Published in final edited form as:

ACS Nano. 2020 June 23; 14(6): 7454–7461. doi:10.1021/acsnano.0c03127.

Chiral Assembly of Gold-Silver Core-Shell Plasmonic Nanorods on DNA Origami with Strong Optical Activity

Linh Nguyen^a, Mihir Dass^a, Martina Ober^a, Lucas V. Besteiro^{b,c}, Zhiming M. Wang^b, Bert Nickel^a, Alexander O. Govorov^{b,d}, Tim Liedl^a, Amelie Heuer-Jungemann^{a,*}

^aFaculty of Physics and Center for NanoScience (CeNS), Ludwig-Maximilians-University, Geschwister-Scholl-Platz 1, 80539 Munich, Germany

^bInstitute of Fundamental and Frontier Sciences, University of Electronic Science and Technology of China, Chengdu 610054, China

^cCentre Énergie Matériaux et Télécommunications, Institut National de la Recherche Scientifique, 1650 Boul. Lionel Boulet, Varennes, QC J3X 1S2, Canada

^dDepartment of Physics and Astronomy, Ohio University, Athens, Ohio 45701, United States

Abstract

The spatial organization of metal nanoparticles has become an important tool for manipulating light in nanophotonic applications. Silver nanoparticles, particularly silver nanorods have excellent plasmonic properties, but are prone to oxidation and are therefore inherently unstable in aqueous solutions and salt containing buffers. Consequently, gold nanoparticles have often been favored, despite their inferior optical performance. Bimetallic, *i.e.* gold-silver core-shell nanoparticles can resolve this issue. We present a method for synthesizing highly stable gold/silver core-shell NRs that are instantaneously functionalized with DNA, enabling chiral self-assembly on DNA origami. The silver shell gives rise to an enhancement of plasmonic properties, reflected here in strongly increased circular dichroism, as compared to pristine gold nanorods. Gold-silver nanorods are ideal candidates for plasmonic sensing with increased sensitivity as needed in pathogen RNA or antibody testing, for non-linear optics and light-funneling applications in surface enhanced Raman spectroscopy. Furthermore, the control of interparticle orientation enables the study of plasmonic phenomena, in particular synergistic effects arising from plasmonic coupling of such bimetallic systems.

Abstract

A.Heuerjungemann@physik.uni-muenchen.de .

Keywords

DNA origami; self-assembly; plasmonic nanoparticles; circular dichroism; bimetallic nanoparticles

Metal nanoparticles (NPs) exhibit excellent optical properties of particular interest in a variety of fields of research including photonics, imaging,¹ medicine,² catalysis,³ biosensing,⁴ and optical data storage.⁵ Anisotropic particles exhibit additional, strongly shape-dependent optical behavior, including localized field enhancement and optical non-

linearities.⁶ The accessibility of different shapes and morphologies by wet chemical synthesis rather than through lithographic techniques has led to high throughput fabrication and commercialization of such NPs. This led to a wide range of potential users and thus allowed for a fast progress in their general application. Especially, the seed-mediated growth method, which was first applied only to nanospheres but later revolutionized the field of anisotropic NPs (*e.g.* NRs or nanostars), has led to synthesis protocols for the production of near-monodisperse particles at high yields.^{7, 8}

In order to manipulate light on the nanoscale, controlling interparticle distance and particle orientation is key. For example, so-called plasmonic hot spots in the gaps between metal particles can lead to strong enhancement of the radiative properties of molecules inside such hot spots by orders of magnitude allowing surface enhanced Raman scattering⁹ on the single-molecule level.¹⁰ The DNA origami technique provides such precise control over metal NPs organization, as demonstrated, *e.g.*, for plasmon-induced circular dichroism, a phenomenon based on the near-field coupling of chiral assemblies of NPs¹¹ that can be employed for sensitive analyte detection.^{12–16}

While the general plasmonic behavior of gold (Au) and silver (Ag) nanoparticles is similar,¹⁷ a Ag shell induces larger near-field enhancement¹⁸ and narrower plasmon linewidth compared to that of pure Au nanorods (NRs) of the same resonance wavelength.¹⁹ Furthermore, Ag exhibits a vastly greater extinction cross-section (AgNPs up to hundred times larger than AuNPs),²⁰ and has the lowest losses of all metals in the visible and near infrared (NIR), which is generally beneficial for plasmonic applications.^{21, 22} Nevertheless, the high chemical stability of AuNPs is often decisive for practical use in spite of better optical properties for AgNPs. Consequently, research thus far focused on AuNPs. While spherical AgNPs have been successfully incorporated into DNA-based assemblies,^{23–25} AgNRs hitherto refused to cooperate with DNA origami. Also, bimetallic Au/AgNPs – which are distinct from a simple combination of the two individual components – and their controlled self-assembly have remained virtually unstudied.

