Optical properties of DNA-hosted silver clusters

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CHAPTER 4

Optical properties of the DNA-hosted silver clusters (Ag:DNAs) on DNA tiles and tubes

In this Chapter we investigate the influence of temperature on fluorescent silver clusters stabilized by single-stranded DNA, both free in solution and as a part of larger self-assembled structures such as DNA tiles and tubes. The emitters we investigate exhibit two fluorescent species, 'green' and 'red', which behave differently during the heating process. Namely, the 'green' species typically shows a fluorescence intensity increase during the heating process in the case of free emitters, emitters on the DNA tubes, and emitters on DNA tiles. However, in the case of the 'red' fluorescence species, the free emitters show a significant decrease of the fluorescence signal, whereas the signal increases for the emitters on the tubes and tiles. By sets of measurements at different temperatures, we explore the origin of these differences which might be associated to the intrinsically different structures and stability of the 'green' and 'red' fluorescent species. One important aspect appears to be that the silver atoms/ions attached to the DNA structure are mobile and can rearrange into the optically active clusters. This mobility seems restricted to distances smaller than the tile dimensions; thus, going from tiles to tubes would not have a significant impact on the fluorescent properties.

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4.1 Introduction

The DNA molecule is an important building block in nanotechnology [23, 24]. Self-assembly of DNA strands enables creation of elaborate DNA scaffolds which are not accessible with top-down techniques. These scaffolds are used for precise positioning of organic molecules [25, 26, 96], quantum dots [26], and plasmonic particles [26, 96, 97], and also for creation of chiral structures [28, 78].

Here we examine the optical properties of a very special kind of emitters, DNA-stabilized silver clusters (Ag:DNAs) [10, 13], both free in solution and on DNA scaffolds such as tiles and tubes. Ag:DNAs can be synthesized directly onto DNA scaffolds following the already published procedure [33]. DNA encapsulated silver clusters have attracted a lot of attention due to their small sizes (10-20 atoms) [19], high fluorescent quantum yields [80], low cytotoxicity [17], and spectral sensitivity to certain heavy metals [16] and even to single base mutations in DNA [98]. Ag:DNAs represent an intermediate size regime between organic dyes and large metal clusters [3]. The sequence of the encapsulating DNA strand determines the optical properties of the silver cluster [13, 52]. From cryogenic microscopy and spectroscopy, it has been concluded that the fluorescence spectra of individual Ag:DNA emitters are very broad, even at 1.7 K ($\sim$ 27 nm) [20] and that their shape is rod-like [19, 99], leading to linearly polarized emission [99]. In general, the chemical yield of fluorescent cluster formation is low, but the samples can be purified [53, 76]. The properties of Ag:DNAs have been discussed in more detail in the recent review articles [100, 101] and in Chapters 2 and 3.

In this chapter we focus on temperature-induced changes in the optical properties of Ag:DNAs. Synthesized on much larger DNA structures such as DNA tubes (where the distance between emitters is smaller than 10 nm) [33] and tiles, their properties, including chemical stability and emission, differ from the properties of their free counterparts. There are several possible reasons for this difference. Emission quenching could take place as a result of a high density of emitters on the tubes. Or, emission enhancement could be caused by coherent collective effects. Also, extended DNA structures might lead to changes due to different strain conditions, different electronic conditions, and/or different mobility of Ag atoms. Furthermore, such larger DNA structures can be a reservoir for Ag ions that are mobile, especially at higher

temperatures.

To investigate the possible causes of DNA scaffold-induced changes on Ag:DNA, we perform temperature-controlled measurements on solutions of free emitters, emitters synthesized on the tubes, and emitters synthesized on tiles which cannot form tubes. Above the melting temperature of the tubes, the emitters are sufficiently far apart in solution such that their potential near-field interactions cannot occur. Our approach is to monitor the change of the emission intensity of the emitters during the heating process.

4.2 Synthesis of Ag:DNAs, DNA tiles and tubes

The optical emitter used in this chapter is the 9C emitter so-called because the encapsulating DNA strand contains 9 cytosines in a single-stranded loop that is closed with a double-stranded stem (see Table 4.1, top row, and Figure 4.1a). To synthesize free 9C emitters, that is, 9C emitters are not attached to a larger DNA scaffold, we dilute native strands 9C-hairpin, in an ammonium acetate/magnesium acetate buffer (8 mM/2 mM). Then we add $AgNO_3$, and after 30 minutes we reduce the silver ions with $NaBH_4$. The final concentrations are 20 μM DNA, 140 μM $AgNO_3$, 40 μM $NaBH_4$ and 2 mM/0.5 mM ammonium acetate/magnesium acetate buffer .

