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Fluorescence spectral properties of cyanine dye-labeled DNA oligomers on surfaces coated with silver particles

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Abstract

We examined the fluorescence spectral properties of DNA oligomers, labeled with Cy3 or Cy5, when bound to quartz surfaces coated with metallic silver particles. Prior to binding of labeled DNA the surfaces were treated with polylysine or 3-aminopropyl triethoxysilane or were coated with avidin for binding of biotinylated oligomers. The fluorescence intensities were increased an average of 8-fold on these surfaces. Despite the increased emission intensity, the photostability of the labeled DNA was the same or higher on the silver-coated surfaces than on the uncoated slides. The time-integrated intensities, that is the area under the intensity plots with continuous illumination, increased an average of 6-fold. In all cases the lifetimes were dramatically shortened on the silver particles, indicating an over 100-fold increase in the radiative decay rates. These results suggest the use of substrates containing silver particles for increased sensitivity of DNA detection on DNA arrays.

The introduction of DNA or “gene chips” has allowed comprehensive studies of gene expression [1–3]. These arrays contain a large number of DNA sequences at known locations and frequently known sequence. These sequences are hybridized with cDNA from a cell or organism, allowing determination of the expressed mRNA sequences. The most common approach is to use cDNA which is labeled with fluorescent probes. The cyanine dyes Cy3 and Cy5 are commonly used for this purpose [4,5].

While fluorescence provides sensitive detection, the need for increased sensitivity exists. This need is particularly urgent for small-copy-number or single-copy detection of biohazards. In these cases the detection limits are determined by the spectral properties of the fluorophores and the amount of background signal from the sample and instrumentation. The signal from a fluorophore is typically limited by photobleaching or photodecomposition. For example, the most stable fluorophores can undergo about 10^6 excitation–relaxation cycles before loss of signal [6–8]. Because of the difficulties of collecting the isotropic emission, a single fluorophore may yield 10^3 or 10^4 observable photons. Another limitation to small-copy-number detection is the finite rate at which a fluorophore can emit photons. The maximal emission rate is near $1/\tau$, where τ is the decay time. A fluorophore with a 1 ns lifetime can emit at most 10^9 photons per second. Finally, background signal from the sample or optical components can result in an inability to detect weak signals.

In recent publications we examined the effects of metallic silver particles on fluorophores [9, 10] and showed that proximity of fluorophores to a conducting metallic surface can result in

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higher quantum yields, decreased lifetimes, and increased photostability. Such spectral changes can be important for DNA arrays because they would allow higher signal levels per molecule and, in the case of saturation illumination conditions, a higher number of photons per second per fluorophore. To evaluate the use of metallic particles on DNA arrays we examined DNA oligomers covalently labeled with Cy3 or Cy5. To be comparable with presently used DNA technology the glass surfaces were derivatized with aminosilane or coated with polylysine [4] or avidin [11]. For testing the effect of silver particles these surfaces were coated with silver island films (SIFs).¹ These films are formed by chemical reduction of silver ions, which form moderately homogeneous silver particles which cover about 20% of the surface [12]. We found that the use of SIF substrates results in an 8-fold or larger increase in the emission intensity of the cyanine-labeled oligomers. The decreased lifetimes on the SIFs indicates that the increased intensity is due in large part to an increased rate of radiative decay. Our results suggest the use of substrates containing silver particles for increased-sensitivity DNA detection.

Theory

The potentially favorable effects of metallic particles on fluorescence can be seen from the basic definitions of the quantum yield (Q) and lifetime (τ). These quantities are given by

$$Q = \frac{\Gamma}{\Gamma + k_{nr}}, \quad (1)$$

$$\tau = \frac{\Gamma}{\Gamma + k_{nr}}, \quad (2)$$

where Γ is the radiative decay rate and k_{nr} is the non-radiative decay rate. This rate (k_{nr}) is typically dependent on the sample condition and local probe environment. Changes in k_{nr} are the origins of the intensity increases of solvent-sensitive probes upon binding to macromolecules. In contrast to k_{nr} , the radiative rate Γ is determined by the extinction coefficient [13] and is not significantly sensitive to the local environment.

