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Making "Sense" of DNA

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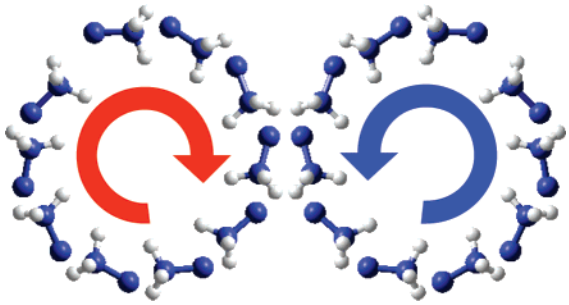
The DNA double helix is emblematic for the basis of all life processes.[1] DNA can exhibit unique molecular recognition properties, many of which are now being exploited in materials synthesis and biodetection schemes that are based on the hybridization (i.e., duplex formation) of oligonucleotides with complementary nucleic acid targets.[2−5] While DNA hybridization in aqueous media is well understood,[6] our molecular-level understanding of DNA duplex formation at surfaces and interfaces is curtailed by the low sensitivity associated with label-free DNA detection methods. Tagging surface-tethered DNA affords important molecular-level information,[8−11] but the signal detected commonly depends on the chemical nature of the label. Molecularly specific and label-free probes for the direct detection of DNA-based structures at surfaces and interfaces[12−16] are thus highly desirable, both from a fundamental science perspective as well as in the context of the demanding engineering aspects associated with high-throughput screening, biochip function, and disease detection.

We recently applied second harmonic generation as a label-free method to obtain the full thermodynamic state information for single-stranded DNA at the silica/water interface.[17] Here, we decipher the molecular structure of surface-tethered oligonucleotides in both single-strand and duplex forms by using polarization-resolved vibrational sum frequency generation (SFG).[18] Taking advantage of the pmp polarization experiment pioneered by Shen and co-workers,[19] we obtain detailed conformational information on surface-bound DNA, including the chirality of individual stereogenic centers in the strands and the secondary structure of the strands upon duplex formation. Probing the CH stretching region using infrared light that is plane-polarized perpendicular to the surface (p), we drive the chiral nonlinear optical response of the oscillators with 800 nm upconverting light fields that are plane-polarized at $m = \pm 45°$ away from the plane of incidence. The difference of the p-polarized SFG spectra may be viewed as a second-order analogue of a vibrational circular dichroism (VCD)[20] spectrum. The key advantage of the SFG approach is that the second-order CD effect can be greatly enhanced over the linear response.[21−23]

We chemically attached 15-mer oligonucleotides to glass microscope slides using established protocols[16] that result in surface coverages of $10^{11}-10^{12}$ strands/cm^2 (see Supporting Information). Each T_{15} strand contains 15 methyl groups from the thymine bases, providing a handle for subsequent interrogation with vibrational spectroscopy (vide infra). To form a surface-bound (sb) duplex with the complementary A_{15} sequence, the T_{15}-functionalized substrate was placed in a 1 μM copious solution of A_{15} in 10 mM PBS buffer (pH 7, 0.3 M NaCl), followed by rinsing with 5 mL of PBS buffer solution and 1 mL of deionized water to remove salt and then drying over N2. Since adenine does not contain methyl groups, the sb-T_{15}:A_{15} duplex still contains only 15 methyl groups; however, the number of deoxyribose units doubles to 30. The vibrational properties of these DNA-functionalized substrates in both single-stranded and duplex forms are then probed using a broadband SFG setup.[24,25]

Scheme 1. Top View of a sb-T_{15}:A_{15} (left) and a sb-A_{15}:T_{15} (right) Duplex with Only the Thymine Methyl Groups (R-CH_3) Visible in the Molecular Packing Diagram, Including Their Sense of Rotational Arrangement, Highlighted in Blue and Red, Respectively (sb = surface-bound)



If a double helix (A or B) were to form between our 3′-surface-bound DNA and its complementary strand, it should be right-handed and antiparallel.[6,7] As our surfaces were first functionalized with T_{15} oligonucleotides, the arrangement of the methyl symmetric stretch modes from the thymine bases should follow a counterclockwise rotation (Scheme 1). Due to the antiparallel duplex DNA configuration, this rotational direction will be reversed if the surface is first functionalized with 3′-amine-terminated A_{15} oligonucleotides and then hybridized with T_{15} oligonucleotides. While these geometrical considerations are irrelevant in isotropic environments, such as an aqueous phase, they become very important in the analysis of surface-bound DNA duplexes.

Figure 1A shows the ssp-polarized SFG spectra, which probe vibrational transitions perpendicular to the interface, of the surface-bound T_{15} single strand (blue trace) and the sb-T_{15}:A_{15} duplex (red trace). While the symmetric and asymmetric methylene stretch modes at 2850 and 2930 cm^{-1}, respectively, are clearly observable in both spectra, the T_{15} single strand does not exhibit methyl asymmetric stretch contributions (2950 cm^{-1}). The methyl symmetric stretch intensity (2875 cm^{-1}) is very small, even though there are 15 methyl groups on the T_{15} single strand. These results suggest a lack of order on the surface, especially with respect to the methyl groups in the thymine single strand. Interestingly, aromatic CH vibrations are either very weak or completely absent in the SFG spectrum (see Supporting Information).

