Corrections

PHYSICS

On page 18727, right column, Eq. 12 should instead appear as

\[
\Gamma[\{\phi_{ab}\}] = \frac{1}{2} \int \frac{dp}{(2\pi)^D} \left( \sum_{ab} (\mu \sqrt{\phi} + \sigma \phi^2) |\phi_{ab}(\rho)|^2 \right. \\
+ m_2 \frac{1}{2} \sum_a \left[ \sum_b |\phi_{ab}(\rho)|^2 \right] \\
+ \sum_{a \neq b} \frac{w_3}{6} \int (2\pi) \phi_{ab}(\rho) \phi_{bc}(\rho) \phi_{cd}(\rho) (-p - p') \\
+ \sum_{a \neq b} \frac{w_2}{6} \int (2\pi) \phi_{ab}(\rho) \phi_{bc}(\rho) \phi_{cd}(\rho) (-p - p'),
\]

On page 18728, right column, Eq. 21 should instead appear as

\[
\lambda = \frac{1}{\rho^2} \int \frac{k_0^3(q)}{g^2(x)} \left[1 - \rho \Delta c(q)\right]^{-3}
\]

The authors note that Tables 1 and 2 appeared incorrectly. The corrected tables appear below.

**Table 1.** Numerical values of the coefficients of the effective action and the physical quantities from the HNC approximation

<table>
<thead>
<tr>
<th>System</th>
<th>T</th>
<th>( \rho_d )</th>
<th>(- w_1 )</th>
<th>(- w_2 )</th>
<th>m_2</th>
<th>m_3</th>
<th>( \sigma )</th>
<th>( \rho )</th>
<th>( \lambda )</th>
<th>( \xi_0 )</th>
<th>G_0</th>
<th>G_i</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-6</td>
<td>1</td>
<td>6.691</td>
<td>3.88 \times 10^{-6}</td>
<td>1.35 \times 10^{-6}</td>
<td>-0.000925</td>
<td>0.000110</td>
<td>0.00195</td>
<td>0.000525</td>
<td>0.348</td>
<td>0.601</td>
<td>224</td>
<td>0.0267</td>
</tr>
<tr>
<td>SS-9</td>
<td>1</td>
<td>2.912</td>
<td>0.0000772</td>
<td>0.0000272</td>
<td>-0.00539</td>
<td>0.000633</td>
<td>0.00163</td>
<td>0.00543</td>
<td>0.353</td>
<td>0.548</td>
<td>34.3</td>
<td>0.0125</td>
</tr>
<tr>
<td>SS-12</td>
<td>1</td>
<td>2.057</td>
<td>0.000275</td>
<td>0.0000973</td>
<td>-0.0116</td>
<td>0.00132</td>
<td>0.00378</td>
<td>0.0152</td>
<td>0.354</td>
<td>0.498</td>
<td>14.2</td>
<td>0.0118</td>
</tr>
<tr>
<td>LJ</td>
<td>0.7</td>
<td>1.407</td>
<td>0.00106</td>
<td>0.000376</td>
<td>-0.0258</td>
<td>0.00290</td>
<td>0.00989</td>
<td>0.0414</td>
<td>0.355</td>
<td>0.489</td>
<td>6.00</td>
<td>0.00833</td>
</tr>
<tr>
<td>HarmS</td>
<td>10^{-3}</td>
<td>1.336</td>
<td>0.00129</td>
<td>0.000465</td>
<td>-0.0336</td>
<td>0.00343</td>
<td>0.00772</td>
<td>0.0779</td>
<td>0.359</td>
<td>0.315</td>
<td>2.82</td>
<td>0.0434</td>
</tr>
<tr>
<td>HarmS</td>
<td>10^{-4}</td>
<td>1.196</td>
<td>0.00165</td>
<td>0.000622</td>
<td>-0.0403</td>
<td>0.00386</td>
<td>0.00819</td>
<td>0.109</td>
<td>0.378</td>
<td>0.274</td>
<td>1.69</td>
<td>0.0632</td>
</tr>
<tr>
<td>HarmS</td>
<td>10^{-5}</td>
<td>1.170</td>
<td>0.00174</td>
<td>0.000663</td>
<td>-0.0416</td>
<td>0.00395</td>
<td>0.00845</td>
<td>0.109</td>
<td>0.382</td>
<td>0.278</td>
<td>1.66</td>
<td>0.0635</td>
</tr>
<tr>
<td>HS</td>
<td>0</td>
<td>1.169</td>
<td>0.00174</td>
<td>0.000664</td>
<td>-0.0418</td>
<td>0.00397</td>
<td>0.00847</td>
<td>0.108</td>
<td>0.381</td>
<td>0.280</td>
<td>1.67</td>
<td>0.0639</td>
</tr>
</tbody>
</table>

