

Supporting Information

Table S1. The gradient conditions in reverse phase-HPLC to separate methylation products catalyzed by WT-PRMT1 and the mutants.

| Time(min) | Flow rate (ml/min) | Column Temperature (°C) | %A | %B |
|-----------|--------------------|-------------------------|-----|-----|
| 0 | 1.0 | 40 | 100 | 0 |
| 15 | 1.0 | 40 | 65 | 35 |
| 40 | 1.0 | 40 | 60 | 40 |
| 41 | 1.0 | 40 | 39 | 61 |
| 44.5 | 1.0 | 40 | 0 | 100 |
| 54 | 1.0 | 40 | 0 | 100 |

Table S2. Data collection and refinement statistics for rat PRMT1 M48L. Values in parentheses correspond to those in the outer resolution shell

| | Rat PRMT1 M48L |
|---|--------------------|
| Data collection | |
| Beamline | Home source |
| Wavelength (Å) | 1.5418 |
| Resolution range (Å) | 28.35-2.20 |
| Outer shell (Å) | 2.28-2.20 |
| No. of reflections | |
| unique | 27,377 |
| total | 130,736 |
| Average redundancy | 4.8 (3.2) |
| Mean I/σ(I) | 8.9 (2.4) |
| Completeness (%) | 94.8 (68.0) |
| R _{sym} (%) ^a | 9.4 (43.7) |
| Space group | P4 ₁ 22 |
| Unit cell dimensions (a,b,c (Å); α=β=γ=90°) | 87.3, 87.3, 143.29 |
| Refinement | |
| R _{work} /R _{free} (%) ^b | 20.1/24.5 |
| Atoms in the structure | |
| protein | 2545 |
| waters | 204 |
| ligands/ions | 27 |
| Average B factors (Å ²) | |
| protein | 39.6 |
| water | 45.9 |
| rmsd bond (Å)/angle (°) | 0.008/1.124 |
| Protein geometry ^c | |
| Ramachandran outliers (%) | 0.0 |
| Ramachandran favored (%) | 96.5 |
| Rotamer outliers (%) | 1.8 |

^a $R_{\text{sym}} = (\sum |I - \langle I \rangle|) / (\sum I)$, where $\langle I \rangle$ is the average intensity of multiple measurements.

^b $R_{\text{work}} = (\sum |F_{\text{obs}} - F_{\text{calc}}|) / (\sum |F_{\text{obs}}|)$ and is calculated using all data; R_{free} is the R-factor based on 5% of the data excluded from refinement.

^c Ramachandran statistics were calculated using the MolProbity server (31).

Table S3. The gradient conditions in reverse phase separation of mass spectrometry analysis of the R3 peptide products

| Time | flow $\mu\text{l}/\text{min}$ | %A | %B |
|------|----------------------------------|----|----|
| 0 | 0.8 | 99 | 1 |
| 7.9 | 0.8 | 99 | 1 |
| 8 | 0.6 | 99 | 1 |
| 20 | 0.6 | 70 | 30 |
| 21 | 0.6 | 20 | 80 |
| 23 | 0.6 | 20 | 80 |
| 24 | 0.8 | 99 | 1 |

Table S4. Steady-state kinetic activity of PRMT1 mutants with large-sized side chains with hnRNP K via ZipTip_{C4} assay

| PRMT1 | % activity of wtPRMT1 |
|----------|-----------------------|
| wt-PRMT1 | 100 |
| M48F | 3.0 |
| M48Y | 0.13 |
| M48W | 0.40 |

Figure S1. Representative 2.2 Å electron density observed at the PRMT1 M48L active site. $2F_o - F_c$ maps are contoured at 1σ .

Figure S2. HPLC analysis of amino acids contained in the R3 peptide. Amino acids in the R3 peptide, Gly, Arg, and Phe, as well as MMA, ADMA, and SDMA standards were derivatized with OPA reagent and separated using RP-HPLC.

