FDPS

PDB:1ZW5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC010004
Entry Clone Source:MGC (I.M.A.G.E. Consortium CloneID 4132071)
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*gh(m)
Host:BL-21(DE3)

Construct

Prelude:
Sequence:

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: Overnight cultures in LB (10 mL with100 µg/mL ampicillin) were used to inoculate 1 L of LB medium containing 100 µg/mL ampicillin. Cultures were grown at 37°C until they reached an OD600 of 0.6-0.8 and then induced with 1 mM IPTG. The temperature was adjusted to 18°C and expression was allowed to continue overnight. The cells were collected by centrifugation.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 mL (GE/Amersham)

Buffers: Binding: 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP; Wash: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, 5% glycerol, 0.5 mM TCEP ; Elution: 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol, 0.5 mM TCEP.

The cell extract was loaded on the column at 1 mL/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 column volumes of Lysis buffer, 10 column volumes of wash buffer, and then eluted with elution buffer at 1 mL/min. The eluted peak at A280 was automatically collected.

Column 2 : Hiload 16/60 Superdex 200 prep grade 120 mL

Buffers : 10 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP.

Extraction

Procedure

Concentration:
Ligand
MassSpec:
Crystallization: Zoledronate, isopentenyl pyrophosphate, and MgCl2 were prepared as 100 mM aqueous stock solutions and added to the protein to a final concentration of 2 mM each. Crystals were grown at 20°C in 300 ?l sitting drops by mixing 150 ?l of protein solution and 150 ?l of precipitant consisting of 14% PEG 6000, 0.7 M LiCl, and 70 mM citrate pH 4.0.
NMR Spectroscopy:
Data Collection:
Data Processing: