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# A DNA origami plasmonic sensor with environment-independent read-out

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## ABSTRACT

DNA origami is a promising technology for its reproducibility, flexibility, scalability and biocompatibility. Among the several potential applications, DNA origami has been proposed as a tool for drug delivery and as a contrast agent, since a conformational change upon specific target interaction may be used to release a drug or produce a physical signal, respectively. However, its conformation should be robust with respect to the properties of the medium in which either the recognition or the read-out take place, such as pressure, viscosity and any other unspecific interaction other than the desired target recognition. Here we report on the read-out robustness of a tetragonal DNA-origami/gold-nanoparticle hybrid structure able to change its configuration, which is transduced in a change of its plasmonic properties, upon interaction with a specific DNA target. We investigated its response when analyzed in three different media: aqueous solution, solid support and viscous gel. We show that, once a conformational variation is produced, it remains unaffected by the subsequent physical interactions with the environment.

## KEYWORDS

DNA origami, plasmonic sensor, molecular detection, gold nanoparticle

## 1 Introduction

Gold nanoparticles (AuNP) exhibit a peculiar opto-electronic feature commonly referred to localized surface plasmon resonance (LSPR). Surface plasmons are collective oscillations of conduction band electrons that can be excited optically by the electromagnetic radiation in presence of a periodic or aperiodic nanostructuring at a noble metal surface [1]. In case of a nanoparticle, surface plasmons are confined to the surface and are not-propagating [2]. The plasmon resonance frequency of isolated AuNP is strictly correlated with the size, the shape and the permittivity of the medium in which the colloids are embedded. When two or more AuNP are separated by less than 2.5 times the particle diameter, the mutual interaction between their plasmons results in an intensity much larger than the linear sum of the single particle plasmons and in a red-shifted frequency [3]. By exploiting this phenomenon, it is possible to create a plasmon ruler which is essentially based on the spectral shift of the scattering spectrum when two plasmonic nanoparticles approach one another. The plasmon ruler concept has been largely employed for example by coupling a metallic particle with a metallic film, demonstrating light absorptions shifts as large as 5 nm of wavelength for every 0.1 nm change in separation

distance [4].

Because of their unique self-recognition properties, DNA molecules can drive the coupling of AuNP ensuring a sub-nanometric control of the interparticle distance by defining the number of nucleotides (0.34 nm each step) [5, 6], thus creating defined plasmonic field hot spots [7, 8] with a finely tuned interparticle distance. More complex DNA architectures have been realized to organize metal particles in quasi crystalline geometries with a sub-nanometric imposition of the lattice parameter [9–11] introducing further degrees of freedom in the design of their optical properties.

Due to its nearly unlimited design flexibility, DNA origami provides an unmatched platform for the realization of structures and devices at the nanoscale [12–14]. The extraordinary properties of nucleic acids of base-pair predictability, biocompatibility, mechanical strength and flexibility allow the accurate synthesis of multi-shaped objects with nanometer resolution [15]. In addition, the annealing of chemical linkers to the DNA origami structure enables the spatial addressability of numerous biological macromolecules and metal particles with nanometric precision. Thanks to its versatility, DNA origami technology was proposed for multiple applications ranging from drug delivery to quantum optics [15]. DNA nanostructures were exploited for cancer therapy by loading intercalating

chemotherapeutic agents in various DNA origami shapes. These drug carriers showed the ability to kill the tumor cells normally resistant to the chemotherapy agent used, demonstrating a good antitumor efficacy also in a mouse model for cancer [16]. Recently DNA origami technology was used for developing novel optical voltage sensing nanodevices that convert a local change of electric potential into optical signals. By using a single pair of FRET dyes, the structural changes upon application of an electric field on DNA structures reversibly immobilized on a nanocapillary tip were successfully monitored [17].

Dynamic origami structures that change conformation in response to an environmental stimulus or an external signal hold great promises in sensing and manipulation in biological environment at the nanoscale [18, 19]. The decoration of DNA origami frameworks with AuNP into fully biocompatible plasmonic DNA nanostructures offers the possibility to realize biosensors in which a biochemical event is transduced in a change in optical properties and it has been applied to enhance Raman scattering [20], fluorescence emission [21], and to develop plasmonic ruler [22, 23].

Plasmonic sensors are then extremely promising candidates for label-free single-molecule analysis not only for the compatibility with the biological environment but also for the sensitive and non-invasive detection system. Origami based plasmonic static and reconfigurable sensors sensitive to pH [24], temperature [25] and circular dichroism [26] were also proposed. However, since a biosensor can be subject to viscous forces, electrostatic forces and surface tension, it should be better independent from the surrounding environment.

In our previous paper, a continuous tuning of the LSPR was obtained localizing two AuNP respectively on the frame and on the movable part of a DNA origami hatch [27], thus resulting in an interparticle gap tuned continuously from 0 to 1.5 times the AuNP diameter. The actuation mechanism was based on DNA hybridization, in particular three different DNA sequences were shown to induce a shift in the absorption maximum wavelength of up to 6 nm. Nevertheless, when deposited on a substrate its motion and final state were strongly influenced by the electrostatic interaction with the substrate. If operated in solution, the structure design would most likely be prone to thermal fluctuations. By using a proper 3D architecture, the limitations connected to the interaction with the surrounding environment should be overcome.

A wireframe geometry benefits from high structural robustness while preserving enough flexibility to allow toggling between alternative configurations. In this paper we report the design, the synthesis, and the response of a tetrahedral DNA origami frame before and after the DNA hybridization of a specific DNA probe-target pair. The origami was actuated in aqueous solution and then its response was analyzed in three different situations: in the same solution, to simulate the operation within a body fluid, running through viscous gel, to simulate the cellular cytoplasm and other high-density physiological environments, and finally adsorbed on a silicon substrate, to emulate hard tissue, such as the core of cancer tissues or bones. For each situation we adopted the most suitable experimental approach, respectively: small angle X-ray scattering (SAXS), optical extinction and scanning electron microscopy. The device response was comparable in all conditions demonstrating a significant robustness against the influence of the external environment during the read-out process.

## 2 Experimental

### 2.1 Sensor design, synthesis and functionalization

The DNA origami structure was designed by using caDNAno software (<http://cadnano.org/>); design schematic is provided in Fig. S1 in the Electronic Supplementary Material (ESM); 3ss structure

contained three gaps in two different pillars in which 4 nucleotide were omitted (Fig. S2 in the ESM), and the correct folding of 0ss and 3ss tetrahedrons were both verified using CanDo [28, 29] software (<https://cando-dna-origami.org/>). 10 nM of M13mp18 scaffold strand was mixed with staple strand mix containing 100 nM of each staple in TAE buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA) with 12.5 mM MgCl<sub>2</sub>. The mixture was subject to a cooling down ramp consisting in a rapid decrease of the temperature from 80 to 60 °C in 20 min and a slow cooling down reaching the room temperature in 40 h. Folded structures were electrophoresed in a 1% agarose gel containing TAE 1× and GelRed 1× at 50 V for 2 h. Bands were visualized with UV light and the concentration of well-folded structures were calculated with ImageJ post-processing software. AuNP were functionalized with thiol-capped oligonucleotides following a published procedure [22, 30]. Freshly prepared DNA origami structures were then incubated with ssDNA-functionalized AuNP considering a fourfold concentration of AuNP with respect to each well folded DNA origami. The annealing was favored by an incubation at 50 °C for 45 min; then the sample was slowly cooled down overnight. The anchoring success was firstly checked with agarose gel electrophoresis; the result obtained can be confirmed by the visible pink/ruby red color of the gel band matching precisely with DNA origami band detected at UV light (Fig. 1(c)). A yield of dimers formation of about 10% was obtained by evaluating DNA intensity, AuNP absorption from the gel and the results of SEM analysis as described in detail in Section 2 in the ESM.

### 2.2 SEM and TEM structure validation

Copper grids covered with a thin carbon layer have been activated with a plasma cleaning treatment for 5 min at 20 W (45 bias). A drop of solution is then deposited on the surface and after 5 min it is removed with a paper wiper and rinsed twice with ultrapure water. Once completely dried, it has been imaged both with SEM and TEM. The sample was first analyzed with field emission gun Supra Zeiss 40 scanning electron microscope at 2 kV of acceleration voltage, a working distance in the 2-to-5 mm range. The same sample has been then imaged with a field-emission transmission electron microscope (JEM 2100f UHR, JEOL) operated at 80 kV.