Primarily the chemical instability of Ag-containing NPs has hampered their wide-spread use in self-assembly based plasmonics. In particular oxidation, disintegration and fast aggregation, are troublesome when ligand exchange reactions need to be performed, which is often required to infer further functionality. DNA is a widely used ligand for the controlled assembly of metal NPs, however, preparing DNA-AgNPs conjugates is not trivial since phosphines, commonly used to produce thiol reactive groups in DNA, interact with Ag ions hindering the conjugation process.²⁶ Moreover, the preparation of AgNRs, with a reliable control over aspect ratio and monodispersity has thus far been strongly limited. Au/Ag core-shell NRs, which offer stability through the Au core as well as cooperative and superior plasmonic behavior,^{19, 27, 28} represent the best compromise so far in synergistic effects for stability and optical performance.

In Au/Ag core-shell systems, the use of large polymeric stabilizing agents, such as polyvinyl pyrrolidone (PVP), has been suggested to avoid aggregation and increase stability in order to resolve issues with conventional stabilizing agents such as cetyltrimethylammonium bromide (CTAB).²⁷ However, while the resulting steric effects provide efficient stabilization,

they also prevent functional molecules from interacting with the particle surface. We speculate that therefore, the functionalization of Au/Ag core-shell NRs with DNA and the controlled assembly of such NRs on a DNA origami substrate could not be explored to date.

Here, we abandon the concept of a two-step reaction, *i.e.* initial synthesis of a Ag shell and subsequent functionalization. We conduct a simple one-pot reaction (Scheme 1) that yields stable DNA-coated Au/AgNRs with good monodispersity.

Results and Discussion

The Ag shells on the AuNRs were synthesized using a protocol by Guyot-Sionnest and co-workers,²⁷ which was modified with the rationale of achieving instantaneous functionalization with thiolated DNA, limiting particle disintegration and oxidation over time. In order to ensure that the NRs are sufficiently stabilized, the thiol-DNA is added in excess. During Ag shell growth, using AgNO₃ as precursor and L-ascorbic acid as reducing agent, the thiol-DNA binds to the Ag surface imparting instantaneous stabilization and functionalization. The lattice constant of Au and Ag have 0.2 % mismatch only allowing for epitaxial Ag growth and formation of monocrystalline Au/AgNRs.²⁷ The Ag-layer thickness can be tuned by the ratio between AuNR and AgNO₃ concentration, *i.e.* a lower ratio leads to thicker shells. It should also be noted that our method is not limited to rod-shaped particles, but can also be further extended to other shapes, *e.g.* spheres, yielding highly stable DNA-coated Au/AgNPs (Figure S2).

Au/Ag core-shell NRs with varying thicknesses of Ag shell were subsequently analyzed by UV-vis spectroscopy and transmission electron microscopy (TEM) (Figure 1). The individual batches exhibited good monodispersity at high production yields resulting in sharp resonance signals in the absorption spectrum (Figure 1 and Figure S3). UV-vis spectra of the Ag/AuNRs were experimentally recorded and simulated theoretically, cf. Figure 1a and 1b.

Prominent features of these spectra are the gradual blue-shift of the longitudinal mode of the LSPR and the overall enhancement of the signal strength with increasing Ag shell thicknesses (Figure 1a and S4). More subtle features are the vanishing transversal LSPRs of the overgrown gold NRs and the small blue-shift of the rising Ag transversal mode. These results can be explained by a combination of two effects, namely the expulsion of the electromagnetic field from the Au core and the decreased aspect ratio of the Ag-shelled NRs for thicker shells.²⁹ The thickness of the Ag shell depends on the Ag⁺ ion concentration during synthesis,¹⁸ therefore the blue-shift of the resonance wavelength of the longitudinal mode can be easily and accurately tuned over a wide spectral range from the visible to the NIR. Here we demonstrate a stepwise shift of the longitudinal plasmon mode from 834 nm for the pristine AuNRs (39.2 × 9.8 nm) cf. Figure S1 to a resonance at 545 nm by using ~ 5 nM AuNRs and AgNO₃ concentrations ranging from 10 to 40 mM which resulted in Ag shell thicknesses ranging from ~ 1.1 to 5.4 nm (Figure 1c).