Figure S3. Michaelis-Menten plots of PRMT1 mutants with the R3 peptide. Proteins were assessed for activity using the R3 peptide. Various concentrations of R3 peptide [25-1000 μM] were used to initiate reactions containing 4 μM mutant PRMT1, 250 μM SAM, 10 nM MTA nucleosidase, 10 μM MnSO_4 , and 50 mM NaPO_4 buffer pH 7.1 at 37 °C. Reactions were performed at least in duplicate, and initial reaction rates were used to assess protein activity. Michaelis-Menten plots for M155A and M48L with the R3 peptide are shown.

Figure S4. Modified Stern-Volmer plot showing the intrinsic fluorescent quenching of PRMT1 by R3 and AdoMet. The data was fit to a line with nonlinear regression where the $y_{\text{intercept}} = 1/fa$, the slope = $1/fa * K_Q$ and the $K_Q = 1/K_D$.

Figure S5. HPLC analysis of automethylated M48L after an 8-hr automethylation reaction. Modified M48L protein was hydrolyzed, and the resulting amino acids were derivatized with OPA reagent and separated using RP-HPLC. Tritiated AdoMet was used as a tracer and peaks were identified as in Figure 2.

Figure S6. MS/MS data for the first methylation of the R3 peptide.

Figure S1.

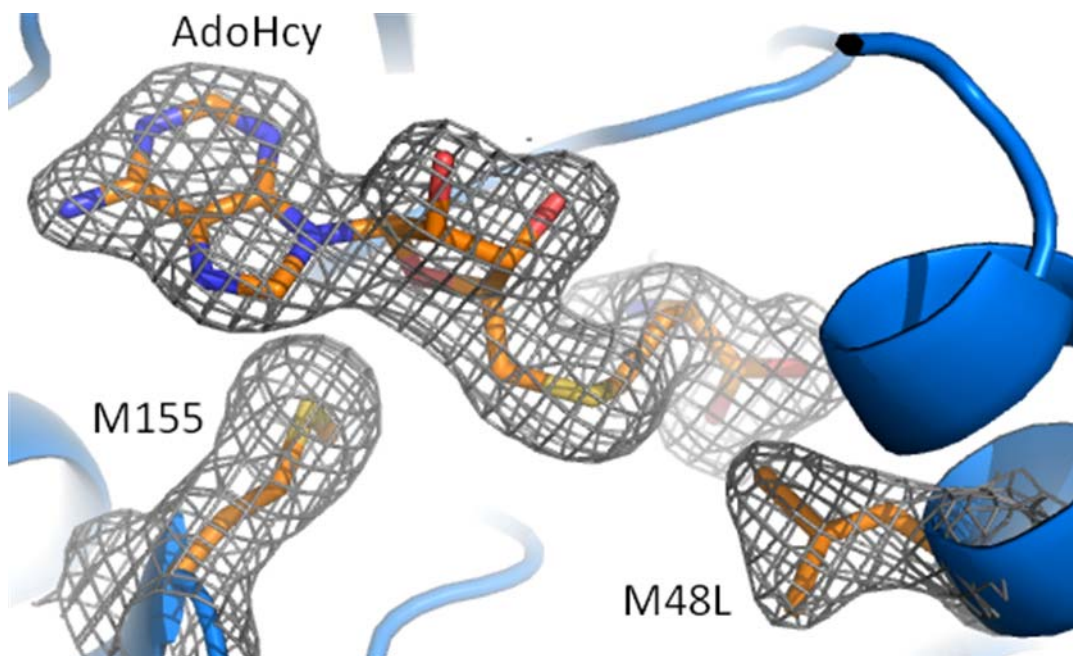


Figure S2.

