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Supporting Information.

Observation of microsecond luminescence while studying two DNA-stabilized silver nanoclusters emitting in the 800-900 nm range.

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MATERIALS AND METHODS

1. Synthesis of DNA841-AgNC and DNA811-AgNC

DNA841-AgNCs and DNA811-AgNCs were prepared by mixing the corresponding hydrated DNA oligonucleotides (DNA841: 5'- GACACGGACC -3', DNA811: 5'- AGTCACGACA -3', Integrated DNA Technologies) with a $AgNO_3$ (Sigma Aldrich, ≥ 99.998%) aqueous solution in 10 mM ammonium acetate (NH_4OAc, Sigma Aldrich, ≥ 98%) at pH 7. After 15 minutes $NaBH_4$ (Sigma Aldrich, ≥ 99.99%) was added to the solutions in order to reduce the silver cations and promote the formation of the silver clusters. For both DNA-AgNCs, the best ratio between the components was found to be [DNA]:[$AgNO_3$]:[$NaBH_4$] = 30 μM: 150 μM: 75 μM.

After synthesis, the solutions were stored in the fridge. For DNA811-AgNCs, the HPLC purification was performed 3 days after the synthesis, while DNA841-AgNCs were purified after a week. In the end, the purified fractions were solvent exchanged to 10 mM NH_4OAc by spin-filtration (3 kDa cut-off membrane filters).

2. HPLC purification methods

For both DNA-AgNCs, the HPLC purification was performed using a preparative HPLC system from Agilent Technologies with an Agilent Technologies 1260 Infinity fluorescence detector, an Agilent Technologies 1100 Series UV-Vis detector, and a Kinetex C18 column (5 μm, 100 Å, 250 × 4.6 mm), equipped with a fraction collector. The mobile phase was a gradient mixture of 35 mM triethylammonium acetate (TEAA) buffer in water (A) and methanol (B). The flow rate was 1 mL/min.

For DNA841-AgNCs the elution gradient ranged from 5% to 95% B. In the first 2 min, it was kept constant to 5% B, then it was linearly increased to 20% B in 15 min, and finally in the 17-20 min interval, the gradient was rapidly varied from 20% to 95% B. The collection was based on the absorbance at 640 nm. As shown in the chromatograms in Figure S1, the purified fraction eluted between 8.5 and 12.5 min (broad peak≈11.5-14.5% B).

For DNA811-AgNCs the gradient was varied as follows: 0-2 min 10% B, 2-17 min linear increase of B until 25%, and 17-20 min from 25% to 95% B. Based on the chromatograms (Figure S2), a single fraction with a retention time around 13 min (≈21% B) was collected by using the absorbance signal at 640 nm.

Both runs were followed by 5 minutes of washing with 95% B to remove any traces of the samples from the column.

3. Spectroscopic measurements

3.1 Absorption measurements

Absorption spectra were measured with a Cary 300 UV-Vis spectrophotometer from Agilent Technologies using a deuterium lamp for ultraviolet radiation and a tungsten-halogen lamp for visible and near-infrared (NIR) radiation.

3.2 Steady-state emission and excitation measurements - FluoTime300 instrument

Steady-state fluorescence measurements for both DNA841-AgNCs and DNA811-AgNCs were performed using a FluoTime300 instrument from PicoQuant. All emission spectra have been corrected for the wavelength dependency of the detector.

Depending on the excitation wavelength, the source used for recording fluorescence spectra was either a picosecond pulsed laser or a Xe arc lamp. Below is a list of wavelengths and sources employed for measuring the spectra reported in the manuscript and supporting information:

- 365 nm (Xe lamp)
- 445.4 nm (LDH-P-C-450B, PicoQuant)
- 507.5 nm (LDH-P-C-510, PicoQuant)
- 634.8 nm (LDH-P-635, PicoQuant)

The excitation spectra were also carried out with a FluoTime300 system equipped with a Xenon arc lamp to monitor the emission at 835 nm for DNA841-AgNCs and 800 nm for DNA811-AgNCs. The spectra were corrected for the wavelength dependency of the detector and for the lamp power.

3.3 Quantum Yield (Q) measurements and calculations

The FluoTime300 system (PicoQuant), used for most measurements, becomes increasingly insensitive beyond 800 nm. Reference-based quantum yield measurements require an accurate determination of the emission spectrum of the reference compound and the DNA-AgNCs of interest. To circumvent the low detector efficiency of the FluoTime300 in the NIR, we used our home-built confocal microscope.[1]

A fiber coupled (NKT Photonics, FD7-PM) continuum white-light laser (NKT Photonics, SuperK EXTREME EXB-6) was used as an excitation source delivering 640 nm wavelength by sending the continuum output through an acousto-optic tunable filter (NKT Photonics, SuperK SELECT). The output of the fiber was cleaned up by a 640 nm band-pass filter (Semrock, LD01- 640BP/8) before it was reflected by a 30:70 beam splitter (Omega Optical, XF122) and sent through an air objective (Olympus, CPlanFLN 10x, NA= 0.3). A standard 1 cm quartz cuvette (Hellma) filled with aqueous solutions of Atto740 (reference dye), DNA841-AgNCs or DNA811-AgNCs was placed on top of the microscope sample stage, and the laser was focused 1 mm into the solutions ensuring that the spectra of the three samples were recorded under identical conditions. The objective that focused the laser into the samples and reference solutions was the same that collected the fluorescence. The laser light was blocked by a filter (Semrock, BLP01-647R), and out-of-focus light was blocked by a 100 µm pinhole. The fluorescence was sent through a spectrograph (SP 2356 spectrometer, 300 grooves/mm, Acton Research) onto a nitrogen cooled CCD camera (SPEC-10:100B/LN-eXcelon, Princeton Instruments) to record the spectra. Finally, the emission spectra were intensity corrected as reported previously.[1]

Prior to fluorescence measurements, the absorption spectra of the clusters and reference compound were carried out using a Cary 300 UV-Vis spectrophotometer.

The quantum yield for DNA841-AgNCs and DNA811-AgNCs was then calculated using the following formula[2]:

$$\mathbf{Q_{NC}} = \frac{\mathbf{F_{NC}}}{\mathbf{f_{A,NC}}} \cdot \frac{\mathbf{f_{A,ref}}}{\mathbf{F_{ref}}} \cdot \frac{\mathbf{n_{NC}^2}}{\mathbf{n_{ref}^2}} \cdot \mathbf{Q_{ref}} \quad \textbf{(1)}$$

where **Q** represents the quantum yield value, **F** is the integrated emission spectrum, $\mathbf{f_A}$ is the fraction of absorbed light, and **n** is the refractive index of the medium where the compound is dissolved in. The subscripts **NC** and **ref** indicate the DNA-AgNC and the reference dye, respectively.
Only one point for the integrated emission counts and one point for the fraction of absorbed light were used in the calculations of the quantum yield of DNA841-AgNCs and DNA811-AgNCs. See Figure S4 for the actual data.

3.4 Steady-state emission measurements – Home-built confocal microscope

The emission spectra shown in Figure 1 were measured on the home-built confocal microscope described above using an oil immersion objective (Olympus, UPlanSApo 100x, NA = 1.4) instead of the air objective (Olympus, CPlanFLN 10x, NA = 0.3). The excitation wavelength selected from the continuum white-light laser was 640 nm for DNA841-AgNCs and 645 nm for DNA811-AgNCs. 25 µL droplets of DNA841-AgNC and DNA811-AgNC solutions were placed on two clean coverslips, and the corresponding fluorescence spectra were recorded. These spectra were both wavelength and intensity corrected, as previously reported, using a calibrated light source.[1]

3.5 Time-correlated single photon counting (TCSPC) - FluoTime300 system

Time-resolved fluorescence and anisotropy measurements for both DNA841-AgNCs and DNA811-AgNCs were performed using a FluoTime300 instrument from PicoQuant with a vertically-polarized 634.8 nm pulsed laser as excitation source.
Additional anisotropy measurements were carried out for DNA811-AgNCs in 95% glycerol and 5% 10 mM NH_4OAc at 5 ºC. The decay curves were measured at 800 nm, exciting at 445.4 nm (repetition rate = 10 MHz), and were recorded at different excitation and emission polarizations (VV = vertical excitation vertical emission, HH = horizontal excitation horizontal emission, VH = vertical excitation horizontal emission, HV = horizontal excitation vertical emission). The area under the curves and the area of the backgrounds were used to calculate the steady-state anisotropy (eq. 2) of the nanosecond NIR emission and that of the microsecond blue-shifted NIR emission, respectively.

