1 hour before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (4,400 x g, 10 min, 4 °C). The resulting cell pellet (20 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 2000 U Benzonase (Merck) and one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 °C.

## Purification

### Procedure

#### Columns

IMAC: Ni-charged 1 ml HiTrap Chelating HP (GE Healthcare)

Gel filtration column: HiLoad 16/60 Superdex 200 Prep Grade (GE Healthcare)

### Procedure

Purification of the protein was performed as a two step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer and gel filtration buffer, respectively. The filtered lysate was loaded onto the Ni-charged HiTrap Chelating column and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the gel filtration column. Fractions containing the target protein were pooled and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using an Amicon centrifugal filter device with 10,000 NMWL (Millipore) to 25 mg/ml in a volume of 2.4 ml.

### Tag removal

The N-terminal histidine tag was proteolytically removed by incubating LMSAHA with His-tagged TEV protease (van den Berg, S., *J. Biotech* **121**, 291-298 (2006)) at a molar ratio of 160:1 at 20 °C overnight. The proteolytic reaction went to completion, as judged by SDS-PAGE. Target protein was purified from tag and protease by passing the reaction mixture over a Ni-charged 1 ml HiTrap Chelating HP column (GE Healthcare) pre-equilibrated with IMAC wash1 buffer. The cleaved protein was concentrated using a centrifugal filter device to 35 mg/ml, resulting in a volume of 0.5 ml, and subsequently dialyzed against GF buffer containing 2 mM TCEP. The identity of the protein was confirmed by mass spectrometry.

## Extraction

### Procedure

The cell suspension was briefly thawed in water. Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) and cell debris was removed by centrifugation (49,100 x g, 20 min, 4 °C). The supernatant was decanted and filtered through 0.45 µm flask filter.

#### Concentration:

#### Ligand

#### MassSpec:

**Crystallization:** Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.1 µl of the protein solution (diluted to 25 mg/ml with GF buffer) including 3 mM NAD was mixed with 0.1 µl of well solution consisting of 0.1 M Bicine, pH 9 and 20% PEG 6000. The plate was incubated at 4 °C and crystals appeared within 1 day. The crystals were quickly transferred to cryo solution containing well solution, 0.2 M NaCl and 20% glycerol and flash frozen in liquid nitrogen.

#### NMR Spectroscopy:

**Data Collection:** Data sets were collected on a single crystal to 2.2 Å resolution at DIAMOND (I03). This data used for the final refinement belonged to P1 space group with cell parameters of  $a = 72.38 \text{ \AA}$ ,  $b = 82.47 \text{ \AA}$ ,  $c = 83.88 \text{ \AA}$ ,  $\alpha = 87.02^\circ$ ,  $\beta = 71.41^\circ$ ,  $\gamma = 74.00^\circ$

**Data Processing:** Data was integrated with XDS, scaled with XSCALE and the structure was solved using MOLREP with PDB ID = 1LI4 as a search model. Four chains were found in the asymmetric unit. The model was improved by successive rounds of manual model building in

COOT and refinement with Refmac5. Final R-values were R= 18.15% and Rfree= 23.16%. Coordinates and structure factors were deposited in the PDB with accession code 3G1U.