Protein Arginine Allylation and Subsequent Fluorophore Targeting

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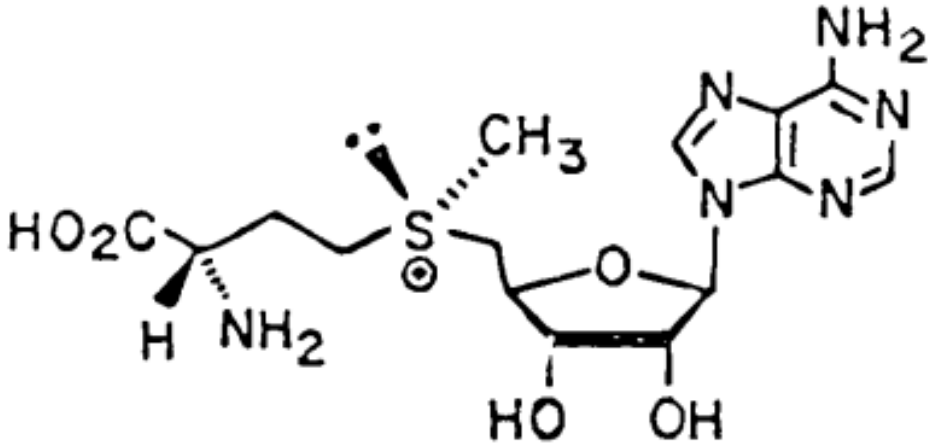
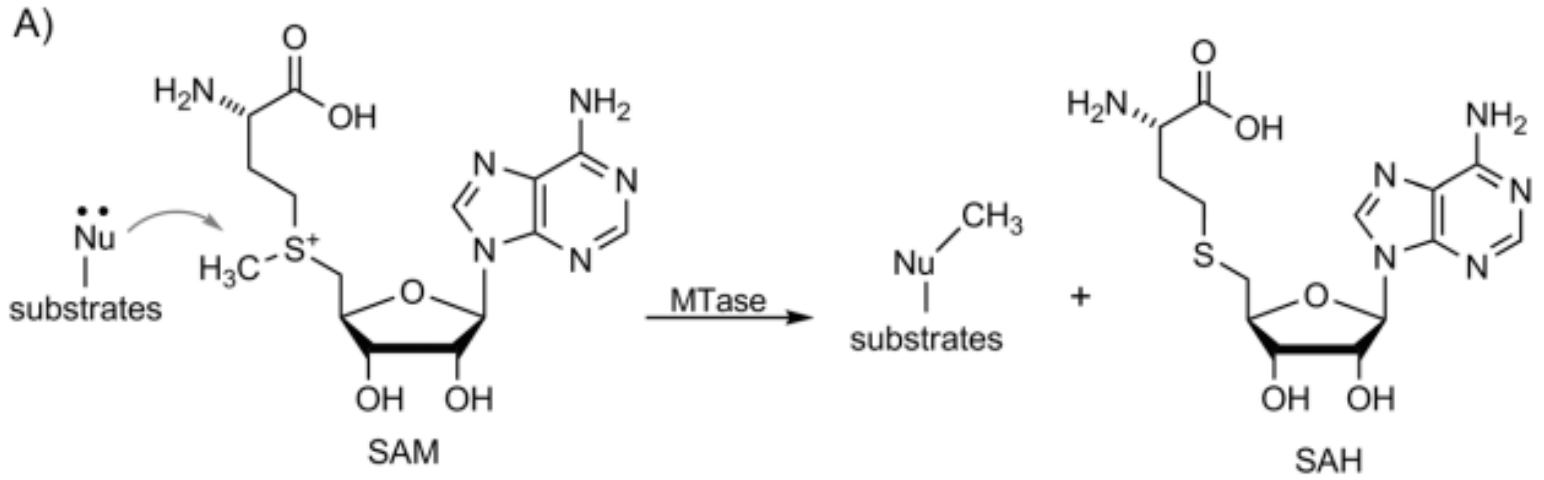
S-Adenosyl-L-Methionine (SAM)