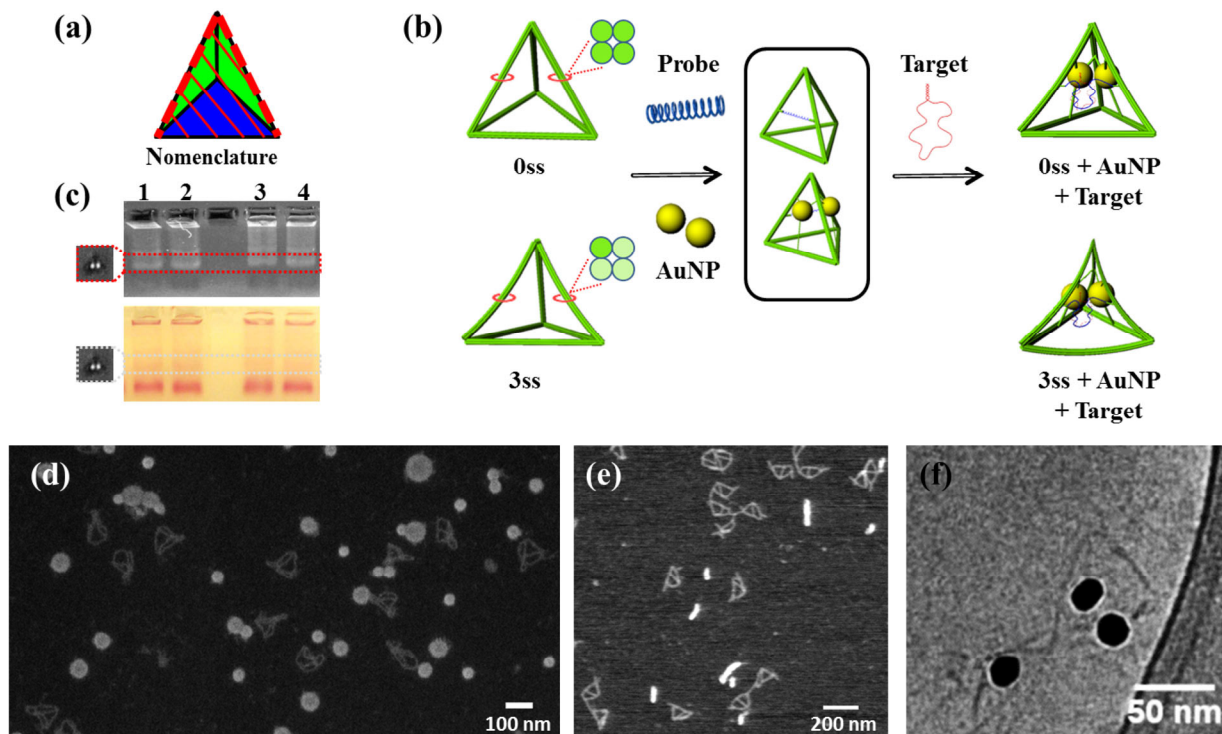
### 2.3 AFM analysis

AFM images have been recorded using a JPK Nanowizard II, operated in air in contact mode. AFM analysis was performed on the 2D DNA origami, depositing a 15 µL of sample in a freshly cleaved piece of mica. After 15 min of incubation, the sample was rinsed with ultrapure water and dried with nitrogen (Fig. S4 in the ESM).

### 2.4 Cryo-EM analysis

The specimens are prepared in standard buffered media and a drop of 4 µL is applied to an EM grid (200-mesh lacey carbon film coated grid, EMS, Hatfield, PA, and Cu-200LN, Pacific Grid-tech, San Francisco, CA). The grid is blotted for 3 s and immediately plunged into a cryogenic liquid (ethane); this step called vitrification, preventing the rearrangement of water molecule into a crystalline lattice [31]. Plunge-freezing of the cryo-EM grids has been performed using Leica EM GP (Leica, Buffalo Grove, IL) that incorporates a chamber to control the humidity (80%) and the temperature (8 °C) for blotting and thus the evaporation. The sample was stored in liquid nitrogen and remains always below -180 °C to avoid devitrification. Hydrated, unstained samples are sensitive to electron irradiation because higher doses lead to progressive alterations of molecular structures [32]. Consequently, cryo-EM images have been recorded at low electron exposures, limiting their signal-to-noise ratio (SNR).

The DNA origami architecture decorated with gold nanoparticle



**Figure 1** Design, synthesis and characterization of the tetragonal DNA origami sensor. (a) Nomenclature of the tetrahedron geometry: the blue facet is the base; the green facets are NP facets; the red striped facet is probe facet; the red dashed corners are probe struts. (b) DNA origami design: 20 nm gold nanoparticles are placed in the center of the AuNP facets so that at rest the center-to-center interparticle distance is 30 nm; a ssDNA probe links the probe struts across the probe facet. The central section of the probe struts is designed in order to have a variable flexibility. In this experiment two probe struts are used: one flexible with three single strands DNA and one double strand segment (3ss) and one with zero single strand and four double strand segments (0ss). The hybridization of a molecular target, an hairpin ssDNA strand, with the probe strand induces DNA origami squeezing, reducing the interparticle gap. (c) Agarose gel electrophoresis of 0ss and 3ss tetrahedrons decorated with AuNP before (lane 1 for 0ss, lane 3 for 3ss) and after probe-target actuation (lane 2 for 0ss and lane 4 for 3ss); the dotted boxes underline the well-folded DNA origami structures in UV/Vis mode. The excess of free staples strands is not visible in the picture because after 2 h it ran beyond the gel boundary. The AuNP band, even if coated with ssDNA, cannot be highlighted by the UV lamp because the fluorescence caused by the intercalation of GelRed requires the presence of double strand DNA. (d) SEM picture of freshly prepared DNA origami tetrahedron without AuNP on carbon TEM grid, white circles are crystallized salt residues. (e) Contact mode AFM topography in air on mica of a 2D DNA origami structures without AuNP designed and synthesized specifically to allow AFM investigations. (f) Cryo-EM image of the hybrid AuNP-DNA origami particles embedded in vitrified ice.

have been imaged with Zeiss Libra 120 transmission electron microscope (Carl Zeiss SMT GmbH, Oberkochen, Germany) under low-dose condition as described [33, 34]. The instrument equipped with a LaB<sub>6</sub> gun operating at 120 kV, an in-column energy filter, and a 4 k × 4 k Gatan UltraScan 4000 CCD camera.

## 2.5 SAXS measurement

AuNP-DNA origami hybrid structures were analyzed through small angle X-ray scattering (SAXS) without previous purification of the sample from excess of AuNP. SAXS data were collected with a Pilatus 1M detector (Dectris, CH) in a  $q$ -range from 0.058 to 5 nm<sup>-1</sup>, at a photon energy of 8 keV [35, 36]. Sample-to-detector distance was 1,381 mm. The liquid samples were filled into flow through glass capillaries of 1.5 mm diameter (WJM-Glas /Müller GmbH, Berlin-Pankow, DE) and then measured at room temperature. Scattering data obtained were corrected for the fluctuations of the primary beam intensity and sample transmission. The background was subtracted with a corresponding buffer measurement. The conversion into 1D SAXS pattern was done with Fit2D. Data fitting analysis was performed using IGOR Pro (Wavemetrics) with custom-made functions based on the literature.

## 2.6 LSPR measurement

UV-vis characterization was performed using the set up described in our previous work [27]. Briefly an inverted optical microscope (Axiovert 200, Zeiss) in transmitted light illumination (HAL 100 illuminator, Zeiss) was coupled with a microscope with 750 mm

long spectrometer (Shamrock SR-750, Andor Technology plc.). The distinguishable agarose gel band representing AuNP-DNA origami hybrid structures were cut out after the electrophoretic procedure and were placed onto clean glass coverslip then mounted on XY sample stage.

The light transmitted through the sample was collected by 100× immersion objective (NA 1.45,  $\alpha$  Plan-FLUAR, Zeiss), directed into a spectrometer, split by a diffractive grating of 600 lines per mm, and finally analysed using TE-cooled EMCCD (Newton DU971-UVB, Andor Technology plc.). The extinction spectra obtained from highly concentrated AuNP were normalized using the extinction spectra of the same gel acquired in a position without AuNP.

## 2.7 Evaluation of the interparticle distance

A 1 × 1 cm of silicon wafer was cut and cleaned firstly with 99% ethanol and then with an oxygen plasma performed in a plasma cleaning machine applying a power of 40 W and a constant flow of oxygen for 2 min. The solution of AuNP-DNA origami was deposited on the activate substrate and incubated over there for 7 min. The excess of sample was removed through two washing steps with deionized water.

The samples were images with field emission gun Supra Zeiss 40 scanning electron microscope at the same conditions described above. The interparticle distance analysis was performed with SmartSEM<sup>®</sup> software, measuring the distance between the NP dimer from center to center to avoid AuNP dimension variability contribution.

### 3 Results and discussion

In order to avoid environmental effects, in the design of a structure should coexist: i) structural stability, ii) flexibility, and iii) functionality. The structure of choice was a wireframe tetrahedral DNA origami composed by six 4-helix bundles with an average length of 90 nm connected by flexible joints. A 120 bases single strand DNA probe connects two of the six struts of the structure and when hybridized with a proper target, in this case represented by a hairpin ssDNA fully complementary with the probe strand, it represents the actuator of the DNA origami device. The functionalization protocol of the 20 nm AuNP, optimized in our lab [22], consisted in the full coverage of the NP surface with thiolated ssDNA oligonucleotides. Two DNA covered-AuNP were trapped in the center of two opposite tetrahedral facets through the extension of oligonucleotides originated from the main structure. In particular each AuNP has three anchor points, or catcher strands, coming out from three different struts of a tetrahedral facet. The length of every catcher strand was accurately calculated to immobilize the AuNP in a configuration which sets the center-to-center interparticle distance to approximately 30 nm and the gap between the two surfaces to approximately 10 nm (Figs. 1(a) and 1(b)).

When the target hybridizes with the correct probe, it shortens its length and pulls toward each other the two struts on which the probe strand is anchored. As a consequence of this deformation the gap between AuNP is also reduced and the optical extinction shifted. Two different wireframes with different rigidity were designed by changing the staple strands used to fold the structures: in one case the struts that were supposed to bend upon the probe-target actuation consisted of a length-fill 4-dsDNA bundle (0ss); in the second case, 3 dsDNA were substituted with 3 ssDNA (3ss) on a 4 nucleotides-long section in the middle of the struts (Fig. 1(b), additional details of the structure in Section 1 in the ESM).

