Supplementary Information

Programming light-harvesting efficiency using DNA origami

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S1 DNA origami design, fluorophore arrangement and attachment

Table 1: Details of the fluorescent dye attachment sites on the DNA origami platform and sequences of the corresponding modified DNA strands. The origami coordinate $n$ denotes the nucleotide number and $h$ the helix number with respect to the origin (0, 0) as defined by the position of A1.

| Name | Label | End | $n$, $h$ | DNA sequence |
|------|-------|-----|----------|--------------|
| A1   | Cy5   | 5'  | 0, 0     | ATTTTTGAGAGCCAGCGAATAATAGAAAGGAA |
| A2   | Cy5   | 5'  | 0, 4     | TTCGACAAAATATAATAAAAGAACAAACGGCA |
| D1   | Cy3   | 5'  | 0, 2     | CTTGCTGATTGAGGATCCAAAGACGAAAATTC |
| D2   | Cy3   | 3'  | 16, 1    | TCACCATCACATTATGGAAACTTTATTAGACG |
| D3   | Cy3   | 3'  | 16, -1   | CATATGTAAATATGATCGGATTATTTGTTT |
| D4   | Cy3   | 5'  | 0, -2    | ATAATGGACCTGATTGGATAACCCGTAATTGA |
| D5   | Cy3   | 3'  | -16, -1  | TGAGTTAAGAAAGGAAACCTACTGATGGC |
| D6   | Cy3   | 3'  | -16, 1   | TTAAGACTTGTGCAAAATCCTCAATAGA |

Figure S1: Chemical structures of Cy3/Cy5 attachment to DNA. a) Cy3 attached to the 3’-end with a 3-carbon linker (arbitrary base shown). b) Cy3 attached to the 5’-end with a 3-carbon linker (arbitrary base shown). c) Cy5 attached to the 5’-end with a 3-carbon linker (arbitrary base shown).
S2 Spectroscopic properties of Cy3 and Cy5

Figure S2: **Ensemble measurements.** a) Normalised absorption (*dotted* lines) and fluorescence emission (*solid* lines) spectra of Cy3-labelled (*green*) and Cy5-labelled (*red* for A1, *pink* for A2) single-stranded DNA (ssDNA), referred to as ‘staples’. The absorption and emission spectra of Cy3 are averaged over all six Cy3-staple strands (D1-D6). b) Steady-state fluorescence emission spectra of the Cy3-donor ring (*green*) and the Cy5-acceptor A1 (*red*) and A2 (*pink*) on the DNA origami platform. The Cy3 molecules were excited at 521 nm, and the Cy5 molecules at 600 nm.

We first characterised the spectroscopic properties of the antenna components without donor-to-acceptor energy transfer (see Supplementary Methods for absorption measurements). In Fig. S2a, normalised absorption and fluorescence emission spectra of the Cy3 and Cy5 molecules conjugated to a single-stranded DNA ‘staple’ are shown. For the Cy3-labelled strands (D1-D6) we obtained average peak absorption and emission wavelengths of 549±1 nm and 564±1 nm, respectively. For the Cy5-labelled strands (A1, A2), the absorption peak values were 645 nm and 648 nm, and the emission peak values 661 nm and 665 nm, respectively. By incorporating the staple strands into three basic DNA origami platforms, the influence of the DNA origami environment on the peak emission wavelength of the Cy3- and Cy5-labelled staples was investigated. The DNA origami platform containing all six
Cy3-dyes (D1-D6) had a peak emission wavelength of 565 nm, while both Cy5-only platforms (A1, A2) had a peak emission wavelength of 660 nm (Fig. S2b). Hence, the peak emission wavelengths of the staple strands in solution and embedded into the DNA origami platform are in good agreement.
S3 Energy transfer efficiency of single donor-acceptor pairs

![Figure S3](image)

Figure S3: **Single-molecule measurements.** Energy transfer efficiencies obtained using single-molecule fluorescence measurements for the single donor-acceptor pairs **a)** A-D1 and **b)** A-D2 representing the two possible next-neighbour distances on the DNA origami platform.

