Plasmon resonance tuning using DNA origami actuation

Luca Piantanida[a], Denys Naumenko[a,b], Emanuela Torelli[c], Monica Marini[c[+]], Dennis M. Bauer[d], Ljiljana Fruk[d], Giuseppe Firrao[c], and Marco Lazzarino[a]

[a] *CNR-IOM Laboratorio TASC, Area Science Park, Basovizza, 34149, Trieste, Italy*
[b] *AREA Science Park, Padriciano 99, 34149 Trieste, Italy*
[c] *Dipartimento di Scienze Agrarie e Ambientali, Università di Udine, via delle Scienze 206, 33100 Udine, Italy*
[d] *Karlsruhe Institute of Technology (KIT) DFG-Centre for Functional Nanostructures (CFN) Wolfgang Gaede Str. 176131 Karlsruhe, Germany*
[+] Present address: King Abdullah University of Science and Technology Physical Sciences & Engineering Division, Kingdom of Saudi Arabia

Electronic Supplementary Information

Materials and chemicals.

Unless otherwise specified, chemicals and reagents were purchased from Sigma-Aldrich. The thiol-capped DNA oligonucleotides were purchased with desalting purification from Sigma-Aldrich. The gel filtration columns used to desalt DNA solution from DTT were illustra™ NAP5 and NAP10 Columns from GE-Healthcare (Buckingamshire, UK). Syringe nylon filters (pore size 0.22μm) were purchased from Teknokroma (Barcelona, ES). D-Tube Dialyzer devices were purchased from Merck-Millipore (Nottingham, UK). Gel Red™ DNA staining solution was purchased from Biotium (Hayward, CA).

Functionalization of nanoparticles.

20 nm gold nanoparticles were purchased from Ted Pella Inc. and they were functionalized as follows. First a coating with BSPP (Bis(p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt) was carried on following published procedures[1]. Briefly 36 mL of colloids solution are left to incubate over night with 14.4 mg of BSPP. Then the particles are centrifuged to remove supernatant before being rinsed, concentrated and resuspended in 2.5 mM BSPP in approx. 2 mL. Second, since the commercial DNA oligoucleotides are protected with disulfide bond, before use, they were reduced with DTT (0.3 M) in a solution with NaCl (0.3 M) and sodium phosphate buffer (0.01 M NaH2PO4/Na2HPO4, pH 7.0) for at least 1 h of incubation. After filtration with NAP5/10 columns the oligonucleotides are mixed with colloids solution (final concentrations ~0.7 nM AuNP, ~2.4 μM oligonucleotide). After 24 h sodium phosphate buffer (10 mM) was added and the solution was incubate over night. In a subsequent salt-ageing process, NaCl solution was added to reach the concentration of 0.1 M in 6 steps during 48 h. To remove free oligonucleotides that didn't react, the solution was centrifuged (8 min at 10000 x g) and the supernatant was removed. The dark red precipitate was solubilized in buffer (TAE 1X, 0.1 M NaCl) and stored at 4°C. Before mixing with DNA origami the NP solutions were concentrated to reach the desired ratio.

2D DNA origami design.

