Plasmon-assisted Förster resonance energy transfer at the single-molecule level in the moderate quenching regime

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1. Spectra

An overview of all spectra, including scattering and absorption of the monomer nanoparticle and absorption and emission of the FRET pair are shown in figure S1. The data for the nanoparticles are computed with the Mie Theory Calculator from Nanocomposix and the dye spectra are from the Atto tec website.

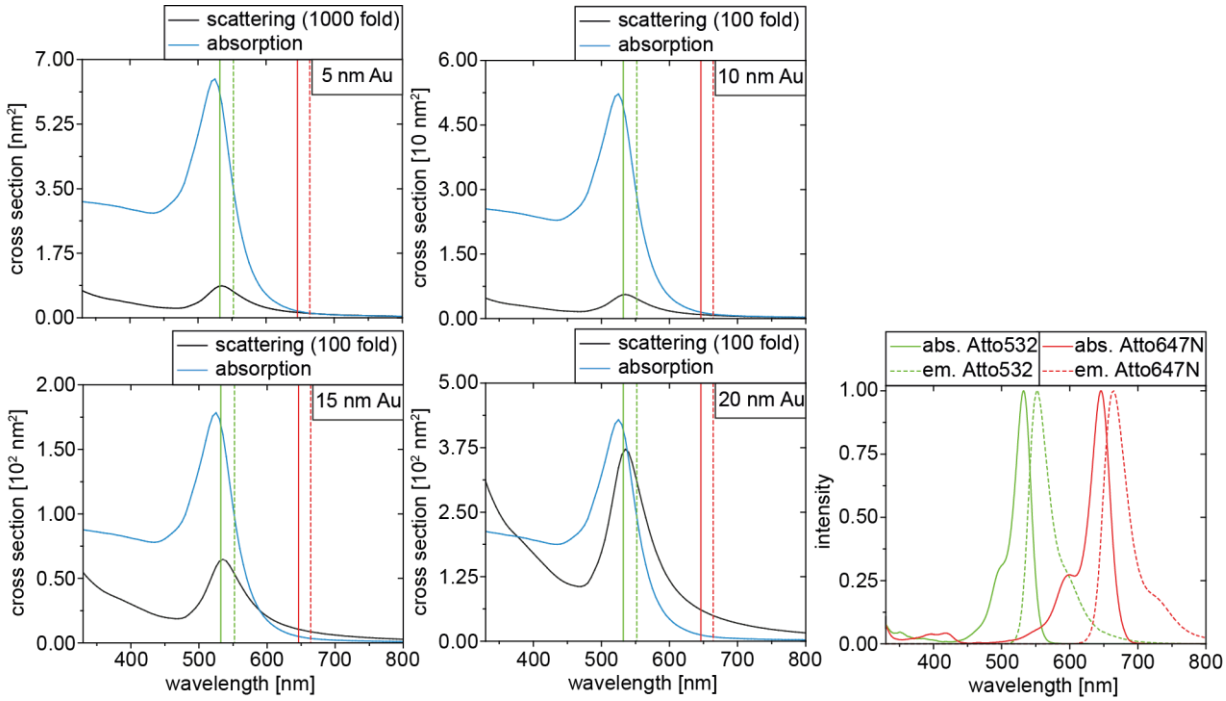

Figure S1: Scattering (black) and absorption spectra (blue) of the employed nanoparticles with the absorption (continuous) and emission maxima (dashed) of the Atto532 (green) and Atto647N (red). In addition, the whole spectra of the FRET pair is diagrammed.