Different and unique effects are possible for fluorophores near metallic particles. The metal particle can amplify the incident light field and increase the rate of excitation of nearby fluorophores, which is called the “lightening rod effect.” Additionally, the excited state acts like an oscillating dipole which interacts with the freely mobile electron in the metal. These interactions have been studied theoretically [14–16]. An interesting prediction is for increased rates of radiative decay for fluorophores 20 to 100 Å from a metallic surface. Suppose the metal (m) increases the radiative decay rate from Γ to $\Gamma_T = \Gamma + \Gamma_m$. The quantum yield (Q_m) and lifetime (τ_m) near the metal (m) become

$$Q_m = \frac{\Gamma + \Gamma_m}{\Gamma + \Gamma_m + k_{nr}} = \frac{\Gamma_T}{\Gamma_T + k_{nr}}, \quad (3)$$

¹Abbreviations used: APS, 3-aminopropyltriethoxysilane; Cy3, *N,N'*-(dipropyl)-tetramethylindocarbocyanine; Cy5, *N,N'*-(dipropyl)-tetramethylindocarbocyanine; FD, frequency domain; SIFs, silver island films; ds, double-stranded.

$$\tau_m = \frac{1}{\Gamma + \Gamma_m + k_{nr}} = \frac{1}{\Gamma_T + k_{nr}}. \quad (4)$$

An increase in the total radiative rate to $\Gamma_T = \Gamma + \Gamma_m$ results in an increased quantum yield and a decreased lifetime. From the point of view of fluorescence detection, the higher quantum yield means more detectable emission. Additionally, a decreased lifetime is expected to result in less photobleaching per emitted photon. This effect is expected because if photobleaching occurs in the excited state, then a shorter lifetime allows less time for photochemical processes. Based on these considerations we examined whether the detectability of labeled DNA could be increased by proximity to metallic particles.

Materials and methods

DNA oligomers

Single-stranded DNA oligomers, labeled with Cy3 and Cy5 on the 5' end, were obtained from Synthetic Genetics (San Diego, CA, USA). The complementary unlabeled oligonucleotides and oligonucleotides labeled with biotin were obtained from the Biopolymer Core Facility at the University of Maryland, School of Medicine (Scheme 1).

Double-stranded (ds) DNA samples (Cy3-DNA and Cy5-DNA) deposited using avidin were previously hybridized by mixing the complementary labeled and unlabeled oligonucleotides in 5mM Hepes, pH 7.4, 0.2M KCl, and 0.25mM EDTA, to a final concentration of 2 μ M, followed by heating to 70 °C for 2 min and slow cooling. For binding to avidin-coated surfaces the unlabeled DNA strand contained a biotin on the 5' end. ds DNA, which was deposited on APS or polylysine-treated slides, was hybridized in 3 $\times$ SSC buffer (45mM sodium citrate, 0.45M NaCl). Concentrations were determined using ϵ (548 nm) = 150,000M⁻¹cm⁻¹ for Cy3 and ϵ (648 nm) = 215,000M⁻¹cm⁻¹ for Cy5. The quantum yields were determined using rhodamine B ($Q = 0.48$) as a reference. The quantum yield of Cy3 and Cy5 in the absence of silver particles were found to be 0.24 and 0.20, respectively.

Surface preparation and silver island films

We used quartz slides as substrates. The use of quartz provided UV transmission and less autofluorescence than glass. The quartz slides were soaked in a 10:1 (v/v) mixture of H₂SO₄ (95–98%) and H₂O₂ (30%) overnight before silver deposition, washed with distilled water, and air-dried prior to use. Prior to silver deposition the slides were coated with amino groups by reaction with 0.1% APS for 10 min or polylysine by spin coating at 3000 rpm 200 μ l of 0.01% polylysine in 0.1 $\times$ PBS. Following these treatments silver islands were deposited by reduction of silver nitrate with D-glucose, as described previously [10].

Other slides were coated with avidin following deposition of the silver island films on clean quartz. The unsilvered and silvered regions of the slides were incubated overnight with 10 μ M avidin (Molecular Probes).

