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COMMUNICATION

Bio-switchable optofluidic lasers based on DNA Holliday junctions†‡

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Bio-switchable optofluidic lasers based on DNA Holliday junctions were demonstrated. Nearly 100% wavelength switching was achieved through reversible conformational change of the Holliday junction controlled by magnesium ionic strength.

Optofluidic lasers synergize microfluidics and laser technology, and have unique capabilities in handling liquid of picoliter to microliter volumes.[1–4] As such, there is currently a great deal of interest in adapting optofluidic lasers for compact, dynamically tunable laser light sources and micro-total-analysis systems. To date, various optofluidic lasers have been demonstrated, including Fabry-Pérot lasers,[5,6] distributed feedback (DFB) lasers,[7,8] and optofluidic ring resonator (OFRR) lasers.[9,10] In those lasers, wavelength switching is usually implemented by flowing liquid droplets (or plugs) containing different dyes through the microfluidic channel, with an excitation laser tuned into the absorption band of each dye.[5,11] Fluorescence resonance energy transfer (FRET) provides another effective means to achieve wavelength switching, in which the donor is excited and transfers energy to the acceptor for it to lase.[9,12,13] As compared to the directly excited laser, the FRET laser is versatile by extending the laser emission range without changing the excitation laser and has a low lasing thresholds due to the significantly reduced self-absorption effect from the dye. The FRET laser can be achieved by co-flowing the donor and the acceptor in free solution through the optofluidic laser cavity.[12,13] More recently, a FRET laser controlled by biomolecules (such as DNA scaffolds) has also been demonstrated, thus opening the door to tune/switch the laser wavelength through a plethora of well-studied biological processes.[9] In the above DNA-controlled FRET laser, the donor and the acceptor were conjugated with linear DNA sequences with a predetermined ratio and spatial configuration. Through DNA hybridization, the donor and the acceptor are brought to close proximity. However, the FRET process and hence wavelength switching demonstrated so far using either free solution or linear DNA sequences are irreversible. Achieving a wavelength reversible bio-controlled optofluidic laser will not only considerably enhance the laser performance and enable development of novel photonic devices, but also have significant implications in bio/chemical sensing applications, as well as in DNA origami, computation and nanomachines.

The DNA Holliday junction, first discovered in 1964, is a critical intermediate in homologous genetic recombination.[14–16] As illustrated in Fig. 1(A), the Holliday junction has four branched double-helical arm structures.[14] Due to the strong electrostatic repulsion among the backbone phosphate groups, the arms of the

