Structure	Human methyltransferase dimer N6AMT1-TRMT112, methyltransferase domain, in complex with S-adenosyl-homocysteine
PDB Code	6PED
Entry clone accession	
Entry clone source	
SGC clone accession	
Tag	N-terminal tag
Construct sequence	MGSSHHHHHHSSGLVPRGSMAGENFATPFHGHVGRGAFSDVYEPAEDTFLLLDALEAAAA ELAGVEICLEVGSGS GVVSAFLASMIGPQALYMCTDINPEAAACTLETARCNKVHIQPVITDLVKGLLPRLTEKVD LLVFNPPYVVTPPQEV GSHGIEAAWAGGRNGREVMDRFFPLVPDLLSPRGLFYLVTIKENNPEEILKIMKTKGLQGTT ALSRQAGQETLSVLKFTKS MHHHHHHSSGRENLYFQGMKLLTHNLLSSHVRGVGSRGFPLRLQATEVRICPVEFNPNFVA RMIPKVEWSAFLEAA DNLRLIQVPKGPVEGYEENEEFLRTMHHLLLEVEVIEGTLQCPESGRMFPISRGIPNMLLSE EETES
Vector	N6AMT1 was cloned into pET28a-LIC, TRMT112 was cloned into pET15a-MHL vector, respectively.
Expression host	BL21 (DE3) Codon plus RIL (Stratagene)
Growth method	The two proteins were co-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 procedure	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.
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, 100 mM NaCl and 1 mM DTT.
Purification procedure	**The proteins were purified by Ni-NTA agarose column and 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 1 mM DTT.**
Protein stock concentration	**The purified protein was concentrated to 10 mg mL-1.**

Crystallization

The complex was crystallized using sitting drop vapor diffusion method by mixing 1 mL protein with 1 mL reservoir solution. The crystals were obtained in 2M Na/KPO4, 7.0. The crystals were cryo-protected in the reservoir solution supplemented with 20% (v/v) glycerol and flash-frozen in liquid nitrogen.