# Comprehensive Assay Approaches for PRMT5 Targeted Drug Discovery

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## Abstract

Protein arginine methyltransferase 5 (PRMT5) is a type II methyltransferase that symmetrically dimethylates arginine residues on histone and non-histone proteins, an essential epigenetic modification that shapes chromatin structure and regulates gene expression. Through these activities, PRMT5 controls key cellular processes, including transcription, RNA splicing, DNA repair, and signal transduction. PRMT5 operates in complex with MEP50, which is required for efficient catalytic activity.

Dysregulated PRMT5 is strongly associated with diseases, particularly cancer. PRMT5 is frequently overexpressed in breast, lung, prostate, and hematologic malignancies, where it promotes tumor growth, metastasis, and therapy resistance. Small-molecule PRMT5 inhibitors have emerged as promising agents that block methyltransferase activity, reverse aberrant epigenetic marks, and disrupt oncogenic pathways. Here, we established comprehensive assay platforms for PRMT5-targeted drug discovery and validated them using five known inhibitors (LLY-283, JNJ-64619178, GSK591, EPZ015666, and GSK33326595).

Our biochemical FlashPlate assay demonstrated potent inhibition of PRMT5/MEP50 activity, with $IC_{50}$ values in the low nanomolar range (0.6–17 nM). Surface Plasmon Resonance (SPR) revealed distinct binding profiles: substrate-competitive inhibitors (EPZ015666, GSK33326595, GSK591) showed weak affinity for apo PRMT5, enhanced by cofactor SAM, whereas cofactor-competitive LLY-283 bound tightly to apo PRMT5 but exhibited >50-fold reduced affinity with the cofactor. JNJ-64619178 displayed similar affinity upon cofactor binding, driven by a slow off-rate consistent with pseudo-irreversible inhibition.

NanoBRET target engagement intracellular assay results indicated that the PRMT5 inhibitors engaged with the PRMT5/MEP50 complex within one hour of incubation in live HEK293 cells, and Western blot confirmed inhibition of histone H4R3me2s methylation, a key substrate of PRMT5, in MV4-11, Jeko-1, 22RV1, and PC3 cancer cell lines. Collectively, these platforms enable identification, optimization, and mechanistic characterization of PRMT5 inhibitors, accelerating development of selective and potent therapeutic candidates.

## Experimental Procedures

### Biochemical FlashPlate Assay

Histone methyltransferases use tritium-labeled SAM as the methyl donor that transfers a radioactive methyl group to the histone substrate. Reaction mixtures containing PRMT5/MEP50 complex, biotinylated histone H4 (1-15) substrate, and 3H-S-Adenosyl methionine were incubated with test compounds, terminated, and transferred to a streptavidin-coated microplate to count bound radiolabeled substrate.

### Surface Plasmon Resonance (SPR)

Biotinylated PRMT5::MEP50 was immobilized on a Series S SA sensor chip to a level of ~5000 RU and data were collected on a Biacore 8K+. Binding of analytes was measured to the apo protein and in the presence of saturating concentrations of MTA, SAH, and SAM. Data were solvent corrected and double referenced and fit using 1:1 Langmuir and/or steady-state affinity model as appropriate based on sample behavior.

