Protein Arginine Methyltransferase 8: Tetrameric Structure and Protein Substrate Specificity

Wei-Chao Lee,† Wen-Ling Lin,† Tsutomu Matsui,‡ Eric S.-W. Chen,† Tong-You Wade Wei,† Wen-Hsuan Lin,† Hao Hu,§ Yujun George Zheng,§ Ming-Daw Tsai,† and Meng-Chiao Ho*,†

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	$P222_1$
Unit-cell	
a / *b* / *c* (Å)	68.2/78.2/203.9
α / β / γ (°)	90/90/90
No. of reflections Measured	13561 (1357)
Completeness (%)	94.8 (96.7)
R_{sym} (%)[a]	27.2 (72.4)
Mean $I/\sigma(I)$	3.9 (2.0)
Multiplicity	2.9 (2.8)
Refinement	
R_{work} (%)	23.1
R_{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] $R_{sym} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

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 quarz 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
Rg (Å) from Guinier	44.31
I(0) from *P(r)*	477.90
Rg (Å) from *P(r)*	44.68
Dmax (Å) from *P(r)*	115.22
Rg (Å) from crystal structure	43.63
Dmax (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
Ab initio analysis	DAMMIF
Validation and averaging	DAMAVER
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).

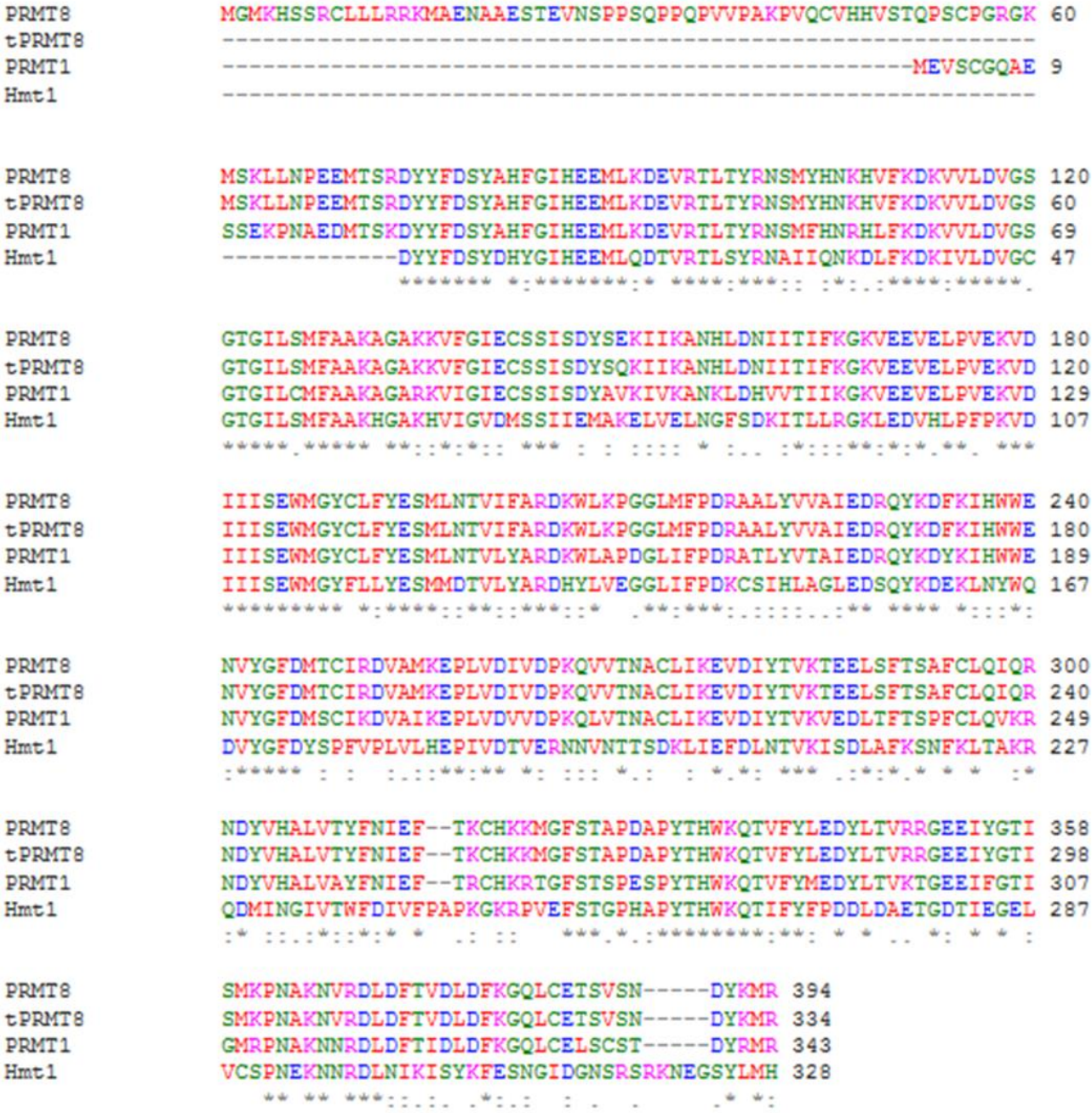

Figure S2: Homotetramerization of tPRMT8: The size exclusion chromatography profile of tPRMT8 at various buffers (left) and salt (right) conditions. The SEC profiles of tPRMT8 were almost identical with varying salt concentration and pH range.

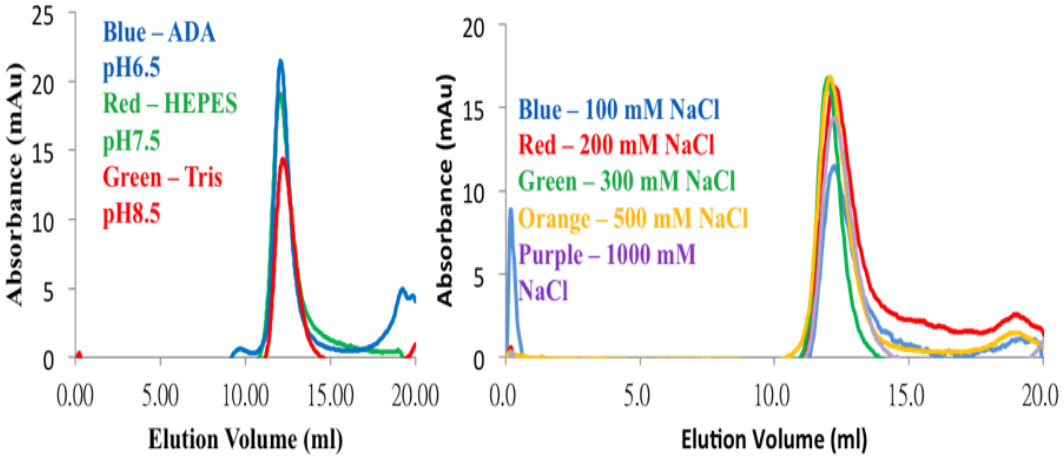

Figure S3: The *in vitro* methylation of NIFK. A) The methylated NIFK by tPRMT8 was digested by Lys-C protease followed by LS-MS/MS which shown 64% coverage. B). In LC-MS/MS spectra, Arg114 and Arg284 were mono-methylated. The Arg244 and Arg245 are close neighbors which leads to difficulties in identifying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

A)

Matched peptides shown in ***bold red***.

