2020 ACS Spring National Meeting & Expo, Philadelphia

[MEDI 268] Biochemical, biophysical and structural studies of hPRMT5 and its peptide–competitive MTA–synergistic small molecule inhibitors for cancer therapeutics

PRESENTER: **Wei Zhou (Ph.D. candidate)**

DIVISION: Division of Medicinal Chemistry

SESSION: General Orals (MEDI)

DATE: Wednesday, March 25, 2020

University of Florida

College of Medicine

Department of Biochemistry and Molecular Biology

Chenglong Li Lab

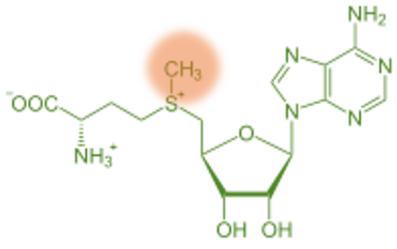
OUTLINE

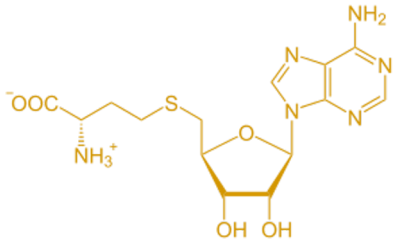
- Background
- Aim
- Methods and Results
- Conclusions
- Future Direction

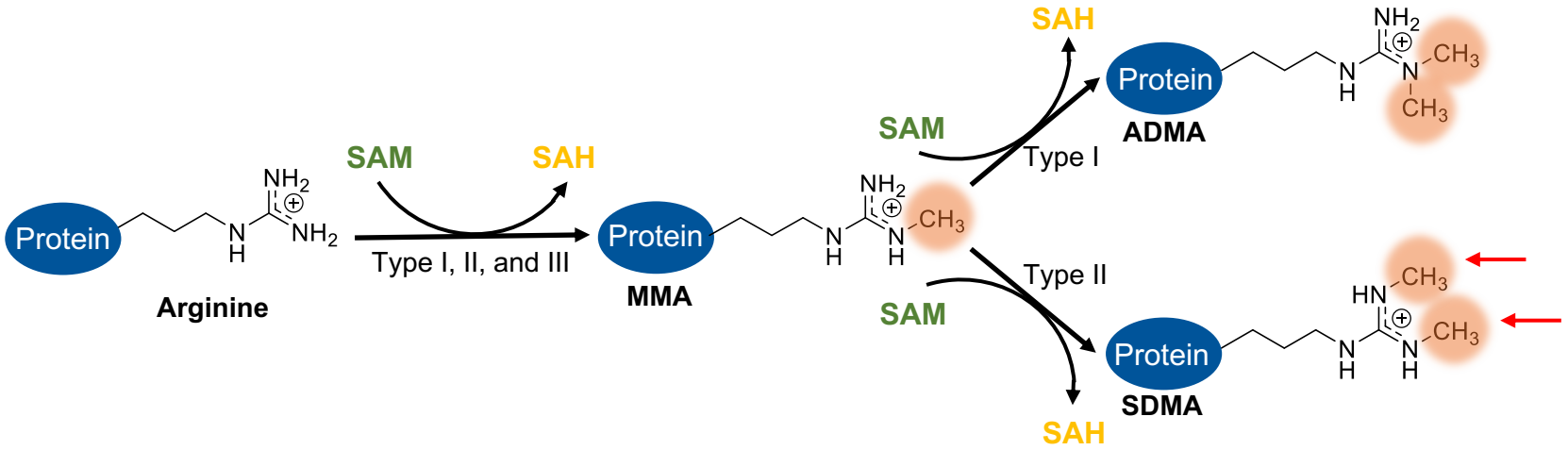
Arginine methylation by PRMT5

PRMT5 (Protein arginine methyltransferase 5)

- The main symmetric dimethylarginine (**SDMA**) methyltransferase (Type II)
- S-Adenosyl-$_L$-Methionine **(SAM)** **==>** S-Adenosyl-$_L$-Homocysteine **(SAH)**
- Major substrates: H4R3, H2AR3, H3R2, H3R8 and non-histone proteins, e.g. p53.







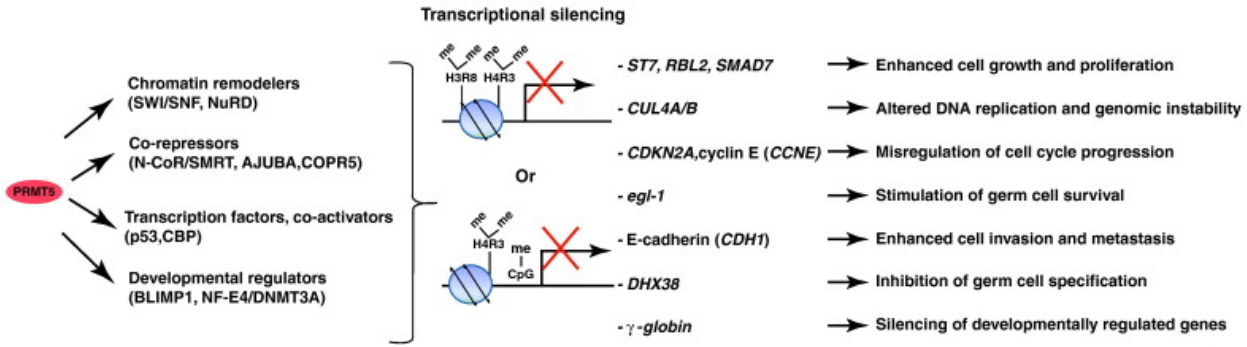
Type I PRMTs (PRMT1,2,3,4,6,8)
Type II PRMTs (**PRMT5**,9)
Type III PRMTs (PRMT7)

ADMA: ω-N^G, N^G-asymmetric dimethylation
SDMA: ω-N^G, N'^{G}-symmetric dimethylation
MMA: ω-N^G-monomethylation

PRMT5 in cancers

- PRMT5 forms a hetero-octameric complex with MEP50.
- PRMT5:MEP50 complex forms large multimolecular complexes with different binding partners.

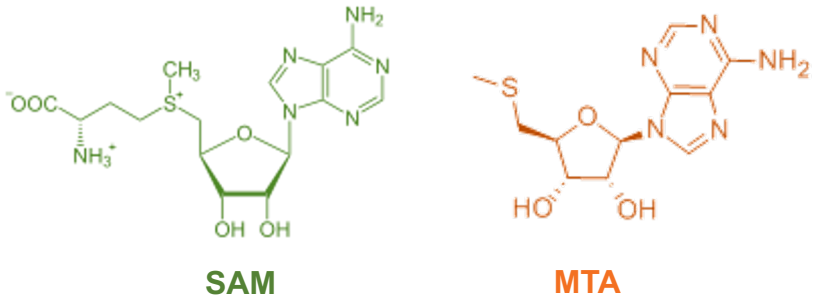
- PRMT5 is overexpressed in many cancers.
- PRMT5 inhibition or depletion induces cell growth arrest and cell death.



Antonysamy et al. *Proceedings of the National Academy of Sciences* (2012). Wei et al. *Proceedings of the National Academy of Sciences* (2013). Karkhanis et al. *Trends in biochemical sciences* (2011).

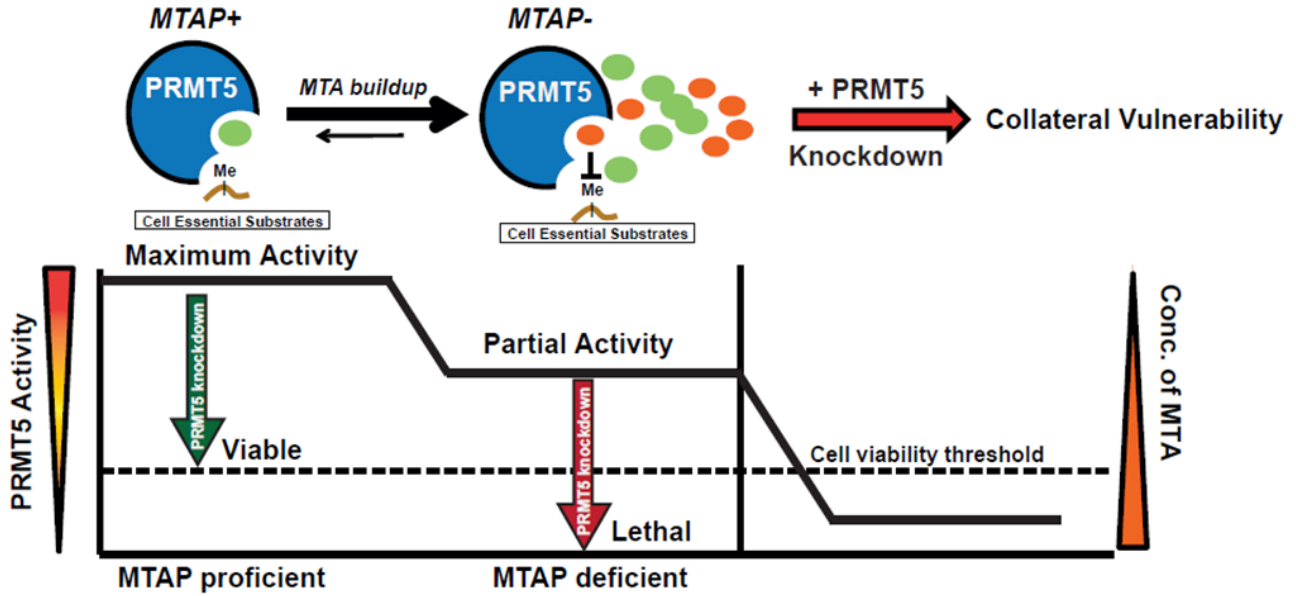
PRMT5 as a novel drug target in MTAP-deleted cancers

- Methylthioadenosine phosphorylase (**MTAP**) **deletion** occurs in ~15% of all human cancers.
- MTAP-deletion in cancer cells disorders methionine metabolism and results in **MTA accumulation** in cells.
- MTA creates a **partially inhibited enzyme state** thus significantly increases their vulnerability to further PRMT5 inhibition.