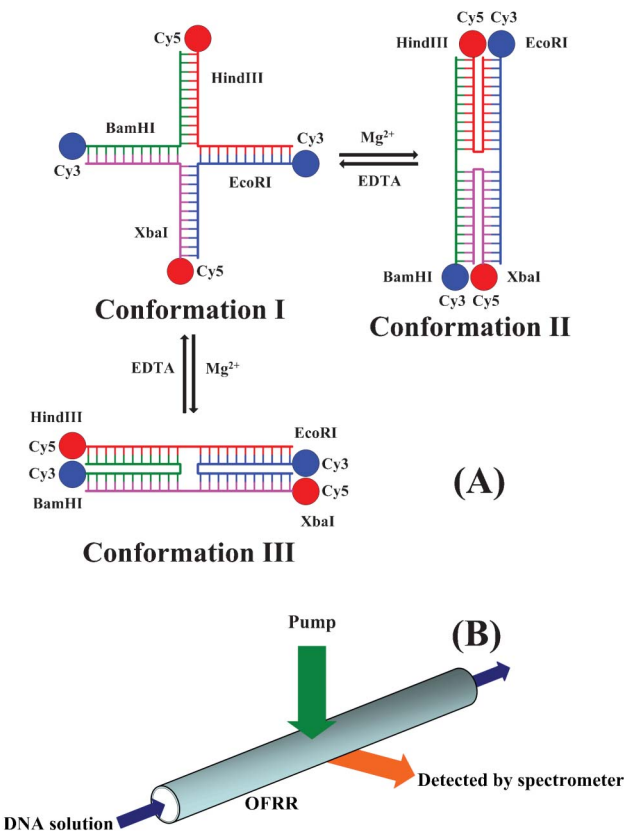

Fig. 1 (A) The ion-dependent folding of the DNA Holliday junction. In the presence of Mg^{2+} ions, the junction folds into the stacked X-structure (Conformation II and III). After Mg^{2+} ions are removed by EDTA, the junction unfolds back to Conformation I. (B) Schematic of the bio-switchable laser based on the optofluidic ring resonator (OFRR).

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junction are fully extended into a planar square conformation.[14–16] However, in the presence of certain kinds of cations, the repulsion is largely suppressed, leading to a folded junction. Group IIA cations (*e.g.*, magnesium) have been shown to be particularly effective in folding the Holliday junction.[14,15,17,18] In this paper, we exploited this unique characteristic of the Holliday junction to develop bio-switchable optofluidic lasers. Nearly 100% wavelength switching was achieved through reversible conformational change of the Holliday junction controlled by magnesium ionic strength.

The Holliday junction used in the experiment is detailed in Table S1 of the ESI.† It was formed by four 40-base single-stranded DNA. Each arm of the Holliday junction was conjugated with Cy3 or Cy5 as the donor/acceptor pair. With the increased Mg^{2+} concentration, the Holliday junction folds gradually, enabling the energy transfer from Cy3 to Cy5. Upon addition of tetrasodium ethylenediamine(tetraacetate) (Na_4 EDTA), Mg^{2+} ions are removed from the Holliday junctions and the Holliday junction unfolds. Note that, for the folded junction, there exist two possible conformations (see Conformation II and III in Fig. 1(A)). The conformation bias depends on the DNA sequences.[17,18] Although the DNA used in our experiments favors Conformation II, the donor and acceptor arrangement in our experiment ensured that efficient energy transfer could occur between Cy3 and Cy5 regardless of the conformation bias. Details of the sample preparation are described in the ESI.†

We achieved the bio-switchable laser using the OFRR based on a thin-walled fused silica capillary (57.5 μm in outer diameter and 2 μm in wall thickness), whose cross section forms the ring resonator that supports the whispering gallery modes (WGMs).[9,10] The WGM ($Q \sim 10^7$)[9,10] circulates along the ring resonator circumference and interacts with the gain medium flowing through the capillary to provide the optical feedback for lasing. The experimental setup is illustrated in Fig. 1(B). The Holliday junction solution was flowed through the capillary at a flow rate of 1 μL min^{-1} and was pumped by an optical parametric oscillator laser at 518 nm (5 ns pulse width and 20 Hz repetition rate), a wavelength within the Cy3 absorption band but far away from that of Cy5. The emitted light was collected by a multimode fiber and was subsequently transmitted to a spectrometer (spectral resolution: 0.4 nm). Details of the experimental procedures can be found in the ESI.†

In the absence of Mg^{2+}, the arms of the Holliday junction were fully extended into a planar square conformation as a result of strong electrostatic repulsion. The distance between Cy3 and Cy5 was approximately 9.6 nm. Based on the energy transfer efficiency given by $R_0^6/(R_0^6 + r^6)$, where R_0 and r are the Förster distance and donor/acceptor distance, we estimate that the energy transfer efficiency between Cy3 and Cy5 was only 5.6% (R_0 = 6 nm for Cy3 and Cy5). Fig. 2 shows the lasing spectra for both Cy3 and Cy5 at a DNA concentration of 100 μM (the lasing threshold curves are shown in Fig. S1). A strong lasing peak emerges at approximately 560 nm, corresponding to the laser emission from Cy3. In contrast, no lasing peak was observed in the emission band of Cy5 due to the insufficient energy transfer from Cy3 to Cy5.

