Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

FRET Enhancement close to Gold Nanoparticle Positioned in DNA Origami Constructs

N. Aissaoui,[a] K. Moth-Poulsen,[a] M. Käll,[b] P. Johansson,[c] L. M. Wilhelmsson,[a] B. Albinsson*[a]

[a]Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, Sweden

[b]Department of Applied Physics, Chalmers University of Technology, Gothenburg, Sweden

[c]School of Science and Technology, Örebro University, Örebro, Sweden

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1. Design of modified DNA origami

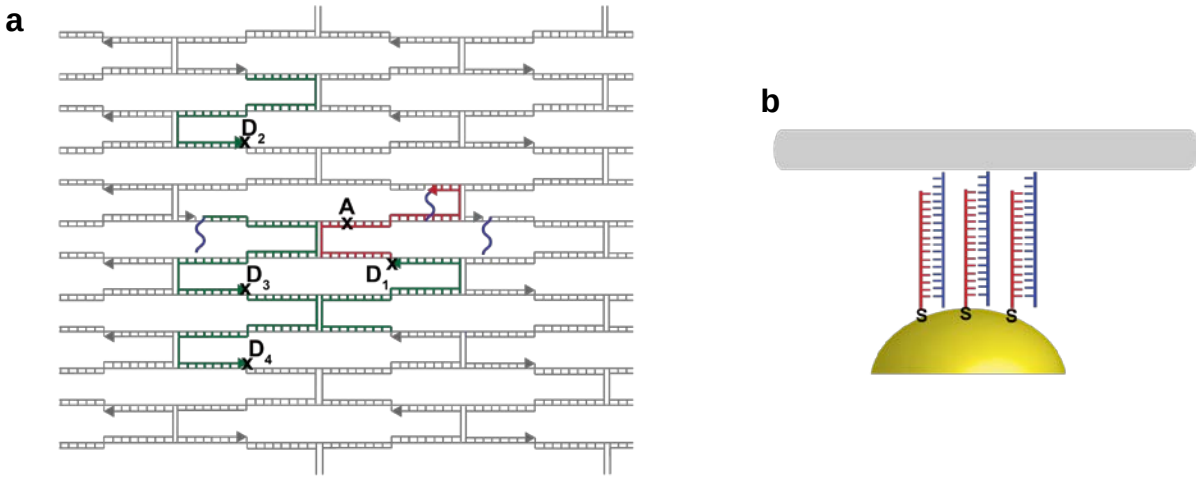


Fig. S1(a) Part of the rectangular DNA origami design, showing internal features with staple strands. The modified staples strands are marked in green or red for donor or acceptor, respectively. The exact position of the fluorophore is marked by a cross. (a) and (b) Three same blue single-stranded 15 bases long are extended from the DNA origami and will fix one AuNP. (b) AuNP functionalized with thiol-modified DNA strands, complementary to the extended 3 capture strands will hybridize to the DNA origami structure.

2. Details of the modified sequences

Modified strands for AuNPs

The unmodified staple strand sequences are from Rothemund's original design.[1]The modified strand sequences given bellow are designed to capture AuNPs bysplitting and merging the region marked bellow in green (5 bases at 3' end) of two adjacent DNA strands: r-3t12f to r-

3t14f, r-5t12e to r-5t14e, and r-5t12f to r-5t14f . The resulting 5’ end is then shifted by half-turn (5 bases) compared to the unmodified strands. The capture is therefore located on the opposite side of the origami compared to the fluorophores locations. 2 thymine bases serving as spacer region are added on the 5’ end and the strand is extended by the 15 capture bases on that end.