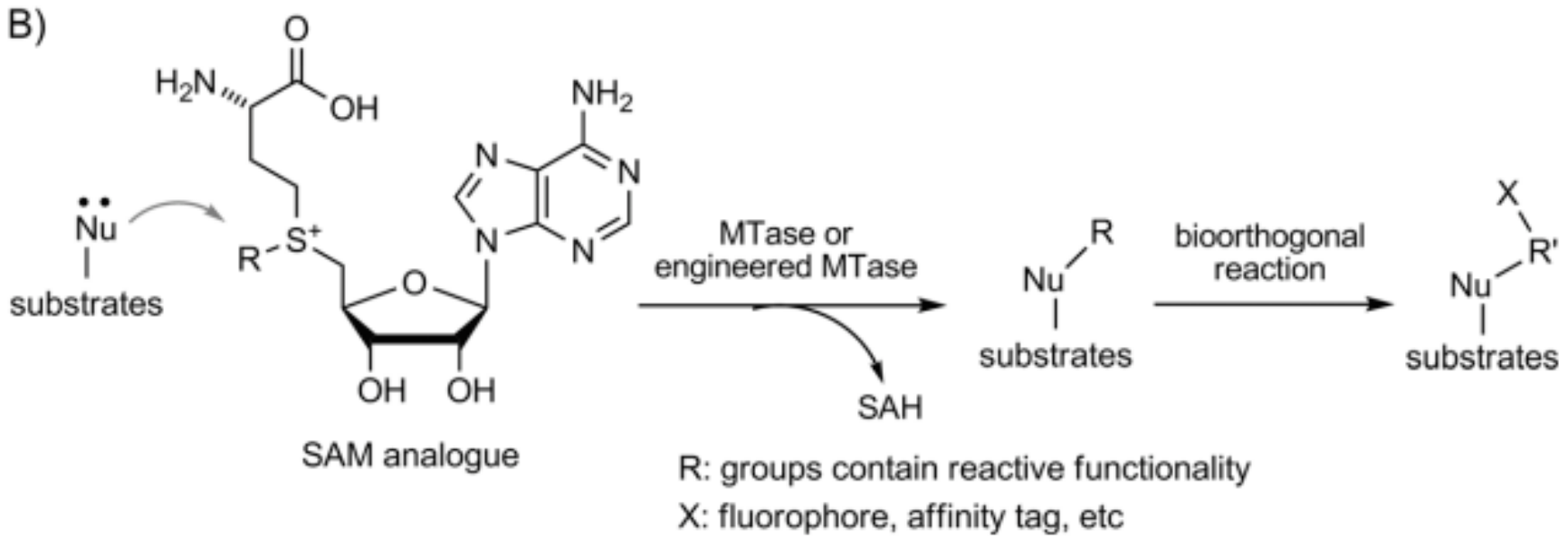


1. Transmethylation
2. Transaminopropylation
3. Transulfur

MTase-catalyzed Transmethylation from SAM



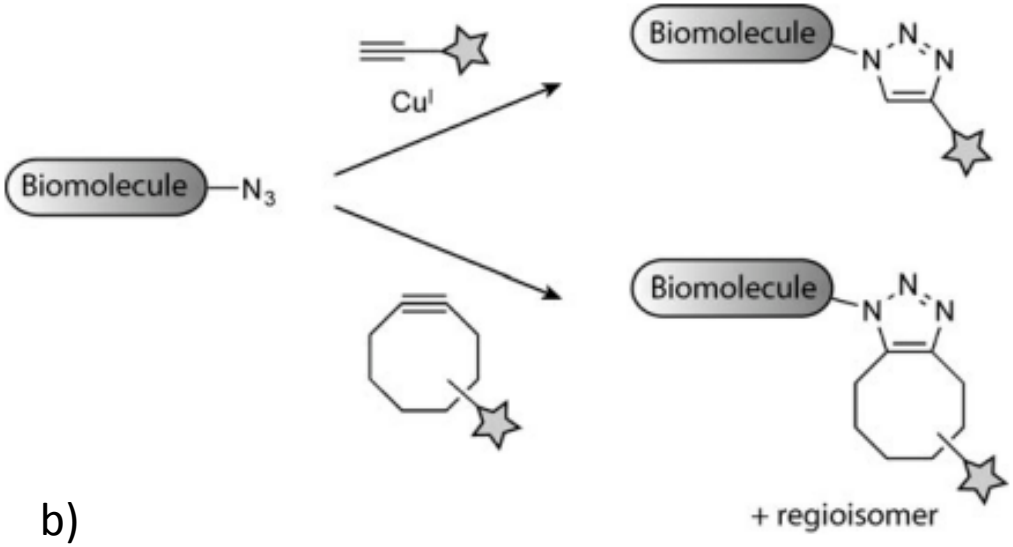
Chemical Biology Strategy for Labeling MTase Substrates with SAM Analogues