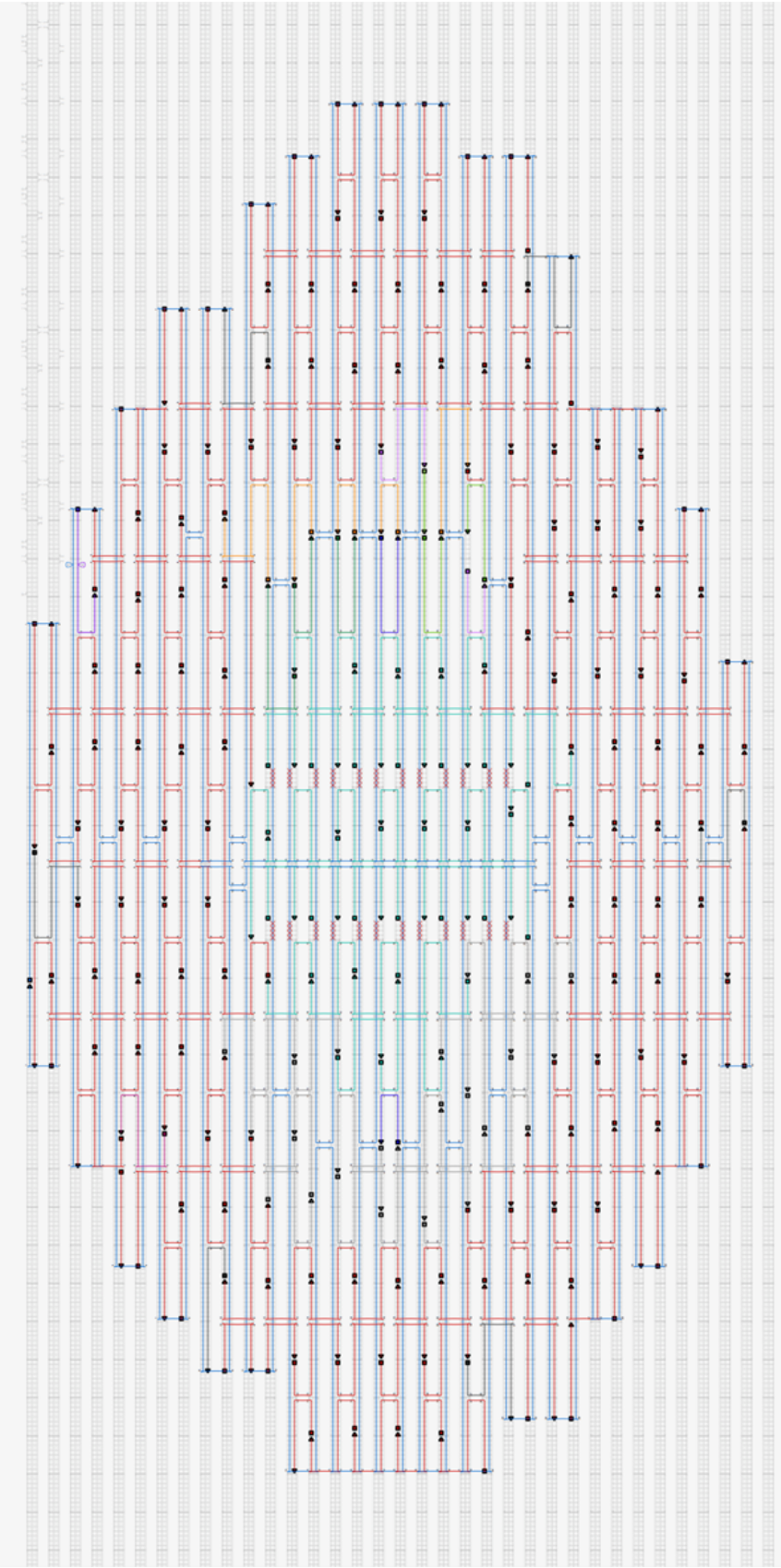
The DNA origami was designed using the square-lattice version of the caDNAno software from ref.[2]. To obtain the 2D circular shape, the entire single-stranded circular DNA of the M13mp18 viral genome (7249 nts) was used as scaffold sequence input with the help of 217 oligonucleotides. In the DNA origami used in this work the previously[3] reported sequences DkD6, Cr77, CrI5, DkD5 and CrI6 were substituted respectively with:

In pink (Figure S1)

Dk_SH: 5`-[Thiol]cTCAGTTGAATACCAAGTACATTTAA-3'
Cr_SH: 5`-TATATTTTCATACAGGCAAGGCACAATTCTACTAATTCAG[Thiol]-3'
In light green (Figure S1)
Cr-SH: 5'-AGTAGTAGCATTA-3'
Cr-SH1: 5'-TTTAAGCGAACCAGACCGGAAAAAGATT-3'
Cr-SH2: 5'-CGCGTTTTAATTCGAGCTTCAAAATATGCAAC-3'

"PROBE"	5'-CGATCCGACCTTCCTCCCTTGTGGTGAAGGTTTCCACACCTCACTGAATTGTCTTTGAACGACAGTTCGAGCTTGTTGTGTTTGAAACTGATTTCCTAAGGCAGCTTCCCGGGCTCGCAG-3'	120 nts
"C120"	5'-GGGCGGGGCGGGGGCGCGGCCTTAGGAAATCAGTTTCAAACACAACAAGCTCGAACTGTCGTTCAAAGACAATTCAGTGAGGTGTGGAAACCTTCACCACAACGCGCCCCCGCCCCGCCC-3'	120 nts
"L84"	5'-GCCTTAGGAAATCAGTTTCAAACACAACAAGCTCGAACTGTCGTTCAAAGACAATTCAGTGAGGTGTGGAAACCTTCACCACAA-3'	84 nts
"S64"	5'-CAAAAGGAAGCATTTGACAGATACGCAGAACTTGTCTGTCACAATACTTT CCAGACAATGCGAC-3'	64 nts
"C64"	5'-GTCGCATTGTCTGGAAAGTATTGTGACAGACAAGTTCTGCGTATCTGTCAAATGCTTCCTTTTG-3'	64 nts

