The ultimate limit for DNA detection

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Background

We present a novel approach to **increase the detection sensitivity** of trace amounts of **DNA** in a sample by employing a combination of multi-pulse pumping, time gated detection and Förster Resonance Energy Transfer (FRET) between intercalating dyes. **Two intercalators** that present efficient FRET were used to enhance sensitivity and improve **specificity** in detecting minute amounts of DNA. Comparison of steady-state acceptor emission spectra with and without the donor allows for simple and specific detection of DNA (acceptor bound to DNA) down to **10 pg/µl**.

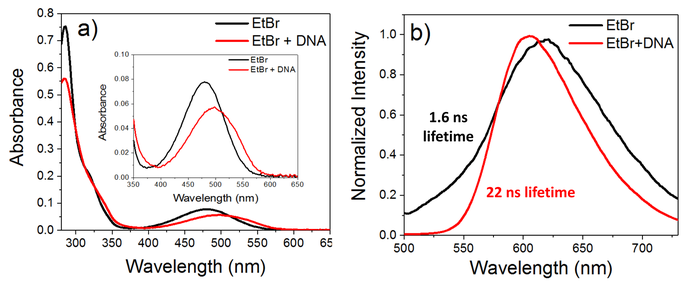


Figure 1. Absorption (a) and normalized emission (b) spectra of free and bound to DNA EtBr.

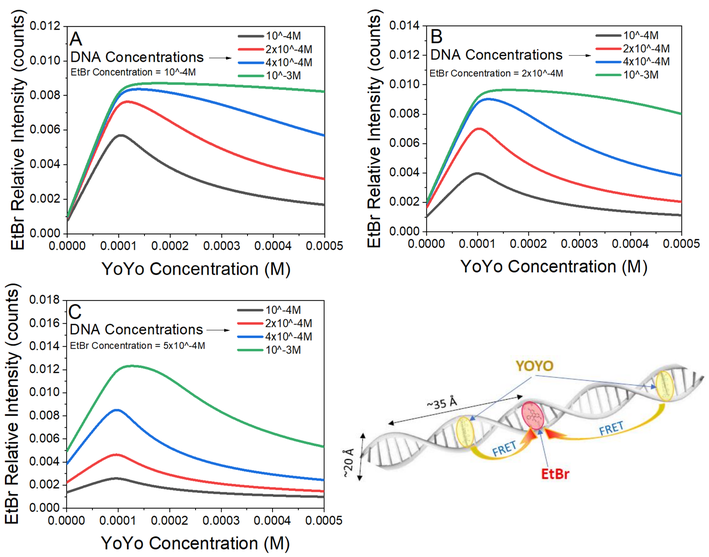


Figure 2. Expected EtBr fluorescence signal measured as a function of YOYO concentration for different concentrations of DNA and EtBr, and schematic of the DNA with EtBr and YOYO intercalated.

Experimental Results

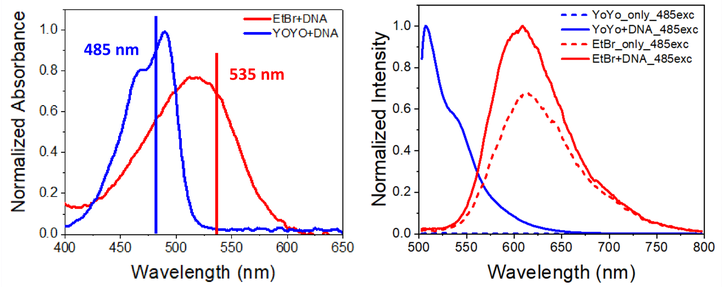


Figure 3. Absorption spectra of fully saturated EtBr and YOYO, and normalized emission spectra of YOYO and EtBr free and with DNA.

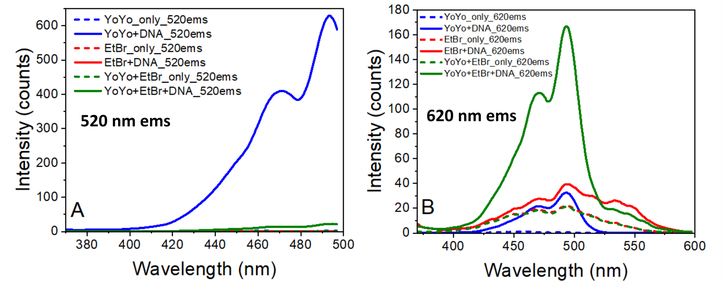


Figure 4. Excitation spectra of YOYO free and bound, EtBr free and bound, and their composition measured at 520 nm and 620 nm observation.

