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Cryo-EM structure-guided selection of computed ligand poses to enhance potency in MTA-synergic inhibition of human protein arginine methyltransferase 5

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ABSTRACT

The potential of using cryo-electron microscopic (cryo-EM) structures of 2.5-4.0 Å resolutions for structure-based drug design was proposed recently, but is yet to be materialized. Here we show that a 3.1 Å cryo-EM structure of protein arginine methyltransferase 5 (PRMT5) is sufficient to guide the selection of computed poses of a bound inhibitor and its redesign for much higher potency. PRMT5 is an oncogenic target and its multiple inhibitors are in clinical trials for various cancer types. However, all these PRMT5 inhibitors manifest negative cooperativity with a metabolic co-factor analog --- 2-methylthioadenosine (MTA), which is accumulated substantially in cancer patients carrying defective MTA phosphorylase (MTAP). To achieve MTA-synergetic inhibition, we obtained a pharmacophore from virtual screen and synthesized a specific inhibitor (11-2F). Cryo-EM structures of the 11-2F/MTA-bound human PRMT5: MEP50 complex and its apo form together showed that the inhibitor binding in the catalytic pocket causes a shift of the cofactor-binding site by 1.5 – 2.0 Å, disfavoring cofactor-binding and resulting in positive cooperativity between 11-2F and MTA. Coarse-grained and full-atomistic MD simulations of the ligands in their binding pockets were performed to compare computed poses of 11-2F and its redesigned analogs. Three new analogs were predicted to have much better potency. One of them, after synthesis, was ~4 fold more efficient in PRMT5 inhibition in the presence of MTA than 11-2F itself. Computational analysis also suggests strong subtype specificity of 11-2F among PRMTs. These data demonstrate the feasibility of using cryo-EM structures of near-atomic resolutions and computational analysis of ligand poses for better small molecule therapeutics.
KEYWORDS:
PRMT5; protein arginine methyl transferase; MTA-inhibitor synergy; cryo-EM structure-based
drug design; computational analysis; catalytic mechanism; drug discovery; docking analysis; en-
ergy minimization.

Abbreviations and acronyms:
PRMT: protein tyrosine methyl transferase
MEP50: methylosome associated protein 50
Cryo-EM: cryogenic electron microscopy
MD: molecular dynamics
MTA: 2-methylthioadenosine
MTAP: MTA phosphorylase
SAH: S-adenosyl-L-homocysteine
SAM: S-adenosyl-L-methionine
SDMA: ω-N^G,N'^G-symmetric dimethylarginine
MMA: ω-N^G-monomethyl arginine
TIM: triosephosphate isomerase
INTRODUCTION

Single particle cryo-electron microscopy (cryo-EM) has undergone a so-called “resolution revo-
lution” and has produced hundreds of cryo-EM structures of 2.5 – 4.5 Å resolutions in the past 10
or so years \(^1-^8\). Structures of many macromolecules complexed with small molecule agonists, an-
tagonsit, or modulators have been published in near-atomic resolutions \(^2,^9\). Although single particle
cryo-EM often lags behind crystallography in resolutions, it was expected to open a new phase in
structure-based drug design, a process that has been successful with high-resolution crystal struc-
tures and fragment-based drug design. Although truly atomic resolutions (~1.2 Å) for high-sym-
metry complexes were achieved recently \(^10,^11\), most of cryo-EM structures of low- or no-symmetry
complexes are resolved to 2.5 to 4.5 Å. With such developments, the potential of cryo-EM struc-
tures for structure-based drug design became quite promising as proposed by multiple groups, and
is tantalizing for many important biological or pharmacological targets; but it is still yet to be fully
materialized, in part because most cryo-EM structures (>80%) are not resolved to 1.0 - 2.5 Å,
where crystal structure-based drug design has been successfully \(^2\). With development of advanced
computational tools for analysis of ligand configurations (poses) \(^12,^13\), it became interesting to test
the combination of virtual screening, molecular docking and energy minimization of ligands with
near-atomic resolution cryo-EM structures to enhance ligand potency. We started this direction in
2015 and selected human protein arginine methyltransferase 5 (PRMT5) as our first target.

PRMT5 is an important epigenetic enzyme \(^14-^16\) because of its critical roles in regulating gene
transcription \(^17\), cell signaling \(^18\), RNA splicing \(^19\), DNA repair \(^20\), chromatin remodeling \(^21\) and cell
cycle \(^22\). It is overexpressed in different types of human cancer \(^23-^28\), and its inhibition causes tumor
suppression or cell death \(^{29,30}\), especially in cancer cells lacking methylthioadenosine phosphorylase (MTAP) activity. MTAP deletion occurs in ~15% of all human cancers and increases significantly vulnerability of cancer cells to PRMT5 inhibition \(^{31-33}\). PRMT5 is therefore a compelling target for drug discovery in cancer therapy \(^{34,35}\).

As the only type II PRMT enzyme well studied so far, PRMT5 is responsible for catalyzing symmetric arginine dimethylation of histone proteins (e.g., H4R3, H3R8) \(^{36}\) and a wide array of non-histone proteins, such as p53 and NF-κB \(^{37,38}\). It utilizes S-adenosyl-L-methionine (SAM) as a cofactor to donate a methyl group and catalyzes formation of an ω-N\(^G\)-monomethyl arginine (MMA), yielding an S-adenosyl-L-homocysteine (SAH) as a by-product. A second SAM molecule is consumed to generate ω-N\(^{G,G}\)-symmetric dimethylarginine (SDMA) as the final product in a distributive fashion \(^{39-41}\). The enzymatic activity of PRMT5 requires a crucial partner named methylosome associated protein 50 (MEP50) \(^{36,42}\), which dramatically augments its activity via increased capability of substrate recognition and presentation \(^{43,44}\). Structural studies of PRMT5 from \(C.\ elegans\), \(X.\ laevis\), and \(H.\ sapiens\) \(^{39,43,45}\) all revealed a conserved triosephosphate isomerase (TIM) barrel at the N-terminal half, a Rossmann-fold domain for cofactor binding and a β-sandwich domain at C-terminal part for substrate binding and dimerization. MEP50, a 7-WD40 repeat β-propeller protein, binds PRMT5 via contacts with the TIM barrel domain, constituting a hetero-octameric complex with four PRMT5:MEP50 heterodimers arranged in a head-to-tail fashion of an apparent D2 symmetry \(^{39}\). The octamer may form larger complexes with other binding partners, such as COPR5 \(^{46}\), SWI/SNF \(^{47}\), pICln \(^{48}\), Riok1 \(^{49}\), Menin \(^{50}\), etc., in order to methylate a broad spectrum of substrates \(^{39}\). Suppressing the elevated PRMT5 activity specifically in cancer cells thus constitutes a potential treatment.
Currently, all PRMT5-targeting small molecule inhibitors fall into three classes based on their sites of action. The first class is cofactor-site competitive inhibitors, which are SAM analogs whose ribose and adenine moieties are strongly favored by the Rossmann-fold domain of PRMT5. The second class is substrate-site competitive inhibitors with high potency and selectivity. The third class is allosteric modulators, which interfere indirectly with PRMT5’s canonical binding sites. Many PRMT5 inhibitors were discovered and designed in the past years. Two molecules, JNJ-64619178 of the first class and GSK-3326595 of the second class, have entered clinic trials for multiple cancer types since 2018. A new molecule, MRTX9768 (from a conference report), had some advantages than these two in preclinical studies (unpublished). Moreover, a nucleoside-based covalent inhibitor could attack a unique cysteine residue (Cys449) to form a covalent adduct in the SAM-binding site, but it faces significant difficulty in achieving high specificity among SAM-binding enzymes. On the other hand, although class 2 inhibitors work differently from the class 1, their function relies on SAM or its analogs, but may not work well in the presence of 5’-methylthioadenosine (MTA). MTAP-/- cancer cells accumulate MTA by >10 folds in cytosol. MTA competes with SAM and reduces the efficacy of many SAM-dependent class 2 inhibitors. For the broad spectrum MTAP-/- cancers, class 2 inhibitors working synergistically with MTA are desired to suppress or kill them specifically.

