Controling the orientation of single fluorescent dyes using the DNA origami technique

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Abstract
Over the last decade, DNA nanotechnology has been increasingly used to self-assemble functional nanostructures. One of the main advantages of this approach is that different species including high quality colloidal nanoparticles and single photon emitters such as fluorophores can be positioned with nm precision and stoichiometric control [1]. In order to fully manipulate the interaction between these species, a key factor for mthe development of nanophotonic devices, it is necessary to not only control their relative position but also their relative orientation. We have recently shown that fluorophore incorporation to DNA origami structures by a covalently bond to the single stranded DNA (DNA Staple) using a single anchoring point leads to a broad distribution of orientations [2]. Alternatively, some fluorophores such as Cy5 can be covalently incorporated to the DNA staples through two anchoring points at the ends. Therefore, fluorophores incorporated in this way could be "stretched" by "pulling" from the ends and thus deterministically oriented along the main DNA double helix. In this work, we study the orientation of Cy5 fluorophores incorporated in this way using two independent measurements carried out in a standard wide-field fluorescence microscope [2]. First, the orientation of the absorption transition dipole of the molecule is determined through a polarization-resolved excitation measurement. Second, the orientation of the DNA origami structure is obtained from a DNA-PAINT nanoscopy measurement (Figure 1). Our results show that single fluorophores attached with two anchoring points can adopt different orientations on a DNA-origami, from perpendicular to aligned with the double-helix depending on the number of bases removed from the complimentary sequence (Figure 2). We consider that this work is a first step towards the orientation control of single fluorophores in DNA origami structures, that will enable the development of more efficient and reproducible self-assembled nanophotonic devices.

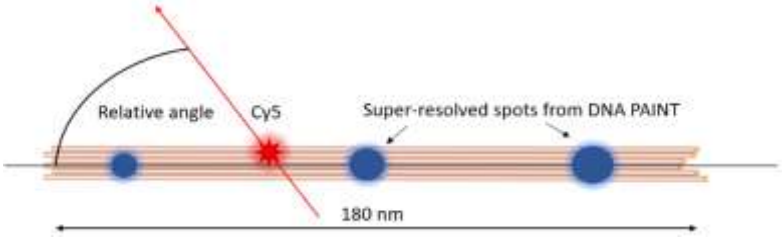

Figure 1. DNA origami sketch with incorporated fluorophore and 3 super-resolved spots from the DNA PAINT measurements. The relative angle is an angle between the DNA origami structure and the transition dipole moment of the Cy5.

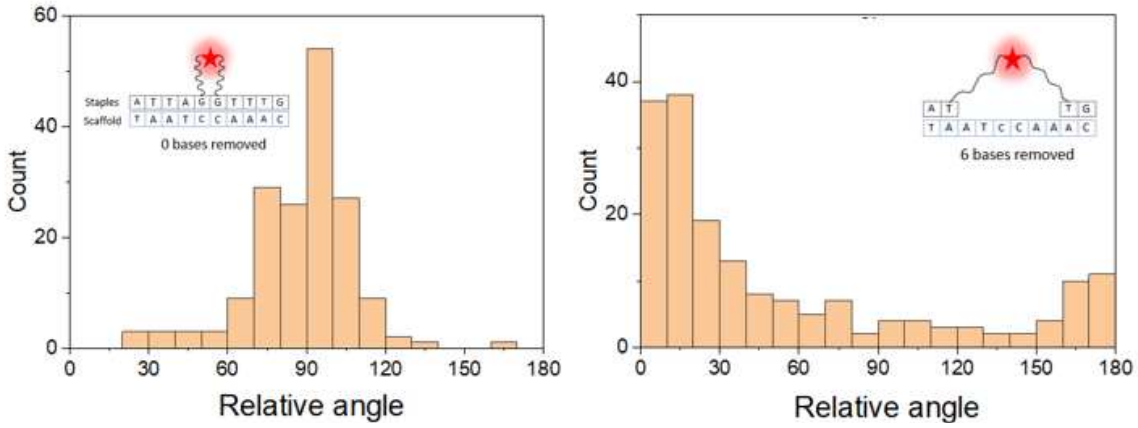

Figure 2. In plane orientation of the excitation transition dipole moment of Cy5 with respect to the DNA origami structure for two different samples where no bases (left) or 6 bases (right) were removed from the sequence and a fluorophore was attached covalently with two anchoring points.

References

[1] Kuzyk, A. et al., ACS Photonics, 5, 1151 (2018), [2] Hübner, K. et al., ACS Nano (2021).