Temperature dependent excited state relaxation of a red emitting DNA-templated silver nanocluster†

Cecilia Cerretani, Miguel R. Carro-Temboury, Stefan Krause, Sidsel Ammitzbøll Bogh and Tom Vosch

The nanosecond excited state temporal and spectral relaxation of a purified, red-emitting DNA-templated silver nanocluster (DNA–AgNC) was characterized as a function of temperature. The findings are explained by introducing a phenomenological electronic structure diagram. The reproducibility and cyclability of the average decay time opens up the possibility of using DNA–AgNCs for decay time-based nanothermometry.

DNA–AgNCs are fluorescent emitters comprising a few silver atoms (typically <25) stabilized by a single stranded DNA scaffold. Their brightness, photostability and wavelength tunability have encouraged their use as sensors and fluorophores in imaging applications. Despite the increase in the number of applications, the relationship between the DNA sequence, the AgNC structure and its final photophysical properties is still an active area of research. In particular, the DNA scaffold appears to have an important influence on the solvation dynamics of the DNA–AgNCs. After the initial sub-picosecond relaxation accounting for most of the Stokes shift, the fluorescence spectra undergo red-shift on a nanosecond time scale. As shown in a previous study, the increase of the average decay time as a function of wavelength in purified DNA–AgNCs can be explained by a spectral relaxation. This phenomenon can be convoluted with other phenomena e.g. multiple emitters in non-purified samples.

Here, we studied the effect of temperature on the nanosecond spectral relaxation dynamics of DNA–AgNCs in 10 mM ammonium acetate (NH₄OAc). Besides giving insight on the spectral relaxation process, this study also demonstrates that the average decay time of DNA–AgNCs could find applications in nanothermometry, where nanoscale probes are used as local thermometers. Therefore, we investigated the performance of the average decay time of DNA–AgNCs as a temperature probe in the temperature range of 5–60 °C together with its stability and cyclability. Although nucleic acid based nanothermometers have been previously reported, to the best of our knowledge, the use of DNA–AgNCs in thermometry applications has not been reported.

In this work we used a cytosine rich DNA sequence 5’-TCTCCACCCACCCCGGCC-3’. This sequence is a shortened version of the sequence 5’-TTCCCACCCACCCCGGCCC-3’ previously studied by Gwinn et al. The removal of the three terminal bases does not seem to change the spectral properties of the DNA–AgNC suggesting that they were not involved in stabilizing the AgNC. After the synthesis of DNA–AgNCs, the sample was purified by HPLC (see Fig. S1, ESI†) where the fraction with a retention time of 9 minutes was obtained. The DNA–AgNCs show an absorption maximum at 573 nm and an emission maximum around 640 nm (Fig. 1). The 2D excitation versus emission plot (Fig. S2, ESI†) shows that the DNA–AgNC appears like a single emissive species. The purified DNA–AgNCs possess good long term stability as evidenced from the very similar absorption spectra measured for a freshly purified sample compared to the same sample one month after synthesis (Fig. S3, ESI†).

Fig. 1 Normalized absorption and steady-state emission at various temperatures. For the emission spectra, the DNA–AgNC sample was excited at 561 nm.
This indicates that temperature mainly affects the excited state relaxation process. An overview of the fluorescence quantum yield, absorption and emission maxima at 5 °C, 25 °C and 40 °C can be found in Table 1.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>λ_{abs} (max)</th>
<th>λ_{em} (max)</th>
<th>1/τ_{w}</th>
<th>QY</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 °C</td>
<td>573 nm</td>
<td>638 nm</td>
<td>2.7 ns</td>
<td>0.88</td>
</tr>
<tr>
<td>25 °C</td>
<td>573 nm</td>
<td>640 nm</td>
<td>2.59 ns</td>
<td>0.80</td>
</tr>
<tr>
<td>40 °C</td>
<td>574 nm</td>
<td>642 nm</td>
<td>2.47 ns</td>
<td>0.70</td>
</tr>
</tbody>
</table>

*Table 1: Absorption and emission maxima, λ_{abs} (max) and λ_{em} (max), weighted average fluorescence decay time, 1/τ_{w}, and fluorescence quantum yield, QY, at different temperatures.*

We explain this effect using a generalized phenomenological AgNC electronic structure model, which is shown in Fig. 3B and is based on previous photophysical studies on DNA–AgNCs.4,6 From the Franck–Condon state (FC) right after absorption of a photon, the system evolves to either the ground state (S0), a dark state (D1) or to the emissive state (S1) on a time scale that cannot be resolved with TCSPC equipment used here, but is demonstrated in literature data.26 The dark state D1 usually has a decay time in the tens of microseconds range and can be used to modulate the fluorescence or generate delayed fluorescence from S1 through secondary absorption of a photon.4,6 However, in this study, we are only monitoring the pathways indicated by the blue arrows. Therefore, we rewrite the measured fluorescence steady state quantum yield (QY) as QY = QY_{S1, quantum} + QY_{D1, delayed}. This model could also help explain the usual nanosecond decay time reported in the literature for DNA–AgNCs, seemingly uncorrelated to the...
corresponding QY and molar absorption coefficient. From the fit to our data, \( \tau_w = 2.86\text{ ns} \) for QY = 1. Since at this point QY = 1 (hence also QYf and QYS1 must be 1), we can calculate the value of \( k_t \) at QY = 1 to be \( k_t^{100\%} = 3.5 \times 10^8 \text{ s}^{-1} \) and plot QYf assuming a constant \( k_t^{100\%} \) (red curve) and QYS1 (green curve) for comparison with our data. The fact that our data was fitted with a linear function of the form QY = a(\( \tau_w \)) - b, where a,b > 0 implies that QYf, k_t = a - b(\( \tau_w \)). Since it is not known a priori which of the values (QYS1 or k_t or both) change with temperature, there are three different possible scenarios that could explain the blue line in Fig. 3A.