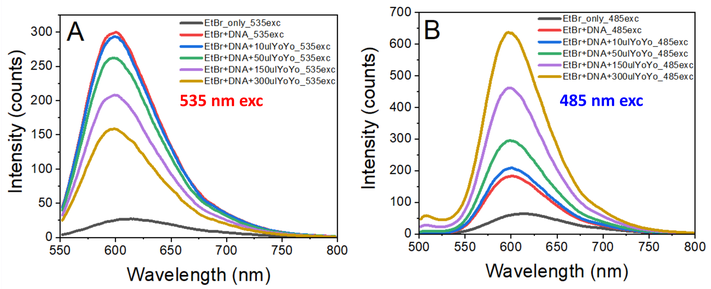
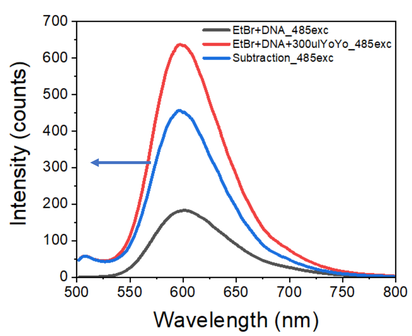


Figure 5. Emission spectra of EtBr and DNA with increasing amounts of YOYO, excited at 535 nm and 485 nm.



Subtracted spectrum = fully bound EtBr

Adding YOYO increases only the signal of bound EtBr

Figure 6. Emission spectra of EtBr and DNA, EtBr, DNA and YOYO, and their subtraction.

Reaching the limit

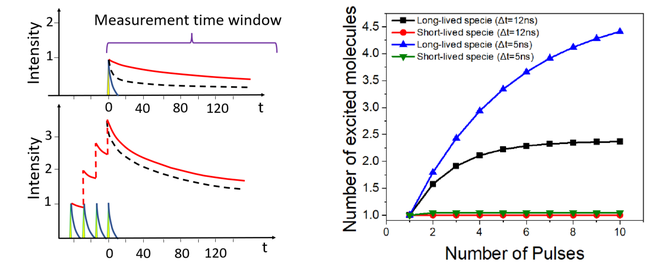


Figure 7. Schematic of multi-pulsing approach and expected number of excited molecules vs. number of pulses.

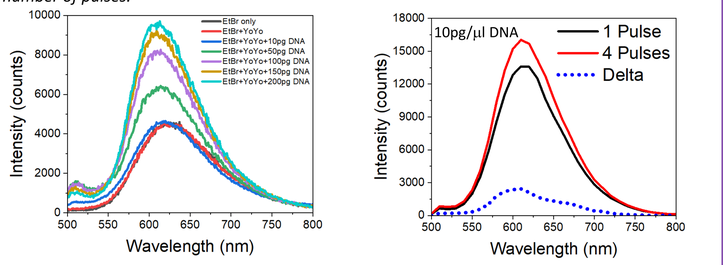


Figure 8. Emission spectra of EtBr and DNA with increasing amounts of YOYO and spectra obtained by "pulses subtraction" on a solution with 10 pg/µl DNA.

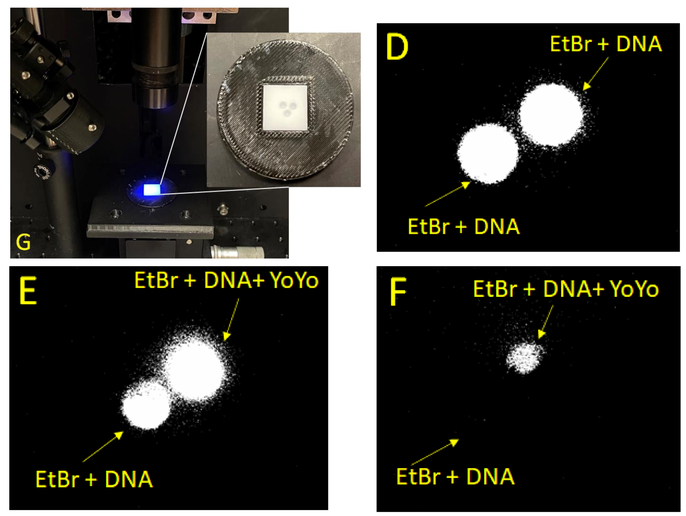


Figure 9. Images obtained with a solution of EtBr and DNA (D), EtBr, DNA and YOYO (E) and their subtraction (F).

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