A Mass Spectrometric Assay for METTL3/14 Methyltransferase Activity

S.M. Buker[1], Z.A. Gurard-Levin[2], B.D. Wheeler[3], M.D. Scholle[2], J.L. Hirsch[3], S. Ribich[1], R.A. Copeland[1], P.A. Boriack-Sjodin[1]

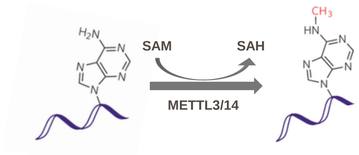
[1]Accent™ Therapeutics, Lexington, MA; [2]SAMDI Tech, Chicago, IL; [3]Confluence Discovery Technologies, St. Louis, MO

ACCENT™ THERAPEUTICS
Extraordinary Science → Life-Changing Therapies

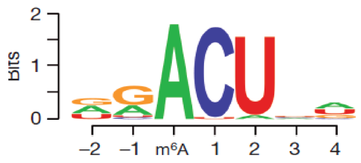
METTL3/14: A Type I RNA Methyltransferase

METTL3, the catalytically active subunit of the METTL3/METTL14 complex, belongs to the Type I methyltransferase superfamily[1] which uses S-adenosyl-L-methionine (SAM) as a substrate. METTL3/14 adds a methyl group to adenosine on a subset of mRNAs containing a specific sequence, most commonly 5'-GGACU-3' also known as a DRACH motif, to create N6-methyladenosine (m^6A)[2]

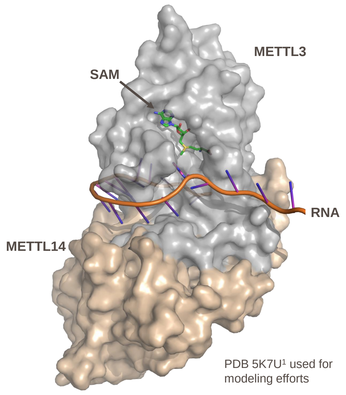
METTL3/14 Catalyzes the Reaction of A to m^6A



METTL3/14 Preferentially Methylates DRACH Sequences[2]



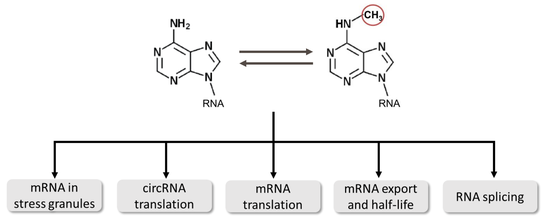
Model of mRNA Binding to METTL3/14-SAM Complex



PDB 5K7U[1] used for modeling efforts

m^6A and METTL3/14 are Implicated in Several Aspects of Cancer Biology

m^6A modulates mRNA turnover and controls the translation of key oncogenes that confer growth advantage and migratory behavior[3-5]. METTL3 is overexpressed in a subset of AML cell lines and m^6A is required for *in vitro* and *in vivo* growth in these cell types. METTL3 knockout also promotes AML differentiation and apoptosis[3-5]. Additionally, m^6A has been shown to be important for differentiation of multiple T-cell lineages, including Tregs[6] and CD4+ cells[7] which are involved in immunosuppression in tumor microenvironments[8].



Due to the increasing validation of m^6A as important in several aspects of cancer biology, there is significant interest in METTL3/METTL14 as a target for drug discovery efforts.

References

[1]Wang et al., Molecular Cell (2016)
[2]Dominissni et al., Nature (2012)
[3]Barbieri et al, Nature (2017)
[4]Vu et al, Nature Medicine (2017)
[5]Wang et al, Cell Stem Cell (2018)
[6]Tong et al., Cell Res (2018)
[7]Li et al., Nature (2017)
[8]Li et al., Nature Rev Immun (2016)
[9]Li, et al., J Biomol Screening (2016)
[10]Will et al., Blood (2015)
[11]Jacques et al., Biochemistry (2016)
[12]Basavapathruni et al., Biochemistry (2016)

Comparison of Radiometric and SAMDI Assay Formats

Radiometric Flashplate Assay

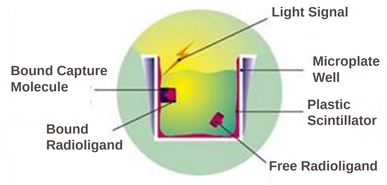
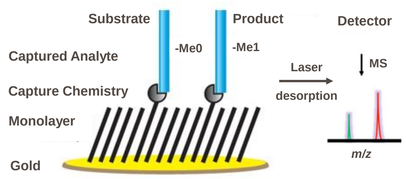


Image adapted from Perkin Elmer website

- "Gold standard" in methyltransferase assays
- 384 well plate format; readout detected by well rate limiting for throughput
- Radioactive assay (^3H SAM required)
- Literature precedent for METTL3 using 27-mer RNA[9]

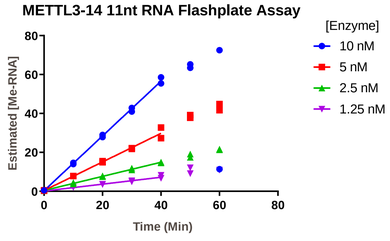
Self-Assembled Monolayer Desorption/ Ionization (SAMDI) Assay



