Fluorescence correlation spectroscopy measurements of DNA unwinding/bending fluctuations with DNA backbone-incorporated dyes.

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Sequence-dependent DNA deformability and dynamics are important factors in how DNA-binding proteins interact and bind to their target sites on the DNA. Numerous studies have reported measurements of DNA conformations and dynamics with FRET labels attached to the DNA with linkers. However, attributing changes in FRET to changes in DNA conformations can be complicated because of the difficulty in separating DNA dynamics from linker/dye dynamics. Recently, we successfully measured unwinding/bending fluctuations in DNA oligomers labeled with the FRET pair ATTO550-ATTO647N placed on either side of a 3 base-pair mismatched site (Ten et al. (2022), J. Biol. Phys. 48:253-272). Equilibrium FRET measurements showed higher FRET in mismatched compared with matched DNA, indicating a more bent/unwound conformation in the presence of the mismatch. Fluorescence correlation spectroscopy (FCS) revealed ~100-300 μs dynamics on mismatched DNA with no dynamics detected for matched DNA. However, fluorescence lifetime measurements on these constructs showed multiple FRET states even for the matched DNA, which we attributed to the likelihood of these positively charged ATTO dyes stacking/unstacking against the DNA. We next tried replacing ATTO550 by the negatively charged ATTO532 as the donor to promote conditions more conducive to free rotation of the attached dye. Surprisingly, with this dye pair we lost the ability to detect any conformational differences between matched and mismatched DNA, and FCS with this pair showed no dynamics on either construct. Here, we explore FRET/FCS measurements using Cy3-Cy5 pair incorporated within the DNA backbone. These rigidly stacked dyes were shown to reduce uncertainty in FRET because of fixed orientation within the DNA molecule (Ranjit et al. (2009), J. Phys. Chem. B 113:7861-7866). Accordingly, we anticipate higher sensitivity in their ability to report on DNA conformational dynamics with FCS.