

Protein Arginine Methyltransferase 8: Tetrameric Structure and Protein Substrate Specificity

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Supplementary Tables and Figures

Table S1: The statistics table for the tPRMT8 X-ray crystal structure

Name	tPRMT8
PDB code	4X41
Data collection	
Resolution (Å)	25-3.49 (3.62-3.49) *
Space group	<i>P</i> 222 ₁
Unit-cell	
<i>a</i> / <i>b</i> / <i>c</i> (Å)	68.2/78.2/203.9
α / β / γ (°)	90/90/90
No. of reflections Measured	13561 (1357)
Completeness (%)	94.8 (96.7)
<i>R</i> _{sym} (%) ^a	27.2 (72.4)
Mean <i>I</i> /σ(<i>I</i>)	3.9 (2.0)
Multiplicity	2.9 (2.8)
Refinement	
<i>R</i> _{work} (%)	23.1
<i>R</i> _{free} (%)	28.2
Geometry deviations	
Bond lengths (Å)	0.005
Bond angles (°)	1.02
No. of atoms / Mean B-values (Å ²)	4998/55.3
Ramachandran plot (%)	
Most favored	93.0
Allowed	7.0
Disallowed	0.0

Values in parentheses are for the highest resolution shell.

$$^a R_{\text{sym}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl).$$

Table S2: Data collection and scattering-derived parameters for tPRMT8

Data Collection Parameters	
Instrument	SSRL BL4-2
Defining slits size (H mm x V mm)	0.3 x 0.3
Detector distance (m)	2.5
Wavelength (Å); energy (keV)	1.127; 11
Beam current (Å)	500
Q range (Å ⁻¹)	0.007-0.46
Exposure time per frame (s)	1
Size of quartz capillary in diameter (mm)	1.5
Frame per FPLC experiment	800
Amount loaded (μl)	100
Sample concentration (mg/ml)	5.0
Temperature (K)	293
SEC column	Superdex 200PC 3.2/3
FPLC flow rate (ml/min)	0.05
Structural Parameters	
Image frame used for analysis	670-699
Q region (Å ⁻¹)	0.013-0.178
I(0) from Guinier	476.70
R _g (Å) from Guinier	44.31
I(0) from $P(r)$	477.90
R _g (Å) from $P(r)$	44.68
D _{max} (Å) from $P(r)$	115.22
R _g (Å) from crystal structure	43.63
D _{max} (Å) from crystal structure	119
Porod Volume estimate from $P(r)$	308,674
Excluded volume from DAMMIF (Å ³)	431,578.95
Dry volume calculated from sequence (Å ³)	210,564
Calculated tetrameric molecular weight	166.23
Software employed	
Primary data reduction	SasTool
Data processing	PRIMUS
<i>Ab initio</i> analysis	DAMMIF
Validation and averaging	DAMAVR
Computation of model intensities	FoXS

Figure S1: Sequence alignment of PRMT1, Hmt1, PRMT8 and tPRMT8. The sequence of the two PRMT8 constructs, PRMT8 and tPRMT8 are aligned with the full length PRMT1 and the yeast PRMT1 homolog, Hmt1. The conserved residues are marked with star and the highly similar and less similar residues are indicated by double and single dots respectively (generated by ClustalW2, EMBL-EBI).

