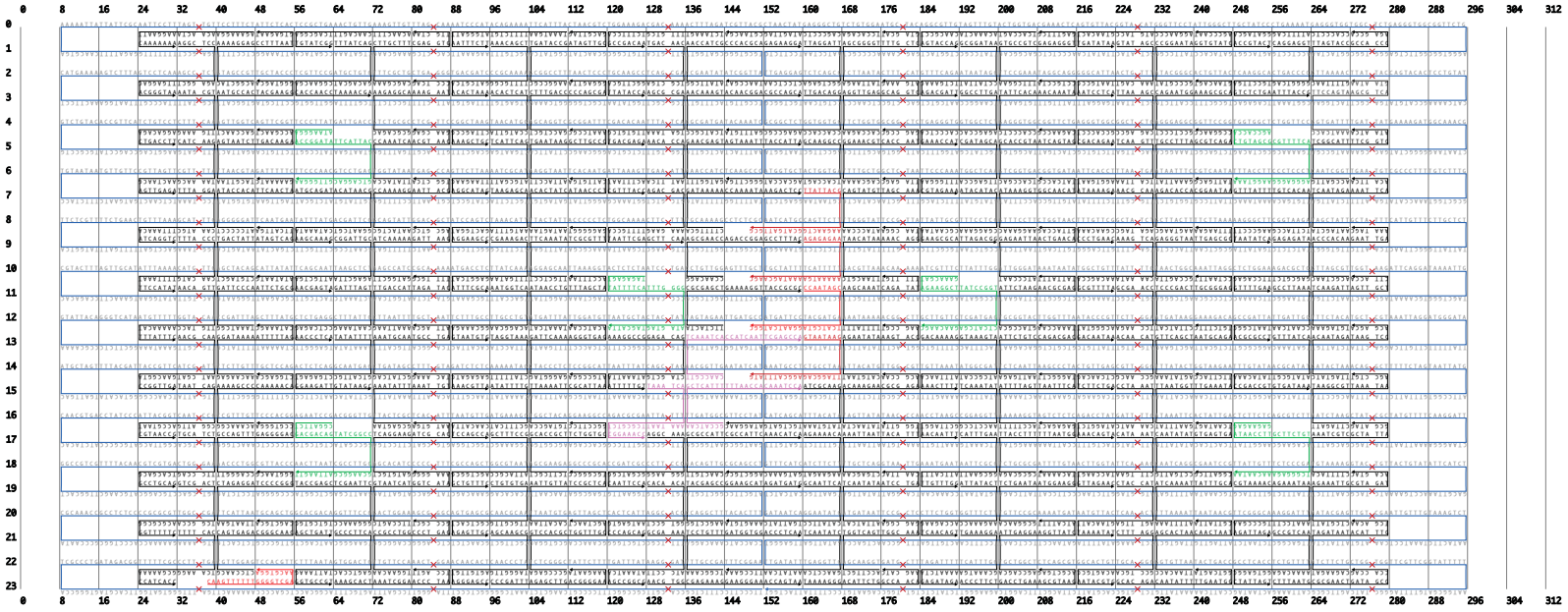
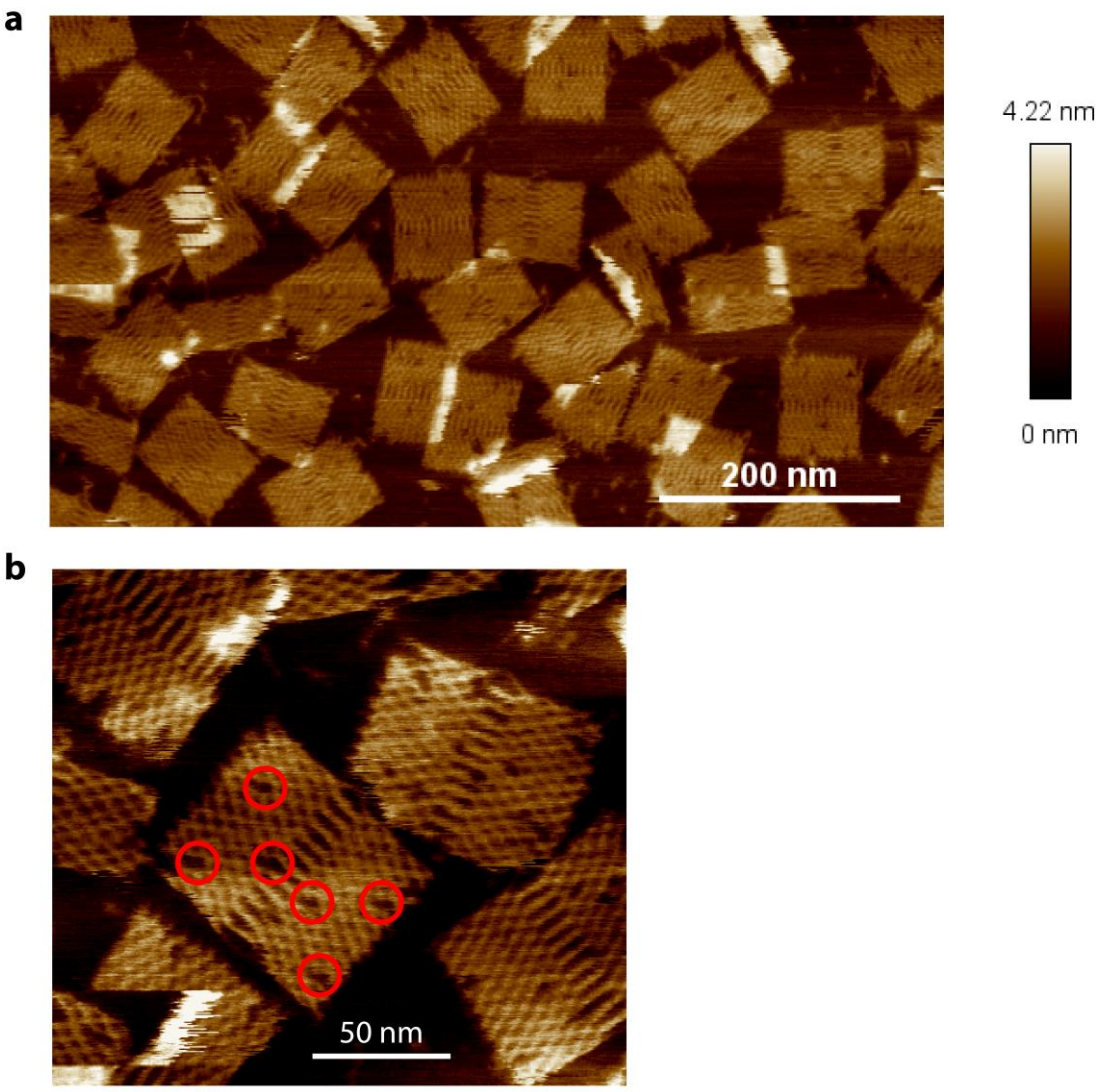
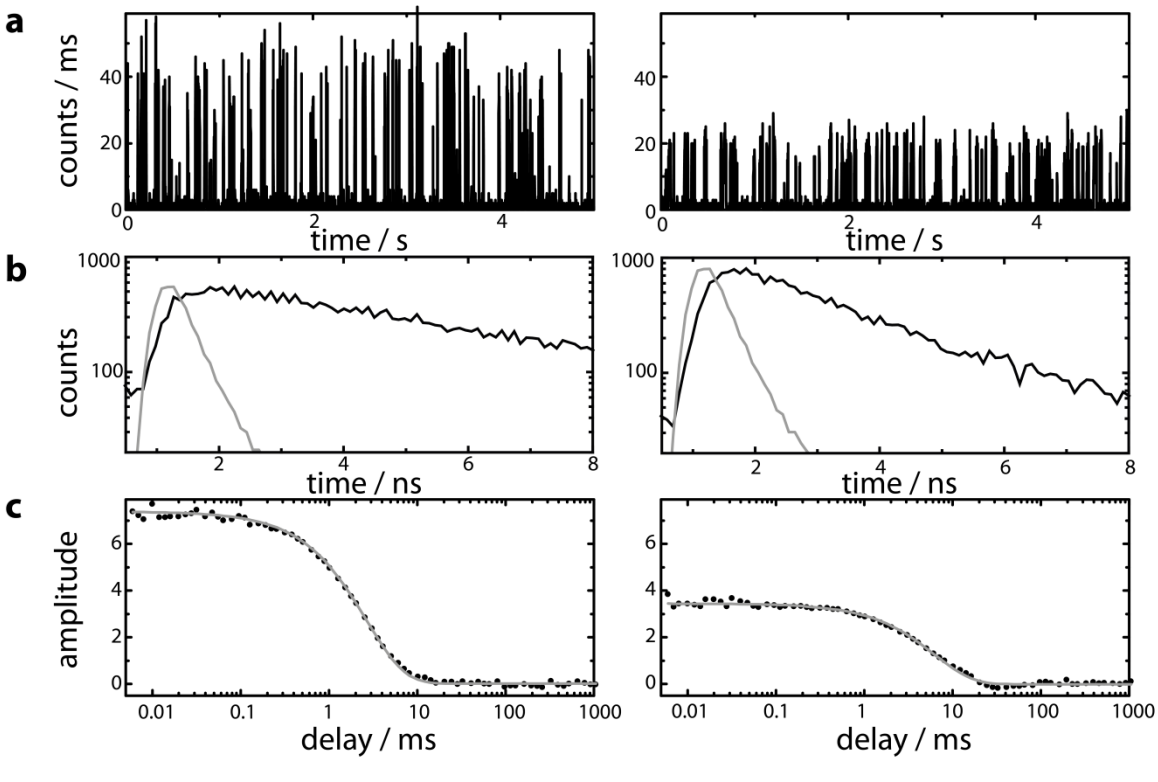


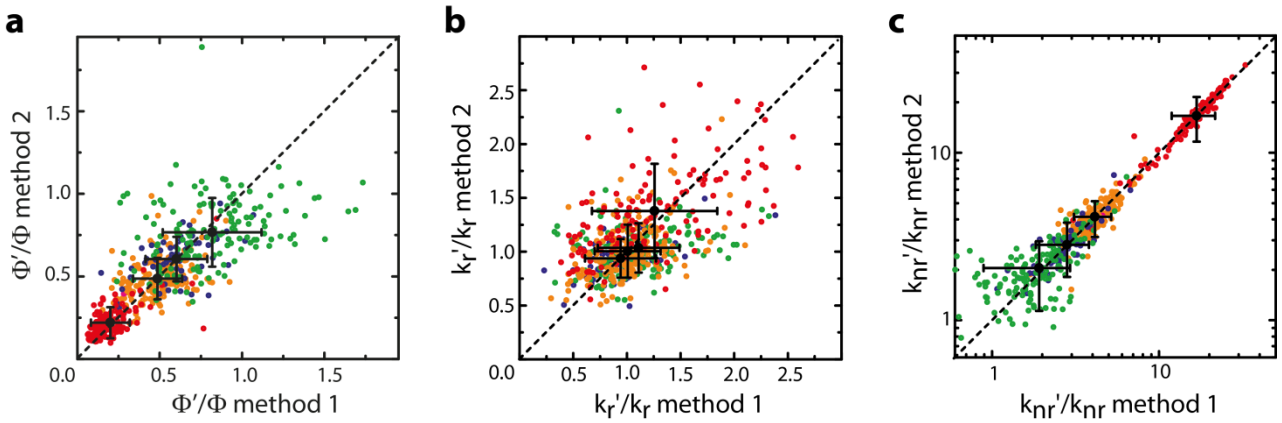
Supplementary Figure 1 | Detailed design of the rectangular DNA origami. The scaffold (blue) is crosslinked into the desired shape by staple strands without modification (black) with biotin t the 5‘ end (green), with a 15A capturing sequence for the NP at the 5‘ end (magenta) and an ATTO647N dye on one of the red staples. The arrows of the individual staples indicate the 5‘->3‘ direction