3.5.1 Acquisition and analysis of fluorescence decays

Single fluorescence decays of DNA811-AgNCs were acquired at λ_{em}= 800 nm at various temperatures (5, 25, and 40 ºC) and in two different media: 10 mM NH_4OAc aqueous solution, and 95% glycerol and 5% 10 mM NH_4OAc. The integration time was chosen between 10 and 20 s in order to reach at least 10,000 counts in the maximum.
Single fluorescence decays of DNA841-AgNCs were carried out at several temperatures, ranging from 5 ºC to 80 ºC. The decay curves were recorded at λ_{em}= 835 nm in 10 mM NH_4OAc aqueous

solution, and 820 nm in 95% glycerol and 5% 10 mM NH_4OAc. The integration time was set to 5 or 15 s in order to achieve at least 10,000 counts in the maximum.
The analysis of time-resolved data was performed with FluoFit v.4.6 from PicoQuant. All decays were fitted globally with a bi- or tri-exponential reconvolution model including the IRF (instrument response function). The intensity averaged decay time $<\tau>$ of every decay was calculated as the intensity-weighted average decay time at the selected emission wavelength.[3]

3.5.2 Low temperature measurements: steady-state and time-resolved emission spectra (TRES)

Low temperature steady-state and time-resolved emission measurements of DNA811-AgNCs and DNA841-AgNCs in 10 mM NH_4OAc were carried out by pouring the solutions into an NMR tube immersed in a transparent Dewar filled with liquid nitrogen (-196 °C), and placing it in a FluoTime300 instrument (PicoQuant). In order to limit the increased scattering, two filters were utilized: a 640 nm band-pass filter (Semrock, LD01-640/8-25) in the excitation path, and a 647 nm long-pass filter (Semrock, BLP01-647R-25) in the emission path. The acquired steady-state emission spectra were corrected for the wavelength dependency of the detector.
Time-resolved emission spectra (TRES) of DNA811-AgNCs and DNA841-AgNCs were constructed by measuring fluorescence decays every 5 nm, in the range 710 - 840 nm and 730 - 830 nm, respectively. In the case of DNA811-AgNCs, every decay was integrated for 30 s, while the integration time for DNA841-AgNCs was 10 s per decay. This integration times were chosen in order to acquire at least 10,000 counts in the maximum at λ_{em}^{max}.
All decay curves were globally fitted with a bi-exponential reconvolution model including the scattered light contribution and the IRF. The obtained TRES were corrected for the detector efficiency and were interpolated with a spline function using the built-in *spaps* MATLAB function with a tolerance of 10^{-10}, *i.e.* forcing the interpolated curve to go through the data points. The average decay time $<\tau>$ at every measured wavelength was calculated as the intensity-weighted average decay time.

3.5.3 Temperature cycles for DNA841-AgNC

Temperature cycles were performed for DNA841-AgNCs in 10 mM NH_4OAc aqueous solution. The cycles consisted of two temperatures, 25 °C and 60 °C or 80 °C. Each heating or cooling step lasted 30 minutes after reaching the target temperature. To ensure a thermal equilibrium the sample was also stirred. The laser was blocked during the waiting period. Absorption, emission spectra and fluorescence decays were recorded at both temperatures for every step. The fluorescence measurements were acquired exciting at 634.8 nm, and the decays were monitored at λ_{em}= 835 nm. Overall, 3 cycles were carried out.

3.5.4 Acquisition and analysis of time-resolved anisotropy data

Time-resolved anisotropy measurements were carried out acquiring both vertically and horizontally polarized fluorescence intensity decays at three different temperatures: 5 °C, 25 °C and 40 °C for DNA811-AgNCs and DNA841-AgNCs in 10 mM NH_4OAc.

The decays were fitted with FluoFit v.4.6 from PicoQuant. A multi-exponential and a mono-exponential reconvolution model (including the IRF) were used, respectively, for the decay time and the rotational correlation time (θ) of both DNA811-AgNCs and DNA841-AgNCs.
The Perrin equation[3] $\theta=\eta V_{hydro}/k_BT$, where η is the dynamic viscosity of the solvent, V_{hydro} is the hydrodynamic volume of the species and k_BT is the product between the Boltzmann constant and the absolute temperature, was used to calculate the hydrodynamic volume of the clusters. For simplicity, the Perrin model assumes that the investigated compound is spherical.

3.6 Steady-state emission and excitation anisotropy measurements

Steady-state emission and excitation anisotropies of DNA811-AgNCs and DNA841-AgNCs were measured in 95% glycerol and 5% 10 mM NH_4OAc at 5 °C using a FluoTime300 instrument from PicoQuant.
Parallel (I_{VV}, I_{HH}) and perpendicular (I_{VH}, I_{HV}) emission spectra were recorded with a 634.8 nm pulsed laser as excitation source. These data were used to calculate the instrumental G factor ($G=I_{HV}/I_{HH}$) and the limiting emission anisotropy (**r**) of the DNA-AgNCs:

$$\mathbf{r} = \frac{\mathbf{I_{VV} - G \cdot I_{VH}}}{\mathbf{I_{VV} + 2 \cdot G \cdot I_{VH}}} \quad \textbf{(2)}$$

Steady-state excitation spectra at diverse polarizations (I_{VV}, I_{VH}, I_{HV}, I_{HH}) were acquired monitoring the emission maxima (λ_{em}= 800 nm and 550 nm for DNA811-AgNCs, and λ_{em}= 835 nm for DNA841-AgNCs) with a Xe arc lamp. G factors and excitation anisotropy values were calculated as described for the emission anisotropy data (eq. 2).[3]
Figure S5 shows three excitation anisotropy traces resulting from different measurements of DNA811-AgNCs. The artifacts around 550 nm (magenta arrow) and above 650 nm (green arrow) were suppressed by using a 325 nm long-pass filter (Semrock, BLP01-325R-25) and a 458 nm long-pass filter (Semrock, BLP01-458R-25), respectively. The resulting excitation anisotropy trace is therefore a combination of the data measured by placing the filters in the excitation path. The same applies for DNA841-AgNCs (data not shown). Examples of I_{VV}, I_{VH}, I_{HV}, I_{HH} for DNA841-AgNCs and DNA811-AgNCs can be found in Figure S6 and Figure S7.

3.7 Microsecond decay measurements of DNA811-AgNC

Microsecond decay measurements were performed for DNA811-AgNCs in 10 mM NH_4OAc using a FluoTime300 instrument (PicoQuant). The sample was excited with a Xenon flash lamp (repetition rate =300 Hz) at 450 and 635 nm. For the latter measurements, a 458 nm long-pass filter (Semrock, BLP01-458R-25) was placed in the excitation path. The IRFs of the Xe flash lamp at the above-mentioned wavelengths are shown in Figure S12.
All decay curves were recorded at λ_{em}= 800 nm at different temperatures (5, 15, 25 and 35 ºC), with an integration time of 10 min. The decays excited at 635 nm are IRF-limited (Figure S13), indicating that no significant microsecond-lived emission is present. The decays measured at λ_{exc}= 450 nm and shown in Figure 6C were tail-fitted mono-exponentially in order to determine the decay time.

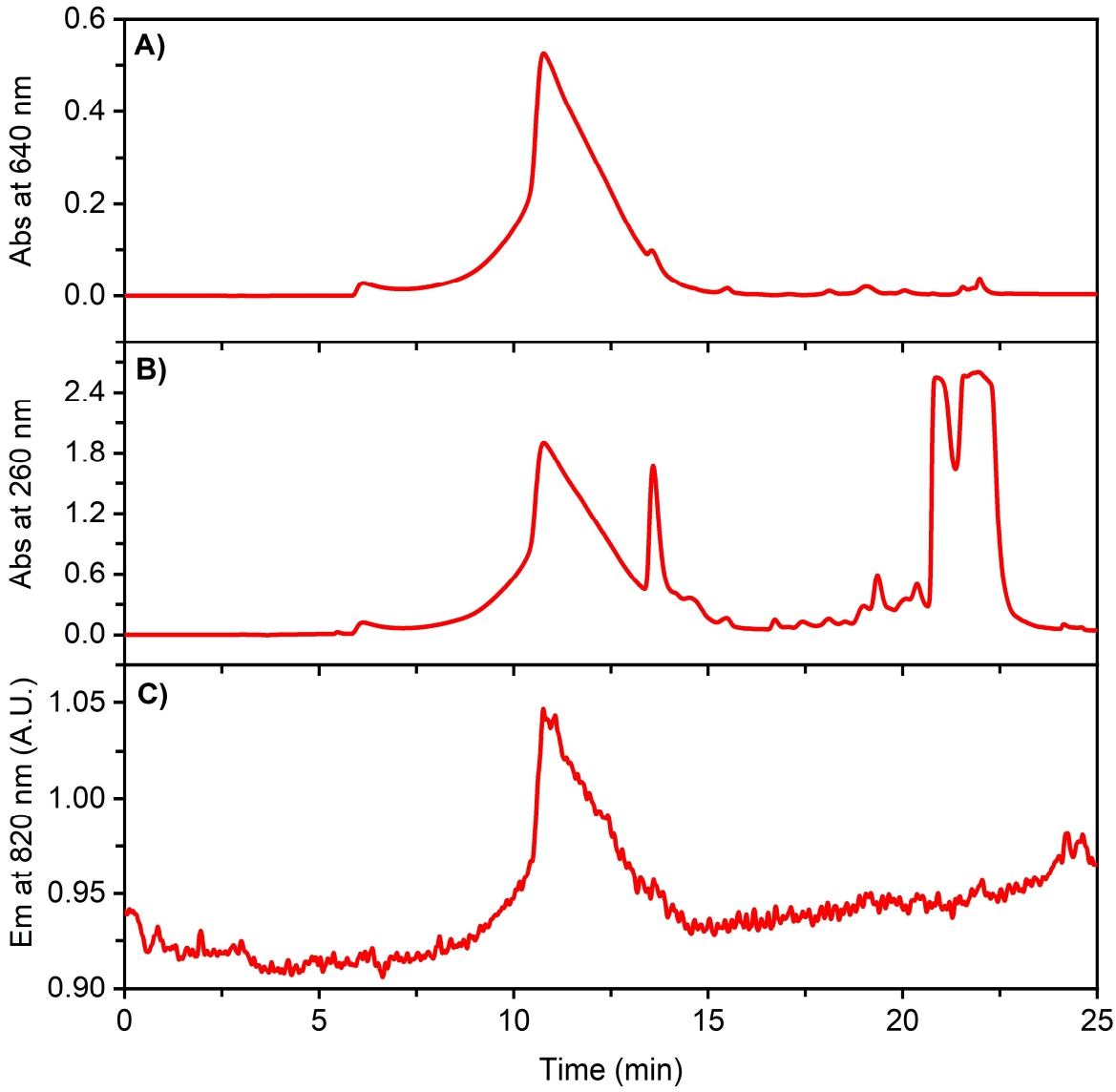

Figure S1. HPLC chromatograms of DNA841-AgNCs **A)** monitoring the main absorption peak of the clusters at 640 nm, and **B)** tracking the DNA absorption at 260 nm. **C)** Chromatogram that monitors the emission of the clusters at 820 nm (λ_{exc}= 640 nm). The fraction collected between 8.5-12.5 min is the sample described in the manuscript.