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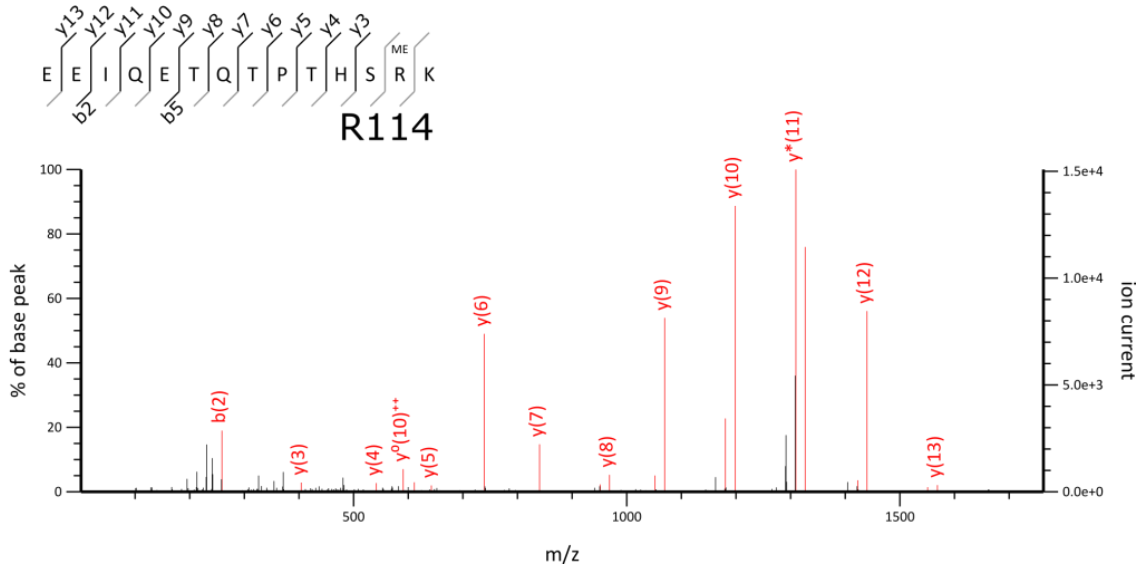
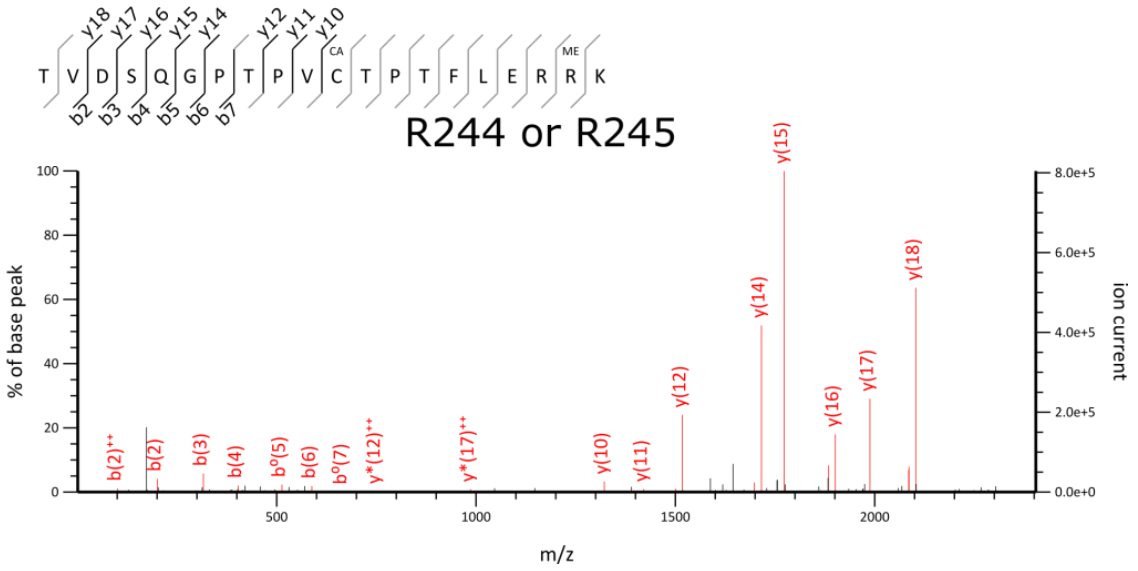
  1 MATFSGPAGP ILSLNPQEDV EFQKEVAQVR KRITQRKKQE QLTPGVVYVR
 51 HLPNLLDETQ IFSYFSQFGT VTRFRLSRSK RTGNSKGYAF VEFESEDVAK
101 IVAETMNNYL FGERLLECHF MPPEKVHKEL FKDWNIPFKQ PSYPSVKRYN
151 RNRTLTQKLR MEERFKKKER LLRKKLAKKG IDYDFPSLIL QKTESISKTN
201 RQTSTKGQVL RKKKKKVSGT LDTPEKTVDS QGPTPVCTPT FLERRKSQVA
251 ELNDDDKDDE IVFKQPISCV KEEIQETQTP THSRKKRRRS SNQ
```

B)




T V D S Q G P T P V C T P T F L E R R K
CA
ME
R244 or R245
% of base peak
ion current
m/z

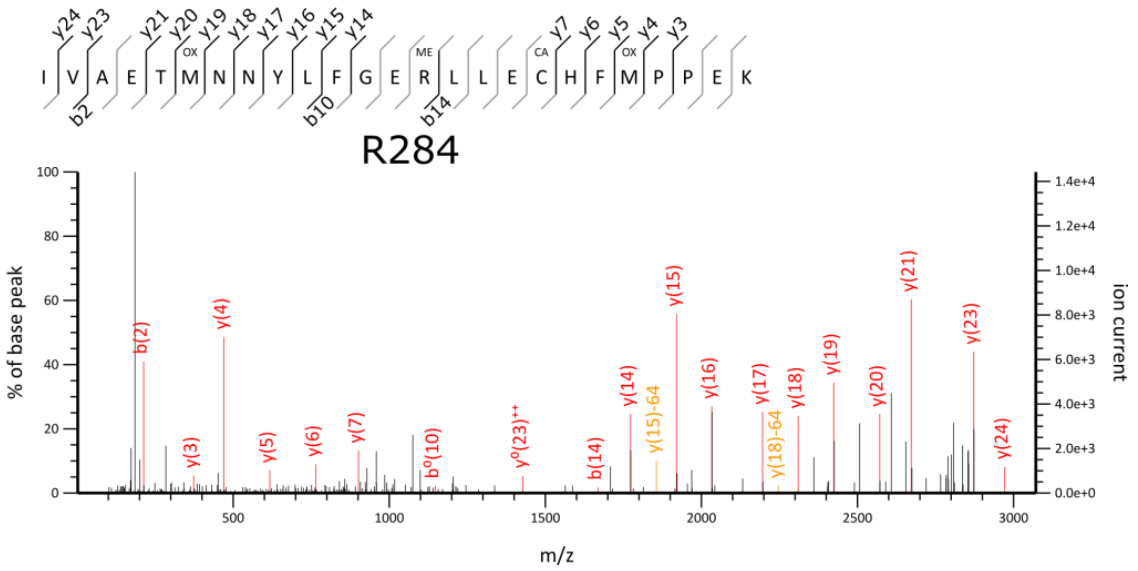
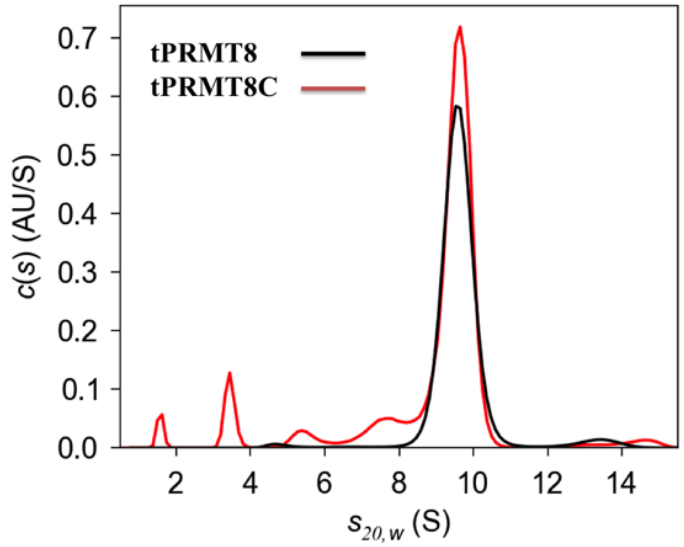

I V A E T M N N Y L F G E R L L E C H F M P P E K
OX
ME
CA
OX
R284
% of base peak
ion current
m/z

Figure S4: tPRMT8C is structurally similar to tPRMT8

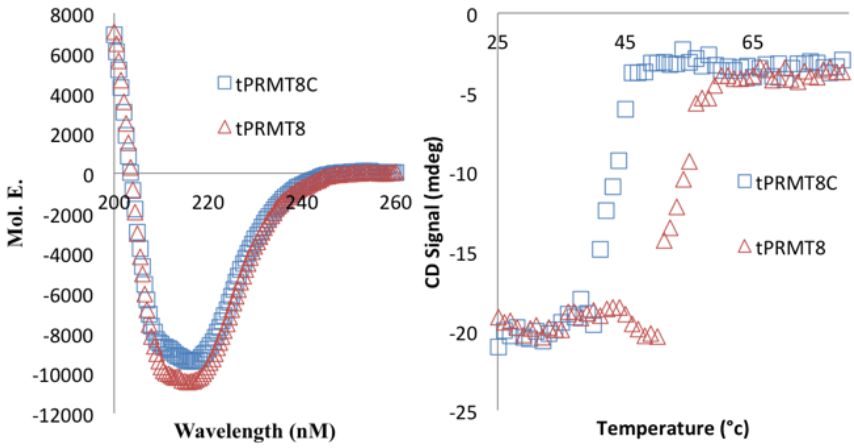
A) The AUC overlay of tPRMT8 (black) and tPRMT8C (in red) indicates a similar profile that represents a major species consistent with a tetrameric form of ~160 kDa.