We found that SIFs were readily formed on the amine-coated slides. From the AFM images we found that the islands were from 100 to 300nm across and near 60nm high (Fig. 1). We believe that some of the larger particles are aggregates of smaller particles. Such particles display a characteristic surface plasmon resonance characteristic of sub-wavelength size silver particles (Fig. 2). Somewhat larger and more aggregated particles were formed when the slides were not coated with amino groups, resulting in a longer absorption wavelength near 470 nm.

Fluorescence measurements

Our sample configuration is shown in Scheme 2. In all cases half of the slide was coated with SIFs and the other half was unsilvered. The coated surface was covered with one part of a demountable cuvette. Similar results on the silvered area were obtained for illumination through the silvered or unsilvered surface.

Emission spectra were obtained using a SLM 8000 spectrofluorometer using 514nm excitation for Cy3-DNA and 605nm excitation for Cy5-DNA. In the observation path we used additional 530-nm and 630-nm long-wave pass filters for Cy3-DNA, and Cy5-DNA respectively. Intensity decays were measured in the frequency-domain using instrumentation described previously [17]. For Cy3-DNA the excitation was obtained from a mode-locked argon ion laser, a 76-MHz repetition rate, and a 100-ps pulse width. The Cy3-DNA emission was observed through a combination of a 565-nm interference filter with a long pass 530 nm filter. For Cy5-DNA the excitation was the 3.8-MHz output of a cavity-dumped R6G dye laser, with 10-ps pulses. The Cy5 emission was observed through a combination of a 665-nm interference filter with a 630-nm long-pass filter. All measurements were performed using front-face geometry in a 0.2-mm demountable cuvette (Scheme 2). The quantum yields were measured in a 10×4 -mm cuvette with square geometry.

Steady-state data were measured with both the excitation and the emission vertically polarized. For frequency-domain measurements the excitation was vertically polarized and the emission observed through a polarizer oriented at 54.7° from the vertical position. The FD intensity decay was analyzed in terms of the multiexponential model

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i), \quad (5)$$

where τ_i are the lifetimes with amplitudes α_i and $\sum \alpha_i = 1.0$. Fitting to the multiexponential model was performed as described previously [18]. The contribution of each component to the steady-state intensity is given by

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j}. \quad (6)$$

The mean decay time is given by

$$\bar{\tau} = \sum_i f_i \tau_i. \quad (7)$$

The amplitude-weighted lifetime is given by

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i. \quad (8)$$

The value of $\langle \tau \rangle$ is proportional to the area under an intensity decay.

Uncertainties in the recovered parameters (α_i and τ_i) were obtained by examination of the χ_R^2 surface. These surfaces were determined by holding the parameter of interest fixed at values different from the optimum value and then fitting of the remaining parameters [19]. This approach accounts for correlation between the parameters which increase the uncertainty of the recovered values.

Results

Cy3-DNA and Cy5-DNA on avidin-coated surfaces

We first describe the spectral properties of the biotinylated oligos on avidin-coated surfaces. From measurements of the peptide bond absorbance we found that roughly the same amounts of avidin were bound to the silvered and unsilvered regions of the slides. Hence we believe avidin binds to both the quartz and silver surfaces (Scheme 3). As a result we expect the population of bound DNA to be heterogeneous, with some DNA bound to the silver particles and some bound between the particles.

Emission spectra of the Cy3- and Cy5-labeled DNA are shown in Fig. 3. In both cases the emission intensity is increased about 10-fold on the silvered half of the slides. The increased intensities seen on the silvered surfaces are useful only if the signal remains higher for a period of time adequate for measurements. If the increased intensities were due to the “lightening rod effect,” then one expects more rapid photobleaching due to increased rates of excitation. We examined the intensities of the labeled DNA with continuous illumination (Fig. 4). The intensity photobleaches more rapidly on the silvered surfaces. However, the signal detectable from these probes is proportional to the time-integrated area under these curves, which is 9.1- and 7.6-fold greater for Cy3 and Cy5, respectively, on the silvered surfaces. Another approach to testing the photostability is to attenuate the incident light on the silvered surface to obtain the same emission intensity as that on the unsilvered surface. In this case photobleaching occurs somewhat more slowly on the silvered surface (Fig. 5). Intuitively it is unclear why the photobleaching is not about 8-fold slower than that seen under identical illumination conditions (Fig. 4). Nonetheless, it is clear that the overall signal can be many-fold larger on the silvered surfaces.