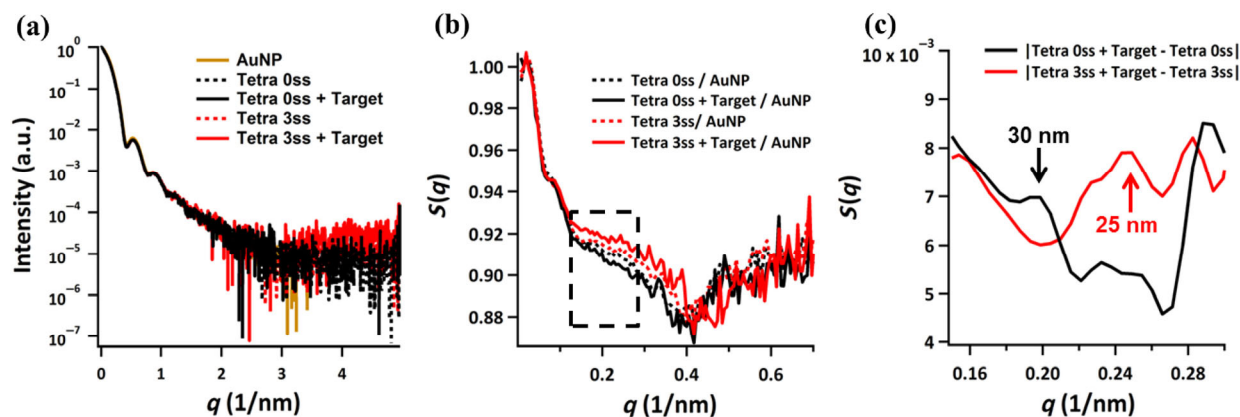
The DNA origami synthesis was validated with agarose gel electrophoresis while the formation of isolated well-folded tetrahedrons was confirmed by SEM (Fig. 1(d)) and TEM analysis (Fig. S4 in the ESM). Atomic force microscopy (AFM) was employed to confirm the structural features by analyzing the length and the diameter of the bundles which agreed with the design. Because of its 3D configuration, the tetrahedron cannot be properly imaged using AFM. By omitting six staples from the staple mix (Fig. 1(e) and Fig. S4 in the ESM), one of the six struts did not completely fold, thus obtaining a 2D origami version with a “kite-like” shape that was

easily imaged by AFM.

The formation of the hybrid DNA-AuNP nanostructure was observed in the gel electrophoresis as a slower band, indicating the effective anchoring of ssDNA-functionalized AuNP to the DNA origami. DNA origami decorated with gold nanoparticles (both 0ss and 3ss) before and after the target addition, produced ruby red gel bands in the agarose gel (Fig. 1(c)). The UV light image of the same gel showing the stained DNA, provides a clear correspondence between the specific bands of the hybrid structure and the DNA origami only (Fig. 1(c)). The more intense red band in the gel is ascribed to free AuNP not bound to the DNA origami.

The proper assembly of the hybrid DNA-AuNP was finally controlled by cryo-electron microscopy (cryo-EM) [33], (Fig. 1(f), and Section 4 in the ESM) to evaluate the effective 3D structure in absence of interaction with substrates. We verified that the insertion of the AuNP does not affect the tetrahedral structure of the origami. The origami ability to recognize the target strand and, consequentially, to modify its original configuration was investigated in different environments exploiting for each of them the most suitable technique. The device behavior was analyzed using SAXS in its synthesis buffer (TAE 1× and 12.5 mM of MgCl<sub>2</sub>) in the excess of free gold nanoparticles which represents a non-perturbing solution. In order to obtain high quality SAXS pattern the free AuNP were not removed since the purification due to the low yield of origami AuNP resulted in pattern with too low statistics for extracting information about the aggregation state.

The measured SAXS pattern (Fig. 2(a)) is mainly determined by the form factor scattering of the single AuNP. To investigate the differences among the working mechanism of 0ss and 3ss in aqueous solution, the structure factor of the AuNP-decorated tetrahedrons (Fig. 2(b)) was determined by dividing all traces with the free AuNP data. In this way the subtle differences among curves can be better resolved. In the following we will focus the discussion on a small portion of the plots corresponding to the  $q$  values that are expected to be affected by the interparticle distance changes. Indeed, the scattering wavevector ( $q$ ) is linked to the characteristic size of the sample ( $d_0$ ) by the simple relation  $d_0 = 2/q$ . Since a single nanoparticle (20 nm) corresponds to  $q = 0.31 \text{ nm}^{-1}$  and a non-actuated dimer interparticle distance (30 nm) corresponds to  $q = 0.21 \text{ nm}^{-1}$  we focused our attention on the  $0.15\text{--}0.30 \text{ nm}^{-1}$  range. For an easier comparison, Figs. 2(a) and 2(b) restricted to this range are shown in Section 5 in the ESM. The absolute value of the difference between the no target curve and the target one, shown in



**Figure 2** SAXS measurements performed in liquid on tetrahedron 0ss and 3ss before and after the probe-target actuation; samples were not purified from excess of AuNP. (a) Scattering pattern of all samples and the DNA-functionalized AuNP reference sample: all the traces mostly superimpose suggesting that the largest contribution to the signal comes from single AuNP form factor. (b) In order to extract the particle-particle interference, the structure factor has been derived by dividing the pattern with the AuNP signal (gold-yellow line). The structure factor of the different tetrahedrons highlights the presence of differences among the samples. The dashed box indicates the region of interest in which we expect to see a change in the structural factor as described in the main text. (c) The absolute value of the difference between the trace of the no target structure (dotted curves) and the traces of target structures (black and red curves) showed that the two samples are peaked at different  $q$ , corresponding to different interparticle distances: 25 nm for 3ss and 30 nm for 0ss. These different peak positions demonstrate that the reduction of the interparticle distance from the initial value of 30 to 25 nm is effectively detected only with the more flexible tetrahedron.