2. Raw Data

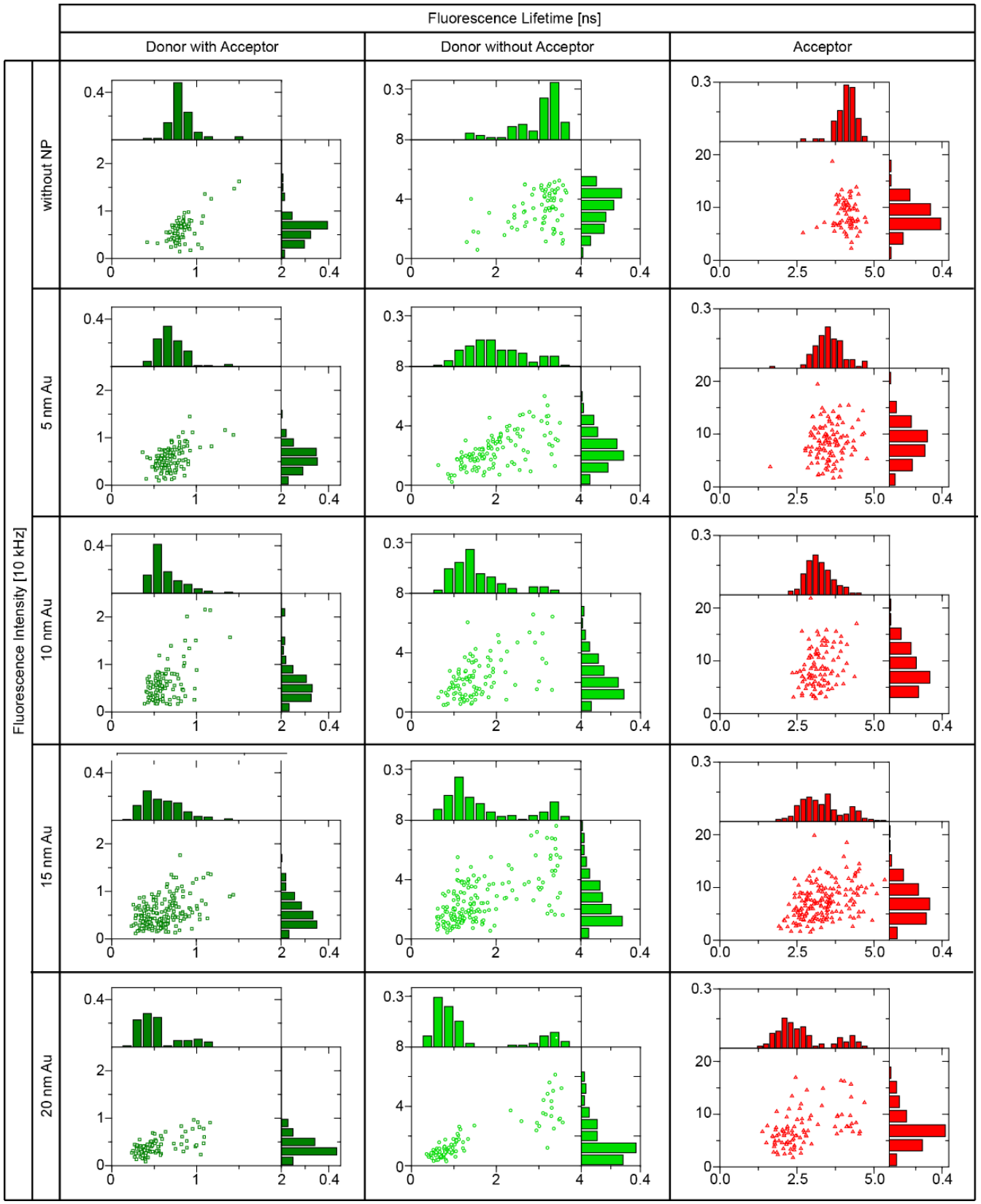

Figure S2: Raw data of the fluorescence lifetime and intensity of all three channels (donor in the presence of the acceptor and after photobleaching of the acceptor and acceptor only) from the measured with and without nanoparticle.

3. Distance calculation between dyes and nanoparticle surface

For the distance between dyes and nanoparticle a, the centroid (S) of the fictive triangle between all possible capturing strands (P_1, P_2, P_3) has to be calculated (see Figure S3).

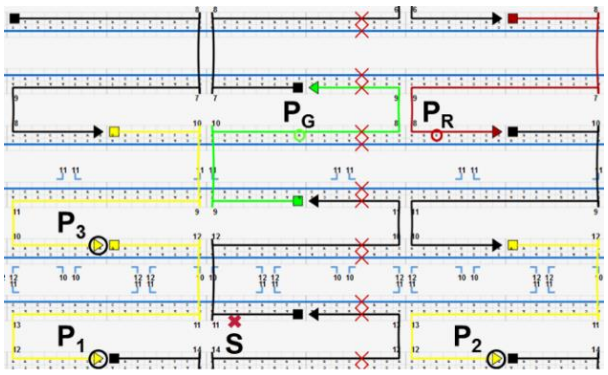

Figure S3: Section from the caDNano images with positions of Atto647N (PR), Atto532 (PG), all capturing strands (P1, P2, P3) and centroid of the capturing strands (S).

With the equations (S1) and coordinates (see table S 1) the centroid S (x_S, y_S) can be calculated.

$$x_S = \frac{x_{P_1} + x_{P_2} + x_{P_3}}{3};\ y_S = \frac{y_{P_1} + y_{P_2} + y_{P_3}}{3} \qquad (1)$$

Table S1: coordinates of Atto647N (P_R), Atto532 (P_G), all capturing strands (P_1, P_2, P_3) and centroid of the capturing strands (S) (the n in the index Indicates a Position, e.g. x_{P_1} stands for the x coordinate of P_1.