We questioned whether the same emission spectrum remained after prolonged illumination. Emission spectra were recorded before and after the illumination shown in Figs. 4 and 5. Except for the intensities, the emission spectra were identical before and after photobleaching (not shown).

Prior to the time-resolved measurements we performed control measurements to test for the possibility of scattered light. Exclusion of scattered light is especially important for measurement of short fluorescence lifetimes. Hence we examined the emission spectra from our samples as observed through the emission filters used for the time-resolved measurements, and then scanning the emission monochromator through the excitation wavelength (Fig. 6). These spectra showed that scattered light of the excitation wavelength contributes less than 1% to the signals observed for the time-resolved measurements.

It is well known that many factors can result in increased intensities from fluorescent probes. These factors typically decrease the nonradiative decay rate, k_{nr} in Eqs. (1) and (2). In contrast, metallic surfaces are expected to increase the radiative decay rate to $\Gamma + \Gamma_m$, so that increased intensities are accompanied by decreased lifetime, (Eqs. (3) and (4)). We measured the frequency-domain intensity decays of the labeled oligos bound to avidin on the unsilvered and silvered surfaces (Figs. 7 and 8). For both Cy3-DNA and Cy5-DNA we observed dramatically decreased lifetimes. The extent of the lifetime decrease is evident from the reconstructed time-dependent decays (Fig. 9). We note that the intensity decays in Fig. 9 are plotted with the time-

zero values normalized to unity. If the area under the curves is normalized, then the intensity decays appear visually to be due to a new component with increased intensity and a short lifetime. At present it is not clear whether to use the average lifetime $\bar{\tau}$ or the amplitude-weighted lifetime $\langle\tau\rangle$ to describe the shortened intensity decays (Table 1). We currently believe that the amplitude weighted lifetime, which is proportional to the time-integrated value of $I(t)$, provides a better representation of the decreased lifetime and/or increased rate of radiative decay.

It is of interest to determine the increase in the rate of radiative decay due to the silver particles. Examination of Eqs. (1) and (2) shows that the radiative rate in the absence of metal is given by

$$Q/\tau = \Gamma. \quad (9)$$

Similarly, in the presence of metal

$$\frac{Q_m}{\tau_m} = \Gamma + \Gamma_m = \Gamma_T. \quad (10)$$

Hence a comparison of these two ratios provides an estimation of the ratio of the total radiative rates near the metal to the radiative rate in the absence of metal, Γ_T/Γ (Table 2). Calculation of the radiative decay rates is straightforward in solution, but difficult for probes on metal surfaces. The difficulty arises from the unknown quantum yield near the metal. The increased intensity can be due to the increased quantum yield or due to the lightening rod effect. Given the 8-fold increase in intensity, and the quantum yields of 0.2–0.24, there must be some contribution of an increased rate of excitation. To obtain an estimate of the radiative rate increase we assumed that quantum yields of Cy3 and Cy5 were unity near the metal particles. Under this assumption, the decreased lifetimes indicate an increase in the radiative rates.

Estimation of the factor increase in the radiative rate (Γ_T/Γ) depends on the accuracy of the recovered amplitude-weighted lifetimes. To estimate Γ_T/Γ reliably, we calculated the confidence interval for the α_i and τ_i values from the χ^2_r surfaces [19]. An example of this calculation is shown in Fig. 10 for the most difficult case (shortest lifetime) of Cy3-DNA-biotin-avidin on silver. For this case we can only estimate an upper limit for the amplitude-weighted lifetime. To estimate the increase in the radiative rates we used the upper limit of the amplitude-weighted lifetimes. For Cy3-DNA and Cy5-DNA bound to avidin on silver island films, the radiative rates increased by at least a factor of 708 and 225, respectively (Table 2).