Fig. 3 shows the evolution of lasing emission spectra for Cy3 and Cy5 when Mg^{2+} was varied. With the addition of Mg^{2+}, the electrostatic repulsion between the arms was suppressed and thus the distance between Cy3 and Cy5 decreased. The increased energy transfer between Cy3 and Cy5 led to emergence of Cy5 lasing

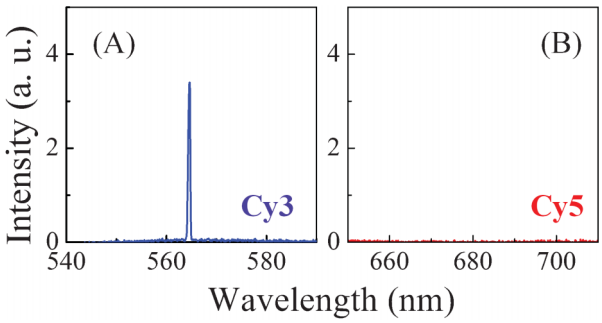

Fig. 2 Emission spectra of the OFRR laser in the absence of Mg^{2+} ions for Cy3 (A) and Cy5 (B). Excitation wavelength: 518 nm.

emission at approximately 680 nm (Fig. 3(A)). Meanwhile, due to the loss of excitation, the lasing intensity of Cy3 declined rapidly. When the concentration of Mg^{2+} reached 7.02 mM (Fig. 3(B)), the maximal folding of the Holliday junction was achieved, in which conformation the two arms formed a 60° angle.[19–21] The corresponding energy transfer efficiency was approximately 32%. Accordingly, the Cy5 lasing emission reached the highest, whereas the Cy3 lasing emission was completely quenched due to the large energy transfer efficiency.

To reverse the lasing wavelength, EDTA was added (Fig. 3(C)–(F)). Mg^{2+} ions react strongly with EDTA to form chelates, thus reducing free Mg^{2+} ions in the Holliday junction solution. In the presence of 1.74 mM of EDTA, the free Mg^{2+} concentration dropped to 5.22 mM. A significant reduction in Cy5 lasing intensity is observed due to the increased distance between Cy3 and Cy5, although the lasing emission from Cy3 is still completely quenched. With the further increase in the EDTA concentration, the lasing emission of Cy3 re-emerges whereas Cy5 lasing emission diminishes. When all the Mg^{2+} ions were removed, strong Cy3 lasing emission was restored with concomitant disappearance of Cy5 lasing, indicating that the Holliday junction fully returned

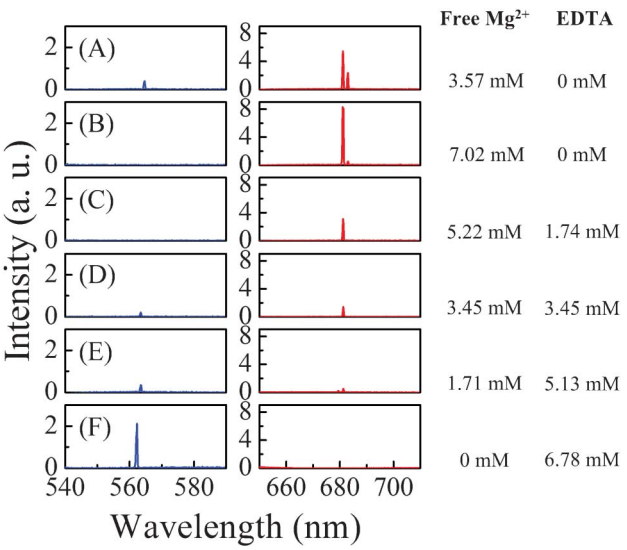

Fig. 3 Emission spectra of the OFRR laser at different concentrations of Mg^{2+} ions. Left column: Cy3. Right column: Cy5. From top to bottom, the concentration of free Mg^{2+} ions was increased to 7.02 mM first, and then gradually decreased to 0 mM after EDTA was added.

back to the original conformation. Note that in the above wavelength switching cycle only very small volumes of magnesium and EDTA solutions were added (about 7% in total volume; see the ESI for details†), which had little effect on the DNA (and hence Cy3/Cy5) concentration. In addition, the Holliday junctions can easily be separated from magnesium/EDTA solution by the ethanol precipitation method; thus the DNA samples can be collected and re-used in the bio-switchable laser.