PRMT8	MSMKHSSRCLLLRKMAENAAESTEVNSPPSQPPQPVVPAKPVQC VHHVSTQPSCPGRGK	60
tPRMT8	-----	
PRMT1	-----MEVSCGQAE	9
Hmt1	-----	
PRMT8	MSKLLNPEEMTSRDYYFDSYAHFGIHEEMLKDEVRTLTYRNSMYHNKHVFKDKVVLVDVGS	120
tPRMT8	MSKLLNPEEMTSRDYYFDSYAHFGIHEEMLKDEVRTLTYRNSMYHNKHVFKDKVVLVDVGS	60
PRMT1	SSEKPNAEEDMTSKDYYFDSYAHFGIHEEMLKDEVRTLTYRNSMFHNRLFKDKVVLVDVGS	69
Hmt1	-----DYYFDSYDHYGIHEEMLQDVRTLSYRNAIIQNKDLFKDKIVLVDVGC	47
	***** *-*	
PRMT8	GTGILSMFAAKAGAKKVFGEICSSISDYSEKIIKANHLDNIIITIFKGKVEEVELPVEKVD	180
tPRMT8	GTGILSMFAAKAGAKKVFGEICSSISDYSQKIIKANHLDNIIITIFKGKVEEVELPVEKVD	120
PRMT1	GTGILCMFAAKAGARKVIGIECSSISDYAVKIVKANLKHVVTTIIGKVEEVELPVEKVD	129
Hmt1	GTGILSMFAAKHGAKHVIGVDMSSIIEMAKELVELNGFSDKITLLRGKLEDVHLPFPKVD	107
	*****-*	
PRMT8	IIISEWMGYCLFYESMLNTVIFARDKWLKPGGLMFPDRAALYVVAIEDRQYKDFKIHWE	240
tPRMT8	IIISEWMGYCLFYESMLNTVIFARDKWLKPGGLMFPDRAALYVVAIEDRQYKDFKIHWE	180
PRMT1	IIISEWMGYCLFYESMLNTVLYARDKWLAPDGLIFPDRAATLYVTAIEDRQYKDYKIHWE	189
Hmt1	IIISEWMGYFLLYESMMDTVLYARDHYLVEGGLIFPDKCSIHLALEDSDQYKDEKLNWQ	167
	*****-*	
PRMT8	NVYGFDMTCIRDVAMKEPLVDIVDPKQVVTNACLIKEVDIYTVKTEELSFTSAFCLQIQ	300
tPRMT8	NVYGFDMTCIRDVAMKEPLVDIVDPKQVVTNACLIKEVDIYTVKTEELSFTSAFCLQIQ	240
PRMT1	NVYGFDMSCIKDVAIKEPLVDVDPKQLVTNACLIKEVDIYTVKVEDLTFTSPFCLQVQR	249
Hmt1	DVYGFDYSPFVPLVLHEPIVDIVERNNVNTTSDKLIEFDLNTVKISDLAFKSNFKLTAKR	227
	*****-*	
PRMT8	NDYVHALVTYFNIEF--TKCHKMGFSTAPDAPYTHWKQTVFYLEDYLTVRRGEEIYGTI	358
tPRMT8	NDYVHALVTYFNIEF--TKCHKMGFSTAPDAPYTHWKQTVFYLEDYLTVRRGEEIYGTI	298
PRMT1	NDYVHALVAYFNIEF--TRCHKRTGFSTSPESPYTHWKQTVFYMEDYLTVKTGEEIFGTI	307
Hmt1	QDMINGIVTWFDIVFPAPKGRPVEFSTGPHAPYTHWKQTFYFPDDLDAETGDTIEGEL	287
	*****-*	
PRMT8	SMKPNAKNVRDLDFTVDLDFKGQLCETSVSN-----DYKMR	394
tPRMT8	SMKPNAKNVRDLDFTVDLDFKGQLCETSVSN-----DYKMR	334
PRMT1	GMRPNAKNVRDLDFTVDLDFKGQLCETSVSN-----DYRMR	343
Hmt1	VCSPNEKNVRDLNLIKISYKFESNGIDGNSRSRKNEGSYIMH	328
	*****-*	

Figure S2: Homotetramerization of tPRMT8: The size exclusion chromatography profile of tPRMT8 at various buffers (left) and salt (right) conditions. The SEC profiles of tPRMT8 were almost identical with varying salt concentration and pH range.

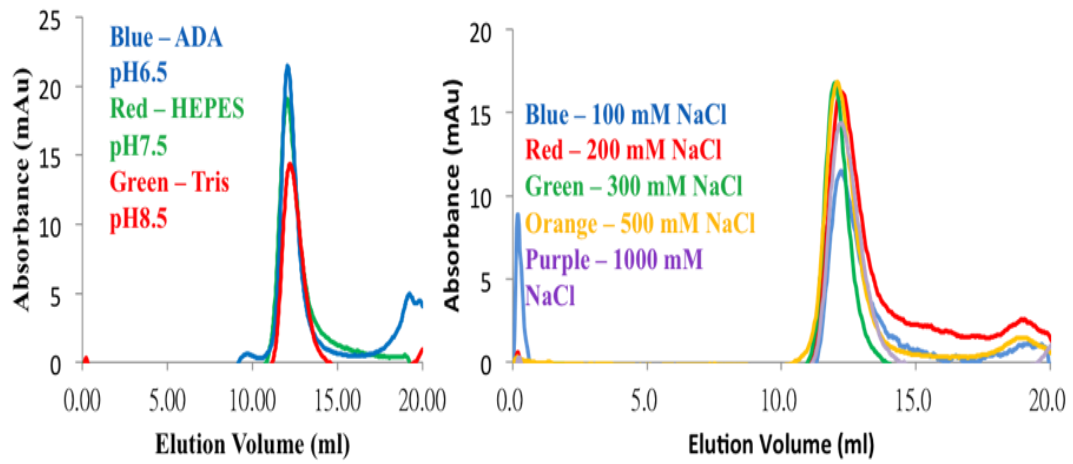


Figure S3: The *in vitro* methylation of NIFK. A) The methylated NIFK by tPRMT8 was digested by Lys-C protease followed by LS-MS/MS which shown 64% coverage. B). In LC-MS/MS spectra, Arg114 and Arg284 were mono-methylated. The Arg244 and Arg245 are close neighbors which leads to difficulties in identifying the methylated arginine and the degree of methylation.