After hybridizing the surface-bound T_{15} single strand with its complementary A_{15} strand, the methyl asymmetric and symmetric stretch signatures of the 15 thymine moieties are clearly apparent (red trace in Figure 1A). This stark change indicates a more ordered methyl group arrangement in the sb-T_{15}:A_{15} duplex, which could be attributed to the formation of a double helix. If this is indeed the case, the handedness of the helix and the directionality imparted

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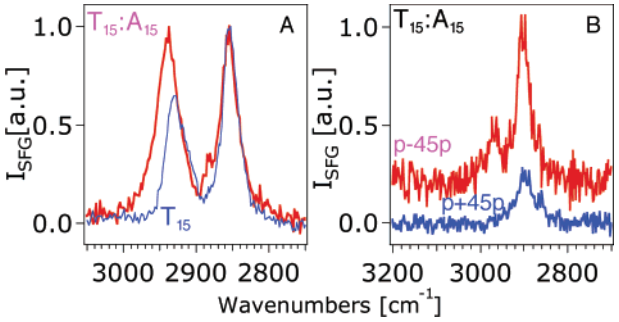

Figure 1. (A) The ssp-polarized SFG spectra of glass substrates functionalized with T_{15} oligonucleotides (thin blue trace) and the sb-T_{15}:A_{15} duplex (thick red trace). (B) The pmp-polarized SFG spectra of glass substrates functionalized with a sb-T_{15}:A_{15} duplex (sb = surface-bound).

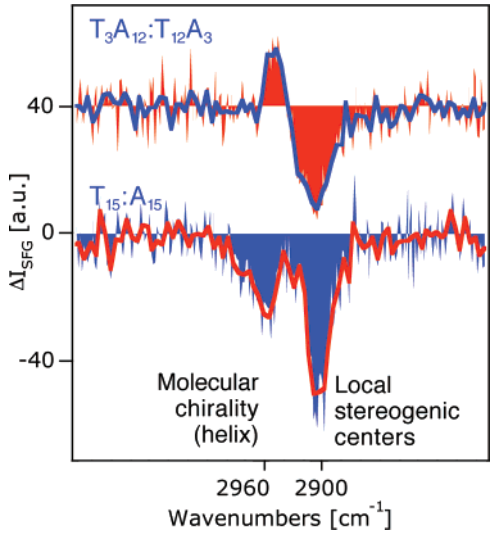

Figure 2. The SFG difference spectra of glass substrates functionalized with a sb-T_{15}:A_{15} duplex (bottom) and a sb-T_3A_{12}:$T_{12}A_3$ duplex (top). The thick solid lines represent a 3-point boxcar average of the difference spectra. Data collection time = 4 min/spectrum.

by the surface should control the rotation direction of the methyl groups in the double helix (Scheme 1).

This hypothesis can be verified using the pmp polarization combination. The sb-T_{15}:A_{15} duplex shows two distinct pmp spectra (Figure 1B), whose spectral difference (P+45p − P−45p) results in a negative methyl asymmetric stretch (2960 cm^{-1}) contribution (Figure 2, bottom). The deoxyribose methine stretch (2900 cm^{-1}) also exhibits negative intensity differences. In contrast, if the surface is functionalized with a T_3A_{12} single strand before being hybridized with an A_3T_{12} complementary strand from solution, the methyl asymmetric stretch contribution from the resulting duplex displays a positive intensity difference. The methine stretches (2900 cm^{-1}) from the 30 ribose groups of the sb-T_3A_{12}:A_3T_{12} duplex still exhibit negative intensity differences, which is not surprising as both duplexes have the same number of ribose sugars whose arrangement within the helix should not depend on the hybridization history. The striking difference in the two spectra shown in Figure 2 arises from the clear intensity differences in the methyl asymmetric stretch contributions. The spectra are consistent with the formation of a double helix upon hybridization that contains methyl groups from the T_{15} strand whose arrangement depends on an external reference point, which is the surface. The pmp polarization combination thus provides information on oscillators associated with stereogenic carbon atoms (methine CH groups, 2900 cm^{-1}) as well as molecular chirality (helically arranged methyl groups, 2960 cm^{-1}). We stress that it would be impossible to observe these stereoscopic differences in isotropic media, such as bulk aqueous solutions.

In summary, we have successfully obtained surface vibrational spectra of surface-bound single-stranded DNA duplexes and verified the highly ordered arrangement of the thymine bases within the double helix formed by Watson−Crick base pairing. These are the first measurements of vibrational signatures from stereogenic carbon atoms in surface-bound DNA duplexes as well as the macroscopic chirality present in these double helices upon hybridization. The high sensitivity and the label-free, molecularly specific nature of our approach should allow for a plethora of fundamental investigations into the nature of surface-tethered biopolymers. The knowledge from these studies should lead to improved biodiagnostic applications and new materials.

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Supporting Information Available: SFG spectra of thymine and thymidine and assignments, pmp studies of achiral control surfaces, χ^3 surface charge density measurements, and synthetic procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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