For each potential, lengths are given in units of \( r_0 \) and energies in units of \( e \), with \( k_b = 1 \). Data at fixed temperature, using density as a control parameter with \( e = \rho_d - \rho \).

**Table 2.** Same as Table 1, but here the data are at fixed density, using temperature as a control parameter with \( e = T_d - T \)

<table>
<thead>
<tr>
<th>System</th>
<th>( \rho )</th>
<th>T_d</th>
<th>(- w_1 )</th>
<th>(- w_2 )</th>
<th>m_2</th>
<th>m_3</th>
<th>( \sigma )</th>
<th>( \rho )</th>
<th>( \lambda )</th>
<th>( \xi_0 )</th>
<th>G_0</th>
<th>G_i</th>
</tr>
</thead>
<tbody>
<tr>
<td>LJ</td>
<td>1.2</td>
<td>0.336</td>
<td>0.00186</td>
<td>0.000663</td>
<td>-0.0361</td>
<td>0.00403</td>
<td>0.0147</td>
<td>0.0572</td>
<td>0.356</td>
<td>0.507</td>
<td>4.56</td>
<td>0.00730</td>
</tr>
<tr>
<td>LJ</td>
<td>1.27</td>
<td>0.438</td>
<td>0.00153</td>
<td>0.000541</td>
<td>-0.0321</td>
<td>0.00370</td>
<td>0.0128</td>
<td>0.0447</td>
<td>0.353</td>
<td>0.536</td>
<td>5.74</td>
<td>0.00771</td>
</tr>
<tr>
<td>LJ</td>
<td>1.4</td>
<td>0.684</td>
<td>0.00108</td>
<td>0.000383</td>
<td>-0.0260</td>
<td>0.00293</td>
<td>0.0100</td>
<td>0.0292</td>
<td>0.355</td>
<td>0.586</td>
<td>8.52</td>
<td>0.00825</td>
</tr>
<tr>
<td>WCA</td>
<td>1.2</td>
<td>0.325</td>
<td>0.00195</td>
<td>0.000686</td>
<td>-0.0389</td>
<td>0.00426</td>
<td>0.0133</td>
<td>0.0607</td>
<td>0.351</td>
<td>0.467</td>
<td>4.37</td>
<td>0.0134</td>
</tr>
<tr>
<td>WCA</td>
<td>1.4</td>
<td>0.692</td>
<td>0.00111</td>
<td>0.000388</td>
<td>-0.0270</td>
<td>0.00301</td>
<td>0.00966</td>
<td>0.0291</td>
<td>0.350</td>
<td>0.576</td>
<td>8.67</td>
<td>0.0106</td>
</tr>
</tbody>
</table>

www.pnas.org/cgi/doi/10.1073/pnas.1309463110
CHEMISTRY, BIOPHYSICS AND COMPUTATIONAL BIOLOGY

The authors note that the following statement should be added to the Acknowledgments: “We also acknowledge funding from the Chemical Sciences, Geosciences and Bioscience Division, Office of Basic Energy Sciences, Office of Science of the US Department of Energy.”

www.pnas.org/cgi/doi/10.1073/pnas.1310422110

NEUROSCIENCE

The authors note that the incorrect term appeared for the mice background that they used. All instances of “C57BL/6J” should instead appear as “C57BL/6.” The locations were:
- On page 15918, left column, line 4 within the Abstract
- On page 15918, right column, first full paragraph, line 5
- On page 15922, left column, second full paragraph, line 2

These errors do not affect the conclusions of the article.

www.pnas.org/cgi/doi/10.1073/pnas.1310560110

IN THIS ISSUE
Correction for “In This Issue,” which appeared in issue 21, May 21, 2013, of Proc Natl Acad Sci USA (110:8315–8316; 10.1073/it2113110).