	P_1	P_2	P_3	S	P_R	P_G
Helix (x_n)	13	13	11	12.3	9	9
Base (y_n)	63	94	63	73.3	89	79

Distances between S and P_R or P_G is calculated by the Pythagoras´ theorem (eq. S2, F indicates the different dyes) with the distance between two oligonucleotides o (0.34 nm), the diameter of a helix d (2 nm) and the crossover between two helix c (1 nm).

$$d_F = \sqrt{((x_S - x_{P_F}) \cdot d + 3c)^2 + ((y_S - y_{P_F}) \cdot o)^2} \quad (S2)$$

The distances are 10.98 nm for S-P_R (d_R) and 9.79 nm for S-P_G (d_G). The height difference, h, is the sum of linker between dye and DNA origami structure (0.5 nm), the diameter of the DNA origami structure (2 nm), the crossover between DNA origami structure and formed linking helix (1 nm), the diameter of the linking helix (2 nm) and linker between linking helix and NP (0.5 nm), so overall 6 nm. By using the Pythagoras´ theorem a second time and subtract the radius r of the NP, a is calculated by Equation (S3).

$$a_{F,r} = \sqrt{((h + r)^2 + {d_F}^2)} - r \qquad (S3)$$

The overall distances are shown in table S2.

Table S2: Distances calulations between NP surfaces and both dyes (Atto647N and Atto532).

r [nm]	$a_{G,r}$ [nm]	$A_{R,r}$ [nm]
5	10.8	11.7
10	10.0	10.8
15	9.4	10.1
20	9.0	9.6

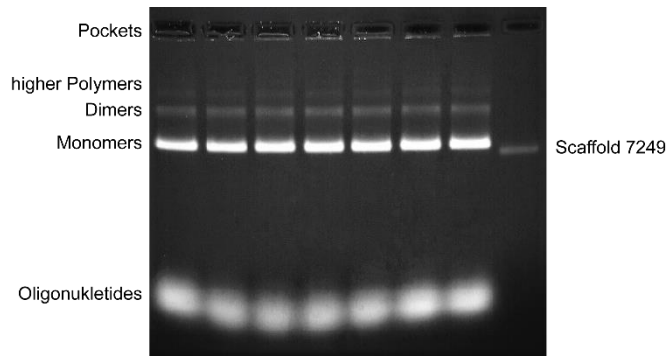

Figure S4: Gel images for the purified rectangular DNA origami structures with monomers, polymers, oligonucleotide and the scaffold as a reference.

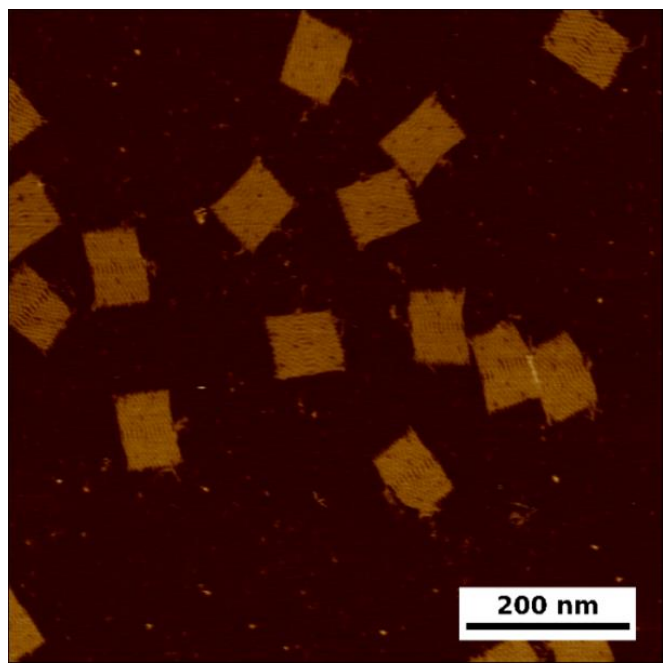

Figure S5: 800×800 µm images of the rectangular DNA origami structure. The holes in the edges and on the left and right side from sprout like center are showing the eight missing oligonucleotides from biotin.

