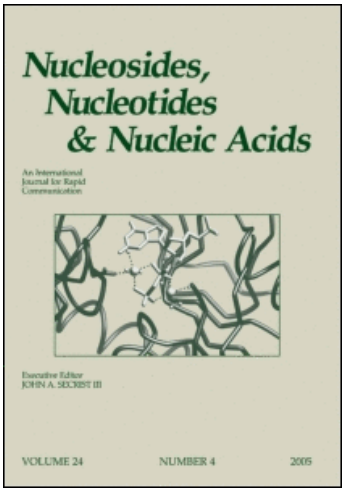

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DNA SELF-ASSEMBLING SYSTEMS: BRANCHED OLIGONUCLEOTIDES AS BUILDING BLOCKS AND MONITORING BY PYRENE EXCIMER BAND FORMATION

Dorthe Lindegaard, B. Ravindra Babu, and Jesper Wengel ▫ *Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M, Denmark*

▫ *The construction of a DNA self-assembling system created by four Y-shaped branched oligodeoxynucleotide building blocks has been studied. The assembly was verified by changes in the fluorescence emission spectra and revealed an additive effect in pyrene excimer band formation during DNA self-assembly.*

Keywords DNA Self-assembly, Branched Oligodeoxynucleotides, Fluorescence Emission Spectroscopy, 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA

INTRODUCTION

We have studied the use 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA[1] to verify formation of a DNA self-assembling system when mixing four different Y-shaped branched oligodeoxynucleotide building blocks. 2′-*N*-(Pyren-1-yl)methyl-2′-amino-LNA monomers are known to result in excimer band formation upon hybridization as a result of interstrand communication when positioned properly in a zipper-like fashion in the two hybridizing strands.[2] We demonstrate herein the applicability of steady-state fluorescence emission spectroscopy to confirm self-assembly as a fast and very simple method compared with non-denaturing gel electrophoresis normally used.

Four Y-shaped branched oligonucleotides were synthesized (ON **A**–ON **D**; Figure 1) and their individually recorded fluorescence emission spectra recorded revealed the typical pyrene monomer fluorescence emission spectra with bands at approximately 380 and 400 nm. In Figure 2, the spectrum of one of these branched building blocks (ON **C**) is shown. Mixing ON **C** and ON **D** resulted in excimer

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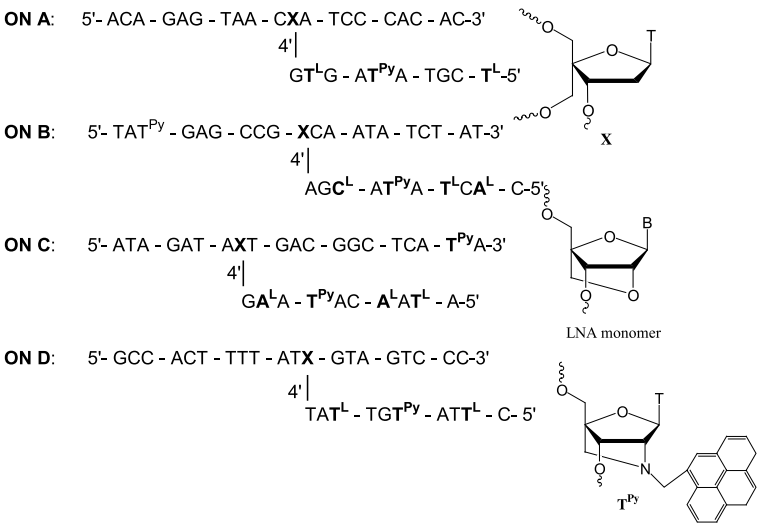

FIGURE 1 Sequences of the four Y-shaped branched oligodeoxynucleotide building blocks. $\mathbf{T^L}$, $\mathbf{A^L}$ and $\mathbf{C^L}$ (5-methylcytosine base) denote locked nucleic acid (LNA) monomers, $\mathbf{T^{Py}}$ denotes a 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA[1] monomer, and **X** a branching point monomer.

band formation at 430–520 nm, whereas mixing ON **B**, ON **C** and ON **D** resulted in the formation of a more intensive excimer band. Further increase in excimer band intensity was observed when mixing all four branched oligodeoxynucleotide building blocks ON **A**, ON **B**, ON **C**, and ON **D** (Figure 2). We validated the

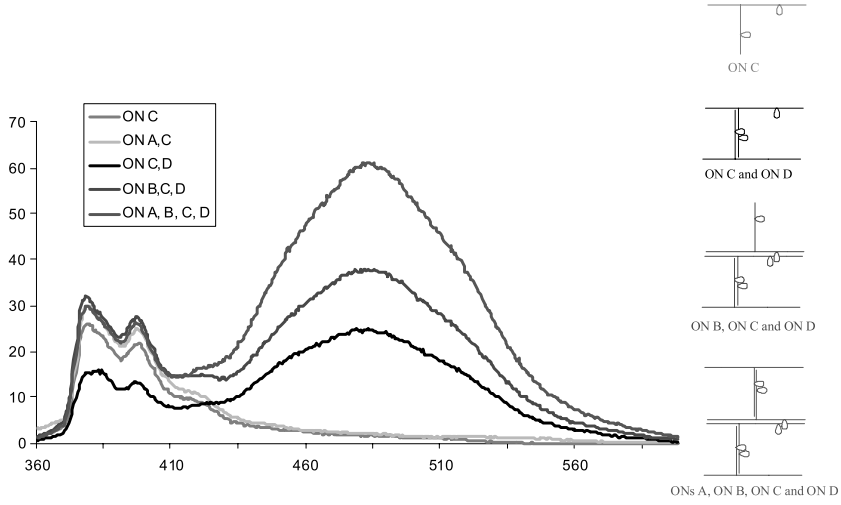

FIGURE 2 Fluorescence emission spectra. The concentration of the branched oligodeoxynucleotide building blocks was 0.15 μM, and a medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, pH 7.0) was used for the hybridization experiments. After mixing the building blocks, the mixture was heated to 80°C and then allowed to cool to 19°C. The measurements were performed at this temperature with excitation at 340 nm. Next to the spectra is shown a schematic representation of the DNA self-assembling systems formed.

method by mixing the non-complementary ONs ON **A** and ON **C**, ON **A** and ON **D**, and ON **B** and ON **D**, and in neither experiment was an excimer band observed. Only the spectrum resulting after mixing the non-complementary pair ON **A** and ON **C** is shown in Figure 2.

We conclude from the additive effect in excimer band formation in the steady state fluorescence emission spectra when mixing the four ON building blocks that the formation of self-assembling systems based on DNA building blocks can be verified by interstrand pyrene excimer band formation between 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA monomers when positioned properly[2] in the hybridizing strands.

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