Unmodified DNA strands

r-3t12f : 5’-CGAGTAGAACTAATAGTAGTAGCAAAC**CCTCA**-3’
r-3t14f : 5’-TATATTTTAGCTGATAAATTAATGTTGTATAA-3’

r-5t12e : 5’-CAATAAATACAGTTGATTCCCAATTTAGAGAG-3’
r-5t14e : 5’-GGTAGCTAGGATAAAAATTTTTAGTTA**ACATC**-3’

r-5t12f : 5’-TCCATATACATACAGGCAAGGCAACTT**TATTT**-3’
r-5t14f : 5’-CAACGCAATTTTTGAGAGATCTACTGATAATC-3

Modified DNA strands

r-3t12f : 5’-CGAGTAGAACTAATAGTAGTAGCAAAC-3’
r-3t14f : 5’-TGACCAATTGACCGATTCCTCATATATTTTAGCTGATAAATTAATGTTGTATAA-3’

r-5t14e : 5’-GGTAGCTAGGATAAAAATTTTTAGTTA-3’
r-5t12e: 5’-TGACCAATTGACCGATTACATCCAATAAATACAGTTGATTCCCAATTTAGAGAG-3’
r-5t12f : 5’-TCCATATACATACAGGCAAGGCAACTT-3’
r-5t14f : 5’-TGACCAATTGACCGATTTATTTCAACGCAATTTTTGAGAGATCTACTGATAATC-3’

Sequence of thiolated strand at the end 3’

5’-TCGGTCAATTGGTCA-3’

Modified strands with donor (D) and acceptor (A) dyes

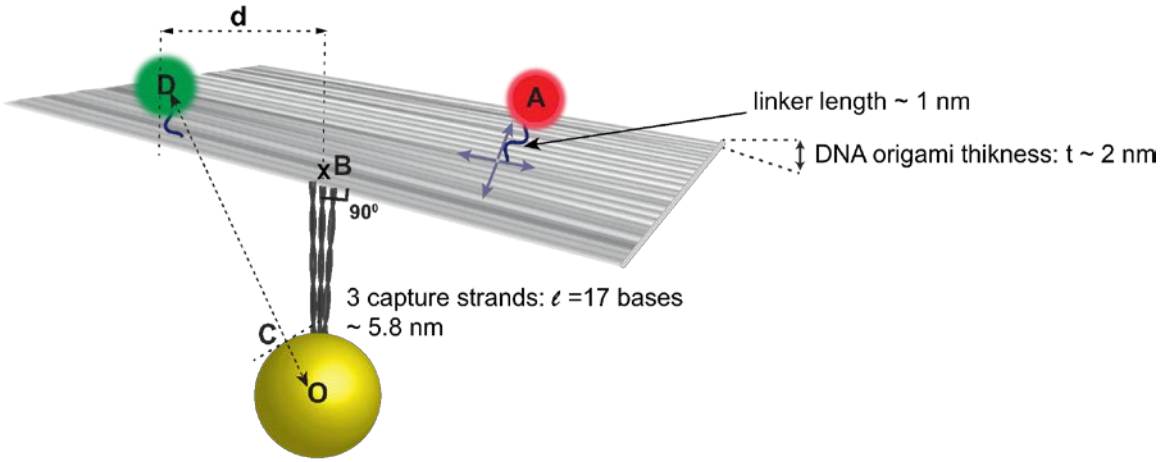
Sequences (from 5’ to 3’)	Modification	labelling
r-5t14e : GGTAGCTAGGAT*AAAAATTTTTAGTTA	Atto 647N	A
r-5t16e : CTTTCATCCCCAAAAACAGGAAGACCGGAGAG*	Alexa Fluor 568	D_1
r-5t10f : CAAAAATCATTGCTCCTTTTGATAAGTTTCAT*	Alexa Fluor 568	D_2
r-5t14f :TGACCAATTGACCGATTTATTTCAACGCAATTTTTGAGAGATCTACTGATAATC*	Alexa Fluor 568	D_3
r-5t16f : AGAAAAGCAACATTAAATGTGAGCATCTGCCA*	Alexa Fluor 568	D_4