Available structural data are insufficient for understanding potential interactions between a substrate-site inhibitor and MTA. The crystal structure (PDB: 3UA4) of the apo C. elegans PRMT5: MEP50 complex determined a decade ago differs significantly from that of the human complex, whereas the apo human complex has been resistant to crystallization. The first co-crystal structure
of the human complex with a SAM analog A9145C and a histone H4 substrate peptide (PDB: 4GQB) reported in 2012 was a milestone and triggered competition in crystal structure-based drug design. 39 However, all published crystal structures of human PRMT5 were obtained in complex with one or more ligands 37 and did not reveal the basis for their lack of synergy with MTA.

The need for PMRT5 inhibitors that work more potently with elevated MTA levels (called MTA-synergistic inhibitors) in cancer cells asks for a new strategy. Although chemical screen against the PRMT5/MTA is a conventional strategy that may provide a practical, yet costly, approach (unpublished data from Mirati Therapeutics) 55, we asked whether the combination of near-atomic resolution cryo-EM structures with computational analysis of compound poses might constitute a different and less expensive approach that would allow us to search and select the right (or most probable) pose of a target compound and use it to guide the design of high-potency binders. The first cryo-EM structure of PRMT5:MEP50 in complex with a cofactor analog (dehydro-sinefungin) was solved at a 3.7 Å resolution in 2018 58, which would be insufficient. We reasoned that structures of near 3.0 Å resolutions may have sufficient structural constraints for large ligands and allow proper docking and selection among possible poses optimized by computational analysis 2,9. The top candidate(s) will be accurate enough for structure-based redesign and reselection, ultimately leading to high potency binders. In this paper, we applied this strategy to a new PRMT5 inhibitor initially discovered by virtual screen, and were able to determine its pose with high accuracy, unravel the chemical basis underlying its synergy with MTA, design and reselect new compounds, and synthesize one of the designed compounds to confirm its higher potency. Our results suggest that this non-crystallographic strategy works, and can be applied more broadly for cryo-EM structure-based molecular therapeutics.
RESULTS

Virtual screening leads to selective inhibitors of PRMT5

Pharmacophores differing from a reported inhibitor (EPZ015666) the Cambridge database were used to screen against PRMT5 and identify a new pharmacophore with comparatively better binding efficiency. The resulting pharmacophore was modified manually based on the crystal structure of the catalytic pocket to increase non-covalent interactions between the inhibitor and the residues lining the binding pocket, and was synthesized in the lab (Fig. 1A). The manual optimization of the molecular design was guided by structure-activity relationship (SAR) and docking of the resulting compound in the PRMT5 catalytic pocket. Compounds with a better binding network were synthesized and tested for in vitro inhibition. Recombinant enzyme (PRMT5:MEP50) was expressed on sf9 cells and purified to biochemical homogeneity (Supplementary Fig. S1). The compounds at varying concentrations were incubated with 100 nM enzyme, 2.0 µM H4-histone peptide substrates and 10 µM SAM. The inhibition of enzyme activity (methylation of H4 peptide) was monitored by measuring luminescence from the MTase-Glow detection reagent. One of the compounds, 11-2F, inhibited PRMT5 strongly (IC\textsubscript{50} = 0.73 ± 0.2 µM). Surface plasma resonance (SPR) studies detected that 11-2F binds to the PRMT5:MEP50 complex with an apparent affinity of 13.6 µM without MTA, but its affinity increased drastically to ~82 nM in the presence of 25 µM MTA (Fig. 1C). How to increase the potency of 11-2F further is a challenging question. Because the 11-2F-bound PRMT5: MEP50 complex could not be crystalized well, it made a good candidate for
obtaining near-atomic resolution cryo-EM-structures and testing the proposed drug-design strategy by selecting compound poses from computation analysis.

A 3.1 Å structure of 11-2F-bound human PRMT5:MEP50 by single particle cryo-EM

The PRMT5:MEP50: MTA:11-2F complex was prepared by mixing 500 µM of MTA and 100 µM of 11-2F and 0.4 mg/ml PRMT5:MEP50 (~0.09 µM) and was incubated on ice for 30 min before being applied to glow-discharged QuantiFoil grids. Movies collected in a Titan Krios/K3 system and were processed as outlined in **Supplementary Fig. S2**, yielding a cryo-EM reconstruction (Coulombic potential map) at a nominal resolution of ~3.1 Å based on a Fourier Shell Correlation (FSC) threshold of 0.143 (**Fig. 2; Supplementary Table 1**). The estimated resolution of the cryo-EM map is in good agreement with the level of visible structural details (**Figures 2A-D**). Local resolutions estimated by ResMap vary in 2.4 – 4.1 Å (**Fig. 2B**). After an X-ray structure (PDB: 6CKC) was modeled and fitted into the cryo-EM map by real-space refinement (**Fig. 2A**), there were clear structural differences between the cryo-EM-map-based model (green) and the crystal structure (orange; **Fig. 2C**). **Fig. 2D** shows the clearly resolved densities corresponding to side chains within two neighboring α-helices. More differences occur within the MEP50 domain at the periphery of the complex (**Fig. 2C**), where all β-sheets were well resolved (**Fig. 2A**). Because MEP50 will not play a significant role in our structure-based analysis of the compound, we will not discuss these structural differences further.

To our satisfaction, the 3.1 Å cryo-EM map clearly shows the density expected for an inhibitor that binds inside the catalytic site of PRMT5 (**Fig. 2E**, where the ligand model is not optimized yet). The fact that the shape of the density can be accounted for by the inhibitor (stick model in
Fig. 2E) suggests that the cryo-EM map at ~3.0 Å probably be suitable for modeling more accurately the binding pose of the compound. In addition, the cryo-EM map shows clear density for MTA, which is smaller than 11-2F (Fig. 3A), but accounts for the density in the cofactor-binding pocket quite well. These structural features in the cryo-EM Coulombic potential map triggered us to test if it is feasible to perform accurate modeling by molecular docking and energy minimization, pose selection, and structure-based design of 11-2F at ~3.0 Å cryo-EM resolutions.

### Feasibility of pose selection at the binding sites

As a positive control for the proposed strategy, we first analyzed the MTA-binding site because we have a crystal structure to check the quality of our results. But cryo-EM maps at 3.0 – 4.0 Å resolutions may have significant uncertainty for small ligands. We first asked whether the cryo-EM density corresponding to MTA is sufficiently good to distinguish its right pose among various possible ones. Different poses of MTA can be generated by software packages for molecular docking, and be ranked relative to each other by minimized binding energy. If the selection of the binding pose based on the cryo-EM map is accurate, the resulted pose is expected to be very similar, if not identical, to that determined by X-ray crystallography at a higher reported resolution.

We first generated 25 million random poses of MTA and used AUTODOCK to introduce rotational freedom around all rotatable bonds and find the one of the lowest binding energy in each run. AUTODOCK clustered these energy-minimized poses for 2,000 runs internally based on a RMSD threshold (Supplementary Fig. S3-1; Supplementary Table S3). The top pose in the 1st cluster represented closely 89% of the 2,000 poses from random starting configurations, suggesting that it was heavily favored. The mean binding energy of the first cluster is significantly lower than the second one. Structural comparison found that the top pose of the first cluster differs
significantly from the ones from other clusters (Supplementary Fig. S3-1). When the top 3 poses from the first cluster were compared with the cryo-EM density, the top one (colored green in Fig. 3B) matches better with the cryo-EM density. When we compared the top pose with the MTA structural model determined by X-ray crystallography (yellow vs. cyan, Fig. 3C) and found that the two overlapped fairly well by using the same set of H-bonds for their binding. These results suggested that the AUTODOCK-optimized top poses selected against the cryo-EM density at ~3.1 Å can lead to an accurate binding pose for a compound as small as MTA.

As more positive controls of our protocol in docking analysis, we ran AUTODOCK to generate top poses of SAH (Supplementary Fig. S3-2; Supplementary Table S4), and SAM (Supplementary Fig. S3-3; Supplementary Table S5) based on the known X-ray structural models, and found that the top pose for each agrees well with the respective X-ray model, except minor differences at flexible tail regions (Fig. 3D and 3E). These comparisons support our general strategy and argue strongly that the predictions from ligand docking and energy minimization in AUTODOCK are relatively accurate for ring-containing compounds, and can be further improved when cryo-EM densities of sufficient resolution are available to constrain them.