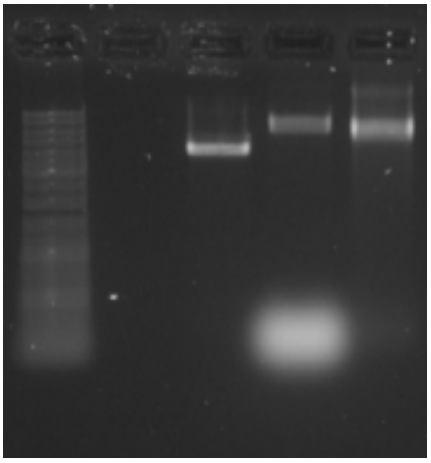
Supplementary Table 1: Sequences of the DNA filament placed in the internal part of the disc as "PROBE" and the sequence of the three different ssDNA targets. The competitor sequence to reclose the system is reported as "C64".



Supplementary Figure 1: 2D DNA origami design obtained with caDNAno software package. The scaffold strand (in blue) is folded with the staple strands starting from the first row on the top. In pink and light green are highlighted the differences with the previously reported works[3-4].

DNA origami synthesis and purification.

The DNA origami was produced by mixing 1.6 nM of circular M13mp18 viral DNA and 16 nM of each staple strands 1x TAEM buffer solution (10x TAEM solution is: 125 mM $MgCl_2$, 400 mM Tris-Acetate, 10 mM EDTA pH 8.0, 20 mM NaCl). The mixture was subjected to a thermal annealing ramp and the folded constructs were purified from staple strands excess, as previously reported by Marini et al.[3-4].

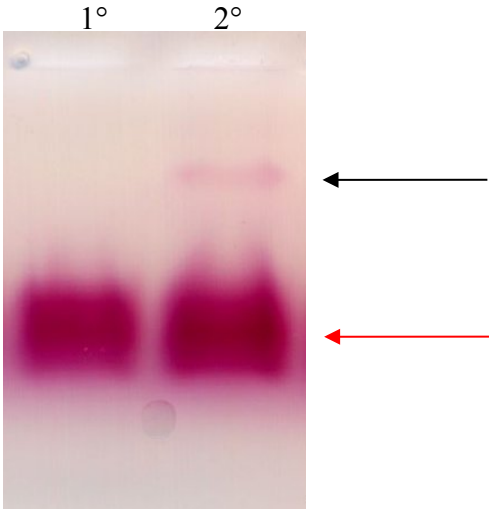


Supplementary Figure 2: Agarose 1% gel electrophoresis of the DNA origami construct. Lanes (from left to right): 1kb ladder / empty / M13mp18 / DNA origami / DNA origami purified from staples.

DNA origami-gold nanoparticles functionalization and purification.

The purified DNA origami solution was mixed with both types of coated nanoparticles with 2:1 DNA/NP ratio, typically in 20 μL: 8 μL of DNA origami (~4 nM), 6 μL of each nanoparticle type (~8 nM). To allow a complete hybridization between DNA strands and a good decoration rate, the solution was incubated at 50° C per 45 minutes and then was left slowly cooling down to room temperature over night. The visualization and purification of the mixed products was carried on by

1% agarose gel electrophoresis, adding <10% v/v loading buffer (60 mM EDTA, 60% glycerin). The collection of the DNA origami was performed cutting the band of interest and extracting the sample using D-Tube Dialyzers modifying the protocol where necessary. Then the sample was concentrated using Amicon Ultra 0.5 mL 100 KDa centrifugal filters (Millipore), modifying the protocol where necessary.



Supplementary Figure 3: Agarose (1%) gel electrophoresis of the DNA origami mixed with the gold functionalized nanoparticles (lane 2); as control, the same solution without the DNA origami is loaded (lane 1). In the lane 2 the light red band (marked by black arrow) represents the DNA construct linked to the nanoparticles, while the intense red band represents the nanoparticles not linked in excess (red arrow). Both these latter bands were extracted and imaged with SEM.