3. Distance calculation

For distance estimation, we consider a distance of 0.34 nm between 2 neighboring base pairs, 3 nm between the adjacent helices,[1] and by taking into account (i) the length of the capture strands 17 bases ~ 5.8 nm, (ii) DNA origami thickness ~ 2 nm, (iii) linker length ~ 1 nm. We also assume the angle between the origami surface and the center of the AuNP attached by the triple "rigid" capturing strands to be 90°,[2, 3] with a vertical distance from the DNA origami surface equal to the radius of the nanoparticle plus the length of the capture strands. To this it should be added the DNA origami thickness of about 2 nm. Hence, the dye to AuNP center distance and its projection on the DNA origami surface form a triangle as shown in Fig. S2. The distance between the dye and the AuNP surface can then be calculated as shown in Figure S2.

The distances between donor-acceptor fluorophores, as well as the distance separating fluorophore molecules to AuNP center are estimated by considering the random linker length, attaching the fluorophore to the DNA strand in the 4 possible directions (shown by arrows in Fig. S2). Minimum and maximum distances were calculated with these assumptions. Distances between fluorophores and AuNP surface are obtained by subtracting the AuNPs radius (2.5 nm).

The source of error in FRET or inter-particle distances could be related to different factors, e.g.size of AuNP, rigidity of the DNA origami structure, dynamic distribution of the linker length, etc.In the calculated distances, we consideredthe uncertainties in positions only related to the linker dynamic keeping the rest of the structure static.



d: number of base pairs * 0.34 OC: AuNP radius r ~ 2.5 nm

OB: ℓ + t +r ~ 10.3 nm DO: distance from the dye to AuNP center = $(OB^2 + d^2)^{1/2}$

DC: distance between dye and AuNP surface = DO - OC

Fig. S2 Schematic representation (not to scale) showing the method of calculation of the distance between the FRET pair dyes and between the dyes and the AuNP-center. The linker flexibility has to be added to the calculated distances considering the extreme orientations indicated by the arrows as described above.

