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Single-Molecule Four-Color FRET Visualizes Energy-Transfer Paths on DNA Origami

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Supporting Information

ABSTRACT: Fluorescence resonance energy transfer (FRET) represents a mechanism to transport light energy at the nanoscale, as exemplified by nature's light-harvesting complexes. Here we used DNA origami to arrange fluorophores that transport excited-state energy from an input dye to an output dye. We demonstrate that energy-transfer paths can be controlled on the single-molecule level by the presence of a "jumper" dye that directs the excited-state energy either to a red or to an IR output dye. We used single-molecule four-color FRET with alternating laser excitation to sort subpopulations and to visualize the control of energy transfer.

The size of photonic components such as fiber optic cables has hampered the development of light-based circuitry that has the potential to overcome the limitations of current electronic circuits. Today, surface plasmon-based photonics ("plasmonics") is considered a key technology to bring photonics to the nanoscale and to overcome the size compatibility problem of optics and electronics.[1] For plasmonic circuits, components such as wires, switches, and connectors are required. Since single emitters can be coupled to plasmonic devices and induce single plasmons,[2] molecular photonic devices such as wires and molecular switches might be included in nanoscale photonic circuitry and take over some of the desired functions. With respect to molecules, fluorescence resonance energy transfer (FRET) offers a means of energy transport that has led to the development of so-called photonic wires.[3−6] Furthermore, functions such as optical switching can be achieved within single molecules.[7,8]

In this Communication, we realize a combination of multistep energy transfer in a photonic wire-like structure using an energy-transfer cascade,[9,10] with the functionality of manipulating the energy-transfer path. We constructed a two-dimensional arrangement of fluorophores that allows for alternative energy-transfer pathways dependent on the incorporation of a "jumper" dye at specific positions. The construct uses two-dimensional DNA origami as a molecular breadboard that allows the precise and programmable arrangement of fluorophores in a grid-like fashion by self-assembly, similar to a circuit board for electronics (see Figure 1).[11] To create the rectangular origami, the ∼7.3k bases long single-stranded DNA genome of phage M13mp18 is folded by hybridization with ∼200 short synthetic DNA "staple" strands. Insertion of fluorophores is achieved using dye-modified staple strands that are incorporated during the self-assembly process (see Supporting Information for details on DNA sequences, modifications, and experimental methods).[11−13]

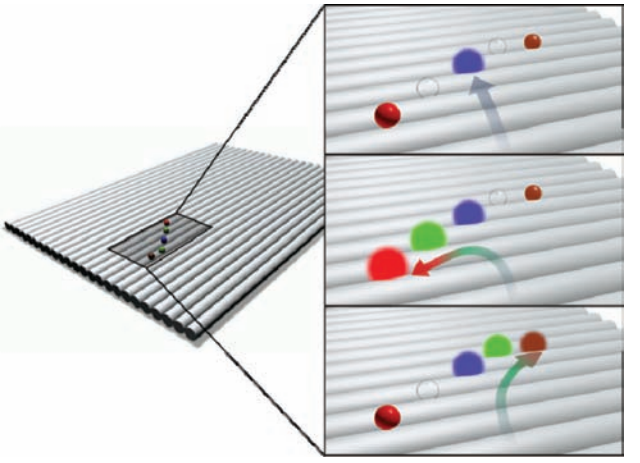

Figure 1. Arrangement of fluorophores on the DNA origami and visualization of alternative energy-transfer pathways with the "jumper" dye guiding the light from the blue input to either the red or the IR output.

On this grid we used a "blue" fluorophore (ATTO488) as the input dye. A "red" fluorophore (ATTO647N) and an "IR" fluorophore (Alexa 750) are used as alternative output dyes in this changeover switch-like arrangement (Figure 1). A "green" fluorophore (ATTO565) serves as the jumper dye that can be placed at two alternative locations, directing the excitation energy either to the red or to the IR dye (see Figure 1, magnified view). Fluorophores are spread over three helices to minimize fluorophore interactions through DNA,[14,15] and care was taken that fluorophores protrude from the same side of the DNA origami. Distances between input and output dyes are of the order of 9 nm to minimize direct FRET but to enable successive FRET with the aid of the jumper dye. We constructed origami samples without jumper dye, with jumper dye to direct the excitation energy to the red output dye, with jumper dye to direct the energy to the IR output dye, and with two jumper dyes.

