Supplementary Information for

DNA’s Chiral Spine of Hydration
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The following Supplementary Information include Theoretical Background for Chiral and Achiral SFG Spectroscopy, and a description of the Self-Referencing method to validate experimental result.

**Theoretical Background for Chiral and Achiral SFG Spectroscopy**

In vibrational sum frequency generation (SFG) experiments,\(^1,2\) a resonant infrared photon and a nonresonant visible photon induce a second-order polarization in the sample, \(P^{(2)}\), emitting a detected photon at the sum frequency:

\[ \omega_{\text{SFG}} = \omega_{\text{vis}} + \omega_{\text{IR}} \]

The SFG beam intensity depends on the second-order polarization’s square magnitude.

\[ I_{\text{SFG}} \propto |P^{(2)}|^2 \]

The polarization itself is dependent on the visible and infrared incident electric fields and the effective second-order nonlinear susceptibility of the sample.

\[ P^{(2)} = \chi^{(2)}_{\text{eff}} E_{\text{vis}} E_{\text{IR}} \]

For vibrational SFG, the second-order susceptibility, \(\chi^{(2)}\), consists of a nonresonant term, \(\chi^{(2)}_{NR}\), and a sum of vibrationally resonant terms, \(\chi^{(2)}_i\), typically described with Lorenzian lineshapes:

\[ \chi^{(2)} = \chi^{(2)}_{NR} + \sum_i \chi^{(2)}_i = \chi^{(2)}_{NR} + \sum_i \frac{A_i}{\omega_{\text{IR}} - \omega_i - i\Gamma_i} \]

where \(A_i\) is the SFG transition moment and \(\Gamma_i\) is the line width of the \(i\)th transition.

Within the \(xyz\) lab frame of reference, there are 27 tensor elements in the second-order nonlinear susceptibility, \(\chi^{(2)}\). For achiral molecules, a reasonable approximation for the overall surface structure is an isotropic achiral surface with \(C_{\infty v}\) symmetry, giving four independent nonzero \(\chi^{(2)}_{\text{eff}}\) elements:

\[
\begin{align*}
\chi^{(2)}_{xxz} &= \chi^{(2)}_{yyz} \\
\chi^{(2)}_{xxz} &= \chi^{(2)}_{yzy} \\
\chi^{(2)}_{xxx} &= \chi^{(2)}_{zyy} \\
\chi^{(2)}_{zzz} &= \chi^{(2)}_{zyy}
\end{align*}
\]

A chiral surface with \(C_{\infty}\) symmetry adds an additional six nonzero (three independent) elements:

\[
\begin{align*}
\chi^{(2)}_{xyy}, \chi^{(2)}_{xyz}, \chi^{(2)}_{yzy}, \chi^{(2)}_{zxy}, \chi^{(2)}_{zyx}, \chi^{(2)}_{zyy}
\end{align*}
\]

At the experimental level, waveplates and polarizers control the polarization of each of the three beams (sum frequency, visible, and infrared) to be parallel (\(p\) for the
German *parallel*) or perpendicular (*s* for the German *senkrecht*) relative to the plane of incidence of the visible and infrared beams on the sample. Polarizations are listed in descending order of photon frequency (e.g. an *ssp* experiment has *s*-polarized SFG, *s*-polarized visible, and *p*-polarized IR beams). Hence, there are eight \( (2^3) \) possible combinations for the polarization settings: *ssp*, *sps*, *pss*, *ppp*, *spp*, *psp*, *pps*, and *sss*.

**Figure S1.** *ssp* polarization SFG setup, *s*-polarized SFG, *s*-polarized visible, and *p*-polarized infrared beams. *s* means perpendicular to the plane of incidence (yellow); *p* denotes parallel to the plane of incidence.

Four of the possible SFG polarization combinations—*ppp*, *ssp*, *sps*, *pss*—measure \( \chi^{(2)} \) elements corresponding to achiral structures and three combinations—*psp*, *spp*, *pps*—measure \( \chi^{(2)} \) elements corresponding to chiral structures. *sss* is completely in the plane of the sample and is zero. A rule of thumb is that the achiral combinations always have an odd number of *p*-polarized beams, while the chiral combinations have an even number of *p*-polarized beams.

The seven second-order susceptibilities at the experimental level can be expressed in terms of the thirteen chiral and achiral \( \chi_{eff}^{(2)} \) elements, the angles of the beams in relation to the surface normal, and the diagonal elements of the Fresnel matrix determined by the two refractive indices of the interface. Under common assumptions, the chiral \( \chi_{eff}^{(2)} \) all depend on the same chiral susceptibility element, \( \chi_{xy}^{(2)} \). Therefore, the choice between the chiral polarization combinations *spp*, *psp*, and *pps* depends only on interface-dependent Fresnel factors, beam angles, and optics.

In this work, we also utilize polarization combinations where the measured SFG beam is linearly polarized at \( \pm 45^\circ \). The second-order susceptibility for the polarization combination \( \pm 45^\circ \) *pp* combines the achiral *ppp* with the chiral *spp*.

\[
\chi_{\pm 45^\circ \ pp}^{(2)} = \cos(\pm 45^\circ) \cdot \chi_{ppp}^{(2)} + \sin(\pm 45^\circ) \cdot \chi_{spp}^{(2)}
\]
where $\varphi$ is the relative phase between the achiral and chiral susceptibilities. In other words, the achiral signal heterodynes the chiral signal. The difference between the $+45^\circ$ and $-45^\circ$ signals is proportional to the chirality of the sample.

