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Full Length Article

# Fluorescence saturation spectroscopy in probing electronically excited states of silver nanoclusters

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ABSTRACT

DNA-based fluorescent Ag clusters attract much attention due to their high brightness and sensitivity to environment, which can be used in chemical sensing and biosensing applications. Low chemical yield of the fluorescent Ag clusters in solution hinders measuring of their absorption cross-section in the ground state and rate constants in the excited states. We applied fluorescence saturation spectroscopy for determining the photophysical constants of an oligonucleotide-stabilized red emitting Ag cluster. Power dependencies of the fluorescence response to the pulse excitation with different pulse duration allowed us to obtain the values of absorption cross-section, dark states formation and deactivation rates of the cluster. The Ag fluorescent cluster exhibited a relatively high (17%) efficiency of a long-lived dark state formation. This feature of the Ag cluster might be further interesting for possible photodynamic and microscopy applications.



## 1. Introduction

Unique structure of DNA offers large possibilities for developing artificial nanostructures for nanophotonics and nanoelectronics applications [1–4]. DNA-stabilized silver nanoclusters have received much attention in past years because of their potential applications in biosensing and bioimaging due to their unique fluorescence properties such as high brightness and photostability [5,6]. For example, Ag clusters are now employed in detection of metal ions [7,8], microRNAs [9–11], target DNA strands [12], and mutations causing human diseases [13,14]. Though a fair amount of DNA-stabilized silver nanoclusters has already been synthesized, their excited state properties, in particular, dark state formation, have been little investigated. Meantime, fluorophores with the dark states have raised increasing interest in fluorescence microscopy in recent years [15]. Dark non-emitting states of Ag nanoclusters have already been successfully used in optically modulated fluorescence imaging [16]. In spite of easy chemical synthesis, which involves formation of a complex of silver ions with nucleobases followed by reduction with $NaBH_4$ in solution, a problem still exist in that the chemical yield of the fluorescent clusters in the mixture does not exceed a few percent. Ag cluster solutions, as prepared, are strongly heterogeneous systems with multiple presence of non-fluorescence species and need further purification to isolate fluorescent clusters. Most studies dealt with unpurified solutions, which hindered probing the photophysical properties of the fluorescent clusters. As a consequence, most of the data reported in the literature on the quantum yields and absorption cross-sections of the clusters are questionable, since the weight of the fluorescent species is unclear.

Here we present a method for both the direct measuring the absorption cross-section of the fluorescent clusters in a mixture of synthesized species and also for determining the rate constants for the dark state of the clusters. In doing so, we modified a method of the fluorescence saturation spectroscopy [17] using three different pulse excitation regimes, namely picosecond and nanosecond pulse duration, and also CW (continuous wave) excitation. We studied Ag clusters emitting at about 620 nm on 12-mer single-stranded DNA 5′-CCTCCTTCCTCC-3′ (S1). The same DNA sequence as a scaffold for Ag clusters has been successfully used in a series of probes for plant microRNA detection [9,10]. Red-emitting cluster on that DNA matrix was synthesized for the first time by Richards et al. [18]. We have determined the absorption cross-section, rates of the dark state formation and deactivation for the synthesized cluster. We show that the quantum yield of the dark state is relatively high (17%) and comparable to the previously obtained one for the cluster on calf thymus DNA [19] with similar spectral, and hence, structural properties.

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## 2. Experimental methods

### 2.1. Clusters synthesis

Synthesis of Ag clusters was performed in a similar way as described in Ref. [18]. Oligonucleotide (S1 strand) 5′-CCTCCTTCCTCC-3′ (BioBeagle ltd.) and $AgNO_3$ aqueous solutions in 20 mM sodium citrate buffer (pH 5) were mixed and stored for 15 min at room temperature. After that, $NaBH_4$ aqueous solution was added followed by vigorous stirring. The final concentrations were $C_{DNA}=20$ μM, $C_{AgNO3}=120$ μM, and $C_{NaBH4}=30$ μM. The sample was kept in the dark about 1 month at 21 °C to reach maximum fluorescence. After that, no spectral change was observed.

### 2.2. Spectral measurements

Fluorescence spectra were obtained on Fluorolog 3 fluorometer at room temperature. All spectra were collected using 0.4 cm quartz cuvette. To remove scattering light, appropriate long wave pass filters were used. Fluorescent emission spectra were corrected for instrument sensitivity as supplied by manufacturers. Blank signals from the solvent, DNA, $Ag^+$-DNA, $AgNO_3$, $NaBH_4$, and reduced $Ag^+$ in the absence of DNA did not exceed 5% relative to the fluorescent maxima of the samples of Ag-DNA over all spectral range. For the fluorescence excitation spectra, the correction was done for the inner filter effect due to high absorbance of the sample in the 260 nm region. To eliminate polarization effects, a vertically oriented polarizer was set into the excitation channel and another polarizer was placed at "magic" angle in the emission channel. Fluorescence polarization degree was calculated as $(I_0-G\cdot I_{90})/(I_0+G\cdot I_{90})$, where $I_0$, and $I_{90}$ – intensities of vertically and horizontally polarized emission beam at vertically polarized excitation light, $G$ – instrument factor calculated as $I_0/I_{90}$ at horizontally polarized excitation light. Fluorescence lifetime was determined using the same fluorometer and a LED as a pulse source (570 nm) with typical FWHM of about 2 ns. Emission bandwidth was set at 15 nm. Circular dichroism (CD) spectra were measured with Jasco J-815 polarimeter. Melting curve were obtained with Specord 210 Plus double-beam spectrophotometer equipped with Peltier cooled cell holder with stirrer.

