REVERSIBLE FLUORESCENCE PHOTOSWITCHING IN DNA: APPLICATIONS IN ADVANCED FLUORESCENCE MICROSCOPY

Darren A. Smith,1 Philipp Holliger,2 Cristina Flors1,3
1EaStChem School of Chemistry, University of Edinburgh, Edinburgh EH9 3JJ, United Kingdom; 2MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom; 3IMDEA Nanociencia, Madrid, Spain
E-mail: cristina.flors@imdea.org

KEYWORDS: photoswitching, super-resolution, optical lock-in detection, DNA, high density labelling

Fluorescence photoswitching is at the core of newly developed fluorescence microscopy techniques, such as super-resolution fluorescence microscopy [1] and optical lock-in detection imaging (OLID) [2]. In these techniques, the ability to label the structures of interest with photoswitchable or photoactivatable fluorophores in high density is critical. While this is easily achievable for proteins, labelling DNA with a high density of photoswitchable fluorophores is still a challenge. Our previous work has shown that photoblinking of intercalating and minor-groove binding cyanine dyes can be used to image isolated and cell DNA using super-resolution microscopy [3-5]. This approach yields super-resolution images of unspecifically stained DNA with a spatial resolution below 40 nm. However, sequence specificity and reversible photoswitching are two desirable properties that would extend the scope of super-resolution imaging of DNA and other advanced techniques. We will show that these two properties can be realized using a new strategy. High density labelling of DNA with cyanine dyes can be achieved by polymerase chain reaction using a modified DNA polymerase that has been evolved to efficiently incorporate Cy3- and Cy5-labeled cytosine base analogues into double stranded DNA, resulting in a new biopolymer termed CyDNA [6]. We have been able to engineer reversible fluorescence photoswitching in CyDNA by using the properties of the Cy3-Cy5 pair, and we show that this strategy can be exploited in OLID. This work also lays the foundations for improved and sequence-specific super-resolution fluorescence microscopy of DNA, with important implications in the study of chromosome structure [7].