Molar extinction coefficients of Au/AgNRs@DNA were experimentally deduced through the relative rise of absorption intensity of the initial AuNRs, which are in good accordance with theoretically calculated values (Table S1).

The optical behavior can be qualitatively described with the Au core being gradually electrically screened by the Ag shell. Numerical simulations reproduce the spectral behavior of the Ag-shelled Au nanorods extremely well (Figure 1b), not only tracking the progressive blue-shift of the main plasmonic peak but also showcasing the increasing relative amplitude of the secondary plasmonic peak, found at higher frequencies. This secondary peak results from the excitation of a transversal mode on the Ag-shelled nanorods, and increases its amplitude as the Ag shell thickens. The field enhancement of this resonant mode is localized in the Ag shell, as it is apparent by observing the field maps in Figure 1d. This implies the formation of charge dipoles between the Au/Ag interface and the outer Ag surface, as we illustrate with schematic charge signs in the right panel of Figure 1d. This does not occur when exciting the longitudinal modes, in which the main peak polarizes the whole structure. So, even though for both modes the relevance of the plasmonic properties of Ag increase as the thickness of the shell grows, as one would expect, their qualitative response differs. Under longitudinal polarization, the most apparent change is the blue-shift of the plasmonic resonance as more Ag is available to carry the longitudinally oscillating currents, due to the higher plasma frequency of Ag with respect to Au. Under a transversal polarization we see the appearance of a resonant mode localized at the shell, at a wavelength that is far from the unscreened transversal resonance of Au at 515 nm (Figure S5).

These differences highlight the interest of studying bimetallic systems with non-spherical geometries, as they can offer richer scenarios for tuning their optical properties. Clearly, the spectra do not arise as the simple sum of the individual components, *i.e.* non-linear effects occur. Similar observations have been reported previously, *i.e.* Ag-involving metallic systems display additional absorption behavior at 340 – 350 nm, this includes bipyramidal AuNPs with a rod-shaped Ag shell,³⁰ AuNRs with an octahedral Ag shell,³¹ as well as AgNRs,³² wires,³³ prisms,³⁴ cubes,³⁵ and more complex shapes.⁹ Opinions are divided over the detailed origin of this signal;^{36–38} therefore, it still remains a topic of investigation to date and more effort needs to be expended in order to understand and particularly to manipulate plasmons in such Ag systems.

Transmission electron microscopy (TEM) analysis reveals the DNA shell as a bright halo around the NRs when stained with uranyl formate, and the Au core can be distinguished from the Ag shell due to the difference in electron density of the two metals (Figure 1c and 1d). Previous studies have shown that the deposition of Ag preferentially occurs on certain Au crystal facets and that two of the four (110) side facets of the freshly growing Ag crystal build up more quickly than the remaining two facets.^{27, 39} Consequently, the position of the Au core becomes geometrically less central as the shell thickness increases (Figure 1c, right panel). Although the microscopic origin of this behavior is not fully understood, it is believed that a selective adsorption of CTAB on different crystal planes of fcc Ag leads to the resulting morphology of the particles.³⁹ Notably, without CTAB, no Ag shell could be grown around the AuNR.³¹ A possible explanation is that the formation of AgBr, where the

Br⁻ ions are provided by CTAB which adsorb onto the facets of Au, is essential for the Ag growth.⁴⁰

