Selective chemical functionalization of N^6-methyladenine in DNA

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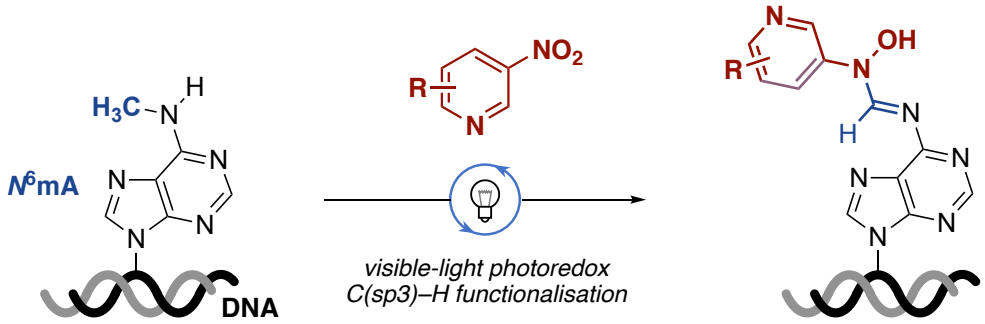

Selective chemical reactions on nucleobases have been key driving forces for the study of base modifications in DNA and RNA.[1] Methylation at adenine N^6 to form N^6-methyladenine (N^6mA) is one of the the most abundant modified bases in mammalian transcriptomes as well as in bacterial genomes. A growing number of studies suggest its presence and potential regulatory roles in the DNA of mammals including humans,[2,3] but the available methods for its detection are not always reliable.[4]

The aminomethyl group in N^6mA being a unique feature in eukaryotic genomes, we explored possibilities to chemoselectively functionalise N^6mA in DNA strands. We were inspired by the dioxygenases responsible for N^6mA demethylation *in vivo*, operating *via* hydrogen abstraction from the N^6-methyl group to form an intermediate 'on-DNA' radical species. Relying on a visible-light-mediated photoredox process to generate a hydrogen abstracting species as well as a radical acceptor, we were able to selectively form an 'on-DNA' radical at N^6mA and intercept this intermediate with the *in-situ* formed radical acceptor.[5]

We further developed an alkynylated probe for downstream functionalisation and demonstrated that we could biotinylate N^6mA in longer single-stranded and double-stranded DNA. This allowed us to enrich for N^6mA-containing DNA fragments from complex DNA mixtures. This work sets the base to further development for chemistry-based methods to map N^6mA in nucleic acid strands.

[1] A. Hofer, Z. J. Liu, S. Balasubramanian, *J. Am. Chem. Soc.* **2019**, *141*, 6420–6429.
[2] Z. Hao *et al.*, *Mol. Cell* **2020**, *78*, 382-395.E8.
[3] Z. Li *et al.*, *Nature* **2020**, *583*, 625–630.
[4] K. Douvlataniotis *et al.*, *Sci. Adv.* **2020**, *6*, eaay3335.
[5] M. Nappi‡, A. Hofer‡, S. Balasubramanian*, M. J. Gaunt*, *J. Am. Chem. Soc.* **2020**, *142*, 21484–21492.