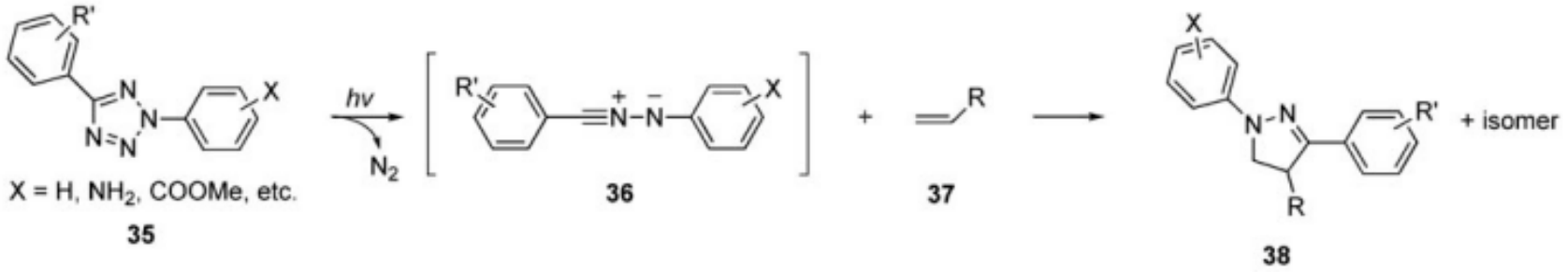
Zhang, Y. X.; Pan, Y. B.; Yang, W.; et al. *ChemBioChem* **2013**, **14**: 1438-1443

Bioorthogonal Reactions

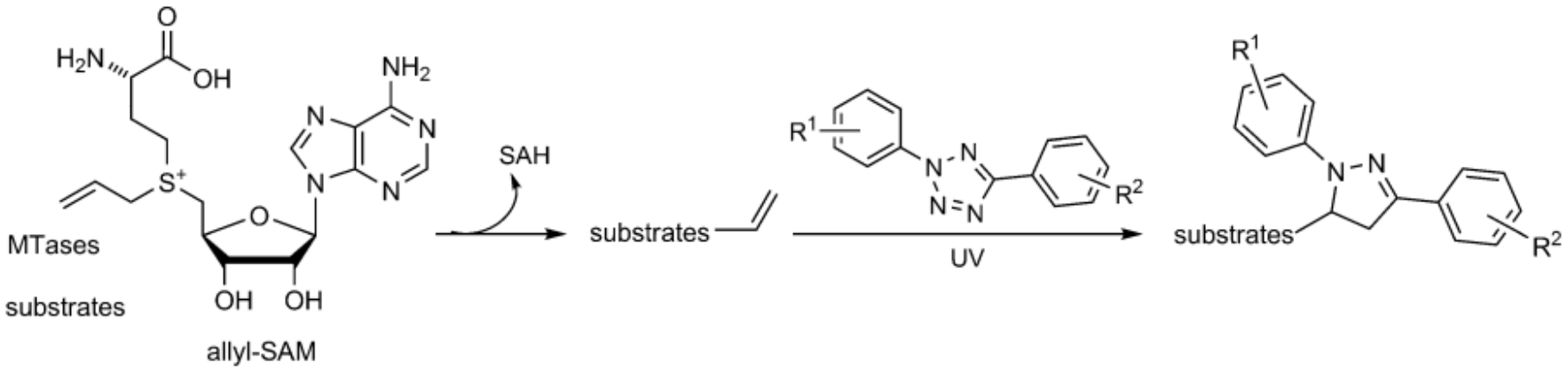
a)