As mentioned above, a further stabilizing ligand besides CTAB is required to ensure colloidal NR stability. DNA provides additional functionality, such as sequence-specific binding. Therefore, we focus in our report primarily on this versatile ligand. In all the coating procedures presented here, DNA was added in large excess, *i.e.* the reaction solution contains many unbound DNA molecules after completion of the Ag shell growth. This excess amount of DNA can either be removed by centrifugation or alternatively be used to achieve higher DNA loading on the particles. Recently, Liu *et al.* showed that very high DNA-loading on AuNPs could be achieved by simply freezing the particles with thiolated DNA.⁴¹ We adapted this strategy for our Ag/AuNRs by optionally freezing the whole reaction mixture after completion of the Ag coating for increased DNA loading as well as for long-term storage and retaining quality. It is noteworthy that after a removal of the excess DNA, performed after completion of the synthesis, the Au/AgNRs@DNA can withstand at least ten freeze-thaw cycles without any changes in colloidal quality and optical properties (Figure S7). Storing of the Au/AgNRs in the frozen state ensures colloidal stability over a very long period of time as the oxidation process of the silver is slowed down, which in turn greatly improves the stability and ease of use of the NPs. In contrast, conventionally stabilized NPs aggregate immediately and irreversibly upon freezing.

At ambient conditions a variety of DNA sequences provide colloidal and chemical stability for at least one month (Figure S8). In order to investigate the impact of sequence length and base content of the coating DNA on the stability of the particles in detail, we employed eight different sequences of varying strand lengths: polyA₁₉, polyT₁₉, polyT₁₅, polyT₈, polyG₁₉, polyC₁₉ as well as two “random” sequences, *i.e.* sequences of heterogeneous base composition (Table S2). For all sequences, the Ag-coating process was successfully verified by a blue-shift of the extinction signal of the longitudinal mode, which was also visible by eye through a color change of the colloidal solution. However, we find that different DNA sequences yield very different degrees of stability, ranging from quickly aggregating to highly stable dispersions (Figure S8).

For particles with Ag shells grown in the presence of polyA₁₉, aggregation occurred under ambient conditions within 2 h (Figure S8g). This could be attributed to Adenine generally exhibiting a high affinity to metal surfaces.⁴² The Adenine bases likely adsorb to the NR surface thus hindering thiol-Ag interactions and thus efficient DNA loading, which would be required for colloidal stability. Similarly, Cytosine also displays a high affinity to metal NPs albeit not as strongly as Adenine. Additionally it has been reported that polyC DNA has the ability to etch Ag⁴³ which may also shorten the stability time-frame. Consequently, Au/AgNRs@polyC₁₉ aggregated within 30 min under ambient conditions (Figure S8h). For polyG₁₉, no aggregation was observed and only a small change in the absorption spectrum became visible after 1 – 2 weeks (Figure S8f). However, repeated centrifugation and re-dispersion gradually resulted in Au/AgNRs@polyG₁₉ aggregation. We attribute this to the fact that polyG sequences are prone to forming G-quadruplexes. If G-quadruplexes are formed intra-molecularly, the surface density should be higher than that of “random coil” DNA, thus higher loading would be possible. However, we hypothesize

that inter-molecular G-quadruplex formation may also occur leading to cross-linking of the particles. For polyT₈, 15, 19, 30 and the two random sequences, no aggregation was observed for at least one month (end of observation time) and even after multiple centrifugation and freeze-thaw cycles (Figure S7). Further, we did not observe any significant difference in stability over time at ambient conditions for the different polyT lengths (Figure S8). The Au/AgNRs@polyT₁₉ and Au/AgNRs@random could also be run on agarose gels where both samples displayed good electrophoretic mobility and striking monodispersity in case of the polyT coating (Figure S10).

In order to test if the thiol reactive group is essential for effective particle stabilization, we employed non-thiolated polyT₁₉ and random sequences in the synthesis. As expected, using non-thiolated DNA did not result in stable particles as can be clearly seen by the formation of aggregates in the wells of the agarose gel (Figure S10).

