Altering the distribution of excited-state lifetimes in aminated GFP chromophores by Ag nanohole arrays

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Abstract. Fluorescence of the modified GFP chromophore diethyl-ABDI-BF2 dispersed in PMMA matrix is studied on top of glass, continuous and perforated optically thin silver films. In polymer, the fluorescence decay kinetics becomes non-exponential and can be described by the distribution of rate constants. The results demonstrate shortening of the excited state lifetime in the presence of silver and broadening of the lifetime distribution caused by the nanoholes.

1. Introduction
Labelling biomolecules via chemical bonding with various fluorescent tags (usually fluorescent proteins or organic dyes) offers unique possibility to visualize molecular structures and study the numerous processes inside living cells. The performance of this technique depends on the intrinsic properties of a fluorophore, such as its fluorescence brightness and photostability. In general, two main approaches exist to enhance these characteristics. The ‘chemical’ one is based on altering the chemical structure of the fluorophore, whereas the ‘physical’ approach relies upon exploiting the amplified optical fields near plasmonic nanostructures.

One of the most widely used fluorescent tags nowadays is the Green Fluorescent Protein (GFP). At the heart of this protein lies a small chromophore molecule, which is responsible for light adsorption and emission. Without protein environment, the GFP chromophore has a very low quantum yield, which hampers its direct application as a fluorescent label [1, 2, 3]. This feature is due to existence of an efficient nonradiative de-excitation channel through the intramolecular rotation around the methine bridge coupled to double bond isomerization. To overcome this obstacle, several modifications of this chromophore have been synthesized and studied. By arresting the rotation of the arylidine fragment of the chromophore via the internal bridge, the researchers were able to drastically increase the quantum yield of the chromophore [4, 5, 6, 7].

Recently, we demonstrated that the brightness of conformationally locked aminated derivative of the GFP chromophore diethyl-ABDI-BF2 can be increased by an order of magnitude by plasmon-induced near fields of optically thin perforated Ag films [8].

Here, by using a time-correlated single-photon counting (TCSPC) technique we show how the presence of Ag nanohole arrays (NHAs) affects the excited state lifetime of the diethyl-ABDI-BF2 molecules, dispersed in thin poly(methyl methacrylate) (PMMA) films.
2. Experiment
The samples are prepared using the previously reported technique [8]. In brief, the short-range ordered NHAs with average hole diameter of 120 nm in 20 nm thick Ag films are fabricated on glass slides of 24 mm diameter using the sparse colloidal lithography approach [9]. Deposition of polystyrene nanospheres on top of triple polyelectrolyte layer is followed by 3 min UV-ozone treatment and a vacuum deposition of thermally evaporated silver. After the lift-off process, the arrays of nanoholes in Ag film are obtained.

Polymer films are produced by dissolving PMMA (MW=350000) in phenetole to a concentration of 5% wt., followed by the addition of diethyl-ABDI-BF2 up to a final concentration of 30 \(\mu\)M. The mixture is then deposited onto the silver nanohole arrays by spin-coating at 4000 rpm. The sample schematics is presented in Fig. 1.

Figure 1. Schematics of the nanostructured AgNHA–PMMA sample, the structural formula of the chromophore and the SEM image of the perforated Ag film.

The control samples are comprised of the same dye-containing PMMA films deposited on glass and on the continuous 20 nm Ag films without nanoholes.

Optical properties of the samples are studied using UV-vis-NIR spectroscopy (Jasco V-770) before and after the polymer deposition. Fluorescence spectra are registered using a custom-built optical setup. Femtosecond laser pulses with a 80 MHz repetition rate, 980 nm central wavelength, and energy up to 25 nJ are generated by a Titanium- Sapphire oscillator (Tsunami, Spectra-Physics). Frequency-doubled pulses at 490 nm are focused by a 40×0.75NA objective lens (UPlanFLN, Olympus) on the sample’s top surface covered with the polymer film. The fluorescence is collected using the same objective lens and filtered using 520 nm long-pass filter and directed to SP300i monochromator connected to PI-MAX 2 CCD camera (Princeton Instruments) for recording the fluorescence emission spectra.

3. Results and discussion
In PMMA matrix, the absorption and emission spectra of diethyl-ABDI-BF2 chromophore are characterized by maxima at 497 and 522 nm, respectively. The Ag NHA samples possess a broad extinction peak between 500 and 650 nm with a maximum at 553 nm. The fluorescence
decay in solution is mono-exponential, with characteristic decay time strongly depending on the dielectric constant of the solvent, changing from 0.83 ns in methanol to 3.2 ns in dioxane.

We find that polymer matrix affects the fluorescence decay kinetics of diethyl-ABDI-BF2, which cannot be adequately described by a single-exponential function. This is anticipated due to hindering of the rotation of diethylamine moiety of the chromophore in PMMA matrix leading to the distribution of the decay times of individual molecules. In such cases the fluorescence signal can be described by the following function:

\[ I(t) = \int_0^\infty H(k)e^{-kt}dk, \]  

with \( I(0) = 1. \)

The recovery of the shape of the distribution function \( H(k) \) from the decay kinetics data is an ill-conditioned problem, which cannot be solved exactly. In the absence of a physical model, the distribution is often approximated by some continuous function, such as Gaussian or Lorentzian. This helps an analysis of the changes observed in various experimental conditions. In this work use the stretched exponential (or Kohlrausch) function:

\[ I(t) = \exp \left[-\left(\frac{t}{\tau_0}\right)^\beta\right], \]  

where \( 0 < \beta \leq 1 \) and \( \tau_0 \) are the parameters, describing the distribution. This simple and relatively flexible function has been successfully used for luminescence decays with underlying distributions [10].

![Figure 2. The normalized distribution functions of the fluorescence lifetime for diethyl-ABDI-BF2 chromophore in PMMA film on the glass (blue), on the continuous Ag film (green) and on the Ag NHAs (red).](image)

The results of fitting the experimental decay data is presented in Fig. 2. In general, the longest excited state lifetimes are observed for the chromophore in PMMA on glass. The presence of silver results in faster decay due to the transfer of excitation to the metal. When nanoholes are present in the metal film, the fluorescence lifetimes become even shorter, and the distribution broadens, which can be explained by different positions of dye molecules in relation to nanoholes.
4. Conclusion
The non-exponential fluorescence decay kinetics observed for the diethyl-ABDI-BF2 chromophore in the PMMA film can be explained by the existence of a distribution of decay rate constants due to hindered rotation of the diethylamine moiety. The experimental data can be well fitted using the stretched exponential function. The observed plasmon-induced shortening of the excited-state lifetime can improve the photostability of the dye. In combination with increased brightness, this can be important for various applications involving the fluorescence life-time imaging.

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