For the synthesis of DNA tube scaffolds, we followed the procedure by O´Neill et al. [33]. Five DNA strands from Table 4.1, SE1-9C, SE2, SE3, SE4 and SE5 (final concentrations 2 μM) were mixed together in ammonium acetate/magnesium acetate buffer (40 mM/10mM), heated to 95°C and then cooled to room temperature over two days. These five strands are programmed to form tiles, 'unit cells' of the tubes. Each tile has four sticky ends, chosen such that the 'diagonal' sticky ends are complementary (Table 4.1). There are typically seven tiles in the circumference of the tube. Upon formation of tubes, we synthesize silver clusters by adding $AgNO_3$ and $NaBH_4$ (2.8 μL of 192 μM $AgNO_3$ and 2.8 μL of 1 mM $NaBH_4$ in 50 μL of DNA tubes). The schematic representation of the tiles and the tube formation is given in Figure 4.1b. To form tubes, it is necessary to neutralize the negatively-charged backbone of the DNA with Mg^{2+} ions. For comparison of the emitters on the tubes to the free emitters, we synthesized the free 9C emitters in a similar buffer.

Because the sticky ends on the tiles are only 5-base long, and because the

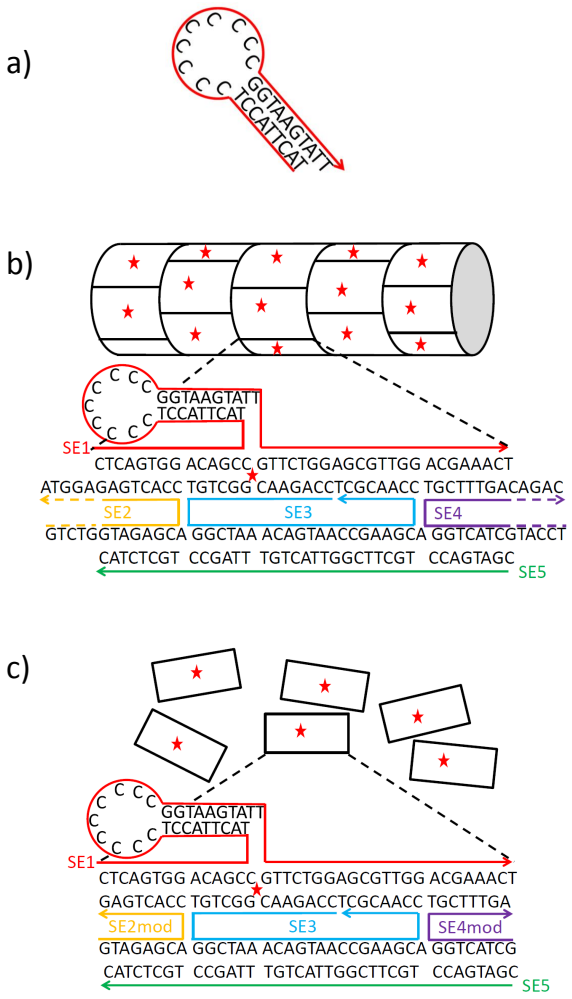

Figure 4.1: Schematic representation of the 9C hairpin (a), DNA tubes (b), DNA tiles (c). a) 9C hairpin consists of 9 cytosines in the loop and 9 base-pair-long stem. b) As demonstrated by O´Neill et al [33], five strands SE1-9C, SE2, SE3, SE4, SE5 can be mixed together such that the tiles with 5-base-long protrusions, sticky ends, are formed (dashed lines of strands 2 and 4). The neighboring tiles are connected, since the sticky ends 'on the diagonal' of the tiles are complementary. 9C hairpin is formed on each of the tiles and protrudes outside the tube. c) Five strands SE1-9C, SE2mod, SE3, SE4mod and SE5 are mixed together forming the tiles without sticky ends. In this case, the absence of the sticky ends prevents the tube formation.

