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Reversible modification of DNA by methyltransferase-catalyzed transfer and light-triggered removal of photo-caging groups†

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Methyltransferases are powerful tools for site-specific transfer of non-natural functional groups from synthetic analogs of their cosubstrate *S*-adenosyl-L-methionine (AdoMet). We present a new class of AdoMet analogs containing photo-caging (PC) groups in their side chain, enzymatic transfer of PC groups by a promiscuous DNA MTase as well as light-triggered removal from the target DNA. This strategy provides a new avenue to reversibly modulate the functionality of DNA at MTase target sites.

Methyltransferases (MTases) modify several classes of biomolecules post-synthetically and modulate their biological function. In many cases, methylation is dynamic and reversible, indicating a regulatory function, as well documented for histone methylation in epigenetics.^{1,2} For DNA, the importance of reversible methylation (m⁵C) for controlling transcriptional activity has recently emerged.^{3,4} In mRNA, the most abundant internal modification was found to be a reversible methylation (m⁶A) with functional implications in mRNA metabolism and translation.⁵

The cosubstrate *S*-adenosyl-L-methionine (AdoMet) serves as nature's methyl donor and synthetic analogs with extended allylic and propargylic groups have proven useful for transferring numerous functional side-chains to specific sites of MTase substrates. The powerful combination of promiscuous or engineered MTases and AdoMet analogs led to a new method for site-specific derivatization of DNA,^{6,7} RNA,^{8–13} proteins,^{7,14,15} and small molecules.¹⁶ Another class of AdoMet analogs, in which the amino acid side chain is replaced by an aziridine, enabled the transfer of the entire cosubstrate.¹⁷ By extending the adenine at the 8 position, transfer of biotin or fluorescent labels directly to DNA was achieved.^{17,18} This approach was applied to optically map individual, long DNA molecules and DNA-binding proteins¹⁸ as well as to identify bacteriophage strain types.¹⁹ In the field of

RNA, AdoMet analogs were used for fluorescent labeling of tRNA and mRNA but also the transfer of photo-crosslinkers and affinity labels was reported.^{8,9,13,20–22}

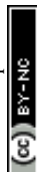
However, while methylation in nature is often a regulatory process that can be reverted, reversibility of the enzymatically introduced non-natural groups has not been reported, impeding our abilities to study the important dynamic aspects of enzymatic modifications and the downstream processes. On the other hand, light is orthogonal to most biological processes and can be used as trigger to remove photo-caging (PC) groups.²³ Oligonucleotides with PC groups have opened the field of optochemical biology, providing a new level of spatio-temporal control in processes at all levels of gene expression, as exemplified by photo-caged siRNAs, plasmids, triplex forming oligonucleotides and aptamers.^{23,24} To date, PC groups are usually installed during chemical synthesis of oligonucleotides and require a combination with ligation strategies to access large nucleic acid molecules such as plasmids in order to bring them under control of light.²⁵

Inspired by the enormous potential of optochemical biology, we thought that MTase-catalyzed introduction of PC groups might provide a straightforward route to modify biomacromolecules post-synthetically and reversibly. We therefore designed and synthesized AdoMet analogs for the enzymatic transfer of typical PC groups. Specifically, we appended the *o*-(ONB) and *p*-nitrobenzyl-(PNB), the 4-acetyl-2-nitrobenzyl-(ANB), and the 2-anthraquinonyl-residues (ANT) to *S*-adenosyl-L-homocysteine, yielding AdoMet analogs **1a–d**, termed AdoONB, AdoPNB, AdoANB and AdoANT, respectively (Fig. 1A). For synthesis of the four AdoPCs, *S*-adenosyl-L-homocysteine was reacted with the corresponding bromides, which were synthesized as described previously (Scheme S1, Fig. S1 and S2, ESI†).^{26,27} All of those AdoMet analogs contain aromatic ring systems as side-chains, which are bulky and might be incompatible with several MTases. However, our recent work on RNA- and DNA-MTases demonstrated that (i) many highly promiscuous MTases are suitable for this purpose and (ii) the benzyl group promotes enzymatic transfer by promiscuous MTases.²⁸ In fact, the PNB group was more efficiently transferred from AdoPNB than the

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of AdoMet analogs containing PC-groups in their side-chain and demonstrated transfer to MTase-target sites of DNA. As expected, PC groups block enzymatic cleavage of plasmid DNA by restriction enzymes. Upon irradiation with light, two of these groups – namely the *o*-nitrobenzyl- and the 4-acetyl-2-nitrobenzyl groups – are efficiently removed and the DNA is again accessible for restriction enzymes. By combining enzymatic transfer, synthetic cosubstrates and photo-caging groups, our strategy presents a new avenue for the reversible modulation of biological functions of DNA that can be readily extended to other biomacromolecules such as RNA and proteins.

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Conflicts of interest

There are no conflicts to declare.

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