The origami coordinates (see Table I) can be translated into theoretical distances between the dyes by assuming a nucleotide-to-nucleotide distance of 0.34 nm along the helix and an average helix-to-helix distance of 2.6 nm. This yields theoretical next-neighbour separations of ~5.2 nm in a perpendicular and ~6 nm in a diagonal orientation with respect to helix direction. Using single-molecule fluorescence measurements, we quantified the energy transfer efficiency $E$ (γ-corrected, see Supplementary Methods) between the acceptor dye and the donor dyes D1 (Fig. S3a) and D2 (Fig. S3b), respectively, representing the two possible inter-dye distances in our design. Assuming a Cy3-Cy5 Förster radius of $R_0 = 5.4$ nm, we could estimate the next-neighbour distances experimentally, yielding $R_{A-D1}=5.2±0.3$ nm (s.d.) and $R_{A-D2}=5.6±0.5$ nm (s.d.). This is in good agreement with the expected theoretical values.
S4 Antenna effect in ensemble fluorescence measurements

Figure S4: **Ensemble measurements.**  
**a)** Intensity of donor emission $I_D(D^*)$ (550-600 nm) and acceptor emission $I_A(D^*)$ (635-700 nm) upon excitation of donors at 521 nm. **b)** Intensity of acceptor emission $I_A(A^*)$ (635-700 nm) upon direct excitation of acceptor (600 nm).
S5 Magnesium dependent fluorescence quenching

Figure S5: **Ensemble measurements.** Change in $I_D(D^*)$ plotted against change in $I_A(A^*)$ at each MgCl$_2$ concentration using three independently prepared samples of the DNA origami platform containing the full donor ring ($N = 6$) and an acceptor in the centre.

We observed that an increase in MgCl$_2$ concentration leads to quenching of the fluorescence of Cy3 and Cy5. To demonstrate that Cy3 and Cy5 were not quenched to different extents at a given salt concentration, we plotted the change in fluorescence emission of the donor ($\Delta I_D(D^*)$) against the change in fluorescence emission of the acceptor ($\Delta I_A(A^*)$) upon direct excitation for each MgCl$_2$ concentration. It can be seen that the scatter points are distributed along the straight line $\Delta I_D(D^*) = \Delta I_A(A^*)$, thus suggesting that the increase in antenna effect is a result of the compaction of the DNA origami platform. The results were obtained from three independently prepared samples of the DNA origami platform containing the full donor ring ($N = 6$) and an acceptor in the centre.
S6 Single-molecule fluorescence measurements

Figure S6.1: **Single-molecule measurements.** a) Histograms showing the single-molecule antenna effect $AE_{\text{sm}}$ fitted with a Gaussian distribution for a set of representative antenna structures. b) Histograms showing the stoichiometry ratio $S$ fitted with a Gaussian distribution for a set of representative antenna structures.

In Fig. S6.1, we provide histograms of the antenna effect $AE_{\text{sm}}$ (Fig. S6.1a) and the stoichiometry parameter $S$ (Fig. S6.1b) obtained from the single-molecule fluorescence measurements. In Fig. S6.2, we provide a summary of $AE_{\text{sm}}$ and $S$-values of all constructs.
measured. The stoichiometry parameter $S$ describes the ratio between donor and acceptor dyes of the sample and is defined as

$$ S = \frac{I_A(D^*) + I_D(D^*)}{I_A(D^*) + I_D(D^*) + I_A(A^*)}, $$

(1)

with $I_A(D^*)$: green excitation, red emission, $I_D(D^*)$: green excitation, green emission, and $I_A(A^*)$: red excitation, red emission. In the case of $\gamma$-values (comprising the detection sensitivity and quantum yields, see Supplementary Methods) of 1, the $S$-value is at 0.5 for a construct with one donor and one acceptor. In our case it is 0.41-0.44 as can be seen from the two 1:1 constructs in Fig. S6.1b (upper two panels). With increasing number of donors, the $S$-value increases. Interestingly, with an increasing number of donors, the whole populations shift to higher $S$-values (lower panels). In all cases the population is very homogeneous and completely shifted to the increased $S$-value, which reflects a defined donor/acceptor ratio. This complete shift of the population is the best proof that the samples have a very high homogeneity and all the individual constructs contain the same number of donors. Within our error bars, we do not see constructs which do not correspond to the correct population. Similarly, the histograms for the $AE_{em}^{sm}$ are mainly shot-noise limited.

Figure S6.2: **Single-molecule measurements.** a) Overview of mean values and standard deviations for $AE_{em}^{sm}$ obtained for each sample using a Gaussian fit as shown in Fig. S6.1a. b) Overview of mean values and standard deviations for $S$ obtained for each sample using a Gaussian fit as shown in Fig. S6.1b.
The histograms always indicate single populations, which shift with an increasing number of donors. We do not have strong indications for significant conformational heterogeneity from the width of the $AE_{tot}^{sm}$ distribution or from the width of related energy transfer distributions (data not shown). Small structural heterogeneity and dynamics faster than the measurement time (which corresponds to the transition time through the laser focus of a few milliseconds) can however not be excluded.