### 2.3. Nonlinear fluorescence saturation spectroscopy

For the picosecond pulse excitation regime, we used Ti:Sapphire 800 nm femtosecond laser (Synergy 20) combined with an amplifier chain (Pulsar, Amplitude Technologies) that gave 2.5 mJ pulse energy and 50 fs pulse duration at 10 Hz. The beam was directed into an optical parametric amplifier (TOPAS-C, Light Conversion). The output pulse energy at 530 nm was about 100 μJ. The pulse was stretched up to ca. 1 ps to avoid efficient stimulated emission from the sample. The output beam was confined to 2 mm in diameter by an iris. For nanosecond pulse excitation, a Q-switched frequency-doubled Nd:YAG laser (Quantel, 532 nm, 10 ns pulse, 10 Hz, 100 mJ) was used. The output beam diameter was confined to 5 mm by a diaphragm. Integral fluorescence intensity within the range of 580–680 nm was measured with a portable spectrometer (Ocean Optics). In the case of CW excitation, we used a pump laser (Finesse, Laser Quantum) with 2 W output at 532 nm. The beam was confined by an iris to 2 mm. A short ~0.2 s single flash was used for excitation to avoid photobleaching. Integral fluorescence was measured using a photodiode. In all cases the luminescence of the sample in 0.4 cm quarts cuvette was collected at right angle. A long pass filter was used to remove scattering light. The excitation intensity was adjusted with neutral glass filters and was measured with a pyroelectric energy meter (Gentec-EO). Solutions were extensively stirred during experiments, and the photobleaching did not exceed 2%.

Saturation curves obtained in the picosecond and CW excitation regimes were fitted analytically with MagicPlot Pro 2.5 software. For simulation of the curves in the nanosecond excitation regime we solved the set of Eq. (1) and integrated $n(t)$ numerically with the step of $2\cdot 10^{-14}\,s^{-1}$ using Runge–Kutta formalism implemented in MatLab. The parameters were varied with appropriate step for the best fit to the experimental points using RMSD criterium.

## 3. Results and discussion

### 3.1. Spectral properties of Ag cluster

Ag clusters synthesized at pH 5 on S1 strand exhibit mostly one type of emitting clusters. Absorption, fluorescence emission, fluorescence excitation, and excitation polarization spectra in a wide spectral range for the clusters with the excitation/emission maxima at 529 nm/620 nm are shown in Fig. 1 and S1. As can be seen from comparison of the excitation and absorption spectra (Fig. S1), chemical yield of the fluorescent clusters appears to be low enough, and most species are dark Ag clusters and nanoparticles. Even at 529 nm, the weight of the red-emitting cluster in the total absorbance is uncertain (Fig. S1). Nethertheless, fluorescence emission spectra practically does not depend on the excitation wavelength, though a minor presence of a green emitting cluster is seen, when exciting at 260 nm (Fig. 1). The 260 nm UV band in the excitation spectrum, practically coinciding with DNA absorption spectrum (Fig. 1), is typically observed for DNA-stabilized Ag clusters due to efficient energy transfer from DNA to the clusters [20]. The difference between the excitation spectrum and DNA absorption spectrum (dashed curve in Fig. 1) evidently reflects intrinsic electronic transitions of the cluster.

A fair amount of dark clusters is a typical for unpurified cluster solutions. Unfortunately, the sample appeared to be unstable during HPLC purification procedure, typically used for DNA-cluster complexes [21]. Typical chromatograms and comparison of the excitation and absorption spectrum after purification are shown in Figs. S2 and S3. Since only one emitting cluster contributed to the fluorescence emission spectrum, when excited at 530 nm, further measurements using fluorescence saturation technique were conducted with unpurified solutions. Fluorescence lifetime for the clusters was

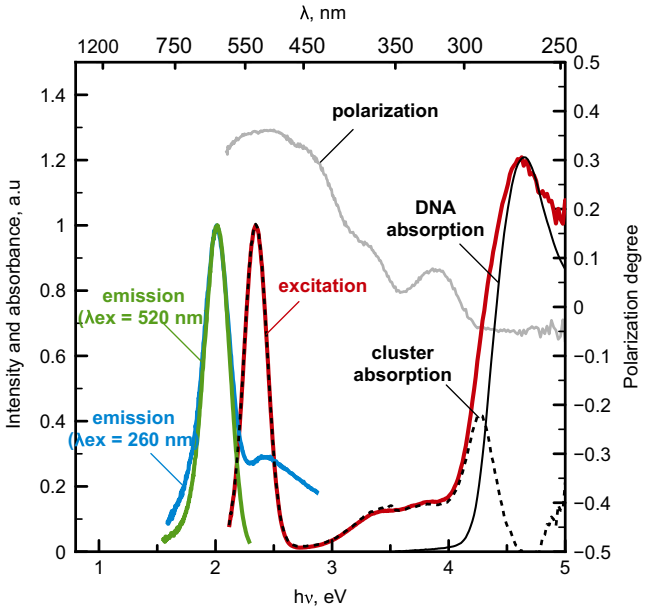


**Fig. 1.** Fluorescence excitation, polarization, and emission spectra of the red-emitting Ag nanoclusters synthesized on S1 DNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)