With the strategy tested positively at the MTA-binding site, we then applied it to 11-2F in the substrate-binding site. The top pose from AUTODOCK (Supplementary Fig. S3-4; Supplementary Table S5) was compared with the cryo-EM map (Fig. 3F), and then refined against the density by all atomistic molecular dynamics calculations (Figs 3G & 3H). It is very close to the final refined model (yellow vs. green, Fig. 3G) with only one rotation of the quinoline group by 180 degrees after refinement, suggesting that the cryo-EM map of 3.1 Å contains sufficient structural
features of the ligand for selecting and refining the top pose generated by computational analysis (Fig. 3H), leading to an accurate model of the ligand, even though individual atoms in two multi-member rings of the quinoline are resolved in the cryo-EM maps.

As a control, we also tested whether different software packages would generate similar or the same top poses for the same compound against the same structural model of the binding pocket. We compared MTA and 11-2F in their respective binding pockets of the cryo-EM structural model of PRMT5 using AUTODOCK, AutoDock Vina and SwissDock. The top poses from the three were very similar to each other (Figs 3I & 3J), probably because of the same chemical constraints for their respective energy minimization processes. Our data so far showcase that the poses generated from computational analysis can be selected and refined against a cryo-EM density of the ligands at ~3.1Å resolution, producing a ligand-binding model that is fairly accurate.

**Structure of the apo human PRMT5:MEP50 complex**

Comparison of the 11-2F/MAT-bound cryo-EM structure with the known X-ray structures containing other ligands suggests that the flexible loop deduced from the structure of the *C. elegans* PRMT5 might be induced into an ordered state and contribute to the compound-binding pocket from the periphery. The flexible loop thus makes an important part for the 11-2F binding pocket, and could be used for structure-based drug design. Moreover, a structure of the apo complex would help verify that the densities assigned to MTA and 11-2F are real. Using a similar procedure, we obtained a 3.2 Å cryo-EM structure of the human PRMT5:MEP50 complex without any ligands (Supplementary Fig. S4, and Fig. 4A). Parameters for data processing and molecular modeling are given in Supplementary Table 1. The local resolutions of the map vary (Fig. 4B), and the
structural model fits the density well after real-space refinement (Fig. 4C), except the flexible loop region (Fig. 4E). As expected, no clear density in the two binding pockets for MTA and the substrate are visible even at a lower threshold level. There is very low density corresponding to most parts of the flexible loop, whereas the density corresponding to the loop was fairly strong in the map of the 11-2F bound complex (yellow, Fig. 4F). In the atomic model for the apo complex, residues 292–294, 304–307, and 312–329 were thus omitted. The modeling of the leftover residues also harbors a high level of uncertainty.

Since in the apo state, both MTA and 11-2F-binding pockets are empty, we compared the volume changes of the two pockets between the two cryo-EM structures. The MTA-binding pocket in the apo state is roughly 28% smaller in the estimated volume than that in the MTA/11-2F bound state. The putative substrate-binding pocket in the apo state is larger because the flexible loop is disordered, leaving an open end. Such differences tell two important points. 1) MTA-binding induces a change in the binding pocket, probably due to induced fit. 2) The substrate-binding pocket in the apo state has a large volume so that a substrate or an inhibitor (11-2F) has significant freedom in testing different poses before becoming securely bound with the flexible loop making part of the pocket (Fig. 4F).

**Structural basis for synergy between MTA and 11-2F**

Our data in Figs 1B and 1C suggest positive cooperativity between MTA and 11-2F. Because it would be ideal to maintain this cooperativity when 11-2F is redesigned, we would like to understand its structural underpinnings. To do that, we aligned the MTA/11-2F-bound cryo-EM structural model with the MTA/H4-bound X-ray model in their N-terminal TIM barrel domains (bottom
part in Fig 5A), and then compare the two catalytic domains. It is obvious that the whole cofactor-binding pocket is pushed upwards by ~2.0 Å when 11-2F is present in the substrate-binding site (Fig. 5B). Published data showed that MTA and H4 peptide had no positive cooperativity, suggesting that the shift of the catalytic domain (Fig. 5B) is probably the root cause for the positive co-operativity between 11-2F and MTA. Fig. 5C shows the shift of MTA together with the walls of its binding pocket as if 11-2F-binding pushes against the cofactor-binding pocket and makes it favor MTA binding. Such a physical shift retains the key residues that stabilize MTA. Inversely, it is equally probable that the shift induced by MTA-binding favors structural changes of the substrate binding pocket for 11-2F to bind into the groove between the β-barrel domain and the Rossman fold. The interactions are therefore mutual, and contribute to the positive cooperativity.

This synergistic mechanism for MTA and 11-2F appears different from the positive cooperativity between SAM and H4 peptide (Fig. 5B) or between SAM and JNJ inhibitor (EPZ015666 in Fig. 5E) because the longer tail of SAM or its analogs (e.g. LLY283 in Fig. 5D) does not favor the shift of the co-factor binding pocket. Alternatively, the physical shift for MTA-binding (Fig. 5C) does not favor the binding of the H4-peptide substrate or the JNJ inhibitor so that they do not show positive cooperativity with MTA. From this line of thinking, a guiding principle for redesigning 11-2F would be to preserve the interactions between the quinoline of 11-2F and residues in the binding pocket, including Glu435, Glu444, Phe327, Trp579, etc., in order to retain the positive cooperativity with MTA.

**Predicted subtype specificity of 11-2F among PRMTs**

With the top poses predicted by computational analysis being very close to the final binding pose
refined against the cryo-EM density (Fig. 3G), it was tempting to ask whether the same analysis of 11-2F among available structural models of six other PRMT proteins would reveal something unique for PRMT5 (Fig. 6). We first identified key residues coordinating 11-2F in the substrate-binding pocket of the PRMT5 that are conserved among PRMTs (Supplementary Fig. S5). Carboxyl oxygen of Asp419 shows a strong interaction (O - N = 2.3 Å) with the nitrogen of the amine group in the quinoline ring. The aromatic ring of quinoline is stacked with those in the sidechains of Phe 327 and Trp579. All residues in the binding pocket, such as Leu312, Thr323, Phe327, Leu336, Gly365, Gly367, Lys393, Glu435 and Ser578, etc., participate in the coordination of the inhibitor by non-covalent interactions (Fig. 6E). The nitrogen atom of the 2-amine group on the quinoline ring and its nitrogen at position 1 form strong H-bonds with catalytic residues Glu 444 & Glu 435 with N---O distances of 2.41Å and 2.86Å, respectively. Other residues, including Gln322, Pro311, Lys 333, Leu437, Val503, Leu312 and Ser310, etc., act in a similar fashion (Figure 6E). Comparison of the crystal structure of EPZ015666-bound PRMT5:MEP50 with our 11-2F-bound cryo-EM structure shows that despite small differences in protein-inhibitor interactions for 11-2F and EPZ015666, PRMT5 takes similar conformational states in the presence of the two. This analysis suggests that it might be feasible to introduce extra interacting groups to 11-2F and enhance its potency.

We used the same docking methods to predict the top poses of 11-2F in the binding pockets of PRMT1-4, and 6-7 (Figs 6A-D, F-G). Alignment of the primary sequences of PRMT1-7 in Supplementary Fig. S5 shows the key residues for ligand-protein interactions are conserved in the substate-binding pockets. Based on the top poses, the binding pockets in PRMT1, PRMT3, PRMT6 and PRMT7 clearly cannot accommodate the quinoline ring in the right position for the
two catalytic Glu residues to interact. The PRMT2 is pretty poor because the alkylated indole ring of 11-2F has very limited interactions with its binding pocket. The main reason appears that the PRMT2 substrate-binding pocket is fairly shallow and cannot accommodate the two parts of 11-2F completely, making its docking energy fairly high (Fig. 6H). PRMT4 is probably the only one that might have a relatively good binding affinity because its Glu266 interacts with the 2-amine on the quinoline ring and its Tyr154 hydroxyl interacts with the N-atom inside the quinoline ring. Other interactions next to the alkylated indole ring help stabilize the tail part of 11-2F (Fig. 6D). The resulted docking energy in PRMT4 agrees with the predicted interactions in the binding pocket (Fig. 6H). These analyses predict that 11-2F is able to differentiate PRMT5 from other PRMTs due to the chemical differences among their binding pockets. It will be interesting to test if mutations in the binding pockets of PRMT4 can enhance 11-2F-binding.