**Comparison of antenna effect between single-molecule and ensemble measurements**

![Figure S6.3: Comparison between single-molecule and ensemble measurements.](image)

Figure S6.3: **Comparison between single-molecule and ensemble measurements.** Antenna effect in dependence of number of donors obtained from single-molecule (*red*, $AE_{tot}^{sm}$) and ensemble (*black*, $AE_{tot}$) measurements. We determined the slopes with a linear fit, yielding $m_{ens} = 0.31$ for the ensemble and $m_{sm} = 0.12$ for the single-molecule measurements, respectively. The error bars correspond to the standard error of the mean.

The values for $AE_{tot}^{sm}$ (single-molecule) are consistently lower than for $AE_{tot}$ (ensemble) (Fig. 3). This can be largely explained by the dependence of the antenna effect on the excitation wavelength; in the two techniques, different excitation wavelengths were used for the acceptor molecule ($\lambda_A^{sm}=640$ nm in single-molecule and $\lambda_A^{ens}=600$ nm in ensemble measurements, respectively), see Fig. 2c. As Cy5 absorbs $\sim 2.8 \times$ more at $\lambda_A^{sm}$ than at $\lambda_A^{ens}$ (Fig. S2a), the extinction coefficient of Cy5 under the conditions of the single-molecule measurements
can be written as \( \Xi_A(\lambda^{sm}_A) = 2.8 \Xi_A(\lambda^{ens}_A) \). The difference in absorptivity for Cy3 at the donor excitation wavelength (\( \lambda^{sm}_D = 532 \text{ nm} \) in single-molecule and \( \lambda^{ens}_D = 521 \text{ nm} \) in ensemble measurements, respectively) is negligible (Fig. S2a), hence we can assume \( \Xi_D(\lambda^{sm}_D) = \Xi_D(\lambda^{ens}_D) \).

The antenna effect for the single-molecule measurements can be expressed as (see Eq. (4) in Methods and S7, S8) \( AE^{sm}_{tot} = N m_{sm} \) where \( N \) is the number of donors and \( m_{sm} = \frac{\Phi^{sm}_D}{\Phi^{sm}_A} E(R) \)

with \( \Phi^{sm}_D / \Phi^{sm}_A = \frac{\Xi_D(\lambda^{sm}_D)}{\Xi_A(\lambda^{sm}_A)} \frac{\lambda^0_D \lambda^{sm}_A}{\lambda^0_A \lambda^{sm}_D} \). Likewise, in ensemble measurements \( AE_{tot} = N m_{ens} \),

where \( m_{ens} = \frac{\Phi_D}{\Phi_A} E(R) \) and \( \Phi_D / \Phi_A = \frac{\Xi_D(\lambda^{ens}_D)}{\Xi_A(\lambda^{ens}_A)} \frac{\lambda^0_D \lambda^{ens}_A}{\lambda^0_A \lambda^{ens}_D} \). As \( \frac{\Xi_D(\lambda^{sm}_D)}{\Xi_A(\lambda^{sm}_A)} = \frac{1}{2.8} \), the relationship between \( AE^{sm}_{tot} \) and \( AE_{tot} \) can thus be written as

\[
AE^{sm}_{tot} = N \frac{\Phi^{sm}_D}{\Phi^{sm}_A} E(R) = N 1.04 \frac{\Phi_D}{\Phi_A} E(R) = 1.04 \frac{m_{ens}}{m_{sm}} AE_{tot} .
\]

The ratio between the slopes is thus expected to be

\[
\frac{m_{ens}}{m_{sm}} = 2.7 .
\]

We verified this by determining the slopes experimentally, as shown in Fig. S6.3. We get a ratio of \( m_{ens} / m_{sm} = 2.6 \), which is in very good agreement with the expected value in Eq. 3.

We can thus conclude that the antenna effect is equivalent in both measurement techniques following the correction accounting for the different excitation wavelengths. Minor sources of errors not taken into account here might arise from differences in set-up parameters such as relative light intensities and detector efficiencies.
**Comparison of energy transfer efficiency between single-molecule and ensemble measurements**

**Figure S6.4:** **Direct comparison between single-molecule and ensemble fluorescence measurements.**  

**a)** Energy transfer efficiency ($E^*$) obtained from single-molecule measurements. We analysed 1-donor (blue), 2-donor (green) and 6-donor (red) samples (Fig. 1b). We screened several thousand molecules for each sample type, and used a Gaussian fit to determine the $E^*$ (data not shown). The error bars correspond to the standard deviation of the Gaussian fit.  

