

Supplementary information

Controlling the dynamics of Förster resonance energy transfer inside a tunable sub-wavelength Fabry-Perot-resonator

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Experimental setup

The microresonator design and the confocal microscope with optical path for excitation and detection is outlined in Figure 1). Due to fast bleaching (1-5 seconds with $20\mu\text{W}$ excitation) of the low concentrated sample (bare donor and FRET-pair), we acquired histograms for more than thousand different diffraction limited spots in free space and in the resonator. Additionally on each location of the resonator a white light transmission spectrum of the Fabry-Perot resonator was acquired in order to determine for each decay curve the on-axis resonance wavelength λ_{res} . The histograms of

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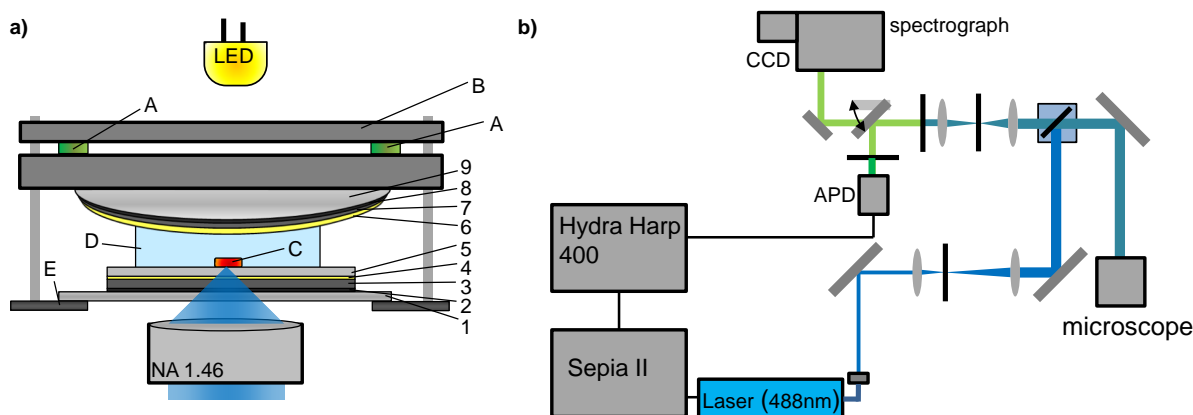


Figure 1: *Scheme of the experimental setup. a) Drawing of the resonator design. The mirrors consist of the following layers: 1) glass cover slide; 2) 1 nm chromium; 3) 40 nm silver; 4) 1 nm gold; 5) 80 nm SiO₂; 6) 1 nm gold; 7) 60 nm silver; 8) 1 nm chromium; 9) glass lens (f=150 mm) hence, the mirror surface can be considered to be parallel within the investigated sample section being less than 100 μm in diameter. The cavity length is tuned by the piezoelectric stacks (A) implemented in a mirror mount (B). The sample (C) is placed about half way between the mirrors on the bottom mirror, which is immersed in the intra-cavity medium (D) (water). The bottom mirror is fixed on a plate (E) which is mounted on a three-axis feed-back controlled scanning stage. b) Scheme of the optical path: The excitation source, a pulsed 488 nm laser diode, is triggered by the Sepia II module which is synchronized with the counter module Hydra Harp 400. The beam is enlarged by a telecentric system and guided by a beamsplitter into the confocal microscope. For excitation and fluorescence collection the same objective (NA=1.46) is used. The fluorescence is deflected through a pinhole and a longpass filter on the detectors, an APD and a spectrograph with equipped thermoelectrically cooled CCD-camera.*

the bare donor show a high signal-to-noise ratio of around 100 while histograms of the donor in the FRET pair show a roughly 20 times lowered signal-to-noise ratio due to the lowered quantum yield of the donor and the bandpass filter in front of the APD additionally reducing the amount of detected donor photons by about 50%.

Sample

As stated in the main article, the thermodynamics and kinetics of the hybridization process of two DNA-strands may lead to false base sequences or incomplete reactions. Hence for ensemble measurements the determining of transfer rates is hampered by averaging over the lifetimes of bare donors and donors with various acceptor distances. To ensure that every measured fluorescence

photon originates from a similarly configured FRET-pair we used a sample design with both chromophores labeled at the same DNA-strand. A second chromophore-less DNA-strand is hybridized to the chromophore labeled DNA-strand to stabilize its conformation. The FRET-pair consisting of Atto488 and Atto590 with the DNA-base sequence is displayed in Figure 2. The hybridized pairs are diluted in Tris-buffer (with $1e-7$ mol/L) and were embedded in a thin PVA film (aqueous 4%PVA solution) by spin-coating (5 min, 6000 rpm) on the substrates. The samples were used after evaporation of the solvent having a concentration of around $10e-9$ mol/L.

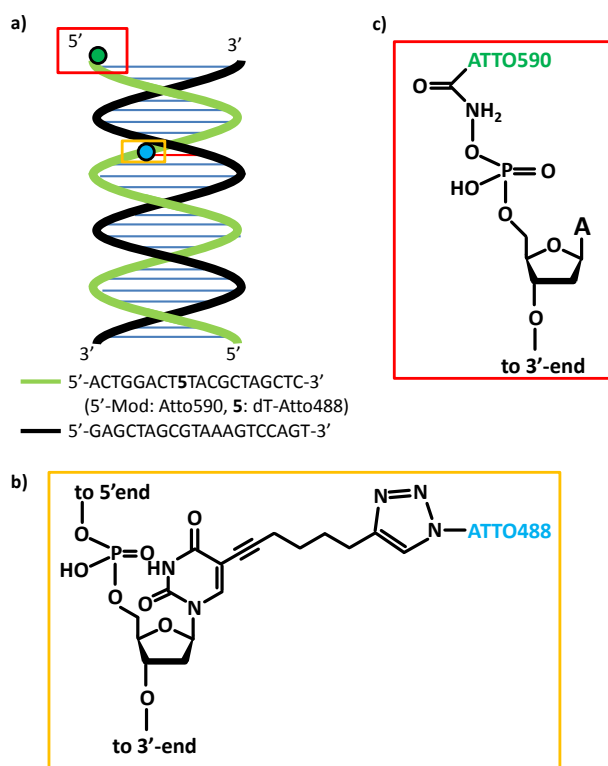


Figure 2: Scheme of the FRET pair. a) Schematic drawing of the hybridized DNA-double strand. The green string is labeled at base number 8 with the fluorophore Atto488 and at the 5'-end with the fluorophore Atto590. The black strand was chosen to contain the complementary base sequence to guaranty conformational stability with the dye-labeled green strand. b) Structure of the binding linker between the base thymine and the fluorophore Atto488. c) Structure of the binding linker between the 5'-end of the green string in a) and the fluorophore Atto590.