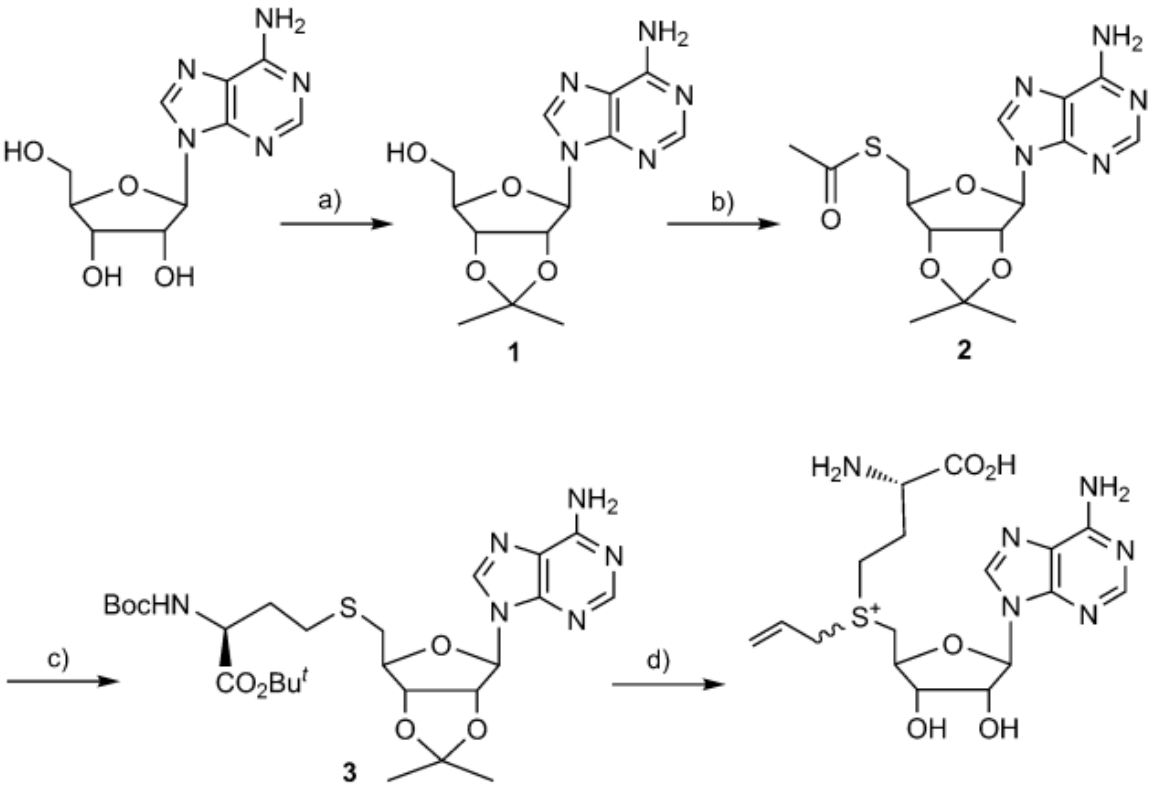
b)