**Structure-based design of 11-2F for higher potency**

The above analysis highlighted three principles that could be considered to enhance the binding affinity of 11-2F analogs to PRMT5. 1) It is important to retain the quinoline ring backbone to maintain the positive cooperativity with MTA. 2) The pi-stacking interactions of Trp579 and Phe327 with the quinoline ring could be enhanced for keeping the inhibitor properly oriented, which could be achieved by introducing small groups (-F, or -CH3, or -NH3+) to the ring. 3) The alkylated indole ring in the tail part of 11-2F is relatively flexible, and could be stabilized by introducing H-bonds or electrostatic interactions with the binding pocket, especially with the residues on the flexible loop. We used these principles to guide the design of dozens of different 11-2F derivatives, and utilized computational analysis to predict their most stable poses before ranking them and selecting the most potent ones. Three 11-2F analogs predicted to have higher potency
introduce more interactions with the binding pockets (Table 1; Supplementary Figs S6 & S7; Supplementary Tables S6 & S7). From the constraints in the cryo-EM model (Fig. 6E), the quinazoline-corresponding parts of these compounds are in almost the same place and orientation as the quinoline of 11-2F. The predicted binding affinity for 11-9F is ~18 nM, and ~1.0 nM for HWIem2104 and 2109 in a similar pose (Table 1, right column). In the binding pocket, the 2,4-di-NH$_2$-quinazoline of 11-9F forms H-bonds with E444, E435, and S439, and its aromatic ring is sandwiched between the sidechains of Phe327 and Trp579. The backbone amino group of F580 also contributes to its binding. When 11-9F was synthesized and assayed, it inhibits PRMT5 enzyme activity 4-5 folds more potently than 11-2F does (Fig. 7E vs. Fig. 1A; more details in a manuscript being prepared by X. Yang, et al.), in good agreements with predictions of the docking analysis and energy minimization (Fig. 7D).

Similarly, the other two compounds (HWIem2104 and HWIem2109) were picked out of different virtual designs, and their top poses are presented in Fig. 7B and 7C, showing the extra interactions of their tail portions with the residues on the flexible loop (Residues 292 – 329) when the added 4-member ring interacts with Thr323 hydroxyl and the carbonyl group in the middle linker region interacts with Ser310. For HWIem2109, AUTODOCK Vina predicted its top pose close to that of 11-2F (Fig. 3F vs. 7C; Supplementary Fig. S6 and Supplementary Table S6). It contains an -NH- in the 6-member alkyl ring fused to the indole ring, which introduces an extra H-bond with Ser 310, albeit it was predicted to have the same potency as HWIem2104 (Fig. 7D). Given that the quinazoline ring in both compounds is constrained as 11-2F, we expect that the high potency for HWIem2104 and HWIdm2109 predicted by computational analysis is very likely a good indicator of successful increase in potency, which still awaits testing after chemical synthesis.
DISCUSSIONS

Near-atomic resolution cryo-EM structures and computational analysis for design of high-potency binders.

We demonstrated that a strategy to redesign and improve potency of an inhibitor for an oncogenic target, PRMT5, by combining a cryo-EM structure with computational analysis of compound poses. Currently, near-atomic resolution structures by single particle cryo-EM are often in the resolution range of 3.0 – 4.5 Å, which were proposed to be useful for structure-based drug design by multiple investigators recently \(^1,2,9,63,64\). Our results showed that a 3.1 Å cryo-EM map of the MTA/11-2F-bound PRMT5/MEP50 complex contains densities in the two ligand-binding pockets with clear shapes that enable the selection of top poses delivered by computational analysis through molecular docking and energy minimization. A key step is the separation of the densities of the two ligands from that of the protein so that the refinement of the protein model does not eclipse the optimization of the ligand models during the refinement. At ~3.1 Å resolutions, two-fused rings (10-atoms) of MTA is sizeable enough for accurate modeling of its binding. The arrangement of the binding pocket residues and the accurate positioning of the adenine ring in MTA or the quinoline ring in 11-2F are important for computational analysis to gain sufficient sensitivity in differentiating the docking energy on a relatively scale and allow the top cluster of compound poses to approximate the true solutions with good accuracy. For 11-2F, the density corresponding to the alkylated indole rings is another determinant for its accurate position. Different software packages for molecular docking generate similar top poses, although their relative energetic levels
vary, probably because of the same stereo chemistry parameters used for proteins and ligands. Expectedly the results will probably be even better with cryo-EM maps of 2.0 - 3.0 Å in resolution, whereby individual atoms of certain multi-member rings in ligands and some of the water molecules at the binding pockets will become recognizable.

The strategies we tested above appear to work well for the redesign of 11-2F based on accurate modeling of the protein-ligand interactions and the computational analysis to select three different ways out of virtual modifications to improve binding affinity. One of them, 11-9F, did show significantly better potency in enzyme inhibition assays, close to the predicted enhancement based on relative binding energy (Table 1; Fig. 7E). Because of the preserved head part (quinazoline) of the compounds, HWIem2104 and HWIem2109 are very probably going to follow the predictions and show even higher potency than 11-9F, even though they still need to be synthesized for experimental tests.

**Catalytic mechanism of PRMT5 and the MTA-inhibitor cooperativity**

Understanding of the catalytic mechanism of PRMT5 may also be helpful in development of potent inhibitors. So far, all well-characterized PRMT5 inhibitors in published studies require the nucleoside component of the cofactor SAM, indicating that they are either SAM analogs or SAM-dependent molecules. Our work demonstrated that the cofactor binding induces a conformational change of the loop region (residues 292–329) by forming a small helix $\alpha_1$ (residues 320–329) which stabilizes the nucleoside via Tyr324, followed by the formation of a “lid” (residues 314–319) and a substrate-binding tunnel (residues 295–313), where Phe327 sandwiches the substrate.
ring against Trp579. These provide a physical connection to achieve positive cooperativity between MTA in the cofactor binding site and the inhibitor at the substrate-binding site. Foreseeably, different SAM analogs other than MTA might be developed to enhance the potency of the compound inhibitor, especially for those MTAP+/+ cancer cells. Given that the MTA and 11-2F binding sites are next to each other, a chimera compound harboring the key groups of the two may be prepared for the same purpose.

Our work demonstrated the feasibility of the proposed strategy in using cryo-EM structures of ~3.0 Å resolutions and computational analysis of compound poses for drug design. It also led to the development of a novel class of substrate-competitive inhibitors that preferentially bind the PRMT5:MEP50 complex with MTA accumulation and may be used to pharmacologically exploit the PRMT5-related vulnerability in MTAP-/- cancer cells. We expect that the same or similar strategy be applicable to other cryo-EM maps of similar resolutions for drug design, which will expand our experimental capacity in developing new molecular therapeutics.

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Author Contributions

C.L. and Q.X.J. initiated the project. Q.X.J designed major steps of the studies and discussed with the rest of the group to implement them. G.P.Y. and W.Z. contributed to cryo-EM grid preparation, grid screening, data collection and analysis as well as manuscript preparation. W.Z. conducted protein expression and purification, enzymatic assay and SPR binding assay. X.Y. performed compound design and synthesis and purification. C.L. helped Q.X.J. to supervise the project and participated in the revision of the manuscript. Q.X.J. supervised all parts for cryo-EM, including grid preparation and evaluation, data collection, data analysis, model building, computational analysis, and molecular interpretation. Q.X.J. wrote the manuscript based on earlier writings by G.P.Y and W.Z. and conducted revisions together with the rest of the group.

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Notes
The authors declare no competing financial interest. The manuscript was written by W.Z. G.Y. and Q.X.J, and revised by Q.X.J. with input from all authors. All authors have given approval to the final version of the manuscript.

Data availability
Cryo-EM density maps of the apo and 11-2F-bound forms of human PRMT5:MEP50 have been deposited in the EM data bank under the accession codes of EMD-20764 and ***, respectively. Atomic coordinates for the molecular models have been deposited in the protein data bank under accession code PDB ID 6UGH and ****, respectively.

Acknowledgements are available online.

Supplementary information is available online.