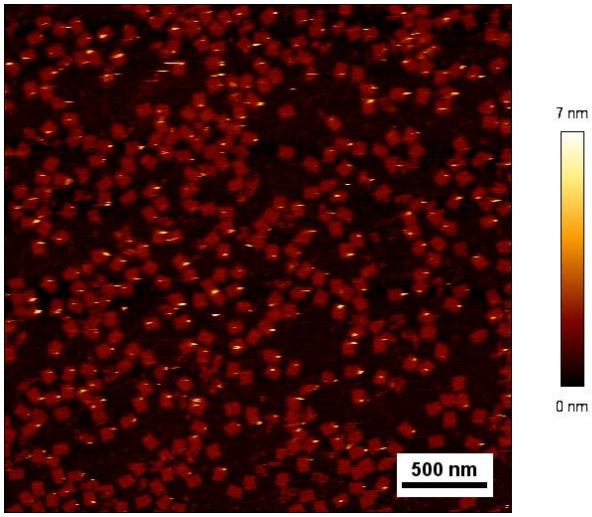

Figure S6: Rectangular DNA origami structure with 5 nm gold nanoparticle with a scale bar ranging from 0 to 7 nm. This DNA origami structure has an height with NP of 2 nm (one helix).

Table S3: Volume of the oligonucleotides with a thiol group at the 3` for nanoparticle with different sizes and materials.

d [nm]	5 Au	10 Au	15 Au	20 Au
V [µL/mL]	95.4	49.5	31.7	24

Table S4: Salting steps.

Step	1	2	3	4	5	6	7
V [µL]	10	10	20	20	20	20	50
Step	8	9	10	11	12	13	
V [µL]	50	50	50	100	100	100	

4. Design of DNA origami structure

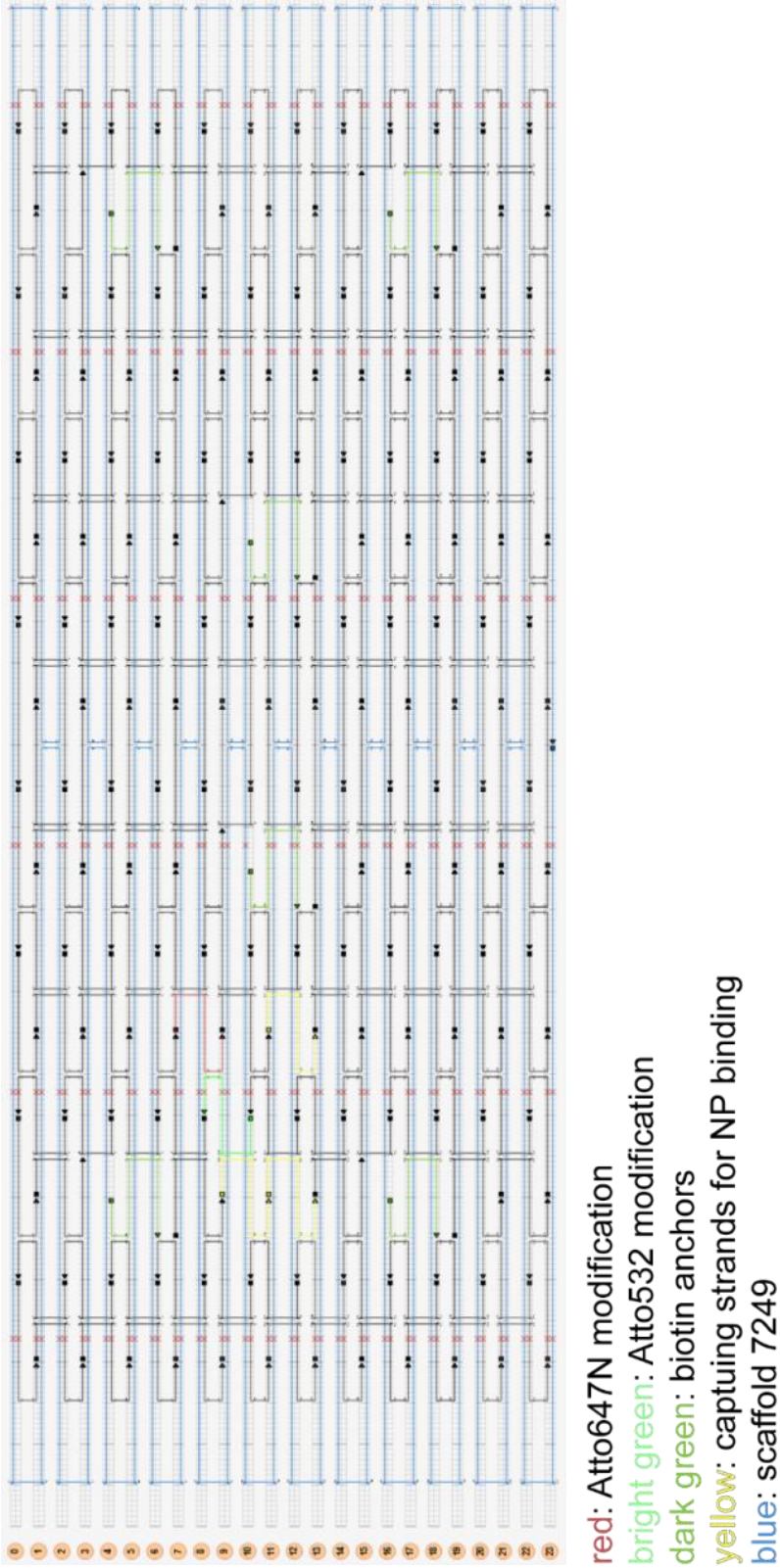

Figure S7: caDNAno image of rectangular DNA origami structure.

