Supplementary Material for "Effect of lesions on the dynamics of DNA on the picosecond and nanosecond time scales using a polarity sensitive probe"

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SUSCEPTIBILITY EMISSION SPECTRA

For quantitative analysis, raw fluorescence spectra must be corrected for wavelength dependent instrument sensitivity (1). In this paper, we initially corrected the spectra to radiometric intensity per wavelength interval $I(\lambda)$ [units = W/nm]. The conversion to photon intensity per frequency interval $I_p(\nu)$ [units = photons/cm^{-1}] is standard (2)

$$I_p(\nu = hc/\lambda) \propto I(\lambda)/\nu^3 . \quad \text{S1}$$

(Because absolute intensities are not important, frequency independent constants are ignored.)

The intensity of fluorescence is intrinsically higher for transitions that are at higher frequency, due to the greater density of electromagnetic modes at higher frequency. This fact is most often expressed in terms of the Einstein A and B coefficients, which correspond to the molecular transition rates of spontaneous emission (fluorescence) and stimulated emission/absorption respectively (3)

$$A \propto \nu^3 B . \quad \text{S2}$$

Although Eq. S2 is widely discussed, few references explicitly relate A and B to experimentally measured spectra. However, careful comparison of the definitions yields

$$A \propto \int_0^\infty I_p(\nu)\, \mathrm{d}\nu \quad \text{S3}$$

$$B \propto \int_0^\infty \chi''(\nu)\, \mathrm{d}\nu . \quad \text{S4}$$

The imaginary part of the molecular susceptibility $\chi''(\nu)$ arises in the treatment of absorption and stimulated emission in classical electrodynamics. It is related to the molar absorption coefficient $\varepsilon(\nu)$, i.e., to the standard absorption spectrum, by (4)

$$\chi''(\nu) \propto \varepsilon(\nu)/\nu . \quad \text{S5}$$

For stimulated emission, the same relationship holds, except that the values of $\chi''(\nu)$ and $\varepsilon(\nu)$ are negative. Combining equations S2-S4 gives

$$\chi''(\nu) \propto I_p(\nu)/\nu^3 . \quad \text{S6}$$

A single electronic transition can be measured through absorption, stimulated emission or fluorescence spectra. This paper deals only with fluorescence spectra, but ongoing work includes absorption and stimulated emission spectra of these systems as well. As the above equations show, any of these spectra can be transformed to a variety of forms that differ by multiplication by factors of the frequency. Choosing the correct form is important at a quantitative level, especially when the spectra lie in different frequency regions (i.e., absorption and fluorescence) or when they have frequency shifts with time (i.e., time dependent Stokes shifts).

We convert our fluorescence spectra to susceptibilities

$$\chi''(\nu = hc/\lambda) \propto I(\lambda)/\nu^6 . \quad \text{S7}$$

The B coefficient and the integral of $\chi''(\nu)$ are directly related to molecular matrix elements without additional frequency factors (3). As a result, absorption and emission susceptibility spectra display mirror symmetry under simple assumptions. The excited state population is proportional to the area of the emission susceptibility spectrum, even if the spectrum shifts in frequency. The susceptibility spectrum is also a standard form for discussing nonlinear optics and line broadening in condensed phases (4).

These are desirable properties. However, the presentation in term of susceptibilities rather than intensities is not essential to any of the results presented in this paper. We present all our results in terms of susceptibility spectra to facilitate future comparisons to other types of spectra and to theory.

FLUORESCENCE LIFETIMES

In addition to fluorescent probes based on solvatochromism, such as the one used here, there are also fluorescent probes that have environmentally sensitive quantum yields (or equivalently environmentally sensitive fluorescence decay rates). In general, quantum yields vary due to environmentally-induced nonradiative decay. The mechanisms of nonradiative decay are complex and difficult to relate to simple properties of the environment. Nonetheless, the environmental sensitivity quenching of 2-aminopurine has often been used to study DNA (5).

In contrast to quantum-yield based probes, coumarins have high quantum yields in a variety of solvents (6). In most cases, the decay of the excited state is uncomplicated and dominated by radiative relaxation. An example is coumarin 102 in methanol, whose single exponential fluorescence decay yields an excited-state lifetime of 4.7 ns (Table S1). Even if the decay is more complex, the shift of the spectrum measured in the TRSS experiment should be independent of the decay of the spectral intensity. Nonetheless, we report the excited-state decay in our samples for completeness.

In the presence of substantial spectral shifting, measuring the excited-state decay rate is not trivial. The fluorescence decay at any single wavelength is affected by the time dependent Stokes shift as well as the excited-state decay (Fig. 3). In broadband measurements, wavelength dependent sensitivity of the apparatus becomes an issue.

However in the course of the TRSS experiments, the excited-state decay was accurately measured. Generating the susceptibility spectra involves making the necessary corrections for variation of instrumental sensitivity and radiative emission rate. The areas of these spectra ω_0 are proportional to the excited-state population.

An example of the excited-state decay is shown in Fig. S1. At long times, the decay is dominated by an 8 ns lifetime, almost twice as long as in a methanol solution. This long lifetime is consistent with the general observation that rigid environments have a reduced nonradiative decay rate.

However at short times, faster decay components are evident (inset Fig. S1). The decay has been fit with a sum of three exponentials.

$$\omega_0(t) = \sum_{i=1}^{3} A_i \exp(-t/\tau_i)\,. \qquad \text{S8}$$

Table S1 gives the fitting parameters for all three sequences. The average decay times τ_{ave} are calculated as the area under the decay divided by the initial amplitude.

The variation in decay rate is much larger than can be accounted for by the wavelength dependence of the radiative decay rate. Thus it appears that the DNA is conformationally heterogeneous and that there are some configurations that result in rapid excited-state decay.

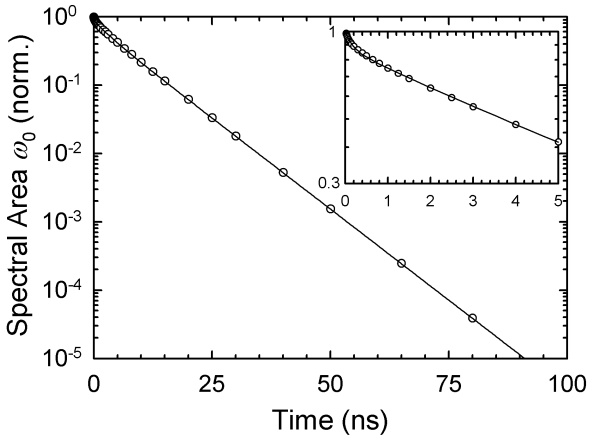

Figure S1. Excited-state decay for sequence 1 (points). The decay has been fit with a sum of three exponentials (solid curve).

Table S1. Excited-state decay parameters for coumarin 102 in methanol and in three oligonucleotide sequences.

	A_1	τ_1 (ns)	A_2	τ_2 (ns)	A_3	τ_3 (ps)	τ_{ave} (ns)
Methanol[a]:	1.00	4.7	–	–	–	–	4.73
Sequence 1:	0.71	8.2	0.17	3.0	0.12	175	6.32
Sequence 2:	0.56	7.9	0.26	4.0	0.18	360	5.54
Sequence 3:	0.54	7.8	0.25	4.0	0.21	230	5.27

[a]475 nm, 6 nm bandpass

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