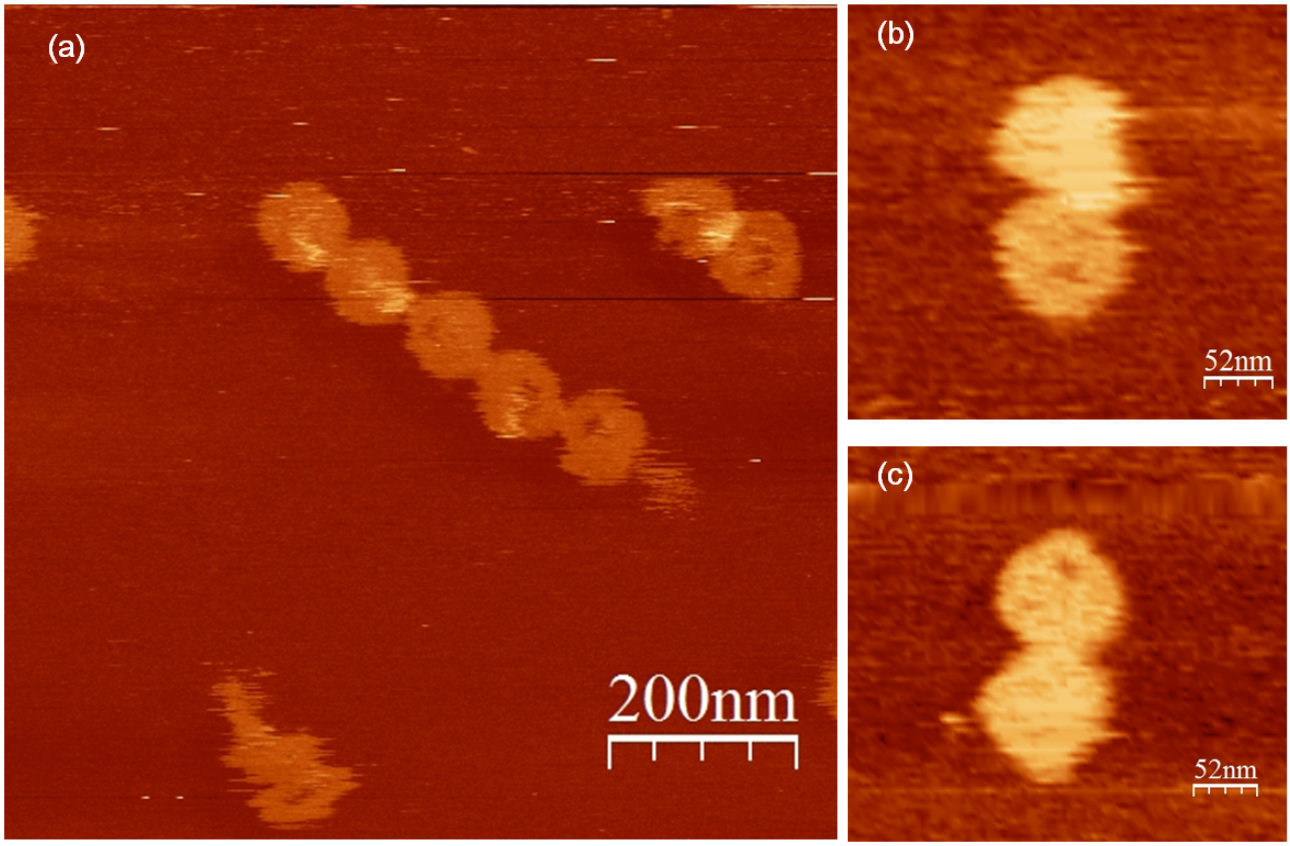
DNA origami morphological characterization.

DNA origami without AuNP: AFM analysis

The DNA origami without AuNP were characterized by AFM to check the proper formation and structure.

A 5 µL droplet of purified DNA origami solution was dispersed on freshly cleaved mica in 1x TAEM buffer to allow the presence of bivalent cations as Mg^{2+} to ensure the adhesion of the negative charged DNA origami structures on the negative mica surface. Samples were let sediment for 10 minutes. The DNA origami images have been obtained using an Asylum MFP3D AFM

system. We used Olympus OMCL-TR400PSA tips with a force constant of 0.08 N/m and a resonance frequency of 34kHz in air. All the AFM measurements were finally performed in liquid in tapping mode. Image analysis was performed using the Gwyddion and WSxM software.