Cy3-DNA and Cy5-DNA on amine coated slides

DNA hybridization is frequently performed on substrates coated with amino groups. Hence it was of interest to examine the effects of SIFs on labeled DNA bound to these surfaces. The quartz surfaces were either treated with APS or coated with polylysine. The samples were prepared in a manner analogous to that used with gene chips, including UV illumination for crosslinking to the surfaces. Emission spectra of Cy3-DNA and Cy5-DNA bound to these surfaces are shown in Figs. 11 and 12. Increased intensities were always observed on the silver surface relative to the unsilvered surface. The magnitude of the intensity increase was variable from 3 to 10, depending on the probe and the surface. Additionally, the lifetimes decreased dramatically on the silvered surfaces (Figs. 13 and 14, Table 3). There seemed to be a correlation between the intensity increase and the decrease in lifetime.

Cy3-DNA shows a larger intensity increase on polylysine than Cy5-DNA (Fig. 9) and Cy3-DNA displays a shorter lifetime on silver (Fig. 13 and Table 3). Similarly, Cy5-DNA shows a larger intensity increase on APS than Cy3-DNA (Fig. 12), and Cy5-DNA displays a shorter lifetime on silver (Fig. 14). These results are consistent with increases in the radiative decay rates of the probes on the silvered surfaces, which increase from 31- to 242-fold depending on the probe and surface coating (Table 2). Our experiments on amine-coated slides were performed on wet samples. In general we found larger intensity increases on wet samples than on dry samples (Fig. 15).

And finally, we examined the excitation spectra of Cy3-DNA on a polylysine-coated slide (Fig. 16). We reasoned that the intensity increase should follow the plasmon absorption of the silver. However, the ratio of the uncorrected excitation spectra is moderately constant from 480 to 560 nm. While these results do not rule out a correlation with the plasmon absorption, such an effect is weak in the present system.

Discussion

We have shown that deposition of silver particles on a substrate is a reliable approach to obtaining increased intensities from fluorophores on DNA arrays. Substantial increases in intensity were obtained whether the DNA was directly bound to amine-coated surfaces or indirectly bound via avidin. We feel the intensity increases and signal stability were somewhat better for the avidin-coated surfaces than for the amine-coated surfaces. This result may suggest that optimal results will be obtained when the probes are not in direct contact with the silver particles, when quenching is expected. While intensity increases from 3- to 12-fold are impressive, we believe that these values are limited by sample heterogeneity. That is, the probes are located randomly and distant near and on the metallic surfaces. It is likely that only a fraction of these probes contribute to the observed intensity increases. Additionally, the SIFs may not be optimal in size and shape for the cyanine dyes. Hence it seems reasonable to expect that additional increases in intensity will result from improved methods of silver deposition and oligonucleotide coupling to the surfaces.

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Fig. 1. AFM image of a silver island film formed on an APS coated quartz plate. Bottom panel shows high profile along the line shown in the image.

Fig. 2. Absorption spectra of silver island films on quartz alone (top), coated with polylysine (middle), and coated with APS (bottom). The spectra on polylysine and APS are slightly blue-shifted.

Fig. 3. Emission spectra of Cy3-DNA (top) and Cy5-DNA (bottom) on avidin-coated slides, with and without silver island films.

Fig. 4. Effects of continuous illumination on the intensities of Cy3- and Cy5-labeled DNA bound to avidin-coated surface. The excitation intensity was the same for each probe on silver or quartz.

Fig. 5. Photostability of Cy3 and Cy5 on avidin-coated surfaces. The incident power was adjusted to obtain the same emission intensity of each probe on silver and quartz.

Fig. 6. Emission spectra of Cy3-DNA-biotin-avidin and Cy5-DNA-biotin-avidin through a combination of filters used in lifetime measurements (—). The dotted lines are the emission spectra observed in a cuvette without the emission filters.

Fig. 7.
Frequency-domain intensity decays of Cy3-DNA on avidin-coated slides.

Fig. 8.
Frequency-domain intensity decays of Cy5-DNA on avidin-coated slides.