Fig. 2(c), allows to amplify the effect of the probe-target actuation in the structure factor, where the effects of the free NP and the single loaded DNA-AuNP are suppressed. The difference pattern shows a peak at different  $q$ -values (Fig. 2(c)), corresponding to different center-to-center interparticle distances: 25 nm for 3ss and 30 nm for 0ss. These differences in the peak positions demonstrate that the reduction of the interparticle distance from the initial value of 30 to 25 nm is detected only with the more flexible tetrahedron. SAXS measurements allow also the determination of the sample clustering through the evaluation of the peak width  $\Delta q$ . The data in Fig. 2(c) show that the width of the peaks is reduced to  $0.04 \text{ nm}^{-1}$  in comparison to the expected value of  $0.11 \text{ nm}^{-1}$  estimated from structure factor of a double sphere model. Therefore, the signal is dominated by structures clustered as observed with cryo-EM analysis (Section 4.4 in the ESM). Nonetheless, these measurements suggest that the clustering of the AuNP tetrahedron is not affecting the working mechanism. We conclude that the designed DNA origami structures are flexible enough to be bent by the target hybridization but stiff enough to maintain the 3D configuration in aqueous environment and in presence of aggregation effects. Further analysis in liquid was performed to optically characterize the AuNP functionalized tetrahedron through sequential measurements of the extinction spectra of aqueous solutions of AuNP with BSPP (bis(p-sulfonatophenyl)phenylphosphinedihydratedipotassium salt, used to stabilize the AuNP), DNA coated AuNP, AuNP-decorated tetrahedrons 0ss and 3ss. The normalized spectra are displayed in Fig. 3(a). The LSPR peak of DNA-covered AuNP is red-shifted with respect to AuNP in BSPP solution due to an increase of dielectric permittivity of the surrounding medium.

A further red-shift for the 0ss and the 3ss is caused by a weak plasmonic coupling between two AuNP connected to the tetrahedral DNA origami.

A larger broadening of the 3ss curve with respect to the 0ss curve can be explained by the higher flexibility of 3ss DNA origami structure leading to a less tight interparticle distance.

As for the SAXS measurements, here the contribution of free AuNP was dominating the spectra, since we did not perform any filtering or purification protocol. Moreover, it was not possible to subtract their contribution with a post processing procedure. Therefore, we did not observe the expected optical shift after the addition of the target as reported in Fig. S10 in the ESM.

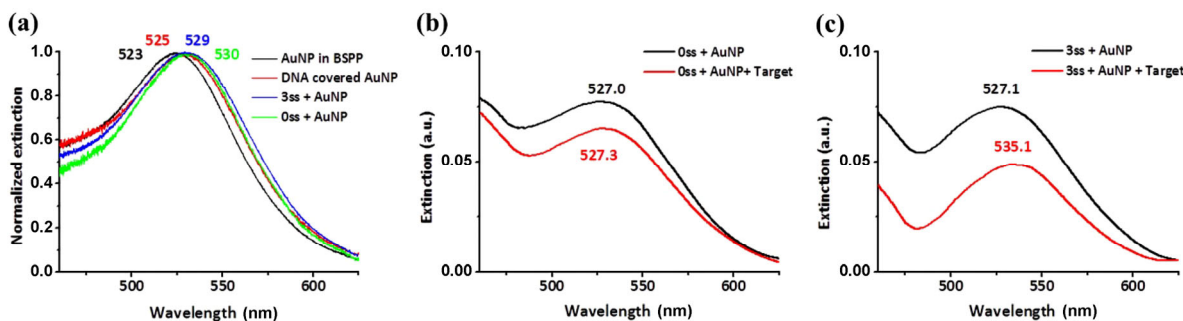
To evaluate the read-out of the tetrahedral DNA origami plasmonic sensor in a viscous environment, which is a better approximation of the extracellular matrix [37, 38] and the cytosol, we purified, concentrated and then measured in agarose gel the same structure, before and after hybridization in solution [30]. Thick agarose gel may scatter light significantly, resulting in poor transmission, thus

hindering UV-vis extinction measurements; therefore, the portion containing the tetrahedral DNA origami plasmonic sensor was cut to the thinnest possible section. Working with agarose gel offers an interesting advantage: running the solution through the gel it is possible to separate the different components composing the solution, and in particular, to get rid of free AuNP and DNA origami, as shown elsewhere [27].

