

## Supporting Information

# DNA-assembled nanoparticle rings exhibit electric and magnetic resonances at visible frequencies

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## Materials and Methods:

### Supplementary Note S1. Assembly of plasmonic ring structures.

*DNA origami folding.* For the DNA origami ring template structure 10 nM of p8634 scaffold, 100 nM of each staple, 10 mM Tris, 1 mM EDTA (pH 8) and 16 mM MgCl<sub>2</sub> were mixed together. This solution was then heated up to 65 °C for 20 min to denature all DNA strands and afterwards slowly cooled down to 25 °C over the course of 40 hours (1 °C per 1 hour). According to the desired design, various sets of elongated handle strands were chosen for nanoparticle attachment (see table 1). After folding, the DNA origami template structures were purified from the excess staple strands by running the samples on a 1.0 % agarose gel in 0.5x TBE buffer with 11 mM MgCl<sub>2</sub>. The bands containing the structures were excised with a razor blade from the gel and then centrifuged with Freeze 'N Squeeze spin columns (BioRad) at 2.600 rcf for 8 min.

*Concentration of AuNPs.* For the functionalization of the DNA-modified AuNPs (10, 20, 30 and 40 nm, 20 ml, BBI Solutions) with single-stranded DNA sequences, the protocol of Schreiber *et al.*<sup>1</sup> was used with minor changes: First the AuNPs were stirred for three days after adding 8 mg of BSPP (Bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt, Sigma-Aldrich). To concentrate the particles, sodium chloride (5 M) was added until the color of the solution changed from red to bluish. Afterwards the solution was centrifuged at 1,600 rcf for 30 min and the supernatant was removed. After dissolving the AuNPs again in BSPP solution (0.8 ml, 2.5 mM in H<sub>2</sub>O) and an equal amount of methanol, the mixtures was centrifuged one more time (1.600 rcf, 30 min). The supernatant was removed and the concentrated AuNPs were dissolved in BSPP solution (0.25 ml, 2.5 mM in H<sub>2</sub>O). An absorption measurement via UV-Vis spectroscopy (Nanodrop) at a wavelength of 530 nm was performed to determine the concentration of the particles ( $c = \text{absorbance}/(\epsilon * d)$ , with the molar extinction coefficient for 40 nm AuNP  $\epsilon = 8.42 * 10^9$  and path length  $d$ ).

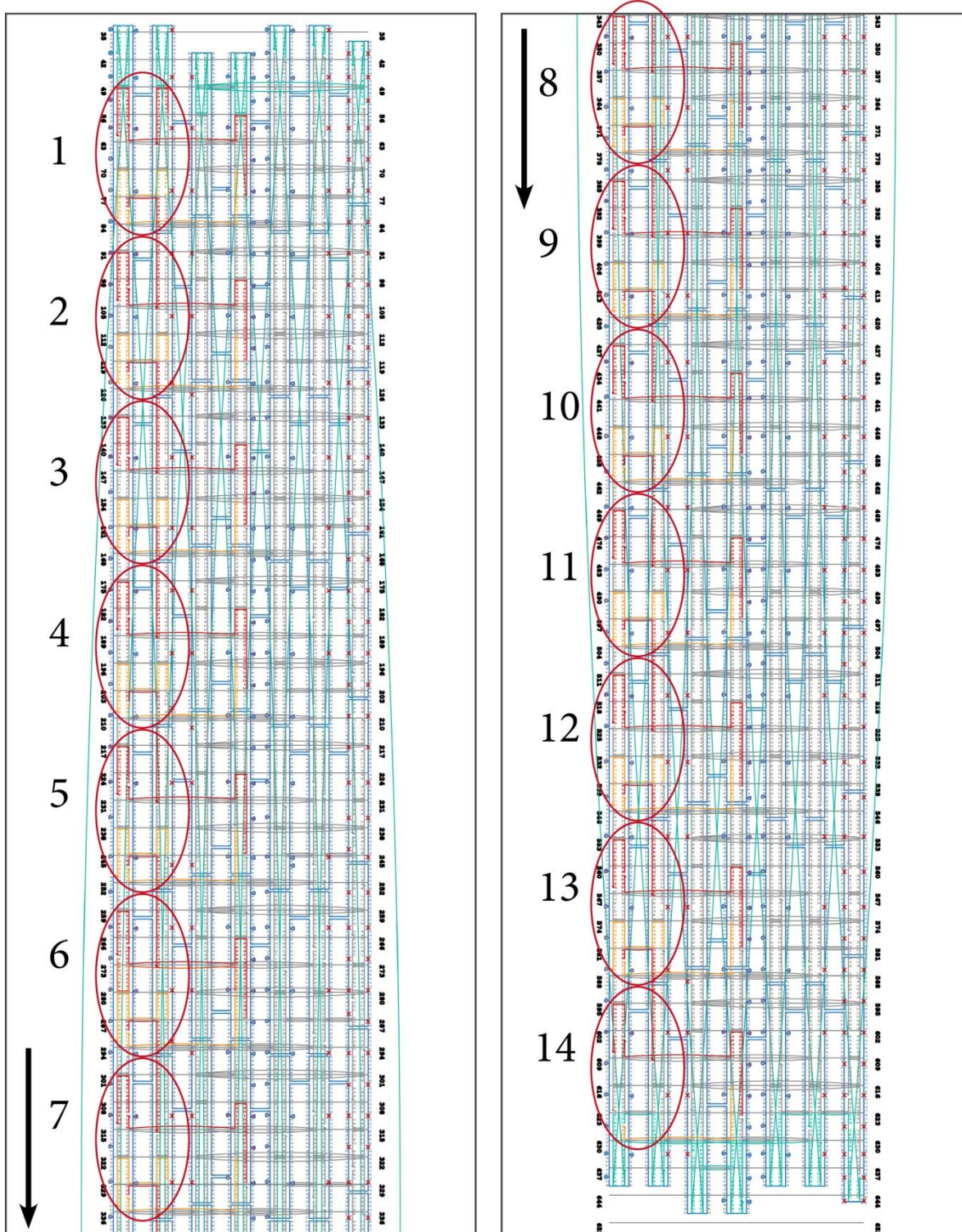
*AuNP-DNA conjugation.* To stabilize the AuNPs against high MgCl<sub>2</sub> concentration that are needed during the DNA origami folding process and to cover the AuNPs with DNA strands for the hybridization to the DNA origami template, the particles were conjugated with thiolated single-stranded DNA (ssDNA) strands (biomers.net, HPLC purified). To reduce possible disulfide bonds, the thiolated ssDNA was incubated with TCEP (Tris(2-carboxyethyl)phosphine hydrochloride), Sigma-Aldrich; 20 mM final concentration) for 30 min. AuNPs and thiolated oligonucleotides were mixed together in 0.5x TBE buffer at a ratio of DNA:AuNP = 4800:1 for 40 nm AuNPs and stirred over 3 days on a shaker. To get rid of the excess of unbound ssDNA strands the mixture was centrifuged with 100 kDa MWCO centrifugal filters (Amicon Ultra, Millipore, 10 min, 8,000 rcf) followed by 8 additional spinning steps with 400 µl of 0.5x TBE buffer added to the filter before each centrifugation step. The centrifugation was done directly before adding the AuNPs to the DNA origami structures so that the least possible amount of unbound oligonucleotides – which can block the attachment sites on the DNA origami structure – are present during the conjugation.

*AgNP-DNA conjugation.* The preparation of silver nanoparticle (AgNP) followed with minor changes the protocol of Pal *et al.*<sup>2, 3</sup> The AgNP (20 nm, BBI Solutions) were concentrated by centrifugation with 100 kDa MWCO centrifugal filter (8,000 rcf, 5 min). Thiolated DNA (biomers.net, HPLC purified) was added in a ratio of DNA:AgNP = 1200:1 and the solution was incubated on a shaker over night. Then the pH was lowered to pH 3 by adding citrate-HCl buffer to a final concentration of 10 mM. After 30 min of incubation, NaCl was added to a final concentration of 30 mM. Then the pH was raised again by adding 10x the sample volume of 0.5x TBE buffer. The excess of unbound DNA strands was removed by 8 times centrifugation of the mixture with 100 kDa MWCO centrifugal filters (Amicon Ultra, Millipore, 10 min 8,000 rcf).

*Functionalization of DNA origami structures with AuNPs (AgNPs).* The stability of the DNA conjugated AuNPs was tested by adding a droplet of AuNPs to a solution containing 20 mM of  $MgCl_2$ . If no color change is observed, the particles are fully covered with DNA and ready for the DNA origami structure functionalization. The DNA-covered AuNPs (AgNPs) were mixed together with the purified DNA origami template structure in a ratio of 3 AuNPs (AgNPs) per attachment site on the DNA origami structure. A four particle ring structure, for example, offers 4 attachment sites and thus the ratio of AuNPs:DNAorigami is 12:1. This excess of NPs is necessary to avoid cross-linking of different DNA origami structures. The solution was kept on a shaker over 24 h at 22 °C. After attachment of the AuNPs (AgNPs) to the DNA origami structures the assembled plasmonic ring structures were purified from excess of unbound AuNPs (AgNPs) via 0.7 % agarose gel in 0.5x TBE buffer with 11 mM  $MgCl_2$ . The structures were extracted from the gel by excising the bands and centrifuging the gel pieces with Freeze 'N Squeeze spin columns (BioRad) at 2.600 rcf for 8 min.