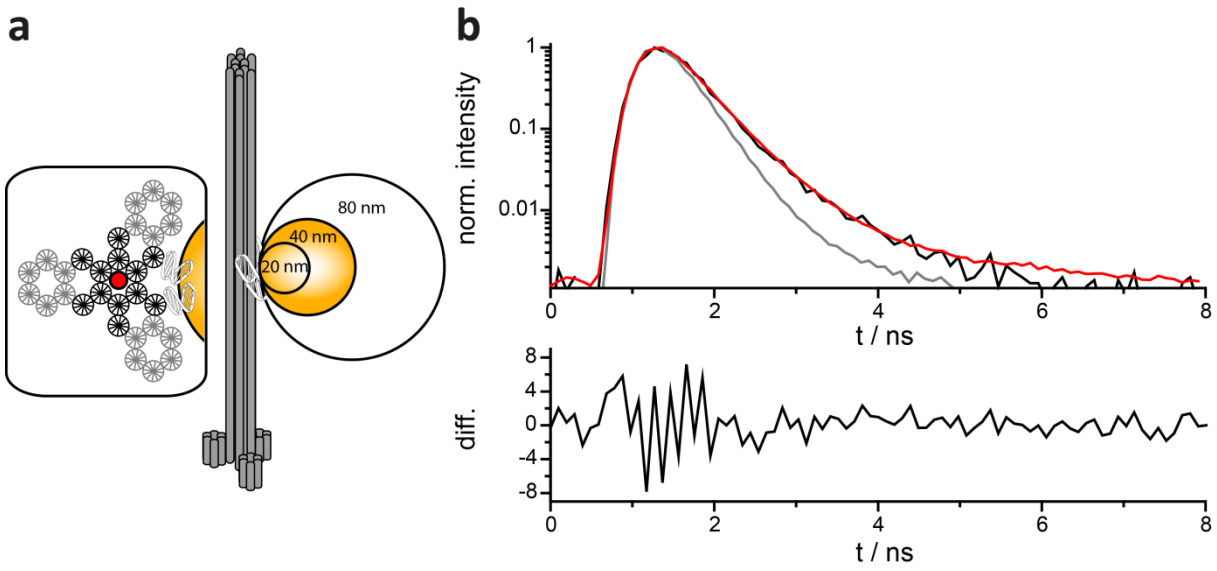
Supplementary Figure 2 | AFM images of rectangular DNA origamis. (a) Overview of area with a multitude of correctly formed rectangular DNA origamis. **(b)** High resolution AFM image of rectangular DNA origamis. The red circles indicate the positions of the six biotin modifications. Since the modified staples are shortened by 5 bases, the positions are visible as gaps in the DNA origami structure.



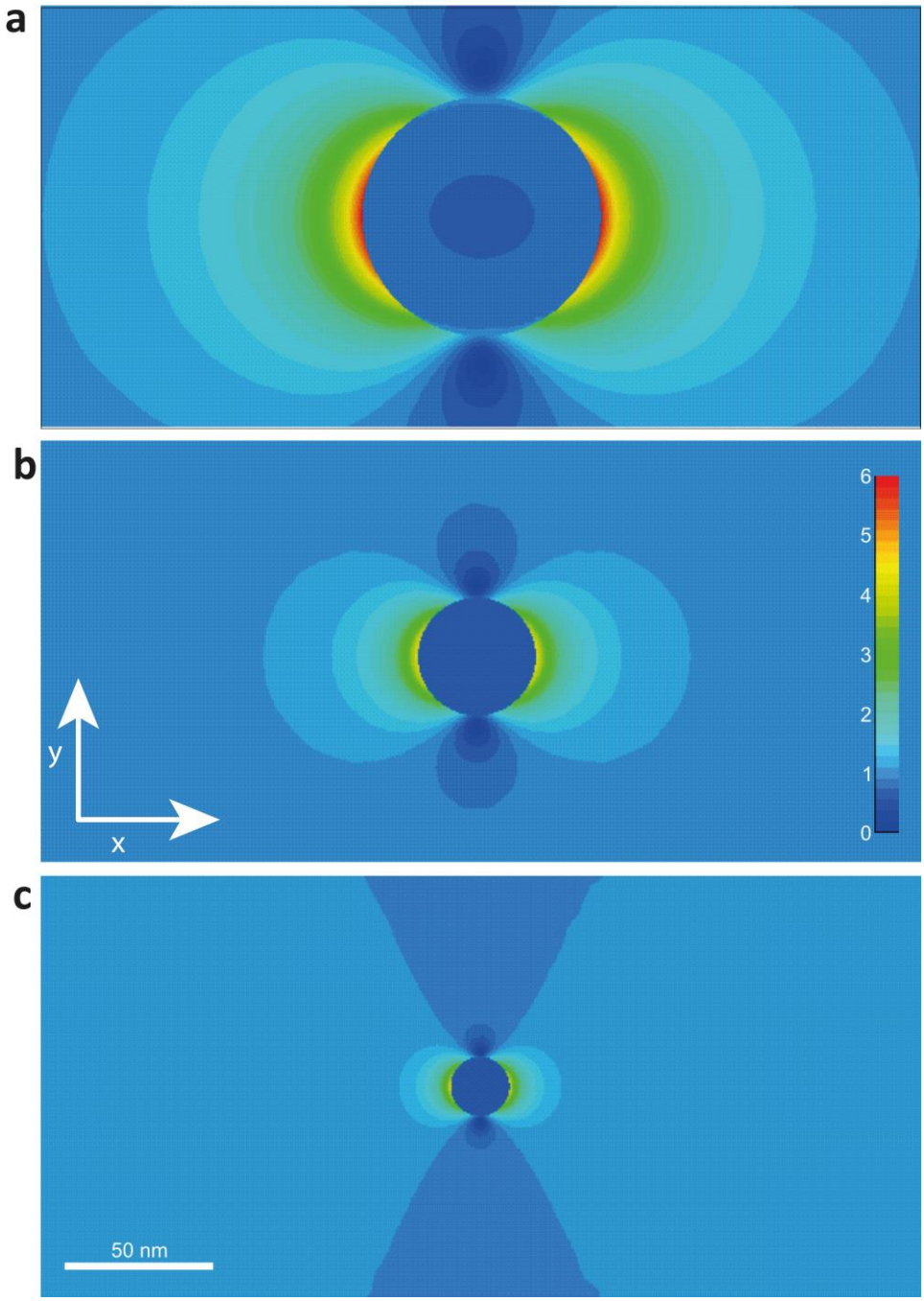
Supplementary Figure 3 | Exemplary fluorescence transients (a) with corresponding fluorescence decays (b) and autocorrelation functions (c) for a dye without NP (left) and with a single 20 nm NP at a distance of 8.3 nm (right). This data is used to extract the parameters τ, I_{on} and t_{on}.



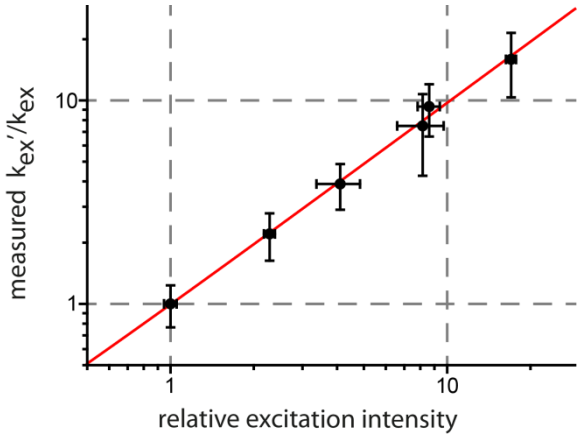
Supplementary Figure 4 | Comparison between two methods to extract changes of photophysical properties. (a) Method 1 assumes constant excitation rate and calculates changes of the quantum yield from $\Phi'/\Phi = I'_{on}/I_{on}$, whereas method 2 uses equation (6) of the main text ($\Phi'/\Phi = (N'_{on}\tau')/(N_{on}\tau)$). **(b)** The radiative rate change is calculated with method 1 as $k'_r/k_r=(I'_{on}\ \tau)/(I_{on}\ \tau')$ or with method 2 and equation (5) of the main text $k'_r/k_r= N'_{on}/N_{on}$. **(c)** In both cases nonradiative rate changes are determined with $k'_{nr}/k_{nr}=(\tau'/\tau - \Phi k'_r/k_r)/(1-\Phi)$ and the intrinsic quantum yield Φ=0.65. Data points represent fluorescent dyes at distances d_1 = 6.6 nm (red, n=140), d_2 = 8.3 nm (orange, n=181), d_3 = 11.2 nm (blue, n=59), d_4 = 15.0 nm (green, n=147) to the NP surface (20 nm diameter) and the respective mean values with standard deviation (black). Dashed lines indicate equivalent results for both methods.



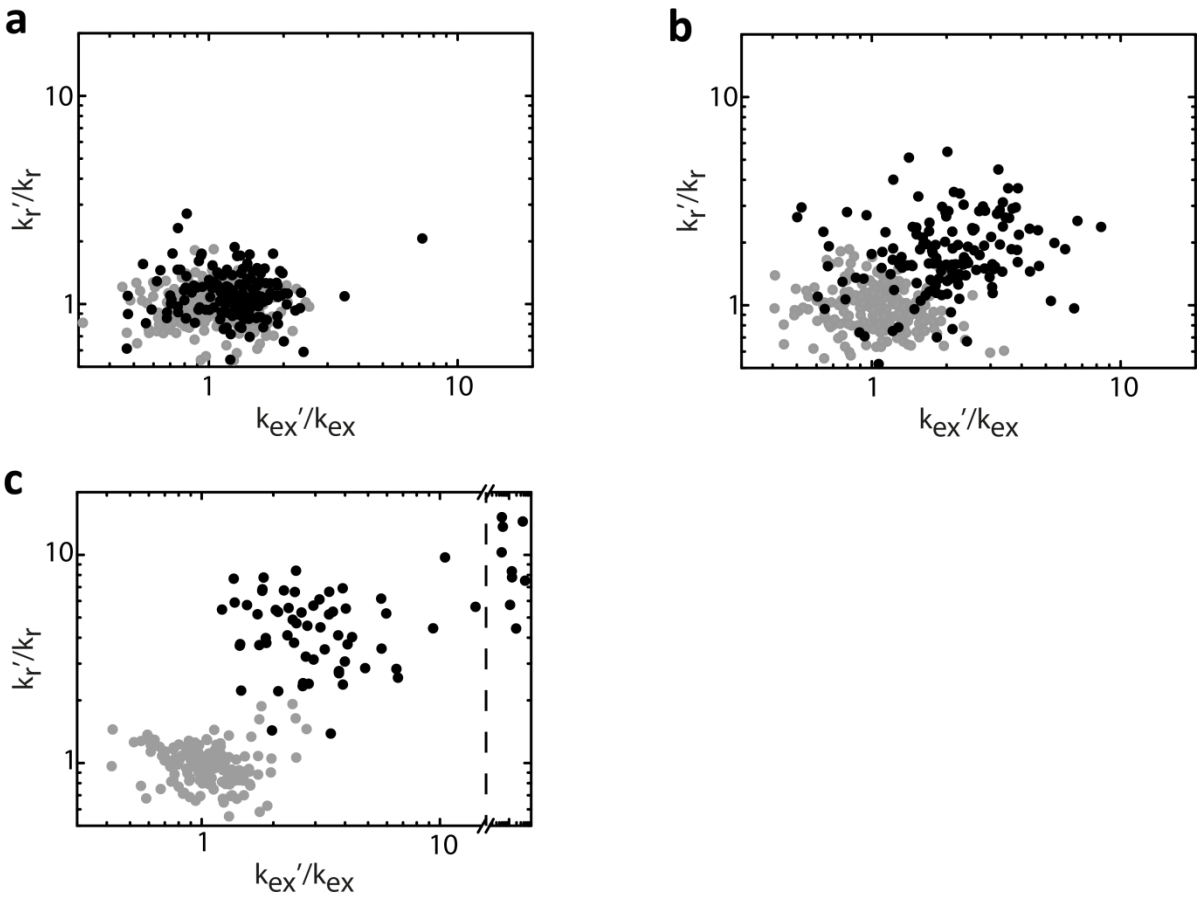
Supplementary Figure 5 | DNA nanopillar sketch and exemplary fluorescence lifetime decay **(a)** Sketch of the DNA nanopillar with the fluorescent dye Atto647N in the center of a 12-helix bundle (inset) and a gold NP of 20, 40 or 80 nm diameter attached to the side of the nanopillar. **(b)** Normalized fluorescence lifetime decay (black) with IRF (grey) and reconvoluted fit (red) for a dye close to an 80 nm Au NP with fluorescence lifetime of 0.46 ns. The lower panel shows the difference (not normalized) between the fit and the experimental decay. This decay is close to the lower bound of lifetimes that could be reasonably fitted.