To visualize the alternative energy-transfer paths, we advanced a previous four-color single-molecule FRET approach with alternating laser excitation.[4,9] This four-color setup allows studying the interaction between six energy-transfer pairs quasi-simultaneously,

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with the potential to measure six distances within a biomolecular complex, and represents an extension of recently developed multicolor schemes.[4,16−21] The setup is based on an inverted confocal microscope and uses the combination of a supercontinuum laser for free wavelength selection with acousto-optical filters (AOTFs) and beamsplitter (AOBS) for excitation alternation and optimized detection sensitivity on all channels. The problem of decreasing transmission of the AOBS at longer wavelengths is circumvented by separating the IR fluorescence with the aid of a dichroic mirror within the microscope (see Supporting Information for details of the setup). Alternating laser excitation enables us to directly probe the stoichiometry of these complex supramolecular constructs by subsequently exciting all four fluorophores.[22]

To obtain statistics on the origami constructs, we identified single-molecule bursts of molecules diffusing through the laser focus and analyzed them with respect to their stoichiometry and energy transfer. Four-color alternating laser excitation was used to probe the presence of the respective fluorophores and to select the subpopulation of interest exhibiting all incorporated dyes. The relatively long diffusion times of about 10 ms for the focus transit allowed collecting an average of ~500 photons per burst, with ~100 photons during blue laser excitation. The extensive information content of the collected data allows discerning the possible combinations of fluorophores for every burst. This is important to exclude incompletely labeled origami structures from FRET analysis. To further refine the burst selection, ES histograms (energy transfer plotted versus dye stoichiometry) were used to exclude molecules where bleaching occurs during diffusion through the laser focus, which is particularly important for the IR dye. Singly labeled origami samples were used to determine the correction factors for leakage (le) into acceptor channels and for direct excitation (dx) of acceptors (see Supporting Information and Figure S2 for details on subpopulation selection and analysis).

Interestingly, we found that the blue, green, and red dyes were incorporated almost quantitatively, with small "donor-only" populations that can supposedly be assigned to premature bleaching. These high incorporation yields demonstrate the reliability and high yield of correctly formed structures of the origami approach. On the other hand, large fractions of missing IR dyes of >50% were detected in all samples (see Figure S2). We assign this sample heterogeneity to an inactive acceptor fraction. Inactive populations are well known for cyanine dyes such as Cy5 (see, e.g., refs 9 and 23). Notably, Alexa 750 is the only cyanine derivative of the dyes used.

To quantify the energy transfer toward a given output (red or IR), FRET-related ratios E^* were calculated as a fraction of the photon counts in the respective output channel divided by the sum of counts in the input and output channels, all during input dye excitation (see eqs S12 and S13 in the Supporting Information). These FRET-related ratios were corrected for direct excitation and leakage of the dyes, to ensure that, e.g., leaking of the red dye into the IR channel does not yield a false positive IR signal. On the other hand, corrections for differing quantum yields or detection efficiencies of the dyes were not taken into account as would be necessary for absolute distance measurements. Figure 2 shows histograms of E^* for the energy transfer from blue to red (left panel) and from blue to IR (right panel) for all of the designed origami constructs. Without the jumper dye, energy transfer from the input to the output dyes is essentially zero, as shown in Figure 2a.