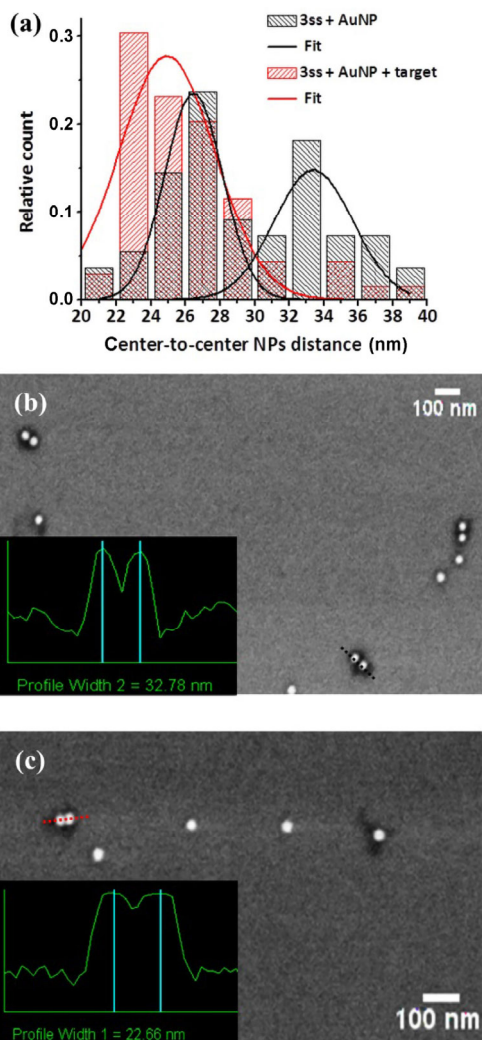
The individual gel bands were cut out of the gel block (Fig. 1(c)) to minimize cross band contamination and the extinction spectra for each band representing 0ss and 3ss, before and after target recognition, can be acquired. The spectra before and after the target addition are shown separately for 0ss in Fig. 3(b) and for 3ss in Fig. 3(c). The two curves representing 0ss before and after target addition are peaked almost at the same position indicating that the force induced by the target is not sufficient to deform the stiffer 0ss frame, as already observed in water. On the contrary, on the 3ss tetrahedron the target triggers a consistent (8 nm) red-shift in the optical response (Fig. 3(c)): as already observed with SAXS measurements, the weakened struts are flexible enough to be deformed upon target recognition; moreover, such deformation is not further modified by the increased viscosity of the read-out medium and remains consistent with SAXS measurements.

We used Mie theory [1, 39–41] in order to evaluate the interparticle gap change in the 3ss DNA origami tetrahedron before and after target addition. First, we obtained the value of the effective dielectric permittivity  $\epsilon_{\text{eff}}$  of DNA coated AuNP in water; this value was corrected in gel (see Section 6.2 in the ESM for more details) to calculate the AuNP surface-to-surface distance in gel network. The obtained values are 9.2 and 5.4 nm for no target and target 3ss tetrahedrons, respectively, which correspond to 29.2 and 25.4 nm of center-to-center interparticle distance, thus reproducing, also numerically, the SAXS results.

The effect of the interaction with a rigid substrate has been successively studied. A small droplet of solution containing the tetrahedral DNA origami-AuNP hybrid structures before and after target addition were incubated on clean silicon substrate that had been previously treated with 2 min oxygen plasma. After 7 min of incubation, the samples were washed twice with MilliQ water and let dry, so that it was possible to image the origami structures with scanning electron microscope (SEM). SEM imaging enables not only the visualization but also the calculation of the center-to-center interparticle distance by analyzing the brightness profile of the images. Its statistical distribution, calculated measuring the interparticle distance of 96 structures for 3ss before (black) and 69 structures for 3ss after (red) the target hybridization, is shown in Fig. 4(a). Before hybridization the distribution is broad and bimodal, peaked at 27 and 33 nm. Indeed, the flexible 3ss structure



**Figure 3** LSPR properties of AuNP-DNA origami hybrid structure. (a) AuNP LSPR analysis in solution. Spectra from AuNP suspended in BSPP solution (black), DNA coated AuNP (red), 0ss (green) and 3ss (blue) origami are displayed. The LSPR peak of DNA-covered AuNP is red-shifted with respect to AuNP in BSPP solution due to an increase of dielectric permittivity of the surrounding medium. A further red-shift for the 0ss and the 3ss is caused by a weak plasmonic coupling between two AuNP connected to the tetrahedral DNA origami. A larger broadening of the 3ss curve with respect to the 0ss curve can be explained by the higher flexibility of 3ss DNA origami structure leading to a less tight interparticle distance. (b) AuNP LSPR analysis in agarose gel, after purification. Spectra from 0ss structures with (red) and without (black) target. No spectral shift is observed. (c) AuNP LSPR analysis in agarose gel, after purification. Spectra from 3ss structures with (red) and without (black) target. A significant red-shift is observed attributed to the interparticle distance reduction due to the probe-target hybridization.