Methods and Materials (details available online):
Expression, purification, and characterization of PRMT5:MEP50 complex.
Preparation of purified PRMT5:MEP50 complex was described in literature 39. Details are available in the Supplementary Information. Protein samples were concentrated to 12 mg/mL in a buffer containing 10 mM HEPES at pH 7.5, 150 mM NaCl, 10% (vol/vol) glycerol and 2.0 mM DTT.
Enzymatic inhibition assay

Enzymatic inhibition activity of inhibitors was determined by a MTase-Glo™ methyltransferase assay (Promega Corporation, V7602). Details are presented in the Supplementary Information online.

Surface plasmon resonance (SPR) binding study

Binding affinity measurements were conducted in a Reichert2SPR system (Ametek) at 25 °C. Details in experimental setup, data collection and analysis are available online.

Cryo-EM grid preparation, data collection and analysis

Details are available online. Grids were prepared in a Vitrobot. Data were collected at both Florida State University (NIH SEM4 consortium) and National Cancer Institute’s National Cryo-EM Facility (NCEF). A general protocol was used for data analysis with details specified in the Supplementary Information.

3D model building and refinement

The crystal structure of an inhibitor-bound (LLY-283) form of PRMT5:MEP50 dimer (PDB: 6CKC) was used as the initial molecular model and docked into the cryo-EM map in Chimera. The model was subjected to real-space refinement in PHENIX \(^{65}\) with secondary structure and geometry restraints, and manually adjusted in COOT \(^{66}\). A molecular dynamics (MD)-based optimization was performed using ISOLDE \(^{67}\) with the technical assistance by Dr. Tristan Croll (CCPEM) \(^{68}\). Overfitting and overinterpretation of the model were monitored by refining the model against one of the two independent half-maps and testing the refined model against the other half-
Cryo-EM data collection and modeling statistics are presented in Supplementary Tables S1.

**Computational analysis of the compounds in the binding pockets of PRMT5.**

**a. Docking analysis using AUTODOCK**

The initial model of a ligand was generated in Chimera and energy-minimized. The model is then docked to overlap with the ligand density in the cryo-EM map, which served as a starting point. The refined protein model based on the cryo-EM map (without the ligands) was used for docking of different inhibitors in AutoDock 4. Top poses from 2,000 runs were clustered into discrete groups using a Root Mean Square Distance (RMSD) threshold. Usually for each ligand we obtained 8-10 clusters and used the pose with the lowest energy from each cluster as its representative one. The top pose means the lowest docking energy ($\Delta G$) among a large number (billions or more) of different configurations.

**b. Comparison of results from three different software packages.**

Similarly, the same scheme was implemented in AutoDock Vina and an online server, SwissDock. The top poses for the same ligand from three different software packages against the same protein structural model were very similar, if not the same, suggesting that the large number of starting positions sampled by this protocol were able to reflect almost all possible configurations of the ligand within a small range of errors.

**c. Refinement of the top pose against the cryo-EM density of the ligand.**

The refinement of the top pose of a ligand was performed by real-space all-atomistic molecular dynamics (MD) calculations implemented in ISOLDE. The refinement may slightly improve the model for better match with the density. For MTA, the refinement introduced small changes of the
ligand model. For 11-2F, the refinement led to a 180° flipping of the quinoline ring around a C-C bond connected to its 6-position.

Figure legends:

**Fig. 1 An inhibitor 11-2F of PRMT5 exhibits positive cooperativity with MTA**

A). Dose-dependent inhibition of enzyme activity by 11-2F. IC50 ~ 730 nM. Errors: s.d., n=3. The chemical structure of 11-2F is showed on the right.  B). SPR of 11-2F binding and unbinding to PRMT5:MEP50 in the absence of MTA, leading to a calculated $K_D$ ~13.6 µM. C). SPR of 11-2F interaction with the enzyme in the presence of MTA. $K_D$ ~82 nM. The apparent positive coupling coefficient between 11-2F and MTA is ~ 166.

**Fig. 2 Cryo-EM structure of the 11-2F-bound PRMT5:MEP50 complex.**

A). A 3.1 Å cryo-EM map of the complex in two different orientations with the atomic model build in the density (PRMT5 in pink and MEP50 in red).  B). Local resolutions of the map estimated by ResMap. C). Comparison of the cryo-EM model (green PRMT5 and yellow MEP50) with the X-ray structure (orange; PDB: 6CKC).  D). Matching of the side chains in two short helices of the cryo-EM model with the density (blue mesh).  E). Density for the inhibitor is well defined in the cryo-EM map. The models of the protein and the inhibitor are in pink.

**Fig. 3 New strategy to use cryo-EM density for selecting top poses of ligands from molecular docking and energy minimization.**
A). Density in the cryo-EM map for MTA. B). Overlay of top three poses from the AUTODOCK analysis with the cryo-EM density for MTA (grey). Clearly the top pose in green fits the density better. C). After refinement, the top pose from the cryo-EM model is almost in exactly the same position as that from the X-ray structure (cyan). D). Top pose of SAH out of AUTODOCK agrees with the model from the crystal structure (cyan). E). Top pose of SAM from AUTODOCK analysis overlaps very closely with the one in the crystal structure (cyan). F). Top three poses of 11-2F out of AUTODOCK analysis overlap relatively well with the cryo-EM map with the tails not well accounted for, which was improved after real-space refinement against the cryo-EM density. G/H). After cryo-EM-density based refinement, the top pose (green) of 11-2F from docking analysis changes slightly with the quinoline ring rotated by 180 degrees around a rotatable bond (in yellow). All-atomistic MD refinement enhances the overlapping of the model (green) with the ligand density (H). I). Comparison of top poses of MTA from three different software packages (AUTODOCK, SwissDock, and AutoDock Vina). J). Top poses of 11-2F from three different docking packages are nearly identical.

Fig. 4  Cryo-EM structure of the apo human PRMT5:MEP50 reveals the disorder of the flexible loop.
A). Cryo-EM map of ~3.2 Å in resolution. B). Local resolutions estimated by ResMap. C). The molecular model. D). FSC for different maps. The corrected map at 3.2 Å was used for modeling. E). The density map with the model for the ligand-bound complex (orange) highlights the disordered loop region not in the density. F). The flexible loop contributes to the binding pocket of the bound ligand at its periphery.
Fig. 5 Structural changes underlying the synergy between 11-2F and MTA.
A). After alignment of the TIM domains (bottom) between the cryo-EM model and the crystal structure of the MTA/H4 bound complex. The ligand-bound domains of the cryo-EM model is shifted upwards, especially in the MTA-binding pocket (red square to be zoomed in B). B) The region marked by the red-square in A is magnified in a stereo view. The red-square marks the MTA-binding pocket. The flexible loop region is also shifted upward. C) MAT-binding pocket is zoomed in and showed in a stereo view. Movement of the MTA-binding pocket in the cryo-EM model with 11-2F bound happens relative to the X-ray model with the H4-peptide substrate (PDB: 5FA5). The push from the 11-2F-binding makes the co-factor binding pocket better for MTA binding. D). The shift of the MTA-binding pocket did not happen in the LLY283-bound structure (magenta). LLY283 is a SAM-analog (PDB: 6CKC). E). The co-factor binding pocket did not shift in the structure of the EPZ/SAM complex (magenta) (PDB: 4X61).

Fig. 6 Computational analysis of 11-2F in structures of different PRMT subtypes suggests subfamily specificity.
A). Top pose of 11-2F in PRMT1; PDB: 6NT7 (A), PRMT2; PDB: 5FUL (B), PRMT3; PDB: 4QQN (C), PRMT4; PDB: 3B3J (D), PRMT5 (E), PRMT6; PDB: 3C05 (F), PRMT7; PDB: 4C4A (G). Some of the key residues contributing to the interactions with the inhibitor are labeled for each PRMT. H). Relatively binding energy of 11-2F to PRMT1-7.

Fig. 7 Cryo-EM structure-based design yields 11-2F analogs of higher potency
Three different compounds were selected based on the docking analysis. 11-9F (A) and other two
HWIem2104 (B) and HWIem2109 (C) are showed in the binding pockets with key residues contributing to their stability.  D). Comparison of relative docking free energy among the four compounds.  E). The chemical structure of 11-9F (left) and its dose-dependent inhibitor of PRMT5: MEP50 enzyme activity, yielding an IC50 ~ 180 nM.