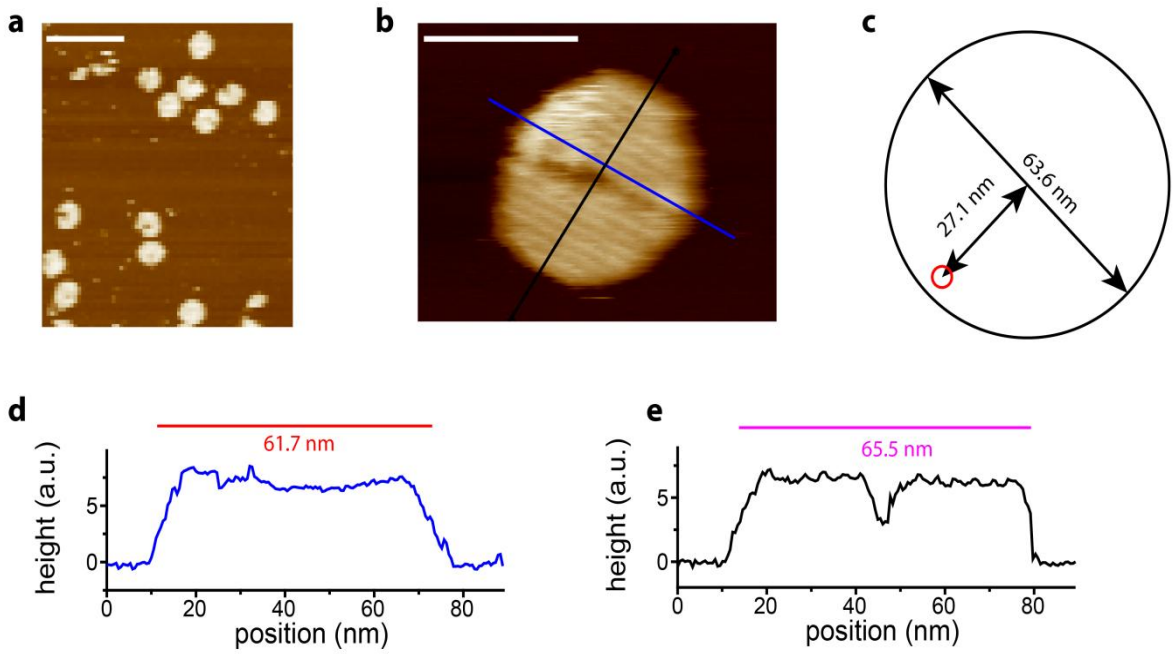
Supplementary Figure 6 | Electric field distributions in the vicinity of gold NPs. Electric field amplitude distribution normalized to the incident amplitude at the equatorial plane of gold NPs of different diameters: 80 nm (a), 40 nm (b) and 20 nm (c). The simulations were carried out with light of 640 nm wavelength polarized in x-direction and propagating in z-direction.



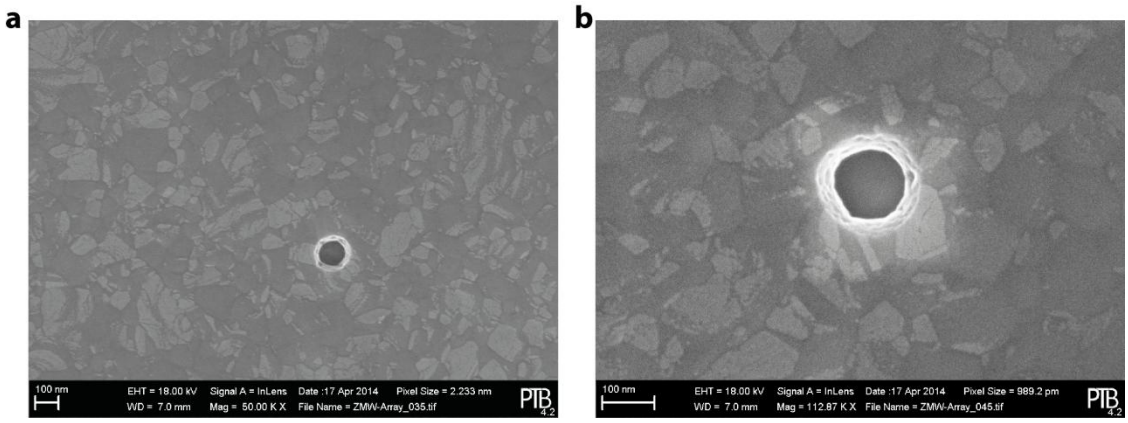
Supplementary Figure 7 | Excitation rate changes can be quantified over at least one order of magnitude. Data points represent the measured change of the excitation rate depending on the laser intensity used (0.29 - 4.89 µW, n=98, 100, 89, 77, 73, 57 with increasing excitation intensity), normalized to the measurement at the lowest laser intensity. Error bars represent the standard deviation of the calculated rate changes and the laser intensity range measured before and after data collection, respectively. The red line is a linear fit to the data which yields an intercept of 0.024 and a slope of 0.970, which proves that the method can reliably detect changes of the excitation rate.



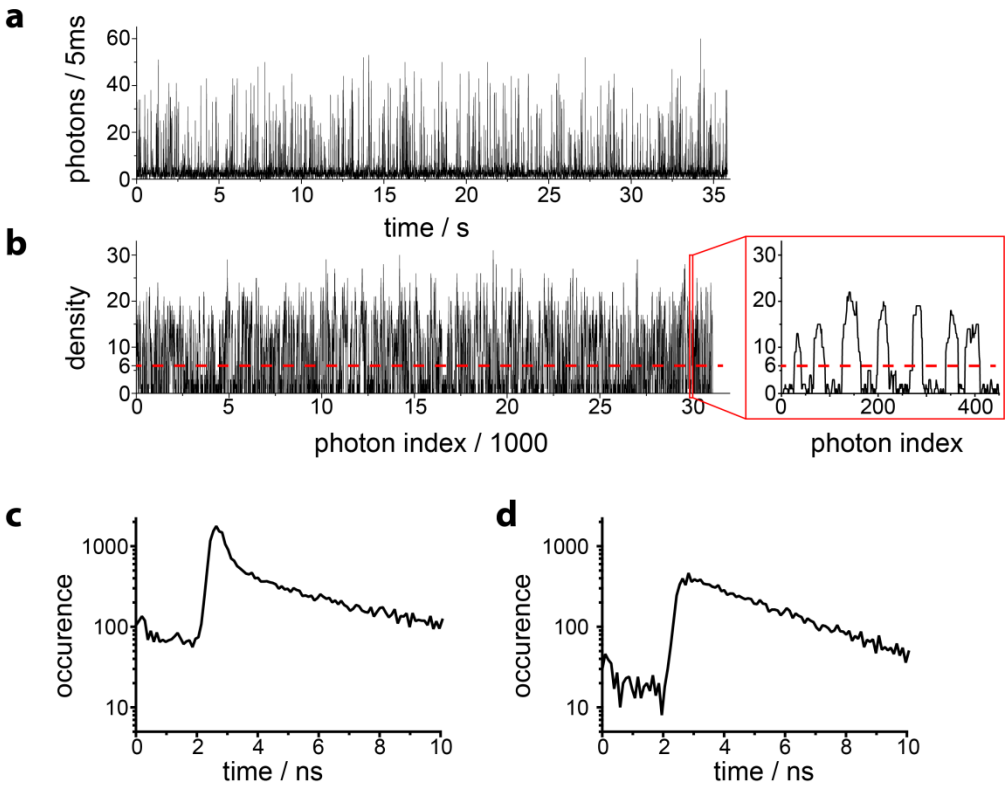
Supplementary Figure 8 | Correlation of radiative and excitation rate with particle size. (a-c) Radiative versus excitation rate constants of molecules with (black) and without NP (grey) normalized to the mean value of the molecules without NP for particles sizes of 20 (a), 40 (b) and 80 nm (c). The data points for one particle size do not exhibit a strong correlation between excitation and radiative rate constant. Data points beyond the break in (c) are not considered for the excitation rate because their fluorescence lifetime could not be determined accurately due to the temporal resolution of our experimental setup. According to the simulations in Figure 4 of the main text, the particle size (and also the distance) should affect both excitation and radiative rate in a similar fashion. Since we observe no strong correlation within the individual samples, we conclude that the size distribution of the gold NPs is not the major contribution for heterogeneous excitation rates. This finding is, however, in agreement with the orientational distribution of the DNA nanopillar as measured with superresolution microscopy [1]. Sample size: n=148, 142 and 69 for NPs of 20, 40 and 80 nm diameter; and n=214, 226 and 154 for the reference molecules without NP.