**Self-referencing**

Second-order nonlinear optical techniques like SFG require broken inversion symmetry.\(^1\) Chiral-specific SFG spectroscopy\(^2\)\textsuperscript{-8} is possible, because chirality breaks inversion symmetry. However, chiral SFG has generally been limited to systems with strong, dense, or highly-ordered chromophores. New methods from our lab, however, have improved the signal-to-noise and robustness of chiral SFG.\(^8\) The use of a waveplate and beam displacer allows simultaneous collection of positively and negatively interfered achiral and chiral signals or alternatively the pure chiral and pure achiral responses. In both cases, the use of self-referencing ensures accurate chiral measurements.

The polarization of the SFG signal impacts the reflection efficiency of the grating. To calibrate for the reflection efficiency, the nonresonant gold signal is rotated and split by the beam displacer into equally intense $+45^\circ$ and $-45^\circ$ signals. Dividing the CCD counts of the two beams reveals the scalar grating efficiency factor.

The grating factor and chiral sensitivity of the experiment can also be confirmed by an achiral sample. In this experiment, the achiral azide monolayer without DNA modification was tested and showed no chirality.

Once the chiral samples are inserted, each sample is individually self-referenced. A polarizer blocks the chiral polarization. The achiral signal is split by the beam displacer into $+45^\circ$ and $-45^\circ$ polarized signals. The equal intensity of the beams verifies the setup's accuracy. Self-referencing each sample increases the robustness of the chiral SFG detection\(^8\). The self-referenced spectrum ensures that the differences in the chiral spectrum are truly signifiers of chirality and not artefacts or errors.

Figure S2 shows the OH stretch around a 24 base pair double-stranded DNA strand consisting of alternating guanine and cytosine bases with and without the self-referencing polarizer. The chirality shown in the left column is evident with the nonzero pure $spp$ (red) and interference $+45^\circ pp - -45^\circ pp$ (gray) chiral SFG intensities. The right column, however, shows no chirality—confirming that the chiral signal is blocked by the polarizer. Furthermore, the achiral $ppp$ signal (blue) is unaffected by the polarizer, confirming that the polarizations are cleanly defined.
Figure S2. The chiral SFG responses for 24 base pair double-stranded DNA strands consisting of alternating guanine and cytosine bases (GCGC) in aqueous 100 mM NaCl. (A) and (C) show the chiral SFG results without self-referencing. (B) and (D) show results with the self-referencing polarizer. (A) demonstrates the chirality of the water around the DNA through the nonzero intensity of the pure chiral spp (red) and in the difference between the of the $+45^{\circ}\text{pp}$ (black), and $-45^{\circ}\text{pp}$ (green) spectra. (C) also demonstrates the same principle with the 5x-magnified view of the pure chiral spp (red), and the interference $+45^{\circ}\text{pp} - -45^{\circ}\text{pp}$ (gray) spectra. (B) shows the effect of the self-referencing through the zero intensity of the pure chiral spp (red) and the identical $+45^{\circ}\text{pp}$ (black), and $-45^{\circ}\text{pp}$ (green) spectra. The achiral ppp spectrum (blue) is unaffected by the self-referencing polarizer. (D) also shows the same principle with the zero pure and interference chiral spectra.

Figure S3 shows the OH stretch around a 24 base pair double-stranded DNA strand consisting of alternating adenine and thymine bases with and without the self-referencing polarizer. The chirality shown in the left column is evident with the nonzero pure spp (red) and interference $+45^{\circ}\text{pp} - -45^{\circ}\text{pp}$ (gray) chiral SFG intensities. The right column, however, shows no chirality—confirming that the chiral signal is blocked by the polarizer. Furthermore, the achiral ppp signal (blue) is unaffected by the polarizer, confirming that the polarizations are cleanly defined.
Figure S3. The chiral SFG responses for 24 base pair double-stranded DNA strands consisting of alternating adenine and thymine bases (TATA) in aqueous 100 mM NaCl. (A) and (C) show the chiral SFG results without self-referencing. (B) and (D) show results with the self-referencing polarizer. (A) demonstrates the chirality of the water around the DNA through the nonzero intensity of the pure chiral \( \text{sp} \) (red) and in the difference between the of the \(+45^\circ \text{pp}\) (black), and \(-45^\circ \text{pp}\) (green) spectra. (C) also demonstrates the same principle with the 5x-magnified view of the pure chiral \( \text{sp} \) (red), and the interference \(+45^\circ \text{pp} - -45^\circ \text{pp}\) (gray) spectra. (B) shows the effect of the self-referencing through the zero intensity of the pure chiral \( \text{sp} \) (red) and the identical \(+45^\circ \text{pp}\) (black), and \(-45^\circ \text{pp}\) (green) spectra. The achiral \( \text{pp} \) spectrum (blue) is unaffected by the self-referencing polarizer. (D) also shows the same principle with the zero pure and interference chiral spectra.

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