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Chemically Induced Hairpin Formation in DNA Monolayers

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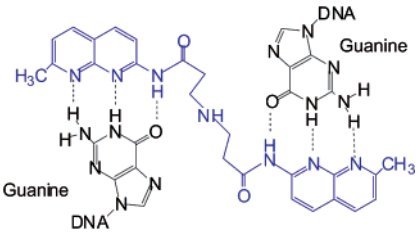
Short DNA molecules that can form hairpin structures are currently used for a variety of biosensor and nanoscale molecular assembly applications. For example, hairpin formation in short, single-stranded DNA (ssDNA) oligomers is currently employed in "molecular beacon" DNA detection methods[1] and in the implementation of DNA computing algorithms.[2] In this contribution, we demonstrate how the sequence-specific binding of a small-molecule ligand to G−G mismatches can be used to chemically induce the formation of hairpin structures in DNA monolayers immobilized onto Au surfaces.

Scheme 1. Structure of the G−G Mismatch Stabilizing Naphthyridine Dimer (in blue) Shown Hydrogen Bonding to Two Guanine Bases



The molecule that is used to induce the formation of the DNA hairpins is a naphthyridine dimer, which is shown in Scheme 1 hydrogen bonding to two guanine bases. Previous papers have demonstrated that this molecule specifically binds to G−G mismatches in double-stranded DNA (dsDNA).[3−5] The binding of the dimer to a short dsDNA sequence that contains a G−G mismatch enhances its free energy of hybridization and raises its melting temperature. The enhanced hybridization of G−G mismatch containing DNA molecules can be observed by the adsorption of ssDNA onto a chemically modified gold surface containing an immobilized oligonucleotide.[5] A DNA microarray can be used to check the G−G mismatch specificity of the dimer. For example, Figure 1a depicts a surface-bound 11-base oligonucleotide that contains one of the four bases at the specified location. The dsDNA formed by the hybridization adsorption of the indicated 11-mer ssDNA from solution creates a duplex containing either zero- or one-base mismatches.

In Figure 1b, surface plasmon resonance (SPR) imaging measurements of a four-component DNA microarray[6] show the adsorption of ssDNA from solution onto the array. SPR imaging is a technique used to study the adsorption of molecules onto chemically

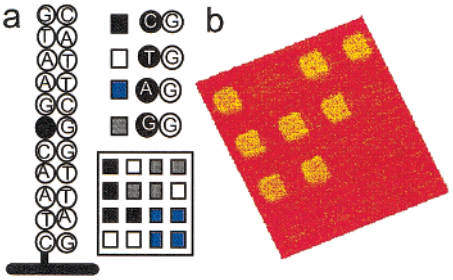
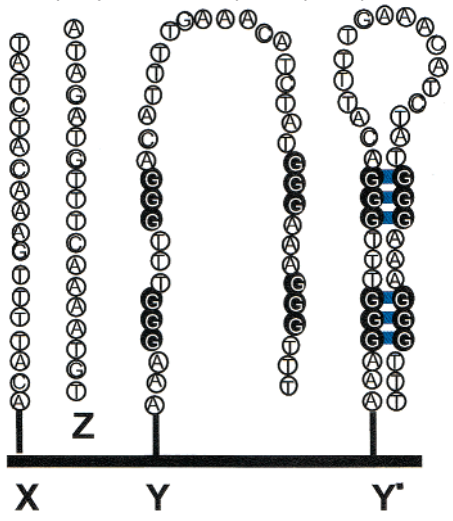


Figure 1. SPR difference image of a four-component DNA array. Each immobilized oligonucleotide differs by one base, indicated by the black circle. The image was taken in the presence of 250 μM naphthyridine dimer with 1 μM DNA complement, shown in the figure legend, at a temperature of 30 °C. The image shows that only the perfect match and the G−G mismatch oligonucleotides hybridize.

modified thin gold films by monitoring changes in the reflectivity that occur as a result of adsorption onto the surface.[7,8] As seen in the figure, in the presence of 250 μM naphthyridine dimer, hybridization adsorption was observed only for the array elements that contain either the perfectly matched sequence or the sequence that formed a G−G mismatch. In the absence of the naphthyridine dimer, hybridization was only observed for the perfectly matched sequence.

The enhanced hybridization of DNA containing G−G mismatches can be used to create DNA sequences that form hairpin structures only in the presence of the dimer. Scheme 2 shows how these "chemically induced" hairpins can be created. Two surface-

Scheme 2. Schematic Representation of the Oligonucleotides Used in Figure 2; Sequence Y Forms a Hairpin Structure in the Presence of Naphthyridine Dimer (blue squares).