Intracellular concentration:

	SAM	MTA
MTAP w.t.	~50 μM	~1 μM
MTAP -/-	~50 μM	~20 μM

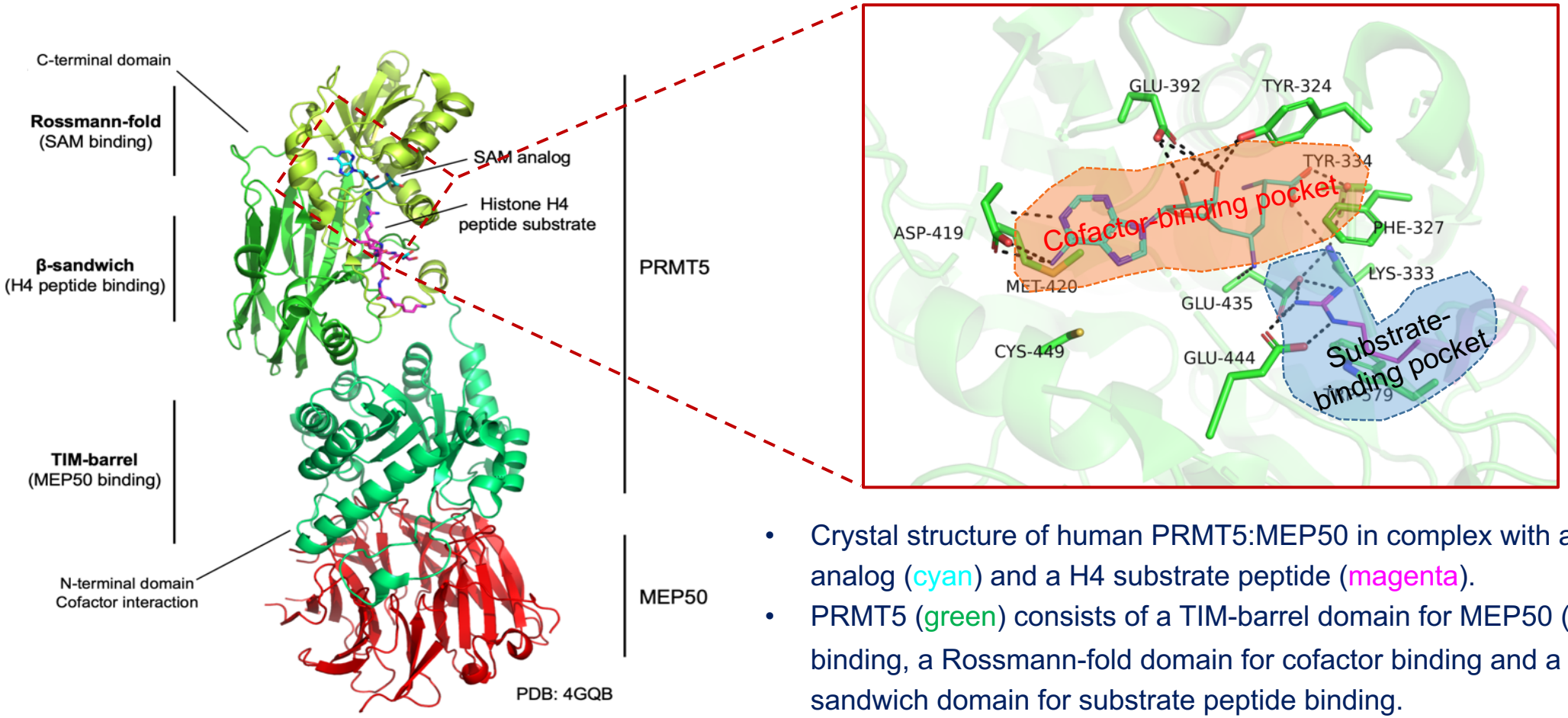


As an oncoprotein in tumorigenesis, PRMT5 emerges as a compelling target for drug discovery in cancer therapy.

Mavrakis et al. *Science* (2016). Kryukov et al. *Science* (2016). Lucia, Angelo, and Peter A. DiMaggio. Data Science for Healthcare. (2019)..

Crystal structure of PRMT5:MEP50

The first crystal structure of human PRMT5:MEP50 was published in 2012.



- Crystal structure of human PRMT5:MEP50 in complex with a cofactor analog (cyan) and a H4 substrate peptide (magenta).
- PRMT5 (green) consists of a TIM-barrel domain for MEP50 (red) binding, a Rossmann-fold domain for cofactor binding and a β-sandwich domain for substrate peptide binding.

Antonysamy et al. *Proceedings of the National Academy of Sciences* (2012).

Limitations of PRMT5 inhibitors in clinical trial

Since 2016, three drug candidates have entered **Phase I clinical trial**:

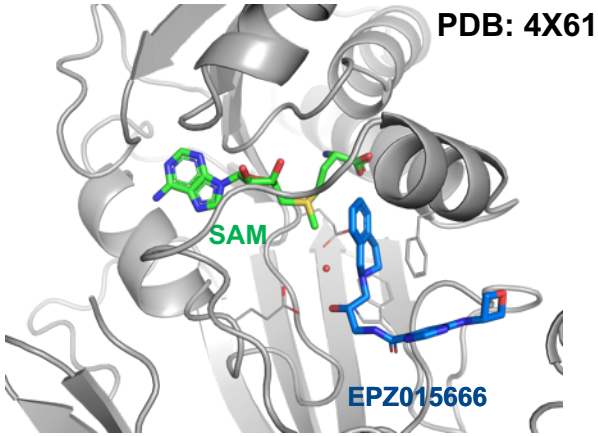
- **Cofactor-competitive inhibitors** (JNJ-64619178 and PF-06939999)

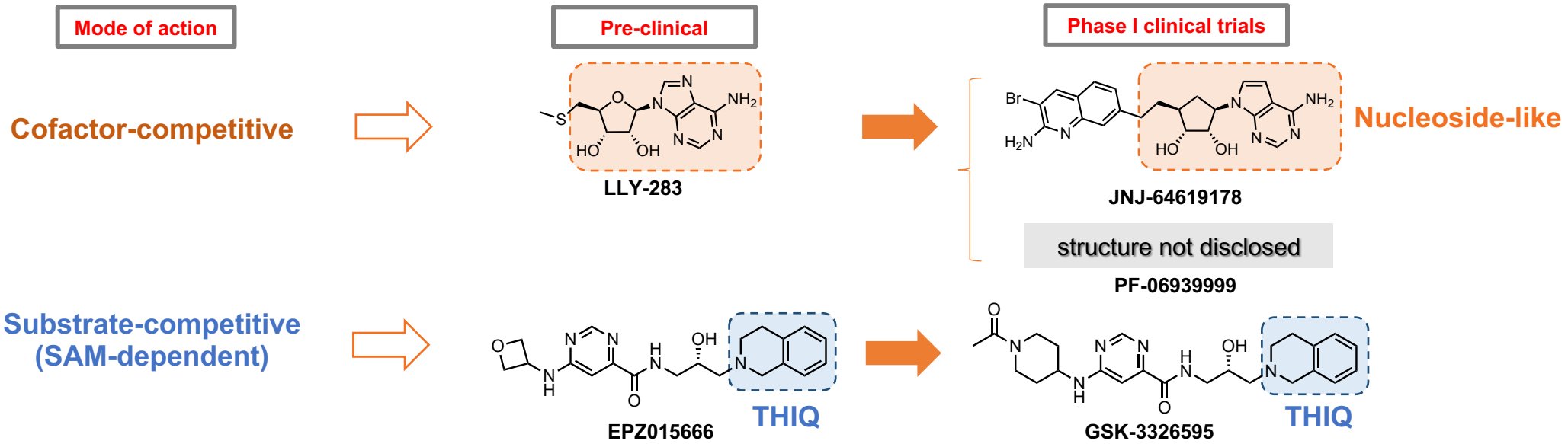
  SAM-analogs may cause off-target effect.

- **Substrate-competitive inhibitors** (GSK-3326595)

  Pre-binding of cofactor SAM is required to form cation-π interactions;

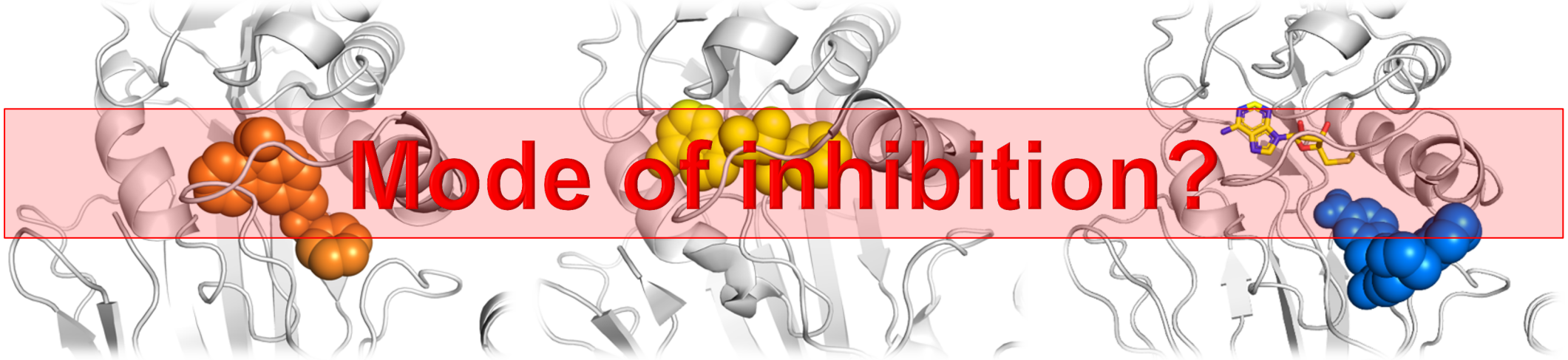
  Low binding affinity to MTA-bound form (MTAP-deleted cancers).