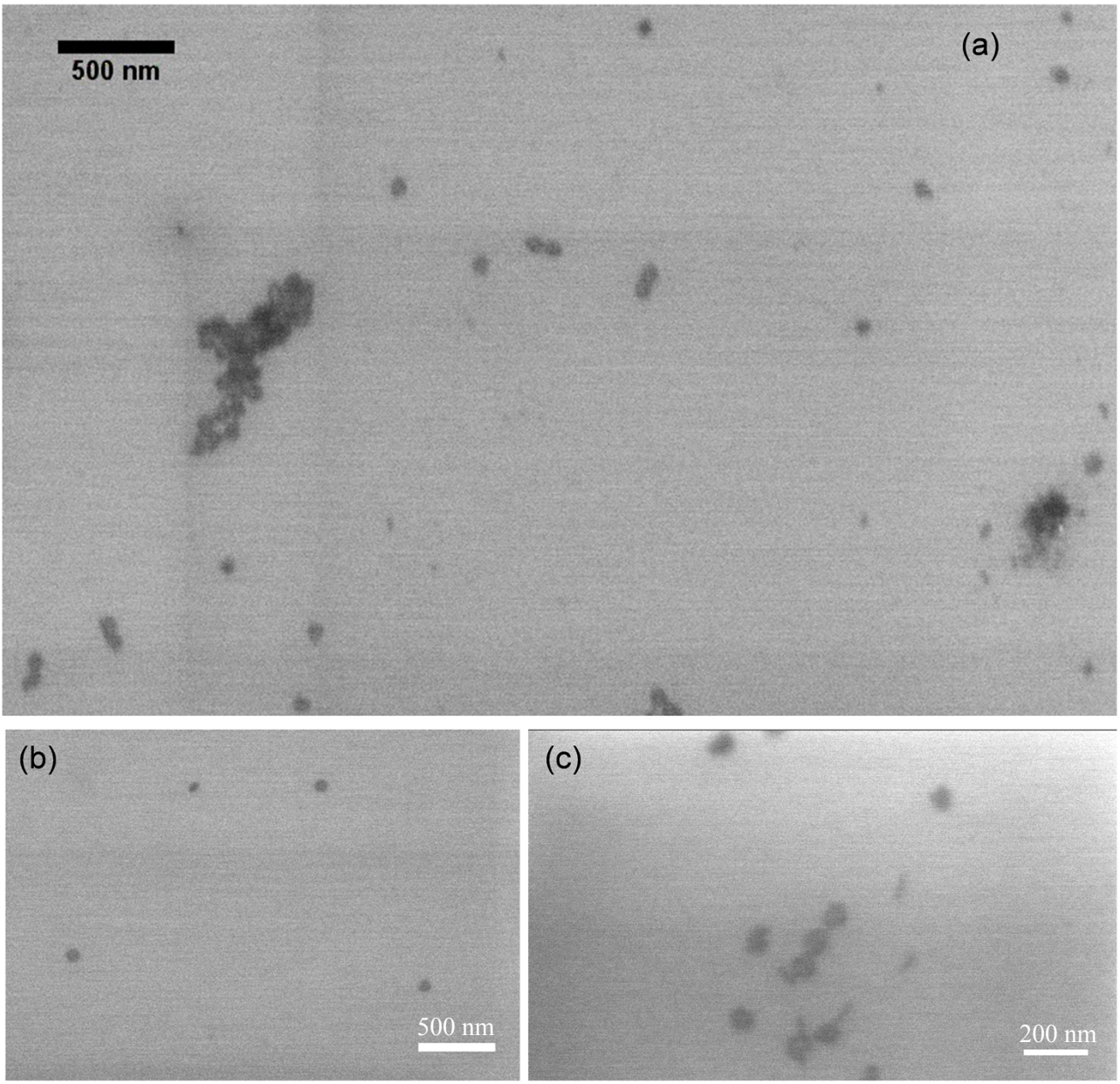


Supplementary Figure 4:
AFM representative images of the DNA origami construct without nanoparticles. (a) The circle shape design tends to aggregate in dimers because of the stacking interaction at the edges. (b-c) High resolution AFM images to better see the center of the structure.

DNA origami without AuNP: SEM analysis

The DNA origami without nanoparticles was also analyzed by SEM to compare it (as control) to the SEM characterization of DNA origami linked with AuNP in Figure S6.