The authors note that within “Measuring telomeres in single cells” on page 8316 the writing credit “C.R.” should instead appear as “C.B.” The online version has been corrected.

www.pnas.org/cgi/doi/10.1073/pnas.1310833110
Probing the relative orientation of molecules bound to DNA through controlled interference using second-harmonic generation

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Contributed by Kenneth B. Eisenthal, February 7, 2013 (sent for review January 12, 2013)

A method is described in which the interference of radiated second-harmonic electric fields generated by a pair of oriented molecules intercalated into double-stranded DNA is controlled and measured. The results show that the relative molecular orientation of the two molecules significantly changes the magnitude of the observed second-harmonic generation intensity, which is described by a simple model that accounts for the interferences of the radiated fields. The technique presented shows promise for future experiments investigating structural changes induced by the formation of a DNA–biomolecule complex.

**Experimental**

The experimental apparatus has been described previously (4, 5). Briefly, a KM Labs Ti-Sapphire oscillator running at 80 MHz, center wavelength of 840 nm, producing 300 mW of average power with a pulse width of ~60 fs was focused into a 2-mm cuvette. The generated SH radiation was collected with a lens, telescopied, and filtered to remove much of the fundamental laser frequency before focusing into an Acton 300i spectrograph coupled to a Princeton Instruments Spec-10 CCD camera. The exposure was set to 1 s; several hundred exposures per titration step were collected to obtain statistics. Each experiment was carried out in triplicate to ensure reproducibility and reduce uncertainties. All quoted uncertainties are given at the 95% confidence interval.

**Results and Discussion**

Daunomycin is an anticancer drug that is commonly used to treat acute leukemia (8–10), and has a somewhat unique property among small-molecule DNA binding agents in that it preferentially binds to triplet sequences in double-stranded DNA (10–14). The work here makes use of the specificity of daunomycin to the sequence TCG to spatially orient the two intercalated drugs on a given DNA duplex. Daunomycin has the effect of lengthening the DNA helical axis by ~3.4 Å and imparting a small unwinding of the DNA helix (~8°) (15–17) relative to non-complexed B-DNA. The sequence of the DNA 33-mer duplex that was used in the experiments was referenced to the sequence

\[ I_{2ω} \propto \left| \sum_k E_{2ω}^k \right|^2, \]

where \( E_{2ω}^k \) is the SH field from a given interfacial molecule, which add up, with its respective phase, with other interfacial molecules to produce the overall SH intensity from an individual particle. The total SHG intensity measured in experiments is the incoherent sum of the coherent SH light generated by the daunomycin pair bound to the DNA on the individual particles in solution. The isotropic bulk medium does not produce coherent SH radiation because the SH field generated by one randomly oriented molecule is, in principle, canceled out by another molecule that has the opposite orientation, leading to an overall destructive interference of the bulk SH signal; although incoherent SH light is generated in the bulk medium by density and orientational fluctuations of the species in bulk solution (hyper-Rayleigh scattering). Experiments have previously found that bulk water is responsible for the incoherent SH background signals (4). In the work reported here, the observed SH intensity generated by a pair of molecules bound to DNA, whose orientations are well defined and controlled, is shown to contain information on the relative orientation of the two molecules, allowing for detailed solution phase investigations of relative molecular orientation.

**Acknowledgments**

This work was supported by the Alfred P. Sloan Foundation (to B.D. and S.W.K.). The authors declare no conflict of interest.

1Deceased November 24, 2012.