Name	DNA sequence (5′-3′)
9C-hairpin	TAC-TTA-CCT-CCC-CCC-CCC-AGG-TAA-GTA-TT
SE1-9C	CTC-AGT-GGA-CAG-CCT-ACT-TAC-CT-CCC-CCC-CCC-A-GGT-AAG-TAT-TGT-TCTGGA-GCG-TTG-GAC-GAA-ACT
SE2	GTC-TGG-TAG-AGC-ACC-ACT-GAG-AGG-TA
SE3	CCA-GAA-CGG-CTG-TGG-CTA-AAC-AGT-AAC-CGA-AGC-ACC-AAC-GCT
SE4	CAG-ACA-GTT-TCG-TGG-TCA-TCG-TAC-CT
SE5	CGA-TGA-CCT-GCT-TCG-GTT-ACT-GTT-TAG-CCT-GCT-CTA-C
SE2mod	GTA-GAG-CAC-CAC-TGA-G
SE4mod	AGT-TTC-GTG-GTC-ATC-G

Table 4.1: DNA strands used for synthesis of the free 9C emitters (9C-hairpin), the 9C emitters on the DNA tubes (SE1-9C, SE2, SE3, SE4, SE5), the 9C emitters on the DNA tiles (SE1-9C, SE2mod, SE3, SE4mod, SE5).

tubes are not ligated (there is a discontinuity in the sugar-phosphate backbone), their melting temperature is $\sim$40°C degrees [32]. Upon heating above the melting temperatures, the tubes are falling apart, making the tiles with the emitters free in solution. The melting temperature of the tiles is significantly higher due to the numerous Watson-Crick pairs between the strands within the tile itself.

4.3 Temperature-dependent fluorescence and absorption spectroscopy

The emission of 9C emitters formed on tubes can be visualized when the tubes are diluted in poly(vinyl alcohol) (PVA) and spin-cast on the glass substrate. For detection we used fluorescence microscope (Zeiss Axiovert 40 CFL) with a 100X oil- immersion objective. Excitation light from a halogen lamp was transmitted through a band pass filter (center: 530 nm, width: 30 nm). A dichroic mirror with edge at 560 nm reflected excitation light and transmitted emission light. Emission light was further directed directed through a second band pass filter (center: 600 nm, width: 75 nm). Figure 4.2 displays the red fluorescence signal (false color) from 9C emitters positioned

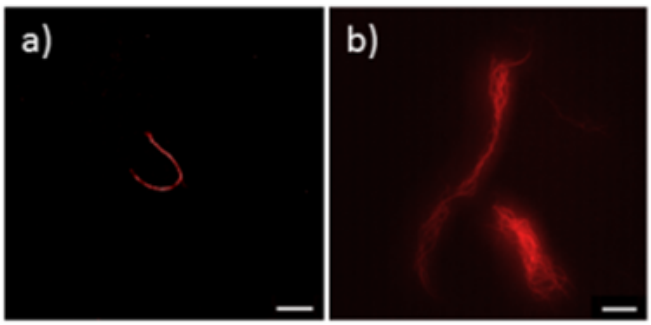

Figure 4.2: Fluorescent images of the tubes immobilized in PVA. a) Single tube(s), and b) tube tangles. Silver ions can mediate the non-Watson-Crick base pairing and cause the tube tangling [33]. In the case of the tiles without sticky ends, the structures could not be detected, but only a faint glow (not presented). Scale bar: 10 μm.

on DNA tubes.

To examine the optical properties of the clusters, we performed fluorescence and absorption measurements using a Cary 50 Bio UV-visible spectrophotometer and Cary Eclipse fluorimeter (Varian). For fluorescence measurements, we held the excitation wavelengths at 460 nm and 560 nm, around the excitation maxima of the 'green' and 'red' fluorescent species of 9C emitters, respectively. The 'green' fluorescent species, which consists of 11 silver atoms encapsulated by DNA, has a quantum yield that is 11 times lower than the 13-silver-atom 'red' fluorescent species [51]. This is the main reason of the large difference in the intensities of the two species. Also, from the electrophoretic mobility of the free emitters in solution and diffusivity measurements, it has been concluded that 'green' and 'red' species have distinct conformations [102]. Namely, the 'red' species most likely retains the form of the hairpin structure, whereas the 'green' species is more compact and reshapes the hairpin structure. Emission spectra were fitted with Gaussian curves, and the peak values were used to extract the emission intensity (Figure 4.3). The temperature of the examined solution of emitters was controlled by a Peltier element from 25°C to 55°C. Emission measurements were performed in several steps. The temperature of the solution was held con-