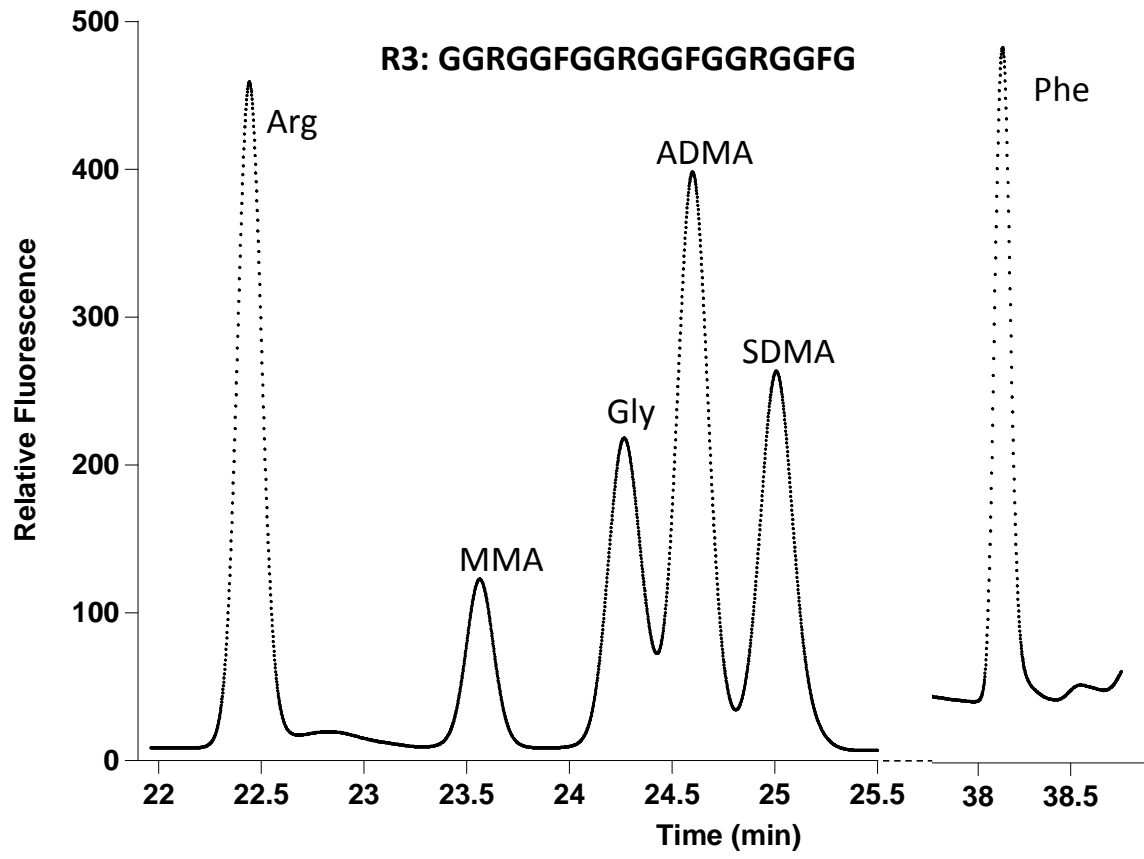


Figure S3.