A)

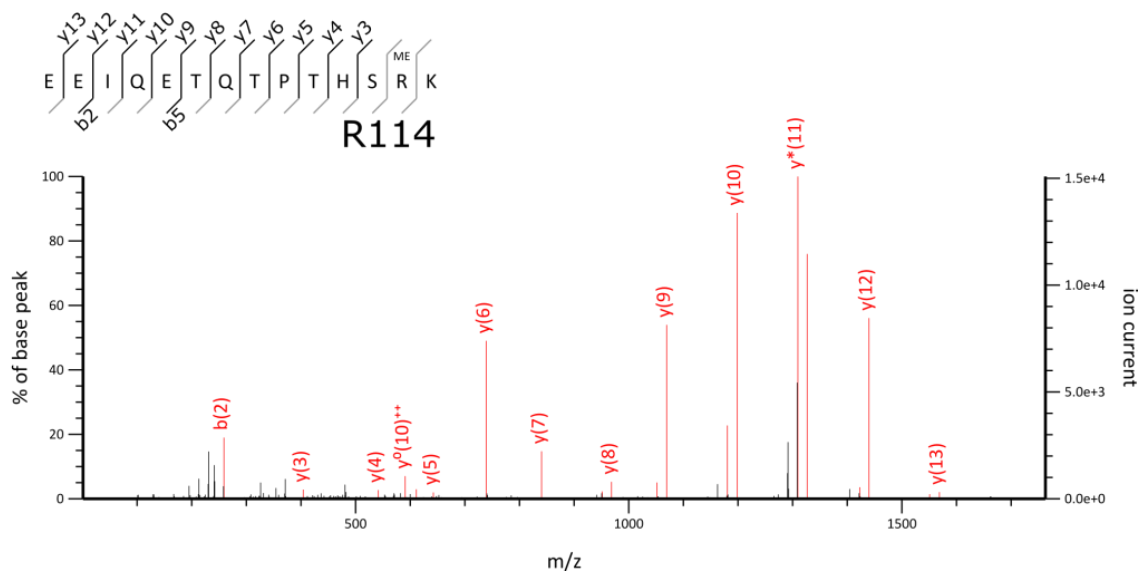
Matched peptides shown in **bold red**.

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1  MATFSGPAGP ILSLNPQEDV EFQKEVAQVR KRITQRKKQE QLTPGVVYVR
51  HLPNLLDETQ IFSYFSQFGT VTRFRLSRSK RTGNSKGYAF VEFESQDAK
101 IVAETMNNYL FGERLLECHF MPPEKVHREL FKDWNIPFKQ PSYPSVKRYN
151 RNRTLQKLR MEERFKKKER LLRKKLAKKG IDYDFPSLIL QKTESISKTN
201 RQTSTKGQVL RKKKKKVSQT LDTPEKTVDS QGPTFVCTPT FLERRKSQVA
251 ELNDDDDKDE IVFKQPISCV KEEIQETQTP THSRKKRRRS SNQ

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B)



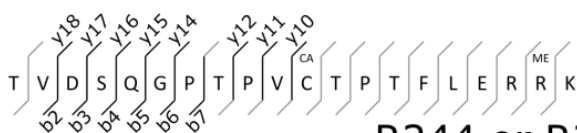
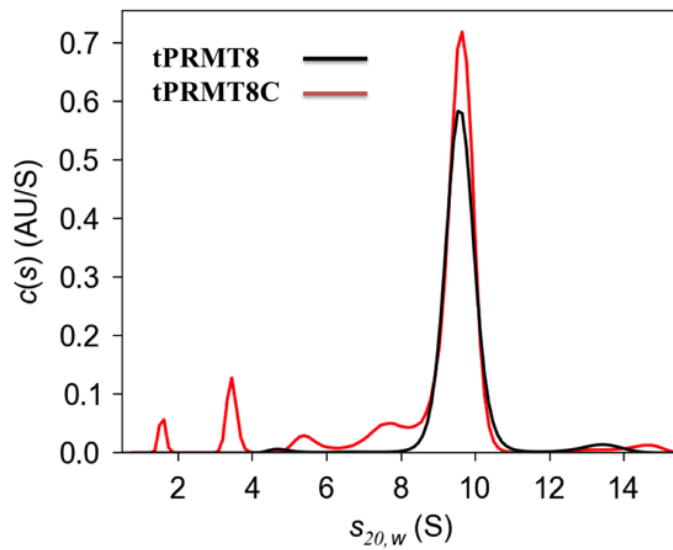


Figure S4: tPRMT8C is structurally similar to tPRMT8

A) The AUC overlay of tPRMT8 (black) and tPRMT8C (in red) indicates a similar profile that represents a major species consistent with a tetrameric form of ~160 kDa.

B) The CD spectra (left) of tPRMT8 (red triangle) overlay with tPRMT8C (blue square) suggests the secondary structures are very similar. The comparison of T_m values (right) for tPRMT8 (red triangle) with tPRMT8C (blue square) suggests tPRMT8c is less stable as the T_m value decreased by ~10°C.

A)



B)