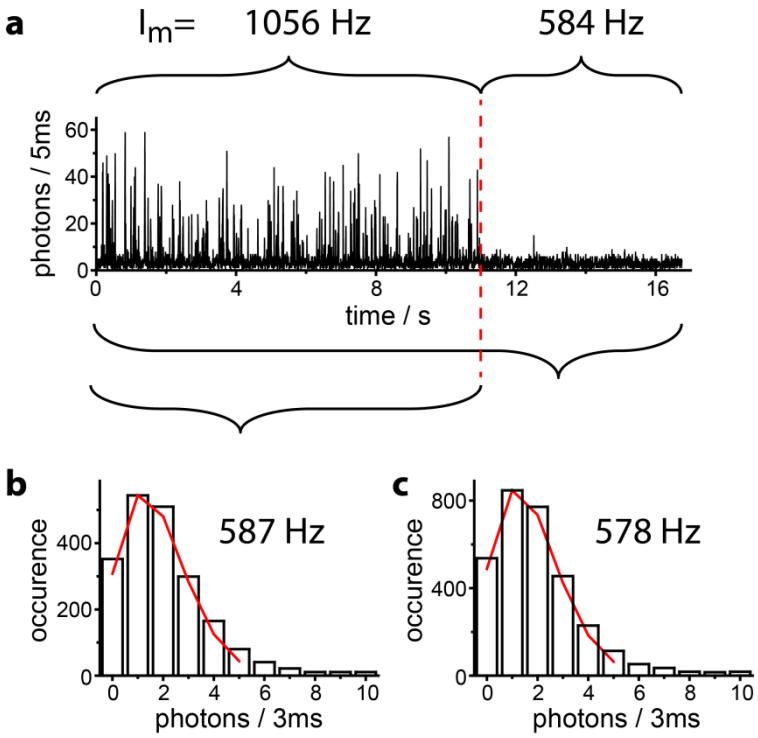
Supplementary Figure 9 | AFM measurements of the DNA nanodisk. **(a)** Low resolution AFM image of several self-assembled DNA nanostructures. Scale bar: 200 nm. **(b)** High resolution AFM image of a single DNA nanodisk. The blue and black lines indicate the cross sections presented in panel d and e. Scale bar: 50 nm**. (c)** Dimensions of the nanodisk used for simulation of the position distribution: the diameter of 63.6 nm is the average of the calculated length along the longest helices (61.7 nm, 0.34 nm/bp) and across the helices (65.5 nm, 2.7 nm helix-helix distance, honeycomb lattice). The distance of the dye to the center of the DNA structure is calculated as 27.1 nm. **(d)** Profile of the DNA nanodisk along one double helix together with red bar indicating the calculated length. **(e)** Profile of the DNA nanodisk perpendicular to the helices together with magenta bar indicating the calculated length.



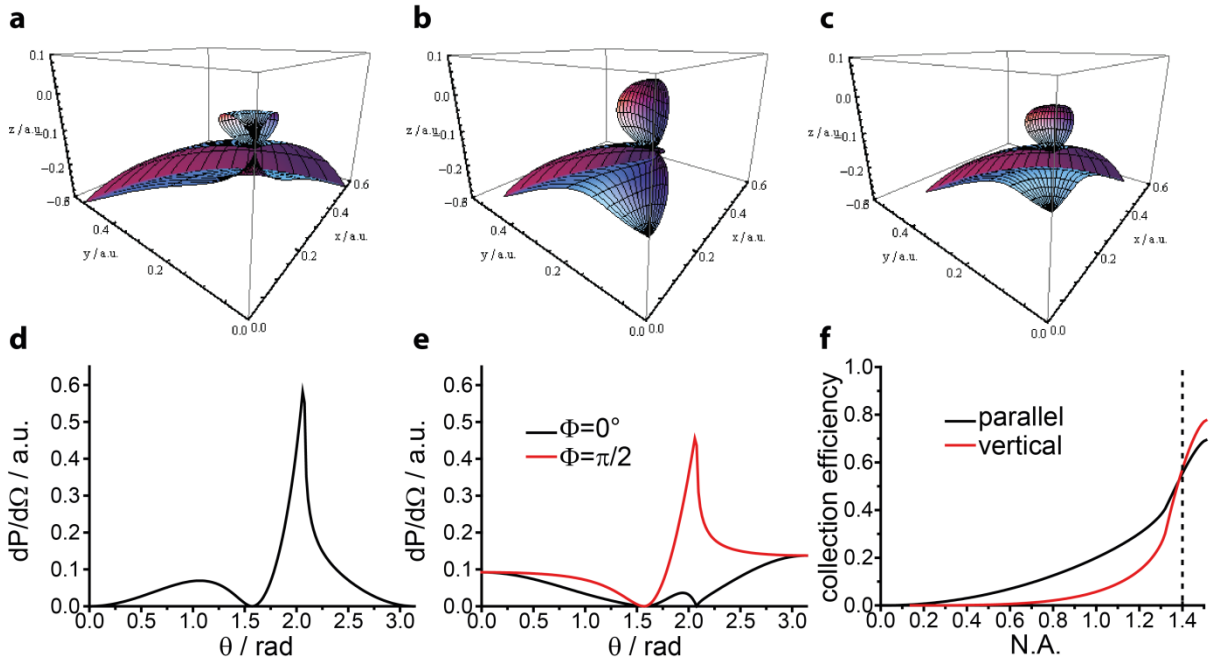
Supplementary Figure 10 | Exemplary SEM images (a) Image of 114 nm ZMW with measured width and height of 116.1 nm and 111.7 nm respectively. **(b)** Image of 136 nm sample with measured width and height of 133.5 nm and 135.5 nm respectively.



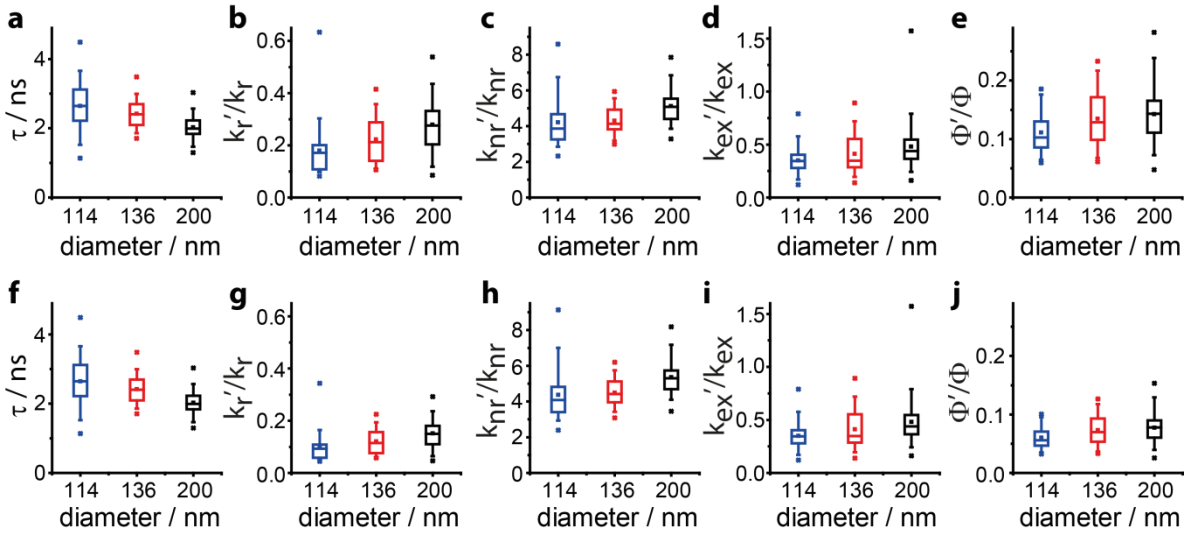
Supplementary Figure 11 | Fluorescence lifetime analysis in presence of high scattering background. **(a)** Fluorescence transient of an Atto647N dye on a DNA nanodisk in a nanoaperture of 114 nm diameter. **(b)** In order to remove photons that are clearly associated with scattering, we determine the number of detected photons in a 2 ms window centered around each photon. This photon density can now be used as a threshold to separate on- and off-times. Unlike intensity thresholding, this approach is insensitive to temporal binning of the fluorescence transient. The right plot shows a magnified range of the photon density of the data in panel (a). The mostly used threshold value of 6 photons per 2 ms window is indicated as dashed red line, in some cases the threshold was adjusted as necessary. **(c)** Fluorescence lifetime decay without thresholding exhibits a large portion of scattering. **(d)** Fluorescence lifetime decay after thresholding as indicated in (b). While the majority of scattering is removed, there is still a scattering component present that needs to be considered for analysis. The fluorescence-to-scattering ratio is however clearly improved.



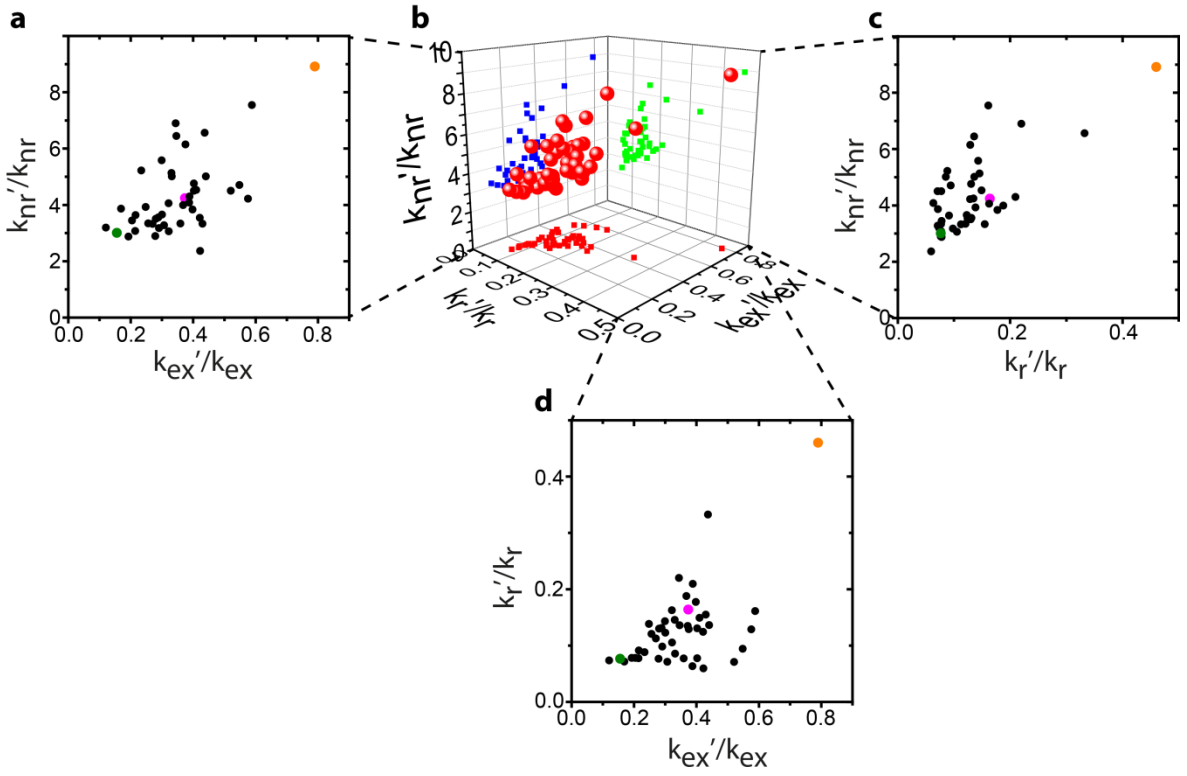
Supplementary Figure 12 | Background determination with poissonian fitting. **(a)** Fluorescence transient of an Atto647N dye on a DNA nanodisk in a nanoaperture of 200 nm diameter. The mean intensities I_m before (1056 Hz) and after bleaching (584 Hz) are indicated. **(b,c)** Intensity histograms of different ranges for 3 ms binning are fitted with a poissonian distribution (red line, numbers indicate mean value of the poissonian). For the fitting procedure both distributions were normalized to their maximum and result in values close to the background intensity after photobleaching. Depending on the background level and intensity of the molecule, the histogram binning and the number of fitted bins were adjusted (1-4 ms and 4-7 bins respectively). The background level for the ZMW measurements was determined by this procedure rather than by measuring the mean intensity after photobleaching, because the lifespan of the dyes in the nanoapertures often exceeded our acquisition time of 30-60 s. The correction of the autocorrelation amplitude for the background intensity is crucial, especially for dim molecules where the average intensity of the transient exceeds the background only slightly.