4. Molecular Structures

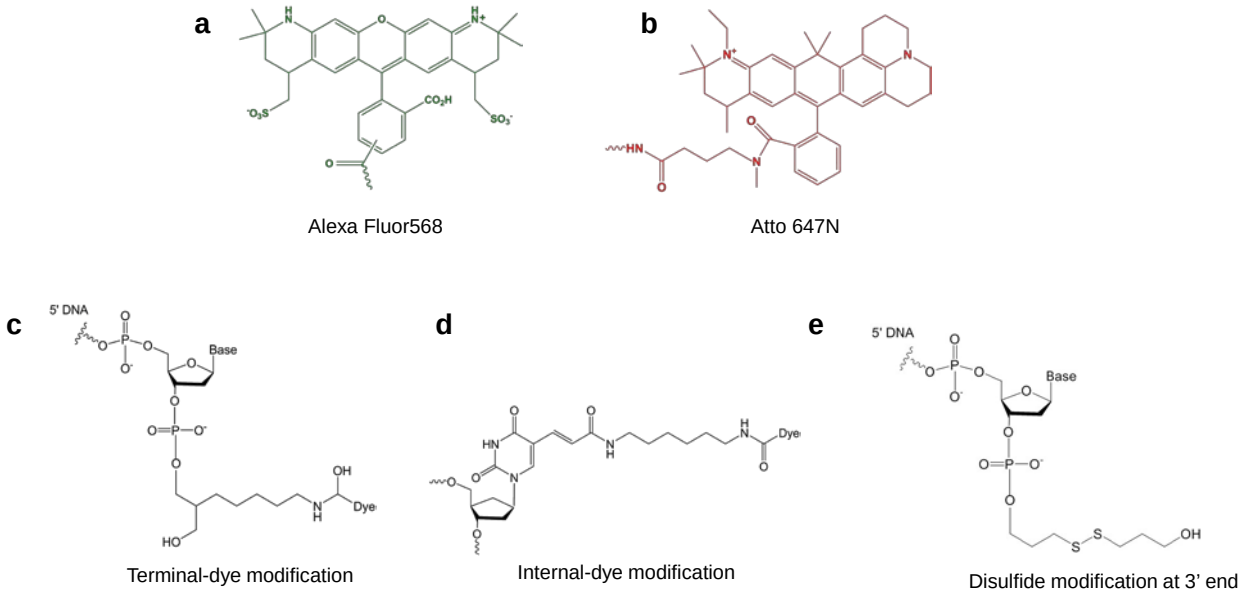


Fig. S3 Molecular structures of the dyes (a) Alexa Fluor 568 and (b) Atto 647N, used as donor and acceptor, respectively. (c)Terminal and (d) internal-dye linker used in the modification of DNA staple strands. (e) Disulfide modification in DNA used for AuNP functionalization.

5. Molar RatiosAuNPs:DNA origami

The influence of AuNPs amount surrounding the dye has been investigated by recording fluorescence lifetime and steady-state emission of the donor fluorophore (Alexa Fluor568) maintained at fixed location, D_1 at the distance ~9 nm from the AuNP surface. The decrease of the donor lifetime as a function of AuNPs:DNA origami ratios (Figure S4a) signals the expected interaction between the fluorophore and the AuNP. This interaction is better illustrated in Figure S4b showing the evolution of the inverse average lifetime ($1/\tau_{av}$) as a function of AuNP:DNA origami ratios. Here, we can observe a progressive increase of $1/\tau_{av}$ as a function of AuNPs concentration to reach a plateau at the 1:1 molar ratio between AuNP and DNA origami, indicating efficient covalent binding of AuNPs to DNA origami. A slight excess of AuNPs (up to the ratio (2:1) between AuNPs and DNA origamis) have no influence

on the donor fluorescence decay and therefore further purification step will not be necessary. In all the following experiments, AuNP-DNA origami conjugation was performed with a molar ratio of (AuNP:DNA origami) = (2:1).

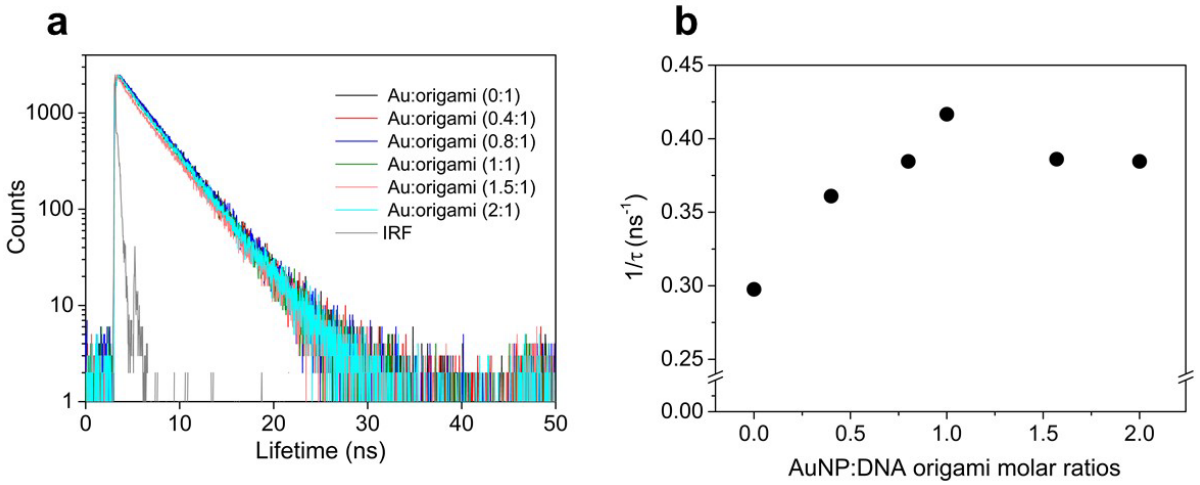


Fig. S4 (a) Fluorescence lifetime decays curves of Alexa Fluor 568 on DNA origami at different AuNPs: DNA origami molar ratios. (b) Corresponding inverse of the fluorescence lifetime obtained from panel (a).

