Investigating the Substrate Specificity of PRMT1 using a Plate-Based Screening Method

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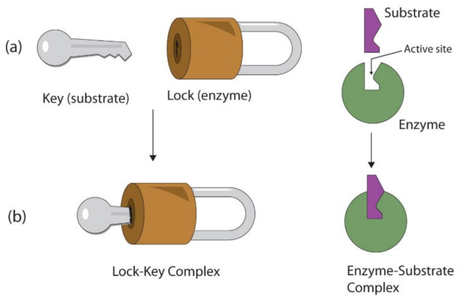
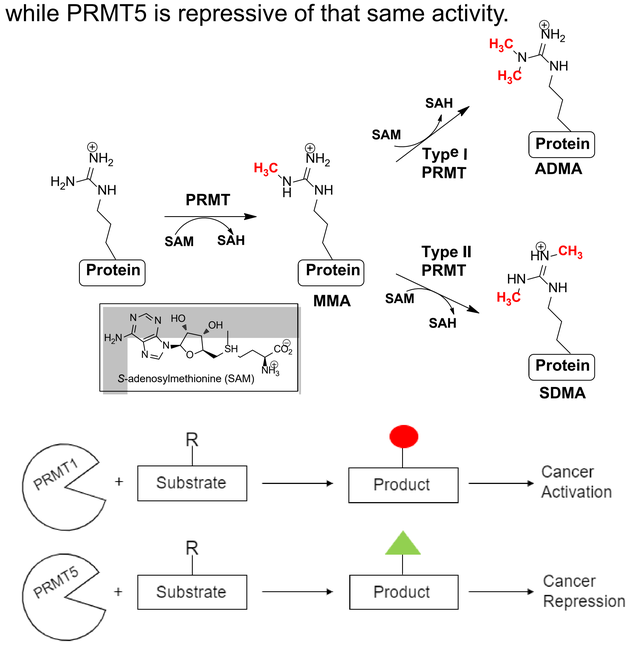
PRMT (Protein Arginine Methyltransferase) is a mammalian enzyme that catalyzes the methylation of arginine residues in a polypeptide chain. PRMT is categorized as Type I, II, or III. The methylation can occur as asymmetric dimethylation (ADMA, PRMT 1 ,3, 4, 6, and 8), symmetric dimethylation (SDMA, PRMT 7, 5 and 9) or monomethylation (MMA, PRMT 7) PRMT1 generates ADMA on arginine residues of the Histone 4 tail, which can lead to transcription of cancer-related genes. Alternatively, PRMT5 can modify the same arginine residue to produce SDMA, which represses the development of those same cancer-related genes. A better understanding of the substrate specificity of these enzymes can assist in the development of novel pharmaceuticals. To identify these differences, we synthesized a 96-well plate of peptides based on the Histone H4 N-terminal tail then screened them against PRMT1 using a previously developed screening method. This screen resulted in seven "hit" compounds, which are currently being validated as true substrates. Further investigations will continue to be conducted to identify the differences in substrates of PRMT1, 4, and 5.

Background

- Enzymes are proteins that are analogous to locks that require a specific key, (substrate) in order to unlock enzymatic activity.

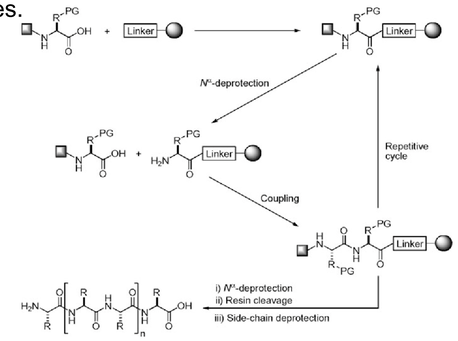


- PRMT1 and PRMT5 target arginine residues on histone tails and methylate the the guanidino nitrogen on arginine asymmetrically or symmetrically, respectively. Overexpression of PRMT1 is associated with the development of certain cancerous genes in the body while PRMT5 is repressive of that same activity.