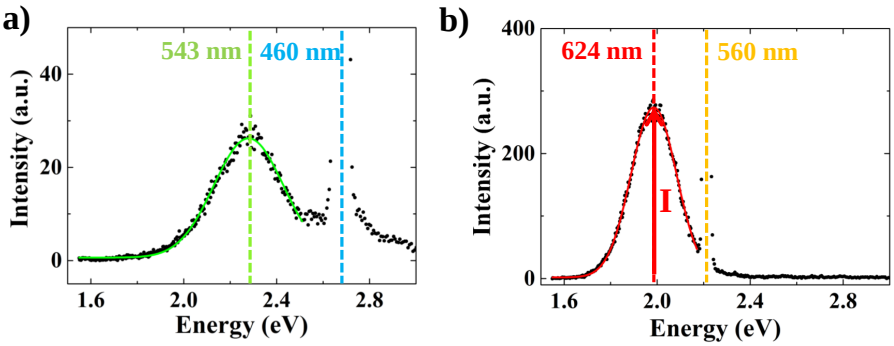

Figure 4.3: Emission intensity of 9C emitters on tubes. a) 'Green' emitters excited at 460 nm (blue vertical line is in the center of the excitation peak), show fluorescence spectra (black squares) which can be fitted with Gaussian peak (green solid line) centered at 543 nm. b) 'Red' emitters excited at 560 nm (yellow vertical line is in the center of the excitation peak), show fluorescence spectra (black squares) which can be fitted with Gaussian peak (red solid line) centered at 624 nm. The peak value of the Gaussian function is taken as a measure for the intensity of the emitter, I, presented by the red arrow. The emission intensity of the 'green' emitters is significantly lower (approximately a factor 10) than in the case of 'red' emitters, mostly due to the difference in the quantum yields.

stant and the intensity was monitored every 5 minutes. After a cycle of 12 points, the temperature was decreased to 25°C (a reference point), and then a new cycle was started after the temperature had been increased by 5°C with respect to the temperature in the previous cycle. The measurements at 25°C enabled us to distinguish permanent changes that took place in the ensemble of emitters from temporary changes due to increased temperature of the solution.

4.4 Characterization of the free 9C Ag:DNA emitters

We first characterize the optical properties of free 9C emitters synthesized in ammonium acetate/magnesium acetate. In Figure 4.4, the emission properties of two ensembles are presented; the lower data set represents the emission intensity of the 'green' fluorescent species excited at 460 nm, whereas the upper data set represents the emission intensity of the 'red' fluorescent species excited at 560 nm.

The emission intensity of 'red' emitters decreases with temperature, while emission intensity of the 'green' species first increases and then decreases. There are at least two reasons for the decrease in emission intensity of the 'red' species. First, the stability of fluorescent Ag:DNA at higher temperatures may be perturbed leading to formation of the non-fluorescent systems or green fluorescent Ag:DNA. Second, a temperature increase also increases vibrational occupation which leads to an enhancement of non-radiative decay processes. It can be notices that the emission intensity of the emitters kept at the constant temperature decreases as the time progresses. To separate the two effects, after each cycle at the constant temperature we cool down the system back to 25°C (red circles). The intensity difference between two neighboring red points (intensity values at 25°C) separated by the constant temperature cycle indicates the destruction of active 'red' emitters during the cycle caused by heating. On the other hand, the difference between the emission intensity at 25°C and the first point in the following cycle provides the information both of the phonons introduced in the system and the reversible changes of the clusters. It is also shown that the emission intensity of the 'red' species decreases slowly when the emitters are kept at 30°C and 35°C and faster at the temperatures above 40°C. The intensity ratio between the highest intensity value at 25°C and the value at the end of the cycle is 1.6.