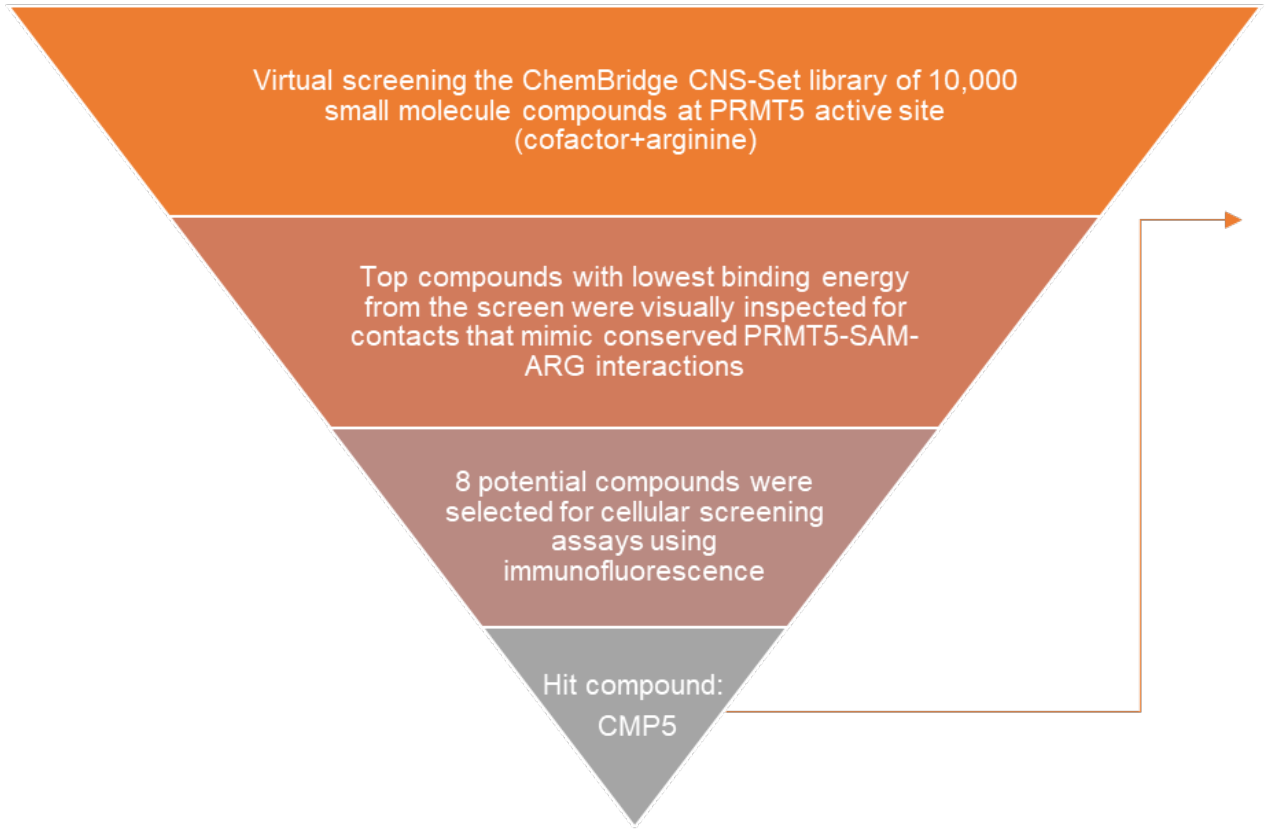
AIM

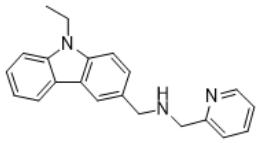
Design a potent, non-nucleoside, SAM-independent small molecule inhibitor targeting PRMT5.



Identification of CMP5 as a 'hit'

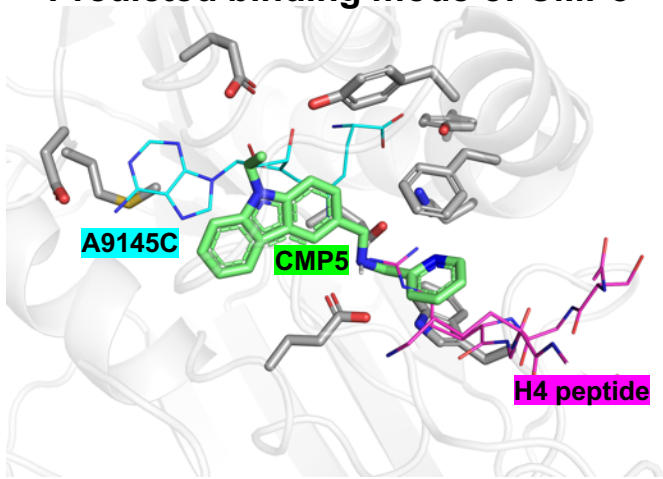
Identification of hit compound CMP5 by virtual screening and cellular assays





CMP5

Predicted binding mode of CMP5

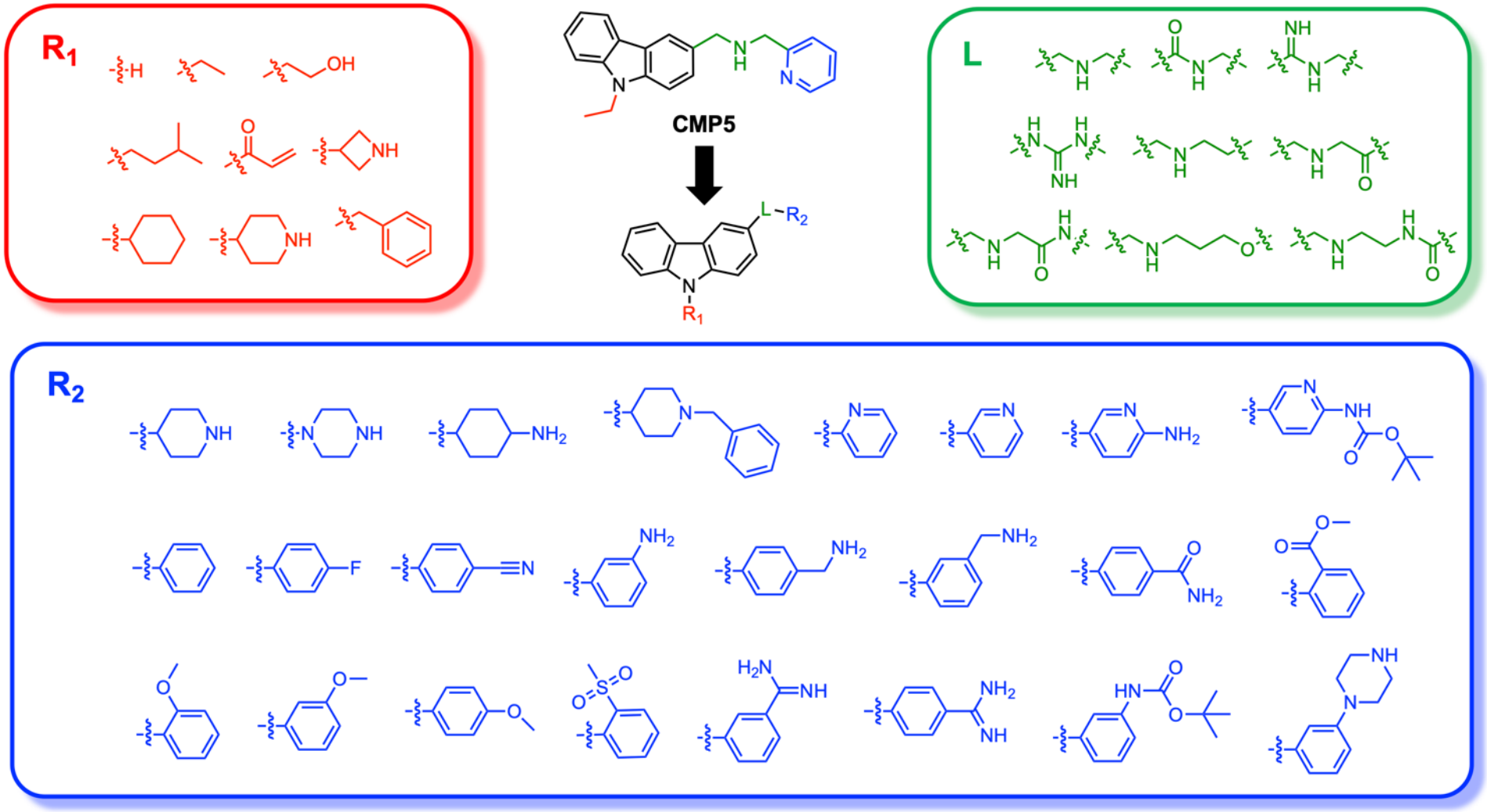


Cellular assays:

- ✓ Histone methyltransferase activity assay using hSWI/SNF-associated PRMT5;
- ✓ Selectivity against other Type I and Type II PRMTs;
- ✓ Cell proliferation assay;
- ✓ Apoptosis analysis;
- ✓ Immunofluorescence;
- ✓ Confocal microscopy;
- ✓ Western blot;
- ✓ Immunoprecipitation (IP).

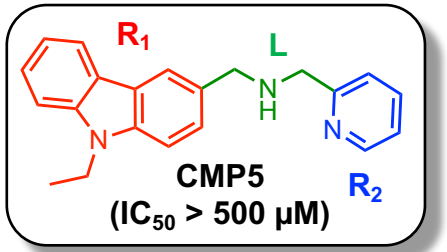
Alinari et al. *Blood, The Journal of the American Society of Hematology* (2015).