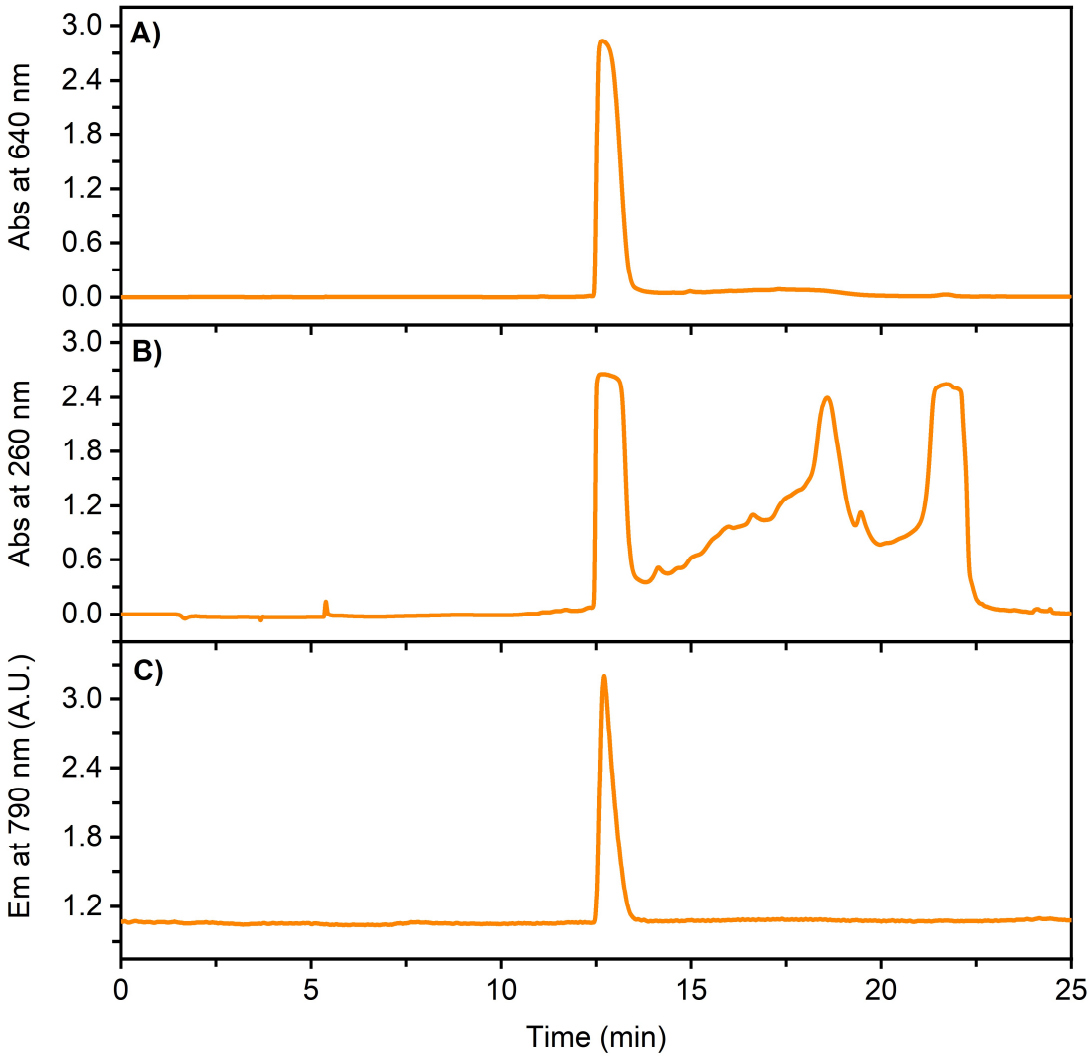

Figure S2. HPLC chromatograms of DNA811-AgNCs **A)** monitoring the main absorption peak of the clusters at 640 nm, and **B)** tracking the DNA absorption at 260 nm. **C)** Chromatogram that monitors the emission of the clusters at 790 nm (λ_{exc}= 640 nm). The fraction collected around 13 min is the sample described in the manuscript.

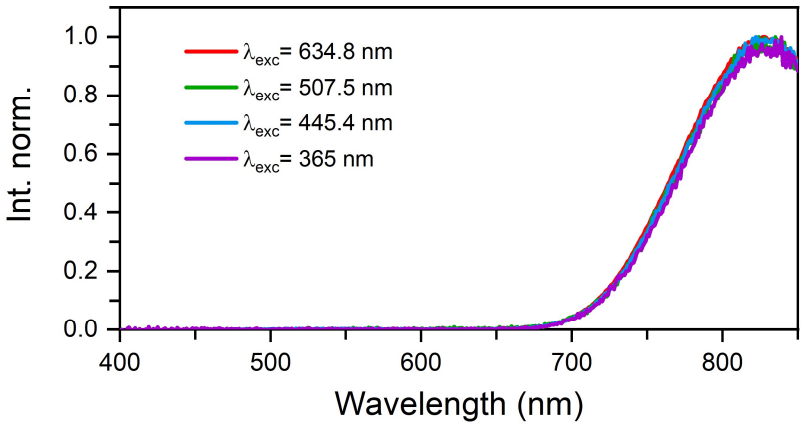

Figure S3. Normalized emission spectra of DNA841-AgNCs in 10 mM NH_4OAc, excited at different wavelengths.

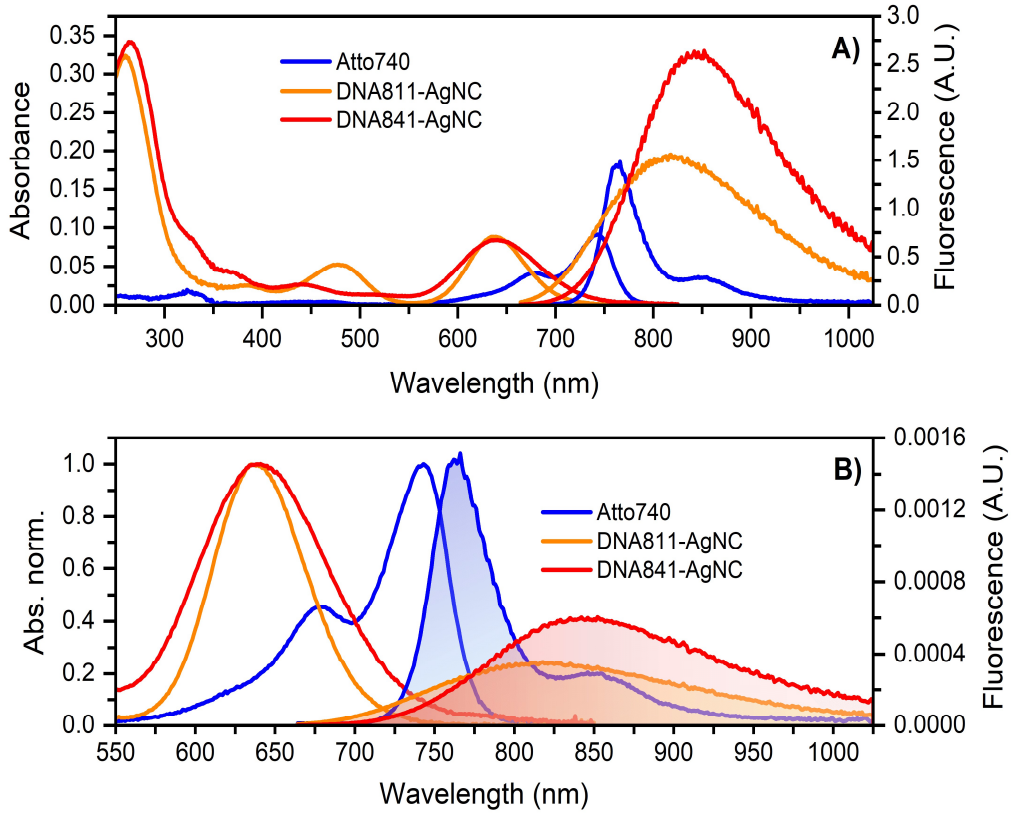

Figure S4. **A)** Absorption and emission spectra (λ_{exc}= 640 nm) that were used to calculate the fluorescence quantum yield of DNA841-AgNC and DNA811-AgNC. Atto740 was used as a reference dye with a reported quantum yield of 0.1 by the manufacturer.[4] For both DNA841-AgNC and DNA811-AgNC a small part of the red edge is missing and hence the actual quantum yields will be slightly higher. The EMCCD camera that was used as detector becomes increasingly insensitive from 1050 nm onwards. **B)** Absorption and emission spectra (λ_{exc}= 640 nm) of Atto740, DNA811-AgNC and DNA841-AgNC. Absorption spectra are normalized to the maximum of the lowest energy transition. For the emission spectra, the area under the curve is normalized to the corresponding quantum yield.