Tab. S 5. sequences of unmodified staples.

Sequence (5'->3')	Length [nt]
TGACAACTCGCTGAGGCTTGCATTATACCA	30
AGAAAACAAAGAAGATGATGAAACAGGCTGCG	32
CTGTAGCTTGACTATTATAGTCAGTTCATTGA	32
TATATTTTGTCATTGCCTGAGAGTGGAAGATTGTATAAGC	40
CTTTAGGGCCTGCAACAGTGCCAATACGTG	30
TTAATGAACTAGAGGATCCCCGGGGGGTAACG	32
TCATCGCCAACAAAGTACAACGGACGCCAGCA	32
TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC	32
CTACCATAGTTTGAGTAACATTTAAAATAT	30
CGAAAGACTTTGATAAGAGGTCATATTTCGCA	32
ATTTTAAAATCAAAATTATTTGCACGGATTCG	32
GCGAAAAATCCCTTATAAATCAAGCCGGCG	30
CTGTGTGATTGCGTTGCGCTCACTAGAGTTGC	32
AGCGCGATGATAAATTGTGTCGTGACGAGA	30
GATGGTTTGAACGAGTAGTAAATTTACCATTA	32
GATGTGCTTCAGGAAGATCGCACAATGTGA	30
TAAATCAAAATAATTCGCGTCTCGGAAACC	30
GACAAAAGGTAAAGTAATCGCCATATTTAACAAAACTTTT	40
CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA	32
CTTATCATTCCCGACTTGCGGGAGCCTAATTT	32
CAGAAGATTAGATAATACATTTGTCGACAA	30
CGTAAAACAGAAATAAAAATCCTTTGCCCGAAAGATTAGA	40
AATACTGCCCAAAAGGAATTACGTGGCTCA	30
ATATTCGGAACCATCGCCCACGCAGAGAAGGA	32
ATACATACCGAGGAAACGCAATAAGAAGCGCATTAGACGG	40
CATCAAGTAAAACGAACTAACGAGTTGAGA	30
TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG	32
AATAGTAAACACTATCATAACCCTCATTGTGA	32
GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA	32
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CTCGTATTAGAAATTGCGTAGATACAGTAC	30
ATTACCTTTGAATAAGGCTTGCCCAAATCCGC	32
GCCGTCAAAAAACAGAGGTGAGGCCTATTAGT	32
AGTATAAAGTTCAGCTAATGCAGATGTCTTTC	32
TGTAGCCATTAAAATTCGCATTAAATGCCGGA	32
CAGCGAAACTTGCTTTCGAGGTGTTGCTAA	30
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GCGGATAACCTATTATTCTGAAACAGACGATT	32
AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC	32
TTAAAGCCAGAGCCGCCACCCTCGACAGAA	30
TTCCAGTCGTAATCATGGTCATAAAAGGGG	30
CACAACAGGTGCCTAATGAGTGCCCAGCAG	30
TCAAGTTTCATTAAAGGTGAATATAAAAGA	30
GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC	32
CCACCCTCTATTCACAAACAAATACCTGCCTA	32
TCAAATATAACCTCCGGCTTAGGTAACAATTT	32
AAAGGCCGGAGACAGCTAGCTGATAAATTAATTTTTGT	38
CTGAGCAAAAATTAATTACATTTTGGGTTA	30
GCGGAACATCTGAATAATGGAAGGTACAAAAT	32
CACCAGAAAGGTTGAGGCAGGTCATGAAAG	30

Sequence (5‘->3‘)	Length [nt]
GAAATTATTGCCTTTAGCGTCAGACCGGAACC	32
GAATTTATTTAATGGTTTGAAATATTCTTACC	32
GTACCGCAATTCTAAGAACGCGAGTATTATTT	32
GTTTATCAATATGCGTTATACAAACCGACCGTGTGATAAA	40
CAACTGTTGCGCCATTCGCCATTCAAACATCA	32
AAAGTCACAAAATAAACAGCCAGCGTTTTA	30
CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCG	40
GTAATAAGTTAGGCAGAGGCATTTATGATATT	32
ATTATACTAAGAAACCACCAGAAGTCAACAGT	32
GAGGGTAGGATTCAAAAGGGTGAGACATCCAA	32
AAGGAAACATAAAGGTGGCAACATTATCACCG	32
TTTTATTTAAGCAAATCAGATATTTTTTGT	30
TAGGTAAACTATTTTTGAGAGATCAAACGTTA	32
ACAAACGGAAAAGCCCCAAAAACACTGGAGCA	32
ATACCCAACAGTATGTTAGCAAATTAGAGC	30
ACCGATTGTCGGCATTTTCGGTCATAATCA	30
CATAAATCTTTGAATACCAAGTGTTAGAAC	30
TATAACTAACAAAGAACGCGAGAACGCCAA	30
ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT	32
TTAGGATTGGCTGAGACTCCTCAATAACCGAT	32
AATTGAGAATTCTGTCCAGACGACTAAACCAA	32
AATAGCTATCAATAGAAAATTCAACATTCA	30
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ATATTTTGGCTTTCATCAACATTATCCAGCCA	32
AGGCTCCAGAGGCTTTGAGGACACGGGTAA	30
GCAAGGCCTCACCAGTAGCACCATGGGCTTGA	32
TTAACACCAGCACTAACAACTAATCGTTATTA	32
GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA	32
TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGA	40
TTGACAGGCCACCACCAGAGCCGCGATTTGTA	32
AGACGACAAAGAAGTTTTGCCATAATTCGAGCTTCAA	37
CGATAGCATTGAGCCATTTGGGAACGTAGAAA	32
ACACTCATCCATGTTACTTAGCCGAAAGCTGC	32
TGGAACAACCGCCTGGCCCTGAGGCCCGCT	30
TTATACCACCAAATCAACGTAACGAACGAG	30
TAATCAGCGGATTGACCGTAATCGTAACCG	30
CGCGCAGATTACCTTTTTTAATGGGAGAGACT	32
GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCA	40
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TGAAAGGAGCAAATGAAAAATCTAGAGATAGA	32
CCTGATTGCAATATATGTGAGTGATCAATAGT	32
CTTAGATTTAAGGCGTTAAATAAAGCCTGT	30
AAGTAAGCAGACACCACGGAATAATATTGACG	32
TTATTACGAAGAACTGGCATGATTGCGAGAGG	32
GGCCTTGAAGAGCCACCACCCTCAGAAACCAT	32
GCCATCAAGCTCATTTTTTAACCACAAATCCA	32
TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT	32
TTAACGTCTAACATAAAAACAGGTAACGGA	30
AGGCAAAGGGAAGGGCGATCGGCAATTCCA	30
ATCCCAATGAGAATTAACTGAACAGTTACCAG	32
AAAGCACTAAATCGGAACCCTAATCCAGTT	30

Sequence (5‘->3‘)	Length [nt]
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TCATTCAGATGCGATTTTAAGAACAGGCATAG	32
GCGAACCTCCAAGAACGGGTATGACAATAA	30
TAAATGAATTTTCTGTATGGGATTAATTTCTT	32
TCACCGACGCACCGTAATCAGTAGCAGAACCG	32
CATTTGAAGGCGAATTATTCATTTTTGTTTGG	32
ACAACATGCCAACGCTCAACAGTCTTCTGA	30
TCACCAGTACAAACTACAACGCCTAGTACCAG	32
GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT	32
GCGCAGACAAGAGGCAAAAGAATCCCTCAG	30
ATTATCATTCAATATAATCCTGACAATTAC	30
AAACAGCTTTTTGCGGGATCGTCAACACTAAA	32
ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG	32
GTATAGCAAACAGTTAATGCCCAATCCTCA	30
AAGGCCGCTGATACCGATAGTTGCGACGTTAG	32
CCTAAATCAAAATCATAGGTCTAAACAGTA	30
CTTTTGCAGATAAAAACCAAAATAAAGACTCC	32
CTTTTACAAAATCGTCGCTATTAGCGATAG	30
CATGTAATAGAATATAAAGTACCAAGCCGT	30
GACCAACTAATGCCACTACGAAGGGGGTAGCA	32
CAGCAAAAGGAAACGTCACCAATGAGCCGC	30
TAAATCGGGATTCCCAATTCTGCGATATAATG	32
AACGCAAAGATAGCCGAACAAACCCTGAAC	30
TAAATCATATAACCTGTTTAGCTAACCTTTAA	32
ATCGCAAGTATGTAAATGCTGATGATAGGAAC	32
AGCCAGCAATTGAGGAAGGTTATCATCATTTT	32
GCCCTTCAGAGTCCACTATTAAAGGGTGCCGT	32
GCTATCAGAAATGCAATGCCTGAATTAGCA	30
GCGAGTAAAAATATTTAAATTGTTACAAAG	30
TATTAAGAAGCGGGGTTTTGCTCGTAGCAT	30
AATACGTTTGAAAGAGGACAGACTGACCTT	30
AAATTAAGTTGACCATTAGATACTTTTGCG	30
TGCATCTTTCCCAGTCACGACGGCCTGCAG	30
TACGTTAAAGTAATCTTGACAAGAACCGAACT	32
ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG	40
CCCGATTTAGAGCTTGACGGGGAAAAAGAATA	32
ACCTTTTTATTTTAGTTAATTTCATAGGGCTT	32
CACATTAAAATTGTTATCCGCTCATGCGGGCC	32
GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT	32
ACAACTTTCAACAGTTTCAGCGGATGTATCGG	32
CTTTAATGCGCGAACTGATAGCCCCACCAG	30
GCACAGACAATATTTTTGAATGGGGTCAGTA	31
AGAAAGGAACAACTAAAGGAATTCAAAAAAA	31
AACAGTTTTGTACCAAAAACATTTTATTTC	30
AGGAACCCATGTACCGTAACACTTGATATAA	31
CCAACAGGAGCGAACCAGACCGGAGCCTTTAC	32
AACGCAAAATCGATGAACGGTACCGGTTGA	30
CAACCGTTTCAAATCACCATCAATTCGAGCCA	32
TTCTACTACGCGAGCTGAAAAGGTTACCGCGC	32
GCCTTAAACCAATCAATAATCGGCACGCGCCT	32
GCCCGTATCCGGAATAGGTGTATCAGCCCAAT	32