To access the homogeneity of the surrounding DNA shell and to obtain the structural details including crystallinity of the Au/AgNRs@DNA, we performed small-angle and wide-angle X-ray scattering (SAXS and WAXS) experiments on 15 nm × 7 nm AuNRs with a thin Ag shell of 5 Å, and a polyT₁₉ as the stabilizing ligand. SAXS probes particle size and particle – particle distances. The SAXS intensity as a function of the scattering vector $q = |\vec{q}| = 4\pi \sin\theta/\lambda$, where λ is the wavelength of the x-rays and θ the half of the scattering angle 2θ , is shown in Figure 2a. From this data one can readily infer that the particles are well dispersed, *i.e.* formation of clusters of particles can be excluded based on the presence of the intensity plateau at small q and the absence of correlation or cluster peaks. Furthermore, the presence of interference effects indicates a well-defined particle shape. For quantitative analysis, we employed a model fit assuming cylindrical particles with two concentric shells, representing Ag and DNA, respectively. The model for the SAXS intensities is described in detail in Note S1 of the Supporting Information. From the SAXS measurements, we obtain an Au core radius $R_{Au} = 3.4$ nm with a variance of the size distribution of ~ 0.4 nm corresponding to a polydispersity (PD) of ~ 0.12 % and a length $L_{Au} = 15.5$ nm with a variance of the size distribution of ~ 4.5 nm corresponding to a polydispersity (PD) of ~ 0.29 %. The Ag and DNA shell thicknesses are $d_{Ag} = 5$ Å and $d_{DNA} = 2.9$ nm, respectively. The shell thickness $d_{DNA} = 2.9$ nm is close to the Flory radius of T₁₉ DNA (4 nm) and somewhat smaller than the highly dense brush regime as observed for DNA on AuNPs (5.7 nm).⁴⁴ Furthermore, all parameters obtained in solvent conditions are in good agreement with the dimensions obtained from TEM images and the NR design.

Crystal structure and crystallinity of the Au/AgNRs@DNA were obtained from WAXS experiments. The WAXS diffraction pattern for different particle compositions (Figure S11) are shown as intensity I vs. scattering angle 2θ in Figure S2b and Figure S12. The WAXS profiles show fcc diffraction peaks with a refined lattice parameter of $a_{Au} = 4.0745$ Å, $a_{Au} = 4.0756$ Å and of $a_{Au} = 4.0770$ Å for AuNRs, Au/AgNRs, and Au/AgNRs@DNA, respectively. The obtained lattice parameters are in good agreement with the literature lattice constant $a_{Au} = 4.0704$ Å and $a_{Ag} = 4.0778$ Å.⁴⁵

Importantly, we observe no features in our SAXS or WAXS data, which would indicate a permeation of DNA through the Ag like porosity of the Ag shell. From this, we derive the DNA being conjugated only on the Ag surface through the thiol-Ag bond (cf. Figure S10).

We hypothesized that our method is not restricted to using thiolated DNA as a ligand, but can be applied more universally using other ligands displaying a thiol group. To demonstrate this we used mercaptopropionic acid (MPA) and O-(2-Mercaptoethyl)-O'-methyl-hexa(ethylene glycol) (mPEG thiol) as ligands. For both ligands, formation of the Ag shell was successful, however, differences in stability of the resulting particles could be observed. The Ag/AuNRs@mPEG-thiol display a slight change in quality after several freeze-thaw cycles as evidenced by UV/vis spectroscopy (Figure S7e). The optical behavior was also monitored over several weeks at ambient condition (Figure S8k) where the Ag/AuNRs display slight changes in the UV/vis spectrum after 7 days. The MPA-functionalized Ag/AuNRs lose their quality upon freezing (Figure S7d). However, they can be stored under ambient conditions for 3 weeks after which they show slight changes in their optical behavior (Figure S8l).

Although such stable Au/AgNRs already possess excellent optical properties as single particles, studying their behaviour in controlled assemblies is of immediate interest. Our method of creating stable DNA-coated Au/AgNRs gives access to assemble these bimetallic particles on DNA origami structures. For conjugating particles to DNA origami, it is essential that all components are stable in buffers containing salt. In order to verify that our particles are stable under typical conjugation conditions, Ag/AuNRs@DNA were dispersed in Tris acetate (TA) or Tris acetate EDTA (TAE) buffer containing increasing amounts of $MgCl_2$. Both the polyT₁₉ and random DNA sequence modified Au/AgNRs displayed good stability in 1×TA buffer containing $MgCl_2$ (Figure S9).

As a proof-of-principle we used two origami templates; a rod-shaped origami (14-helix bundle, 14 HB) with anchor strands along the long axis, and a flat square origami (44 HB, double-layer sheet), designed to align two Ag/AuNRs@DNA in a cross-like geometry as illustrated in Figure 3. Each origami template was mixed with the Ag/AuNRs@DNA in a ratio of ~ 15 rods per binding site in 1×TA buffer containing 6 mM $MgCl_2$ to ensure hybridization of the anchor strands. The mixture was exposed to a temperature ramp and the Au/AgNRs@DNA@origami conjugates were subsequently purified from excess NRs by agarose gel electrophoresis (Figure 3, left panels). The two fastest moving bands were assigned to excess Au/AgNRs@DNA and NR dimers, respectively. The slowest band contained the desired Au/AgNR@DNA conjugated with the DNA structure as confirmed by TEM (Figure 3, right panels and Figure S13). In each assembly one can discern the Ag shell on the AuNR as well as the dense DNA shell surrounding the rod and connecting it to the respective DNA origami.