Structure-based drug design and structure-activity relationship study



Unpublished data

Compound optimization and mode of inhibition (MOI) study



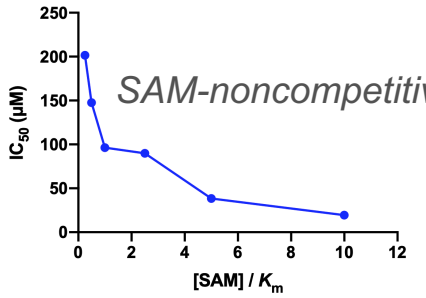
CMP5
(IC_{50} > 500 μM)

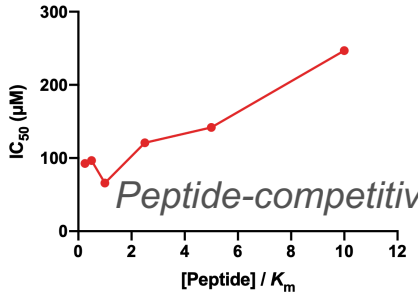
> 5-fold

Optimizations on R_1 and R_2

6-1F (IC_{50} = 99.4 ± 5.1 μM)
1-ET-1F (IC_{50} = 107.7 ± 11.7 μM)

MOI of 1-ET-1F





~100-fold

SAR on R_2

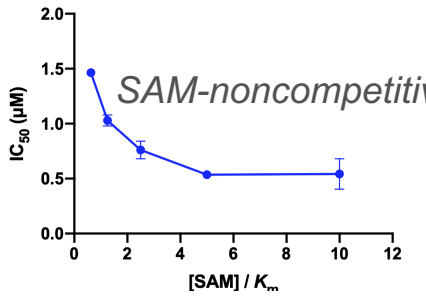
11-3F (IC_{50} > 500 μM)
11-5F (IC_{50} = 7.2 ± 0.2 μM)
11-6F (IC_{50} > 500 μM)
11-9F (IC_{50} = 179.5 ± 10.4 nM)

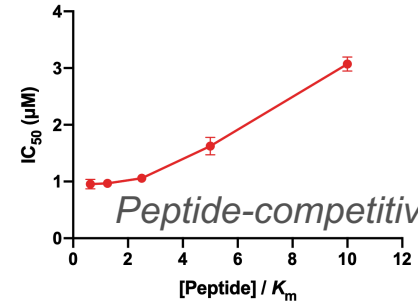
~5-fold

Optimizations on L

11-1F (IC_{50} = 1.3 ± 0.2 μM)
11-2F (IC_{50} = 0.7 ± 0.2 μM)

MOI of 11-1F





~5-fold

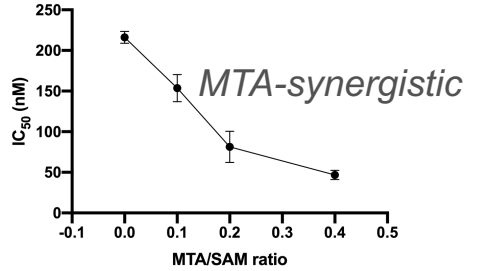
SAR on R_2

15-2F (IC_{50} < 150 nM)
15-3F (IC_{50} > 100 μM)
15-4F (IC_{50} > 100 μM)
15-5F (IC_{50} = 29.6 ± 1.3 μM)

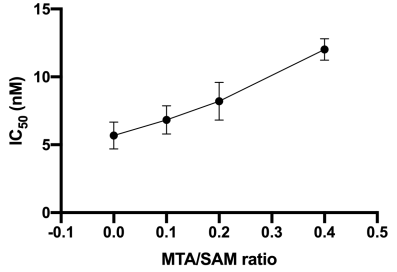
Optimizations on R_1

15-1F (IC_{50} = 233.8 ± 15.8 nM)

15-1F + MTA/SAM



GSK-3326959 + MTA/SAM

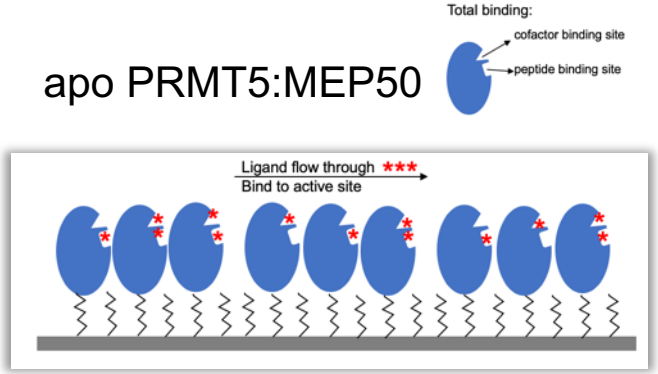


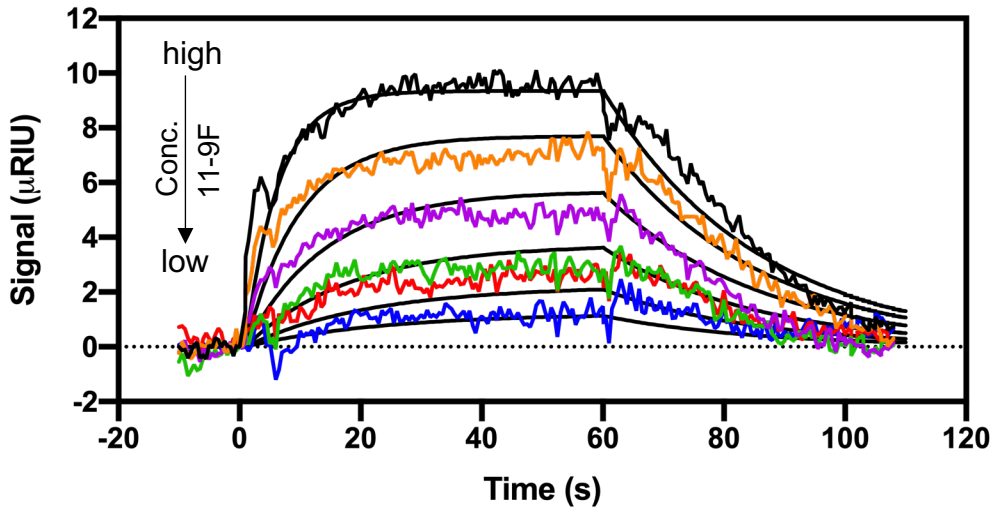
Unpublished data

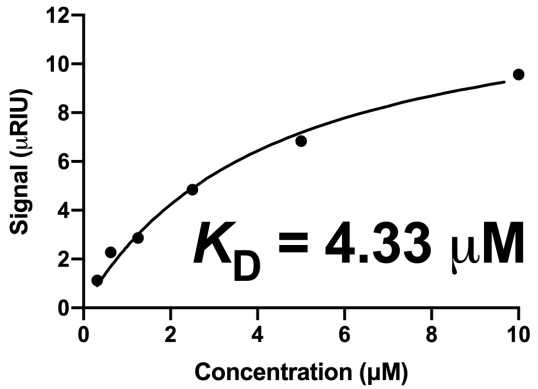
Binding preference study by SPR

Protein immobilized | **Sensorgram** | **K_D (affinity fitting)**

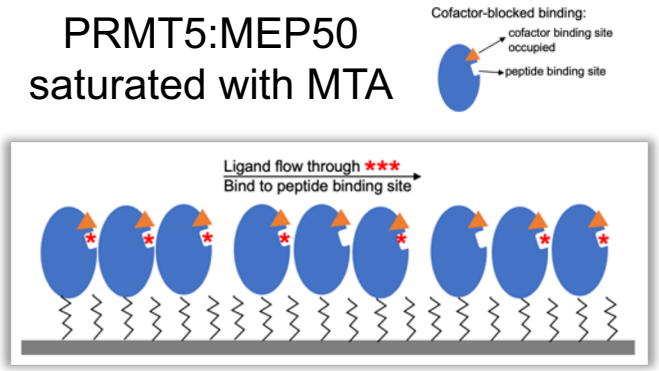
apo PRMT5:MEP50

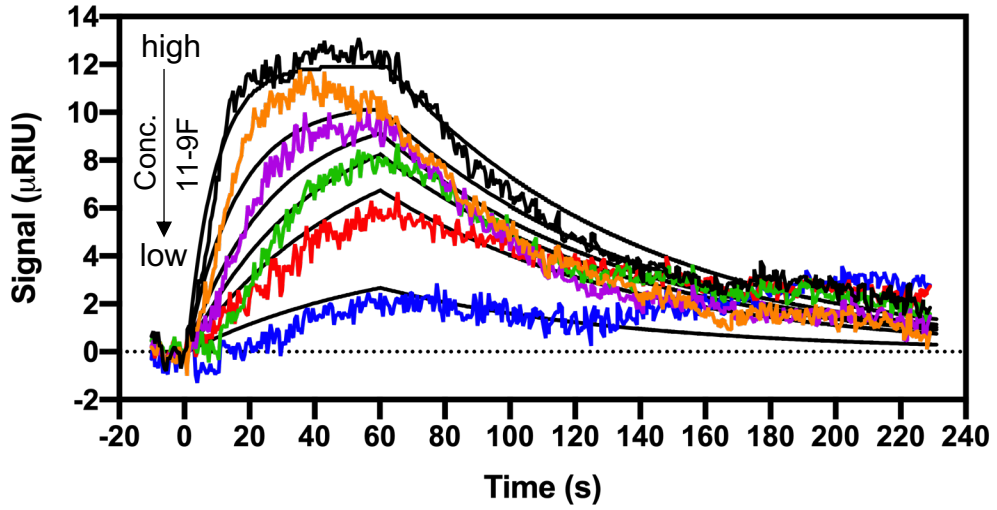


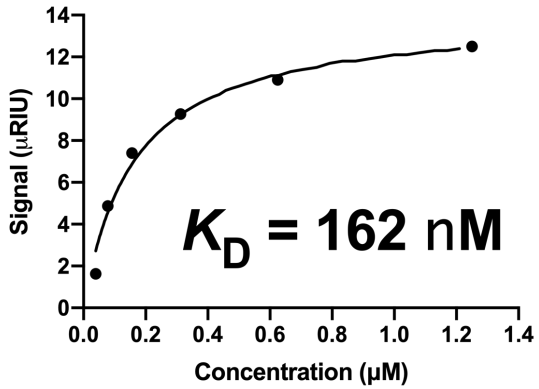




PRMT5:MEP50
saturated with MTA







Unpublished data

Binding preference study by SPR

Compound name	Enzymatic IC_{50} (μM)	Binding affinity K_D (μM) on different receptors			
		Apo P:M	P:M + MTA	P:M + SAH	P:M + SAM
11-1F	1.3	15.7	0.256	1.67	1.32
11-2F	0.7	12.1	0.095	0.895	0.692
11-3F	> 500	N.B	L.B	N.B	N.B
11-5F	7.2	N.B	N.B	N.B	2.84
11-6F	> 500	N.B	L.B	N.B	N.B
11-9F	0.180	4.33	0.162	0.191	0.732
15-1F	0.234	7.32	0.038	0.218	0.106
GSK-3326959	0.006	N.B	5.9	0.794	< 0.1