Table 1. Comparison of 11-2F and its designed analog compounds based on docking calculations using the cryo-EM structural model

| Inhibitors         | Estimated binding energy (kcal/mol)(Vina) | Estimated Ki (nM) | RSMD (Å) | Some key residues in non-covalent interactions (D…H distance, Å)                                                                 |
|-------------------|-------------------------------------------|-------------------|----------|---------------------------------------------------------------------------------------------------------------------------------|
| 11-2F             | -9.89 (-10.1)                             | 55.6              | 6.91     | Glu444:OE1 … 11-2F:N (1.87)  
Glu435:OE1 … 11-2F:N (1.87)  
Ser578: HG … 11-2F:O2 (1.99)  
Lys333:HZ3 … 11-2F:O2 (1.77)  
Thr323:OG1 … 11-2F:H25 (1.87)  
Phe327:O … 11-2F: H26 (2.09) |
| 11-9F             | -10.56 (-10.6)                            | 18.1              | 1.92     | Glu444:OE1 … 11-9F: N (1.87)  
Glu435:O … 11-9F:NH (1.87)  
Ser439:OG … 11-9F:HN (2.1)  
Ser439:OH … 11-9F:O (2.1)  
Phe580:NH … 11-9F:HO (2.0) |
| HWIem2104         | -12.34 (-11.7)                            | 0.9               | 2.45     | Glu444:OE1 … 2104:N (1.87)  
Glu435:OE1 … 2104:N (1.87)  
Glu312:O … 2104:NH (1.99)  
Ser310:OH … 2104:O (1.88)  
Glu323:OE1 … 2104:N (1.87) |
| HWIem2109         | -12.21 (-11.4)                            | 1.1               | 2.34     | Glu444:OE1 … 2109:N (1.87)  
Glu435:OE1 … 2109:N (1.87)  
Glu312:O … 2109:NH (1.99)  
Ser310:OH … 2109:O (1.88)  
Glu580:OE1 … 2109:N (1.87) |
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Fig. 1

**A**

Enzymatic activity (%) vs. Log [concentration (μM)]

IC$_{50}$ = 0.73 ± 0.20 μM

**B**

PRMT5:MEP50 + 11-2F

$K_D$ = 13.6 μM

$k_{on} = 6.19 \times 10^3$ M$^{-1}$s$^{-1}$

$k_{off} = 8.44 \times 10^{-2}$ s$^{-1}$

**C**

PRMT5:MEP50 saturated with 25 μM MTA + 11-2F

$K_D = 82.1$ nM

$k_{on} = 2.46 \times 10^5$ M$^{-1}$s$^{-1}$

$k_{off} = 2.02 \times 10^{-2}$ s$^{-1}$
Fig. 2
Fig. 5

A. Cryo-EM (green) and X-ray (magenta) structures aligned in TIM barrel domains

B. Shift of MTA in stereo [cryo-EM (yellow) vs X-ray (white)]

C. Cryo-EM (green) vs LLY283 (magenta)

D. Cryo-EM vs EPZ015666 _SAM
Fig. 7
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**Detailed Methods and Materials:**

**Expression, purification, and characterization of PRMT5:MEP50 complex.**

PRMT5:MEP50 protein complex was prepared as described in Antonysamy, S., et al. Full-length human PRMT5 (residues 1–637, NP_006100) and human MEP50 (residues 2–342, NP_077007) were co-expressed in Sf9 cells using a Bac-to-Bac expression system (Invitrogen). The cells were harvested and lysed. After removal of cell debris, supernatants were collected for FLAG-affinity chromatography (Sigma; A2220) and fractionated by size exclusion chromatography in a 10/300 Superose 6 column (GE Life Sciences). The purified PRMT5:MEP50 complex was characterized by SDS-PAGE and sedimentation velocity analytical ultracentrifugation (SV-AUC) using ProteomeLab™XL-I (Beckman Coulter) ([Supplementary Figure 1](#)). Protein samples were concentrated to 12 mg/mL in a buffer containing 10 mM HEPES at pH 7.5, 150 mM NaCl, 10% (vol/vol) glycerol and 2.0 mM DTT and were stored at -80°C until the time of use.

**Enzymatic inhibition assay**

Enzymatic inhibition activity of 11-2F was determined by MTase-Glo™ methyltransferase assay (Promega Corporation, V7602). 11-2F was serial diluted by 5-fold from 500 µM to 6.4 nM in DMSO then added into reaction buffer (30 mM Tris-HCl at pH 7.4, 500 mM NaCl, 2 mM MgCl₂, 2 mM TCEP, 0.1% (wt/vol) BSA and 0.01% (vol/vol) Tween-20) with final DMSO concentration at 5% (vol/vol). The enzymatic inhibition assay is performed in a solid white low-volume 384-well plate (Greiner, #7784075) with total reaction volume of 16 µl and in the presence of 100 nM PRMT5:MEP50 enzymes, 10 µM SAM (Sigma-Aldrich, A4377), 2 µM substrate histone H4 (1-21) (ANASPEC, #AS-62499) and 11-2F at indicated concentrations. Reactions without enzyme are conducted as negative control and reactions without 11-2F are included as positive control in every experiment. Methyltransferase reaction is started by adding 4 µl of SAM/H4 substrate mixture to each well that contains 8 µl enzyme and 4 µl 11-2F which are pre-mixed and incubated for 10 min. The reaction is performed at room temperature for 60 min followed by the addition of 4 µl 5X MTase-Glo Reagent to produce SAH and concomitantly convert it to ADP. Mix the plate by shaking for 2 min, and incubate at room temperature for 30 min. Then, 20 µl room temperature MTase-Glo Detection Solution is added and mixed well before incubating for another 30 min and recording luminescence. Luminescence is measured using the Synergy Neo2 HTS multimode
microplate reader (BioTek). Each data point represents the average of three replicates; the error bars represent the standard deviation. Data are analyzed in GraphPad Prism 8. For inhibitor studies, IC$_{50}$ is determined by nonlinear regression (curve fitting) using the equation for the sigmoidal dose response (variable slope).

**Surface plasmon resonance (SPR) binding study.**

Binding affinity measurements were conducted on a Reichert2SPR instrument (Ametek) at 25 °C. PRMT5:MEP50 proteins were directly immobilized onto the 500,000 Da carboxymethyl dextran sensor chip at pH 5.5 using the standard amine coupling approach. Small molecule analyte 11-2F was injected at a flow rate of 30 µL/min at different concentrations in the running buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v Tween-20, and 5% DMSO). The association and dissociation times were set to be 1 min. In the competitive binding experiment, 25 µM MTA was added into the running buffer to saturate the cofactor-binding pocket of immobilized PRMT5:MEP50 protein before the injection of analyte. The dissociation time for 11-2F binding in MTA containing running buffer was elongated to be 3 min to achieve complete dissociation. Sensorgram data was processed using TraceDrawer software to calculate the equilibrium dissociation constant $K_D$, association rate $k_{on}$ and dissociation rate $k_{off}$ values.

**Cryo-EM grid preparation and imaging**

The frozen protein sample was thawed and diluted by 1:30 (vol/vol) with a buffer containing 10 mM HEPES at pH 7.5, 150 mM NaCl and 1.0% (vol/vol) DMSO to a final concentration of 0.4 mg/ml. Aliquots of 4.0 µL of the diluted protein were applied to plasma cleaned (Solarus, GATAN model 950 advanced plasma system) 300 mesh holey carbon-coated gold grids (Quantifoil R1.2/1.3, Electron Microscopy Sciences #Q325–AR1.3). After sample loading, the grids were incubated for 30 seconds at 100% humidity in a plunge-freezing device (Vitrobot Mark III, FEI) before being sandwich-blotted for 4.0s with a blot force of 8.0. Grids were plunge-frozen in liquid nitrogen-bathed liquid ethane and were transferred into grid-boxes and stored in liquid nitrogen before cryo-EM imaging. Grids were first screened and checked in a CM120 microscope before data collection in a Titan Krios. CryoEM grids for the compound bound form of the PRMT5:MEP50 (PRMT5:MEP50:11-2F) were prepared similar way as of apo form except the
protein was incubated with inhibitor for 30 min at 1:5 protein: inhibitor molar ratio and final protein concentration to 0.05 mg/ml prior to applying the protein solution to the grids.