Assemblies such as the ones shown in Figure 3b furthermore display interesting optical properties in the form of circular dichroism, owing to their chiral arrangement (Figure 4). The characteristic bisignate spectra arise from the plasmon interactions of the individual NRs in each metamolecule when excited by circularly polarized light, resulting in the rise of distinct symmetric and antisymmetric modes with opposite chirality. The symmetric mode

is blue-shifted while the antisymmetric mode is red-shifted compared to the longitudinal resonance. Corresponding with the shift in the longitudinal resonance peak of individual NRs, the CD spectra as a whole was also blue shifted for structures assembled with Au/AgNR compared to AuNR assemblies (N.B.: The same AuNRs were used for the assembly and the formation of the Au/AgNRs used for assembly).

The anisotropy factor, also known as the g-factor is used as a measure of the structural asymmetry of chiral structures^{46, 47} (for further details, see Supporting Information). As seen in Figure 4, the g-factor of Au/AgNR assemblies exceeds that of uncoated AuNR assemblies by a factor of 7 (see Figure S13 for representative TEM images) and is most importantly also $3 \times$ stronger than current state-of-the-art for self-assembled systems,⁴⁸ which proves both the excellent assembly capability as well as strong plasmonic capabilities of the bimetallic rods.

Conclusion

We have presented a simple and fast one-pot method to synthesize monodisperse Au/AgNRs and their instantaneous functionalization with DNA. The size and aspect ratio of the Au/AgNRs and especially the Ag shell thickness can be precisely controlled allowing us to tune the LSPR as desired. Their improved shelf life and storability make them an ease-of-use product in the laboratory. Further, the DNA functionality of Au/AgNRs@DNA allows for their organization on a DNA origami platform, which as of yet has not been accessible using such bimetallic NPs or AgNRs. With this methodology, plasmonic structures built with DNA origami will be extended from mono- to bimetallic systems. Particularly the cooperative and synergistic effects of bimetallic structures, will not only be of fundamental interest but also essential for optical applications. The bimetallic chiral assemblies shown here display strongly increased CD responses in comparison to their Au counterparts, which could hold great promise for plasmonic sensing with increased sensitivity as urgently required for antibody or pathogen RNA testing. In prospective work our presented method may be also employed to create other particle geometries to further expand the variety of such ordered plasmonic systems.

Methods

Synthesis of AuNRs

The synthesis of AuNRs was carried out following the protocol by Murray *et al.*⁴⁹

Ag coating of AuNRs and DNA-functionalization

AuNRs were re-dispersed in 0.1 M CTAB resulting in a concentration of 5 – 50 nM of AuNRs. To 5 mL of this AuNR solution 22.5 mL of 0.1 M CTAB and 2.5 mL of 100 μ M of thiol-modified DNA was added (for chiral Au/AgNRs assemblies a mixture of polyT₁₉ and polyT₈ DNA in the ratio of 1:9 was used). Note that tris(2-carboxyethyl)phosphine (TCEP) is often used to cleave the disulfide bond of thiol-modified DNA, however, strongly interacts with Ag and disturbs the Ag-coating process. Since CTAB crystallizes at room temperature, the mixture was stirred and heated to 35 °C and kept under this temperature during the whole procedure to ensure the dissolution of CTAB. Subsequently, 4 mL of 10 – 40 mM

AgNO₃ and 625 μ L of freshly prepared 0.2 M L-ascorbic acid was added. In a last step 1.25 mL of 0.2 M NaOH was injected under vigorous stirring to increase the pH and the reduction potential of L-ascorbic acid and thus to initiate the reaction. To obtain a high degree of monodispersity the formation of foam by CTAB needs to be avoided. Immediately a color change can be observed. The reaction is completed a few minutes to an hour after the color change depending on the AgNO₃ concentration that influences the Ag growth rate. The obtained Au/Ag core-shell nanorods were isolated from the reaction solution by 4-times centrifugation and re-dispersion in 0.1 % SDS. Optionally, the NRs can be re-dispersed in 1 $\times$ TA buffer or water.

