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

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Bioorthogonal Reactions

a) 

Biomolecule $\text{-} \text{N}_3$

\[ \text{Cu}^+ \]

$\rightarrow$

Biomolecule

$\rightarrow$

Biomolecule

$+ \text{regioisomer}$

b) 

$\text{N}_2$

$\rightarrow$

$35$

\[ \rightarrow \left[ R' \text{-} \text{N} \equiv - C \equiv + \text{N} \equiv - \text{N} \equiv R \right] \]

$36$

$\rightarrow$

$37$

$\rightarrow$

$38$

$\text{X = H, NH}_2, \text{COOMe, etc.}$

$\text{36}$

$\text{37}$

$\text{38}$

$\text{+ isomer}$
Strategy for Fluorophore-targeting MTase Substrates
Synthesis of Allyl-SAM

a) p-TsOH, acetone, room temperature, 95 %;
b) PPh₃, DEAD, AcSH, THF, -10°C then 0°C, 77 %;
c) N-Boc-g-tosyl-homoserine-tert-butylester, CH₃ONa, MeOH, -20°C then room temperature, 32%;
d) allylbromide, AgClO₄, 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

<table>
<thead>
<tr>
<th>Modification site[a]</th>
<th>Sample 1[b]</th>
<th>Sample 2[b]</th>
<th>Allylation state</th>
<th>Methylation state</th>
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<tbody>
<tr>
<td></td>
<td>Run 1[c]</td>
<td>Run 2</td>
<td>Run 1</td>
<td>Run 2</td>
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<tr>
<td>1</td>
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<td>–</td>
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</table>

[a] Location of alkyl–arginine residues in Npl3 was shown as below. RSNR284GGFR288GR290GGFR294GGFR298GGFR302GGFSR307GGFGGPR314GGFGGPR321GGYGGYSR329GGYGGYSR337GGYGGSR344GGYDSPR351GGYDSPR358GGYSR363GGYGGPR370NDYGPRR377GSYGGRSR384GGYGPR391GDDGPRRD. [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.