Strategy for Fluorophore-targeting MTase Substrates



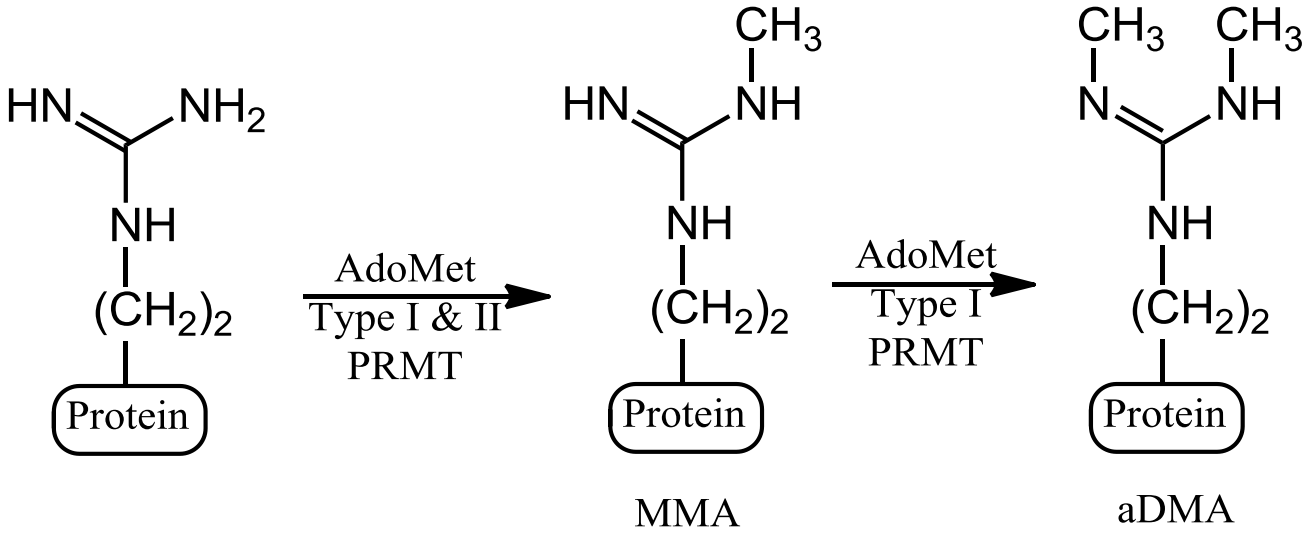
Synthesis of Allyl-SAM



a) *p*-TsOH, acetone, room temperature, 95 %;
b) PPh_3, DEAD, AcSH, THF, -10^{0}C then 0^{0}C, 77 %;
c) *N*-Boc-g-tosyl-homoserine-*tert*-butylester, CH_3ONa, MeOH, -20^{0}C then room temperature, 32%;
d) allylbromide, $AgClO_4$, 0^{0}C then room temperature, 55 %.