L.B.: very low binding response; N.B.: no binding

Unpublished data

Binding preference study by SPR

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11-1F	1.3	15.7	0.256	1.67	1.32	
11-2F	0.7	12.1	0.095	0.895	0.692	
11-3F	> 500 (inactive)	N.B	L.B	N.B	N.B	No binding
11-5F	7.2	N.B	N.B	N.B	2.84	
11-6F	> 500 (inactive)	N.B	L.B	N.B	N.B	No binding
11-9F	0.180	4.33	0.162	0.191	0.732	
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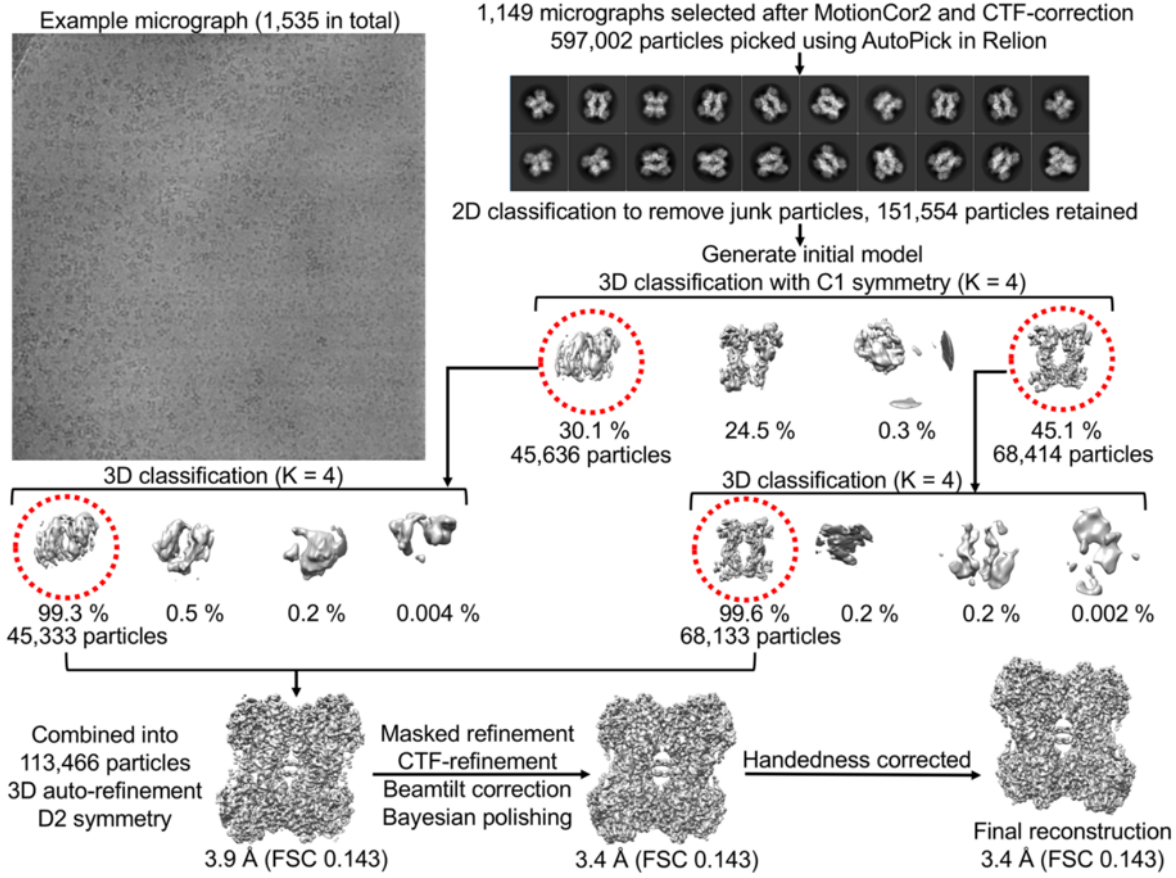
L.B.: very low binding response; N.B.: no binding

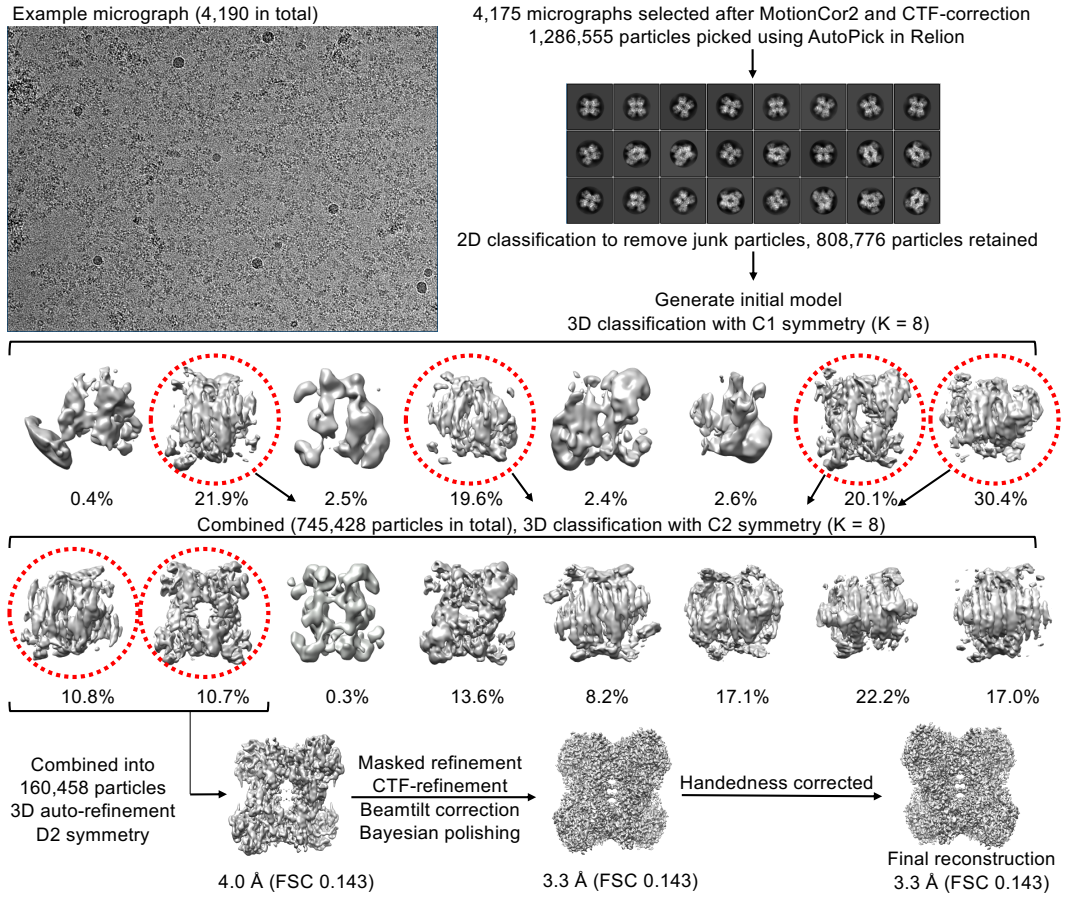
Unpublished data

Structure determination by cryo-EM

The *apo* form of PRMT5:MEP50



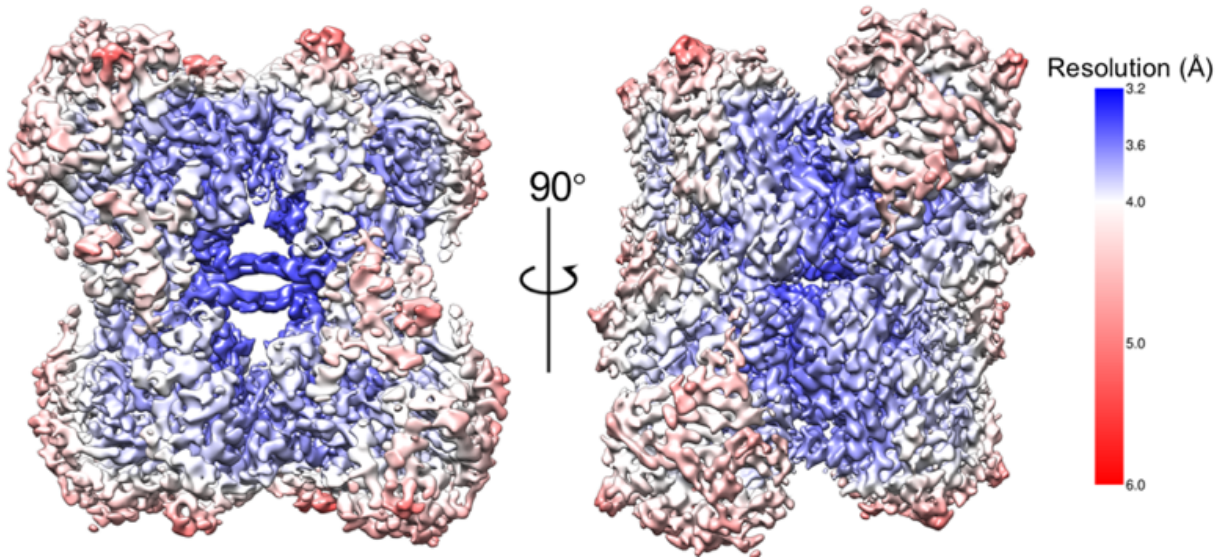
PRMT5:MEP50 in complex with MTA and 11-2F



