Towards employing fluorescence anisotropy to measure the binding constant of hybridizing oligonucleotide DNA strands

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Fluorescence anisotropy provides a sensitive tool to measure the binding constant between two interacting molecules and has been used in case of ligand-protein or protein-DNA interaction. Here we use fluorescence anisotropy to determine the binding constant between two DNA strands. One of them is labeled with a fluorophore. The fluorophore is excited with polarized light. The emission polarization anisotropy depends on the rotational diffusion of the fluorophore during its excited state. If one strand binds to another, the anisotropy changes due to the changes in mobility of the fluorophore. We first designed a setup and then measured the binding constant among several 16 bp DNA strands, including different number of mismatches in different positions.