Upon insertion of the jumper dye between the blue and the red dyes, the FRET-related ratio E^*_{br} increases from 0 to 0.34 ± 0.12, indicating a two-step energy transfer from blue to red enabled by the green jumper dye (see Figures 1 and 2b). The mean FRET-related ratio between the blue and IR dyes is unchanged, and the histogram is slightly broadened due to reduced photon numbers in the donor detection channel and larger correction terms in the respective acceptor channel. Alternatively, the jumper dye can be inserted between the blue and IR dyes. This causes the energy transfer to increase from blue to IR from 0 to $E^*_{bir} = 0.25 \pm 0.13$. Again, the alternative energy-transfer path remains at a mean value of zero, with a broadened histogram due to shot noise (Figure 2c). In the case of two inserted jumper dyes, both FRET-related ratios increase from 0 to $E^*_{br} = 0.36 \pm 0.17$ and $E^*_{bir} = 0.27 \pm 0.18$, respectively.

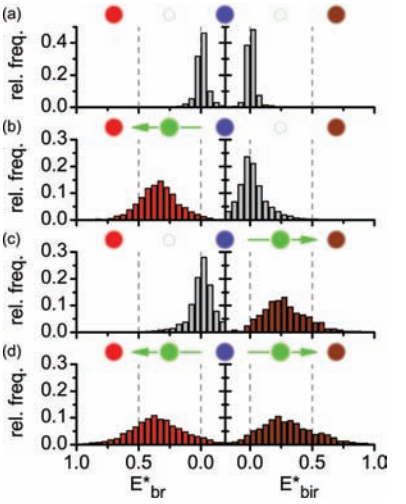

Figure 2. FRET-related ratios from blue to red, E^*_{br}, and from blue to IR, E^*_{bir}, for the four different origami samples. For each of the graphs, the colored spheres indicate the presence of the respective fluorophores on the DNA origami, and the white sphere marks the absence of the green jumper dye.

To quantify the reliability of the readout of the changeover switch, we characterized the output ratio, defined as the number of photons in the red output channel divided by the number of photons in the red and IR output channels, all during blue excitation and corrected for direct excitation and leakage (see Figure 3 and eq S17 in the Supporting Information). For the origami sample with the green jumper dye between the blue and red dyes, the histogram of the output ratio is centered at 1, indicating that photons are mainly emitted at the red output. For the sample with the jumper dye guiding the light toward the IR dye, the histogram of the output ratio is centered at 0, showing that photons are mainly emitted at the IR output. Most notably, there is almost negligible overlap between these two histograms, which are colored red and brown, respectively, in Figure 3. For an average of 82−122 collected photons during blue excitation for the different samples and a threshold of 10 photons for the sum of the output channels, the probability for correct assignment of the jumper position is >98.5%. For the origami sample with two green jumper dyes, the output ratio assumes an intermediate value of ~0.5, since light is emitted equally at both of the outputs.

In summary, our data clearly show that the energy-transfer path depends on the position of the jumper dye on the origami construct. The jumper dye determines the direction of the energy transfer, and different output dyes provide an easily read output signal. DNA origami might hence serve as a circuit board for photonic devices beyond the diffraction limit down to a molecular

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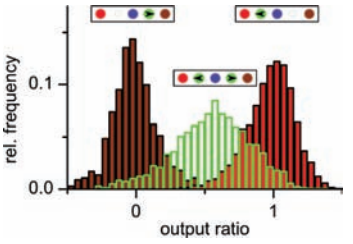

Figure 3. Histograms of the output ratios from single molecules. The number of photons in the red output channel divided by the number of photons in the red and IR output channels (all during blue excitation) are plotted for the three origami samples with jumper dyes. The fluorophore arrangement is indicated schematically above each of the histograms. Values exceeding the range of 0−1 are due to correction factors (see eq S17 in the Supporting Information).

scale. DNA nanostructures additionally offer ways for integration with other photonic structures, such as nanoscale plasmonic waveguides, since metallic nanoparticles and rods are readily modified with DNA.[24,25] The concept can also be extended to three dimensions.[26] Replacing the jumper strand by sensor strands[27] that, for example, report on binding opens interesting possibilities to exploit the control of energy-transfer paths for nanoscale sensors or molecular computing. Finally, single-molecule four-color FRET with alternating laser excitation has proven valuable to disentangle biomolecular complexes of increasing complexity.

■ ASSOCIATED CONTENT

Supporting Information. Experimental methods, data analysis, and DNA sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

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