Sequence (5‘->3‘)	Length [nt]
TCCACAGACAGCCCTCATAGTTAGCGTAACGA	32
TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA	31
AACAAGAGGGATAAAAATTTTTAGCATAAAGC	32
AGAGAGAAAAAAATGAAAATAGCAAGCAAACT	32
TCAATATCGAACCTCAAATATCAATTCCGAAA	32
CCACCCTCATTTTCAGGGATAGCAACCGTACT	32
GTCGACTTCGGCCAACGCGCGGGGTTTTTC	30
GTTTTAACTTAGTACCGCCACCCAGAGCCA	30
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GCAATTCACATATTCCTGATTATCAAAGTGTA	32
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AAGCCTGGTACGAGCCGGAAGCATAGATGATG	32
AACGTGGCGAGAAAGGAAGGGAAACCAGTAA	31
CCAATAGCTCATCGTAGGAATCATGGCATCAA	32
ACGCTAACACCCACAAGAATTGAAAATAGC	30
TGTAGAAATCAAGATTAGTTGCTCTTACCA	30
CAAATCAAGTTTTTTGGGGTCGAAACGTGGA	31
TCGGCAAATCCTGTTTGATGGTGGACCCTCAA	32
TTTTCACTCAAAGGGCGAAAAACCATCACC	30
CTCCAACGCAGTGAGACGGGCAACCAGCTGCA	32
TTTACCCCAACATGTTTTAAATTTCCATAT	30
GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAA	32
TTTAGGACAAATGCTTTAAACAATCAGGTC	30

Tab. S 6: Modified staples with dyes, biotin and capturing strands for NP.

Sequence (5‘->3‘)	Length [nt]
TAAGAGCAAATGTTTAGACTGGATAG-Atto647N-AAGCC	32
GATGGCTTATCAAAA-Atto532-GATTAAGAGCGTCC	30
Biotin-CGGATTCTGACGACAGTATCGGCCGCAAGGCGATTAAGTT	40
Biotin-AGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAA	40
Biotin-ATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAA	40
Biotin-GAGAAGAGATAACCTTGCTTCTGTTCGGGAGAAACAATAA	40
Biotin-TAGAGAGTTATTTTCATTTGGGGATAGTAGTAGCATTA	38
Biotin-GAAACGATAGAAGGCTTATCCGGTCTCATCGAGAACAAGC	40
AATGGTCAACAGGCAAGGCAAAGAGTAATGTGAAAAAAAAAAAAAAAAAAAA	52
GATTTAGTCAATAAAGCCTCAGAGAACCCTCAAAAAAAAAAAAAAAAAAAAA	52
CGGATTGCAGAGCTTAATTGCTGAAACGAGTAAAAAAAAAAAAAAAAAAAAA	52

Oligonucleotide sequence for nanoparticle from 5’ to 3’:
TTTTTTTTTTTTTTTTTTTTTTTTT-Thiol