- Assay measures both substrate and product mass
- 1536 plate format for detection; rapid readout by MALDI mass spec
- Precedent for methyltransferase enzymes[10-12]

11-mer RNAs are Suitable METTL3/14 Substrates for Assay Development

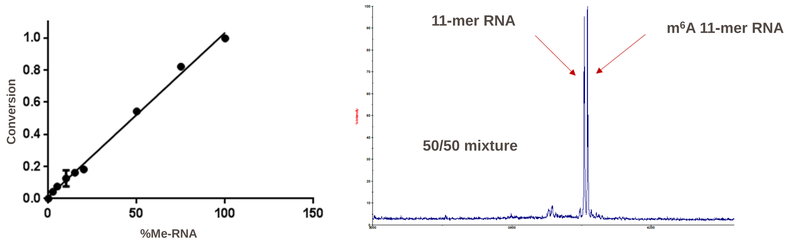
11-mer RNA is Active in METTL3/14 Radiometric Assay



Development of a MALDI compatible assay required identification of an RNA substrate active in the METTL3 biochemical assay for which both substrate and product ionized well (≤5000 Da; ≤15-mer) and had linear detection of product conversion. An 11-mer RNA containing a central DRACH sequence met both criteria:

5'-UCU**GGACU**AAA-3'-biotin

Detection Efficiency of 11-mer RNA is Amenable to MALDI Technology



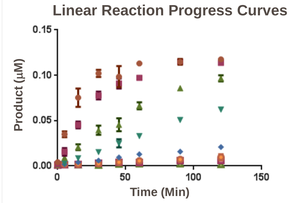
Conclusions

- Robust assays developed for METTL3/14 using two orthogonal assay technologies
- First example of SAMDI technology applied to RNA modifying proteins
- 11-mer RNA identified as a suitable substrate for METTL3/14
- Multiple assay formats provide flexibility in assessing compound screening options and opportunities for validation of chemical matter using orthogonal methods

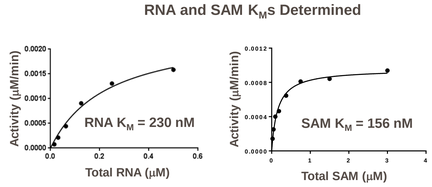
Radiometric and SAMDI Assays Developed in Parallel for METTL3/14

SAMDI Assay Developed for METTL3/14 with 11-mer RNA

Linear Reaction Progress Curves



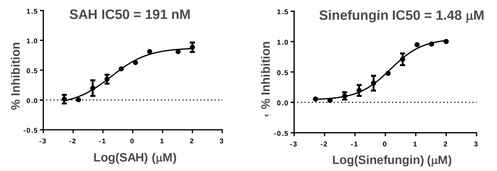
RNA and SAM K_Ms Determined



Good Plate Uniformity

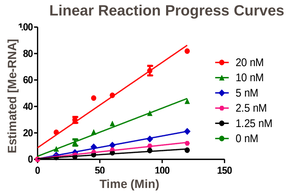
	Mean	SD	Z' Factor
Controls	0.327	0.020	0.756
100mM SAH	0.009	0.005	

Methyltransferase Inhibitor IC50s Measured

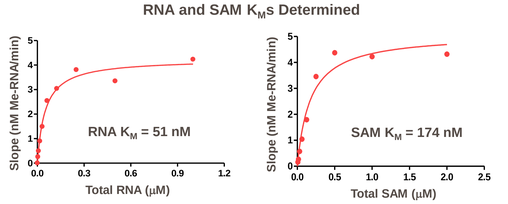


Radiometric Assay Developed for METTL3/14 with 11-mer RNA

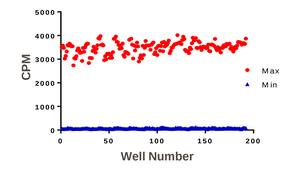
Linear Reaction Progress Curves



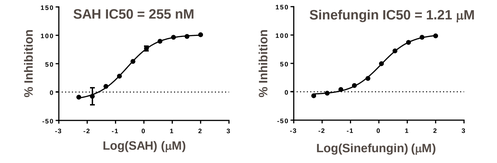
RNA and SAM K_Ms Determined



High Quality Data Obtained



Methyltransferase Inhibitor IC50s Measured



SAMDI and Radiometric Assays Determine Comparable Kinetic and IC50 Values

Parameter	SAMDI Assay (11-mer)	Accent Rad Assay (11-mer)	Literature Rad Assay[8] (27-mer)
SAM K_M	156 nM	174 nM	102 nM
RNA K_M	230 nM	51 nM	22 nM
SAH IC50	191 nM	255 nM	900 nM
Sinefungin IC50	1480 nM	1210 nM	Not measured

Good correlation seen for inhibitor IC50 values from orthogonal assay formats with same RNA substrate

Acknowledgements

The authors would like to thank Dr. Allen Sickmier at Accent for help with METTL3/14-RNA modeling efforts