Fig. 9.
Time-dependent intensity decays of Cy3-DNA and Cy5-DNA on avidin-coated slides.

Fig. 10. Confidence interval analysis for the shortest component in Cy3-DNA-biotin-avidin intensity decay on a silver island film.

Fig. 11. Emission spectra of Cy3-DNA (top) and Cy5-DNA (bottom) on polylysine-coated slides, with and without silver island films.

Fig. 12. Emission spectra of Cy3-DNA (top) and Cy5-DNA (bottom) on APS-treated slides, with and without silver island films.

Fig. 13.
Frequency-domain intensity decay of Cy3-DNA and Cy5-DNA on polylysine-coated slides, with and without silver island films.

Fig. 14.

Frequency-domain intensity decays of Cy3-DNA and Cy5-DNA on APS-treated slides, with and without silver island films.

Fig. 15.
Emission spectra of Cy3-DNA on a polylysine-treated slide when wet or dry.

Fig. 16.

Excitation spectra of Cy3-DNA on a polylysine-coated slide. The insert shows the ratio of the excitation spectra. In this separately prepared slide there was about 25% fewer silver islands deposited which result in lower enhancement.

(biotin)-5'-TCC ACA CAC CAC TGG CCA TCT TC-3'
 3'-AGG TGT GTG GTG ACC GGT AGA AG-5'-**Cy3**

biotin-DNA-Cy3

(biotin)-5'-TCC ACA CAC CAC TGG CCA TCT TC-3'
 3'-AGG TGT GTG GTG ACC GGT AGA AG-5'-**Cy5**

biotin-DNA-Cy5

Scheme 1.

Chemical structures and base sequences of the labeled DNA oligonucleotides. Biotin was present in the oligos used with avidin and not present when using the APS- or polylysine-coated slides.

Scheme 2.

Experimental configuration for fluorescence enhancement measurements. The sample is deposited on a quartz (Q) plate half-coated with silver islands (S). The quartz plate is assembled with 0.2-mm demountable cuvette (Starna, Inc.) filled with a buffer solution. The entire cuvette is mounted on a precise translator.

Scheme 3.
DNA oligomers bound to an avidin-coated surface.

Table 1
Multiexponential analysis of avidin-bound Cy3-DNA and Cy5-DNA monolayers intensity decays on quartz and silver island film

Compound/conditions	$\bar{\tau}$ (ns)	$\langle \tau \rangle$ (ns)	a_i	f_i	τ_i (ns)	χ^2_R
Cy3-DNA, quartz	1.85 ^a	1.53 ^b	0.443 (0.420–0.470) ^c	0.213 (0.195–0.235)	0.73 (0.70–0.76)	0.7
			0.557 (0.530–0.580)	0.787 (0.705–0.875)	2.16 (2.10–2.21)	
Cy3-DNA, silver	0.23	≤0.009	0.987 (0.977–0.999)	0.436 (0.385–0.485)	0.002 (0.000–0.004)	1.1
			0.001 (0.000–0.002)	0.179 (0.167–0.192)	0.909 (0.845–0.970)	
Cy5-DNA, quartz	1.53	1.31	0.012 (0.002–0.019)	0.385 (0.355–0.420)	0.166 (0.150–0.176)	1.2
			0.528 (0.480–0.570)	0.323 (0.255–0.385)	0.80 (0.75–0.85)	
Cy5-DNA, silver	0.36	≤0.029	0.472 (0.430–0.520)	0.677 (0.630–0.720)	1.88 (1.80–1.95)	1.8
			0.945 (0.925–0.970)	0.295 (0.265–0.330)	0.006 (0.002–0.010)	
			0.051 (0.031–0.068)	0.455 (0.425–0.480)	0.175 (0.165–0.185)	
			0.004 (0.003–0.006)	0.250 (0.235–0.265)	1.105 (1.050–1.150)	

^a $\tau = \sum_i f_i \tau_i$.

^b $\langle \tau \rangle = \sum_j a_j \tau_j$.

^cThe confidence intervals in parentheses were calculated from the χ^2_R surfaces.

