NTMT1

PDB:5E1B

Revision

Revision Type:created
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Entry Clone Accession:GI:56676399
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SGC Clone Accession:APC005_1A01
Tag:N-terminal: MGSSHHHHHHSSGLVPRGS
Host:BL21 (DE3) Codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPRGSTSEVIEDEKQFYSKAKTYWKQIPPTVDGMLGGYGHISSIDINSSRKFLQRFLREGPNKTGTSCALDCG
AGIGRITKRLLLPLFREVDMVDITEDFLVQAKTYLGEEGKRVRNYFCCGLQDFTPEPDSYDVIWIQWVIGHLTDQHLAEFLRRCKGS
LRPNGIIVIKDNMAQEGVILDDVDSSVCRDLDVVRRIICSAGLSLLAEERQENLPDEIYHVYSFALR

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:NTMT1 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 Âµg/mL of kanamycin. Cell were grown at 37 ÂºC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.2 mM, and incubated overnight at 16 ÂºC.

Purification

Procedure
The supernatant was loaded into Ni-NTA and washed the non-specific proteins with 20 mM Tris pH 7.5, 200 mM NaCl, 20 mM imidazole and 4mM BME. The target protein was eluted by 20 mM Tris pH 7.5, 200 mM NaCl, 250 mM imidazole and 4mM BME. The elution was diluted to 20mM Tris pH 7.5, 30mM NaCl and passed through a HiTrap Q HP. The peak fractions were pooled and subjected to gel filtration (SuperdexâÂ□¢ 200 10/300 GL, GE Healthcare). The buffer for gel filtration contained 20 mM Tris, pH 7.5, 150 mM NaCl, and 0.5 mM TECP.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For the purification the cell paste was thawed and resuspended in lysis buffer The cells were lysed by sonication (Virtis408912, Virsonic) on ice. The lysate was centrifuged at 15000rpm for 1h.
Concentration:37 mg/mL.
Ligand
MassSpec:
Crystallization: To crystallize the complex, NTMT1 was first incubated with substrate peptides at a molar ratio of 1:1.5 for 1h on ice, and the crystals were grown at 277K using sitting-drop vapor diffusion method by mixing 1µl complex solution with 1µl reservoir consisting of 23-28% PET3350 and 14-18% Tacsimate pH 6.0. The crystals were flash frozen in liquid nitrogen after soaking in cryo-protectant solutions containing the reservoir solution supplemented with 20% (v/v) glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: