Detection of DNA by Sequence Specific Fluorescent Polyamides

Thesis by

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Dedicated to Lily, My Parents, Pyrrhus, and Ludwig Boltzmann

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Abstract

This thesis covers the use of hairpin polyamides to achieve, most notably, HIV-1 LTR gene regulation and fluorescent detection of double stranded DNA. In Chapter 2 we discuss our collaboration with Professor David Margolis to study integrated HIV-1 latency in quiescent T-lymphocytes. Understanding latency in HIV-1 infection is of paramount importance for developing anti-HIV-1 therapeutics. Chapter 3 deals with the characterization of a special case of 2-□-2 polyamide binding in the minor groove, and we discuss the use of (S)-2,4-diaminobutyric acid to influence polyamide specificity and orientation. In Chapter 4 we present data concerning the use of hairpin polyamides that, when unbound to DNA, quench the fluorescence of the xanthene fluorphore to which they are covalently attached. We cover experiments aimed at exploring the uses of this fluorescence phenomenon to optically detect double stranded DNA in a sequence specific manner, an issue of great importance as shown in the literature by the numerous denaturing assays for oligo detection by hybridization. In Chapter 5, the fruits of a collaboration with Alexander Dunn of Professor Gray's group, we attempt to define the mechanism whereby polyamides quench tetramethyl rhodamine fluorescence.

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