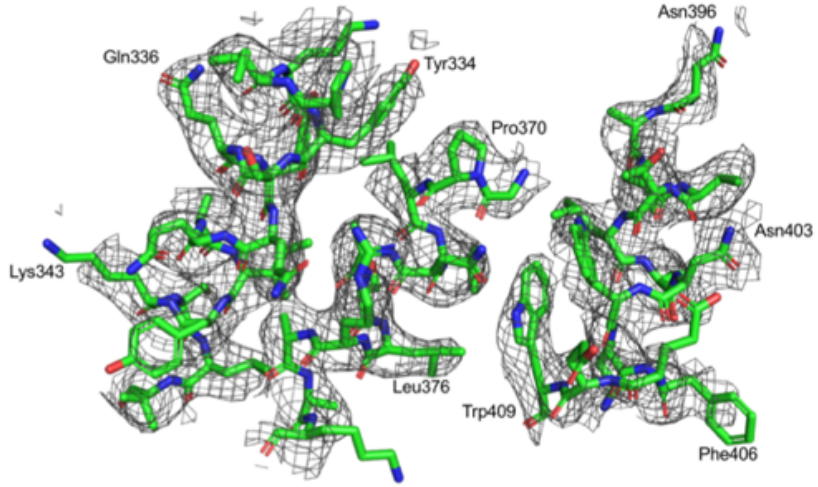
Unpublished data

Structure determination by cryo-EM

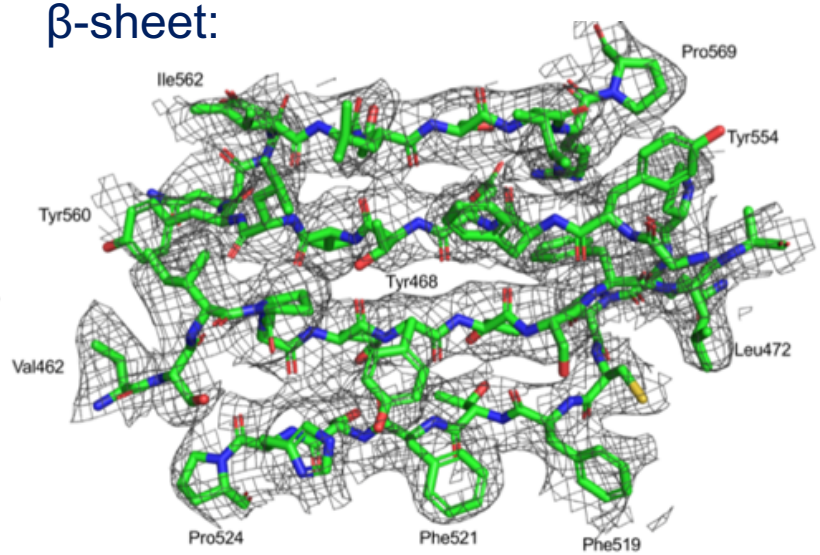
Local resolution of *apo* PRMT5:MEP50 complex:



α-helix:

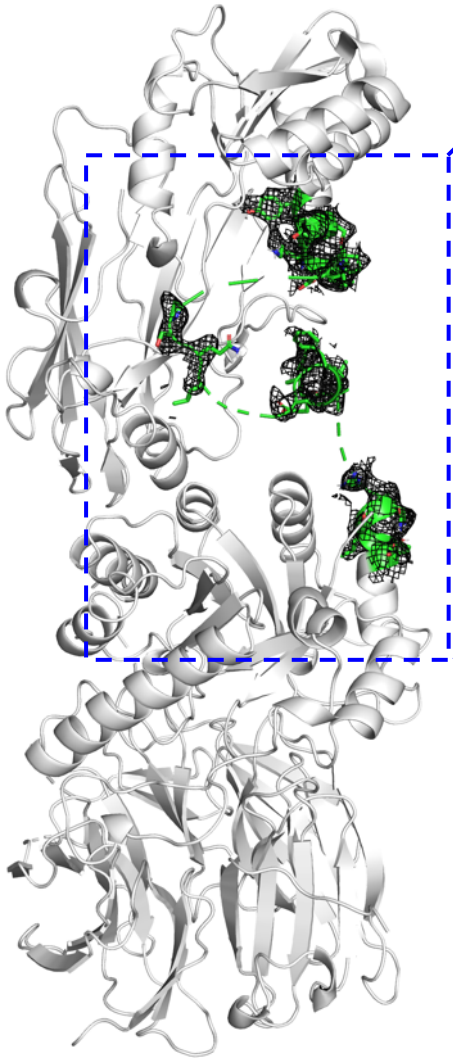


β-sheet:

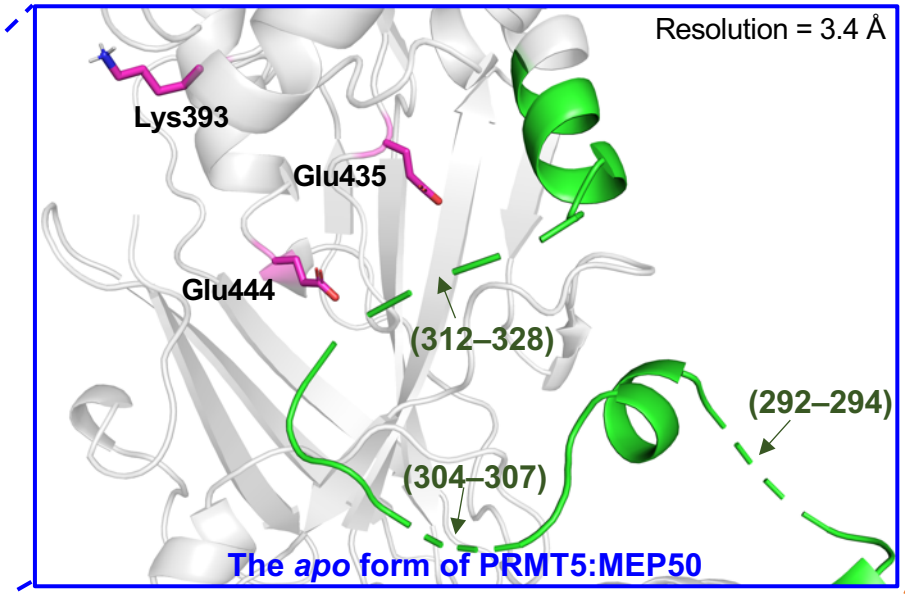


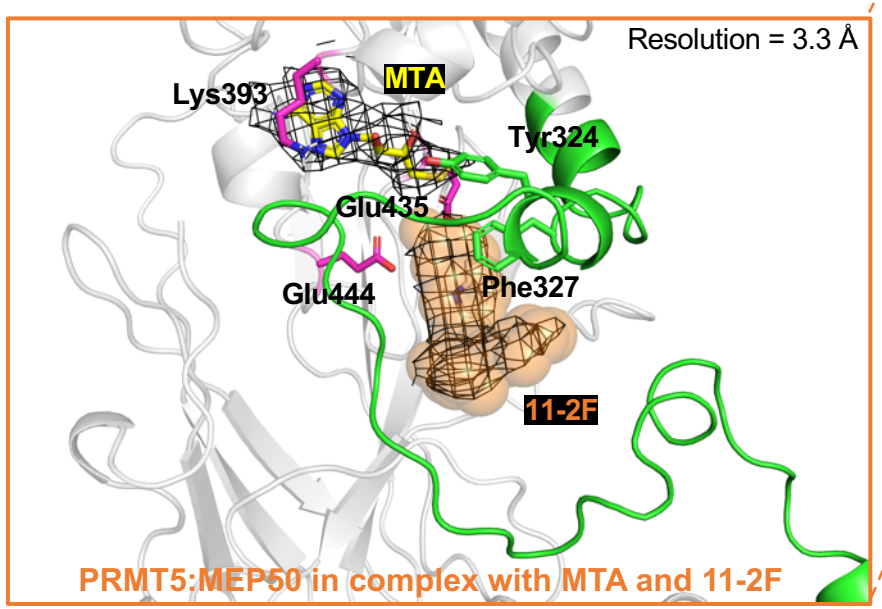
Unpublished data

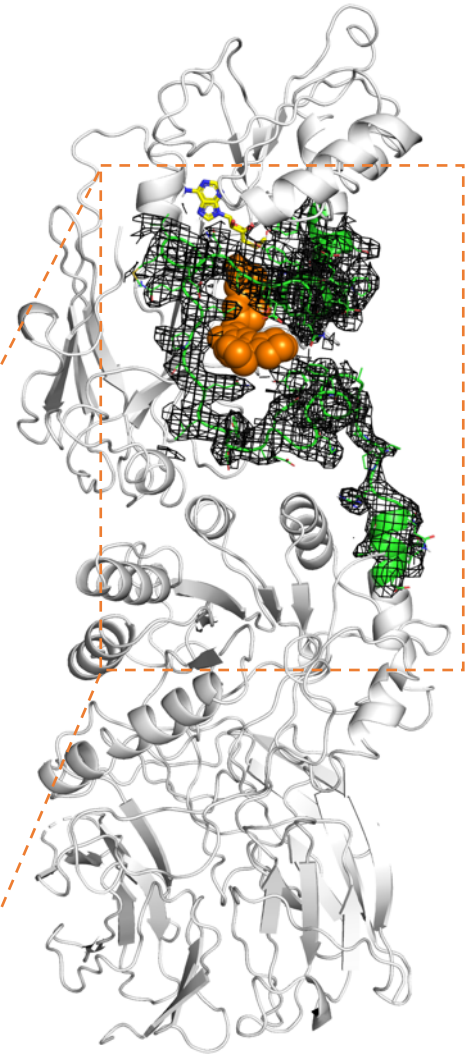
Structure comparison of apo- and holo- forms of PRMT5:MEP50



Apo PRMT5:MEP50
(EMDB-20764) (PDB 6UGH)



