= Title =
Crystal Structure of mouse protein arginine methyltransferase 7 in comlex with SGC8158 chemical probe

= Keywords =
PRMT7, SGC8158 chemical probe, methyltransferase

= PDB Code =
6OGN

= Ligands =
MJ7, UNX

= Entry clone accession =

= Entry clone source =

= SGC clone accession =
PBC015-H01

= Tag =
N-terminal His6-tag with TEV cleavage site

= Construct comments =

= Construct sequence =
gKVFCGRANPTTGSLEWLEEDEHYDYHQEIARSSYADMLHDKDRNIKYYQGIRAAVSRV
KDRGQKALVLDIGTGTGLLSMMAVTAGADFCYAIEVFKPMAEAAVKIVERNGFSDKIKVI
NKHSTEVTVGPDGDLPCRANILITELFDTELIGEGALPSYEHAHKHLVQEDCEAVPHRATV
YAQLVESRRMWSWNKLFPVRVRTSLGEQVIVPPSELERCPGAPSVCDIQLNQVSPADFTV
LSDVLPMFSVDFSKQVSSSAACHSRQFVPLASGQAQVVLSWWDIEMDPEGKIKCTMAPF
WAQTDPQELQWRDHWMQCVYFLPQEEPVVQGSPRCLVAHHDDYCVWYSLQRTSPDEN
DSAYQVRPVCDCQAHLLWNRPRFGEINDQDRTDHYAQALRTVLLPGSVCLCVSDGSLLS
MLAHHLGAEQVFTVESSVASYRLMKRIFKVNHLEDKISVINKRPELLTAADLEGKKVSLL
LGEPFFTTSLLPWHNLYFWYVRTSVDQHLAPGAVVMPQAASLHAVIVEFRDLWRIRSPCG
DCEGFDVHIMDDMIKHSLDFRESREAEPHPLWEYPCRSLSKPQEILTFDFQQPIPQQPMQS
KGTMELTRPGKSHGAVLWMEYQLTPDSTISTGLINPAEDKGDCCWNPHCKQAVYFLSTT
LDLRVPLNGPRSVSYVVEFHPLTGDITMEFRLADTLS

DNA sequence has been verified by sequencing

= Vector =
pFBOH-MHL

= Expression host =
Spodoptera frugiperda

= Growth method =
Shaker

The recombinant donor vector pFBOH-MHL: mPRMT7 was transformed into DH10Bac E. coli cells (Invitrogen) to generate recombinant viral DNA.
Sf9 cells (Invitrogen) were transfected with Bacmid DNA using jetPRIME® transfection reagent

(PolyPlus Transfection), and recombinant baculovirus particles were recovered.
The recombinant virus sequentially amplified from P1 to P3 viral stocks. Sf9 cells grown in I-Max Insect medium (Wisent Biocenter, Cat.# 301-045-LL) to a density of 4mln/mL and with viability not less than 97% were infected with 10 mL of P3 viral stock for each 1 L of cell culture. Cultured cells were collected when cell viability dropped to 75-80%, normally after 4 days of post-infection time

= Extraction buffers =
20mM Tris pH 8.0, 500 mM NaCl with 5% glycerol

= Extraction procedure =
Native cell pellet was resuspended in extraction buffer and the cells disrupted by sonication for 10 mins at 5" on 10" off duty cycle at 90W output power.

= Purification buffers =
loading buffer: 20mM Tris pH 8.0, 500 mM NaCl with 5% glycerol
Washing Buffer: 20mM Tris pH 8.0, 500 mM NaCl with 5% glycerol, 5 mM imidazole
Elution Buffer: 20mM Tris pH 8.0, 500 mM NaCl with 5% glycerol, 250 mM imidazole

= Purification procedure =
The crude extract was cleared by centrifugation. The lysate was loaded onto (1ml/L) Talon Metal Affinity Resin (Cat# 635504 Clontech).
The resin was washed with 50 CV of loading buffer (20mM Tris pH8.0, 500 mM NaCl with 5% glycerol) and 5 CV washing buffer with 5mM imidazole.
The protein was eluted with elution buffer (loading buffer with 250 imidazole). The protein was further purified by Superdex200 (GE Healthcare) using 20 mM Tris pH7.5 plus 250 mM NaCl. His tag was removed overnight at 4 degrees.
The third purification step was the Source Q using 20mM Tris pH7.5 (buffer A) with 1M NaCl (buffer B).

Protein yield: 2.6 mg/L

= Protein stock concentration =
Concentration used for crystallization : 8.5 mg/mL

= Mass spec =
N/A

= Functional multimerization =
Monomer

= Crystallization =
Crystallization of the PRMT7 in complex with SGC8158 chemical probe was performed in 96-well vapor diffusion sitting drop plates by mixing 1:5 ratio of PRMT7 with SAH and incubated for 30 min on ice, then setup plates at room temperature. Diffraction quality crystals were obtained in 20% PEG 3350, 0.04 M Citric Acid, and 0.06 M Bis-TRIS propane [pH 6.4].
For soaking with SGC8158 chemical probe, mPRMT7 crystals were then transferred into 1 ul drop containing the same precipitant solution supplemented with 1 mM SGC8158 and 20% (v/v) glycerol for 5 days at 20 °C. Soaked crystals were then cryo-cooled in liquid-nitrogen.