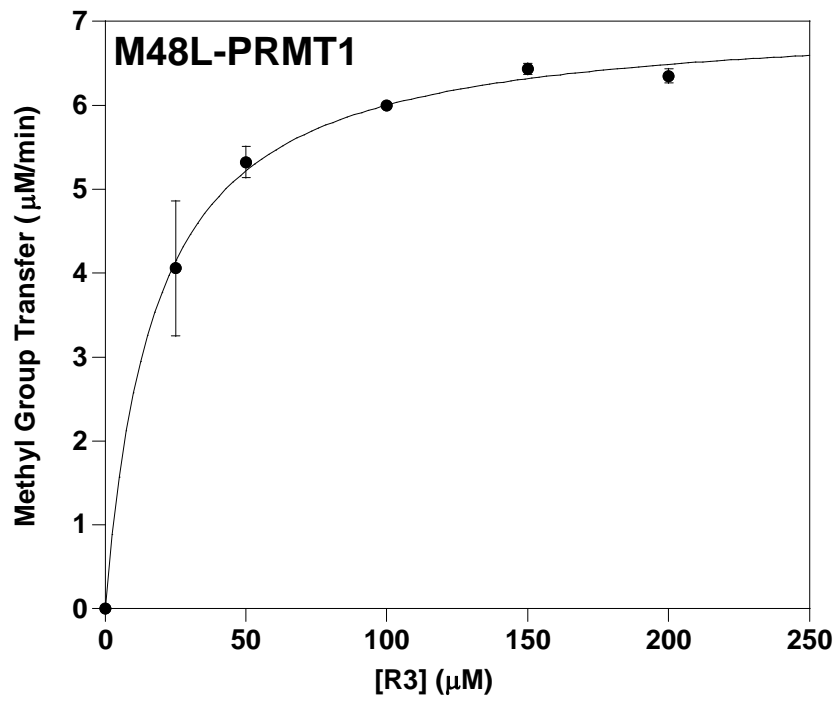
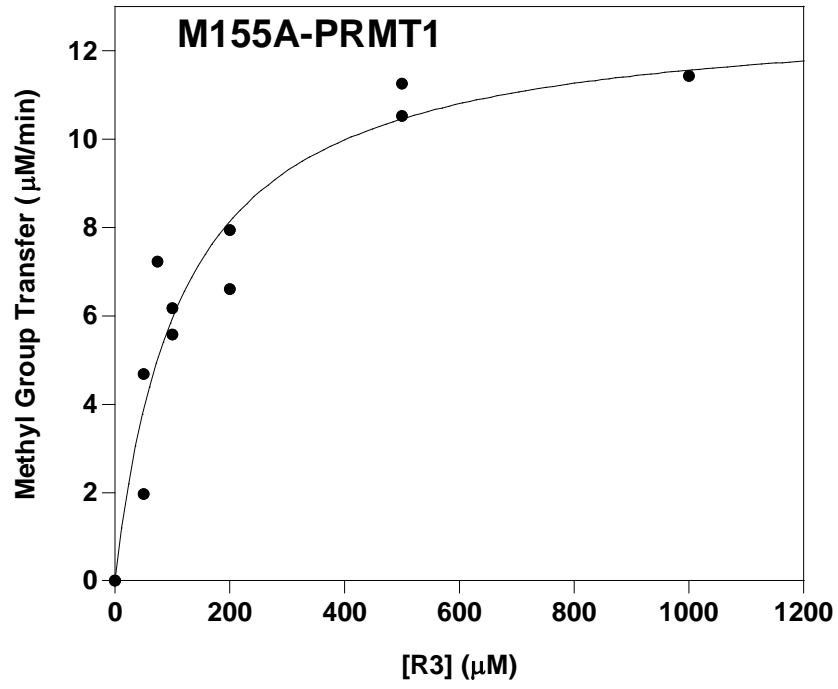


Figure S4.

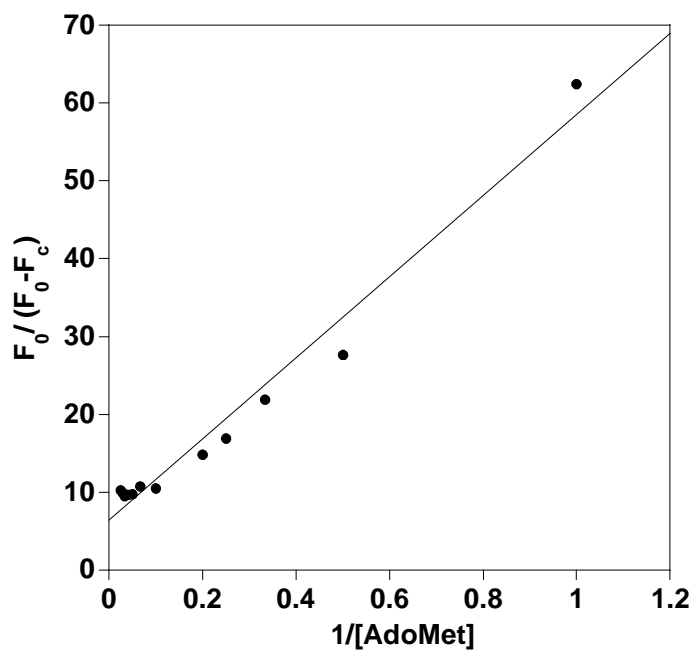
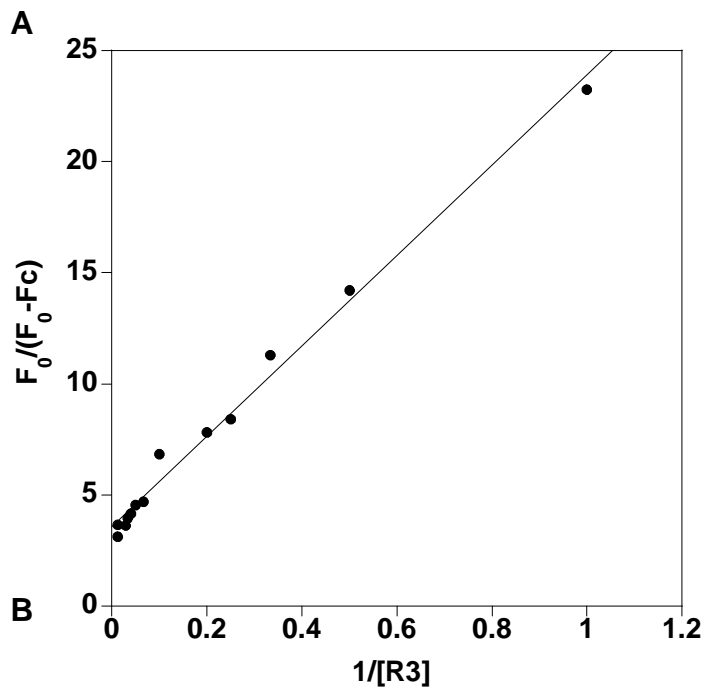