Table 2

Relative intensities of Cy3- or Cy5-labeled DNA oligomers on quartz slides or silver island films

Sample	Relative intensity (silver/quartz)	Photostability (silver/quartz)	I_T/I^a
Cy3-DNA-biotin-avidin	12.6	9.1	708
Cy5-DNA-biotin-avidin	10.0	7.6	225
Cy3-DNA on polylysine	9.5	8.1	242
Cy5-DNA on polylysine	2.7	2.5	199
Cy3-DNA on APS	5.0	4.8	31
Cy5-DNA on APS	7.4	6.5	88

^aCalculated using Eqs. (9) and (10) and the amplitude-weighted lifetimes in Tables 1 and 3. The quantum yields of Cy3-DNA and Cy5-DNA in quartz were taken as 0.24 and 0.20, respectively. The quantum yields of Cy3-DNA and Cy5-DNA on SIFs were taken as 1.0 for both probes.

Table 3
Multiexponential analysis of the fluorescence intensity decays of Cy3-DNA and Cy5-DNA

Compound	$\bar{\tau}$ (ns)	$\langle \tau \rangle$ (ns)	a_i	f_i	τ_i (ns)	χ^2_R
Cy3-DNA on Poly-Lys, Q	1.526	1.107	0.419 ^d (0.412–0.428)	0.115 (0.109–0.122)	0.305 (0.280–0.320)	0.9
			0.581 (0.572–0.590)	0.885 (0.820–0.930)	1.685 (1.660–1.720)	
Cy3-DNA on Poly-Lys, S	0.134	≤0.019	0.970 (0.960–0.975)	0.700 (0.630–0.755)	0.011 (0.008–0.013)	1.0
			0.028 (0.023–0.034)	0.182 (0.155–0.210)	0.098 (0.080–0.105)	
Cy5-DNA on Poly-Lys, Q	1.738	1.398	0.002 (0.001–0.003)	0.118 (0.110–0.125)	0.917 (0.830–1.005)	1.3
			0.291 (0.280–0.305)	0.067 (0.058–0.078)	0.323 (0.285–0.360)	
Cy5-DNA on Poly-Lys, S	0.913	≤0.035	0.709 (0.695–0.720)	0.933 (0.920–0.945)	1.839 (1.810–1.870)	2.1
			0.923 (0.920–0.930)	0.235 (0.220–0.245)	0.017 (0.014–0.019)	
Cy3-DNA on APS, Q	1.345	1.057	0.051 (0.049–0.053)	0.175 (0.165–0.185)	0.224 (0.200–0.250)	1.4
			0.026 (0.024–0.028)	0.590 (0.580–0.600)	1.474 (1.430–1.520)	
Cy3-DNA on APS, S	0.532	≤0.140	0.362 (0.335–0.395)	0.111 (0.088–0.140)	0.325 (0.275–0.380)	1.9
			0.638 (0.600–0.665)	0.889 (0.860–0.910)	1.472 (1.420–1.530)	
Cy5-DNA on APS, Q	1.560	0.833	0.674 (0.665–0.685)	0.151 (0.130–0.170)	0.028 (0.022–0.033)	1.4
			0.269 (0.260–0.275)	0.394 (0.375–0.415)	0.183 (0.170–0.195)	
Cy5-DNA on APS, S	0.411	≤0.047	0.057 (0.052–0.061)	0.455 (0.440–0.470)	1.003 (0.965–1.055)	1.6
			0.614 (0.608–0.620)	0.159 (0.150–0.165)	0.215 (0.205–0.230)	
			0.386 (0.380–0.395)	0.841 (0.834–0.848)	1.815 (1.780–1.850)	
			0.923 (0.915–0.930)	0.438 (0.405–0.470)	0.019 (0.017–0.022)	
			0.069 (0.065–0.075)	0.287 (0.270–0.300)	0.169 (0.155–0.185)	
			0.008 (0.007–0.009)	0.275 (0.265–0.285)	1.353 (1.310–1.415)	

^aThe confidence intervals in parentheses were obtained from the χ^2_R surfaces.