Supplementary Figure 13 | Determination of collection efficiency of a fluorophore at the glass/water interface with and without ZMW. (a) Calculated emission pattern for a dipole emitter oriented vertical (z-direction) at an interface (z=0) between glass (below, n_g=1.52) and buffer (above, n_w=1.33). All calculations follow reference [2]. **(b)** Emission pattern for a dipole oriented in x-direction (parallel to interface). **(c)** Emission pattern of rotating dye (average of emitters oriented in x-, y- and z-direction). **(d)** Angular power density dP/dΩ (θ) for vertically oriented dipole, with standard spherical coordinates (θ=0 is the z-axis). **(e)** Angular power density dP/dΩ (θ, Φ) for horizontally oriented dipole at selected polar angles. The collection efficiency is calculated by comparing the total emitted power $P_{\mathrm{all}} = \int_0^{2\pi} \mathrm{d}\,\Phi \int_0^{\pi} \sin\theta \,\mathrm{d}\theta \frac{\mathrm{dP}}{\mathrm{d}\Omega}$ with the collected power $P_{\mathrm{coll}} = \int_0^{2\pi} d\Phi \int_{\theta_{\mathrm{m}}}^{\pi} \sin\theta \,\mathrm{d}\theta \frac{\mathrm{dP}}{\mathrm{d}\Omega}$, where θ_{m} is defined by the numerical aperture (in our case NA=1.40). **(f)** Collection efficiency for a horizontal and vertical oriented emitter depending on the NA. For our setup, the collection efficiency of a randomly oriented dipole is η = 54 %. For the ZMW measurements, we make the assumption, that primarily the emission into the upper half space is suppressed, while the emission into the glass remains identical. We therefore only have to relate to the power emitted into the lower half space $P_{\mathrm{low}} = \int_0^{2\pi} \mathrm{d}\Phi \int_{\pi/2}^{\pi} \sin\theta \,\mathrm{d}\theta \frac{\mathrm{dP}}{\mathrm{d}\Omega}$. This gives a collection efficiency of η' = 75 % and therefore the relative collection efficiency α = 0.73.



Supplementary Figure 14 | Photophysical rate changes in ZMWs for extreme cases of collection efficiency. (a-e) Results for the case that the collection efficiency with and without ZMW is identical (no change in the emission pattern, $\alpha = 1$). **(f-j)** Results for the extreme case that all emitted fluorescence is collected by the objective ($\alpha = 0.54$). This primarily affects the calculated radiative rate (b, g) and quantum yield (e, j) and has a minor influence on the nonradiative rate (c, h). The trends between ZMW of different diameter remain, however, unchanged as compared to results presented in Figure 7 of the main text (with $\alpha = 0.73$). Sample size: n=45, 45 and 90 for ZMWs of 114, 136 and 200 nm diameter.



Supplementary Figure 15 | Correlation plots of the photophysical rates for dyes on 114 nm ZMWs. (b) 3D scatter plot reveals correlation between all three relevant rates (n=45). The projections show correlation plots between two respective rates. **(a)** Correlation between the non-radiative and excitation rate. **(c)** Correlation between the non-radiative and radiative rate. **(d)** Correlation between the radiative and excitation rate. The exemplary molecules from Figure 6 (main text) are marked in orange, magenta and olive.

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1[224]	3[223]	GTATAGCAAACAGTTAATGCCCAATCCTCA	30	
22[207]	20[208]	AGCCAGCAATTGAGGAAGGTTATCATCATTTT	32	
6[111]	4[112]	ATTACCTTTGAATAAGGCTTGCCCAAATCCGC	32	
23[160]	22[176]	TAAAAGGGACATTCTGGCCAACAAAGCATC	30	
3[128]	5[127]	AGCGCGATGATAAATTGTGTCGTGACGAGA	30	
13[160]	14[144]	GTAATAAGTTAGGCAGAGGCATTTATGATATT	32	
16[271]	14[272]	CTTAGATTTAAGGCGTTAAATAAAGCCTGT	30	
16[111]	14[112]	TGTAGCCATTAAAATTCGCATTAAATGCCGGA	32	
22[175]	20[176]	ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA	30	
14[175]	12[176]	CATGTAATAGAATATAAAGTACCAAGCCGT	30	

21[160]	22[144]	TCAATATCGAACCTCAAATATCAATTCCGAAA	32	
5[96]	7[95]	TCATTCAGATGCGATTTTAAGAACAGGCATAG	32	
0[79]	1[63]	ACAACTTTCAACAGTTTCAGCGGATGTATCGG	32	
18[271]	16[272]	CTTTTACAAAATCGTCGCTATTAGCGATAG	30	
3[32]	5[31]	AATACGTTTGAAAGAGGACAGACTGACCTT	30	
17[160]	18[144]	AGAAAACAAAGAAGATGATGAAACAGGCTGCG	32	
3[192]	5[191]	GGCCTTGAAGAGCCACCACCCTCAGAAACCAT	32	
13[120]	15[127]	AAAGGCCGGAGACAGCTAGCTGATAAATTAATTTTTGT	38	
22[79]	20[80]	TGGAACAACCGCCTGGCCCTGAGGCCCGCT	30	
5[160]	6[144]	GCAAGGCCTCACCAGTAGCACCATGGGCTTGA	32	
14[79]	12[80]	GCTATCAGAAATGCAATGCCTGAATTAGCA	30	
10[111]	8[112]	TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT	32	
20[47]	18[48]	TTAATGAACTAGAGGATCCCCGGGGGGTAACG	32	
4[175]	2[176]	CACCAGAAAGGTTGAGGCAGGTCATGAAAG	30	
0[239]	1[223]	AGGAACCCATGTACCGTAACACTTGATATAA	31	
11[32]	13[31]	AACAGTTTTGTACCAAAAACATTTTATTTC	30	
1[96]	3[95]	AAACAGCTTTTTGCGGGATCGTCAACACTAAA	32	
13[224]	15[223]	ACAACATGCCAACGCTCAACAGTCTTCTGA	30	
6[175]	4[176]	CAGCAAAAGGAAACGTCACCAATGAGCCGC	30	
7[192]	9[199]	ATACATACCGAGGAAACGCAATAAGAAGCGCATTAGACGG	40	
0[271]	1[255]	CCACCCTCATTTTCAGGGATAGCAACCGTACT	32	
4[143]	3[159]	TCATCGCCAACAAAGTACAACGGACGCCAGCA	32	
21[96]	23[95]	AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC	32	
7[32]	9[31]	TTTAGGACAAATGCTTTAAACAATCAGGTC	30	
11[256]	13[255]	GCCTTAAACCAATCAATAATCGGCACGCGCCT	32	
17[224]	19[223]	CATAAATCTTTGAATACCAAGTGTTAGAAC	30	
15[32]	17[31]	TAATCAGCGGATTGACCGTAATCGTAACCG	30	
18[239]	16[240]	CCTGATTGCAATATATGTGAGTGATCAATAGT	32	
10[207]	8[208]	ATCCCAATGAGAATTAACTGAACAGTTACCAG	32	
6[143]	5[159]	GATGGTTTGAACGAGTAGTAAATTTACCATTA	32	
9[224]	11[223]	AAAGTCACAAAATAAACAGCCAGCGTTTTA	30	
0[175]	0[144]	TCCACAGACAGCCCTCATAGTTAGCGTAACGA	32	
2[143]	1[159]	ATATTCGGAACCATCGCCCACGCAGAGAAGGA	32	
9[96]	11[95]	CGAAAGACTTTGATAAGAGGTCATATTTCGCA	32	
13[256]	15[263]	GTTTATCAATATGCGTTATACAAACCGACCGTGTGATAAA	40	
14[271]	12[272]	TTAGTATCACAATAGATAAGTCCACGAGCA	30	
7[128]	9[135]	AGACGACAAAGAAGTTTTGCCATAATTCGAGCTTCAA	37	
4[47]	2[48]	GACCAACTAATGCCACTACGAAGGGGGTAGCA	32	
12[175]	10[176]	TTTTATTTAAGCAAATCAGATATTTTTTGT	30	
16[239]	14[240]	GAATTTATTTAATGGTTTGAAATATTCTTACC	32	
4[111]	2[112]	GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA	32	
10[175]	8[176]	TTAACGTCTAACATAAAAACAGGTAACGGA	30	

17[96]	19[95]	GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC	32	
14[207]	12[208]	AATTGAGAATTCTGTCCAGACGACTAAACCAA	32	
2[271]	0[272]	GTTTTAACTTAGTACCGCCACCCAGAGCCA	30	
3[224]	5[223]	TTAAAGCCAGAGCCGCCACCCTCGACAGAA	30	
5[192]	7[191]	CGATAGCATTGAGCCATTTGGGAACGTAGAAA	32	
10[79]	8[80]	GATGGCTTATCAAAAAGATTAAGAGCGTCC	30	
13[64]	15[71]	TATATTTTGTCATTGCCTGAGAGTGGAAGATTGTATAAGC	40	
8[111]	6[112]	AATAGTAAACACTATCATAACCCTCATTGTGA	32	
14[111]	12[112]	GAGGGTAGGATTCAAAAGGGTGAGACATCCAA	32	
11[96]	13[95]	AATGGTCAACAGGCAAGGCAAAGAGTAATGTG	32	
17[192]	19[191]	CATTTGAAGGCGAATTATTCATTTTTGTTTGG	32	
magenta				
16[143]		AAAAAAAAAAAAAAAAGCCATCAAGCTCATTTTTTAACCACAAATCCA	47	
15[128]		AAAAAAAAAAAAAAAATAAATCAAAATAATTCGCGTCTCGGAAACC	45	
14[143]		AAAAAAAAAAAAAAAACAACCGTTTCAAATCACCATCAATTCGAGCCA	47	
green				
4[63]	6[56]	ATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAA	40	5’ biotin
4[255]	6[248]	AGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAA	40	5’ biotin
10[191]	12[184]	GAAACGATAGAAGGCTTATCCGGTCTCATCGAGAACAAGC	40	5’ biotin
16[63]	18[56]	CGGATTCTGACGACAGTATCGGCCGCAAGGCGATTAAGTT	40	5’ biotin
10[127]	12[120]	TAGAGAGTTATTTTCATTTGGGGATAGTAGTAGCATTA	38	5’ biotin
16[255]	18[248]	GAGAAGAGATAACCTTGCTTCTGTTCGGGAGAAACAATAA	40	5’ biotin
red				
staples used for individual DNA origamis with dye at five different positions:				
replaces	distance			
7[160]	d1	TTATTACGAAGAACTGGCATGATTGCG	27	3’ ATTO647N
9[160]	d2	AGAGAGAAAAAAATGAAAATAGCAAGC	27	3’ ATTO647N
11[160]	d3	CCAATAGCTCATCGTAGGAATCATGGC	27	3’ ATTO647N
13[160]	d4	GTAATAAGTTAGGCAGAGGCATTTATG	27	3’ ATTO647N
23[32]	d5	TCAAGTTTTTTGGGGTCGAAACGTGGA	27	5’ ATTO647N

Supplementary Table 1 | Structure details for rectangular DNA origami. The design is based on the original rectangular structure (7249 nt scaffold derived from M13mp18), but base deletions were included every 48nt along the length of the structure to correct for twist [4,5].

