Structure	Crystal structure of human alpha N-terminal protein methyltransferase 1B
PDB Code	5UBB, 6DUB
Entry clone accession	NP_001129579
Entry clone source	SGC clone accession JMC119-E02
Tag	N-terminal tag: mkkhhhhhhhhhssglvprgsmsdsevnqeakpevkpevkpethinlkvsdgsseiffkikkttplrrlmeafakrqgkemdslrflydgiriq adqtpedldmedndiieahreqiggtenlyfqg Mkkhhhhhhhhhssglvprgsmsdsevnqeakpevkpevkpethinlkvsdgsseiffkikkttplrrlmeafakrqgkemdslrflydgiriq adqtpedldmedndiieahreqiggtenlyfqg
Construct sequence	TSQVINGEMQFYARAKLFYQEVPATEEGMMGNFIELSSPDIQASQKFLRKFVG GPGRAGTDCALDCGSGIGRVSKHVLLPVFNSVELVDMMESFLLEAQNYLQVKG DKVESYHCYSLQEFTPPFRRYDVIWIQWVSGHLTDKDLLAFLSRCRDGLKENGIII LKDNVAREGCILDLSDSSVTRDMDILRSLIRKSGLVVLGQEKQDGFPEQCIPVWMFALH
Vector	pET28-MKH8SUMO
Expression host	BL21 (DE3) Codon plus RIL (Stratagene)
Growth method	NTMT2 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin. Cell were grown at 37 ºC 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 ºC. Cell pellets collected by centrifugation and frozen at -80 ºC.
Extraction buffers	**Lysis buffer**: **20 mM Tris-HCl pH 7.5, 400 mM NaCl, 5% glycerol and 2 mM beta-mercaptoethanol**
Extraction proc	Frozen cell pellet was thawed and suspended in lysis buffer. The cells were lysed by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 5 sec pulse at half-maximal frequency (5.0), 7 second rest, for 10 minutes total sonication time per pellet. The lysate was centrifuged at 15000rpm for 1h.

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Purification buffers	**Wash buffer**: 20 mM Tris pH 7.5, 400 mM NaCl, **5% glycerol** and 25 mM imidazole; **Elution buffer**: 20 mM Tris pH 7.5, 400 mM NaCl, **5% glycerol** and 300 mM imidazole; **Gel filtration buffer**: 20 mM Tris-HCl pH 7.5, 150 mM NaCl and 0.5 mM TCEP
Purification procedure	**Cells were lysed in 20 mM Tris-HCl pH 7.5, 400 mM NaCl, 5% glycerol and 2 mM beta-mercaptoethanol buffer and purified by Ni-NTA agarose chromatography. The SUMO tag was cleaved by TEV protease at 4 °C overnight and removed by reloading onto the Ni-NTA. The protein was diluted and applied onto HiTrap Q HP anion exchange chromatography column (GE Healthcare) equilibrated with 20 mM Tris-HCl pH 7.5, 25mM NaCl and 0.5 mM TCEP (tris (2-carboxyethyl) phosphine). The proteins were eluted with a linear gradient of 0-50% elution buffer (20 mM Tris-HCl pH 7.5, 1M NaCl and 0.5 mM TCEP). The proteins were further purified by gel filtration Superdex 200 10/300 (GE Healthcare). The gel filtration buffer contains 20 mM Tris-HCl pH 7.5, 150 mM NaCl and 0.5 mM TCEP.**
Protein stock concentration	**The purified protein was concentrated to 20 mg mL-1** using 15 mL concentrators with a 10,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore).
Crystallization	NTMT2 purified from *Escherichia coli* contains endogenous methyl donor SAM/SAH, so we did not add any additional SAM/SAH during the crystallization. The NTMT2-SAM complex was crystallized in 20% (*w/v*) PEG3350 and 0.2 M sodium acetate via sitting drop vapor diffusion by mixing 1 microL protein and 1 microL reservoir solution at 4°C. To get the ternary complex crystal, the protein was incubated with SPKRIA peptide (from 100 mM stock) at a molar ratio of 1:1.5 for 1 h on ice before setting up the crystallization trial. The crystals of NTMT2 in complex with SPKRIA were obtained in 30% PEG2000 (*w/v*) and 0.1 M potassium thiocyanate at 18 °C. The crystals were cryo-protected in the reservoir solution supplemented with 20% (*v/v*) glycerol and flash-frozen in liquid nitrogen.