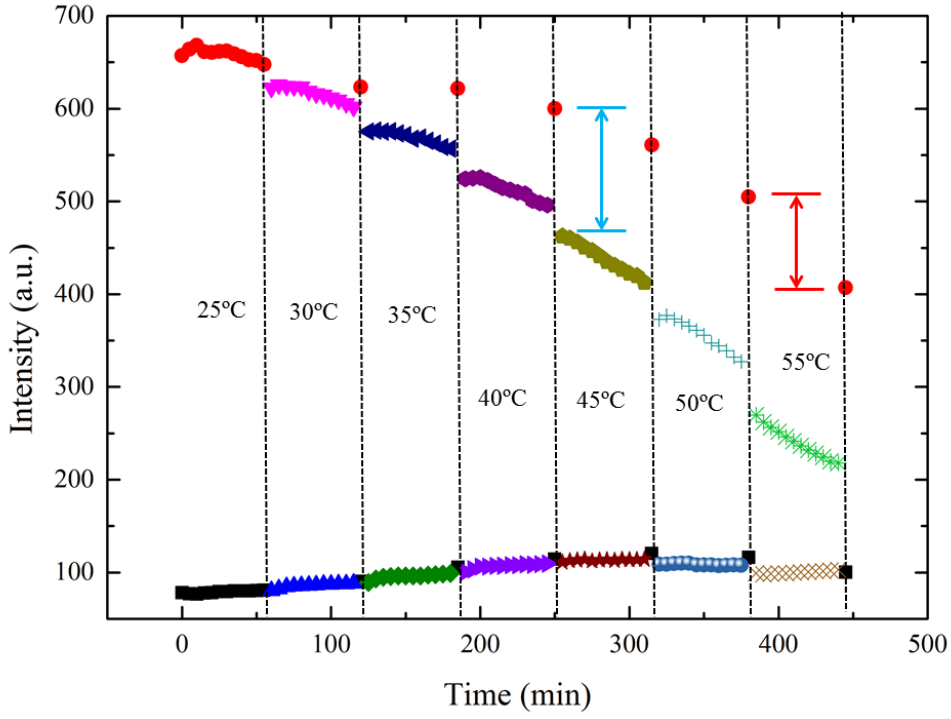

Figure 4.4: Emission intensity of free 9C emitters. The emission intensity of the 'green' ensemble excited at 460 nm (data set at the bottom) increases slightly when the temperature of the solution is increased from 25°C (black squares) to 55°C. The emission intensity of the 'red' ensemble excited at 560 nm (data set at the top) decreases as the temperature is increased from 25°C to 55°C. After each one-hour cycle at the constant temperature, the temperature is returned to 25 °C (black squares for 460 nm excitation and red circles for 560 nm excitation). The difference between the intensity values at 25°C and the first data point in the following cycle (blue arrow) indicates that the new phononic degrees of freedom provide non-radiative decay channels. The intensity difference between two neighboring data points taken at 25°C separated by the cycle at the constant temperature (red arrow) represents the heat-induced degradation of emitters.

Based on Figure 4.4, the emission intensity of the 'green' fluorescent species is slightly increasing as the temperature increases to 45°C and then slowly decreases at higher temperatures. After each cycle at a constant temperature, the temperature of the system was brought back to 25°C (black squares). It appears that the 'green' species is less susceptible to vibrational degrees of freedom, as the intensity differences between the points at 25°C and the points at the end and beginning of the new cycle are similar. The emission intensity of the green species at the end of the process increases 1.3 times with respect to the initial value.

It has been shown previously that the 'green' and 'red' species are connected with the chemical reaction in which the disappearance of the 'red' emitters directly relates to the appearance of the 'green' emitters [102]. Thus, it is likely that in the heating process, 'red' emitters are (at least partially) transformed into 'green' emitters. Since the 'red' emitters contain 13 silver atoms and green emitters 11 silver atoms, it seems reasonable that two end point Ag atoms (ions) get detached.

4.5 Fluorescent properties of Ag:DNAs on DNA tubes

We now present optical measurements on the tubes (Figure 4.5). The emission intensity of the 'green' fluorescent species increases 1.9 times in the heating process with respect to its initial value. The intensity initially decreases at the temperatures lower than 40°C, but starts increasing at the higher temperatures. In the case of the 'red' species the difference between the free PC emitters and the 9C emitters on tubes is more dramatic than for the 'green' species. Whereas the free emitters steadily decreased in intensity as function of temperature, the emitters on the tubes significantly increase in intensity, especially at the temperatures above 40°C, which corresponds to the temperatures at which the tubes break [32]. As mentioned in section 4.2, the synthesis of the tube is a two-day process starting at 95°C, therefore after tubes have been broken into the tiles, they will not significantly reassemble into tubes on the time scales and temperatures of the current experiments. At the end of a process, we obtained an overall intensity increase of ~ 1.5 times with respect to value before heating. The emission intensity of the emitters increases in the heating process up to 50°C, but starts slowly decreasing at the temperatures above. In this case 9C hairpins are part of a much larger structure, a tube, so

