FLUORESCENCE ENHANCEMENT FROM SINGLE DNA MOLECULES CONFINED IN SiO₂ NANOCHANNELS

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ABSTRACT

We demonstrate that the detected emission intensity from YOYO-labeled DNA molecules confined in 180 nm deep Si/SiO₂ nanofunnels changes significantly and not monotonically with the width of the funnel, an emission enhancement that is only detected for emitted light polarized parallel to the channel. We explain the enhancement effect as being due to optical phenomena in the channels. The enhancement effect may be of importance for quantitative fluorescence microscopy and for experiments with a tight photon budget.

KEYWORDS: Single molecule, DNA, Fluorescence microscopy, Nanofluidics

INTRODUCTION

When a DNA molecule is confined in a channel with two dimensions smaller than its radius of gyration it will extend along the channel.[1,2] The extension scales linearly with the contour length of the DNA and coarse genetic information can thus be read from the DNA.[3] Nanochannel studies of DNA elongation can also be used to study DNA/protein interactions [4,5] and to obtain a deeper understanding of general polymer physics [6]. The theory for the extension is generally explained by the models developed by deGennes and Odijk.[7,8] Tapered nanochannels were recently introduced to expose DNA molecules to a gradual change in confinement[9] in a way comparable to standard force spectroscopy techniques like optical or magnetic tweezers [10] but at a lower force regime and where studies of DNAs with different topologies, such as circular DNA, are possible. Nanofluidic chips allow for high parallelization in Lab-on-a-Chip applications.

A main obstacle when studying single DNA molecules, and single molecules in general is the low photon-flux. Therefore ways of improving the number of detected photons are of great interest. We here demonstrate that the detected emission intensity from fluorescently labeled DNA is significantly enhanced at certain dimensions of the nanochannels.[11]

EXPERIMENTAL

All experiments were conducted with λ-phage DNA (48.5 kbp, New England Biolabs). The DNA was stained with the fluorescent dye YOYO®-1 (YOYO, Invitrogen) at a binding ratio of one dye molecule per ten base pairs. The DNA was dissolved in a 0.5x TBE buffer or a 1x TBE buffer. 3% β-mercaptoethanol was added to suppress photobleaching and phonticking.

Unpolarized DNA imaging was performed using an epifluorescence video microscopy system consisting of a Nikon Eclipse TE2000-U inverted microscope, 60x water immersion (NA 1.0, Nikon) objective (with a 1.5x additional magnification lens for a total magnification of 90x) coupled to a cooled back-illuminated EMCCD camera (Andor Technology, iXon DV 887-EC5 BV). Polarization-sensitive imaging of DNA was carried out on another microscope setup consisting of the same model of microscope and objective but with a back-illuminated EMCCD camera.

Figure 1: Cartoon showing the principles of the experiment. Top: DNA is confined in a nanofunnel with a width ranging from 50 to 650 nm. As the channel becomes narrower the DNA becomes more and more extended. Bottom left: schematic drawing showing the principal chip design with a series of nanofunnels connected to microchannels that are used for sample transport. Bottom right: schematic drawing of the cross section of the nanofunnel. The channel width (w) ranges from 50 to 650 nm and the height (h) is 180 nm. There is a 50 nm thick SiO₂ layer on three sides of the channel and the fourth side is a 550 m thick borosilicate lid.
RESULTS AND DISCUSSION

Figure 2 shows how the detected emission intensity from single YOYO-labeled DNA molecules varies when the width of 180 nm deep channel increases from 50 to 650 nm. There is a distinct peak at a width of approximately 180 nm. The wavelength of maximum emission of the dye used is 510 nm, which corresponds to 380 nm in water. The wavelength of the emitted light is thus on the same length-scale as the nanostructures. We therefore believe that the enhancement is due to optical phenomena in the channel. The enhancement can be caused by either (1) a local enhancement of the excitation intensity, (2) an enhanced quantum yield and/or emitter rate or (3) a modified radiation pattern where more of the emitted light is directed towards the detector.[12] Note that since the refractive index of water and SiO$_2$ is similar meaning that the main reflecting interface in the chip is the Si/SiO$_2$ interface and the “optical width” of the channel is thus 100 nm larger than the physical channel width. The broadness of the peaks in Figure 2 can be explained by that we have a large number of emitting dyes on every DNA (~5000) that are located throughout the width of the channel and hence experience slightly different optical environments. The fact that the emitted light is collected through a 500-550 nm bandpass filter also contributes to the broadness of the peak.