**b)** Energy transfer efficiency ($E^*$) obtained from ensemble measurements. We analysed 1-donor (blue), 2-donor (green) and 6-donor (red) samples (Fig. 1b). Each sample was prepared in three independent replicates. The error bars correspond to the standard error of the mean.

We have determined the energy transfer efficiency $E^*$ from both single-molecule and ensemble measurements. The energy transfer efficiency is defined as

$$E^* = \frac{I_A(D^*)}{I_A(D^*) + I_D(D^*)},$$  

where $I_A(D^*)$ corresponds to the fluorescence emission of the acceptor dye (Cy5) upon excitation of the donor(s) (Cy3) and $I_D(D^*)$ to the fluorescence emission of the donor dye(s) upon direct excitation. For the single-molecule measurements, the donor excitation wavelength is $\lambda_{D^{sm}} = 532$ nm. For the ensemble measurements, the donor excitation wavelength is $\lambda_{D^{ens}} = 521$ nm. While the donor and acceptor fluorescence can be detected in two separate
channels in the single-molecule measurements, it is necessary to decompose the emission spectrum into the spectral components of donor and acceptor fluorescence in the ensemble measurements. Here, we integrated over the wavelengths 550-600 nm to obtain \( I_D(D^*) \) and 635-700 nm to obtain \( I_A(D^*) \). Please note that \( E^* \) does not include a \( \gamma \)-correction (see Supplementary Note S3). In Fig. S6.4, we provide a direct comparison between \( E^* \) obtained from the single-molecule (Fig. S6.4a) and ensemble (Fig. S6.4b) measurements as described above. It can be observed that overall the absolute values are in the same range. There is no systematic change in \( E^* \) with number of donors as expected. The single-molecule measurements show more distinct differences between structures with different donor-acceptor distances (see e.g. samples 1 and 2) while having very similar \( E^* \)-values when the donor-acceptor distances are the same (see e.g. samples 1 and 3, and samples 2 and 6). A possible reason for the discrepancies between the results obtained from the two techniques is that is that undesired samples not showing FRET (i.e. donor and acceptor only populations) are filtered out in single-molecule spectroscopy.
S7 Antenna effect in ensemble measurements: analysis without fitting parameters.

Figure S7: Antenna effect ($AE_{tot}$) as a function of the number of donor dyes. $AE_{tot}$ measured experimentally (black), theoretical $AE_{tot}$ using linear fit (red) and $AE_{tot}$ calculated using Eq. (7) with experimental quantities (blue).

The antenna effect ($AE_{tot}$) can be expressed in terms of the number of donor dyes $N$, the energy transfer efficiency $E(R)$, the molar extinction coefficient of the donor (acceptor) dye $\Xi_D(\lambda)$ [$\Xi_A(\lambda)$], and the intensity $I_{inc}^{D}$ ($I_{inc}^{A}$) of the external pump exciting the donor (acceptor) at the wavelength $\lambda_D$ ($\lambda_A$), see also S8:

$$AE_{tot} = NE(R)\frac{\Xi_D(\lambda_D) \omega_0^{A} I_{inc}^{D}(\lambda_D)}{\Xi_A(\lambda_A) \omega_0^{D} I_{inc}^{A}(\lambda_A)}$$

(5)

where $\omega_0^{D}$ ($\omega_0^{A}$) is the resonant frequency of the donor (acceptor). In the main text (see Model) we have studied the behaviour of the $AE_{tot}(N)$ as a function of $N$ ($N=1,...,6$) assuming an average transfer efficiency $E(\bar{R})$ and linear fitting of the experimental antenna effects ($y$), $y = \bar{\alpha}x + q$, $x = NE(\bar{R})$ and $\bar{\alpha} = \Phi_D/\Phi_A$,

$$\frac{\Phi_D}{\Phi_A} = \frac{\Xi_D(\lambda_D) \lambda_0^{D} I_{inc}^{D}(\lambda_D)}{\Xi_A(\lambda_A) \lambda_0^{A} I_{inc}^{A}(\lambda_A)}$$

(6)
From the linear fit we obtained $\tilde{\alpha} = \Phi_D/\Phi_A = 0.606$ and $q = 0.006$ (see Model in the main text).