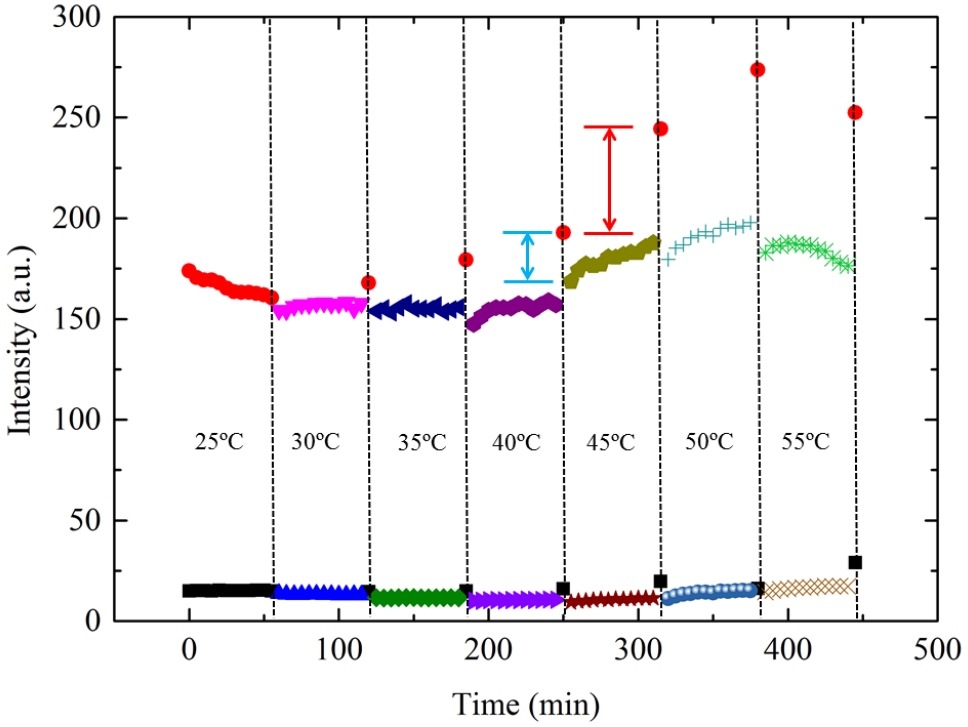

Figure 4.5: The emission intensity of 9C emitters formed on the tubes as a function of temperature. The emission intensity of the ensemble excited at 460 nm (data set at the bottom) slightly decreases at temperatures below 45°C, whereas it increases at temperatures above 45°C. The ensemble excited at 560 nm (data set at the top) shows increase ot the emission at the temperatures below 40°C. For the temperatures above 40°C, the intensity increase is more significant. However, at 55°C the intensity decrease is obvious, due to the degradation of the emission species. Black squares ('green' species) and red circles ('red' species)after each cycle represent the intensity values at 25 °C, which is taken as a reference value. The intensity increase (red arrow) between two neighboring red points (intensity values at 25°C) separated by the constant temperature cycle shows that the new are being formed at the temperatures up to 50°C, whereas at 55°C the emitters are being destroyed . On the other hand, the difference between the emission intensity at 25°C and the first point in the following cycle gives the information about the phonons introduced in the system and the reversible changes of the clusters (blue arrow).

their structural stability is significantly improved with respect to the case of free emitters where the temperature can induce reconfiguration of the clusters into non-fluorescent species. However, at the temperatures above 50°C, they start degrading slowly. This suggests that breaking of the tubes into tiles plays an important role.

4.6 Fluorescent properties of Ag:DNAs on DNA tiles

In the previous section we demonstrated that the temperatures at which the tubes break into tiles under the influence of temperature increase has a significant effect on the emission of Ag:DNA emitters.

To investigate the phenomena further, we synthesized emitters on modified tiles. The procedure is similar to the previous case, except that we now use two modified strands, SE2mod and SE4mod (Table 5.1). The modified strands are created by removing 10 bases from the original strands (SE2 and SE4), 5 bases from $5^{'}$ end and 5 bases from $3^{'}$ end. In this case the tiles can be formed but not the tubes, since the removed sticky ends are crucial for connecting the neighboring tiles. The heating and measurement procedures are repeated and the results are presented in the Figure 4.6.

The emission intensity of the 'green' fluorescent species is very low and the absolute value of the intensity change with the temperature increase is also very small. We observe that the intensity increases in the heating process such that the emission intensity at the end of the process is 1.9 times higher than at the beginning. The 'red' species also shows an emission increase after each temperature cycle. The final intensity is 1.6 times higher than the initial intensity. The fluorescent 9C emitters show a very similar behavior as the emitters on the tubes. This additional set of measurements indicates that the breaking of the tubes is not the dominant effect in the change of optical properties. Rather, it seems that the temperature increase influences modifications of the emitters on the tiles, such that the emitters form more efficiently on the tiles. This is not connected to the distance between the emitters, since each tile only hosts at most one 9C emitter, but to the state of the clusters encapsulated by the DNA. The emission intensity increases with heating, indicating that DNA-cluster systems undergo a structural change which leads to the intensity increase. Since each tile can be seen as a net that can have several Ag atoms weakly bound to its structure, those atoms might migrate and support

