Shape Selective Recognition of the DNA Minor Groove by Hairpin Polyamides

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For my Parents

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Abstract

Polyamides composed of N-methylpyrrole (Py), N-methylimidazole (Im), and 3-hydroxy-N-methylpyrrole (Hp) are crescent-shaped ligands that bind predetermined DNA sequences with affinities and specificities rivaling naturally occurring proteins. Inherent limitations of the thymine-selective Hp residue, including reduced affinity, diminished stability in aqueous solution, and loss of specificity in N-terminal pairings, have restricted the array of DNA sequences that can be specifically targeted with polyamides. The work described in this thesis addresses two major areas of research: the development of fluorescent conjugates of minor groove-binding polyamides as tools for genomic analysis and expansion of the minor groove recognition code by designing internal and N-terminal replacements for Hp.

Fluorophore-polyamide conjugates were designed using different fluorescent probes, different sites of probe attachment with respect to the polyamide, and different chemical linkers separating the above moieties. Ring conjugates, connecting tetramethylrhodamine or cyanine probes to the N-methyl position of Py rings exhibited reasonable affinities and specificities for the cognate DNA sequences, and displayed fluorescent enhancement upon association with the minor groove. The cyanine conjugates, though less quenched than their TMR counterparts, also demonstrated the capacity for fluorescence resonance energy transfer (FRET). The advantages offered by polyamides relative to oligonucleotide-

based probes for DNA detection suggest that polyamides might be useful tools for genomic analysis.

The utility of polyamides as diagnostic tools or as therapeutic agents would be greatly enhanced by the development of novel thymine-specific residues. Efforts toward this end have employed two general design strategies for Hp replacement. One approach has sought to remove the hydroxyl recognition element in favor of purely shape selective discrimination of the T•A base pair, while other efforts have examined alternative hydroxy-substituted aromatic scaffolds that possess greater stability than Hp. Both of these approaches are discussed in the context of N-terminal, internal, and multiple recognition of T•A base pairs.

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