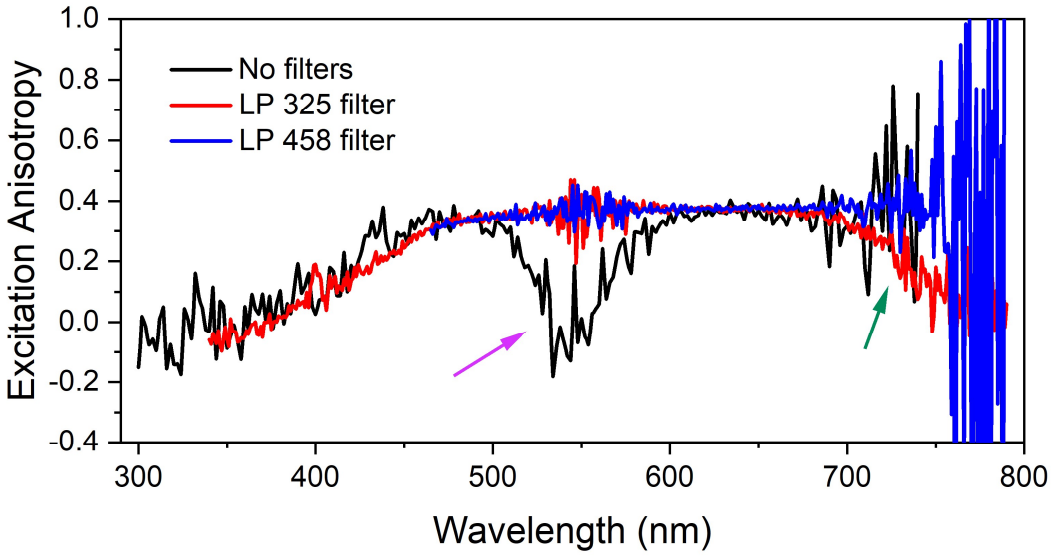

Figure S5. Excitation anisotropy traces of DNA811-AgNCs in 95% glycerol and 5% 10 mM NH_4OAc at 5 ºC (λ_{em}= 800 nm), measured without filters (black), with a 325 nm long-pass filter (red), and with a 458 nm long-pass filter (blue). The magenta arrow indicates the artifact around 520-580 nm that was suppressed by using a 325 nm long-pass filter, while the green arrow points to the artifact above 650 nm, which was removed by placing a 458 nm long-pass filter in the excitation path.

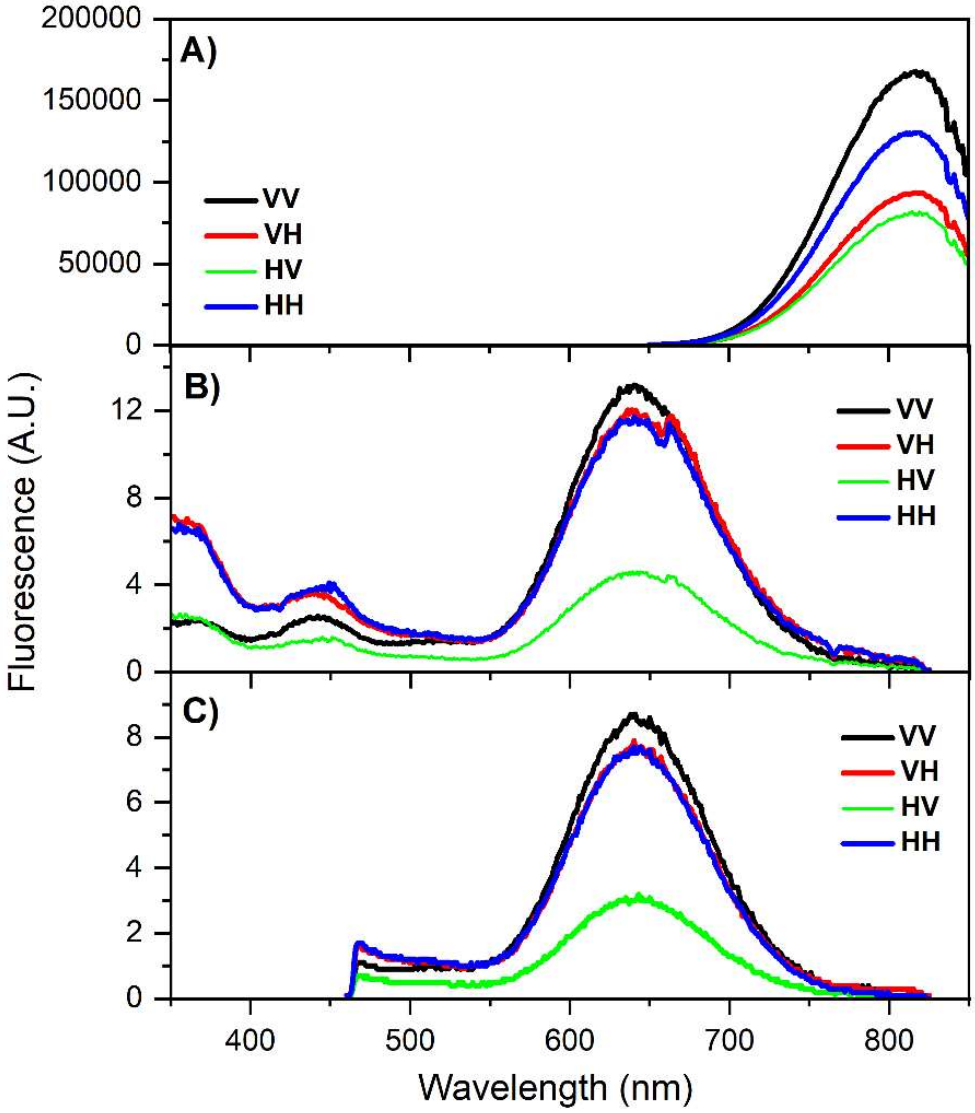

Figure S6. Raw spectra of DNA841-AgNCs in 95% glycerol and 5% 10 mM NH_4OAc at 5 °C. **A)** Emission spectra at different emission polarizations (vertical and horizontal), exciting with vertically and horizontally polarized 634.8 nm laser. **B)** Excitation spectra at different excitation polarizations (vertical and horizontal), monitoring the emission with vertically and horizontally polarized light at 835 nm (Xe lamp). A 325 nm long-pass filter was placed in the excitation path. **C)** Same excitation spectra (I_{VV}, I_{VH}, I_{HV}, I_{HH}) as (B), but placing a 458 nm long-pass filter in the excitation path.