6. Donor alone and donor-AuNP at different locations in DNA origami structures

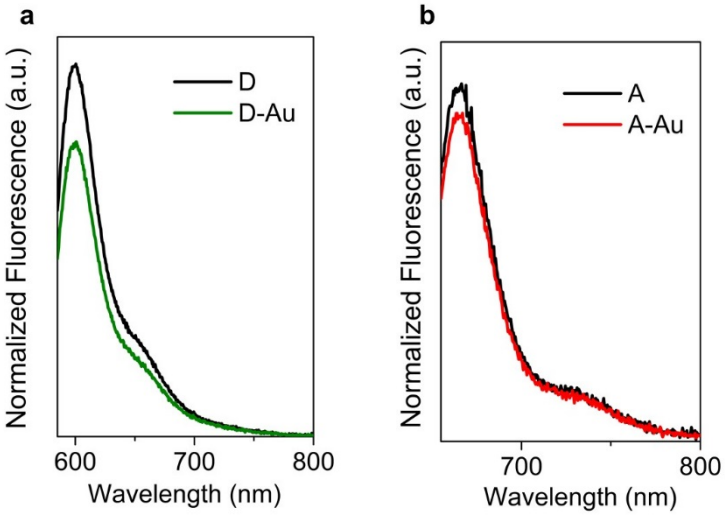


Fig. S5(a) and (b) Steady-state fluorescence intensities decrease of the donor and the acceptor dyes in the presence of AuNP at distance separation Dye-Au ~ 9 nm: the donor is at the position D_1 and the acceptor is at the fixed location A.

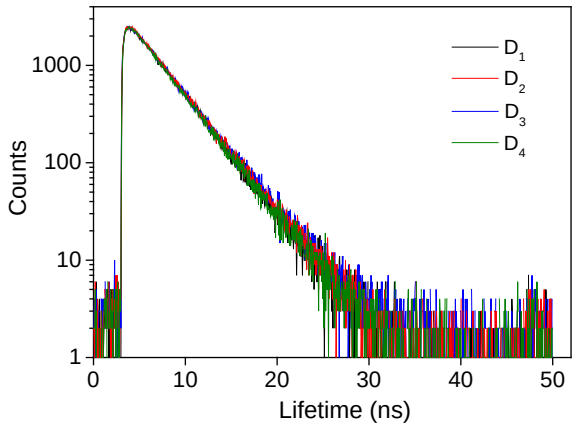


Fig.S6 Fluorescence decay curves of the donor Alexa Fluor568 at different positions on DNA origami.