*Transmission electron microscopy (TEM) grid preparation, dark-field scattering spectroscopy and scanning electron microscopy (SEM).* The gel-purified structures were immobilized on a carbon-formvar-coated grid and stained with 1 % uranyl acetate for 20 seconds. The TEM measurements were performed using a JEOL JEM-1100 electron microscope at 80 kV. For dark-field scattering spectroscopy the gel-purified structures were 100x diluted with 0.5x TBE buffer and 11 mM  $MgCl_2$  and deposited on a glass slide. First, scattering spectroscopy was performed in liquid in a dark field configuration using an oil immersion dark field condenser with NA=1.2 (Zeiss) and Achroplan 100x water objective (NA=1.0, Zeiss). After drying the samples with a nitrogen flow, the samples were further studied with an Epiplan-Neofular 100x air objective (NA=0.9, Zeiss). The illumination source was a 100 W halogen lamp (Zeiss) and the spectra were detected with an Acton SP2500 spectrometer (Princeton Instruments). Subsequently the glass slides were carbon sputtered and SEM images were taken with a Gemini Ultra Plus SEM (Zeiss). The absorption spectrum of the gel-purified structures in solution was taken with a Jasco V-650 spectrometer.

**Supplementary Note S2. CaDNano<sup>4</sup> image of the DNA origami ring structure.** The scaffold path (blue), the staple oligonucleotides (grey), the connection staples to close the ring (turquoise) and the attachment sites (red and orange, encircled in red) are shown in this schematic picture. The folded ring template structure has a diameter of around 60 nm and offers 14 attachment sites for AuNPs or AgNPs, each site consisting of 5 single stranded 15 nucleotide (nt) long extensions on the 3' end of the origami staples. The AuNPs are bound via hybridization to the DNA origami template by covering them with ssDNA of complementary sequence to the attachment sites. To specifically attach AuNPs of different sizes as shown in Fig 1b of the manuscript, different DNA extension sequences were used for the attachment sites as well as for covering the nanoparticles.

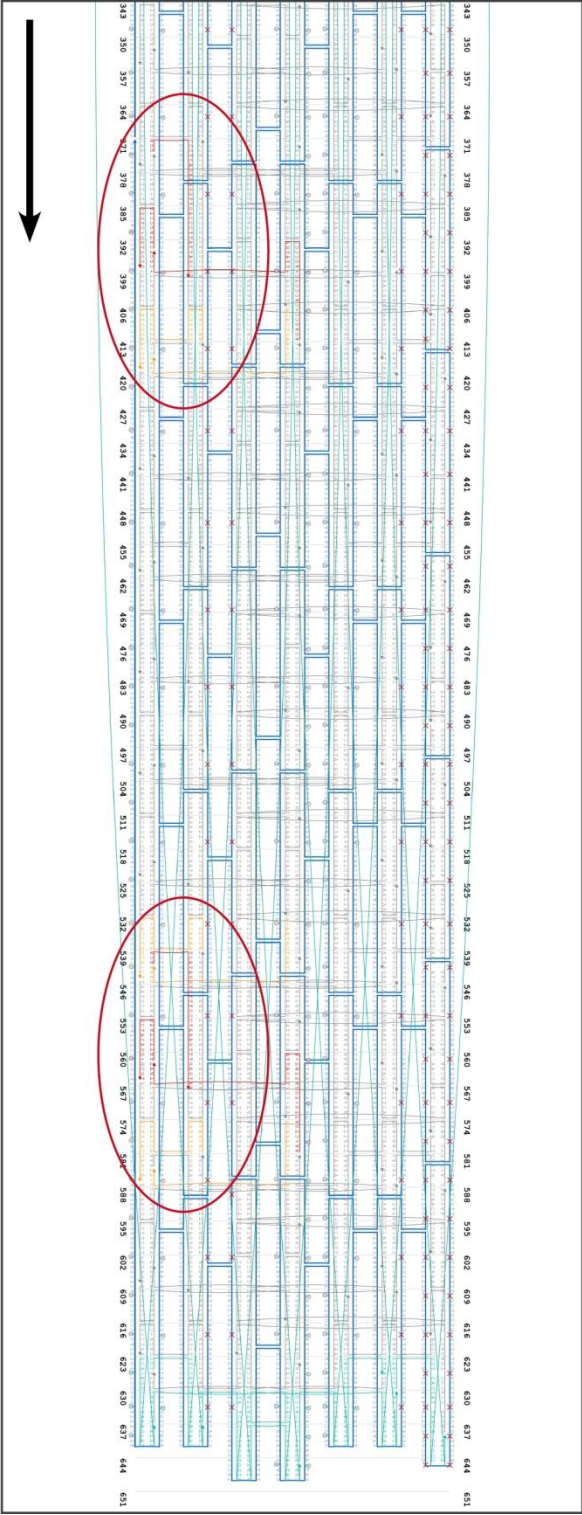
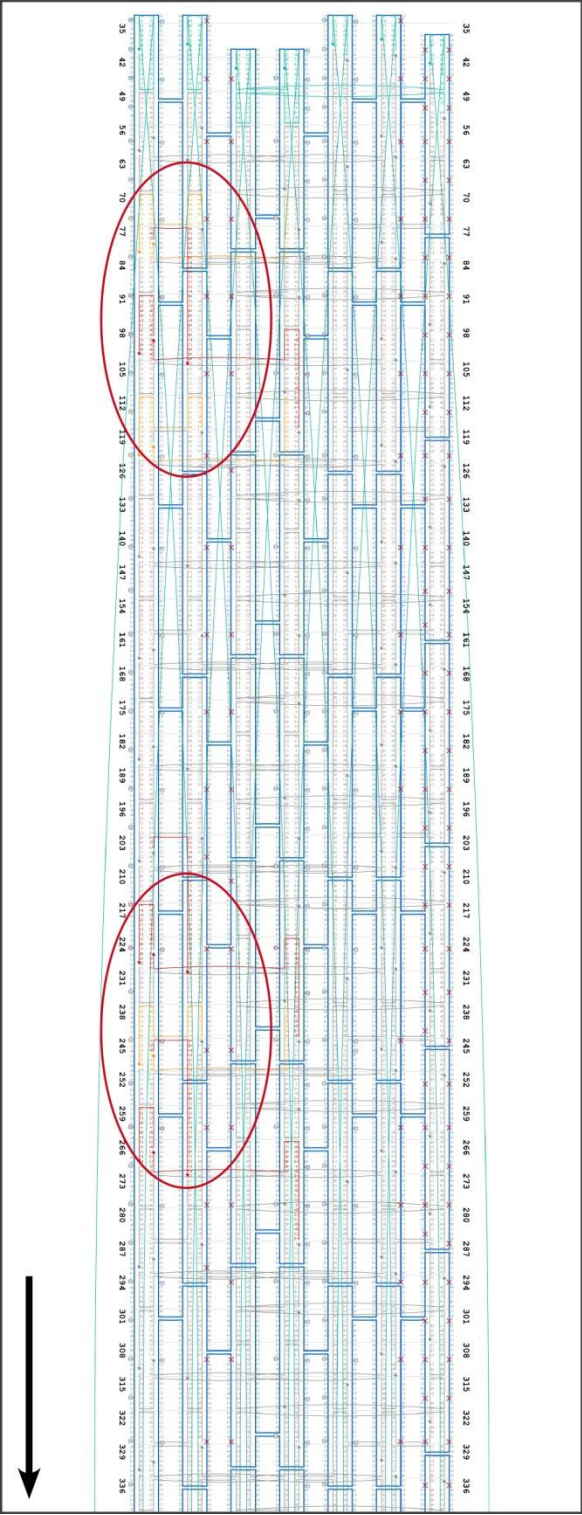


**Supplementary Note S3. Activated attachment sites on the DNA origami template for different plasmonic ring structures.** The number of the attachment sites and the colors correspond to the definition given in S2. All red attachment sites consist of 3 elongated ssDNA strands; all orange attachment sites consist of 2 elongated ssDNA strands (this is due to design and space considerations). All in all the structure offers specific addressable attachment sites every 21 base pairs.

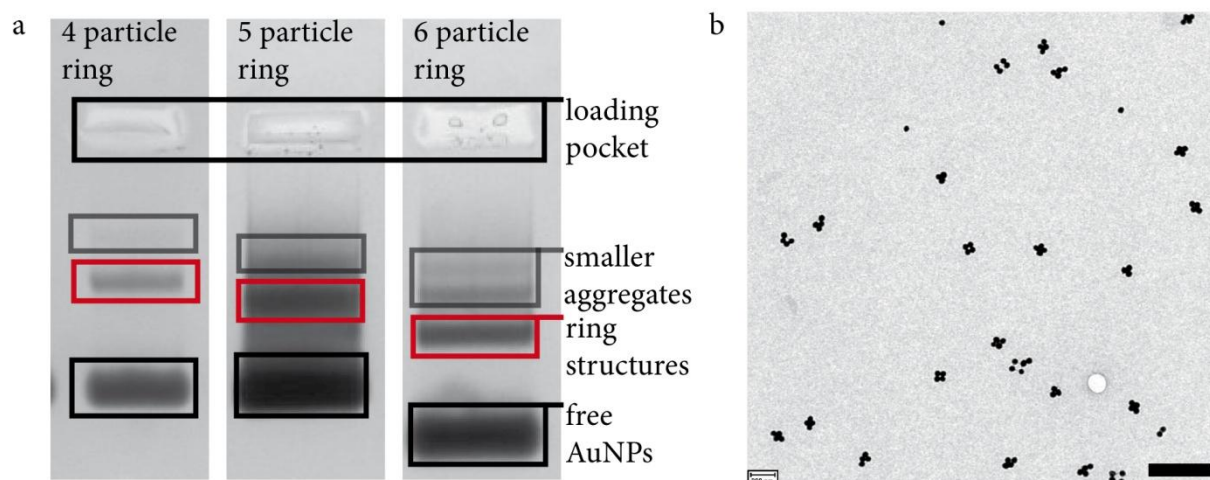
Attachment site #	SRR	8x AuNP Dimer	8 AuNP ring	6 AuNP ring	5 AuNP ring	4 AuNP ring
1			orange	orange	red orange	orange
2		red orange	red	red		red orange
3	red orange	red orange	orange			
4	red orange			red orange	red orange	
5	red orange	orange	red orange			red orange
6	red orange	red orange		orange		red
7	red orange	red	red	red	red	
8	red orange		orange			
9	red orange	red orange	red	red		red orange
10	red orange	red orange	red		red	
11	red orange			red orange		
12	red orange	orange	red orange		orange	orange
13		red orange		orange	red	red orange
14		red	red	red		



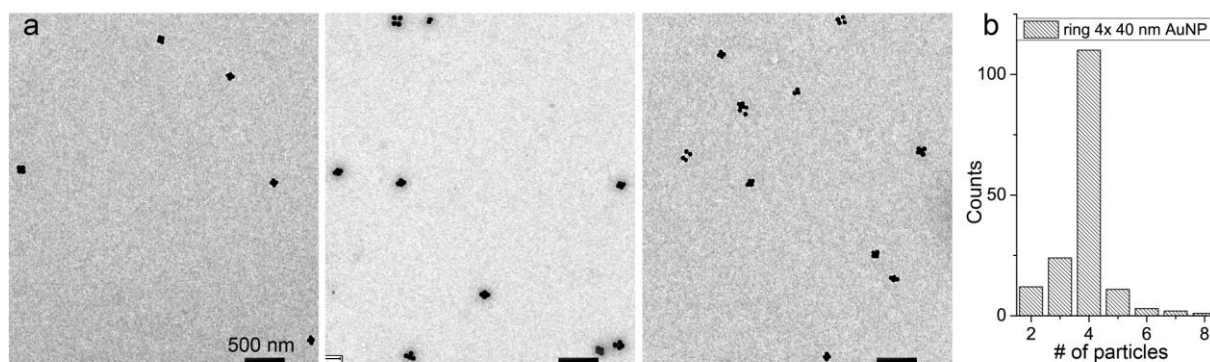
**Supplementary Note S4. CaDNAno image of the DNA origami ring structure with four attachment sites for (metal) nanoparticles.** The attachment sites used for a ring structure consisting of four metal nanoparticles are shown (red and orange, encircled in red).



**Supplementary Note S5. a**, Purification of the plasmonic ring structures by gel electrophoresis from free AuNPs and smaller aggregates. **b**, Wide field TEM image of plasmonic ring structures with four 40 nm AuNPs attached. Scale bar 500 nm.

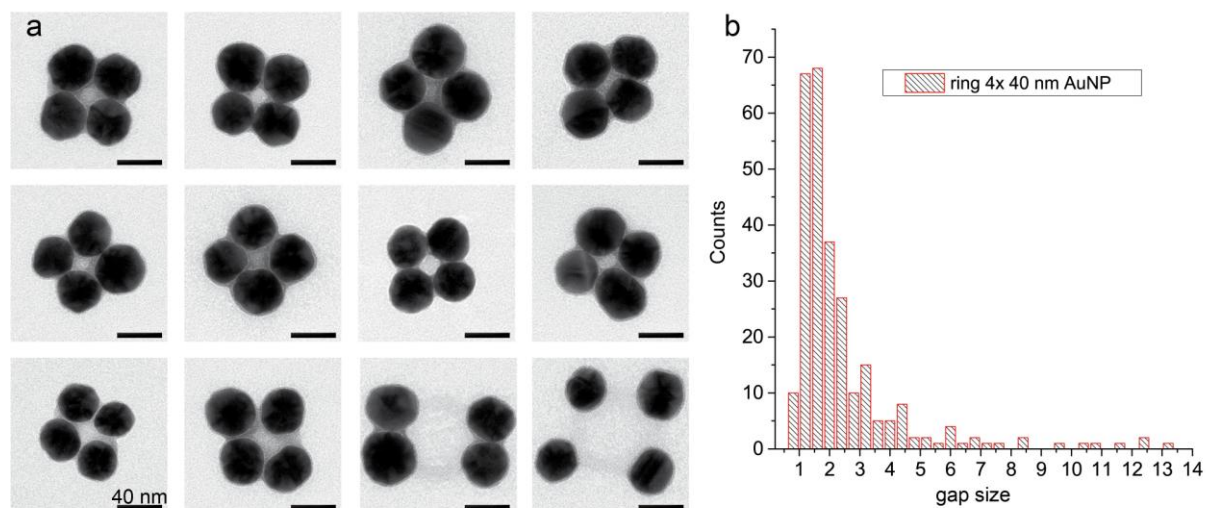


**Supplementary Note S6. a**, Wide field TEM images of plasmonic ring structures with four 40 nm AuNPs attached. **b**, Statistics of the number of bound AuNPs per four-particle-ring-structure after gel purification. The four ring assembly yield was determined by electron microscopy analysis. Out of the analyzed structures up to 68 % were assembled with four particles attached, 10 % of the structures are consisting out of five and more particles or smaller aggregates and 22 % have only three or two particles attached.

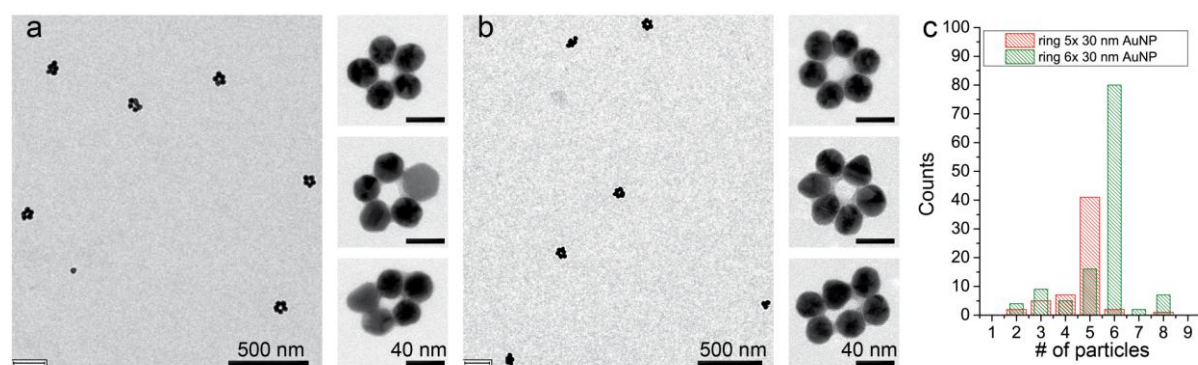




**Supplementary Note S7. a**, Additional TEM images of ring structures with four 40 nm AuNPs attached. **b**, The gap size (surface-to-surface distances) of 256 neighbouring AuNPs was measured, the median value is 1.7 nm, the mean value 2.4 nm ( $\pm 1.9$  nm SD). Structures where the AuNPs are arranged on the outside of the DNA origami were excluded from the measurements (15 %). Two of such structures are shown in the lower right part of panel a. The larger gap sizes (more than 4 nm) appear in structures which were immobilized on the TEM grid in a slightly asymmetric arrangement due to the distortion during the adsorption and drying process on the TEM grids (*cf.* the two examples in the lower of panel a).

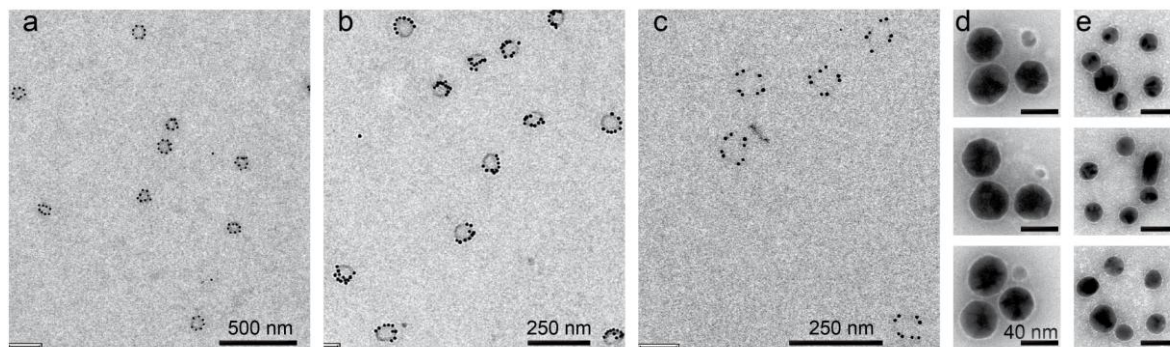


**Supplementary Note S8. a**, Wide field TEM and zoom in images of plasmonic ring structures with **a**, five 30 nm AuNPs and **b**, six 30 nm AuNPs attached. **c**, The assembly yield was determined by electron microscopy for five bound 30 nm AuNPs to 73 % and for six bound 30 nm AuNPs to 65 %. Structures with a distorted geometry, which can occur during the adsorption and drying process on the TEM grids, are shown in the lower right of **a** and **b**.

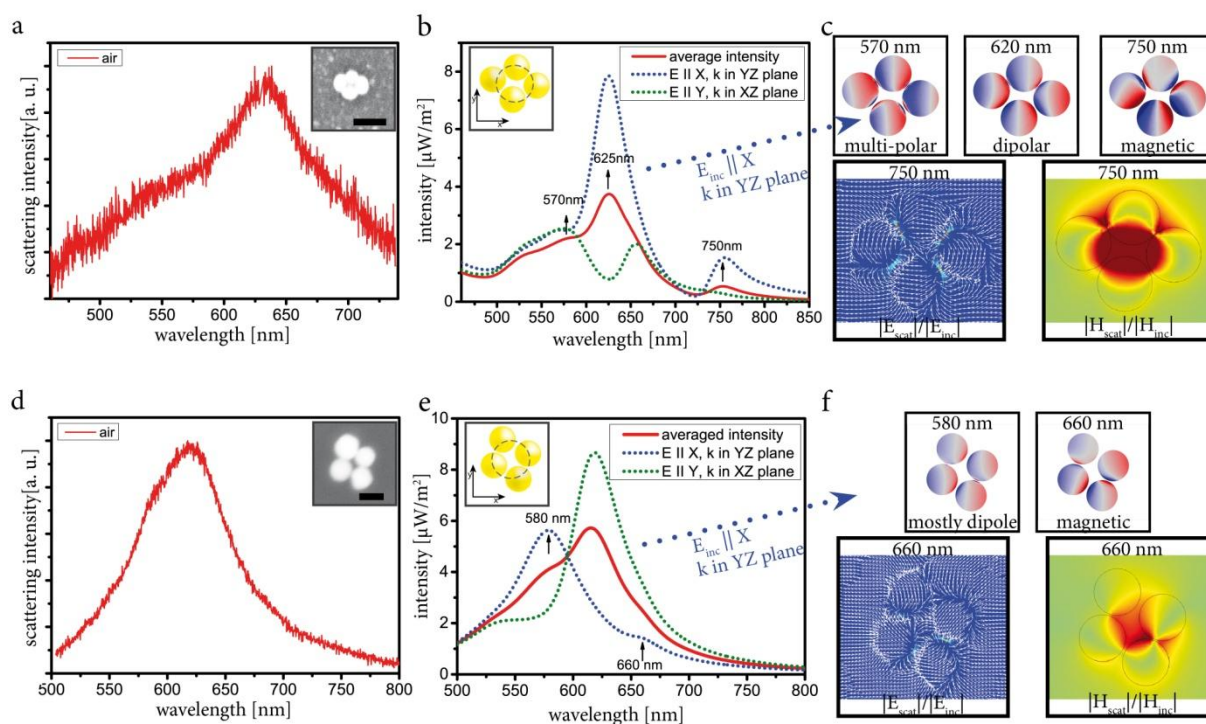




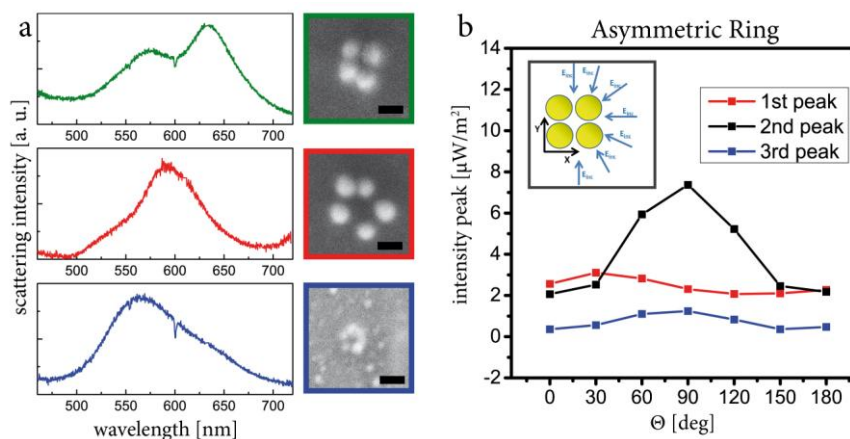
**Supplementary Note S9.** Wide field TEM images of a rings with eight 10 nm AuNPs attached, **b**, rings with 10 nm AuNPs attached in a SRR conformation, **c**, rings with eight 10 nm AuNPs attached in pairs of two and **d**, additional images of asymmetric ring structures with three 40 nm AuNPs and one 20 nm AgNP, **e**, five 20 nm AuNPs and one 30 nm AuNP. Note that for the latter two particle arrangements the concentrations were too low to find more than one structure per wide field image.



**Supplementary Note S10. Additional dark-field scattering spectra and corresponding simulations.** Scattering spectra of single structures immobilized on glass substrate (**a, d**) and corresponding simulation (**b, e**). The simulation of the surface charge distribution for all peaks as well as the electric and magnetic field distribution (**c, f**) reveals the origin of the peaks at dipole, multi-polar and magnetic interaction.



**Supplementary Note S11. a, Dark-field scattering spectra of individual ring structures consisting of four, five and six AuNPs.** Ring of four 40 nm AuNP diameter, ring of five 40 nm AuNPs and ring of six 30 nm AuNPs. The corresponding SEM images are shown in the right panel. Scale bars: 50 nm. **b, Scattering intensity as a function of the angle of the incident electric field.** The simulation of the peak intensities of the structure in S10a as a function of the angle of the incident light illustrates the anisotropic response of a not perfectly symmetric ring structure.



**Supplementary Note S12. FDTD simulation.** FDTD simulations of AuNP ring structures in solution (particle diameter 40 nm, gap size 10 nm) were performed using Lumerical FDTD solutions (Lumerical Solutions, Canada). Linear polarized light was injected from all three directions with a total-field scattered-field source and the calculated absorption cross sections were averaged to reproduce the experimentally measured bulk absorption measurement. Convergence was reached with a mesh size of 0.4 nm.

**Supplementary Note S13. Comsol simulation.** To simulate the dark-field scattering spectra Comsol Multiphysics ([www.comsol.com](http://www.comsol.com)) was used. The permittivity of the matrix regarding the simulation of samples in air, dried on a glass plate has been modified to  $\epsilon = 1.75$  regarding to samples in solution on a glass plate to  $\epsilon = 2.15$ .

## References:

- (1) Schreiber, R.; Do, J.; Roller, E.-M.; Zhang, T.; Schuller, V. J.; Nickels, P. C.; Feldmann, J.; Liedl, T. *Nat. Nanotechnol.* **2014**, *9*, 74-78.
- (2) Zhang, X.; Servos, M. R.; Liu, J. *Chem. Commun.* **2012**, *48*, 10114-10116.
- (3) Pal, S.; Deng, Z.; Ding, B.; Yan, H.; Liu, Y. *Angew. Chem. Int. Ed.* **2010**, *49*, 2700-2704.
- (4) Douglas, S. M.; Marblestone, A. H.; Teerapittayanon, S.; Vazquez, A.; Church, G. M.; Shih, W. M. *Nucleic Acids Res.* **2009**, *37*, 5001-5006.