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

A)



B)



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Supplemental Information – Materials and Methods

MS analysis for *in vitro* methylation of NIFK- The recombinant methylated NIFK was separated from tPRMT8 by electrophoresis followed by in-gel digestion with Lys-C (Wako. Chemicals USA, VA). Digested peptides were extracted and dried. Peptide samples were reconstituted in 0.1% FA and analyzed on Waters Synapt G2 HDMS (Waters, Milford, MA). The C18 column was maintained at 35 °C and the bound peptides were eluted with a gradient of 5-35 % of CAN in the presence of 0.1% FA. Data acquisition was performed using data directed analysis (DDA) method. The DDA method included one full MS scan (m/z 350-1600, 1 s/scan) and three MS/MS scans (m/z 100-2000, 1 s/scan). Analysis of all MS/MS samples was performed using Mascot (Matrix Science; version 2.4.0). Mascot was set up to search the Swiss-Prot human database. Database search against Mascot was performed with a fragment ion mass tolerance of 0.05 Da and a parent ion tolerance of 25 ppm. Two missed cleavages were allowed during trypsin digestion. Oxidation (Met), carbamidomethyl (Cys) and arginine methylation (methyl and dimethyl) were specified as variable modification.

Circular dichroism analysis

Both tPRMT8 and tPRMT8C were purified as described and samples were prepared to the desired concentration of ~10 μM in 50 mM sodium phosphate, pH7.5, 100 mM sodium chloride. All CD spectra were collected with an Aviv CD 202 spectrometer (Lakewood, NJ) at 25 °C. The samples were placed in a 1 mm path length cuvette for CD spectra from 190 to 260 nm wavelength of step of 0.5 nm. For thermal denaturation experiments, the sample was monitored at 215nm from 25 to 95 °C with a 2 °C interval. The data curves were analyzed using SigmaPlot 8.02 (SPSS Inc.).