2To whom correspondence should be addressed. E-mail: kbe1@columbia.edu.
used where only one recognition site was present: 5′-CTC AAG TGA ACT CAA GTG AAT CCA ATC GAA GTT-3′; the recognition site is highlighted and the complementary strand is implied for succinctness. To change the orientation angle between the two drugs on a DNA duplex, the distance between two recognition sites was varied by changing the number of base pairs separating the two recognition sites. For instance, the DNA used where the spacing was 20 bases apart was 5′-CTC AAT CGA ACT CAA GTG AAT CCA ATC GAA GTT-3′.

Representative binding isotherms collected in the experiment are shown in Fig. 2. It was observed that the SH intensity at the maximum density of bound daunomycin, which is the plateau of the isotherm, changed as the spacing between the two recognition sequences was varied. The orientation angle between the two daunomycin molecules is given by $\Phi = n \times 36^\circ$, where $n$ is the number of base pairs separating the two recognition sites. Therefore, when $n = 5, 15, 25$, etc., the two binding sites are antiparallel and the SHG will destructively interfere; similarly, when $n = 10, 20, 30$, etc., the intercalated drugs are aligned parallel and will constructively interfere, as observed from the SH binding data in Fig. 2.

To quantify the modulated SHG intensity, the signal intensity at the isotherm plateau was averaged and plotted vs. number of base pairs separating the two binding sites in Fig. 3. This plot more clearly illustrates the oscillatory behavior that was expected to occur by systematically rotating one daunomycin relative to another by changing the number of base pairs separating them, and collecting the resulting SH light. The relative orientation is depicted in Fig. 3 as arrows representing the individual drug molecules.

A simple model was implemented to quantify the interference of radiated SH fields from the daunomycin molecules on a DNA duplex, which is given below. A coordinate system is defined according to Fig. 4 where two generated SH fields, $E_a$ and $E_b$ are defined relative to the $x$ axis by the angles $\phi_a$ and $\phi_b$ for $E_a$ and $E_b$, respectively (bold type indicates vector quantities). Similarly, the angle out of the $x$-$y$ plane is defined as $\theta_a$ and $\theta_b$. The SH intensity due to the intercalated daunomycin molecules, $a$ and $b$, is the coherent sum of their individual SH fields and is written as follows:

$$I_{2\omega} \propto |E_a + E_b|^2 = |E_a|^2 + |E_b|^2 + 2E_a \cdot E_b,$$

[2]

where each SH field can be written explicitly in terms of vector components to give the following:

$$E_a = E_a \left( \sin \theta_a \cos \phi_a \hat{x} + \sin \theta_a \sin \phi_a \hat{y} + \cos \phi_a \hat{z} \right).$$

[3]

The DNA’s helical axis is defined along the $z$ axis in this coordinate system so the DNA bases lie parallel to the $x$-$y$ plane. Based on the crystal structure of the daunomycin–DNA complex, daunomycin is known to intercalate parallel to the DNA’s bases (15–17) yielding the approximation, $\theta_a \sim \theta_b \sim 90^\circ$. Applying this approximation to Eq. 3 and substituting into Eq. 2 yields the following:

$$I_{2\omega} \propto |E_a|^2 + |E_b|^2 + 2|E_a||E_b|\left( \cos \phi_a \cos \phi_b + \sin \phi_a \sin \phi_b \right).$$

[4]

Simplifying and noting that the intensity is proportional to the orientational average of the intercalated daunomycin–DNA complex on a given particle gives the following:

$$I_{2\omega} \propto |E_a|^2 + |E_b|^2 + 2|E_a||E_b|\left( \cos (\phi_a - \phi_b) \right).$$

[5]

Setting $\Phi = \phi_a - \phi_b$ and noting that $|E_a| = |E_b| \equiv |E_{2\omega}|$ yields the following:

$$I_{2\omega} \propto 2|E_{2\omega}|^2 \left[ 1 + \langle \cos \Phi \rangle \right].$$

[6]

where $|E_{2\omega}|^2$ represents the generated SH intensity of one daunomycin molecule bound to DNA. The SHG signal from a single silica particle is the coherent summation of all of the individual daunomycin–DNA complexes attached to the particle. The total SHG signal is the incoherent sum of the SHG from the individual particles. The orientational average in Eq. 6 contains the variation in daunomycin orientations (e.g., tilt angles, etc.) relative to the DNA helical axis. To fit the

![Fig. 1.](image1.png) The relative orientation of two daunomycin molecules (orange with black arrows) bound to DNA is sketched here (omitting the amino sugar for clarity).