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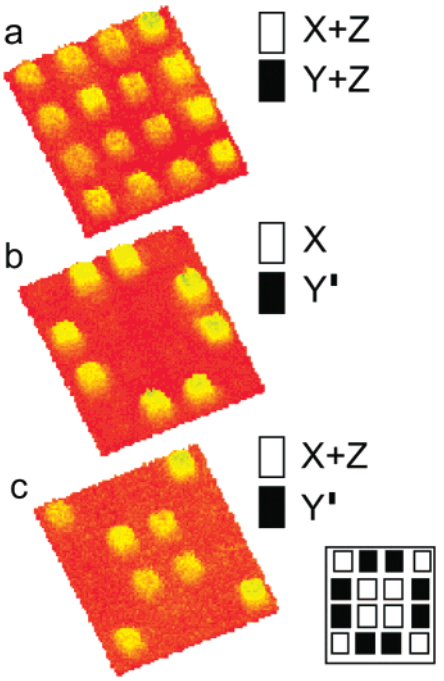

Figure 2. SPR difference images of a two-component DNA array obtained from the SPR images before and after: (a) The introduction of 2 μM complementary DNA sequence Z at 27 °C. This sequence hybridized to both immobilized strands X and Y and was subsequently removed by exposing the surface to 8 M urea. (b) The addition of 250 μM naphthyridine dimer to the ssDNA array showing binding to sequence Y. (c) The introduction of 2 μM DNA sequence Z to the dimer-exposed surface created in (b). Sequence Z only hybridized to sequence X, indicating the formation of a hairpin in sequence Y.

bound DNA sequences are shown (X and Y); both sequences X and Y contain an 18-base sequence that is complementary to the sequence of an 18-mer probe (Z) in solution. In addition, molecule Y has two 12-base pair sequences flanking the region complementary to probe Z. These flanking regions can act as the stem of a hairpin structure, and contain six complementary base pairs and six G−G mismatches. The presence of the naphthyridine dimer can be used to greatly enhance the hybridization of these flanking sequences and will induce the formation of a hairpin structure that prevents hybridization of molecule Z.

SPR imaging measurements are used to demonstrate the formation of chemically induced DNA hairpins in oligonucleotide monolayers at chemically modified gold surfaces. Figure 2 shows SPR difference images of a two-component DNA array composed of molecules X and Y immobilized onto a gold surface. In the absence of the dimer, hybridization adsorption is observed to both sequences X and Y (Figure 2a). This demonstrates that no hairpin formation is observed in sequence Y; the presence of the six G−G mismatches prevents hybridization in the stem region. The surface was regenerated to the original ssDNA array by exposure to 8 M urea.

The surface was then exposed to the naphthyridine dimer. The SPR difference image in Figure 2b shows an increase in reflectivity only for the sequence Y spots when the surface is exposed to the dimer. This increase is attributed to hairpin formation and results from the change in index of refraction of the monolayer due to the presence of the dimer molecules and any conformational changes that occur in the oligonucleotide. The surface density of the attached DNA is estimated from the SPR imaging signal and fluorescence wash-off measurements to be approximately 10^{11} molecules/cm^2, corresponding to an area of 1000 nm^2 per molecule. At this surface coverage, it is unlikely that the sequence-Y molecules are interacting in an interstrand fashion.

Figure 2c shows the SPR difference image after the subsequent exposure of the surface to the complement molecule Z. In the presence of the dimer, hybridization adsorption is only observed to the sequence-X array elements. This demonstrates that the formation of a hairpin structure in sequence-Y renders the region complementary to sequence-Z inaccessible to hybridization. If the dimer is present in the complement solution, then the surface shown in Figure 2c is stable. In the absence of the dimer in the complement solution, the surface reverts to that shown in Figure 2a in approximately 20 min. This demonstrates the reversibility of dimer intercalation and the formation of the hairpin structure.

We envisage a number of potential applications for chemically induced sequence-specific hairpin formation as described in this communication. The presence of the dimer can "turn off" or make inaccessible various DNA sequences to hybridization. This chemical control can be used to direct the programmable fabrication of nanoscale structures via sequential DNA hybridization, and to create "switchable" DNA array elements whose hybridization properties can be varied over time.

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Supporting Information Available: Complete DNA sequences used in this study (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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