**Cryo-EM data collection for apo form (PRMT5:MEP50)**

Cryo-EM data was collected at both Florida State University (NIH SEM4 consortium) and National Cancer Institute’s National Cryo-EM Facility (NCEF). A total of 1,535 micrograph movies were recorded inside a Titan Krios operated at 300 kV utilizing a Gatan K2 Direct Electron Detector (Gatan Inc.) in the super-resolution electron-counting mode at a nominal magnification of 105,000×. The calibrated pixel size under such conditions was 0.66 Å, and the defocus range was set in -0.75 to -3.0 μm. Each movie was collected in 12 s with an exposure time of 0.3 s per frame, resulting in 40 frames per movie. The total electron dose rate was approximately 40 e-/Å² per movie.

**Cryo-EM data collection for compound bound form (PRMT5:MEP50:11-2F)**

A total of 4153 micrograph movies were recorded inside a Titan Krios operated at 300 kV utilizing a Gatan K3 Direct Electron Detector (Gatan Inc.) in the super-resolution electron-counting mode at a nominal magnification of 105,000×. The calibrated pixel size under such conditions was 0.56 Å, and the defocus range was set in -0.75 to -3.0 μm. Each movie was collected in 12 s with an exposure time of 0.3 s per frame, resulting in 40 frames per movie. The total electron dose rate was approximately 40 e-/Å² per movie.

**Cryo-EM data processing for APO form (PRMT5:MEP50)**

The movie stacks were motion-corrected, dose-weighted and binned by a factor of 2 using MotionCor2², resulting in a pixel size of 1.32 Å. Parameters of contrast transfer function (CTF) for each movie were estimated by CTFFind4³. Relion3.0⁴ was utilized for particle picking, extraction, classification and refinement. LoG-based particle picking mode was utilized to select an initial set of particles as templates for the following template-based auto-picking procedure. The auto-picked 597,002 particles were subjected to three rounds of 2D classification to remove junk particles, yielding a sub-set of 151,554 particles in 3D classification. An initial de novo 3D model was generated in Relion3.0. Two rounds of 3D classification were performed to identify distinct conformational states or sub-stoichiometric assemblies of the PRMT5:MEP50 complexes.
Unbinned particles from good 3D classes exhibiting 1:1 stoichiometry and high-resolution structural features were re-extracted. A cleaned dataset of 113,466 particles were auto-refined in Relion3.0, yielding a 3.9 Å resolution structure based on the gold-standard FSC (Fourier shell correlation)\(^5\) with the 0.143 criterion with D2 symmetry imposed. The particle stack was then subjected to 2 iterations of CTF refinement and beam tilt correction, resulting in a 3.8 Å resolution structure. The resolution was improved to 3.6 Å by applying a soft mask around the entire protein complex during 3D auto-refinement. Bayesian polishing implemented in Relion3.0 and another iteration of CTF refinement further improved the final resolution to 3.4 Å. The refined particles were extracted and further refined in the cisTEM\(^6\) which slightly improved the resolution to 3.17 Å. The final map was sharpened with a soft mask with an automatically calculated B-factor of -79.7 Å\(^2\). The local resolution of the \textit{apo} form of PRMT5:MEP50 was assessed using ResMap\(^7,8\) and colored in Chimera\(^9\).

**Cryo-EM data processing for compound bound form (PRMT5:MEP50:11-2F/MTA)**

The movie stacks were motion-corrected, dose-weighted and binned by a factor of 2 using MotionCor2\(^2\), resulting in a pixel size of 1.11 Å/pixel. Parameters of contrast transfer function (CTF) for each movie were estimated by CTFFind4\(^3\) and micrographs with good CTF fitting were selected(1918). CisTEM\(^4\) was utilized for particle picking, extraction, classification and refinement. The auto-picked 866,240 particles were subjected to two rounds of 2D classification to remove junk particles, yielding a sub-set of 461,119 particles in 2D classification. Three rounds of 3D classification were performed to identify distinct conformational states or sub-stoichiometric assemblies of the PRMT5:MEP50 complexes using apo map as a reference. A cleaned dataset of 213,221 particles were auto-refined in cisTEM, yielding a 3.14 Å resolution structure based on the gold-standard FSC (Fourier shell correlation)\(^5\) with the 0.143 criterion with D2 symmetry imposed. The final map was sharpened with a soft mask with an automatically calculated B-factor of -90.0 Å\(^2\). The local resolution of the compound bound form of PRMT5:MEP50:11-2F was assessed using ResMap\(^7,8\).

**3D model building and refinement**
The crystal structure of an inhibitor-bound (LLY-283) form of PRMT5:MEP50 dimer (PDB: 6CKC) was used as the initial model and docked into the density map using chimera. The model was subjected to real-space refinement using PHENIX 10 with secondary structure and geometry restraints and manually adjusted in COOT 11. A molecular dynamics (MD)-based optimization was performed using ISOLDE 12 with the technical assistance by Dr. Tristan Croll (CCPEM) 13. Overfitting and overinterpretation of the model were monitored by refining the model against one of the two independent half-maps and testing the refined model against the other map. The final structure was assessed in MolProbity and optimized to minimize clashes 14. Cryo-EM data collection and modeling statistics are summarized in Supplementary Table 1 for the apo and inhibitor bound PRMT5 complexes.

**Molecular docking analysis**

Molecular docking was performed mainly by using AutoDock 4.2.6 15. The X-ray crystal structure of human PRMT5 in complex with a substrate and a co-factor (PDB codes: 5FA5, 4X61, & 4X63), structures of different PRMTs (PDB codes: PRMT1:6NT7, PRMT2:5FUL, PRMT3:4QQN, PRMT4:3B3J, PRMT6:3C05, PRMT7:4C4A) and the 3D structures of MTA, SAM and SAH were retrieved from the RCSB Protein Data Bank (www.rcsb.org) 16. All input files were prepared using the AutoDockTools (ADT) 1.5.4 package. To carry out the docking simulations, a 50 Å × 50 Å × 50 Å grid with a lattice spacing of 0.375 Å was defined with its center at the 11-2F position that was modelled and refined in cryo-EM map. The grid fully enclosed the catalytic center of PRMT5. The AutoGrid program was used to construct the grid maps for energy scoring. The three-dimensional locations and orientations of the various inhibitor configurations were analyzed from 25 million configurations from randomly sampled seed parameters using a Lamarckian genetic algorithm (LGA) 17, 18. After energy minimization, the best poses from 2000 runs were generated, which represent 2,000 typical configurations of the inhibitor and were grouped into clusters by a root mean square deviation (RMSD) threshold of 2.0 Å. These clusters were ranked by the relative binding energy of their best poses. The poses of the lowest energy (optimal configurations) and the cluster of the largest size (suboptimal conformation) were chosen for further analyses in the PyMOL molecular graphics system (https://pymol.org/2/). Usually at least 3-5 poses were examined.
Supplemental Figures:

Supplementary Figure S1. PRMT5:MEP50 complex purification and characterization.

(A). Size exclusion chromatography of the PRMT5:MEP50 complex after anti-FLAG affinity column. Fractions at the indicated area were collected and concentrated. (B) An SDS-PAGE gel is shown for the collected fractions of PRMT5:MEP50 with a purity greater than 95%. (C) Sedimentation velocity analytical ultracentrifugation (SV-AUC). The PRMT5:MEP50 complex has an estimated mass of 445 kDa.
Supplementary Figure S2. Procedures for processing cryo-EM data of the PRMT5: MEP50/11-2F/MTA complex. The final map has an overall estimated resolution of 3.14 Å (FSC = 0.143). The last steps of refinement were performed in cisTEM. The presentations were prepared in Chimera and PyMOL.
Supplementary Figure S3-1 Top 8 poses of MTA out of computational analysis.

For each of the 2000 runs, 25 million seed positions of the ligand were used as starting points for movements and energy minimization. The energy-minimized poses from 2000 runs were then clustered into 8 groups based on their RMSD. The best one in each cluster is showed to represent the respective cluster.
Supplementary Figure S3-2 Top 10 poses of SAH out of computational analysis.

Similar to S3-1, the same analysis was performed for SAH, leading to 10 clusters.
Supplementary Figure S3-3 Top 10 poses of SAM out of computational analysis.

Similar to S3-1, the same analysis was performed for SAM, leading to 10 clusters.

Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
Cluster 9
Cluster 10
Supplementary Figure S3-4 Top 10 poses of 11-2F out of computational analysis.