Figure S5.

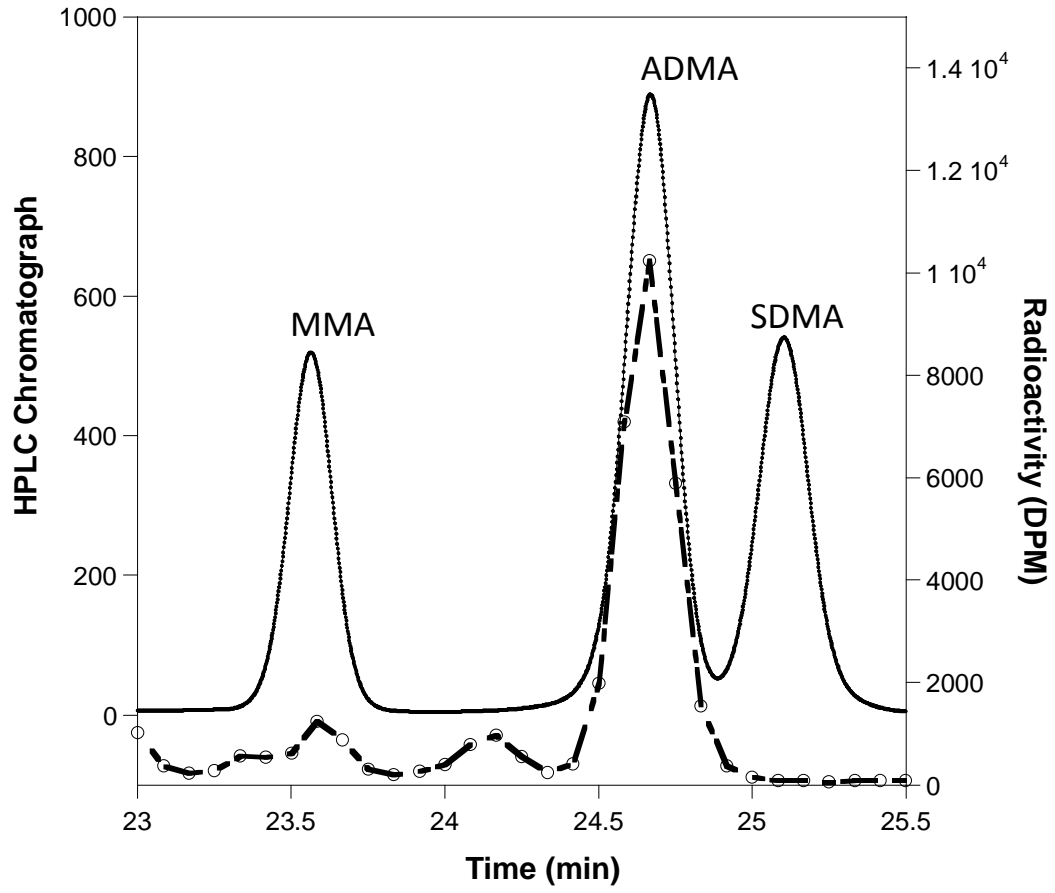


Figure S6.

