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J. Am. Chem. Soc., **2003**, 125 (33), 9908-9909 • DOI: 10.1021/ja036431+ • Publication Date (Web): 30 July 2003

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Noncontact Temperature Measurement in Microliter-Sized Volumes Using Fluorescent-Labeled DNA Oligomers

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Temperature is always fundamental, yet problematic to measure in ultrasmall volumes of fluid. This problem pertains especially to microfluidic and MEMS-based methods of fluid control,^{1–3} where the presence of any external sensor would produce inordinately large heat loss to that sensor. Here, we demonstrate a simple, rationally generalizable new method to sense temperature, based on the large temperature sensitivity of the fluorescence lifetime of fluorescent-labeled DNA oligomers. Through two-photon excitation from a tightly focused laser, a tiny excitation volume ($\sim 10\text{--}15\ \mu\text{L}$) is probed without contribution from surface quenching.

The main idea of using fluorescent-labeled DNA as a temperature indicator is that DNA bases quench fluorescence with variable efficiency: adenine (A) = 0 (no quenching), cytosine (C) < guanine (G) \leq thymine (T).⁴ Therefore, the spacing between a fluorescent dye and a designed sequence of DNA bases is modulated by conformational changes of a DNA chain, and the ability of dye molecules to fluoresce is also modulated: the higher the temperature, the faster the conformational changes, so the shorter the fluorescence lifetime. The fluorescence lifetime measurement, made at the magic angle, has the additional advantage that it conveniently does not depend on the fluorescence intensity. While a sample's fluorescence intensity depends on the power of the incident light and also is adulterated by photobleaching, the fluorescence lifetime is unaffected by laser power or photobleaching.⁵ Because of this, the fluorescence lifetime measurement is reproducible and does not require laborious calibration to convert it to temperature.

We tested this idea using the previously studied rhodamine-labeled DNA oligomer,⁶ 5'-RhG-GATGATGAGAAGAAC-3', in which the rhodamine-G dye was separated from the main chain by a sequence of 6 methylene units. To excite fluorescence, we used two-photon excitation by a femtosecond Ti:Sapphire laser and focused it away from the surface to avoid surface quenching. Amidine-terminated polystyrene latex particles of diameter 40 ± 10 nm were also employed for a control experiment; they are positively charged in deionized water, with a surface charge density of $3.2\ \mu\text{C}/\text{cm}^2$. The DNA concentration in solution was dilute, $\sim 2\ \mu\text{M}$. Fluorescence lifetime and rotational correlation time constants were calculated as explained elsewhere.⁷

To illustrate temperature sensitivity, in Figure 1 the fluorescence intensity in deionized water is plotted against time on the nanosecond time scale at temperatures from 15 to 35 °C. Examples of anisotropy decay are contained in the Supporting Information. A detailed analysis of these curves shows that they obeyed two rotational correlation time constants, for example, 0.421 and 2.385 ns at 25 °C. The shorter time constant comes from the fast rotation of the carbon linker, and the longer time constant comes from the slower rotations that include the DNA backbone. This means that

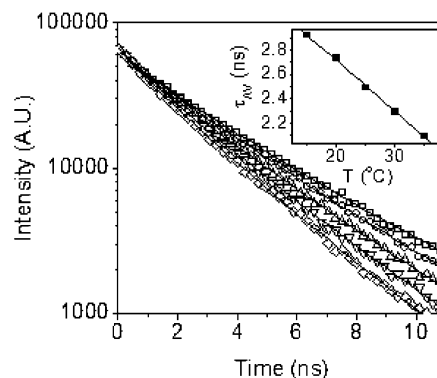


Figure 1. Fluorescence intensity plotted logarithmically against linear time on the nanosecond time scale, for 5'-RhG-GATGATGAGAAGAAC-3' at $2\ \mu\text{M}$ concentration in deionized water at various temperatures: \square 15 °C, \circ 20 °C, \triangle 25 °C, ∇ 30 °C, \diamond 35 °C. In the inset, the average fluorescence lifetime constant is plotted against temperature. Error bars are smaller than the size of the symbols.

there are two possible routes for the collision of DNA and dyes, and this is why all fluorescence lifetime decay curves in Figure 1 were best fitted by double exponential functions.

Because both affected the fluorescence lifetime, the average fluorescence lifetime, τ_{AV} ($\tau_{\text{AV}} \equiv A_1\tau_1 + A_2\tau_2$, $A_1 + A_2 = 1$), was used for further analysis. The inset of Figure 1 shows that τ_{AV} decreased linearly with temperature, with a slope of 42 ps/°C. When one compares to a more detailed analysis the changes of rotational correlation time of the DNA backbone (40 ps/°C) and of the carbon linker (6.4 ps/°C), it is evident that the overall change of fluorescence lifetime by 42 ps/°C was closer to that of the DNA backbone rotation. This implies that rotation of the DNA backbone was the main barrier for the fluorescence quenching. The apparent activation energy was $12.5\ \text{kJ K}^{-1}\ \text{mol}^{-1}$.