![Fig. 2.](image2.png) Representative binding isotherms collected with different numbers of base pairs separating two daunomycin molecules bound to DNA (listed on Right).
The SH intensity is plotted vs. base pair separation on the lower axis, and orientation angle on the upper axis. The relative orientation of the bound daunomycin molecules is sketched as arrows that are associated with each data point.

The experimental data, the orientational average above is replaced with an empirical parameter, \( g \), and summing over the particles yields the following:

\[
I_{\text{2ω}}(\text{total}) \propto \sum_{k} I_{\text{2ω}, k} = 2[1 + g \cdot \cos \Phi] \sum_{k} |E_{\text{2ω}, k}|^2. \tag{7}
\]

The experimental data in Fig. 3 was fit to Eq. 7 to yield \( g = 0.53 \pm 0.17 \) and \( \sum_{k} |E_{\text{2ω}, k}|^2 = 101 \pm 15 \) arbitrary units (arb. units). The value of \( \sum_{k} |E_{\text{2ω}, k}|^2 \) extracted from the fit is in agreement with the SH intensity that was observed in experiments with one recognition site (120 \( \pm 17 \) arb. units), as expected. The agreement between the fit and the measured intensity from the binding of daunomycin to one recognition site serves as an internal consistency check to ensure the model and fit retrieve meaningful parameters.

The retrieved value of \( g \) indicates that the interference “efficiency” is \( \sim 53\% \). The deviation from ideal SH interference might be due to the ensemble distribution of orientations that the two drugs can take relative to one another, resulting in altered relative orientations, e.g., the daunomycin molecules might be twisted or tilted slightly from one another when intercalated into the DNA. Imperfect interference could also occur from structural and orientational fluctuations occurring on timescales much faster than the measurement. The slight unwinding of DNA by daunomycin could affect the interference of the SHG fields, which would be observed in Fig. 3 as a phase shift (i.e., the maxima and minima would not coincide with 0° or 180°, respectively). Because the data are well described by Eq. 7 (Fig. 3), it is surmised that daunomycin-induced changes in the DNA structure are within the experimental error of the results reported here. Despite its simplicity, the model successfully recovered the correct oscillation frequency of the SH intensity vs. base pair separation.

**Conclusions**

The results presented here demonstrate that the interference of second-harmonic fields generated by a pair of molecules complexed to double-stranded DNA can be used to probe their relative orientation. The straightforwardness of the experimental approach to control and measure relative molecular orientation, e.g., the anticancer drug daunomycin complexed with DNA investigated in this study, presents opportunities to probe structural aspects of biomolecular complexes in solution. For instance, it could be possible to investigate structural changes in DNA due to complex formation with proteins or drugs, e.g., one would measure a phase shift in the SH intensity vs. number of base pairs separating the intercalated daunomycin molecules, and thus, their relative orientation angle would differ before and after complex formation of DNA with the ligand of interest.

**ACKNOWLEDGMENTS.** We acknowledge many useful conversations with Dr. Joseph R. Roscioli and Dr. Louis H. Haber. K.B.E. acknowledges generous funding from National Science Foundation (NSF) Eager Award CHE-1041980, NSF Grant CHE-1057483, and Defense Threat Reduction Agency Grant HDTRA1-11-1-0002. N.J.T. received funding from NSF Grants CHE-11-1398 and DMR-02-17374.


**Fig. 3.** The SH intensity is plotted vs. base pair separation on the lower axis, and orientation angle on the upper axis. The relative orientation of the bound daunomycin molecules is sketched as arrows that are associated with each data point.

**Fig. 4.** Depiction of the relative angles between SH fields generated by the \( a \) and \( b \) daunomycin molecules bound to DNA.