We now study the relation $AE_{\text{tot}} = AE_{\text{tot}}(N)$ without using any fitting parameter, evaluating the ratio $\Phi_D/\Phi_A$ using experimental values for the molar extinction coefficients. The molar extinction coefficients of Cy3 (D) and Cy5 (A) at maximum absorption wavelengths ($\lambda_D^0 = 548$ nm and $\lambda_A^0 = 646$ nm) are $\Xi_D(\lambda_D^0) = 136.000$ and $\Xi_A(\lambda_A^0) = 250.000$ ([M$^{-1}$ cm$^{-1}$]), respectively$^4$ and have been corrected to take into account the excitation wavelengths ($\lambda_D = 521$ nm and $\lambda_A = 600$ nm) used in the ensemble measurements. Assuming equal photon fluxes $F (F = I_{\text{inc}}/h\omega)$ for excitation of the dyes, $F_D(\lambda_D) = F_A(\lambda_A) \implies I_{\text{inc}}^D(\lambda_D)/I_{\text{inc}}^A(\lambda_A) = \lambda_A/\lambda_D \approx 1.15$, so that

$$AE_{\text{tot}} = N_D E \frac{\Phi_D}{\Phi_A} = N_D E \frac{\Xi_D(\omega_D)}{\Xi_A(\omega_A)} \frac{\omega_A^0}{\omega_D^0} \frac{I_{\text{inc}}^D}{I_{\text{inc}}^A} = N_D E \frac{\Xi_D(\lambda_D)}{\Xi_A(\lambda_A)} \frac{\lambda_A^0}{\lambda_D^0} \frac{\lambda_D}{\lambda_A}. \quad (7)$$

Exciting both dyes at their maximum absorption wavelengths ($\lambda_A^0 = \lambda_A$, $\lambda_D^0 = \lambda_D$), Eq. (7) becomes $AE_{\text{tot}} = N_D [\Xi_D(\lambda_D)/\Xi_A(\lambda_A)]$. Using Eq. (7) with $E = E(R) = E(\bar{R})$ (see Model in the main text) we can then work out the ratio $\Phi_D/\Phi_A = \frac{\Xi_D(\lambda_D)}{\Xi_A(\lambda_A)} \frac{\lambda_A^0}{\lambda_D^0} \frac{\lambda_D}{\lambda_A} = 0.89$ and thus calculate $AE_{\text{tot}}(N_D)$. This is shown in Fig. S7 (blue line) together with the experimental $AE_{\text{tot}}$ values (black) and the linear fit (red line) already presented in the main text. The discrepancy between the measured antenna effect and that evaluated using Eq. (7) ($\approx 45\%$) could be explained by the change in the extinction coefficients occurring when the dyes are bound to the origami platform compared to their values measured in solution and used to estimate the $AE_{\text{tot}}$ using Eq. (7).
S8 Detailed model

We model the energy transfer from the antenna complex to the common acceptor core using a set of rate equations governing the dynamics of the populations of the donor and acceptor chromophores, under external laser excitation and hetero-FRET interaction. This treatment assumes that only one particle (i.e. one excitonic quasiparticle in this case) is present in the system at any time, which is valid for the low excitation conditions under which the experiments have been carried out.

Let then $\rho_A, \rho_D$ be the populations of the excited states of the acceptor ($A$) and the donor ($D$), respectively. For a single donor-acceptor pair, the temporal evolution of the exciton population is then described by the following rate equations:

\begin{align}
\dot{\rho}_D &= -\Gamma_{DA}\rho_D - \Gamma_D\rho_D, \\
\dot{\rho}_A &= \Gamma_{DA}\rho_D - \Gamma_A\rho_A,
\end{align}

where $\Gamma_{DA}$ is the pairwise hetero-FRET rate constant between the donor and the acceptor dyes, and $\Gamma_D (\Gamma_A)$ the radiative recombination rates of the donor (acceptor), respectively. The FRET rate $\Gamma_{DA}$ depends on the lifetime of the donor excited state ($\tau_D = 1/\Gamma_D$) and the donor-acceptor separation ($R$):

$$\Gamma_{DA} = \frac{1}{\tau_D} \left(\frac{R_0}{R}\right)^6,$$

where $R_0$ is the Förster radius, i.e. the donor-acceptor separation corresponding to a FRET efficiency $E(R)$ equal to 50%:

$$E(R) = R_0^6/(R_0^6 + R^6), \quad R_0 = 0.2108[\kappa^2 n^{-4} QY_D J],$$

where $\kappa^2$ is the dipole orientation factor, which is equal to 2/3 for quasi-random dipole orientations, $n$ is the refractive index of the medium, $QY_D$ is the fluorescence quantum yield.
of the donor and $J$ is the spectral overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor.