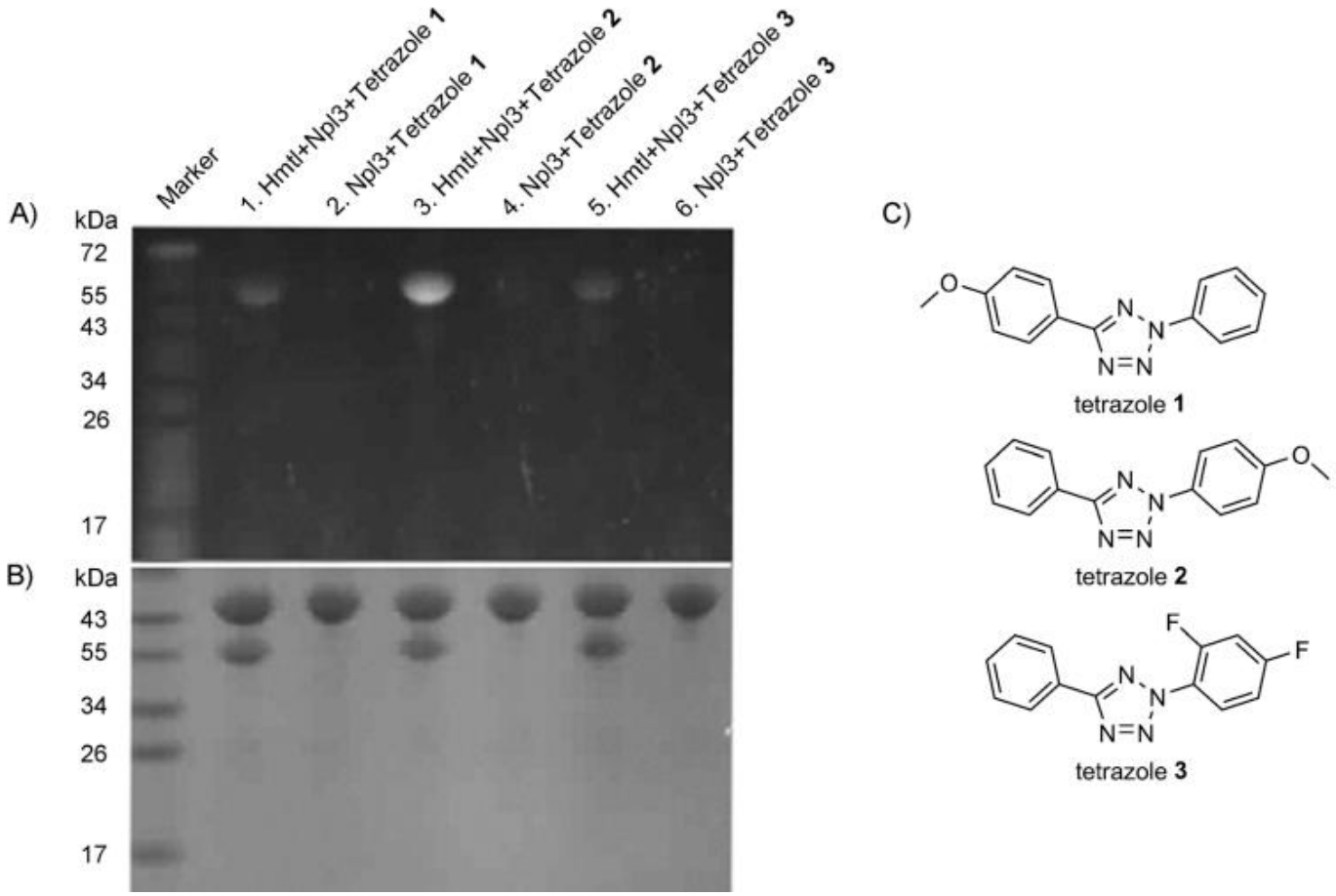
Hmt1 and Npl3

- Hmt1 (Type I PRMT)



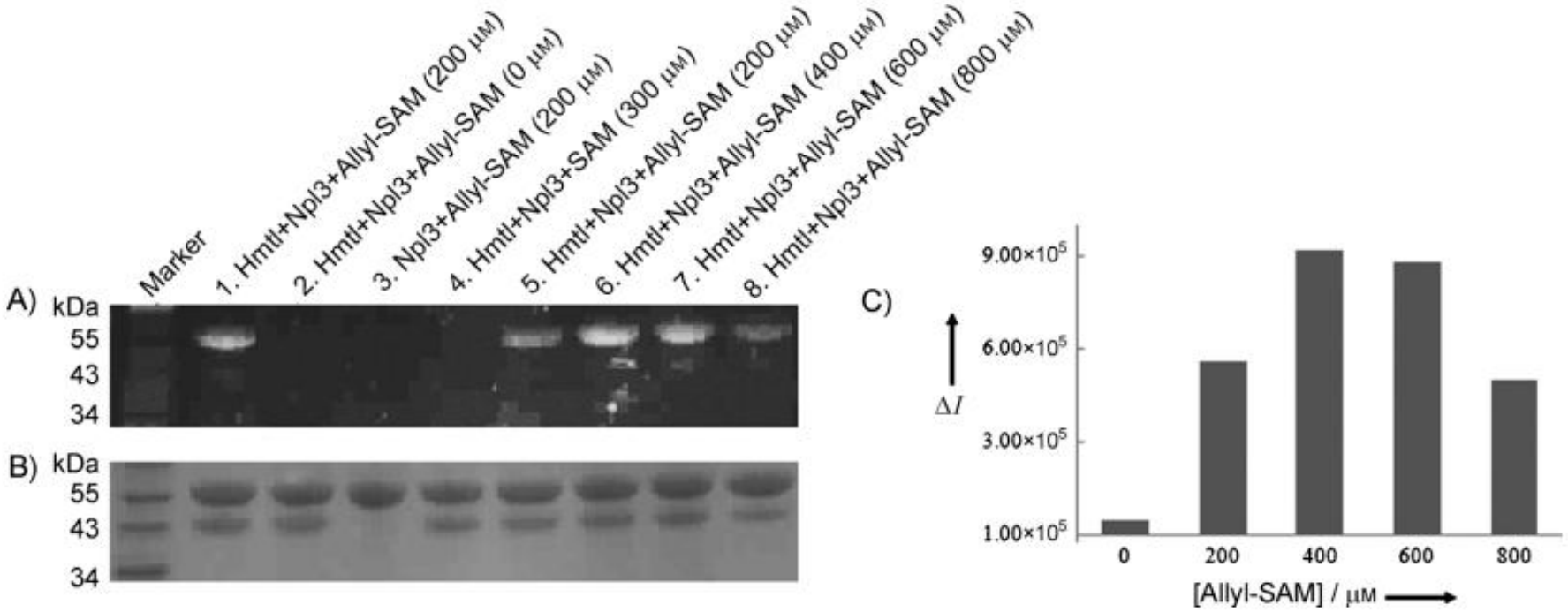
- Npl3 is a yeast mRNA-binding protein that has up to 17 potential arginine methylation sites.