To test if this reflected conformational changes of DNA chemically linked to the fluorescent label, control experiments were performed. First, the fluorescence lifetime of unattached rhodamine-6G dyes was measured (not shown here); they showed a single lifetime time constant regardless of temperature. Second, the labeled DNA was allowed to adsorb onto larger colloidal particles to experimentally separate motion of the DNA backbone from that of the short carbon chain that linked the dye to the DNA. As DNA and these nanoparticles possessed opposite charge, they formed a charge-charge complex in which the adsorbed DNA backbone could not rotate on the nanosecond time scale. In Figure 2, the time-resolved fluorescence decay is plotted at several temperatures. The inset shows that the average lifetime was nearly temperature-independent. To observe this constant lifetime confirms that the τ_{AV} of labeled DNA in free solution resulted from intrinsic fluorescence quenching by DNA residues. Parenthetically, we note

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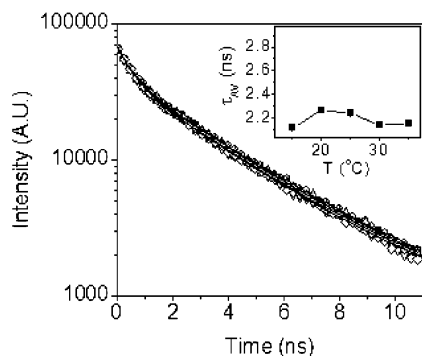


Figure 2. Fluorescence intensity plotted logarithmically against linear time on the nanosecond time scale, for 5'-RhG-GATGATGAGAAGAAC-3' adsorbed onto colloidal particles of opposite charge as described in the text, at various temperatures: \square 15 °C, \circ 20 °C, \triangle 25 °C, ∇ 30 °C, \diamond 35 °C. In the inset, the average lifetime constant is plotted against temperature. The temperature independence in this control experiment confirms that temperature sensitivity in Figure 1 results from conformational changes of the DNA chains.

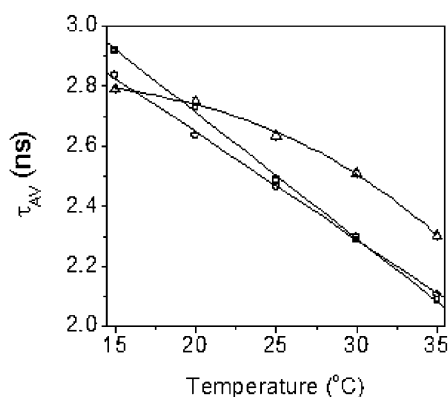


Figure 3. The average fluorescence lifetime (τ_{AV}) plotted against temperature for the following: \square ssDNA in deionized water, \circ ssDNA in TE buffer, and \triangle dsDNA in TE buffer. Error bars are smaller than the size of the symbol.

that the reason for somewhat faster decay at the earliest times is likely surface quenching.

Other control experiments concerned the fluorescence lifetime when this single-strand DNA (ssDNA) was hybridized with the complementary sequence, resulting in stiffer chains of double-stranded DNA (dsDNA). This operation, performed in TE buffer solution with 50 mM concentration of NaCl, also increased the fluorescence lifetime. Figure 3 compares the average fluorescence lifetime of ssDNA in deionized water, ssDNA in TE buffer, and dsDNA in TE buffer. Initially, we had expected that because dsDNA is stiffer, fluorescence quenching would be less probable and the fluorescence lifetime would be longer over the entire temperature range. The nonlinear dependence that we in fact observed, indicating that enhanced quenching occurred at the lower temperatures, can

probably be explained as the tradeoff between the larger volume of the dsDNA chain and its slower chain dynamics, but no quantitative explanation is offered at this time. The main point is that the temperature sensitivity of ssDNA was insensitive to ionic strength, the slope being 42 ps/°C (deionized water) and 36 ps/°C (buffer). It is, anyway, something of a moot point because in the applications we envision, the buffer concentration will be held constant.

In summary, the fluorescence lifetime of labeled DNA is shown to be a useful, generalizable method to measure temperature. This method is noninvasive and, using the methods of two-photon excitation employed in this study, can be applied to microliter-sized volumes. Alternatively, the method can be implemented using confocal single-photon fluorescence methods. As a hypothetical example of applicability, one can imagine a miniaturized PCR (polymerase chain reaction) in a microfluidic channel; because the efficiency of the PCR reaction is so sensitive to temperature,⁸ successful implementation of the PCR reaction would require knowing the temperature with precision, so data of this kind would be of value. Also, in the study of aqueous lubrication, an unmet need is to know the temperature in micrometer-thin films.⁹

Although the strategy of temperature measurement presented here is based on measurements using a specific sequence of DNA, whose specific temperature sensitivity depends on having that specific sequence, it can also be generalized to a dye attached to any other intrinsic quencher of fluorescence whose conformation changes with temperature.

Acknowledgment. This work was performed at the University of Illinois and supported by the U.S. Department of Energy, Division of Materials Science, under Award No. DEFG02-02ER46019 through the Frederick Seitz Materials Research Laboratory at the University of Illinois at Urbana-Champaign.

Supporting Information Available: Time-resolved fluorescence anisotropy decay for ss-DNA in deionized water (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA036431+