The antenna effect is given by $AE_A^D = \frac{I_A^R(D^*)}{I_A^R(A^*)}$, where $I_A^R(D^*)$ and $I_A^R(A^*)$ are the acceptor fluorescence intensities following the excitation of the donor and the acceptor dye at given excitation wavelengths, respectively. The fluorescence intensities are proportional to the occupation of the excited acceptor states,

$$I_A^R(D^*) \propto \Gamma_A\tilde{\rho}_{A,D^*}, \quad (12)$$

$$I_A^R(A^*) \propto \Gamma_A\tilde{\rho}_{A,A^*}, \quad (13)$$

so that $AE_A^D = (\Gamma_A\tilde{\rho}_{A,D^*})/(\Gamma_A\tilde{\rho}_{A,A^*})$, with $\tilde{\rho}_{A,D^*}$ and $\tilde{\rho}_{A,A^*}$ being the steady-state populations of the acceptor dye, following the excitation of the donor and the acceptor, respectively. These steady-state populations are straightforwardly obtained as the steady-state solutions of the coupled Eqs. (8-9) when a term describing the excitation of the donor dye by an external light source is included in r.h.s. of Eq. (8) to obtain $\tilde{\rho}_{A,D^*}$, or in the r.h.s. of Eq. (9) to find $\tilde{\rho}_{A,A^*}$. Such a pump term can be written as

$$P = \alpha(\omega)\frac{I_{inc}}{\hbar\omega_0}, \quad (14)$$

where $\alpha(\omega)$ is the absorption coefficient, $\omega_0$ the resonant frequency of the donor/acceptor molecule, and $I_{inc} = \frac{1}{2}\varepsilon_0cE_0^2$ is a cycle averaged intensity of the incident radiation driving the oscillator $\omega_0$.

We then get:

$$\tilde{\rho}_{A,D^*} = \frac{\Phi_D\Gamma_{DA}}{\Gamma_A(\Gamma_{DA} + \Gamma_D)}, \quad (15)$$

$$\tilde{\rho}_{A,A^*} = \frac{\Phi_A}{\Gamma_A}, \quad (16)$$

where $\Phi_D = (\alpha_D(\omega_D)I_{inc}^D)/\hbar\omega_0^D$ and $\Phi_A = (\alpha_A(\omega_A)I_{inc}^A)/\hbar\omega_0^A$, with $I_{inc}^D$ ($I_{inc}^A$) the intensity
of the applied external pump exciting the donor (acceptor) at frequency $\omega_D$ ($\omega_A$), while $\omega_D^0$ ($\omega_A^0$) is the resonant frequency of the donor (acceptor). The antenna effect for the single donor-acceptor pair thus reads

$$AE_D^A = \frac{\Phi_D}{\Phi_A} = \frac{1}{1 + \Gamma_D/\Gamma_{DA}}$$

$$= \frac{\Phi_D}{\Phi_A} E(R)$$  

The antenna effect can be written in terms of the molar extinction coefficient $\Xi(\omega)$, where $\alpha = c_m \Xi$, $c_m$ being the molar concentration:

$$AE_D^A = E(R) \frac{\Phi_D}{\Phi_A} = E(R) \frac{\Xi_D(\omega_D) \omega_A^0 I_{inc}^D}{\Xi_A(\omega_A) \omega_D^0 I_{inc}^A} = E(R) \frac{\Xi_D(\lambda_D) \lambda_A^0 I_{inc}^D}{\Xi_A(\lambda_A) \lambda_D^0 I_{inc}^A}. \quad (19)$$

This is the most general expression of the antenna effect. If one assumes equal incident photon fluxes ($F = I_{inc}/\hbar \omega$) for the excitation of donors (at wavelength $\lambda_D$) and acceptors (at wavelength $\lambda_A$), $F_D(\lambda_D) = F_A(\lambda_A)$ and thus $I_{inc}^D(\lambda_D)/I_{inc}^A(\lambda_A) = \lambda_A/\lambda_D$, which yields

$$AE_D^A = E(R) \frac{\Phi_D}{\Phi_A} = E(R) \frac{\Xi_D(\lambda_D) \lambda_A^0 \lambda_D}{\lambda_A^0 \lambda_D}. \quad (20)$$