SDS-PAGE Analysis of Hmt1-catalyzed Allylation of Npl3



A) Fluorescence image, B) Coomassie Blue staining, C) Structures of tetrazole compounds used in the photo-click reaction

Allyl-SAM Concentration Dependence of Hmt1-catalyzed Allylation of Npl3



A) Fluorescence image, B) Coomassie Blue staining, C) Relative fluorescence intensity (DI) of allylated Npl3 samples by using different concentrations of allyl-SAM.

Allylation and Methylation Sites of Npl3 Based on LC–MS/MS Analysis and Literature

	Modification site[a]	Sample 1[b] Run 1[c]	Run 2	Sample 2 Run 1	Run 2	Allylation state	Methylation state
1	Arg284	–	–	–	M	M	M
2	Arg288[d]	–	–	–	–	–	M
3	Arg290[d]	–	–	–	–	–	D
4	Arg294	M	D	–	D	M/D	D
5	Arg298	M	D	–	D	M/D	D
6	Arg302	D	D	M	M	M/D	D
7	Arg307	M	–	M	M	M	D
8	Arg314	D	M	–	D	M/D	D
9	Arg321	M	M	D	M	M/D	D
10	Arg329	M	M	D	M	M/D	D
11	Arg337[d]	–	–	–	–	–	D
12	Arg344[d]	–	–	–	–	–	M
13	Arg351	–	–	–	D	D	M
14	Arg358	M	M	M	D	M/D	M
15	Arg363	D	M	–	–	M/D	D
16	Arg370	M	D	D	M	M/D	–
17	Arg377	D	D	D	M	M/D	M
18	Arg384	D	M	–	M	M/D	M
19	Arg391	D	D	M	–	M/D	–

[a] Location of alkyl–arginine residues in Npl3 was shown as below. RSNR^{284}GGFR^{288}GR^{290}GGFR^{294}GGFR^{298}GGFR^{302}GGFSR^{307}GGFGGPR^{314}GGFGGPR^{321}-GGYGGYSR^{329}GGYGGYSR^{337}GGYGGSR^{344}GGYDSPR^{351}GGYDSPR^{358}GG YSR^{363}GGYGGPR^{370}NDYGPPR^{377}GSYGGSR^{384} GGYDGPR^{391}GDYGPPRDA. [b] Two sets of parallel samples were evaluated with Hmt1 (10 μM), Npl3 (10 μM), and allyl-SAM (400 μM). [c] All samples were run twice in parallel by LC–MS/MS; –/M/D represent no/monoalkyl/dialkyl modification. [d] Allylation of Arg288, Arg290, Arg337, and Arg344 was not observed in these sets of samples but were detected in the other samples when using different amounts of allyl-SAM.

Summary

- The authors developed an alternative procedure for the preparation of allyl-SAM
- arginine residues of Npl3 were extensively modified by a Hmt1-catalyzed allylation reaction with allyl-SAM.
- The allylated protein was further labeled by using a photoinducible cycloaddition reaction, leading to formation of protein-attached fluorescent products.

Technique: Creation of SAM Analogues

- High Sensitivity.
- High throughput, but only for screening one methyltransferase at a time against many substrates.
- Relative quantification.
- Safe and easy to visualise.
- Need to develop an analogue for each PMT; this process can be time-consuming.
- Methylation sites cannot be directly localised.