Start	End	Sequence	Length	Modification
57[98]	59[97]	TAGTAAAACGAGAAATAAGAGAGAGTACCTTTAATCGAAGAC	42	
50[118]	43[108]	CTGAAAAGGTGGCACAATAAATATTTCATATT	32	
19[18]	18[18]	GCATAAAGTGCCACACAACA	20	
43[109]	35[111]	TTAGATCTACAGCAAACTTCGCATCAATAGG	31	
18[111]	7[111]	AATCAAATTATCTCACCTGGACGTTAGTATTCGCATAACGCT	42	
41[109]	28[108]	GAGTTATACCCAACGGCAAAGACATGCTTTCCTTT	35	
34[65]	22[66]	CTGCGTGGCGTCAGGGCATTAAAGCGCTGGTTGCCCTTTGTC	42	
20[149]	13[156]	ATATTTGCTCCCCGTATGGGGTCAAATCCTCTGGCCTTGAT	41	
10[97]	13[90]	AGAGTGAAATCGAAGCGAAAATACATACAACCGAT	35	
53[77]	50[84]	GAAACAAACCTACCAATATCAAACCCTAAGCCAGC	35	
38[34]	45[34]	TCAGTGACGTTGTAAGACAAAAGATTAA	28	
22[34]	29[34]	GCGGGGATTTTCTTCTTTCCATCTAAGA	28	
29[35]	30[35]	ACGCGAGGTAGGAATTGTTCCTCACCCA	28	
60[132]	52[129]	GCAAAGCGACTATTCCCTCAACAATACTGAGA	32	
6[97]	9[90]	TATTATCGGTTTTGCAGAGGGAAGGTAATTACCAT	35	
33[56]	34[56]	CAATAATTAAACAACGGCGAAGCAAGTG	28	
1[84]	9[76]	TCCATACTCAATGAGTTAGAGTCTGAGCTCCGGTGTGAAACCCACCAGT	49	
53[67]	41[66]	TACAAATCGCTTGAATTTGCTTCTATCAAAAAACTATAGTGA	42	
19[112]	23[125]	TTGGTAAGGAGGTTGAACCGCCACCCTCCATAGTTGAAGACG	42	
9[77]	6[83]	AGCACCAATATTGAAGTCTAATGAAGTCTTGCAGAC	36	
32[128]	25[129]	CGTAAACAGCAAAAAAGCTGTATGCAGCCCTAGAG	35	
46[62]	53[55]	CCCTTCTGACCTGATAGCCCTCGAATTATACAGTA	35	
50[128]	42[126]	GGGCAAAGAATACTTTTGTAATGTAGAGGGT	31	
59[60]	51[66]	AGTATTAGACATAATACTATCTTTTTGGCAACTTG	35	
53[140]	56[133]	AGCAACAAAAACCAAGATACAGTAGCTCAACATGTATTGCTGTGAATCC	49	
18[97]	21[90]	GAGCAGCGACGCAGTCAACATAAAAACAATAGCTA	35	
30[139]	37[139]	CTGGCGATTCCGGCTTTTGCGAGACTTT	28	
8[107]	3[122]	CCAATCACCGCGTTTGCGTAGCGCGTTTTCATCGG	35	
23[126]	14[126]	CCTGGTCACTGCGTGATTGTGGTGTTGAGTATTGCTTATCAA	42	
34[55]	41[55]	TAGCGGTCTCGTTATGCGTTAGAAATAC	28	
1[60]	0[60]	GTTGTGCAGAAAAAAGATTG	20	
61[119]	59[132]	TTACCCTGGATTGCCGAGCTTCAAAGCG	28	
9[91]	10[98]	TAGCAATAATCAAACCAGAACTAAAAGATCAAAGG	35	
34[97]	37[90]	TTTGAGGGCTCATTAACGGAATCATAATAAAGTAC	35	

25[35]	26[35]	AATAAGACGAGCGTTTCACCACTGTTTG	28	
17[119]	18[112]	CAGGAGTTATTTCGAGTAAGCGCTTGAAACAGCAA	35	
50[139]	57[141]	TATTTTCGACCATTAAATAGCGCGGAATCG	30	
51[67]	46[63]	CTGAGGTGAGCGCCATTGATAGAA	24	
34[139]	41[139]	ACGTTGGCGTCTGGTAAAACAATTGTGT	28	
53[34]	52[31]	AACGTCAGGAAATTGCGTA	19	
10[118]	17[118]	CGCTGGCAACTATCCTGAATTGATGATA	28	
29[161]	30[161]	TCAGCGGGCGAATATTAACCAGGTGCGG	28	
31[109]	19[111]	AGGGATTAAGGTTGTAACCAGGCTTCCGATAGGCAATGCCATGAA	45	
34[76]	41[76]	GCGCTAGGCTAAACAAAAGCCATAAGGC	28	
21[77]	18[84]	AATAGCAGGGAAGCCGCTCACTGCCCTTGTCTCGA	35	
34[183]	41[177]	TCTCCGTGGGAGCGAGACGAAGGAAC	26	
44[166]	53[156]	CTTGCCGGCCGGAAAATCGGTTGTACCGCCTCAGACGGAACAAC	44	
25[140]	26[140]	CATTTTCTAGCATTGTTGTCGCGCAACA	28	
54[83]	61[76]	CAATATCTGGTCAGAGGAGCATATCATCCATTATC	35	
28[107]	21[108]	AATAGACGTTCGATCTAACCCTCATAGTACCGAGG	35	
7[49]	14[56]	GCATACAGAATTATGGTTTACCAGCGCCAGAAACGTTGCCCTCACTCGC	49	
0[97]	3[90]	ATGAGTATGTTTCTTCAGGCCGGAAACGGTTTGCC	35	
26[160]	33[160]	TAGAAGAATGCGTAATAATTTACAATGA	28	
32[23]	20[22]	TCAATCCCATCGCGCCCCCGGTATGAGCCTATTTATCCATTGAG	44	
3[63]	1[83]	AATCAGTAGCGACAGAATCAATCACCAATCTTGTC	35	
49[119]	50[119]	TAAGAACAGTTGAGGGCAAGGGCGCGAG	28	
53[119]	54[119]	GGAATTATTTTGCAAGTAGATATGCAAC	28	
21[46]	8[42]	TAACAGAAGGCAAAAGAACCACGGTTCATATCACCGTC	38	
37[13]	38[15]	AATCGCCAGCTCAACATGGTTGGAGTAA	28	
30[160]	37[160]	GCCTCTTCGGAAACTTGCAGGTCCATTA	28	
54[118]	61[118]	TAAAGTATTGCGGAAAACAGTCAGGTCT	28	
58[82]	60[65]	GAGCCGTCAATAGTTTACAACGAACGTTATTA	32	
48[107]	41[108]	CCAACCTTATATTTCAAAGAGTAACGCATAGAACG	35	
51[35]	42[45]	GCAGCAAATGAAAACACCGCCGAACTGAAAGCGTAATTATTTCAGC	46	
29[140]	30[140]	GCTAAACTTGAAAATCGCATTACGCCAG	28	
33[140]	34[140]	CGATAGTAGGCCGCACCGCTTATAGGTC	28	
3[91]	6[98]	TTTAGCGTCAGACTCATCTTTTTACATAGGTTGAG	35	
25[56]	26[56]	TAACGTCCCTGAATAGCTGATTTGCCCC	28	
28[44]	21[45]	ATCGCGTTTTACGCTAAAACGATTCCTGAACGAGT	35	
53[91]	54[98]	TTCGCAATGCAGATAGAGGGGTGATTCCTGGAAGT	35	
41[35]	42[35]	AAATTTAAATCGCAGCAATACTGCAACA	28	
26[55]	33[55]	AGCAGGCAACAAGATTTTTATAACCAAT	28	
52[128]	45[129]	GGCCGAGGCAATTCATCTGGCTCAAATTGGGGGAT	35	

37[56]	38[56]	CATTTTCTATCATAGAATCAGGCCAGAA	28	
31[130]	26[119]	GCTAAGGGGGCCAAGCTAAAAATGCGCCGCC	31	
21[140]	22[140]	GCGGGGTGCCCGGATGCTGGAATTAACT	28	
13[91]	14[98]	TGAGGCCACCAGAGCCAGTAACAAGACCAGAGTTT	35	
17[31]	18[35]	AACGGAATACCAAACCGAGGAGATGATCCGCT	32	
31[46]	22[45]	CGGTGAACCAAGTTTGGGAAAATCGTGAGACGAAT	35	
29[119]	30[119]	GAATTTTGCTCCAAGCCAGTGATGTGCT	28	
34[34]	41[34]	CCACCACGAGCACGAGTATAACTGACCT	28	
25[161]	26[161]	CAATAGGCAGTACAGCCAGGATCAGAAA	28	
6[118]	0[98]	GTACTCAAACATCGAACATTGCAAGGAGTTTATAA	35	
21[56]	22[56]	AATAAGAAATTAACTAACTCACCAGCTG	28	
30[118]	37[118]	GCAAGGCAAGATCGAGCAGCGTACAGAG	28	
42[125]	49[118]	AGCTATTTTTGAGAAATGCAATGGTTTAGCGATTT	35	
30[23]	22[18]	TTGGATAGGGTTCCGAAGGGTGGTGAGGCGGTTT	34	
45[91]	46[98]	TTTTTCCTTCATCACTTTAATCCTCATAACGCAAG	35	
30[149]	19[150]	ATTCACCCTCATTACAGGACTTGTGAGCGAATGAA	35	
22[139]	29[139]	CTCAGGCGTTCAGGCCACAGAGGATTTT	28	
46[34]	44[23]	GTGGCACAGTCTTTCTGAGCAAAAGAAGAAACATCTCCTT	40	
6[143]	13[139]	GAGTCTGTAGTGTCGTCTCGCCACCGCAGGTC	32	
22[118]	29[118]	CAGAGCAGATGAACAGCGTAAAGTAAAT	28	
29[12]	30[9]	AAGGCTTATAATAGCAAGCCCGAGGGTCGAGGTGC	35	
46[118]	53[118]	AAGCCTTTCATACAATTTAGGGCCAAAA	28	
21[109]	8[108]	CTGTTATTCTGGCTTTTTACCGTTCCGCCGCGCCG	35	
37[35]	38[35]	AACATGTTCTTACCTATAACGTTTATAA	28	
17[67]	2[63]	CCTATATAAAAAAGACATATTCATGCAAAATATCGATA	38	
45[140]	46[140]	CAAATCACAGAACGAAGATTCTTATGAC	28	
49[35]	50[32]	CAAAATTCAATTACAATGCGCTGCAACAGTG	31	
41[91]	42[98]	AACACACAAAGTACGCTGGCTAGTCTGGAAAGGCT	35	
11[126]	6[119]	TTACGCACATATGAATTTCGCGTCAGATGATGACC	35	
39[25]	30[24]	AACGGCCACCCTTTGACACCCGCCAGGGAGCTTTT	35	
50[151]	39[150]	CCTGTCAATAAAAAAAACAAAAAGGGTAGCTGATGTC	37	
33[14]	34[10]	AAATAATACAATAGACCCTAAGCGCTTAATGC	32	
18[34]	25[34]	CACAATTTAAAGCCGAGGGTACAATCCA	28	
17[77]	14[84]	CAGTATGTTAAGAACCGGGTACCGAGGAAGAACCC	35	
41[161]	42[161]	TGTTACTAGGGAACACCCCGGATATTCA	28	
29[67]	17[66]	CGGATTTTATAAAAATGACGGGAGGCAAGAACAGATAGGACT	42	
26[184]	33[177]	ACATCATTGAAGGGATTCAACTAACAC	27	
37[91]	38[98]	CGACACGAGGGTAGAAGCGCGTTTTAACTAAATTT	35	
35[112]	39[125]	AACGCCACCAGCGAATTTGTATCATCGCGTGTACATCGATGA	42	
38[160]	45[166]	GATTGTACATATGTCGAACTGAAAGCTGCTCATT	34	