**Figure 4** SEM analysis of the interparticle distance. (a) Statistical distribution of interparticle distance (center-to-center) of 3ss dimers before (black) and after (red) the hybridization of target. The interparticle gaps were evaluated measuring the brightness profile of the SEM pictures through SEM SUPRA software tool. Before hybridization the distribution is broad with a bimodal character peaked at 27 and 33 nm. After target hybridization, the distribution become monomodal peaked at 23–24 nm. (b) and (c) Representative SEM image of not purified DNA origami samples. Well-folded 3ss tetrahedron decorated with two gold nanoparticles are easily identified and distinguishable from the free AuNP also present in the solution. In the inset a representative profile used to evaluate the interparticle gap of 3ss before (b) and after (c) probe-target actuation.

toggles between a broad range of configurations, ranging from the AuNP contact to a separation of 20 nm, peaked around its equilibrium configuration of 30 nm. The four facets of the tetrahedral structures have the same probability to get in contact to the silicon solid surface, but the two surfaces with the AuNP have a much smaller contact area and thus a lower sticking probability. Moreover, in this case the two AuNP would appear superimposed in the SEM image and they would not be considered in the statistics. If the facet containing the probe is interacting with the substrate, the electrostatic interaction will freeze it in the configuration it had last assumed while in solution, which in absence of a probe-target interaction is the one with the largest AuNP distance (33 nm). On the other hand, the docking of the fourth facet exposes the flexible parts to the capillary forces developed during drying which shrinks the structure to an interparticle distance of 27 nm. The docking of these two facets has similar probability, hence the bimodal distribution. When the target hybridizes before the docking, the distribution becomes monomodal and peaked at 25 nm. The addition of the

target pulls the struts closer and stiffens the whole structure in a shrunk configuration, independently from the facet docking, and in agreement with the LSPR and SAXS analysis. These data show that the interaction with a solid surface after hybridization does not influence the configuration, which depends only on the presence of the target.

These results together demonstrate that the designed 3ss tetrahedron maintains the structural modification induced by target hybridization irrespective of the environment in which it is measured: aqueous solution, highly viscous matrix, deposited and dried on a solid substrate. In particular, 3ss tetrahedron shows the capability to switch its configuration by reducing the interparticle gap in a controlled way, without being clearly perturbed by capillarity, viscosity, adhesion forces and surrounding pressure. It can still be slightly affected by thermal fluctuations in aqueous solution, as shown in LSPR analysis in graph in Fig. 3(a), where the 3ss shows a wider peak than the 0ss. The 0ss instead does not change its configuration, thus highlighting the higher rigidity of the “fully double strand” structure, which hinders the proper response when stimulated by the target. In addition, the plasmonic properties of the sensor could promote the fast and non-invasive LSPR detection of numerous targets of medical interest not only *in vitro* but also *in vivo*, improving the actual diagnostic in term of target localization and clinical invasiveness.

#### 4 Conclusions

In this work we described a DNA origami-based plasmonic device in which a biomolecular reaction produces a structural reconfiguration. The device has a tetrahedral structure with rigid struts connected by flexible joints. To increase its flexibility, in one design, we introduced 4-nucleotides gaps in three helices of the bundle positioned in a specular way in the two struts connected by the actuator strand. The detection strategy relies on the plasmonic response of two AuNP strategically positioned on two opposite facets of the tetrahedron: any relevant structural change, which may result in a nanometric change of the interparticle gap, can be monitored through extinction measurements. The actuation, triggered by the hybridization with a molecular target, induces a clearly detectable red-shift of the peak position of AuNP LSPR spectrum proportional to the interparticle distance reduction. The optical extinction measurements of target hybridized and not hybridized structures highlighted the presence of an LSPR shift in the target hybridized 3ss samples only, confirmed by in-depth analysis of conformational changes performed using SAXS detection. We investigated the effect of the environment in which the switch of the configuration is evaluated, by performing SAXS measurements in aqueous environment, LSPR measurements in a highly viscous agar gel matrix and SEM imaging with the DNA origami tetrahedron interacting with a solid substrate, and in all environments a consistent gap reduction between AuNP after the probe-target recognition was observed. The tetrahedral DNA origami has demonstrated the environment independence for which it has been originally designed, paving the way to its application in the field of molecular recognition in different conditions. Moreover, its biocompatibility due to both its tetrahedral shape, compatible with most cell uptake mechanisms [42, 43], and its material, DNA and AuNP, suggests that the wireframe tetrahedron presented in this paper could be taken in consideration for *in vivo* analysis, for example as an injectable biosensor or as a contrast medium for biomarker detection. The new technology is not restricted only to the recognition of DNA targets. The substitution of the DNA probe with antibodies and aptamers would allow the detection of antigens or protein markers both *in vitro* and *in vivo*, enabling the target diagnosis directly in the native environment.

## 5 Materials

Unless otherwise specified, chemicals and reagents were purchased from Sigma-Aldrich. The oligonucleotides custom design for the folding of the tetrahedral structure with an average length of 32 nts and the 3' thiol-capped DNA oligonucleotides for the AuNP coverage were purchased with HPLC purification from Sigma-Aldrich. M13mp18 is a M13 lac phage vector long 7249 nts (250 µg/mL) and was purchased from New England BioLabs Inc. GelRed™ DNA staining solution was purchased from Biotium. Gold colloids (AuNP), 20 nm in diameter with a coefficient of variation < 8% supplied in water, and Muscovite Mica grade V1 were purchased from Ted Pella, Inc. AFM imaging was performed using Olympus OMCL-TR400PSA AFM tips with a pyramidal tip (spring constant 0.02–0.08 N/m).

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