In Fig. 4, we analyze the wavelength switching ratio, $\Gamma = (I_{Cy3} - I_{Cy5})/(I_{Cy3} + I_{Cy5})$, where I_{Cy3} and I_{Cy5} denote the emission intensity of Cy3 and Cy5, respectively. A ratio of 1 or −1 refers to a complete wavelength switching from Cy3 to Cy5 or *vice versa*. According to Fig. 4, complete wavelength switching can be accomplished with a change of only 5 mM of Mg^{2+}. Fig. 4 also plots Γ for conventional fluorescence of the same Holliday junction (see Fig. S2, ESI†). Conventional FRET using fluorescence has been routinely employed in characterizing conformational changes in a biomolecular structure, where $\gamma = I_{Acceptor}/I_{Donor}$ is usually used as the sensing signal (see Fig. S3, ESI†),[22] which is related to Γ by $\gamma = (1 - \Gamma)/(1 + \Gamma)$. Comparison of Γ between the laser and fluorescence shows that the laser signal is approximately 16 times more sensitive to the Mg^{2+} change (*i.e.*, $\Delta\Gamma/C$, where C is the Mg^{2+} concentration) and hence DNA conformation change than fluorescence, because any small change in fluorescence (*i.e.*, gain) can be significantly amplified through the optical feedback provided by the laser cavity.[23]

In conclusion, we have experimentally demonstrated a bio-switchable optofluidic laser based on the DNA Holliday junction by controlling the concentration of Mg^{2+} in the solution. Nearly 100% reversible wavelength switching was achieved. We also showed that the lasing signal is over one order of magnitude more sensitive to the DNA conformational change than the conventional fluorescence signal.

The bio-switchable laser reported here represents significant improvement over the previous optofluidic laser based on linear DNA scaffolds. Both laser gain media (*e.g.*, Cy3 and Cy5 in the current work) are co-located on the same DNA structure and the reversible wavelength switching can be achieved easily at room temperature by simply inducing DNA conformational changes. The gain media can be recycled by a simple DNA purification process. The continuous adjustability and 100% reversibility of the laser emission demonstrated here will impart a series of new functions to the optofluidic laser that can certainly be utilized in development of novel photonic devices. The bio-switchable laser can also be exploited in biosensing applications, and in characterization of DNA origami and nanomachines.[24–27] The Holliday junction laser provides a robust, simple, and well-studied model for us to investigate more complicated systems (such as protein–protein interactions, which are essential in many cellular functions and signaling).[22] Its large lasing intensity swing provides a much more sensitive tool than the conventional fluorescence method to study small conformational changes in DNA/protein structures triggered by environmental changes.[22] Finally, as all the materials used are bio-compatable, the bio-switchable optofluidic lasers may be realized in a cell, which is one step closer to intracellular sensing, cytometry and imaging.[28]

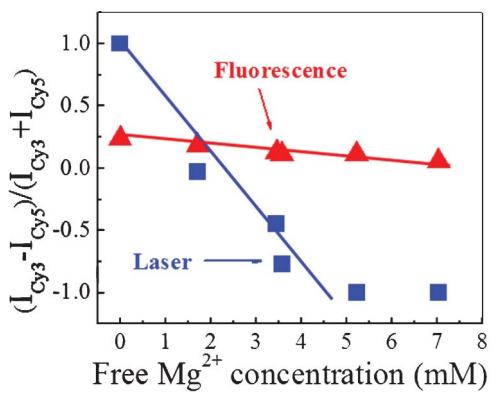

Fig. 4 Γ for both laser and fluorescence signal at various concentrations of free Mg^{2+} ions based on the spectra in Fig. 3 and Fig. S2. Solid lines are the linear fit for the linear part of response.

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