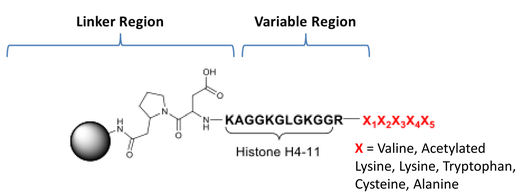


Library Synthesis and Screening

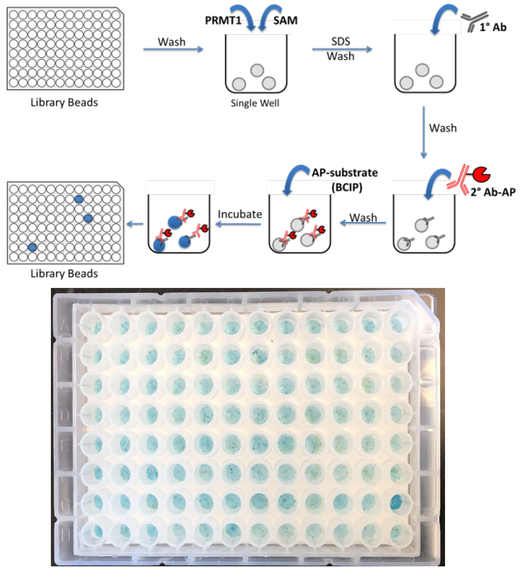
- Solid Phase Peptide Synthesis (SPPS) "couples" individual amino acid residues together with the use of coupling agents and the removal of protecting groups. Substrates were developed by utilizing SPPS in order to build the possible substrate peptides.



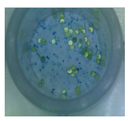
- The H4-11-R Linker (KAGGKGLGKGGR) was synthesized within a syringe and then the beads were divided evenly into a 96 well plate for the same process to be completed as in the syringe, but with each well having a variable region of five residues.



- The peptides were again divided into separate 96-well plates and then washed and screened against PRMT1 to identify possible substrates



(a) Photo of completed plate after PRMT1 Screening



(b) Non-Hit (c) Hit (Blue)

Results

- After screening against PRMT1, there were 7 "hit" compounds.

Hits	
Well Location	Sequence N to C
B7	AMKVV
D5	VKVCM
C6	KAMKK
F8	MKCKV
G2	MCCVA
G7	MWWAA
C1	MKVAA

V=Valine **A**=Alanine **K**=Lysine **M**=Acetylated Lysine
C=Cysteine **W**=Tryptophan

- Consensus sequence libraries were generated for the PRMT1 "hit" sequences and are pictured below. The consensus sequence was typed C to N terminus, as it was coupled.

Current & Future Directions

- Synthesize a series of peptides based on the consensus sequence to validate these are true substrates. The kinetic values will be measured using a standard methyltransferase assay.

Sequence Name	Sequence N to C
ValPep1	**AKCKM**-RGGKGLGKGGAK
ValPep2	**AKKKM**-RGGKGLGKGGAK
ValPep3	**VVCKM**-RGGKGLGKGGAK
ValPep4	**VVKKM**-RGGKGLGKGGAK

- Screening the same peptide library against other PRMT isozymes, such as PRMT4 and PRMT5, to determine differences in the substrate specificity amongst PRMT family members.
- Develop more specific PRMT inhibitors using this information.

References

I. Mann, Sarah A.; Salsburg, Andrew; Causey, Corey P.; Knuckley, Bryan; The development and characterization of a chemical probe targeting PRMT1. *Bioorganic & Medicinal Chemistry*. **2019**;27(1):224-229.
II. Nguyen, Hao C.; Wang, Min; Salsburg, Andrew; Knuckley, Bryan; Development of a Plate-Based Screening Assay to Investigate the Substrate Specificity of the PRMT Family of Enzymes; *ACS COMBINATORIAL SCIENCE*. **2015** 17(9):500-505
III. Maude, Tai S.; Davies, L.R.; Liu, R.P.; Harris, B.; Kocieński, S.A.; Aggeli, A. P.J.; Peptide synthesis and self-assembly. *Topics in current chemistry.* **2011** *310*, 27-69 .

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