When more than one donor is present, as in the ring antenna system examined here, the system Eqs. (8-9) scales up with total number $N$ of donor dyes $D_i$ ($i = 1, ..., N$):

$$\dot{\rho}_{D_i} = -\Gamma_{D_iA} \rho_{D_i} - \Gamma_{D_i} \rho_{D_i}, \quad i = 1, ..., N \quad (21)$$

$$\dot{\rho}_A = \rho_A \sum_i \Gamma_{D_iA} - \Gamma_A \rho_A. \quad (22)$$

In the case of identical donors ($\Gamma_{D_i} = \Gamma_D \forall i$) located at a same distance from the acceptor $A$ ($\Gamma_{D_iA} = \Gamma_{DA} \forall i$), the cumulative antenna effect ($AE_{tot}$) simply scales with $N$,

$$AE_{tot} = N AE_D^A. \quad (23)$$
In the analysis developed above we have not considered the homo-FRET interaction between
the donor dyes. We have assumed that the (identical) donor dyes are (i) equally spaced within
the ring, so that the homo-FRET rates between a dye and its first nearest neighbours are
the same ($\Gamma_{D_{i-1}D_i} = \Gamma_{D_iD_{i+1}} = \Gamma_{DD} \forall i$) and (ii) all positioned at the same distance from the
acceptor, so that the hetero-FRET rate $\Gamma_{DA}(R = R_{DA})$ is the same for each donor-acceptor
pair. Under these conditions, the homo-FRET rate $\Gamma_{DD}$ does not affect the final expression
for the antenna effect $AE_{tot}$. This can be demonstrated finding the steady-state populations
of Eqs. (21-22) in the presence of the additional terms describing the homo-FRET interaction
between neighbouring donors: $\dot{\rho}_{D_i} = -\Gamma_{D_iA}\rho_{D_i} - \Gamma_{D_i}\rho_{D_i} - 2\Gamma_{DD}\rho_{D_i} + \Gamma_{DD}(\rho_{D_{i+1}} + \rho_{D_{i-1}})$,
$\Gamma_{DD}$ being the homo-FRET rate.
S9 Circular vs. linear antenna geometry (theory)

Figure S8: **Theoretical model.** Simulated antenna effect ($AE_{tot}$) as a function of the number of donors. Ring with the acceptor placed at its centre (red) and wire with acceptor placed at the end (black). The inset shows the magnification of the $AE_{tot}$ curve for the wire configuration with the acceptor placed at the end.

Here, we simulate the antenna effect ($AE_{tot}$) in two different configurations: (i) a ring with $N$ identical donors and a common acceptor placed at its centre, the configuration which has been discussed throughout this Letter, and (ii) the photonic wire, a one-dimensional linear chain consisting of $N$ identical donor dyes and an acceptor at one end, see Fig. S8. The two configurations have been modelled using a formalism based on rate equations describing the dynamics of the excited states of donor and acceptor dyes in presence of external laser excitation, see Methods. We have included the pairwise hetero-FRET rate constant $\Gamma_{D_iA}$ ($i = 1, \ldots, N$ with $N$ being the number of donors) between the donor and the acceptor dyes as well as the homo-FRET rate $\Gamma_{DD}$ between each donor and its first nearest neighbours.

For the ring structure, we assume that all the donors $D_i$ are (i) located at the same distance $R$ from the acceptor $A$, so that $\Gamma_{D_iA}(R_i) = \Gamma_{DA}(R) \forall i$ (see Fig. S8) and (ii) equally spaced on the ring so that $\Gamma_{D_iD_{i+1}} = \Gamma_{D_{i-1}D_i} = \Gamma_{DD} \forall i$. The ring-shaped antenna assembled on the DNA origami plate is a good approximation of such an idealised geometry.

For the wire configuration, we consider again equally spaced dyes with inter-dye sep-
aration equal to $R$. In this case the separation between each $i$-th dye and the acceptor is $R_i = iR$, $i = 1, ..., N$ (see Fig. S8), and the hetero-FRET decay rates are given by $\Gamma_{D_iA}(R_i) = \Gamma_{DA}(R)i^{-6}$ $i = 1, ..., N$. When the donors are equally separated from the acceptor (as in the ring configuration) and the donor-acceptor energy transfer is thus described by the same hetero-FRET rate $\Gamma_{DA}$, the total antenna effect $AE_{tot}$ does not depend on the homo-FRET rate $\Gamma_{DD}$ and simply scales with $N$, see Eq. (4) in Methods. However, in the wire configuration, each $i$-th dye has a different hetero-FRET rate $\Gamma_{D_iA}(R_i)$ and $AE_{tot}$ does depend on the homo-FRET interaction $\Gamma_{DD}$.