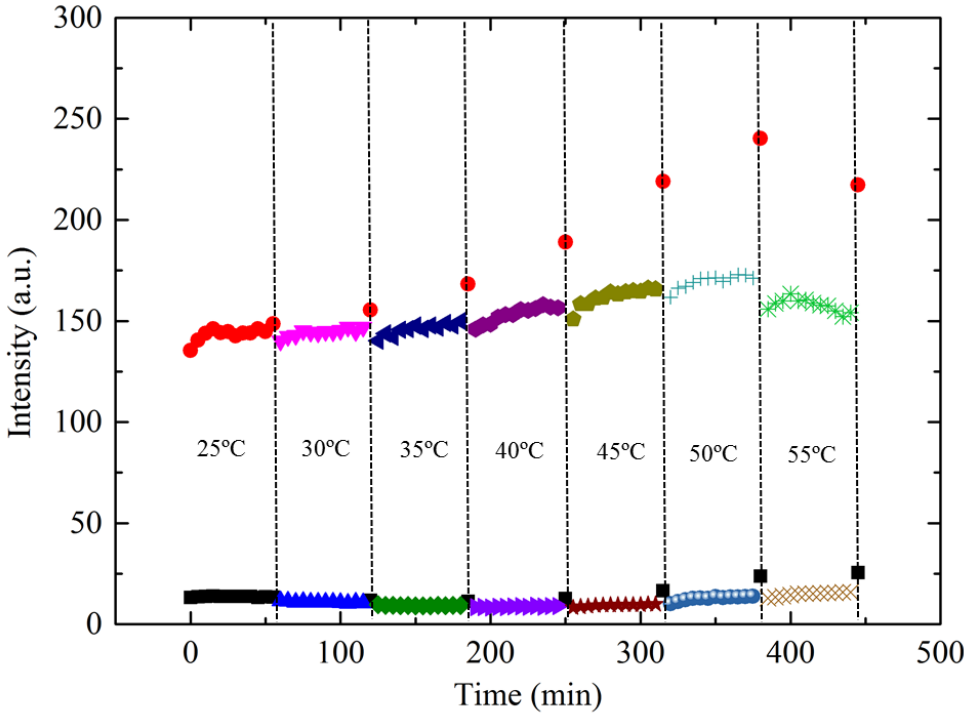

Figure 4.6: The emission intensity of 9C emitters on the tiles without sticky ends as a function of temperature. The emission intensity of the ensemble excited at 460 nm (data set at the bottom) slightly decrese at the temperatures lower than 40°C, whereas it increases at the higher temperatures. The emission intensity of the ensemble excited at 560 nm slowly increases with the heating process up to 50°C, and then the intensity decreases as a consequence of the degradation of emitters. Black squares (bottom data set) and red circles (top data set) represent the emission intensity value at 25°C after each heating cycle.

the formation of the 'green' and 'red' optically active clusters.

4.7 Absorption properties of Ag:DNAs

Clearly, multiple physical and chemical processes have to be taken into consideration as possible explanations for the observed effects. In order to gain additional insight we also performed absorption measurements before and after heating periods of the sample. Absorption measurements were per-

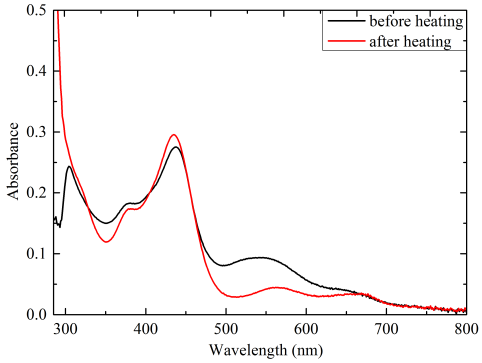

Figure 4.7: Absorption measurements of free 9C emitters. We measured the absorbance of the emitters at 25°C as a function of wavelength before heating (black solid line) and after heating to 55°C (red solid line). After heating, the absorbance remains almost similar around 460 nm, whereas the absorption peak around 560 nm reduces, which is in good agreement with the fluorescence spectra (Figure 4.4).

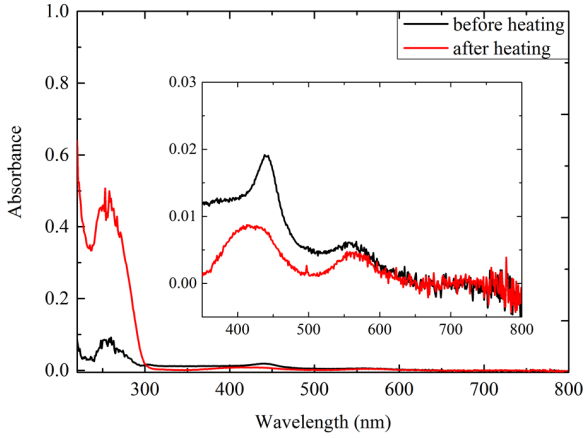

Figure 4.8: The absorption of 9C emitters formed on the tubes. Before heating (black solid line) the absorption peak around 260 nm, which corresponds to the absorption of DNA is 5 time lower than after heating (red solid line). Also, the change in the absorption in the visible range (inset) suggests the structural change of the fluorescent species.