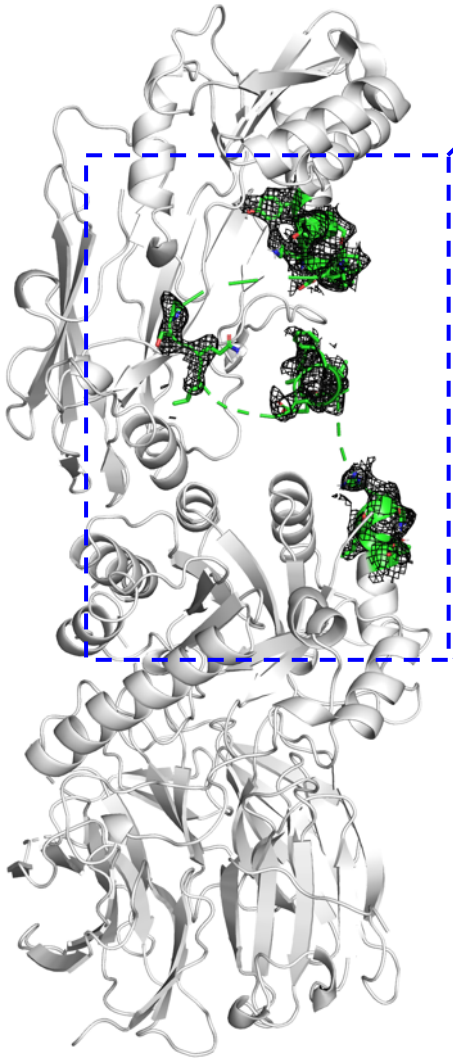




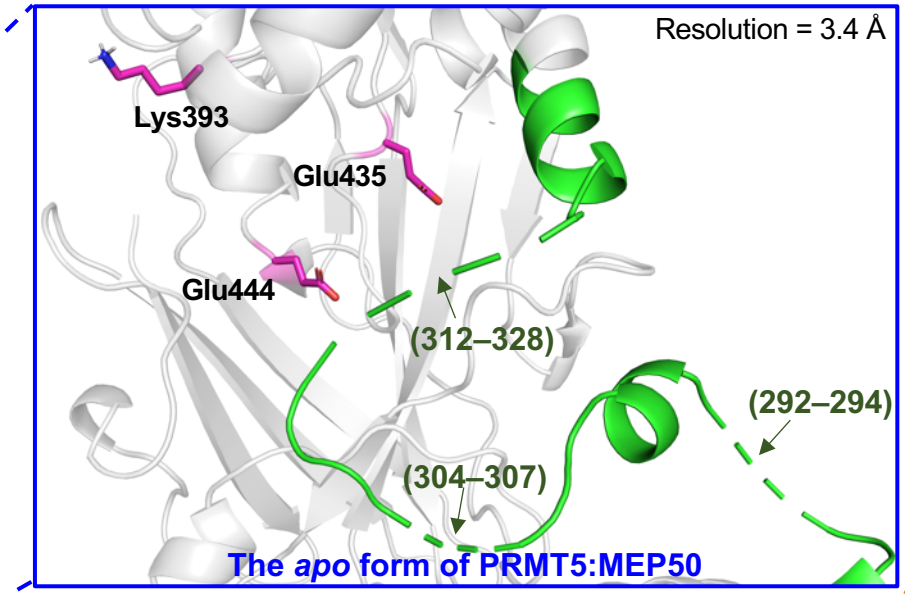
Holo PRMT5:MEP50
(EMDB/PDB: not deposited)

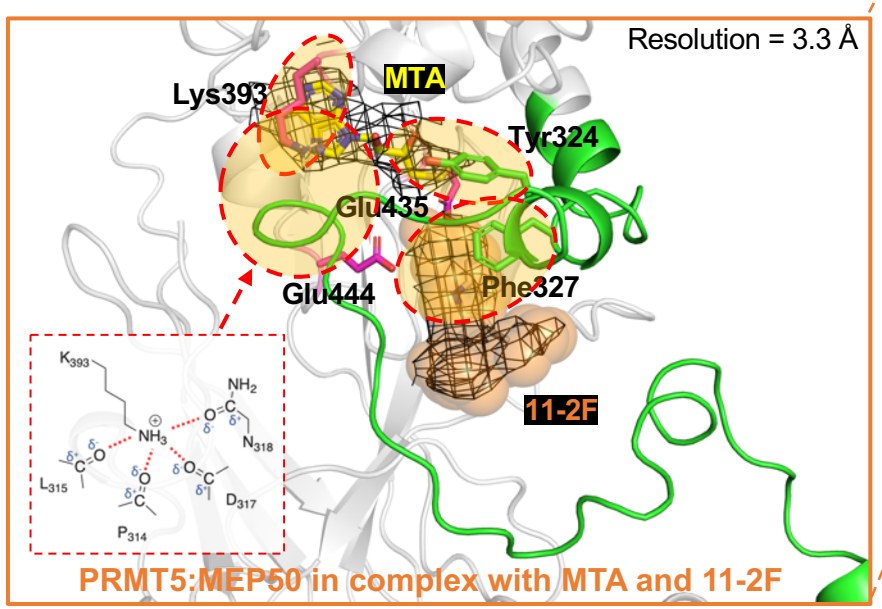
Unpublished data

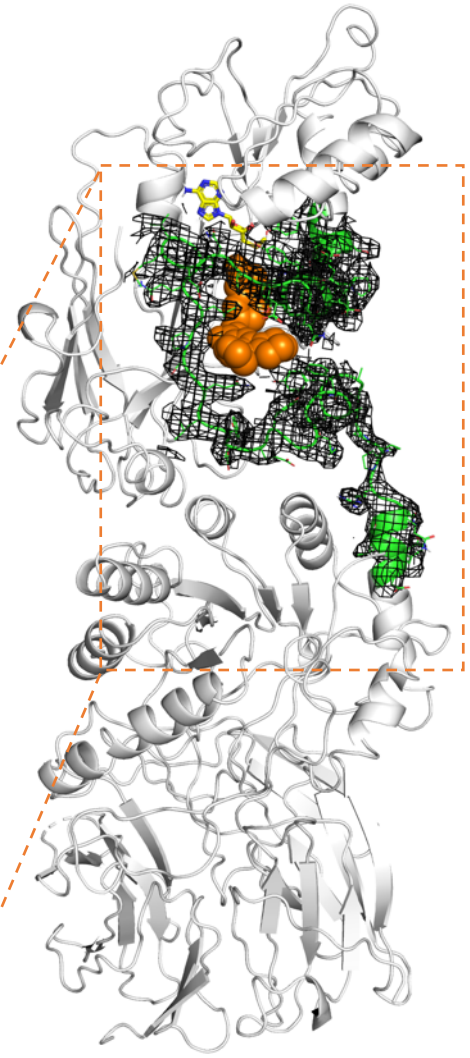
Structure comparison of apo- and holo- forms of PRMT5:MEP50



Apo PRMT5:MEP50
(EMDB-20764) (PDB 6UGH)





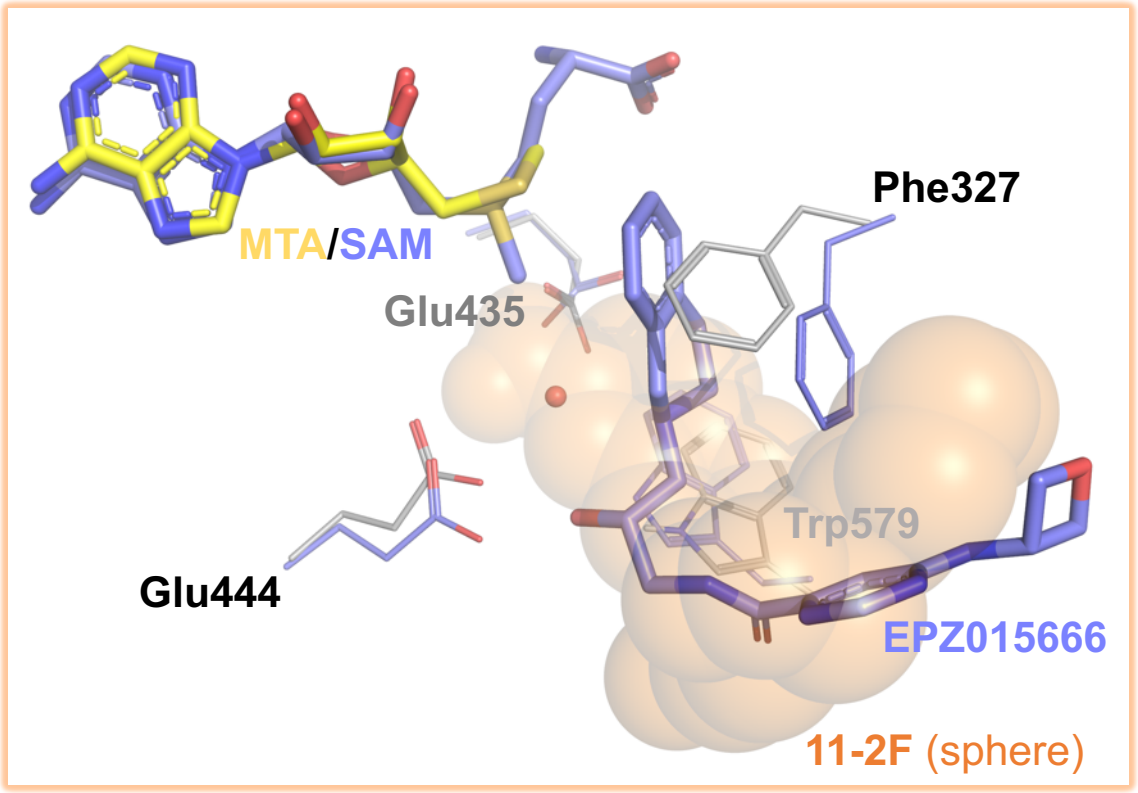


Holo PRMT5:MEP50
(EMDB/PDB: not deposited)

Unpublished data

Structure alignment of bound-forms of PRMT5:MEP50

[11-2F + MTA]-bound form (grey) is aligned with [EPZ015666 + SAM]-bound form (purple)



In [11-2F + MTA]-bound form, 11-2F:

- kicks out the **H_2O** molecule in EPZ015666-bound form by forming H-bonds with **Glu435** and **Glu444**,
- forms π-π interactions with **Phe327** and **Trp579**.