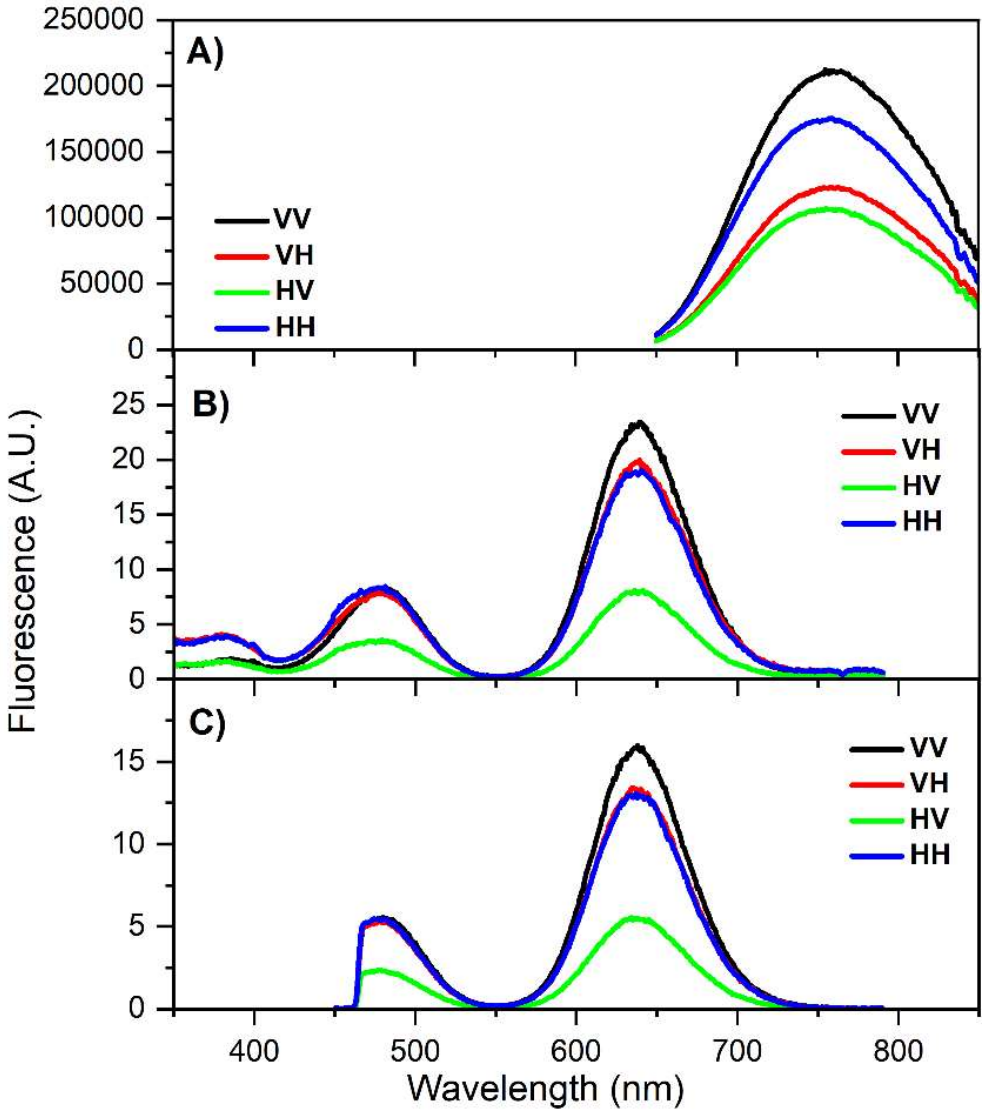

Figure S7. Raw spectra of DNA811-AgNCs in 95% glycerol and 5% 10 mM NH_4OAc at 5 °C. **A)** Emission spectra at different emission polarizations (vertical and horizontal), exciting with vertically and horizontally polarized 634.8 nm laser. **B)** Excitation spectra at different excitation polarizations (vertical and horizontal), monitoring the emission with vertically and horizontally polarized light at 800 nm (Xe lamp). A 325 nm long-pass filter was used in the excitation path. **C)** Same excitation spectra (I_{VV}, I_{VH}, I_{HV}, I_{HH}) as (B), but placing a 458 nm long-pass filter in the excitation path.

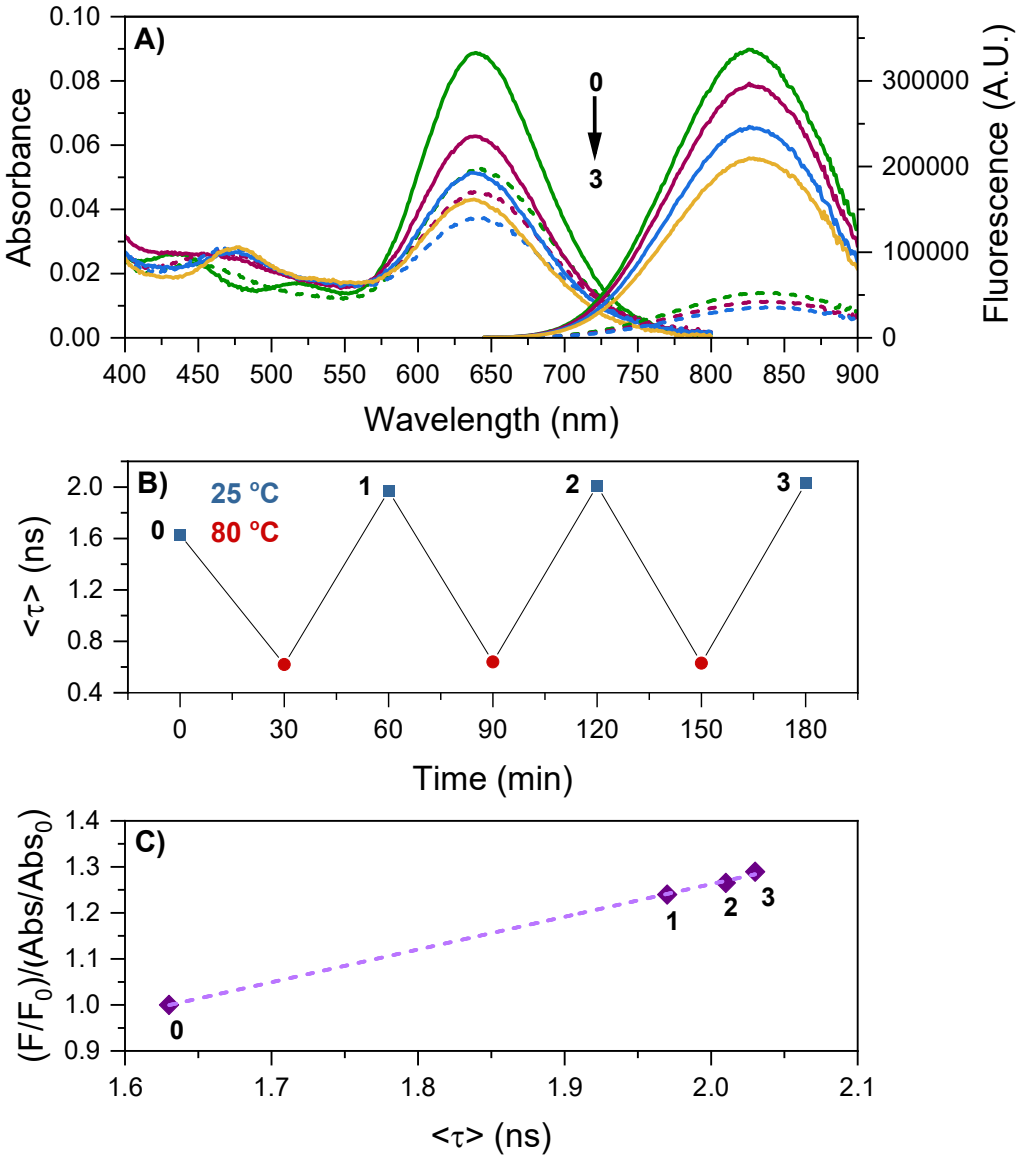

Figure S8. Cycles 0→3 of DNA841-AgNCs in 10 mM NH_4OAc between 25 °C and 80 °C. To ensure thermal equilibrium at each temperature, every measurement was carried out 30 minutes after the target temperature had been reached. The excitation source was blocked between measurements. **A)** Absorption and emission spectra (λ_{exc}= 634.8 nm) recorded at 25 °C (solid lines) and 80 °C (dashed lines) for cycles 0→3. **B)** Intensity averaged decay time <τ> of DNA841-AgNCs in 10 mM NH_4OAc as a function of time for different temperature cycles: blue squares represent the lifetimes at 25 °C, whereas red dots define the decay times measured at 80 °C. **C)** Apparent quantum yield change expressed as the ratio between fluorescence (F) and absorbance at the excitation wavelength (Abs) normalized to the starting condition at t=0 (F_0/Abs_0). The apparent quantum yield change is plotted as a function of <τ> for the 25 °C data. Cursory inspection shows a linear trend, as would be expected for a classic two-level system where $Q = k_f \cdot \tau$, indicating that the changes are mainly due to changes of k_{nr}.[3]

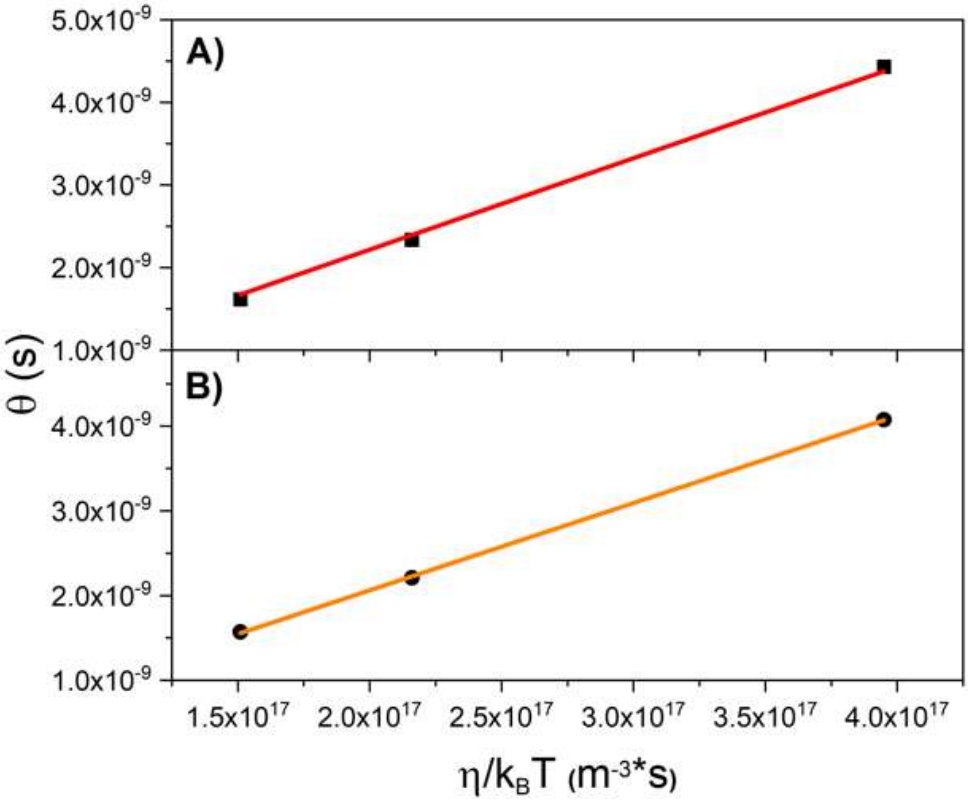

Figure S9. Linear fit of the rotational correlation times (θ) as a function of η/k_BT for **A)** DNA841-AgNCs and **B)** DNA811-AgNCs in 10 mM NH_4OAc. Data points were collected at 5 °C, 25 °C and 40 °C. The slope represents the hydrodynamic volume (V_{hydro}). DNA841-AgNC has a V_{hydro} of 11.07 nm^3, while V_{hydro} of DNA811-AgNC is 10.31 nm^3.