To further increase the DNA loading, the Au/AgNRs@DNA were not isolated from the reaction mixture but frozen at -20 °C. After thawing, the NRs were purified by centrifugation and re-dispersion in 0.1 % SDS or 1 $\times$ TA buffer. For a long-term storage the Au/AgNRs@DNA were kept in the frozen state.

Conjugation of DNA-functionalized Au/AgNRs with DNA origami

The NRs were added in a ~10-times excess per binding site to the origami solution with a final concentration of 1 – 2 nM origami and 6 mM MgCl₂ in 1 $\times$ TA buffer. Under gentle shaking, the mixture was exposed to a temperature ramp starting at 45 °C followed by slow cooling to 20 °C (rate: 1°C/10 min), repeated a total of 4 times. The Au/AgNRs@origami structures were isolated from excess Au/AgNRs by gel electrophoresis (Figure 3).

Supporting Information

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

L.N. acknowledges financial support from the Deutsche Forschungsgemeinschaft (DFG) through the excellence cluster “e-conversion”. T.L. acknowledges funding through the European Research Council under the European Union’s Horizon 2020 research and innovation program (Grant agreement No. 818635, DNA Funs). M.D. has received funding from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 765703. M.O., B.N., T.L. and A.H-J acknowledge the financial support of the DFG through SFB1032 “Nanoagents”, projects A06/A07. A.O.G. acknowledges the support from the United States-Israel Binational Science Foundation (BSF). L.V.B. was supported by the Institute of Fundamental and Frontier Sciences, University of Electronic Science and Technology of China and China Postdoctoral Science Foundation (2017M622992 and 2019T120820). Z.M.W. was funded by the National Key Research and Development Program (No. 2019YFB2203400) and the “111 Project” (B20030). The authors would also like to thank Maximilian Urban for synthesis and imaging of spherical Au/AgNPs and Susanne Kemper for help with TEM imaging.

This document is the Accepted Manuscript version of a Published Work that appeared in final form in ACS Nano, copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see <https://pubs.acs.org/doi/abs/10.1021/acsnano.0c03127>.

References

1. Cognet L, Tardin C, Boyer D, Choquet D, Tamarat P, Lounis B. Single Metallic Nanoparticle Imaging for Protein Detection in Cells. *Proc Natl Acad Sci USA*. 2003; 100 :11350–11355. [PubMed: 13679586]

2. Boca SC, Potara M, Gabudean A-M, Juhem A, Baldeck PL, Astilean S. Chitosan-Coated Triangular Silver Nanoparticles as a Novel Class of Biocompatible, Highly Effective Photothermal Transducers for *In Vitro* Cancer Cell Therapy. *Cancer Lett.* 2011; 311 :131–140. [PubMed: 21840122]
3. Priece P, Salami HA, Padilla RH, Zhong Z, Lopez-Sanchez JA. Anisotropic Gold Nanoparticles: Preparation and Applications in Catalysis. *Chin J Catal.* 2016; 37 :1619–1650.
4. Nath N, Chilkoti A. Label Free Colorimetric Biosensing Using Nanoparticles. *J Fluoresc.* 2004; 14 :377–389. [PubMed: 15617380]
5. Gu M, Zhang Q, Lamon S. Nanomaterials for Optical Data Storage. *Nat Rev Mater.* 2016; 1 :16070.
6. Lamarre J-M, Billard F, Kerboua CH, Lequime M, Roorda S, Martinu L. Anisotropic Nonlinear Optical Absorption of Gold Nanorods in a Silica Matrix. *Opt Commun.* 2008; 281 :331–340.
7. Jana NR, Gearheart L, Murphy CJ. Wet Chemical Synthesis of Silver Nanorods and Nanowires of Controllable Aspect Ratio. *ChemComm.* 2001 :617–618.
8. Jana NR, Gearheart L, Murphy CJ. Wet Chemical Synthesis of High Aspect Ratio Cylindrical Gold Nanorods. *J Phys Chem B.* 2001; 105 :4065–4067.
9. Mulvihill MJ, Ling XY, Henzie J, Yang P. Anisotropic Etching of Silver Nanoparticles for Plasmonic Structures Capable of Single-Particle SERS. *J Am Chem Soc.* 2009; 132 :268–274.
10. