Functionalizing large nanoparticles for small gaps in dimer nanoantennas

Carolin Vietz, Birka Lalkens, Guillermo P Acuna and Philip Tinnefeld

Institute for Physical & Theoretical Chemistry, and Braunschweig Integrated Centre of Systems Biology (BRICS), and Laboratory for Emerging Nanometrology (LENA), Braunschweig University of Technology, D-38106 Braunschweig, Germany

E-mail: g.acuna@tu-bs.de and p.tinnefeld@tu-bs.de

Keywords: gold nanoparticles, DNA origami, plasmonics, fluorescence enhancement

Abstract

The process of functionalizing gold nanoparticles with DNA commonly competes with nanoparticle aggregation, especially for larger particles of more than 80 nm diameter. Longer DNA strands reduce the tendency for aggregation but commonly lead to larger gaps when applied in certain geometrical arrangements such as gap nanoantennas. Here, we demonstrate that reversing the polarization of one of the strands for hybridization (yielding a zipper-like geometry) is sterically possible with uncompromised yields. Using the single dye molecule’s fluorescence lifetime as an indicator of the proximity of the nanoparticle in combination with electrodynamic simulations, we determine the distance between the nanoparticle and the dye placed in a DNA origami pillar. Importantly, compared to the common shear geometry smaller distances between the connected structures are obtained which are independent of the length of the DNA connector. Using the zipper geometry, we then arranged nanoparticles of 100 and 150 nm diameter on DNA origami and formed gap nanoantennas. We find that the previously reported trend of increased fluorescence enhancement of ATTO647N with increasing particle size for 20–100 nm nanoparticles is stopped. Gap nanoantennas built with 150 nm nanoparticles exhibit smaller enhancement than those with 100 nm nanoparticles. These results are discussed with the aid of electrodynamic simulations.

1. Introduction

Colloidal gold and silver nanoparticles (NPs), with sub-wavelength dimensions, are known to exhibit collective electron oscillations upon light excitation in the visible range [1]. These so-called localized surface plasmons have been used for a realm of optical applications ranging from biosensing [2] and thermoplasmonics [3] to light confinement [4]. The interaction between light and NPs can be understood in terms of two parameters: the absorption cross section and the scattering cross section [5]. The former, with an approximated \( d^3 \) dependence (with \( d \) the NP’s diameter) is related to the NPs energy absorption and can be exploited for thermo-plasmonic applications. The latter has an approx. \( d^6 \) dependence and it is related to near field effects arising from the NP’s polarization such as the electric field enhancement in the vicinity of the NP’s surface [6]. Thus, while small NPs show a small scattering cross section and a low absorption cross section, large NPs can exhibit absorption and scattering cross sections which are even bigger than their geometrical cross section. Furthermore, when two or more NPs are brought into close proximity, a nonlinear coupling between the NPs occurs leading to a stronger NP polarization and an increment of the electric field enhancement at the NPs gap upon light excitation [7].

In order to exploit these effects and self-assemble functional plasmonic structures, it is necessary to arrange NPs with nanometer precision. Since the pioneering work of Mirkin [8], thiolated single DNA strands (among other compounds such as NHS esters [9] and lipoic acid [10]) were employed to functionalize metallic NPs and build dimer structures. The development of the DNA origami technique [11] and its further extension to three dimensions [12] lead to a breakthrough in the field of self-assembly of...
hybrid nanostructures. Several species such as spherical gold [13] and silver NPs [14], gold nanorods [15], quantum dots [16] and fluorophores [17] were incorporated into DNA origami structures with nanometer precision and precise stoichiometric control. Thus, by employing the DNA origami as a breadboard, complex hybrid structures were fabricated [18]. Among them, DNA origami based antennas [19] gain considerable attention for SERS [20] and for fluorescence applications [21] motivated by the fact that single molecules of interest such as fluorophores can be positioned at the antenna hot spot. In order to further develop these applications it would be desirable to employ large NPs (> 80 nm) and to reduce the inter-particle separation of dimer structures below 15 nm. The problem, however, arises since larger particles have a stronger tendency for irreversible aggregation during functionalization [22]. As NaCl is added during the functionalization process in order to screen the negative charges of the DNA and the gold nanoparticle surface, gold nanoparticles tend to aggregate. Thus, gold nanoparticles precipitate resulting in a transparent solution losing the characteristic reddish color and can not be restored with ultra-sonication. This aggregation tendency has been addressed using longer DNA sequences for particle functionalization (bigger than 20 nucleotides [23]) setting a minimum distance for the inter-particle gap. Two approaches were followed in order to circumvent difficulties [24]: gold NPs were grown after incorporation to the DNA origami structure decreasing therefore the inter-particle gap or a long single-stranded sequence was employed for functionalizing the metallic NPs while a shorter strand (complimentary to only a fraction of the sequence used for the NPs) is employed protruding out of the DNA origami for hybridization. While successful for chirality applications [24], the chemicals involved in gold growing can irreversibly affect the fluorescence properties of dyes and it is not always clear that the grown shell yields the expected shape and plasmonic properties. In addition, the non-hybridized fraction of the DNA sequence employed for the NP functionalization can lead to a rather ‘loose’ binding of the NPs to the DNA origami introducing further uncertainties on the NP position.

In this contribution, we introduce a method for functionalizing and incorporating large nanoparticles to DNA origami structures in order to produce dimer antennas with a reduced inter-particle distance. To the best of our knowledge, we bind for the first time 150 nm gold NPs to a pillar-shaped DNA origami structure. This origami structure is modified with a single fluorophore so that the distance between the fluorophore and the incorporated NP can be estimated based on fluorescence lifetime measurements [21, 25] and a comparison to numerical simulations. Our results confirm not only that larger NPs can be functionalized and incorporated but also that the inter-particle gap can be reduced. Finally and as an example, we build dimer antennas for fluorescence enhancement applications [26].

2. Experimental design

Figure 1 shows a sketch of the dye-labeled (ATTO647N) DNA origami pillar with a single AuNP (monomer) attached through three DNA strands (poly A/poly T). The poly T ssDNA strands are bound to the AuNP with a covalent thiol-gold bond, for further details we refer to the Methods section. The functionalization is realized by adding salt to the DNA/AuNP mixture because the positive ions help to overcome electrostatic repulsions of the two reactants. Still, the salting process might also cause aggregation of the AuNP [23]. Experiments showed that the functionalization becomes more stable when using longer poly T ssDNA [23] while shorter poly T ssDNA leads more often and faster to particle aggregation. This proved true especially for bigger particles from 80 to 150 nm. We failed, for example, in modifying 150 nm AuNPs with 15 nt single-stranded DNA due to nanoparticle aggregation.

Traditionally, the distance between the nanoparticle and the DNA origami structure to which the nanoparticles are hybridized scales with the length of the gold nanoparticle functionalization sequence (upper inset in figure 1). In this shear approach, the nanoparticle is e.g. attached to the 5’ end of one DNA strand and the 5’end of the complementary strand is attached to the DNA origami structure [24, 26]. To reduce the distance between the nanoparticle and the DNA nanostructure we suggest to reverse the polarity of one of the two strands so that they bind in the zipper geometry (see lower inset of figure 1: 3’ end labeled nanoparticle; zipper and shear here refer to the different forces necessary to pull the DNAs apart [26]). This approach might appear less intuitive as it is sterically more demanding. If successful, however, the distance between nanoparticle and DNA structures should be smaller for the zipper than for the shear approach and, importantly, it should become rather independent of the length of the single-stranded DNA used to modify the nanoparticles.

The influence of the ssDNA length (ranging from 20 nt to 25 nt) on the final NP binding distance for the zipper and the shear geometry was studied with two particle sizes of 100 and 150 nm by analyzing the fluorescence lifetime change of a fluorophore bound to the DNA origami (red sphere in figure 1).
fluorophore thus functioned as a probe to sense the proximity of the nanoparticle [25]. Additionally, our results were compared to numerical simulations.

3. Results and discussion

3.1. Monomer measurements

Following sample preparation and immobilization onto a glass surface, confocal fluorescence images were obtained (further details on the confocal measurements and data analysis are included in the Methods section). We employed a rather low laser power (1 μW) to avoid significant heating of the nanoparticles, which could damage the DNA origami structures (see the methods section for a calculation of the nanoparticle's temperature increase). Figure 2(A) includes a fluorescence intensity image with its corresponding fluorescence lifetime image for a 100 nm monomer sample. The intensity image shows spots with different intensities. The corresponding fluorescence analysis is included in figure A1. In agreement with previous works [19], a single spherical gold nanoparticle can strongly affect the fluorescence properties of fluorophores. The size of the particle, the distance between the fluorophore and the nanoparticle, the relative orientation to the incident electric field polarization together with the fluorophore's orientation and intrinsic quantum yield will determine whether the final outcome results in a fluorescence enhancement or quenching [27]. The inset on the top right shows the fluorescence intensity transient for a selected monomer and its corresponding fluorescence lifetime decay (bottom right). The blinking in the intensity transient clearly demonstrates that also the bright spots are related to fluorescence from individual molecules. Since the interaction between a plasmonic structure and a fluorophore also strongly quenches its fluorescence lifetime, the presence of a NP bound to the DNA origami structure can be inferred [21]. We noticed that the binding yield of nanoparticles was typically over 50% not notably dependent on the DNA sequence length or the binding geometry (zipper versus shear).

Monomer structures with unquenched fluorescence lifetime (no AuNP bound) were considered as a reference to calculate the relative fluorescence lifetime of the quenched monomer structures. We used numerical simulations [28] to estimate the NP to dye distance from the fluorescence lifetime changes. Figures 2(B) and (C) show the results of the simulations for different fluorophore-AuNP orientations (tangential and radial) and distances. Besides the radial and tangential orientation, we also approximate an average calculated as two times the tangential orientation plus one time the radial orientation (normalized) [19]. Based on a comparison between the lifetime measurements and the numerical simulations, we estimated the distance between the fluorophore and the AuNP surface (dye-particle distance). The results of
the fluorescence lifetime and its standard deviation for more than 250 monomer structures (per data point) are included in figures 2(B) and (C). For figure 2(B), 100 nm AuNPs functionalized with 20T oligomers and 25T oligomers were bound in zipper (z) and shear (s) configuration to a DNA origami offering three 20A capturing strands. The analogous analysis was performed for the first time for 150 nm AuNPs as shown in figure 2(C).

The results indicate that in every case the zipper conformation leads to lower relative fluorescence lifetimes and therefore closer dye-particle distances. The shear conformation obviously leads to a 0.3 nm larger dye-particle distance compared to the zipper conformation. Although the assignment of an absolute dye-particle distance is challenging, the fact that for the zipper conformation a consistently lower relative fluorescence lifetime is measured indicates that the AuNP is closer to the fluorescent dye as compared to the shear conformation. For the scheme depicted in figure 1, a stronger difference was expected for the two binding modes as one nucleotide corresponds to a DNA contour length of 0.34 nm. Twenty nucleotides, for example would correspond to a distance of about 6.6 nm. Clearly, this distance difference is not reflected in the data indicating that the binding geometry for the shear conformation also occurs at a comparably flat angle probably depending on where the DNA strands binding to the DNA origami structure are located on the AuNP surface. In the case of the zipper (shear) geometry fluorophore-particle distances of around 6 nm (6.25 nm) and 6.5 nm (6.75 nm) were obtained for 100 nm and 150 nm particles respectively. The fluorophore is placed at the center of the DNA origami pillar which consist of a six-helix bundle with an overall diameter of approximately 7.5 nm. Thus, for
the zipper geometry, these results are in good agreement with the distance estimation from geometric considerations for a completely hybridized zipper considering 3.75 nm between the dye and the origami outer surface and one more double helix of approximately 2.5 nm. However, it is surprising that the distance for the shear geometry is only larger by about 0.25 nm, which further supports our interpretation that the binding in shear configuration occurs at a flat angle.

The shear 25T and the shear 20T show the expected tendency of larger NP-dye distances for the 25T oligonucleotides on the DNA (figures 2(B) and (C), data points 20 T s, and 25 T s). As three 20A binding sites are offered by the DNA origami the expected quantitative distance difference is hard to estimate. It will depend on the distribution of the exact binding positions and the flexibility of the single-stranded DNA region. Importantly, for the zipper geometry (figures 2(B) and (C), data points 20 T z, 25 T z) the NP-dye distance is small and uncorrelated to the length of the hybridizing DNA. This supports our assumption that the zipper geometry can substantially simplify binding of large particles in small distance gap antenna configurations.

We also note that the absolute distances between the NP and the dye are about 0.5 nm larger for the 150 nm AuNPs than for the 100 nm AuNPs, which could be due to stronger electrostatic repulsion but the difference might also be within the scope of possible systematic errors in the simulations as the plasmonic properties are drastically changing for the 100 and 150 nm configurations (see below).

Our results represent a step towards the reduction of the interparticle gap of dimer nanoantennas built out of large AuNPs. Based on the symmetry of the DNA origami pillar design another AuNP can be incorporated to form a dimer AuNP antenna [26]. Therefore, the dye-particle distance is a direct measure to estimate the interparticle gap. For 100 and 150 nm dimer AuNP antennas the resulting interparticle gap with the zipper conformation is 12 nm and 13 nm, respectively.
3.2. Dimer measurements

As anticipated in the previous section, we set out to build more complex structures involving two AuNPs forming a dimer nanoantenna. We modified the structure depicted in figure 1 and added three extra capturing strands on the opposite side as in [26] and bound two AuNPs in zipper conformation with 20 nt ssDNA. Confocal fluorescence measurements were performed as in the previous section in order to extract the fluorescence enhancement. First the population with and without AuNPs was identified based on the quenched and unquenched fluorescence lifetime, respectively, and the corresponding average fluorescence intensity of the unquenched species was set as a reference. Thus, for each dimer nanoantenna the fluorescence enhancement was calculated, see figures 3(A) and (B) for 100 nm and 150 nm AuNP nanoantenna, respectively.

In the case of the 100 nm AuNP nanoantenna, we obtained a very high binding efficiency with a maximum fluorescence enhancement of approximately 222 fold. This measurement, which was performed with circularly polarized light, is in good agreement with previous results [26] in which slowly rotating linearly polarized light was employed and thus approximately twice the enhancement was observed. For the 150 nm AuNP nanoantenna, we followed the same sample preparation procedure with a similar optical density (OD) of the AuNP solution. This translates into a lower AuNP concentration, nevertheless we still obtained high binding efficiency (over 50%) even for extremely large 150 nm AuNPs. The maximum fluorescence enhancement is in this case approximately 93 fold. This effect is expected due to the fact that large nanoparticles start to exhibit retardation effects and multipole excitation [1]. In addition, dimer structures of large nanoparticles with a reduced interparticle distance (<15 nm) show a localized surface plasmon resonance peak which is strongly red-shifted [29]. Figures 3(C) and (D) show the simulated absorption and scattering cross section for the dimer structures, respectively. The absorption wavelength peak is not strongly dependent on the particle size. However, the scattering cross section shows a strong particle size dependence. For the 150 nm AuNP, the resonance peak is red-shifted to approximately 875 nm. This is considerable bathochromic compared to the laser excitation wavelength at 640 nm and also not well aligned with the spectrum of the dye used. In most reported experiments, the highest fluorescence enhancement was obtained when the excitation wavelength is slightly red-shifted with respect to the plasmon resonance [6].

4. Conclusions

In this work, we have addressed one of the major challenges for the self-assembly of complex hybrid plasmonic nanostructures: the functionalization and incorporation of large AuNPs with sizes exceeding 80 nm. This is achieved by extending the length of the ssDNA used to functionalize AuNPs in order to avoid NP aggregation. To overcome the expense of a higher interparticle gap distance and position uncertainty when building gap nanoantennas we introduced the zipper conformation. Smaller distances between DNA nanostructures and Au nanoparticles were confirmed in a detailed comparison of both the zipper and the standard shear conformation. Based on fluorescence lifetime measurements we determined that the former approach leads to shorter binding distances, which are independent of DNA lengths. Finally, we assembled dimer nanoantennas with 100 nm and 150 nm AuNPs with an estimated interparticle gap of approximately 12 nm and 13 nm respectively and determined the fluorescence enhancement. In the case of the 100 nm AuNP antennas we measured a fluorescence enhancement of more than two orders of magnitude whereas in the case of the 150 nm AuNP antennas size-related effects such as retardation and plasmon resonance red-shift lead to reduced fluorescence enhancement of approximately 93 fold.

The presented technique enables the precise assembly of spherical AuNP up to 150 nm in size. These particles which show an absorption cross section which is higher than the geometrical cross section can be employed as nano sources of heat in thermo-plasmonic applications [30]. Besides large spherical AuNPs, we believe this approach can be extended to other plasmonic nanoparticles of different geometries and materials such as rods, stars and silver spherical nanoparticles in order to build complex functional nanostructures.

5. Methods

5.1. Gold nanoparticle functionalization

Gold nanoparticles of 100 nm and 150 nm diameter (BBI solutions) were functionalized with single stranded polyT DNA-oligonucleotides of different lengths (15 to 25 nt) incorporating a thiol modification either on the 3' or the 5' end (Ella Biotech GmbH). The functionalization process was modified from Mirkin [8]. 2 ml of
nanoparticle solution were mixed with 20 μl Tween20 (10%, Polysorbate20, Alfa Aesar), 20 μl of a potassium phosphate (4:5 mixture of monobasic and dibasic potassium phosphate, Sigma Aldrich) and an excess of the desired oligonucleotide solution (50 nM, 18.4 μl) and stirred overnight. Afterwards, the nanoparticle oligonucleotide mixture was heated to 40 °C and over an hour salt was added each 5 min stepwise with increasing amounts up to a concentration of 750 mM using PBS buffer containing 3.3 M sodium chloride. For purification the mixture was diluted 1:1 with 1xPBS containing 10 mM NaCl, 2.11 mM P8709, 2.89 mM P8584, 0.01% Tween20 and 1 mM EDTA and spun down. The supernatant was pipetted off and the particle dilution was diluted in the PBS buffer mentioned above. This spinning process was repeated 6 times to completely purify nanoparticles from free oligonucleotides.

5.2. DNA origami
The DNA origami pillar consists of 8064 bp and was designed with the software CaDNAno [31]. p8064 scaffold was extracted from M13mp18 bacteriophage [31]. Staple strands (see table B1) were purchased from Eurofins Genomics. For folding a ten-fold excess of each staple strand was added to 10 nM of the scaffold in 1xTE (10 mM Tris, 1 mM EDTA; pH 8.0) buffer with 14 mM MgCl2. In the annealing process the folding mixture was heated and slowly cooled down, see table B2. Afterwards the folded DNA origami pillar was purified from excess staple strands by Amicon filtering (Amicon Ultra—0.5 ml, Ultracel®- 100 K Membrane, Millipore) washing 3 times with 1xTE buffer containing 14 mM MgCl2 and centrifuging each time at 10 krcf speed for 5 min at 20 °C. To recover the DNA origami pillar the Amicon filter was flipped into a new tube and centrifuged 3 min at 1 krcf speed at 20 °C.

5.3. Sample preparation
The DNA origami pillars were immobilized on a glass surface coated with BSA-Biotin (Sigma-Aldrich) and Neutravidin (Sigma-Aldrich) by the strong interaction of Neutravidin to the Biotins on the base of the DNA origami pillar. The nanoparticle solution was diluted to an absorption of 0.1 on the UV/vis-Spectrometer (Nanodrop 2000, Thermo Scientific) with 1xTE containing 12 mM MgCl2 and 100 mM NaCl. Subsequently, the immobilized DNA origami pillars are incubated with the diluted nanoparticle solution for 48 h at 4 °C. Confocal measurements were performed after washing the sample with 1xTE containing 12 mM MgCl2 and 100 mM NaCl in this buffer.

5.4. Confocal measurement and analysis
A home-built confocal microscope based on an Olympus IX-71 inverted microscope was utilized for single molecule fluorescence measurements. For excitation a 80 MHz pulsed laser at 640 nm (LDH-D-640, Picoquant) was used. The laser intensity was tuned with a variable neutral density filter and circular polarization was achieved using a linear polarizer followed by a quarter wave plate. A dual-band dichroic beam splitter (Dualband 532/633, AHF) is used to couple the laser onto the oil-immersion objective (UPlanSApo 60XO/1.35 NA, Olympus) and the laser is focused to the sample with a final power of 1 μW. The position of the sample can be varied by a Piezo-Stage (P-517.3CL, Physik Instrumente GmbH&Co. KG) resulting in a scan image where molecules can be selected and positioned in the laser focus for time-resolved analysis (see figure 2(A)). Resulting fluorescence is collected by the same objective, separated from the excitation light by the dichroic beam splitter and two spectral filters (ET 700/75m, AHF and RazorEdge LP 647, Semrock) after focussing on a 50 μm pinhole (Linos). The fluorescence signal is detected by a Single-Photon Avalanche Diode (µ-SPAD-100, Picoquant) and registered by a time-correlated single-photon counting PC card (SPC-830, Becker&Hickl). The raw data is processed using custom made LabVIEW software (National Instruments). The molecules were selected by single-step photobleaching transients and the fluorescence lifetime decays of those molecules were fitted monoexponentially and deconvolved from the instrument response function using the program FluoFit (PicoQuant). Unquenched fluorescence lifetimes were employed as a reference to obtain relative fluorescence lifetimes for the quenched species. The distribution of relative fluorescence lifetimes was then histogrammed and fitted with a log-normal distribution to determine the average relative fluorescence lifetime and its standard deviation. For the calculation of the fluorescence enhancement, the intensity values were corrected according to Acuna et al [19] to compensate for APD saturation.

5.5. Numerical simulations
All simulations were performed using a commercial FDFD software (CST). For the cross section of the dimer structures the incident light polarization was set along the dimer orientation. In the case of the relative change of
the fluorescence lifetime, we followed the procedure introduced in [28] and considered the fluorophore’s intrinsic quantum yield according to [32].

5.6. Heating of gold nanoparticles and temperature increase

Gold nanoparticles exhibit a significant absorption cross section in the optical range. Therefore, under laser illumination, the energy absorbed will lead to a temperature increase. According to [33] this temperature increase can be calculated for pulsed illumination as:

$$\Delta T = \frac{\varepsilon}{V/\rho_{\text{Au}}C_{\text{Au}}}$$

with $\varepsilon$ the energy absorbed per pulse, $V$ the nanoparticle’s volume, $\rho_{\text{Au}} = 19320$ kg m$^{-3}$ and $C_{\text{Au}} = 129$ J/(kg K) the gold mass density and specific heat capacity respectively. $\varepsilon$ can be estimated based on the average laser power (1 $\mu$W), the diameter of the confocal spot (~300 nm) and the absorption cross section. In our experiment, we use a 640 nm laser, which is red-shifted from the absorption peak of gold nanoparticles (typically around 550 nm depending on the size). If we consider figure 3(C), an absorption cross section of approximately $1 \times 10^{-4}$ nm$^2$ for the 100 nm dimer structure is simulated whereas for the monomer an even smaller cross section is simulated (around 6000 nm$^2$, not shown). For these values we obtain $\varepsilon < 2 \times 10^{-9}$ J which leads to a temperature increase around 1.5 K for 100 nm particles.

Acknowledgments

This work was supported by a starting grant (SiMBA, EU 261162) and by a proof of concept grant (safeDNA, EU 620300) of the European Research Council (ERC), and the Deutsche Forschungsgesellschaft (AC 279/2-1 and TI 329/9-1). CV is grateful for a scholarship from the Studienstiftung des Deutschen Volkes. We thank Pablo del Pino for fruitful discussion.

Appendix A

Figure A1. Fluorescence enhancement measurements of 100 nm (A) and 150 nm (B) AuNP monomer structures (NP bound with 20 T in zipper conformation) show a maximum enhancement of 34 for 100 nm dimers and 12 for 150 nm dimers.

Appendix B
Table B1. Unmodified and modified staples from the 5’ to the 3’ end for the DNA origami pillar.

| Sequence (5’ to 3’) | Length | Modification |
|---------------------|--------|--------------|
| TTAGGGGTAGTGCCGCAGAATAAGAAATTTCTGCTAGGATA | 42 | |
| TATGACCTTTATACGTATTTTTTTTTATGAAAAAGTACACCCGTT | 42 | |
| AATAAAACGGAACTATGACCCACCACCAAGC | 28 | |
| CTCATGCTGATGAGCAGCCATGAAAACAAATTCTGCTAGGATA | 35 | |
| CCCCACGATACAGGCAGCCAAATTCAAAAAGCTACTAGTACAT | 42 | |
| CTCAATTGCTGAGATTGCAAATTAATGCAAAAATAATATA | 42 | |
| TTTAGCTGATACCAACGGCGTTA | 21 | |
| AAAGAGCTTCTGATACCCCCAGCATACGCCACAGCTGCTGAC | 42 | |
| CCCCGCTAGGGCAACAGCTGCGGAAGGGAATTGCAGGATAA | 42 | |
| GCCGATAGCTAGGCGGAGAG | 21 | |
| CTTTGCCAAATTTGATTCAGGGAACGAGATTGCAATGCCC | 42 | |
| TTAAATTGCGAGGAAATGTT | 21 | |
| AAAGATGATAGAGACGAGGGAGAGATTCATACTGCAATGAAACCA | 42 | |
| ACGAACATACAGGGCTTCTTATAGAAGACTCACTCGGAGATAA | 42 | |
| GCCGATAGCTAGGCGGAGAG | 21 | |
| TGCTGCCCAGGATCAMTAGATCGATTTGAGAT | 31 | |
| TAGCTCGAAGCATACCCGTT | 21 | |
| AGCAAAAAATGCAAGAAATATCCAAATCTGCCGTCAGGGA | 42 | |
| AGCGTCTGGCAAGACTCCCTCAAGAAGAAAAGATTTCCGGAAC | 38 | |
| ACCTGACGCGGAAAGCCGCCGACCAATAAGTCTGCCGCTGTCG | 42 | |
| CCGTAAATCTGAGATCCAGAACATCTTAATTATTCATTCATAAAGG | 42 | |
| GAACTGCGCTATTACAACCTTATCTCTTGAGATTTACTTA | 42 | |
| GAGTTAAAGGGTATGATGCGCCTATATCGAGGAACTGAACCC | 46 | |
| AGTTTCCACACATTATACATTTTAC | 25 | |
| ACGCGAGAGAAAGGCATATAATGATTGCCCCGCTTAAATTGAGAAATTCGC | 48 | |
| ACTAATGGCAGATACGAAATCAA | 21 | |
| CTGTATGGGATTACCGTTATCA | 25 | |
| TAGCTACGTTTCTACCAAATTAACGCT | 28 | |
| CTCGTTTATCCAGAAACAAA | 21 | |
| TTACGCCCAGAGGAAACACCCAGAAAATGCGGTAGAATTATTTAA | 45 | |
| TGAGTTAAGGATAAGTTTAGCTATATCATAGACCCATTAGATA | 42 | |
| Sequence (5’ to 3’) | Length | Modification |
|---------------------|--------|--------------|
| ATTTCCCTATTACATGATGGCTTTAAAGAGCCTAAAA | 42 |          |
| CCAGCCCTCGATCTCATCGGGA | 24 |          |
| ATGAAAGGCTAAAGTTCAGTTGCGAGAGGCCATGCCGCTGCCATG | 42 |          |
| TAAGGGCTGATGATTAAAAGAA | 21 |          |
| TAAATAAGAACGACCTGCTTTCTGCCACT | 28 |          |
| GTCGCCAGAACAACTTAAATTGGCC | 24 |          |
| GTTAAAGGAAAGACAGCAGACTGCTATATTAAGAGGCAGGAGGTATA | 46 |          |
| AAGGGCTCCAAAAAGAGCCTTTTATATTTTTCAGTCCTACAGTCACCCT | 49 |          |
| TAAACGACATTTTTTACAGGCCAAGAACAGTTACAGAAGACCCAAA | 45 |          |
| AAGGGATATTTCTACCTTTAAGACCTATAGGCT | 31 |          |
| TTTGCCATGCATGTGGCCGACATGCTTTTCAGGCTGTA | 42 |          |
| AATATCGTAAAGAGCAGAAAGCAGATTTGGAATAAAAATGCGCTTTT | 45 |          |
| AAAATGACGTAAATATGAGTTTATCTAACGGAATAACCGTGAG | 42 |          |
| ACCGCCACTCTAGAAACCGAGTACTCTAGGGA | 31 |          |
| TCTCGGGGTTTCTGGCAGGCGCTGAGATCC | 31 |          |
| ATGACCGGCTTACCAACATTGTCAGCTTTAAAGTAAAGTAAATTTC | 45 |          |
| CCCGGGTTGATAAAGACGCTGAACT | 24 |          |
| AGTAGTATATGCGGTTTACA | 24 |          |
| CTGGCATTAGGAGAATAAAATGAGAAAGAAAGTTTTTTCAGTATA | 42 |          |
| CGAACACCAAATAAATAATGAGAGCAGAATGCTTTAGCCGCTAGA | 45 |          |
| TGCTAAATGGGGGAGCCCGCTATTTAAGAGCTACAGAAGACATT | 42 |          |
| CGGCTCAGAGTAAATAAAGGGCAGATTTCTCAGTAGAGACCTTAG | 42 |          |
| AAGACAAATACAGTGCTCTACTAGATGCGACA | 32 |          |
| TTTCGCCGATGATACGCGGCTTTAAAGCCCTGTAGCAAGCAGCA | 42 |          |
| ACATAAATGAGAAAAATAAGAGAAGGAAGAGAAAGAGATGTCAT | 39 |          |
| CAAAATCCCGGGAAAAGCAAGGAGATTTTGTCACACATCACAC | 42 |          |
| GCCTGTTAGTAGGCTTAAATTG | 21 |          |
| TTATAAGGGTTATGGAATAATATCCTACAATATA | 31 |          |
| ATAGGGACGAGGCTATCATAACCCAAATCCCAAGAAATTTCTCATCCTCAT | 49 |          |
| CGAAGAAAAGTGGTTAAGACATGCGCCTGATGGAAACCA | 39 |          |
| TTTAGATTCACAGTCACAGAGCCCGGCTGCTTTCCCAGA | 42 |          |
| CGAGCCAAATAGGAAACCCCGCTCACCCCGAA | 31 |          |
| AGCTTTACCGAGAAGGCAATA | 21 |          |
| CATTGGATAAACCCGAAAGAACATG | 26 |          |
| AAAACCCCAAAATTCGCTAAACAAATATCCGCTGCT | 35 |          |
| ACGAGGGGCGGCTCGAGCCAGGCCCCGATTTAGATTGAAAAA | 42 |          |
| Sequence (5' to 3') | Length | Modification |
|---------------------|--------|--------------|
| TAAAACCGTTAAAGAGTCTGCTCATCAGAAGGACACACAAATC | 42     |              |
| TATTACGATTAAACAAATACGATATGGT | 32     |              |
| GAAGGAGCGGAATTATCATCATATATCATATTACATAGCACAAT | 42     |              |
| CCTCGCTTTCGCCAACCAGGAACGCGCTCCCTCA | 34     |              |
| ACCAGAGCGCTATACGAAGAAGCCGC | 25     |              |
| AGAAATCGTTGAGATCACCCTTTTTTAAGGCGTTGATGACCATTTTGCA | 45     |              |
| AGTTTAGTTGATCATATAAGCTTGATGATACCTGATTTTTGCA | 45     |              |
| ATTTGAGATTCTAGCTCATACATGTTTTA | 31     |              |
| GAGAACAATATACAAATACGGCAGAGGCAGATTGGACAAATCTTTGAA | 49     |              |
| GTGGGCTGCTTGGAAATTGTCAAAG | 25     |              |
| GAAACGCGCCACCTCCATATCATACC | 25     |              |
| CGCGCGCCACAGAACAGAGGCCTAAAAGGTGCAA | 35     |              |
| AAAAGCCTAGATAGCTGCTGAGCAGGCTGCCAACCTGCTTTG | 42     |              |
| GGCAGAAGCGGCAGGGAAGGGCCTGTGTGTTGA | 31     |              |
| GAGGCCAACGGTTGAAATACAGAGGCCCAGGTTTCCAGCTGATGCG | 42     |              |
| TGAAAATCCGGTCGTAAACACTCTAAATTTGACTCTTTT | 35     |              |
| TATTGAAAGAAATGAGGTAG | 21     |              |
| GAGAATTTATCCTGAGTTAAACAGTGACTCCTT | 31     |              |
| ATCGTGCAGATATTCTCAAACACGAAAG | 31     |              |
| GTAAGAACGGCCACCTACCACTAAATAGCCGC | 32     |              |
| GGCAGAGCGGTACATGTGGGATGCTCCATGTGTTG | 39     |              |
| TTTTGCGAGTCTGGACAAAAGGTATTTTGAATGTTTGCAAAAGAAGTT | 49     |              |
| AAGGCGCTGTTATGATCAGGATGCTACTACCTTC | 31     |              |
| CGAGGGAATCTTTTCTAGTGACCAGGCGTGAAATGACGCAGGCCACACC | 46     |              |
| AGCTTTGAGATGGGAGATGGCACGCGGGAAT | 32     |              |
| TTGAGAACATTATTTAAATGCAAC | 25     |              |
| ACCAAGCGAGCAGAATAATTGACG | 22     |              |
| GGCAGAACCAGGCTCTATGAGTGAGCTCAACACAGATAGGGT | 42     |              |
| ATCGATGCTGAGATCTCAAACAGAGAGGAGGCAACACAGGAA | 42     |              |
| ACCAAACCAACAAAATTCATTTTCTTGGAATTACCGAGG | 42     |              |
| GCAGAAAATCCGGTAGAAAAAGGCCTGGTGCTGTCATACCGGGGTCCG | 45     |              |
| GAATTGCAGCTCCTGGGTTGGCTTGCTCAATTCATGGGACACGG | 32     |              |
| AATGACATTACACCTCATAGCCACGGACG | 32     |              |
| CCTGGCGCTGGAGGGGAAAGGAGAGGAGGGGCGGCGG | 42     |              |
| CAAATTATCATTTAATTATGCTGAGTA | 28     |              |
| Sequence (5′ to 3′) | Length | Modification |
|---------------------|--------|--------------|
| AATTGTTGCGAAATCCGGCACACAAACGGAGATTTGATCAA | 42     |              |
| TGGCTGTCTCGGTTTGTGTACATCG | 24     |              |
| AACCGTGTCTTGAACGGTGCAAATATATTTTTAATGAAAGGCTG | 42     |              |
| TGGCTTTTACGCTGAAATGAAAGCG | 27     |              |
| CGTACAGCGCGCCCTAACGCTGCCCGGTACCAGAGGCTTCG | 39     |              |
| AAGAAAGGCCGCTAATCCTAAATTCTAAGGAAAGCCTTCG | 42     |              |
| TTCCATGGGTCATTTCGCTATACAAATCAA | 28     |              |
| CCTAATTTAAACACCTCTAATCTGATTCCGCTATCTC | 42     |              |
| AAACTCACAGAAACGGTACGCTAGCTAAGAAAAGGGGTCGAGGAA | 42     |              |
| AATTCTTAAAACCGGCTTAAATTGGTGCCGCGGCTATATA | 42     |              |
| GAGAAGGCATCTGCAATGGGATAGTCAACAAAC | 32     |              |
| CCAATGTTTAAGTACGGCTGTCGCAA | 25     |              |
| AATTCAGCTCATTAAAAACATATTGTTAATAATTCGCTATTA | 42     |              |
| TTTCAGTGTTCCCGGCGCTACCTACCCGCACCCAGGCTATGCCTCAGT | 42     |              |
| CGTAAATAGAAAACAAATTATTTTGCACTAAGAACCTTAAGGA | 39     |              |
| GTAATTATTTAAGTATGGAAGGGCGATCTGGTGCGGCAA | 42     |              |
| TAAAGCCTCAGTACCTGATAGTTAAACG | 28     |              |
| AGGGAGCCGCTCCAGGGAAGGGATAGGCAACATCACATCTCTG | 45     |              |
| TGAGTGGTCGCAAGCGCTCTGAGCGCTATGGTCTAAAT | 39     |              |
| TTGGGGCGCTTTTCGGCGAAATTTGCTCT | 28     |              |
| CGGCACGTTGCTAATTTAATTTTTGAGTGGACGTCTTTTCCAGA | 49     |              |
| CCATAAATGCCCGCAGCTATACAGGCGGCCAGCATCTA | 35     |              |
| TGAGGGCCGCTCATTAAAATTTATTTTTGCAATGCTATCCACA | 42     |              |
| ACTAAAGAGCAAGGGAAATCTCCACCACCACCACTAAGAAGAA | 46     |              |
| AGACGGCAGAAACGGGAAGGGAAATAATCCGAGTGACAGTAAAT | 46     |              |
| TTTCAGTGCCGCAAAACCTCCTGCTATAAC | 28     |              |
| TTGGCGATATGTTATCGGAGGCTGAGTTCAATTTTGACG | 42     |              |
| TGAGAATATTTTACGCGCGTACGGTAAATTTTGG | 42     |              |
| ATAAAGCTTTTTCTTCTATCTC | 21     |              |
| AGGACAGATGGAAGGGTGTAACATAGGAACCGAAGAT | 39     |              |
| CAGCGCGCGCCCATAGAATGAAACCGGCATATATATTGGCCAT AACAG | 46     |              |
| AACACAGGGAAGCCGCTCCTTTGCTGTAATATCCGAAAGCG | 42     |              |
| ACAACGGCGTCGAGCTATTACGTTAGGAAAGAG | 31     |              |
| CGCTGTGATAAATAACCTCGCGCTGATG | 28     |              |
| AGAATTTTAGGGAAGAAACATATACCGGCGACTGCTATT | 42     |              |
| AGAAATGGCTCTTTTATGACGCTCTAATTAGGGTTGGCCA | 45     |              |
| CATCGAGATACGCGCTAAACATATAGGACCGAAAGAATTT | 38     |              |
| Sequence (5′ to 3′) | Length | Modification |
|---------------------|--------|--------------|
| TTACCATTAGCAAGGCCCTTGAAATTAGAGCCAGCCCGACTTGAGC | 44 | |
| GACAATTTAGCAAGGCCATTTTCGAG | 26 | |
| AATATCCATTGAATCTCGATGAGATAGCGTTCAAT | 35 | |
| TTAACCTGGAATTAGTAAATACTATTATGTGAGTGATTCT | 42 | |
| CGTGTCAATCCATAGCTTAATTAGGTTT | 31 | |
| TATGCAGGGGGAATTGCGGAGGATTACCGCT | 42 | |
| GGAATAATTAGCAATGATAGCATAGTCTATGTTAGTCTAGTAC | 31 | |
| TAAAGTTTTCAGCCATTGGAGTATAGTCTAGGTTT | 21 | |
| ATTGGCTTGCATTGGCTCAACAATTCAATTACCTTTCGCTGA | 45 | |
| GCCTGCCCATTAGGAGCCTTACATGTACCTGGTGA | 21 | |
| CGTACTATGTTAACCACATGCTTATTAAATGGGGAATGATACAC | 42 | |
| ACUGGCGAGTATCTTGTGAGTTTATTATTGAGCTAACCC | 42 | |
| CAAAAGGAACCAGGGAATTAAGAAATTACAGG | 38 | |
| TCACAGGCCTACTCGGTGGAGGATAGCTAAGAGCAGGAGG | 42 | |
| TAAACCATCATAAATGCAAGAGGCTACATACATTAGAAG | 42 | |
| CAGCAGCCCGCTTGTATTACACGGTCTCAGGAAA | 35 | |
| TACGCCGGAAATAGGTAATTAGGTAATGTGGCCTGGA | 35 | |
| AATGGGGGAAACTGCGTTTATTCACTGAGATTCAAC | 42 | |
| ATITCCAACAAAAATATCTAAATTAGGAACCATTCTTGCAGGCGGAGC | 49 | |
| TGCTGATTGGCTGTGTCATACACATGCGAGG | 32 | |
| GGCTAAAACTCCAGAGAAGGTTTGCCGAGGATAGAACC | 38 | |
| GAGGCTGATTGTTTATATAATACATATACACCAC | 35 | |
| AATGGTCATTGGGAAAGAGCGGAAGGCTACGATTGAGA | 42 | |
| CCGAATAGAAAGGATGCCTTGCAAAACACTTCCAC | 38 | |
| CTACAGCTATTGGAACACGATCTGCTGTATTAGAAGATACAA | 42 | |
| ACTGGGCTGACTTGGCATACACAGAATAGCTGGCCAGAGC | 42 | |
| CAGGGACAACAATCTTTACATCTT | 24 | |
| CTTCATCCACCAGGCGCTTCTCGCTATTACCGCCAGTTCG | 42 | |
| TGAGCAATTATTACAGGAAATAACTCATCATTGCTGAGTCT | 42 | |
| AATAGAAAAAAATATAACGTTGCAAGGAATATAGGAACACACTATGAT | 49 | |
| ATTGACGATAAAATTGCGACTTTAGGGGAGACAGC | 39 | |
| GCTGGCTTGGTCCAGGCGGATACTCGGCGCTTACTGAGC | 42 | |
| CGTGTGTAAGTGCTAGGGCTTCTTGTGGAATTGTTTCTGAGCT | 42 | |
| ACATAAGCTTTACACTGTTGGCTTAATTGTG | 35 | |
| TGGCTATTCCACAGGCGCGGTTTACTCTGGCGGCTTATTAG | 42 | |
| GCCAGCGTGGGGGCAATACGCGGTTTCTCGCTGCGGTTG | 42 | |
| Sequence (5′ to 3′)                                                                 | Length | Modification                      |
|-----------------------------------------------------------------------------------|--------|-----------------------------------|
| TACGACGGTGGAGGTGGCGACCTCGTCAGTTGGCTCCGGCA                                     | 42     |                                   |
| GAGAGATGACCTTTACGGGAGTCA                                                      | 25     |                                   |
| TGCAATTAGAGGGCTCCAGCTCACGGGCCAGGGCA                                          | 42     | only for Monomer                  |
| AGCCGAGCTTCAAACGTAATTCTAGGTCAGGGGAAAATT                                    | 38     | only for Monomer                  |
| GCGTCACTATTACCCAGGAATGCTACTGAGCC                                          | 34     | only for Monomer                  |
| ATTAGCGGCGGTTTCTGCAGTCAAGGCCGCAAGAGCTG                                      | 41     | 5′-Biotin                         |
| TGCCCGTATAAAACAGTGCTTTCTGTA                                              | 30     | 5′-Biotin                         |
| AGAAAGGAGATGGACCTAAATCTACGCCCTCAAATTGTTTA                                 | 44     | 5′-Biotin                         |
| ATACGAATGCTATAAAATGCTTAGGATAATAT                                          | 30     | 5′-Biotin                         |
| AGGAATCGAGTACCCTTTTTATAGTACC                                             | 30     | 5′-Biotin                         |
| GATAGAAGTAGCTCCTTTACCTCAAGGG                                              | 28     | 5′-Biotin                         |
| CCTTAATGAAAGTAGGGGAGGCTCACAG                                               | 30     | 5′-Biotin                         |
| GCATGTAGAAGAAGAATCCTAGAGTCTCAG                                              | 30     | 5′-Biotin                         |
| TGGTGGTTGTTCCAGTTTGGAACA(A)15 or (A)20                                       | 57/62  | NP b., only for Dimer             |
| AGCCGAGCTTCAAACGTAATTCTAGGTCAGGGGAAAACCT(A)15 or (A)20                     | 53/58  | NP b., only for Dimer             |
| GCGTCACTATTACCCAGGAATGCTACTGAGCC(A)15 or (A)20                             | 49/54  | NP b., only for Dimer             |
| TGGTTGGTTGGCTTTAGTGGAACA(A)15 or (A)20                                       | 39/44  | NP binding                        |
| GGATGTTGGTCCGCCAGGCA(A)15 or (A)20                                          | 36/41  | NP binding                        |
| CGCTTTCCAGGTAGCTGTGTTAAAGAACGT(A)15 or (A)20                               | 44/49  | NP binding                        |
| AGAGAAAATCCAGAGATGGCAGCAAATC                                                | 29     | ATTO647N-3′                       |
Table B2. Folding program for the DNA origami pillar.

| Temp (°C) | 65 | 64 | 63 | 62 | 61 | 60 | 59 | 58 | 57 | 56 | 55 | 54 | 53 |
|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Time (s)  | 120 | 180 | 180 | 180 | 180 | 900 | 900 | 1800 | 2700 | 3600 | 4500 | 5400 | 5400 |
| Temp (°C) | 52 | 51 | 50 | 49 | 48 | 47 | 46 | 45 | 44 | 43 | 42 | 41 | 40 |
| Time (s)  | 5400 | 5400 | 5400 | 5400 | 5400 | 5400 | 5400 | 5400 | 4500 | 3600 | 2700 | 1800 | 1800 |
| Temp (°C) | 39 | 38 | 37 | 36 | 35 | 34 | 33 | 32 | 31 | 30 | 29 | 28 | 27 |
| Time (s)  | 1800 | 900 | 480 | 480 | 480 | 480 | 480 | 480 | 480 | 120 | 120 | 120 | 120 |
| Temp (°C) | 26 | 25 | 20 |
| Time (s)  | 120 | 120 | ∞ |

References

[1] Coronado E A, Encina E R and Stefani F D 2011 Optical properties of metallic nanoparticles: manipulating light, heat and forces at the nanoscale *Nanoscale* **3** R402–39
[2] Raschke G, Kowarik S, Franzl T, Sönntichsen C, Klar T A, Feldmann J, Nichtl A and Kürzinger K 2003 Biomolecular recognition based on single gold nanoparticle light scattering *Nano Lett.* **3** R15–8
[3] Stehr J et al 2008 Gold nanostoves for microsecond DNA melting analysis *Nano Lett.* **8** 619–23
[4] Hoppener C, Lapin Z I, Bharadwaj P and Novotny L 2012 Self-similar gold–nanoparticle antennas for a cascaded enhancement of the optical field *Phys. Rev. Lett.* **109** 017402
[5] Bharadwaj P, Deutsch B and Novotny L 2009 Optical Antennas *Adv. Opt. Photon.* **1** 438–83
[6] Darvill D, Centeno A and Xie F 2013 Plasmonic fluorescence enhancement by metal nanostructures: shaping the future of bionanotechnology *Phys. Chem. Chem. Phys.* **15** 15709–26
[7] Li K, Stockman M I and Bergman D J 2003 Self-similar chain of metal nanospheres as an efficient nanolens *Phys. Rev. Lett.* **91** 227402
[8] Mirkin C A, Letsinger R L, Mucic R C and Storhoff J J 1996 A DNA-based method for rationally assembling nanoparticles into macroscopic materials *Nature* **382** 607–9
[9] Zhang J, Fu Y, Chowdhury M H and Lakowicz J R 2007 Metal–enhanced single-molecule fluorescence on silver particle monomer and dimer: coupling effect between metal particles *Nano Lett.* **7** 2101–7
[10] Spelring R A and Parak W J 2010 Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles *Phil. Trans. R. Soc. A* **368** 1333–83
[11] Rothemund P W and Folding D N A 2006 to create nanoscale shapes and patterns *Nature* **440** 297–302
[12] Douglas S M, Dietz H, Liedl T, Hogberg B, Graf F and Shih W M 2009 Self-assembly of DNA into nanoscale three-dimensional shapes *Nature* **459** 414–8
[13] Sharma J, Chhabra R, Andersen C S, Gohdolf K V, Yan H and Liu Y 2009 Toward reliable gold nanoparticle patterning on self-assembled DNA nanoscaffolds *J. Am. Chem. Soc.* **130** 7820–1
[14] Pal S, Deng Z, Ding B, Yan H and Liu Y 2010 DNA-origami-directed self-assembly of discrete silver–nanoparticle architectures *Angew. Chem. Int. Ed. Engl.* **49** 2700–4
[15] Pal S, Deng Z, Wang H, Zou S, Liu Y and Yan H 2011 DNA directed self-assembly of anisotropic plasmonic nanostructures *J. Am. Chem. Soc.* **133** 17066–9
[16] Bui H, Onodera C, Kidwell C, Tan Y, Grausgnard E, Kuang W, Lee J, Knowlton W B, Yurke B and Hughes W L 2010 Programmable periodicity of quantum dot arrays with DNA origami nanotubes *Nano Lett.* **10** 3367–72
[17] Samanta A, Zhou Y, Zou S, Yan H and Liu Y 2014 Fluorescence quenching of quantum dots by gold nanoparticles: a potential long range spectroscopic ruler *Nano Lett.* **14** 5052–7
[18] Stein H, Steinhaus C and Tinnefeld P 2011 Single-molecule four-color FRET visualizes energy-transfer paths on DNA origami *J. Am. Chem. Soc.* **133** 4193–5
[19] Kuzyk A, Schreiber R, Fan Z, Pardatscher G, Roller E M, Hogle A, Simmel F C, Govorov A O and Liedl T 2012 DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response *Nature* **483** 311–4
[20] Schreiber R, Do J, Roller E M, Zhang T, Schuller V J, Nickels P C, Feldmann J and Liedl T 2014 Hierarchical assembly of metal nanoparticles, quantum dots and organic dyes using DNA origami scaffolds *Nat. Nanotechnol.* **9** 74–8
[21] Acuna G P, Roller E M, Holzmeister P, Beater S, Lalkens B and Tinnefeld P 2012 fluorescence enhancement at docking sites of DNA-directed self-assembled nanoantennas *Science* **338** 506–10
[22] Prinz J, Schreiber B, Olejko L, Oertel J, Rackwitz J, Keller A and Bald I 2013 DNA origami substrates for highly sensitive surface-enhanced Raman scattering *J. Phys. Chem. Lett.* **4** 4140–5
[23] Thacker V V, Herrmann L O, Sigle D O, Zhang T, Liedl T, Baumberg J J and Keyser U F 2014 DNA origami nanoantennas for surface-enhanced Raman scattering *Nat. Commun.* **5** 4448
[24] Kuhler P, Roller E M, Schreiber R, Liedl T, Lohmuller T and Feldmann J 2014 Plasmonic DNA-origami nanoantennas for surface-enhanced Raman spectroscopy *Nano Lett.* **14** 2914–9
[25] Acuna G, Grohmann D and Tinnefeld P 2014 Enhancing single-molecule fluorescence with nanophotonics *FEBS Lett.* **588** 3547–52
[26] Holzmeister P, Acuna G P, Grohmann D and Tinnefeld P 2014 Breaking the concentration limit of optical single-molecule detection *Chem. Soc. Rev.* **43** 1014–28
[27] Pellegrotti I V, Acuna G P, Puchkova A, Holzmeister P, Gietl A, Lalkens B, Stefani F D and Tinnefeld P 2014 Controlled reduction of photobleaching in DNA origami–gold nanoparticle hybrids *Nano Lett.* **14** 2831–6
[28] Zhang X, Servos M R and Liu J 2012 Instantaneous and quantitative functionalization of gold nanoparticles with thiolated DNA using a pH-assisted and surfactant-free route *J. Am. Chem. Soc.* **134** 7266–9
[29] Fujita M, Katashichi Y, Ito K, Kanayama N, Takarada T and Maeda M 2012 Structural study on gold nanoparticle functionalized with DNA and its non-cross-linking aggregation *J. Collid Interface Sci.* **368** 629–35
[30] Zhang X, Gouryute T, Goeken K, Servos M R, Gill R and Liu J 2013 Toward fast and quantitative modification of large gold nanoparticles by thiolated DNA: scaling of nanoscale forces, kinetics, and the need for thiol reduction *J. Phys. Chem. C* **117** 15677–84
[31] Schreiber R et al 2013 Chiral plasmonic DNA nanostructures with switchable circular dichroism *Nat. Commun.* **4** 2948
[32] Acuña G P et al 2012 Distance dependence of single-fluorophore quenching by gold nanoparticles studied on DNA origami *ACS Nano* **6** 3189–95
[26] Puchkova A, Vietz C, Pibiri E, Wunsch B, Sanz Paz M, Acuna G P and Tinnefeld P 2015 DNA origami nanoantennas with over 5000-fold fluorescence enhancement and single-molecule detection at 25 µM Nano Lett. 15 8354–9

[27] Anger P, Bharadwaj P and Novotny L 2006 Enhancement and quenching of single-molecule fluorescence Phys. Rev. Lett. 96 113002

[28] Taminiau T H, Stefani F D and Hulst N F V 2008 Single emitters coupled to plasmonic nano-antennas: angular emission and collection efficiency New J. Phys. 10 105005

[29] Yu X, Lei D Y, Amin F, Hartmann R, Acuna G P, Guerrero-Martínez A, Maier S A, Tinnefeld P, Carregal-Romero S and Parak W J 2013 Distance control in-between plasmonic nanoparticles via biological and polymeric spacers Nano Today 8 480–93

[30] Baffou G and Quidant R 2013 Thermo-plasmonics: using metallic nanostructures as nano-sources of heat Laser Photon. Reviews 7 171–87

[31] Douglas S M, Marblestone A H, Teerapittayanon S, Vazquez A, Church G M and Shih W M 2009 Rapid prototyping of 3D DNA-origami shapes with caDNAno Nucleic Acids Res. 37 5001–6

[32] Bharadwaj P and Novotny L 2007 Spectral dependence of single molecule fluorescence enhancement Opt. Express 15 14266–74

[33] Baffou G and Rigneault H 2011 Femtosecond-pulsed optical heating of gold nanoparticles Phys. Rev. B 84 035415