Similar to S3-1, the same analysis was performed for 11-2F, leading to 10 clusters.
Supplementary Figure S4 Procedures for processing cryo-EM data of the apo PRMT5: MEP50 data. The final map has an overall estimated resolution of 3.14 Å (FSC = 0.143). The presentations were prepared in Chimera\textsuperscript{9} and PyMOL\textsuperscript{19}. 
Supplementary Figure S5  Sequence alignment of PRMT1-7 focusing on the catalytic domains. Key residues in the catalytic units are high-lighted.
Supplementary Figure S6 Top 10 poses of HWIem2109 from computational analysis.

Similar to S3-1, the same analysis was performed for the redesigned ligand, HWIem2109, leading to 10 clusters.
Supplementary Figure S7 Top 10 poses of HWIem2104 from computational analysis.

Similar to S3-1, the same analysis was performed for HWIem2104, leading to 10 clusters.
Supplementary Tables S1- S7:

Supplementary Table 1. Cryo-EM data collection and model statistics

| Data collection and processing               | PRMT5:MEP50:11-2F | PRMT5:MEP50 |
|---------------------------------------------|-------------------|-------------|
| Magnification                               | 105,000           | 105,000     |
| Voltage (kV)                                | 300               | 300         |
| Electron exposure (e⁻/Å²)                   | 40                | 40          |
| Defocus range (μm)                          | -0.75 to -3.0     | -0.75 to -3.0 |
| Pixel size (Å)                              | 0.66              | 0.66        |
| Symmetry imposed                            | D2                | D2          |
| Initial number of particle images (no.)     | 866,240           | 235,210     |
| Final number of particle images (no.)       | 207,392           | 101,707     |
| Map sharpening B factor (Å²)                | -68               | -79.7       |
| Overall map resolution (Å)                  | 3.14              | 3.17        |
| FSC threshold                               | 0.143             | 0.143       |

**Refinement**

| Initial model used (PDB code)               | Apo PRMT map      | Generated in Relion3.0 |
|---------------------------------------------|-------------------|------------------------|
| d FSC model (0/0.143/0.5) Å                | 3.1/3.2/3.4       | 2.9/3.2/3.6            |
| Model composition (per asymmetric unit)    |                   |                        |
| Chains                                      | 2                 | 2                      |
| Non-hydrogen atoms                          | 7384              | 7265                   |
| Protein residues                            | 932               | 925                    |
| Ligands                                     | 2                 | 0                      |
| B factors (Å²) (min/max/mean)               | 24.99/75.31/47.74 | 61.67/137.13/86.91     |
| R.m.s. deviations                           |                   |                        |
| Bond angles (°)                             | 0.566             | 0.645                  |
| Bond distances (Å)                          | 0.005             | 0.007                  |

**Validation**

| MolProbity score                            | 0.78              | 1.05                    |
| Clashscore                                   | 0.69              | 0.89                    |
| Poor rotamers (%)                            | 0.00              | 0.00                    |
| Ramachandran plot                            |                   |                        |
| Favored (%)                                  | 97.73             | 97.58                   |
| Allowed (%)                                  | 2.16              | 1.87                    |
| Outliers (%)                                 | 0.11              | 0.55                    |
| EMringer Score                               | 3.02              | 2.84                    |
### Supplementary Table S2. Clusters of energy-minimized poses for MTA

| Cluster Rank | Lowest Binding Energy | Run | Mean Binding Energy | Num in Clus |
|--------------|-----------------------|-----|---------------------|-------------|
| 1            | -6.63                 | 1556| -6.03               | 1781        |
| 2            | -5.95                 | 241 | -5.39               | 104         |
| 3            | -5.62                 | 1994| -5.54               | 48          |
| 4            | -5.49                 | 1083| -5.45               | 32          |
| 5            | -5.21                 | 1555| -5.17               | 4           |
| 6            | -5.18                 | 107 | -5.18               | 1           |
| 7            | -5.06                 | 526 | -4.94               | 24          |
| 8            | -4.97                 | 837 | -4.97               | 6           |

### Supplementary Table S3. Clusters of energy-minimized poses for SAH

| Cluster Rank | Lowest Binding Energy | Run | Mean Binding Energy | Num in Clus |
|--------------|-----------------------|-----|---------------------|-------------|
| 1            | -8.71                 | 30  | -8.11               | 7           |
| 2            | -8.54                 | 36  | -7.91               | 13          |
| 3            | -8.02                 | 40  | -7.60               | 15          |
| 4            | -7.61                 | 49  | -7.33               | 3           |
| 5            | -7.57                 | 24  | -7.57               | 1           |
| 6            | -7.48                 | 13  | -7.48               | 1           |
| 7            | -7.07                 | 3   | -7.04               | 2           |
| 8            | -6.93                 | 37  | -6.93               | 1           |
| 9            | -6.93                 | 33  | -6.93               | 1           |
| 10           | -6.92                 | 23  | -6.80               | 2           |
Supplementary Table S4. Clusters of energy-minimized poses for SAM

| Cluster Rank | Lowest Binding Energy | Run | Mean Binding Energy | Num in Clus |
|--------------|-----------------------|-----|---------------------|-------------|
| 1            | -8.72                 | 3   | 8.15                | 6           |
| 2            | -8.69                 | 36  | 8.24                | 4           |
| 3            | -8.34                 | 22  | -7.65               | 10          |
| 4            | -8.02                 | 46  | -8.02               | 1           |
| 5            | -7.89                 | 43  | -7.55               | 3           |
| 6            | -7.88                 | 10  | -7.57               | 12          |
| 7            | -7.71                 | 7   | 9.59                | 2           |
| 8            | -7.64                 | 45  | 7.17                | 4           |
| 9            | -7.56                 | 9   | -7.56               | 2           |
| 10           | -7.54                 | 6   | 7.54                | 1           |

Supplementary Table S5. Clusters of energy-minimized poses for 11-2F

| Cluster Rank | Lowest Binding Energy | Run | Mean Binding Energy | Num in Clus |
|--------------|-----------------------|-----|---------------------|-------------|
| 1            | -10.99                | 301 | -10.18              | 49          |
| 2            | -10.92                | 825 | -10.43              | 70          |
| 3            | -10.38                | 875 | -9.72               | 10          |
| 4            | -10.38                | 1353| 9.56                | 540         |
| 5            | -10.36                | 234 | 9.68                | 24          |
| 6            | -10.21                | 512 | 9.74                | 173         |
| 7            | -10.13                | 304 | 9.67                | 20          |
| 8            | -10.10                | 1757| 9.28                | 11          |
| 9            | -9.92                 | 1551| -9.37               | 22          |
| 10           | -9.89                 | 217 | -9.28               | 114         |
### Supplementary Table S6. Clusters of energy-minimized poses for HWIem2109

| Clus Rank | Lowest Binding Energy | Run | Mean Binding Energy | Num in Clus |
|-----------|-----------------------|-----|---------------------|-------------|
| 1         | -12.26                | 725 | -11.56              | 420         |
| 2         | -11.69                | 879 | -11.06              | 290         |
| 3         | -10.98                | 1254| -10.48              | 911         |
| 4         | -10.83                | 631 | -10.14              | 44          |
| 5         | -10.28                | 1034| -9.81               | 58          |
| 6         | -10.27                | 1291| -9.72               | 21          |
| 7         | -10.24                | 1561| -9.96               | 173         |
| 8         | -10.22                | 169 | -10.22              | 1           |
| 9         | -10.21                | 939 | -9.86               | 6           |
| 10        | -10.12                | 754 | -10.12              | 1           |

### Supplementary Table S7. Clusters of energy-minimized poses for HWIem2104

| Clus Rank | Lowest Binding Energy | Run | Mean Binding Energy | Num in Clus |
|-----------|-----------------------|-----|---------------------|-------------|
| 1         | -12.67                | 968 | -11.98              | 1117        |
| 2         | -12.64                | 1222| -11.54              | 160         |
| 3         | -11.60                | 6   | -10.62              | 284         |
| 4         | -11.29                | 531 | -10.17              | 4           |
| 5         | -11.24                | 394 | -10.75              | 14          |
| 6         | -11.23                | 23  | -10.57              | 5           |
| 7         | -10.93                | 1715| -10.93              | 1           |
| 8         | -10.82                | 446 | -10.53              | 34          |
| 9         | -10.63                | 1067| -10.31              | 15          |
| 10        | -10.23                | 1373| -10.03              | 10          |
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