### NanoBRET Target Engagement Intracellular PRMT5 Assay

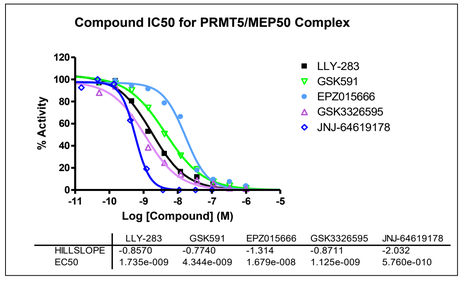
HEK293 cells were transfected with the NanoLuc-PRMT5 fusion vector and MEP50 expression vector using FuGENE HD Transfection Reagent. Testing compounds were delivered into a 384-well assay plate using the Echo 550 liquid handler. Transfected cells were harvested, incubated with NanoBRET TE PRMT5 Tracer, and dispensed into 384-well plates. The plates were incubated at 37°C in a 5% CO2 cell culture incubator for 1 hour. NanoBRET Nano-Glo Substrate was added to each well and incubated for 20 minutes. Emission wavelengths at 460 nm (donor) and 600 nm (acceptor) were measured using the Envision 2104 Multilabel Reader. The BRET Ratio was calculated, and IC50 curves were generated, with IC50 values determined using GraphPad Prism based on a sigmoidal dose-response equation.

### Western Blot

MV4-11, 22RV1, PC3 and Jeko-1 cells in the logarithmic growth phase were seeded into 12-well plates. Next day, the cells were treated with the PRMT5 inhibitors LLY-283, JNJ-64619178, GSK591, EPZ015666, and GSK33326595 for 72 hours. The cells were lysed with 1X SDS Lysis Buffer. Cell lysates were subjected to SDS-PAGE on 12% Bis-Tris gels and transferred onto nitrocellulose membranes using the iBlot dry blotting system. The membranes were blocked with 3% milk and probed with anti-Histone H4R3me2s (symmetric) and anti-Histone H4 antibodies. Anti-rabbit IgG IRDye 680RD secondary antibodies were used for detection. The membranes were scanned using the LI-COR Odyssey Fc Imaging System.

## Results

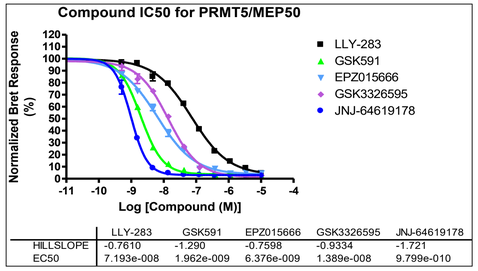
### Biochemical FlashPlate Assay




| | LLY-283 | GSK591 | EPZ015666 | GSK3326595 | JNJ-64619178 |
|---|---|---|---|---|---|
| HILLSLOPE | -0.8570 | -0.7740 | -1.314 | -0.8711 | -2.032 |
| EC50 | 1.735e-009 | 4.344e-009 | 1.679e-008 | 1.125e-009 | 5.760e-010 |

**Figure 1.** PRMT5/MEP50 complex, biotinylated histone H4 (1-15) substrate, and 3H-S-Adenosyl methionine were incubated with five known PRMT5 inhibitors (LLY-283, GSK591, EPZ015666, GSK33326595, and JNJ-64619178) for 20 minutes. Bound radioactivity in the FlashPlate was measured using a scintillation counter.

### NanoBRET TE Intracellular PRMT5 Assay




| | LLY-283 | GSK591 | EPZ015666 | GSK3326595 | JNJ-64619178 |
|---|---|---|---|---|---|
| HILLSLOPE | -0.7610 | -1.290 | -0.7598 | -0.9334 | -1.721 |
| EC50 | 7.193e-008 | 1.962e-009 | 6.376e-009 | 1.389e-008 | 9.799e-010 |

**Figure 3.** HEK293 cells transiently co-expressing NanoLuc-PRMT5 fusion protein and co-factor MEP50 protein were seeded into 384-well plates. The cells were pre-treated with the PRMT5 Tracer and treated with various concentrations of PRMT5 inhibitors (starting at 10 µM, with 10 doses and 3-fold serial dilutions) for 2 hours. Intracellular PRMT5 target engagement by the inhibitors was assessed using the NanoBRET assay.