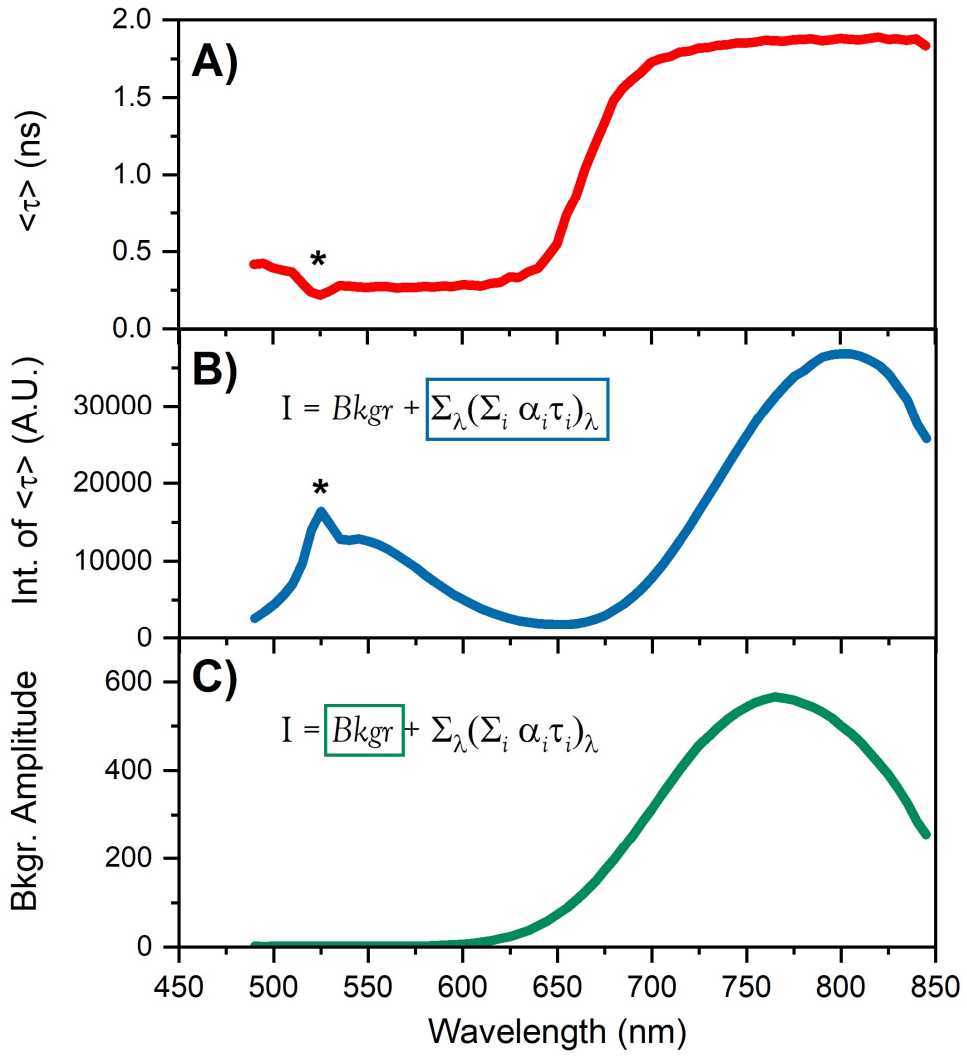

Figure S10. DNA811-AgNCs in 10 mM NH_4OAc aqueous solution at room temperature. Global tri-exponential fit of fluorescence decays with an overall reduced χ^2 of 1.21. The fluorescence decays were acquired from 490 to 845 nm, every 5 nm, exciting at 445.4 nm. Given the complexity and variety of decay components over the whole wavelength range, we only present a global overview of the results. **A)** Intensity averaged decay time $<\tau>$, **B)** emission intensity calculated from τ_1, τ_2 and τ_3 and the corresponding amplitudes (α_1, α_2, and α_3), and **C)** background amplitude of the decays in the fit (*Bkgr*) as a function of emission wavelength. The background amplitude is a proxy for the microsecond-lived emission. * represents an artifact due to the Raman scattering of water.

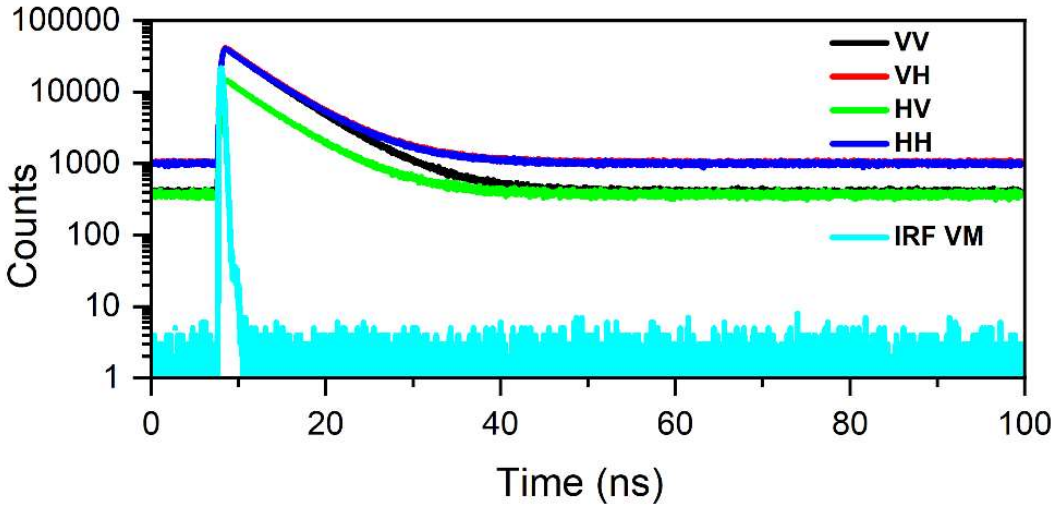

Figure S11. Anisotropy measurements of DNA811-AgNCs in 95% glycerol and 5% 10 mM NH_4OAc at 5 ºC. The decay curves were measured at 800 nm (λ_{exc}= 445.4 nm), and were recorded at different excitation and emission polarizations (VV, HH, VH, HV). These data allowed to distinguish between the nanosecond (decay) and the microsecond (background) contribution to the NIR emission. Although the total signal gives an excitation anisotropy of 0.25, in line with the value in Figure 3B, the nanosecond component gives an anisotropy of 0.36, while the microsecond component has an anisotropy value of 0.012 (which is close to the random orientation value of 0).

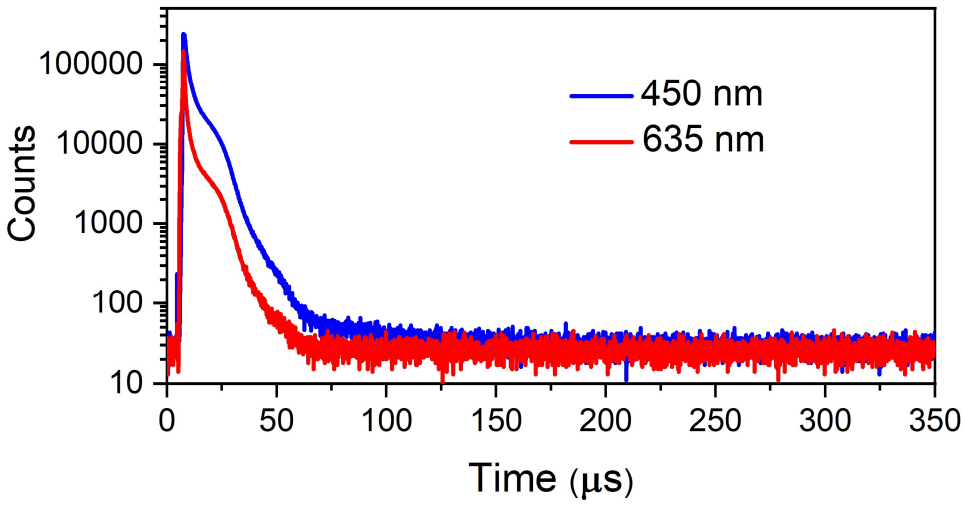

Figure S12. IRFs of the Xenon flash lamp (repetition rate = 300 Hz) at 450 nm (blue curve) and 635 nm (red curve).