7. Steady-state data analysis

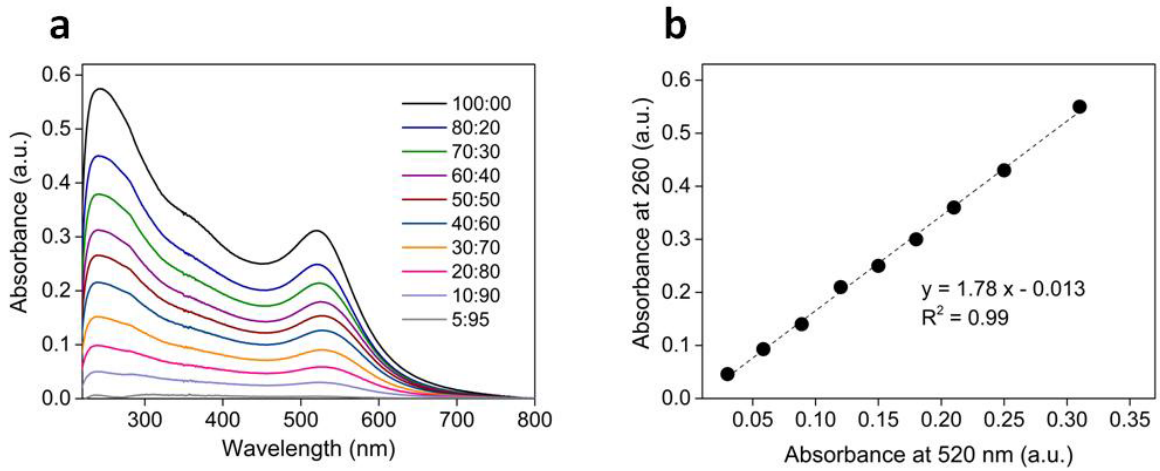


Fig. S7 (a) UV-vis spectra of AuNP 5 nm solutions diluted in TAEx1 buffer at different volume ratios between AuNP and buffer solutions, and varying between (100:00 and 5:95). (b) Au absorption peak at 260 nm as a function of the absorption peak at 520 obtained from UV-Vis spectra in (a).

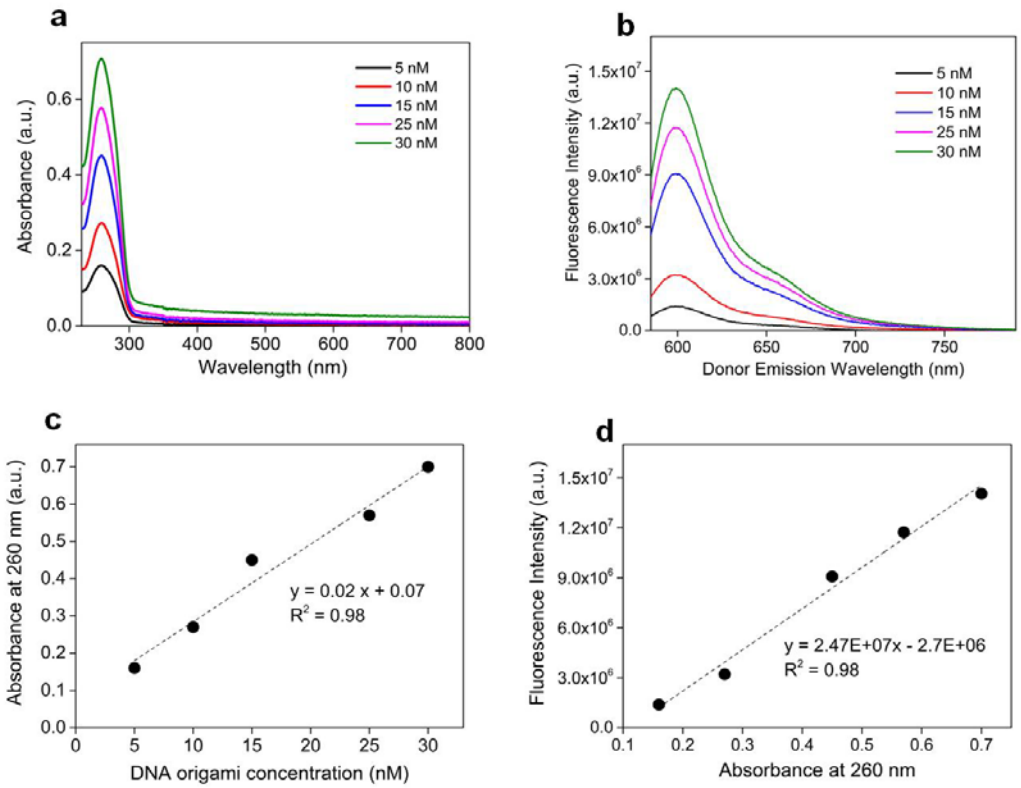


Fig. S8(a) and (b) UV-vis and correspondingFluorescence emission spectra of different concentrations of DNA origami structures labelled with Alexa Fluor568 dye. (c) DNA absorbance values at 260 nm from UV-vis spectra as a function of DNA origami concentration. (d) Fluorescence intensities values at the maximum absorption peak of Alexa Fluor568 at 601 nm obtained from (b) as a functionofDNA absorbance peakvalues at 260 nm obtained from (a).

