

## **Using fluorescence as a tool for studying biomolecular dynamics**

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### **ABSTRACT:**

Fluorescence of an organic fluorophore, when observed at the single molecule level, can give information on dynamic heterogeneities and intermediate stages in biological reactions, which are otherwise lost to ensemble averaging in bulk experiments. Utilizing this idea, we have studied DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) hybridization using cyanine fluorophore probes tagged at one end of one strand of double stranded DNA/RNA structures. A fluorescence microscope in the Total Internal Reflection (TIRFM) geometry was used to quantify fluorescence dynamics with 30 milli seconds time resolution, with the help of an emCCD camera. The TIRFM geometry resulted in a good signal to background ratio of fluorescence signal when compared to a normal bright-field geometry. We find sequence sensitive quenching of fluorescence of the cyanine dyes tagged to DNA/RNA when the complementary strand has a Guanine base in it. By tracking fluorescence fluctuations, the base-pairing breathing dynamics at room temperature as well as fluorescence quenching could be quantitatively analyzed, as well as heterogeneities tracked. We also present comparative studies with cyanine dye tagged nucleic acids where the fluorescence is not quenched, and propose some simple applications as biomolecular sensors by making use of this single molecule fluorescence quenching protocol.