Fig. S8 shows the simulated antenna effect $AE_{tot}$ in both the ring and the wire configurations, estimated as a function of the number $N$ of donor dyes, with $N = 6$ as in the experimental configuration investigated in this Letter.

In this simulation we used: $R = \bar{R}$, where $\bar{R} = 5.4$ nm is the average donor-acceptor distance in the ring structure as estimated from the experimental efficiencies measured by single-molecule spectroscopy; $\Phi_D/\Phi_A = 0.606$ as obtained from the linear fit modelling $AE_{tot}$ as a function of $N$ (see Methods); Förster radius $R_0 = 5.4$ nm; $\Gamma_{DD} = 10\Gamma_D$, $\Gamma_D$ being the total decay rate of the donor. The results in Fig. S8 clearly show that the ring design outperforms the wire: having an ensemble of donors placed at the same distance from a common acceptor ensures an overall transfer of energy larger than that possible with a line of equally-spaced dyes. In this latter configuration, the collection of energy at the end-acceptor is mainly determined by hetero-FRET, with transfer rates which decrease for increasing donor-acceptor separations. This also explains why in the wire configuration, even in presence of the diffusive homo-FRET, the antenna effect rapidly saturates as the number of donors increases.
S10 Estimated error from averaging over different distances

Figure S9: **Theoretical model.** Difference between the averaged $AE_{\text{tot}}(\bar{R})$ and the exact expression $AE(R_1) + AE(R_2)$ for increasing differences $\delta R$ between the single donor-acceptor separations $R_1$ and $R_2$ ($\delta R = R_2 - R_1$).

To analyse the cumulative antenna effect ($AE_{\text{tot}}$) in the two-donor configuration (see Model in Methods) we introduced the average donor-acceptor separation $\bar{R}$, where $R_1 = \bar{R} + \delta R \gtrsim R_2 = \bar{R} - \delta R$. Then, we approximated $AE_{\text{tot}} = AE(R_1) + AE(R_2)$ with $AE_{\text{tot}}(\bar{R})$, see Eq. (5) in Methods. Figure S9 shows how the averaged $AE_{\text{tot}}(\bar{R})$ differs from the exact expression $AE(R_1) + AE(R_2)$ for increasing differences $\delta R$ between the single donor-acceptor separations $R_1$ and $R_2$ ($\delta R = R_2 - R_1$). In this simulation we considered a Förster radius $R_0 = 5.4$ nm, $R_1 = 5.2$ nm, increasing values of $R_2$ from $R_1$ up to 6.2 nm and $\Phi_D/\Phi_A = 0.606$ as obtained from the linear fit modelling $AE_{\text{tot}}$ as a function of $N$ (see Methods). These results indicate that approximating the cumulative antenna effect with $AE_{\text{tot}}(\bar{R})$ yields a relative error $\gtrsim 2\%$ only for large differences $\delta R \gtrsim 1$ nm. For the range of estimated donor-acceptor separations of our samples, $\delta R = 0$ ($R_1 = R_2 = R$ with $R = R_{D_1A}=5.2$ nm or $R = R_{D_2A}=5.6$ nm) or $\delta R = 0.4$ nm ($R_1 = R_{D_1A}=5.2$ nm and $R_2 = R_{D_2A}=5.6$ nm) and the error is thus negligible.
Supplementary Methods

Absorbance measurements in bulk

Absorbance measurements were performed using a Cary 300 Bio UV-Visible Spectrophotometer (Agilent Technologies). The Cy3- and Cy5-labelled staple strands were diluted to a final concentration of \( \sim 500 \text{ nM} \) in \( 1 \times \text{TE} \) in a low volume cuvette \( (\sim 100 \mu\text{l}) \) (Sigma-Aldrich). Absorbance spectra were recorded over a wavelength range of 350–700 nm and normalised with the blank solution \( (1 \times \text{TE}) \).

Data analysis of single-molecule fluorescence measurements

The data received from the fluorescence measurements are analysed by using a burst search algorithm.\(^7\) We subtracted the background signals from the photon counts and extracted the leakage value from the donor only population and the direct excitation factor from the acceptor-only population according to N. K. Lee \textit{et al.}\(^8\) The \( E \)-values are corrected with the detection correction factor \( \gamma \) to take into account differing detection efficiencies and quantum yields of the dyes. The \( E\)-\( S \)-histograms are further filtered by using ALEX-2CDE and FRET-2CDE filters.\(^9\)

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