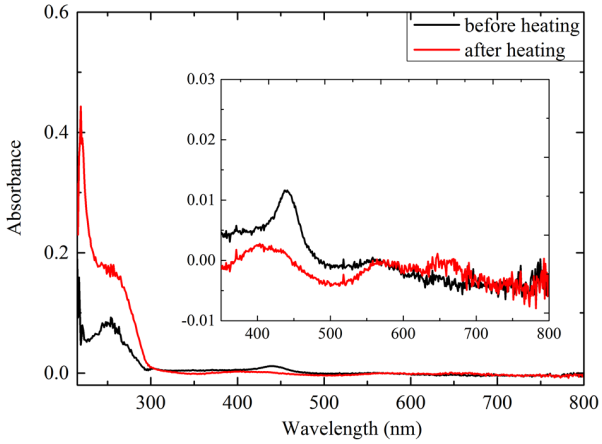

Figure 4.9: Absorption of 9C emitters formed on the tiles without sticky ends. The absorption peak around 260 nm increases approximately 2 times after the heating process. Also, in the visible spectral range, the absorption spectra change indicates the change of the emitters.

formed at 25°C.

From the absorption spectra for the free 9C emitters presented in Figure 4.7, we realize that after heating the most significant changes occur around 560 nm approximately the absorption peak of the 'red' 9C emitters (a decrease of $\sim$ 2 times) and below 320 nm. The absorption below 320 nm is typically associated to DNA itself and in this chapter we focus on the emission around the Ag cluster wavelength of 460 nm for 'green' and 560 nm for 'red' species. The change in absorption around 560 nm roughly corresponds to the decrease of the emission spectra of $\sim$ 1.6 times from Figure 4.4 . The small discrepancy is caused by the fact that the absorption spectra contain also the contribution of other non-emissive species which make the analysis more difficult, whereas the emission spectra give a clear Gaussian distribution.

Figure 4.8 presents absorption measurements of the emitters on tubes before and after tube heating to the temperature of 55°C . Since the concentration of the emitters is very low ($\sim$10 times lower than in the case of free 9C emitters), the absorption values are very low in the visible range. That is why we consider the absorption results relevant only as a proof that the absorption

spectra qualitatively change below 300 nm and in the visible range (see the inset of Figure 4.8). This data set is difficult to analyze due to the low signals compared to an overall background level. Especially before heating, there seems to exist almost a uniform absorption level (from 300 nm to 500 nm). This lifts the 'before heating' curve (black solid line) compared to the 'after heating' curve (red solid line) where this overall background level is significantly reduced. Perhaps before heating there are various sizes of Ag:DNA clusters that are not emissive, but do contribute to absorption over a broad wavelength range. Only after heating, optically active emitters become more prominent and the background absorption reduces. Of course, there is another effect that is clearly visible and that is the huge increase in absorption below 300 nm. As mentioned above, this absorption is typically due to DNA itself. It seems that after heating this absorption is strongly enhanced due to the 'integration' of Ag atoms into the DNA. Additional studies have to be carried out to investigate this mechanism and in this chapter the goal was to focus on the typical 9C Ag emission of the 'green' and 'red' fluorescent species.

In Figure 4.9 we show that the absorption properties of the emitters on the tiles before and after heating show similar character as in the case of the emitters formed on the tubes.

4.8 Conclusion

The combined fluorescence and absorption measurements on free 9C emitters, 9C emitters on the DNA tubes and DNA tiles indicate that the lower emission intensity of the emitters on the tubes before breaking is not a consequence of the emission quenching due to a high density of emitters. This was supported by performing the experiments on the 9C emitters formed on the modified tiles, which cannot form tubes. Instead, the fact that the emitters formed on the modified tiles behave similarly, indicates that the intensity increase is not related to the mutual distance between the emitters. A naive picture would be that the tiles represent the 'nets' which can catch silver atoms and ions. At elevated temperatures those atoms would be more mobile and could migrate to the hairpin loops to form the fluorescent clusters. The heating process is in this case an annealing process in which the new emitters are formed through the changes of the silver-DNA structure. An additional new

effect has been identified, namely strong enhancement of the typical DNA absorption (below320 nm) after heating suggesting an important role for Ag atoms also in this process.