ENZYME-DIRECTED LABELLING OF DNA

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Our work focusses on the application of enzymes as molecular machines that can deliver targeted modifications to the DNA molecule. We apply this chemistry as a tool for microscopy and particularly single-molecule and super-resolution imaging of DNA.

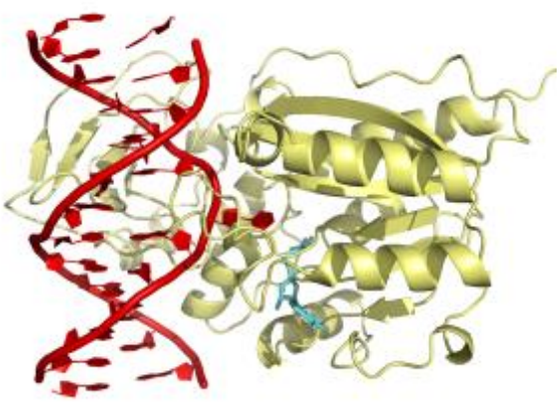

Figure 1- The HhaI DNA methyltransferase enzyme bound to its target recognition sequence (5'-GCGC-3') and the cofactor analogue s-adenosyl-L-homocysteine.

The DNA methyltransferases direct sequence-specific DNA labelling[1] and can be used to create maps, or barcodes, of the DNA molecule. We have found that a subset of enzymes, such as that shown in Figure 1, are capable of catalysing the transfer of groups larger than –methyl to the DNA[2] and that the most malleable of all the enzymes tested is M.TaqI, which targets adenine within the four-base sequence, 5'-TCGA-3', for modification. We show that M.TaqI is capable of the direct transfer of both chemically reactive groups and select fluorophores to DNA. We demonstrate a simple route to a range of enzyme-catalysed modifications.

We are applying this approach for fluorescent DNA labelling in a range of applications from DNA mapping- a technology for rapid identification of species using their genomes- to the development of new probes for fluorescence in-situ hybridisation and other molecular diagnostic tests.

Here, we present an overview of the chemistry we use to label the DNA molecule and its emerging application in the direct visualization of the DNA sequence using super-resolution microscopy.

[1] Dalhoff, C., Lukinavicius, G., Klimasauskas, S. and Weinhold, E., Direct transfer of extended groups from synthetic cofactors by DNA methyltransferases, *Nat Chem Biol*, **2**, 31–32 (2006).
[2] Vranken, C., Deen, J., Dirix, L., Stakenborg, T., Dehaen, W., Leen, V., Hofkens, J. and Neely, R.K., Super-resolution optical DNA Mapping via DNA methyltransferase-directed click chemistry, *Nucleic Acids Res.*, **42**, e50–e50 (2014).