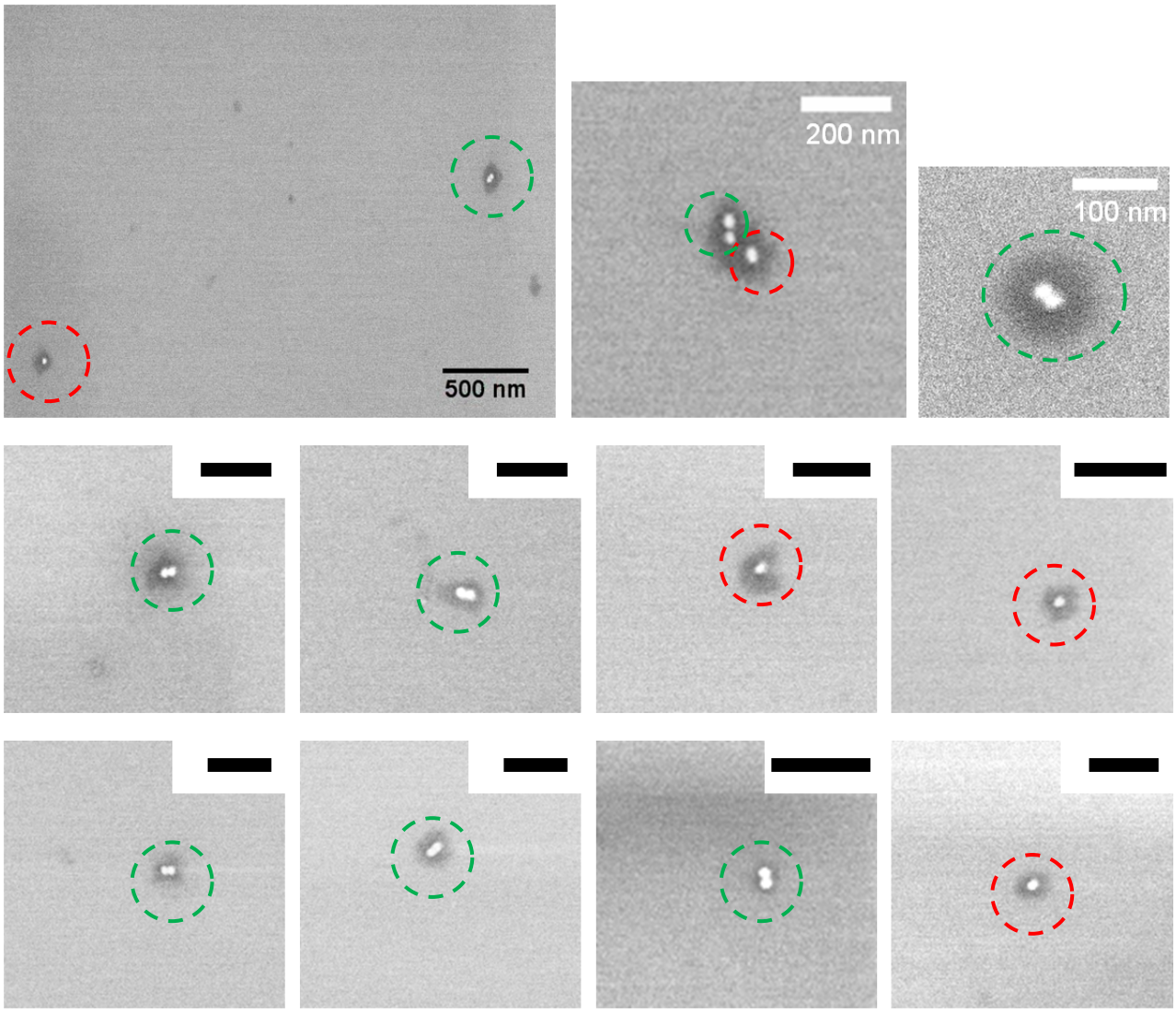
A clean piece of silicon with a layer of silicon oxide of 200 nm was previously treated with oxygen plasma to make the surface more hydrophilic and then a droplet (5 µL) of purified DNA origami solution was left to adsorb for 7 minutes and washed with water to remove salt crystals. After drying, the sample was imaged with SUPRA 40 (Zeiss) scanning electron microscope at 4 kV.



Supplementary Figure 5:
SEM images of the DNA origami construct before the nanoparticles linking. The DNA origami appear in a darker circular shape that can be compare with Figure S6 where AuNP are linked on the structure.

DNA origami with linked AuNP: SEM analysis

The same procedure was adopted to image DNA origami with nanoparticles: a 5 µL droplet of extracted DNA origami with nanoparticles solution was left to adsorb for 7 minutes and washed.