5. Numerical calculations

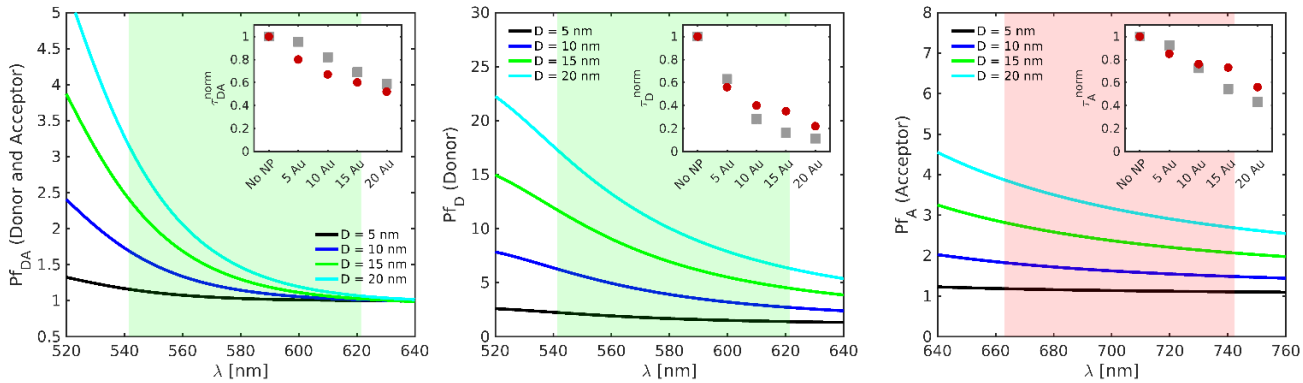

Figure S8: Numerical Purcell factor, Pf, spectra for the donor in presence (left) and absence (center) of the acceptor, and for the acceptor in isolation (right). Calculations for the four Au NP sizes considered in the experiments are shown (D indicates the NP diameter). The insets show normalized lifetimes calculated from the spectral averaging (taken within the colored range in the main panels) of the Pf spectra and using Equation (4). Experimental and theoretical results are plotted in red circles and grey squares, respectively.

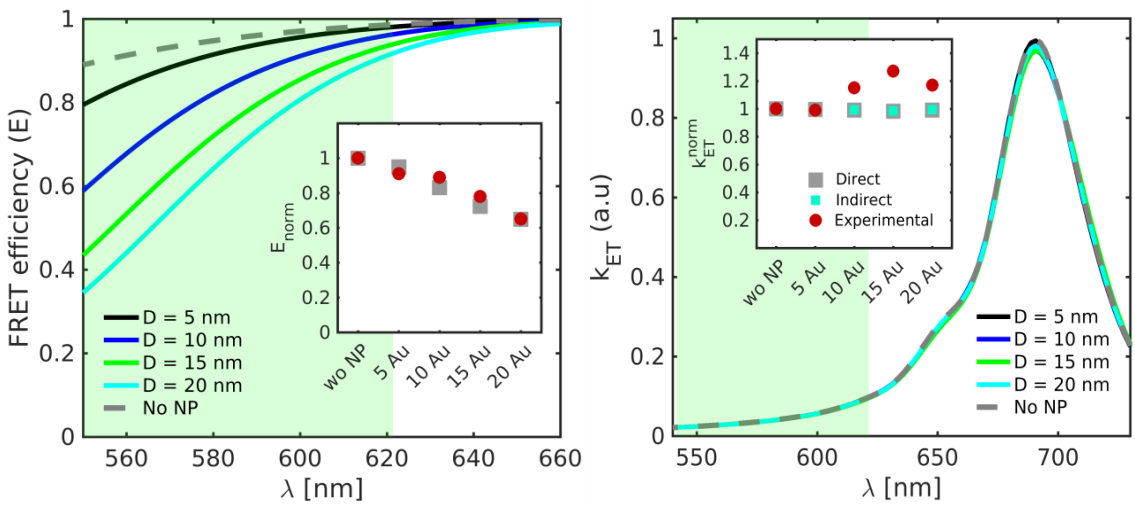

Figure S9: Theoretical predictions for the FRET efficiency and rate. Right: $E = 1 - Pf_D/Pf_{DA}$ (note the equivalence with Equation (3)) as a function of the donor emission wavelength. The inset (grey squares) plots the efficiency obtained from the spectral averaging within the green window. Left: $k_{ET} \propto V^{-1} \int |E_{DA}|^2 dV$ as a function of the donor emission wavelength. The inset (grey squares) shows the rate obtained from the spectral averaging within the green window. For comparison, the indirect prediction obtained from the evaluation of Equation (2) with numerical results in the insets of Figure S8 is shown in cyan squares. In both panels, red circles correspond to experimental data.