14[83]	21[76]	AATATTACATAACATGGATCCAAGTAAGACAATGA	35	
14[162]	21[166]	TTGTTGTTCCCGTGAAAACAGTGAGTACCAGGCGGA	36	
60[97]	60[98]	CCCGATATTAAATCGGAACAAAGAAAACCATAAATGAGGAAG	42	
14[139]	21[139]	ACTCCTGTAAACTGTAATGCCAGGATTA	28	
59[98]	51[111]	TTCAAATATCGCGTGGATTAGGTCATTTCGGTGTCCAATTCT	42	
41[67]	29[66]	TAATGTTTAGGAGCCAGACGACAACGGCTGTGAACAAGCTTG	42	
38[118]	45[118]	GTTAAAAAAGAGAAGACCAGGTCTTGAC	28	
42[83]	49[76]	GGCCTTGCTGGTAAACACGACATAACCTACCTTTT	35	
50[97]	53[90]	TACTAATTAACAGTGTGGAAGGGTTAGATAACGGA	35	
12[128]	9[144]	AGCGGTTGAGCTCAGAGACCGGAACCGCCTCCCTCA	36	
29[56]	30[56]	CTCCCGACAAGCCGGTCCACTGATGGCC	28	
49[91]	50[98]	ACATATTGTGAATTCATTCAATAACATCTCAATTC	35	
29[91]	30[98]	AAGATCGTCTTTCCTGTATCGTCACGACTTGGGTA	35	
38[55]	43[62]	TCCTGAGATTAGTAGCTGATGTCAATAGTGAATTTGTAAATCCAGATTC	49	
57[77]	54[84]	GATTATAGCGGAATCTAACAACTAATCTCATCAAT	35	
38[97]	41[90]	TTGTTAAGCCTGAGGAAACCTCCGGCTTAAGAATA	35	
42[139]	49[139]	ATGCCGGGTAGGTAAGTAGTATTATACC	28	
18[139]	25[139]	AAACTGCCTGGAACATAGGTGCCACCCT	28	
30[55]	37[55]	CACTACGGGAAAGCCATGTTCGCAGAGG	28	
10[83]	17[76]	TCCCCATTCTGCAACAAAACATGGCAACTATTACG	35	
49[140]	50[140]	AGTCAGGTTACAGGAATTAAGTTAGCTA	28	
19[151]	11[151]	CGGCGCTAACACGAAGTGCCATCCTTGGA	29	
43[168]	34[171]	GACAGTCCAATATGTTGATAACCCAAAATAAATGTGAAC	39	
14[97]	17[90]	CTGCGGCGTATTACGACCGAAGCCCTTTTTAGCAA	35	
25[91]	26[98]	AGAATAGAACCGCCAAGTTTTGTGGCGAATATCTG	35	
45[130]	32[129]	ATTATGAACGCTGATAACTCATCTGGACTAAGGAT	35	
30[34]	37[34]	AATCAAGCCCCGATGCGCCTGCAACGCC	28	
2[123]	10[119]	CCCCCTTATTAGGAACCAGAGCCACCCCACCACATGCTGATGTCTGA	47	
46[97]	49[90]	GATAAAAGTAGCATCTCTGATTGCTTTGAAACAGT	35	
43[63]	34[66]	ACCAGTCTATCCAGCACTTGCACGGTACAGCGGGAGGCG	39	
29[77]	26[77]	TGAAGCCACCGCACACTCCAACGTCATTTTAGTTGCAGCAAG	42	
58[132]	50[129]	GAAGCAAGAGCTTATTTAAATTTAGTTTATTT	32	
30[97]	33[90]	ACGCCAGGACGACAAAAAAGGTAAAGTACCAAGAA	35	
37[161]	38[161]	AACGGGTCTAAAACTCAACATACAGGAA	28	
17[56]	18[56]	TGATTAACCGAACAGAGTCACTGTTTCC	28	
42[97]	45[90]	ATCAGGTTTAGAACCAAATCAATATATGGACTACC	35	

42[44]	31[45]	CATTTCTTTGAAGTGTTTGCTTTCCACGCTGTTGA	35	
21[119]	22[119]	TCAAGAGGTACTCACACCATCGAAGCGG	28	
32[170]	25[177]	TCGCATCGCCAGGAATTAGTGAGATCGTCACAACCCATGTA	41	
17[91]	18[98]	ACGTAGTCATACATGAAACATGAAGGTGACTCAAC	35	
6[82]	13[76]	ATCACGAAGGTGTGTTTATACGGAAATAAAGGGC	34	
22[55]	29[55]	CATTAATGGGCAACCTTACCAAGCGAAC	28	
22[97]	25[90]	ACTGGGGTGTGAGTGTTAGTTGCTATTTTACAGAG	35	
38[76]	45[76]	GACAGGACTGAGTAGTTATATTCATAGG	28	
52[48]	45[45]	AAATAAAATGAATATTCATTTAATTACATTAATTAAGAA	39	
33[35]	34[35]	TACGAGCGCAGAACTTAGAGCCGCGTAA	28	
61[65]	53[66]	AGTAAATATTCCATCCTGAAATTATTCTTT	30	
8[144]	17[139]	AGAACTCACAGCCAGGTGAGTATCTGTCGCTATCCAGAATTAATAAG	47	
55[123]	60[112]	TTAACTCCAACAGGTCATTTAATTATCAAAA	31	
13[140]	14[140]	AGACGATATTAAAGTACGGGGTGGGAAG	28	
25[77]	22[84]	CAGCCTTTGCACCCTGGCCCTGAGAGGTATGCTTT	35	
46[139]	53[139]	CCTGTAATTAGCAATAGAAAGTAGTAAG	28	
42[55]	49[55]	TTACCGCACATTGGGTCGCTATTTAACA	28	
15[161]	25[160]	GACGGAAGAACGAGGATATTGGAAAGAATTGATATGCAAGCC	42	
41[13]	42[19]	AGTTAATTAGAAAACTCACGCAGG	24	
30[76]	37[76]	CCGTCTAAGAAAGGCCAGACGTAATAAG	28	
18[55]	25[55]	TGTGTGAAGTGAGCTGAACACTTTTGTT	28	
26[118]	33[118]	TGAACCAAACGACGAAGGAGCGAGGTGA	28	
33[91]	34[98]	CGGGTTTATCAGCTGCATCGGGTATCGGCTGCCAG	35	
15[28]	22[35]	GTTTTATGGAAACGAACCCACAAGAATTAAAGTCATGGGGTGCCAACGC	49	
25[130]	12[129]	CCATATCACCAAGGATTCCCTGCCGTACTGGGGAA	35	
57[53]	58[60]	CATCAATATATGATTATTCTAAAAATT	27	
41[56]	42[56]	CGACCGTTGTAAATATAACATAACAATA	28	
10[62]	17[55]	GAAATGACTGATACACGATACCAAAGACACTGGCA	35	
26[97]	29[90]	CCACTCATTCCCAGGTATTAAACCAAGTTTAAATC	35	
46[82]	53[76]	TCTGGCCAACAGAAAAAATAAGTTACAATCGGGA	34	
7[112]	11[125]	TGTGAAACCTCAGACAGCATTGACAGGAGCAGTCTGACATCA	42	
37[140]	38[140]	TTCATGAAATACACCCTTCCTATATTTA	28	
22[65]	10[63]	GTGCATTAATTCATAGCAGGAGAAATCCTCGCTCTGTATGTGCGA	45	
61[77]	58[83]	ATTTTGCCTTTGCCACAATTCGACAACTTGAGATTA	36	
34[160]	41[160]	TTGACCGGCTTTCAGAAAGAGTGCTCCA	28	
53[56]	54[52]	ACAGTACTGCACGTGCATCACATCAACAGTTG	32	
0[121]	1[120]	ACATACATTGCTGATACCG	19	
14[125]	21[118]	GCACTGCACTGGTGGGATGGGGAACCTAAGACTCC	35	

49[77]	46[83]	TTAATGGAATACCACCGAACGAACCATAATGGACAT	36	
39[151]	30[150]	AATTAAGCAAGTAGCCATAATGGGCTGGTGCCGCT	35	
21[91]	22[98]	TCTTAAAGTATTAAGCCACCCACCTTTTCATGACG	35	
33[151]	20[150]	CCGTTTCACGAACTTTCACGCCTGAGGGATAAAGT	35	
20[166]	29[160]	AGAGGGACCAGCAGAGAGGTTCGAAGTGCAAGTTAACTACAAACAGTT	48	
9[42]	7[62]	GCCATTTGGGAATTAGAGCCATAAAGGTAATATTC	35	
54[97]	57[97]	TTCATTCCCTTTTGTGCCACCAGAAGGACTTCTGAATAATAA	42	
7[63]	6[49]	CATGAAGTTATGTAGATGAAGGTATAGA	28	
30[184]	37[178]	TGTTGGGAAGTTCGCCAGATATATGTAA	28	
33[161]	34[161]	CAACAACCTGAGGCCAGGCAACGGCGGA	28	
51[112]	55[122]	GCGAACGAAAGAAGGACTGGATAGCGTCATGCTTTTGGC	39	
60[111]	48[108]	AGATTAACAAAAATTCAGAAAATGTTTATTTTGCCACATAACAATA	46	
45[46]	28[45]	GAGCAAATCCATGGTTTTACAAATAATTTAGAGCTAATATGTAGATTTC	49	
13[77]	10[84]	GACATTCATAAAGGTATAGATGATTATTGAACAAA	35	
42[160]	49[164]	ACCGTTCTGAGAAACTGACGAGAAGAAAAATC	32	
52[155]	40[150]	ACGACGATACTATCATAACATTAACGTTGGGAAACACACGTAACACCA	48	
49[56]	52[49]	ATTTCATGCAGAGGAAAACATGCGGTCAGTATTAAATCTAAAAAAACAG	49	
41[77]	38[77]	GTTAAATAGGTTGGGAAGAACTCAAATTATAAAGGGATTTTA	42	
26[76]	33[76]	CGGTCCAAACGTGGTCATCGACTTTCCT	28	
42[34]	49[34]	GGAAAAAATCGTCTCTTAGAAAAGAAAA	28	
45[77]	42[84]	TCTGAGATGAGTGACAGTAATAAAAGTTCACTATC	35	
40[177]	32[171]	GTCAATCATATAGCCGGCACCAACAAAATACTCGG	35	
41[140]	42[140]	CGAAATCTTGAAAGACTAGCATAAATTA	28	
18[83]	25[76]	ATTCGTAATCATGGTGCGTTGGCATTAGAAAATAG	35	
25[11]	26[9]	AGCCATATTAATTTGCCGGCGCCAATCGGCAAAATC	36	
40[149]	33[150]	ACTCGCGACCGCAAAAGGGAAGTTGAGTTAATGCG	35	
26[139]	33[139]	CAGCAATTTATTATTCTCCAATTGATAC	28	
14[55]	21[55]	GGGGATTCACTTCCAAGTTACGCCCAAT	28	
22[44]	10[38]	CGGCCTAATGAATTGTTATAAATGTATTTTAGGTGGAACGTGCAAAATT	49	
17[140]	18[140]	TTTTAACAAACAGTGAAAGCAAGTAGGG	28	
50[83]	57[76]	AGAAGATAAAACAGAACCTCAATATCAATTGTTTG	35	
34[170]	23[173]	AAAAGCGCCAGGCGATCACAGTTCTTCAGCAGGATCT	37	
45[35]	46[35]	GACGCTGATTTTCCGAAATGGAGAATAC	28	
39[126]	31[129]	ACGGTAAAACGTTATAATTCGTGTAGATGCCA	32	
13[31]	14[28]	TCAATAGAAAAAATAAGTCGAGTACTCTGAACTCGCTAC	39	