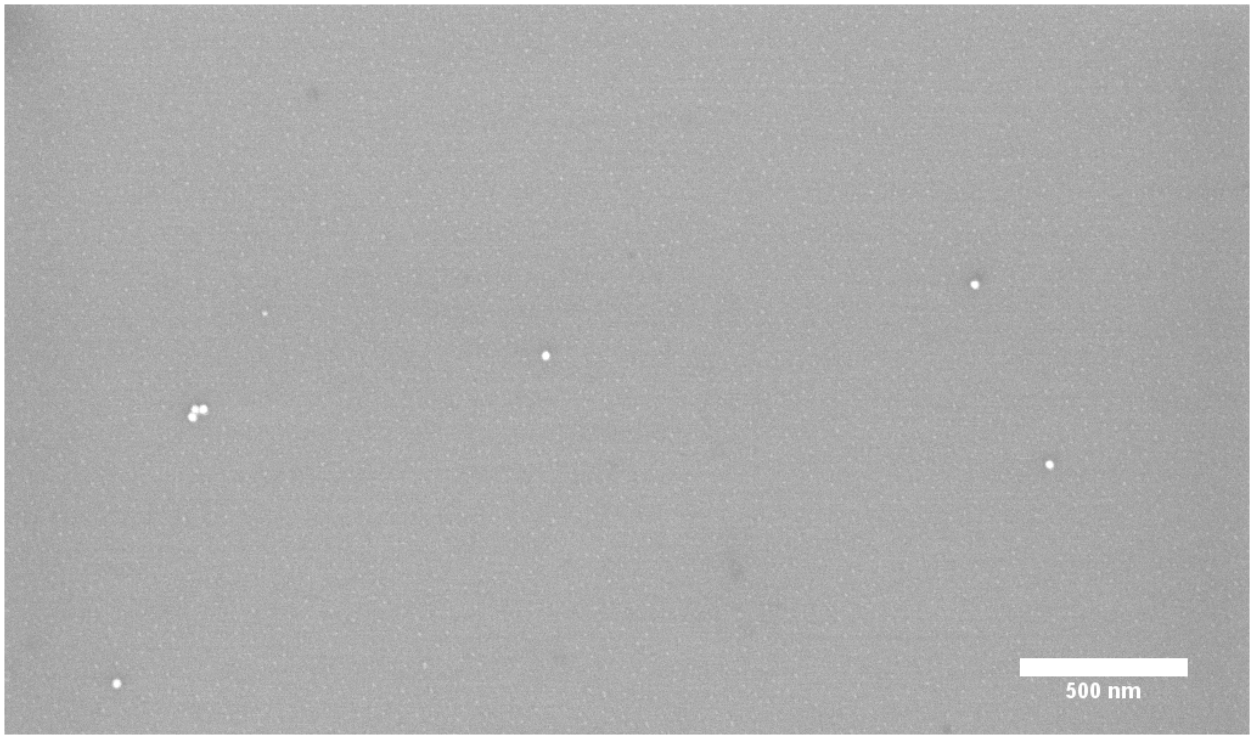


Supplementary Figure 6:

SEM images of the DNA origami with nanoparticles extracted from the gel band (black arrow of Suppl. Fig. 3). Since the density in the field of view is too low we reported more images with single origami. There are both single (green circle) both double (red circle) nanoparticles placed on the DNA disc structures (200 nm black scale bars).

Nanoparticles excess: SEM analysis.

The same procedure was adopted to image nanoparticles excess (red arrow of Suppl. Fig. 3) as control of the separation: a 5 μL droplet of extracted nanoparticles solution was left to adsorb for 7 minutes and washed.



Supplementary Figure 7:

SEM image of the nanoparticles not linked to the DNA origami extracted from the intense red band (red arrow of Suppl. Fig. 3). The representative image shows that DNA origami are not present neither with nor without AuNP linked.

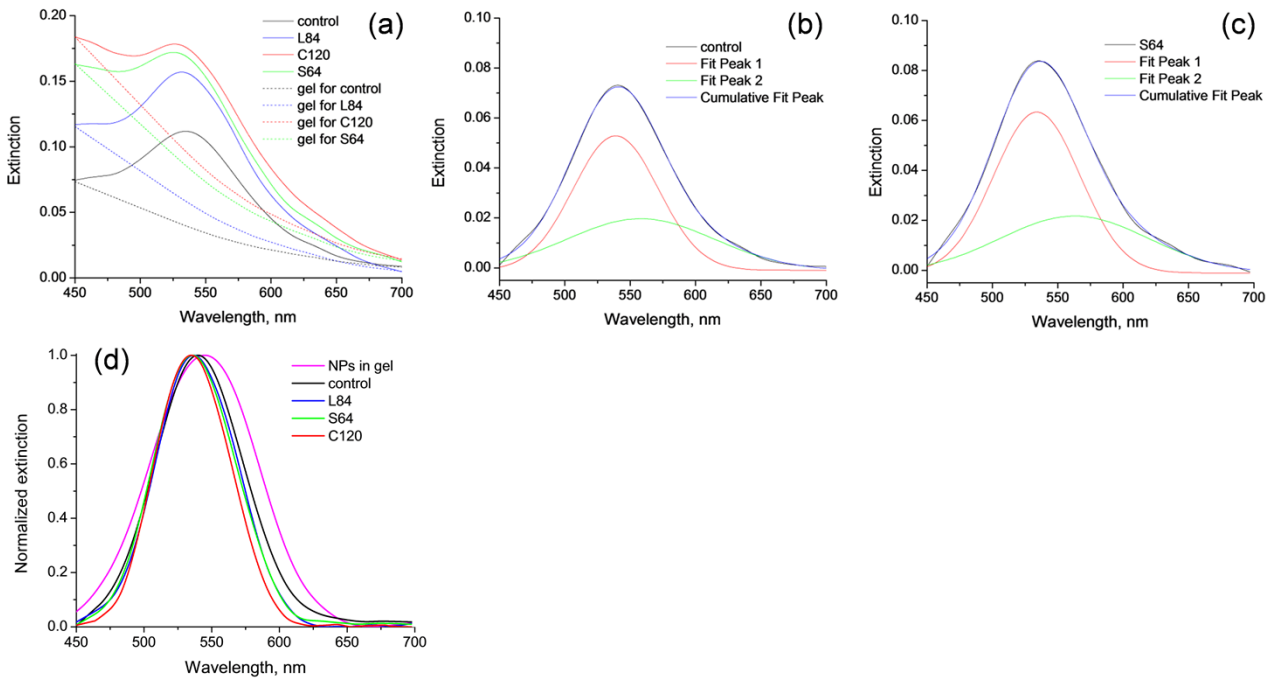
Direct gel absorption measurements.

UV-Vis characterization was performed on inverted optical microscope (Axiovert 200, Zeiss) in transmitted light illumination (HAL 100 illuminator, Zeiss) coupling a microscope with 750 mm long spectrometer (Shamrock SR-750, Andor Technology plc.). The agarose gel resulting from the electrophoretic procedure with distinguishable bands was placed onto clean glass coverslip that was mounted on XY sample stage. The light transmitted through the sample was collected by 100x immersion objective (NA 1.45, α Plan-FLUAR, Zeiss), directed into a spectrometer, split by a diffractive grating of 600 lines/mm, and finally analyzed using TE-cooled EMCCD (Newton DU971-UVB, Andor Technology plc.). The raw extinction spectra (Fig. 2d and 3d) are plotted using the equation:

$$E = (I_0 - I_i)/(I_0 - I_{bg}) \quad (1)$$

where I_i is the corresponding intensity of light passed through each band displayed in Fig. 2c (lanes 3-6), and I_0 is the intensity of light passed through the clean gel in a position without origami. I_{bg} is a background dark signal of CCD. At least 5 different positions along each lane were characterized and finally averaged resulting in the spectra displayed in Fig. 2d and 3d.

The resulting spectra show a peak in the range of 525-535 nm which is attributed to the LSPR in single gold NPs or dimers. The spectra also demonstrate a different extinction at low wavelengths complicating an estimation of LSPR position. Since the gel density, gel hydration and AuNP size in each series of experiment are supposed to be constant the difference in the extinction is caused by a variable gel thickness in a position of the lanes. To determine the LSPR position we applied two steps procedure. At first step, the gel thickness corrected background was plotted for each spectrum (Fig. S8a) and then subtracted. Second, the resulted spectra were fitted with two Gaussian functions in the range of 500-600 nm considering i) LSPR in spherical gold NPs at about 530 nm[5], and ii) an additional peak in the range of 560-600 nm. The last one is caused either by a non-sphericity of gold NPs[6] or possible plasmon coupling effect[7], which are inseparable in our case. An example of such fitting for closed and open origami is shown in Fig. S8b,c. This allowed to determine the LSPR positions for three series of experiments resulting in 6.0±1.0 nm, 5.7±0.5 nm, and 3.6±0.7 nm blue shifts in respect to the control sample for C120, S64, and L84 targets respectively, and summarize the data in the box plot displayed in the inset of Fig. 2d. For comparison Fig. 2d is re-plotted after background subtraction and normalization as Fig. S8d. The figure shows the broadening of LSPR peak in case of closed origami or highly packed NPs in gel due to plasmon coupling effect (dimer effect) and highlights that the determination of actual extinction peak position requires the fitting procedure.



Supplementary Figure 8:

(a) The raw extinction spectra of lane 3-6 displayed in Fig. 2c together with corresponding gel thickness corrected backgrounds. (b,c) An example of fitting procedure for control origami (b, closed configuration) and actuated structure (c, open) with two Gaussian functions in the range of 450-700 nm. (d) The extinction spectra after background subtraction and normalization procedures.

References.

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