In Scenario I (Fig. 3A and Fig. S5, ESI†), the value of k_t is assumed to be constant and has the value \( k_t^{100\%} \). This implies that QYS1 (green line) decreases as a function of temperature faster than QYf (red line) and is responsible for a faster decrease of the measured total QY. Interestingly, this would allow the observed \( \tau_w \) to remain in the nanosecond range, even close to a QY of zero. The non-radiative rate \( k_{nr} = 1/(\tau_w) - k_t \) also increases as a function of temperature in this scenario. In Scenario II (Fig. S6, ESI†), QYS1 is constant, likely at or close to 1 (since we measured a value of QY = 0.9 at 5 °C), and the decrease in k_t and the increase in k_{nr} are responsible for the QY trend. In Scenario III (Fig. S7, ESI†), both variables (QYS1 and k_t) change as a function of temperature in such a way that the linear relationship between QY and \( \tau_w \) holds. The latter seems unlikely. We currently believe that Scenario I is the most plausible. However, further investigations are needed for a more conclusive answer. In order to assess the reproducibility and cyclability of the temperature response, we measured TRES data while cycling the temperature between 5 °C, 25 °C and 40 °C four times in different orders and in a time span of 25 hours (Fig. 4A). Note that the sample was stored in a fridge between cycle 3 and cycle 4. The average decay time spectra (Fig. 4B) of the four cycles show a very similar slope for the three temperatures, and a temperature dependent offset. The average decay times were extracted from a tri-exponential fit with linked decay times. The spectral shift of the emission maxima was interpolated from the TRES.

These relaxation dynamics of the emission maximum usually comprise a fast component in the order of hundred picoseconds and slower components in the nanosecond range (see Fig. 4C). This is in agreement with previous studies. The spectral shifts measured while cycling the temperature are shown in Fig. 4C. The time resolved emission maxima clearly decrease as a function of increasing temperature, in line with the steady state measurements (Fig. 1). The starting value of the emission maxima at \( t = 0 \) also decreases as a function of temperature. As the absorption remains constant over this temperature range, the emission maxima at \( t = 0 \) at all the temperatures should in principle be the same. However, in our TCSPC experiments we cannot observe the initial fast spectral relaxation due to the IRF.
limited response of the equipment \( \approx 150 \) ps. This is in agreement with previous ultrafast spectroscopy studies which show that the electronic relaxation from the absorbing state to the emissive state occurs on a sub-picosecond timescale.\(^{14,26,27} \) The time-resolved measurements demonstrate the reproducible and reversible temperature response of the AgNCs. It also validates the thermal stability of the DNA–AgNCs in the probed temperature range of 5–40 °C, as shown in Fig. 4. Together with previous studies in the literature, this indicates that the nanosecond spectral relaxation could be a common feature in DNA–AgNCs.\(^{15,17–19} \) Next, we studied the temperature dependent change of (\( \tau \)) in more detail. For this, a new batch of the same DNA–AgNCs was synthesized under the same conditions. Now, only single decay curves at \( \lambda_{\text{exc}} = 561 \) nm and \( \lambda_{\text{em}} = 633 \) nm were measured while varying the temperature from 5 °C to 60 °C, in steps of 5 °C (Fig. S8, ESI†). The curves were fitted with a bi-exponential fluorescence decay model in order to determine (\( \tau \)). Fig. 4D shows (\( \tau \)) plotted as a function of temperature (black circles) which can be fitted with a linear function (solid black line) in this extended temperature range. The decay time values from the previous sample (Fig. 4B) at \( \lambda_{\text{em}} = 635 \) nm, plotted in the same graph (Fig. 4D, square markers) fall close to the fit, indicating a high degree of reproducibility. Using the linear fit in Fig. 4D as a calibration curve for thermometer applications, and using the formula \( \Delta(\tau)/\Delta T = \langle(\tau)\rangle_{\text{max}}^{\mathrm{31}} \) we can estimate a sensitivity value with respect to the highest decay time of \( S = 0.0027 \) °C\(^{-1} \) at a temperature range of 5–60 °C. This is about five times less than that of the well known Rhodamine B example which has \( S = 0.0127 \) °C\(^{-1} \) at a temperature range of 10–70 °C (see ESI†).\(^{20} \) Since this is the first reported example of DNA–AgNCs, it is likely that there might be other examples with higher sensitivity and biocompatibility, opening up possibilities for \textit{in vitro} and \textit{in vivo} thermometry.\(^{5} \)

In conclusion, we have studied the fluorescence decay of DNA–AgNCs as a function of temperature, and found that they can be used as decay time based nanothermometers. Further systematic studies with different sequences may improve the performance of such thermometers and could explain if viscosity also plays a role.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

27. Usually this is done for fluorophores that decay mono-exponentially, but here we have assumed that the average decay can also be a representative value.