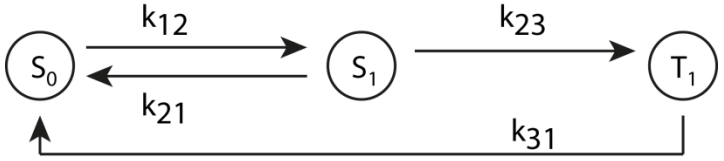
26[34]	33[34]	ATGGTGGTTGAGTGTCATTACCCTAATT	28	
45[119]	46[119]	AAGAACCCTTGAGATGCCTGAGCGGGAG	28	
22[83]	29[76]	CCAGTCGGGAAACCCACCGCCAGCTACAGAGGTTT	35	
45[23]	32[24]	AGCTTGAACGCGTCATCTTAGCCAACTATTTAATTTA	37	
38[139]	45[139]	AATTGTATCGTAAAAGGACAGCATTACC	28	
33[77]	30[77]	TATCATTATTCTGTAAGGGAAGAAAGACGGAAGGGCG AAAAA	42	5'-biotin
33[119]	31[108]	ATTTCTTCACCCTCCACTCCAGGGCGCATCGTAACCGTG CATCCTC	46	5'-biotin
37[119]	38[119]	GCTTTGATTGACCCTCAAAAAATATTTT	28	5'-biotin
37[77]	34[77]	AGAATATTACTAGAAGGAGGCCGATTCAGGCGAAAGG AGCGG	42	5‘-biotin
47[22]	39[24]	GCTATTAGACAATACGCTCACGCTCATAATT	31	3‘-Atto647N

Supplementary Table 2 | DNA origami structure details for DNA nanodisk. The DNA nanodisk is designed from an 8634 nt long M13mp18 derived scaffold and generally consists of four layers in a honeycomb lattice. The table presents the full list of staple strands, modified staple strands are grouped at the end.

Thermocycler Program
80°C for 4 minutes
80°C for 24 sec -0.1°C per cycle 200 times
60°C for 12 minutes -0.1°C per cycle 400 times
5°C for ever
End

Supplementary Table 3. Detailed folding program for the DNA origami nanodisk

Supplementary Note 1 - Autocorrelation analysis with derivation



We consider a three level system with rate constants k_{mn} for transition from state m to state n. The three states are the singlet ground state $S_0(t)$, the first excited singlet state $S_1(t)$ and the triplet state $T_1(t)$. The system is fully described by the following set of homogeneous first order differential equations:

$$\frac{\mathrm{d}}{\mathrm{dt}}\vec{X}(t) = \frac{\mathrm{d}}{\mathrm{dt}}\begin{pmatrix} \mathrm{S_0(t)} \\ \mathrm{S_1(t)} \\ \mathrm{T_1(t)} \end{pmatrix} = \begin{pmatrix} -\mathrm{k12} & \mathrm{k21} & \mathrm{k31} \\ \mathrm{k12} & -\mathrm{k21}-\mathrm{k23} & 0 \\ 0 & \mathrm{k23} & -\mathrm{k31} \end{pmatrix}\begin{pmatrix} \mathrm{S_0(t)} \\ \mathrm{S_1(t)} \\ \mathrm{T_1(t)} \end{pmatrix}$$

Considering that radiative and non-radiative processes occur on a significantly faster timescale than the intersystem crossing, one can assume k21>>(k23+k31). With this, the solution to this set of equations with the molecule initially in its ground state $\vec{X}(0) = (1,0,0)$ can be simplified to:

$$\mathrm{S_0(t)} = \frac{\mathrm{k21\,k31}}{\mathrm{k21\,k31 + k12(k23 + k31)}} + \frac{\mathrm{k12}}{(\mathrm{k12 + k21})}\,\mathrm{Exp}[-(\mathrm{k12 + k21})\mathrm{t}] + \frac{\mathrm{k12\,k23\,k21}}{(\mathrm{k12 + k21})(\mathrm{k21\,k31 + k12(k23 + k31)})}\,\mathrm{Exp}\left[-\left(\frac{\mathrm{k12\,k23}}{\mathrm{k12 + k21}} + \mathrm{k31}\right)\mathrm{t}\right]$$

$$\mathrm{S_1(t)} = \frac{\mathrm{k12\,k31}}{\mathrm{k21k31 + k12(k23 + k31)}} - \frac{\mathrm{k12}}{(\mathrm{k12 + k21})}\mathrm{Exp}[-(\mathrm{k12 + k21})\mathrm{t}] + \frac{(\mathrm{k12})^2\mathrm{k23}}{(\mathrm{k12 + k21})(\mathrm{k21\,k31 + k12(k23 + k31)})}\,\mathrm{Exp}\left[-\left(\frac{\mathrm{k12\,k23}}{\mathrm{k12 + k21}} + \mathrm{k31}\right)\mathrm{t}\right]$$

$$\mathrm{T_1(t)} = \frac{\mathrm{k12\,k23}}{\mathrm{k21\,k31 + k12(k23 + k31)}} - \frac{\mathrm{k12\,k23}}{(\mathrm{k21\,k31 + k12(k23 + k31)})}\mathrm{Exp}\left[-\left(\frac{\mathrm{k12\,k23}}{\mathrm{k12 + k21}} + \mathrm{k31}\right)\mathrm{t}\right]$$

and describes the probability that an individual molecule resides in the S_0, S_1 or the T_1 state at a certain time t after it is reset to its ground state at t=0 [6]. The constant term is the steady state probability, while the terms with decay constants τ_2= (k12+k21)$^{-1}$ and τ_3= (k12 k23/(k12+k21) +k31)$^{-1}$ represent the antibunching and triplet dynamics, respectively.

Following ref. [7], the normalized autocorrelation function (AC) for three states can be defined as

$$\mathrm{G'(\tau)} = \frac{\langle \mathrm{I(t)I(t+\tau)} \rangle}{\langle \mathrm{I(t)} \rangle^2} = \frac{\sum_{\mathrm{m=1}}^{3}\sum_{\mathrm{n=1}}^{3}\mathrm{I_m I_n P(m)P(m,t|n,t+\tau)}}{(\sum_{\mathrm{m=1}}^{3}\mathrm{I_m P(m)})^2}$$

where P(m) is the probability of detecting a molecule in state m and P(m,t|n,t+τ) the probability of subsequently detecting it in state m at time t and in state n at time t+τ. The formula greatly simplifies, since in our case only state 2 (the excited singlet state S_1), is fluorescent ($I_m I_n = I_2^2 \delta_{m2} \delta_{n2}$, with Kronecker delta δ). P(2) is the steady state probability of the S_1 population $S_1(\infty)$ and because after each detected photon the system is reset to its groundstate, P(m,t|n,t+τ) can simply be expressed as $S_1(\tau)$ presented above. This can be summarized to

$$G'(\tau) = \frac{S_1(\tau)}{S_1(\infty)} = 1 - \frac{k12}{(k12+k21)} \frac{k21\,k31 + k12(k23+k31)}{k12\,k31} \; \mathrm{Exp}[-(k12+k21)\tau]$$
$$+ \frac{(k12)^2 k23}{(k12+k21)} \frac{1}{k12\,k31} \; \mathrm{Exp}\left[-\left(\frac{k12\,k23}{k12+k21} + k31\right)\tau\right]$$

$$= 1 - 0 + \frac{k12\,k23}{(k12+k21)} \frac{1}{k31} \; \mathrm{Exp}\left[-\left(\frac{k12\,k23}{k12+k21} + k31\right)\tau\right]$$

$$= 1 + A_3 \; \mathrm{Exp}\left[-\frac{\tau}{\tau_3}\right]$$

We ignore the second term, since these antibunching dynamics occur on shorter timescales than observed in our autocorrelation analysis and therefore $\mathrm{Exp}[-(k12+k21)\tau] = 0$. Because we only measure monoexponential autocorrelation functions, we now omit the index “3” of the amplitude and decay time in the following for simplicity. The parameters of the autocorrelation function generally relate to the macroscopic on- and off-times as follows:

$$\tau = (k_{off} + k_{on})^{-1}$$

$$A = \frac{k_{off}}{k_{on}}$$

$$t_{off} = k_{on}^{-1} = \tau(1+A)$$

$$t_{on} = k_{off}^{-1} = \tau\left(1 + \frac{1}{A}\right)$$

The experimental observables relate to the rate constants from Figure 1 of the main text as

$$k_{off} = \frac{k12\,k23}{k12+k21} = \frac{k_{ex} k_{ISC}}{k_{ex} + (k_r + k_{nr})} = k_{ex} k_{ISC} \tau_{fl} = t_{on}^{-1}$$

$$k_{on} = k31 = k_t$$

Here τ_{fl} is the fluorescence lifetime of the excited state and we employ the low excitation regime approximation ($k_{ex} << \tau_{fl}^{-1}$). The expression for the off-rate equals equation (1) of the main text.

Experimentally, the correlations G (τ) are calculated from the fluorescence transients I(t) as

$$G(\tau) = G'(\tau) - 1 = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2} - 1 = \frac{\langle \delta I(t)\delta I(t+\tau)\rangle}{\langle I(t)\rangle^2}$$

where <> denotes temporal averaging and $\delta I(t) = I(t) - \langle I(t)\rangle$. Compared to the conventional AC definition, we subtract the constant offset of value 1. The AC is fitted with the monoexponential decay model presented above. We multiply the background correction factor C to the amplitude of the decay to account for the influence of uncorrelated background signal[8]. The time-averaged background signal B is determined for each molecule after the fluorophore bleached (NP measurements) or from a poissonian fit to an intensity histogram (ZMW measurements, see Supplementary Figure 10).

$$C = \frac{\langle I(t)\rangle^2}{(\langle I(t)\rangle - B)^2}$$

Together with the mean intensity $I_m = \langle I(t)\rangle$ we extract the number of detected photons before blinking occurs N_{on}. The intensity I_{on} during t_{on} represents the intensity in the fluorescence transient if no blinking was present.

$$I_m = B + I_{on} \cdot \frac{t_{on}}{t_{on} + t_{off}}$$

$$N_{on} = I_{on} t_{on} = (I_m - B) \cdot (t_{on} + t_{off})$$

$$I_{on} = (I_m - B) \cdot \frac{(t_{on} + t_{off})}{t_{on}}$$

While this derivation describes triplet blinking as employed for the ZMW measurements, it is also valid for monoexponential radical blinking used for the NP measurements. When the triplet state is 100% reduced to a radical state (i.e. only single component in AC function), the definitions of the on-time and on-counts do not change. Only the off-time is changed since radical states have a generally longer off-time compared to triplet states, but the off-states are irrelevant for the analysis presented here.

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