### Table of IC50 Value Summary Across Assays

| Assay Type | Biochemical Flashplate Assay | Surface Plasmon Resonance (SPR) | | NanoBRET TE Intracellular Assay |
|---|---|---|---|---|
| | IC50 Value (M) | Kd Value (M) | Kd Value (M) | IC50 Value (M) |
| Compound/Target | PRMT5/MEP50 + SAM | PRMT5/MEP50 (Apo) | PRMT5/MEP50 + SAM | PRMT5/MEP50 |
| LLY-283 | 1.74E-09 | 1.68E-09 | 1.08E-07 | 7.19E-08 |
| GSK591 | 4.34E-09 | 4.92E-05 | 1.21E-10 | 1.96E-09 |
| EPZ015666 | 1.68E-08 | 8.58E-05 | 4.56E-10 | 6.38E-09 |
| GSK33326595 | 1.13E-09 | 2.07E-05 | 6.13E-11 | 1.39E-08 |
| JNJ-64619178 | 5.76E-10 | 1.24E-11 | 9.22E-12 | 9.80E-10 |

### Surface Plasmon Resonance (SPR) Analysis

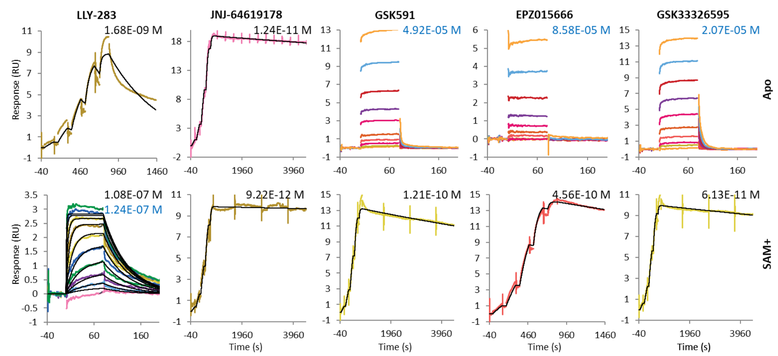



**Figure 2.** Biotinylated PRMT5::MEP50 was immobilized on a Series S SA sensor chip. Binding of five known PRMT5 inhibitors (LLY-283, GSK591, EPZ015666, GSK33326595, and JNJ-64619178) was measured to the apo protein (top) and in the presence of saturating concentrations SAM (bottom) on a Biacore 8K+. Data were fit using 1:1 Langmuir (black lines) or steady state affinity fits, with resultant $K_D$s in black and blue, respectively.

### Western Blot Analysis

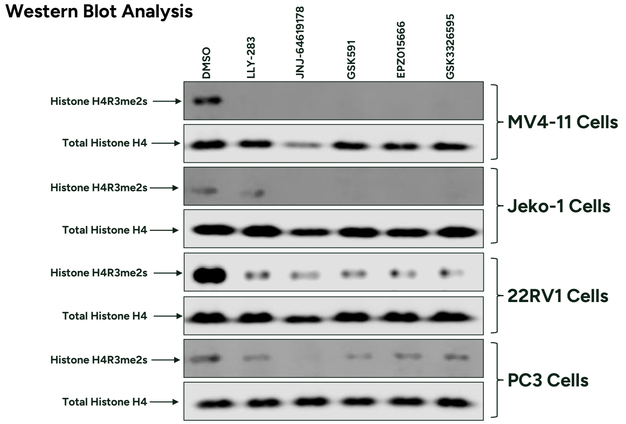



**Figure 4.** MV4-11, 22RV1, PC3 and Jeko-1 cells were treated with the PRMT5 inhibitors LLY-283, GSK591, EPZ015666, GSK33326595, and JNJ-64619178 for 72 hours. The cell lysate samples were subjected to Western blot analyses with anti-Histone H4R3me2s (symmetric) and anti-Histone H4 antibodies.

## Conclusion

Reaction Biology provides a comprehensive suite of assays for PRMT5-targeted drug discovery, including biochemical FlashPlate assays, Surface Plasmon Resonance (SPR), NanoBRET target-engagement cellular assays, and Western blot analysis.

## Contact Information

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