11-2F as a substrate-competitive inhibitor:

- does not rely on the pre-binding of SAM
- desired MOI in MTA-accumulated MTAP-deleted cancers

EPZ015666 (IC_{50} = 21.7 ± 5.0 nM)

11-2F (IC_{50} = 0.7 ± 0.2 μM)

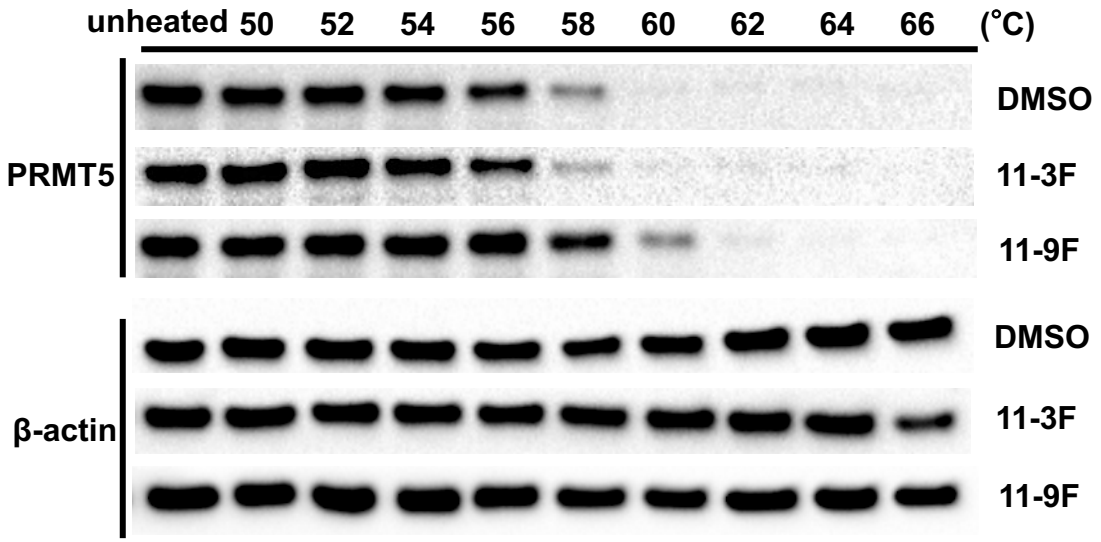
Unpublished data

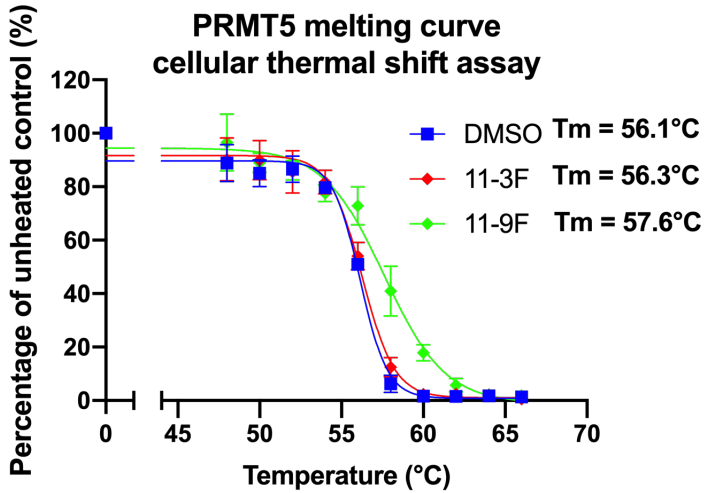
Target engagement study by CETSA

CEllular Thermal Shift Assay (CETSA)

11-3F (IC_{50} > 500 μM)

11-9F (IC_{50} = 179.5 ± 10.4 nM)



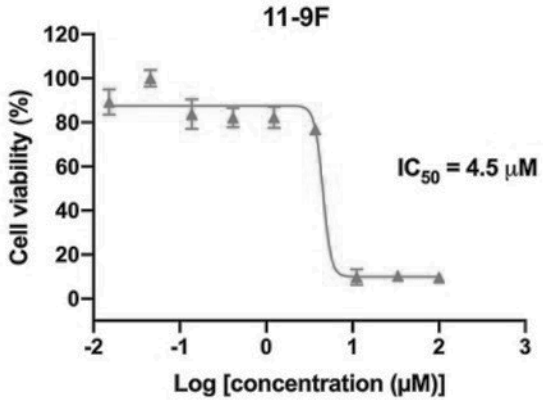


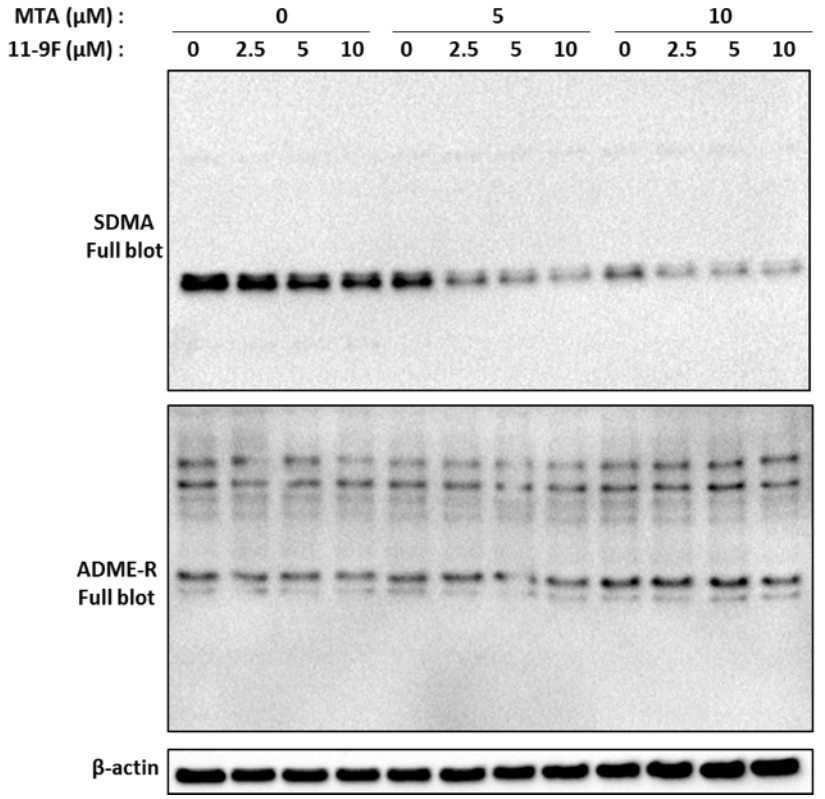
Unpublished data

Protein methylation inhibition and selectivity in cells by western blot

Cellular effects of 11-9F in MDA-MB-231 breast cancer cell line (96 h)

- Concentration-dependent inhibition of cellular protein arginine symmetric dimethylation **(SDMA)** level was shown by western blot.
- MTA improved the inhibition activity of 11-9F, suggesting a synergistic effect.
- Unaffected protein arginine asymmetric dimethylation level (**ADME-R**) indicated a decent selectivity of 11-9F against Type I PRMTs.
- 11-9F showed anti-proliferation effect in MTT assay.





Unpublished data

CONCLUSIONS

- ✓ The potency of designed inhibitors has been improved by more than 3000-fold.
- ✓ Designed inhibitors show a substrate-competitive mode of action in enzymatic assays.
- ✓ 11-9F binds to both apo- and cofactor-bound forms of PRMT5:MEP50 and shows binding preference to MTA-bound form.
- ✓ The flexible loop region at the active site of PRMT5 undergoes a huge conformational change upon cofactor binding.
- ✓ Interaction between 11-2F and PRMT5 is different from current inhibitors, making it a novel series of PRMT5 inhibitor.
- ✓ Treatment of 11-9F in combination with MTA improves its inhibition activity in cellular assays.

CONCLUSIONS

11-9F is a potent and selective PRMT5 inhibitor that binds to PRMT5 at substrate-binding site and shows synergistic effect with MTA *in vitro*.

FUTURE DIRECTION

- Compound optimization (potency↑, toxicity↓)
- Inhibition selectivity against other protein methyltransferases
- *In vitro* cellular assays to further investigate the anti-cancer effect of designed inhibitors
- Compound activity profile in cancer cell lines with different MTAP-expression levels
- *In vivo* evaluations in mice models

ACKNOWLEDGEMENTS

Li Lab Members:

Xiaozhi Yang (medicinal chemistry)
Mohammad Rezaei, PhD
Daniel Schultz
Chen Zhou
Gustavo Seabra, PhD
Iram Hyder, PhD
Dejun Li, PhD
Chenglong Li, PhD (PI)

Previous Members:

Guqin Shi, PhD (computation)
Linsen Li, PhD (medicinal chemistry)
Hongshan Lai (medicinal chemistry)

Jiang Lab Members:

Gaya Yadav, PhD (cryo-EM)
Qiu-Xing Jiang, PhD (PI)

Committee Members:

Prof. Chenglong Li (Chair)
Prof. Qiu-Xing Jiang
Prof. Robert Mckenna
Prof. Jianrong Lu
Prof. Hendrik Luesch

UF Center for Structural Biology:

Mavis Agbandje-McKenna, PhD

Cryo-EM Data Collection:

UF Interdisciplinary Center for Biotechnology Research UNIVERSITY of FLORIDA

THE FLORIDA STATE UNIVERSITY CHEMISTRY & BIOCHEMISTRY

NIH NATIONAL CANCER INSTITUTE NCIatFrederick

National Cryo-Electron Microscopy Facility

Funding:

NIH/NCI R01CA212403
UFHCC Shands fund
NIH (R21GM131231, R01GM111367, R01GM093271, 1U24GM116788)
CF Foundation (JIANG15G)
ROF2019 pilot award from UF

NIH National Institutes of Health

UF Department of Biochemistry and Molecular Biology College of Medicine UNIVERSITY of FLORIDA

UF College of Pharmacy UNIVERSITY of FLORIDA

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