Normalization to DNA absorbance peak

For quantitative evaluation of steady-state measurements, fluorescence intensities of the dyes on DNA origami samples have been normalized to the DNA absorbance peak at 260 nm. The absorbance peak of the dyes at 575 nm and 645nm for Alexa Fluor568 and Atto647N, respectively are too weak to be observable (only one dye per ~7250 bp DNA origami), i.e. the molar absorptivity of the dyes (Ɛ ~0.93 × 10^5 and 1.2 x 10^5 M^{-1} cm^{-1} for

Alexa Fluor568 and Atto647N, respectively) are significantly weaker comparing to the molar absorptivity of the DNA origami estimated from Figure S8c to be ~10^8 M^{-1} cm^{-1} in agreement with the literature.[4]Furthermore, the linear relationship between the dye fluorescence intensity and the DNA absorbance suggests that the single DNA origami are singly labelled with the dye moleculeand the structural integrity of the DNA origami nanostructures.[3] This linear relationship,allowed usto quantitativelyevaluate the steady-state measurements of the different labelled DNA origami samples by normalizing the dye emission peak at 601 nm to the DNA absorbance peak at 260 nm of the same sample.

Contribution of gold nanoparticles absorption

Another big challenge in the quantitative evaluation of steady-state results is the presence of gold nanoparticles. The molar absorptivity of 5 nm gold nanoparticles at the plasmonic resonance peak (Ɛ ~10^7 $L.mol^{-1}.cm^{-1}$ at 520 nm[5]) is 2 orders of magnitude higher than those of the dyes at their respective maximum absorption. To eliminate absorption contribution from the NPs, fluorescence spectra of Au-DNA origami structures were determined by subtracting the AuNP absorbance at 260 nm. This strategy was employed by Jennings et al. to eliminate absorption contribution of AuNP from dye absorption spectra.[6]

8. Radiative and non-radiative rates

In the following, the steady state fluorescence measurements and the average lifetimes were used to calculate the average radiative and non-radiative decay rates. The fluorescence quantum yield and lifetime of a fluorophore, D, are expressed as:

$$\Phi_F(\mathrm{D}) = \frac{k_F(D)}{k_F(\mathrm{D}) + k_{nr}(D)} \qquad (1)$$

$$\tau_F(\mathrm{D}) = \frac{1}{k_F(\mathrm{D}) + k_{nr}(\mathrm{D})} \quad (2)$$

In the presence of a metallic nanoparticle, additional pathways are introduced in the relaxation mechanism of the excited states, such that the effective rate constants of the radiative and non-radiative pathways become ($k_{F(D\text{-}Au)}$) and ($k_{nr\ (D\text{-}Au)}$), respectively. Thus, the modified expressions for quantum yield $\Phi_{F(D\text{-}Au)}$ and average lifetime $\tau_{F(D\text{-}Au)}$ are:

$$\Phi_{F(\mathrm{D\text{-}Au})} = \frac{k_{F(D-Au)}}{k_{F(D\text{-}Au)} + k_{nr\ (D-Au)}} \quad (3)$$

$$\tau_{F(\mathrm{D\text{-}Au})} = \frac{1}{k_{F(D\text{-}Au)} + k_{nr\ (D-Au)}} \quad (4)$$

where Φ_F´s are the relative donor fluorescence quantum yields with and without the AuNP that could be estimated from the peak intensities of the respective donor emissions at 601 nm (Fig. 5a).

Notes and references

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