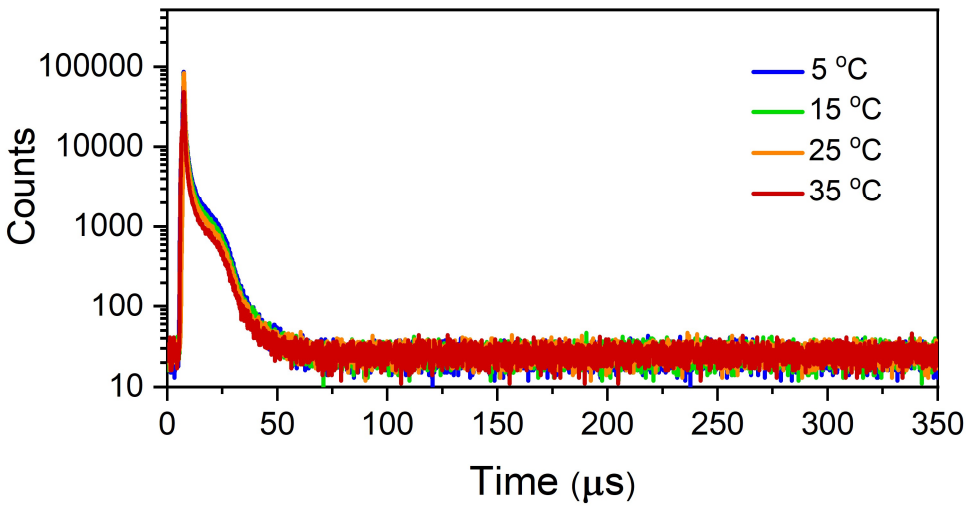

Figure S13. Decay curves (λ_{em}=800 nm) at different temperatures for DNA811-AgNCs in 10 mM NH_4OAc, exciting at 635 nm with a Xenon flash lamp (repetition rate = 300 Hz).

4. Steady-state and time-resolved information on the DNA-AgNC stabilized by 5'–CCACCTTTTC–3'

4.1 Synthesis and HPLC purification

This 10-base DNA-embedded AgNC was synthesized as described in section 1. The optimal ratio of the components was also [DNA]:[$AgNO_3$]:[$NaBH_4$] = 30 μM: 150 μM: 75 μM in 10 mM NH_4OAc solution (pH= 7). The sample was stored in the fridge for 6 days, and then purified with the same HPLC system, column, buffer and flow rate mentioned in section 2. The mobile phase gradient was varied from 15% to 95% 35 mM TEAA buffer in MeOH (B) in 25 min, *i.e.* from 85% to 5% 35 mM TEAA in H_2O (A). In the first 2 min the elution gradient was kept constant to 15% B, after that it was linearly increased to 35% B in 20 min, and finally in the 22-25 min range, it was rapidly changed from 35% to 95% B. The run was followed by 5 min of washing with 95% B.

Based on the chromatograms shown in Figure S14, a single fraction with a retention time around 11 minutes (≈24% B) was collected by using the absorbance signal at 470 nm.

As for DNA841-AgNCs and DNA811-AgNCs, the solvent was exchanged to 10 mM NH_4OAc by spin-filtration after HPLC purification.

4.2 Steady-state absorption, excitation and emission measurements

The absorption spectrum was acquired with a Cary 300 UV-Vis spectrophotometer from Agilent Technologies using a deuterium lamp for ultraviolet radiation and a tungsten-halogen lamp for visible and near-infrared radiation.

Steady-state emission and excitation measurements were performed using a FluoTime300 instrument from PicoQuant. Emission spectra were recorded exciting with a picosecond-pulsed laser at 445.4 nm, while excitation spectra were acquired with a Xenon arc lamp, monitoring the emission at 550 and 730 nm. The 2D emission vs excitation plot (Figure S16) was constructed from the emission spectra excited from 420 to 650 nm, in steps of 5 nm, with a Xe arc lamp. When monitoring the green emission, a 420 nm long-pass filter was placed in the excitation path. All fluorescence spectra were corrected for the wavelength dependency of the detector, and the excitation spectra and 2D graph were additionally corrected for the lamp power.

4.3 Time-resolved measurements and analysis

All time-resolved data was recorded using a FluoTime300 instrument (PicoQuant).

4.3.1 Luminescence decays

Time-Correlated Single Photon Counting (TCSPC) and microsecond decay measurements were performed at different temperatures, spanning from 5 ºC to 65 ºC, every 10 ºC. Nanosecond decays were monitored for the green emission at 540 nm, exciting at 445.4 nm with a picosecond-pulsed laser. Microsecond decay curves were acquired at 730 nm (NIR emission) using a Xenon flash lamp (repetition rate= 300 Hz) at 450 nm. All decay curves were analyzed using FluoFit v.4.6 software from PicoQuant. The fluorescence decay curves were fitted using a tri-exponential

reconvolution model including the IRF and the scattered light, while the microsecond luminescence decays were fitted with a bi-exponential tail-fit function. The obtained intensity averaged decay times, $<\tau>_{GREEN}$ (ns) and $<\tau>_{NIR}$ (μs), are reported in Table S1.

4.3.2 Time-resolved emission spectra

Time-resolved emission spectra (TRES) were constructed for the nanosecond green emission and the microsecond-lived NIR emission at diverse temperatures: 5 ºC, 15 ºC, 25 ºC, and 35 ºC (Figure S17). Fluorescence decays were recorded in the interval 480 - 670 nm, every 5 nm, exciting the sample at 445.4 nm with a pulsed laser. The integration time was chosen to be 15 s per decay to achieve at least 10,000 counts in the maximum at λ_{em}^{max}. Microsecond decays were acquired in the 650 - 800 nm range, in steps of 5 nm, using a Xenon flash lamp (repetition rate= 300 Hz) at 460 nm. A 420 nm long-pass filter was placed in the excitation path.

The fluorescence decay curves were analyzed globally using a tri-exponential reconvolution model including the IRF, whereas the longer luminescence decays were fitted globally using a bi-exponential tail-fit function. The obtained TRES were corrected for the detector efficiency and transformed to wavenumber units by multiplying with the Jacobian factor ($10^7/v^2$).[5] Then the spectra were interpolated as described in paragraph 3.5.2 with a wavenumber step equivalent to 0.001 nm wavelength. The overall intensity-weighted decay time $<\tau_w>$ was calculated as the average of $<\tau>$ (paragraph 3.5.1) over the emission spectra, weighted by the steady-state intensity. The resulting decay times $<\tau_w>$ (data not shown) are consistent with the intensity averaged decay times $<\tau>$ obtained from the single decay measurements.

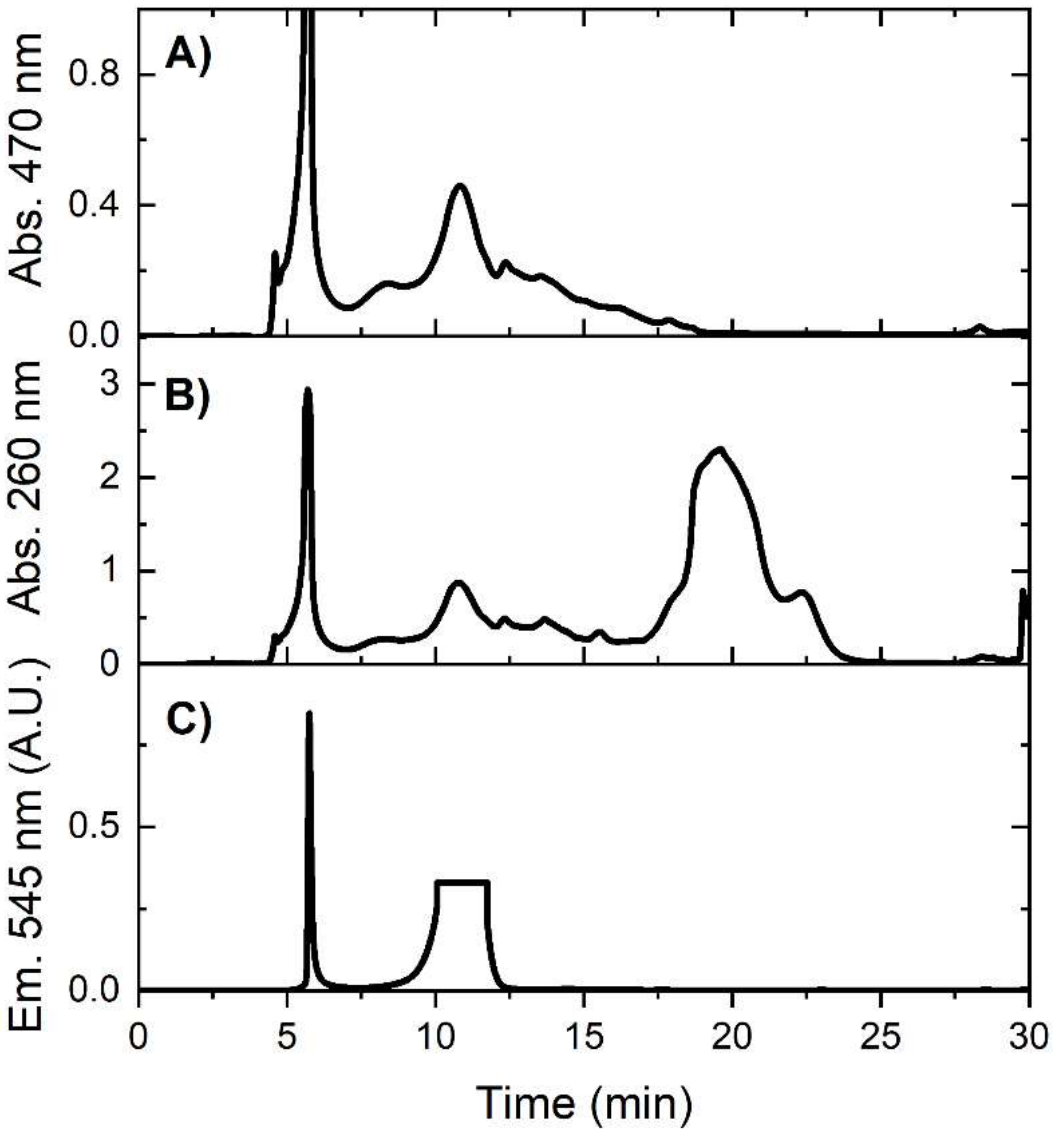

Figure S14. HPLC chromatograms of DNA-AgNCs stabilized by 5'-CCACCTTTTC-3' **A)** monitoring the AgNC absorption peak at 470 nm, **B)** monitoring the DNA absorption at 260 nm, and **C)** monitoring the DNA-AgNC emission at 545 nm (λ_{exc}= 470 nm). The fraction collected between 9.8 and 11.7 min is the investigated sample. (The plateau of the peak around 9.8 - 11.7 min in the fluorescence chromatogram is due to detector saturation).