To further elucidate the phenomenon, we imaged the detected emission parallel and perpendicular to the channel simultaneously. Figure 3 shows that the enhancement effect is only seen for emitted light polarized parallel to the channel, while the emission intensity perpendicular to the channel is unaffected by the channel width. This observation agrees well with that the effect seen is due to optical phenomena caused by the nano-confinement. Two perpendicular dimensions, one confined and the other unconfined, should lead to radically different emission profiles when the confinement is on the size of the wavelength of the emitted light.

To exclude instrument- and experiment related errors Figure 2 and 3 were recorded at different microscopes and using different ionic strengths.

In each frame the molecule is automatically located and fitted by a rectangle where all sides are set to the position where the intensity is 50% of the maximum intensity within the molecule after background has been subtracted. The detected emission intensity value is then the sum of all pixel values within this rectangle. Each datapoint in Figure 2 and 3 corresponds to the average total intensity from 400 images and the error bars correspond to one standard deviation.

The chip consists of 180 nm deep funnels with a width that varies continuously from 50 nm to 650 nm. The total length of each funnel is 600 µm. The rate of change in the width along the channel is 1 nm/µm, so that locally the DNA is exposed to a channel with more or less straight walls. The DNA is manipulated in the micro and nanochannels using pressure driven flow. The channels were defined by electron beam and UV lithography followed by CF$_4$/CHF$_3$ based reactive ion etching. Finally a thin layer (50 nm) of dry thermal oxide was grown to render the surfaces hydrophilic. The devices were sealed with a 550 µm thick borosilicate glass lid. The depth of the channel was chosen to minimize the aspect ratios when changing the width of the funnel in the optically interesting regime 50 – 650 nm. With our 180 nm deep channels, the aspect ratio varies from 1:3.6 to 3.6:1. We define the optical width of the channels as the distance between the two main reflecting surfaces, i.e. the SiO$_2$/Si interfaces (the width of the channel plus twice the thickness of the SiO$_2$ layer). For more details on data analysis and chip fabrication, see ref [2].

from another manufacturer (Photometrics, Cascade II 512). Polarization-sensitive imaging was achieved by placing a Dual-View$^\text{TM}$ (DV-2) unit (from Photometrics) on the collecting side. This splits the light into two paths, which enables us to visualize the two polarization directions simultaneously.

The detected emission intensity is due to optical phenomena seen in 0.5x TBE buffer. The black lines connecting the data points are drawn to guide the eye. Each trace is vertically shifted 5 a.u. for clarity and the lowest one corresponds to actual baseline subtracted data.

Figure 3: Detected emission intensity for light polarized perpendicular (full symbols) and parallel (open symbols) to the channel for four individual YOYO-labeled DNA molecules at different positions in a 180 nm deep funnel in 1 × TBE buffer. The black lines connecting the data points are drawn to guide the eye.
Changing the ionic strength changes the extension of the DNA so that at a higher ionic strength the DNA molecule will be less extended.[13] Since the peak in the detected emission intensity does not vary with ionic strength we concluded that the effect seen is not caused by the intrinsic polymer physics of the DNA but rather the nanoconfinement. This is corroborated by the observation of similar effects when using fluorescent nanospheres (see ref [11]). Furthermore, the fact that the detected emission intensity does not vary with the confinement in the perpendicular polarization direction rules out any artifacts due to photo bleaching.

The fact that the detected emission intensity varies significantly with the confinement is of importance for quantitative studies of DNA interactions with drugs or proteins. An increase in detected emission at a certain confinement, caused by the actual confinement, can easily be misinterpreted in as an increased binding affinity. Special care must be taken when comparing the two polarization directions, e.g. when studying the local orientation of confined DNA [14], since the enhancement only appears in one polarization direction. On the other hand, the effect can be used in future when designing nanofluidic chips to maximize the signal to noise ratio. By tuning the dimension of the channels with the photophysical properties of the dye, the detected emission can be increased significantly (in this case at least three fold). It should be noted that the main reflecting surface is the Si/SiO$_2$ interface, the degree of confinement and the size of the optical cavity can be varied independently and it should therefore be straight-forward to combine a high photon flux and an efficiently stretched DNA.

CONCLUSION

We have demonstrated how the detected emission intensity varies significantly when the width of 180 nm deep channels increases from 50 to 650 nm, and that the enhancement is only seen for emitted light polarized parallel with the channel. We explain the enhancement as being due to optical phenomena caused by the nanostructures that has dimensions similar to the wavelength of the emitted light. We believe that this effect is of critical importance for future design of nanochannel structures and for single molecule experiments with a tight photon budget.

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