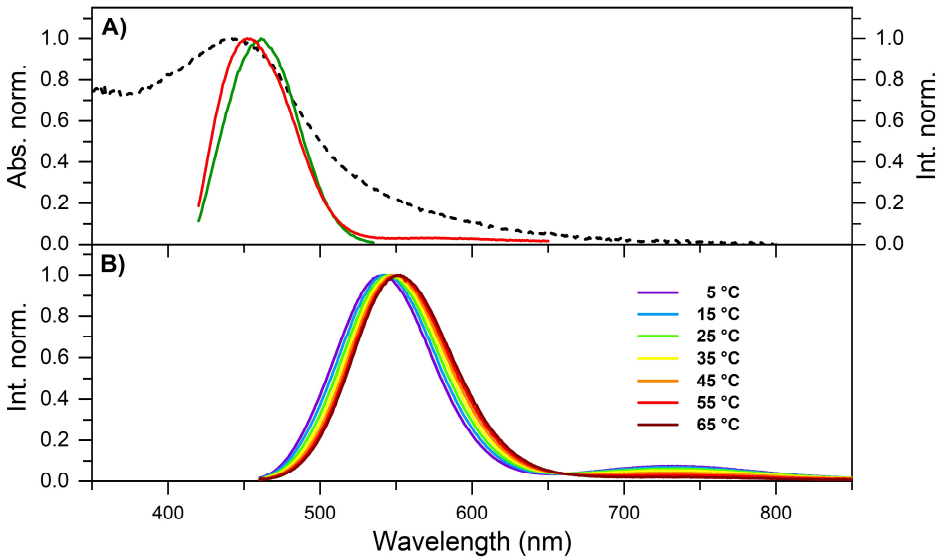

Figure S15. Normalized steady-state data of HPLC-purified DNA-AgNCs stabilized by 5'-CCACCTTTTC-3' in 10 mM NH_4OAc. **A)** Absorption spectrum (dashed black), and excitation spectra monitored at λ_{em}= 730 nm (red) and λ_{em}= 550 nm (green) at room temperature. The excitation spectra were recorded by placing a 420 nm long-pass filter in the excitation path The absorption spectrum is much broader than the excitation spectra, which indicates that the collected sample contains a range of additional products. **B)** Emission spectra, exciting at 445.4 nm, acquired at different temperatures.

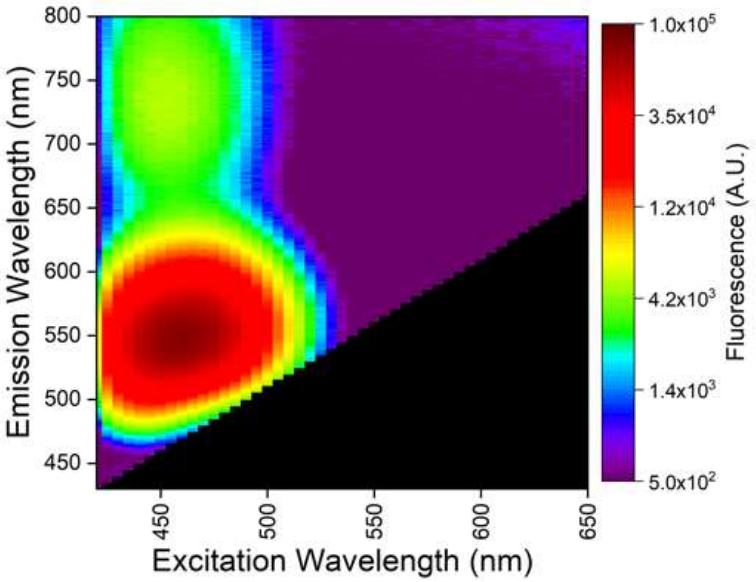

Figure S16. 2D emission vs excitation plot of the purified 10-base DNA-AgNCs (5'-CCACCTTTTC-3') in 10 mM NH_4OAc, with a logarithmic fluorescence intensity scale. A 420 nm long-pass filter was placed in the excitation path.

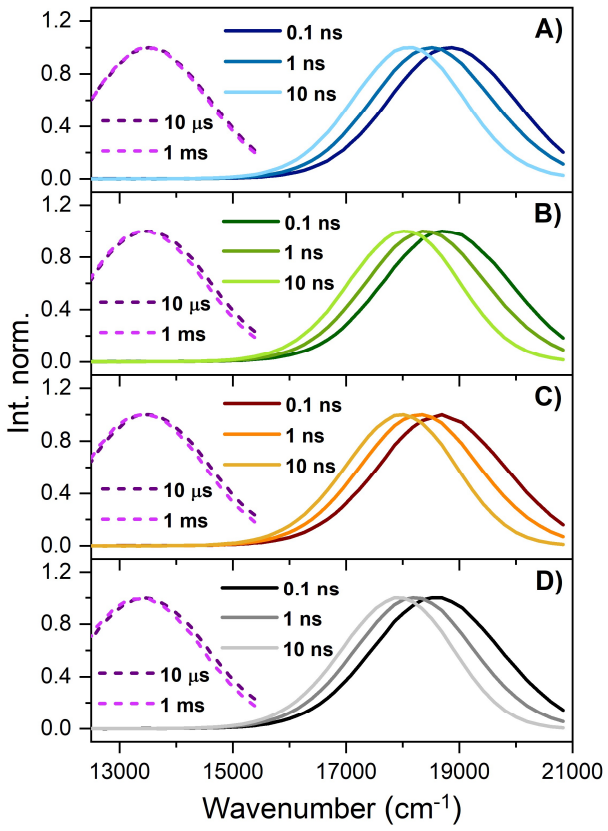

Figure S17. Normalized time-resolved emission spectra (TRES) of the green fluorescence (solid lines) and NIR emission (dashed traces) for DNA-AgNCs (5'-CCACCTTTTC-3') in 10 mM NH_4OAc at **A)** 5 °C, **B)** 15 °C, **C)** 25 °C, and **D)** 35 °C.

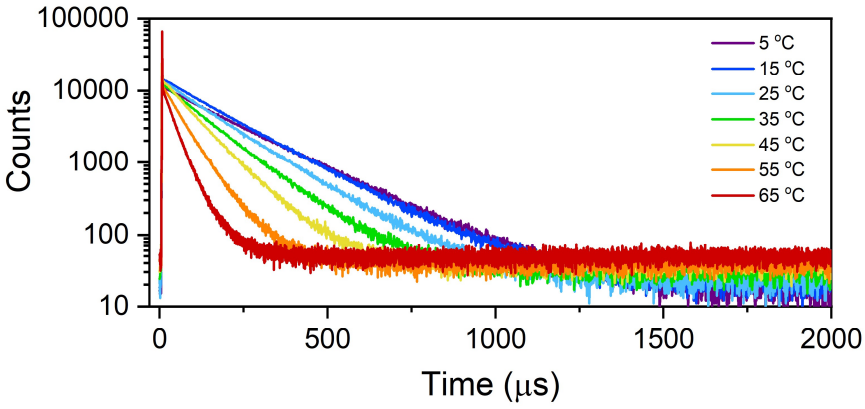

Figure S18. Decay curves (λ_{em}=730 nm) at different temperatures for DNA-AgNCs (5'-CCACCTTTTC-3') in 10 mM NH_4OAc, exciting at 450 nm with a Xenon flash lamp (repetition rate = 300 Hz). The difference in the baseline is due to diverse integration times (30-240 s).

Table S1. Emission maxima of the GREEN and NIR emission, and intensity averaged decay times $<\tau>$ at 540 nm (GREEN) and 730 nm (NIR) at different temperatures. All steady-state data and nanosecond decay curves of DNA-AgNCs (5'-CCACCTTTTC-3') in 10 mM NH_4OAc were recorded exciting at 445.4 nm with a picosecond-pulsed laser, while a Xe flash lamp (λ_{exc}= 450 nm) with a repetition rate of 300 Hz was used to measure the microsecond decays. Microsecond decay curves are shown in Figure S18. Reduced χ^2 of the nanosecond decay fits range from 0.99 to 1.13, whereas reduced χ^2 of the microsecond decay tail-fits are comprised in the 0.97-1.10 interval.

Temp. (°C)	**5**	**15**	**25**	**35**	**45**	**55**	**65**
$\lambda_{em\ GREEN}$ (nm)	541	543	545	547	549	551	551
$\lambda_{em\ NIR}$ (nm)	732	731	731	732	729	729	[a]
$<\tau>_{GREEN}$ (ns)	2.71	2.60	2.49	2.35	2.21	2.03	1.81
$<\tau>_{NIR}$ (µs)	192	172	147	120	89.8	60.3	38.8

[a] Too low intensity for accurate determination.

References.

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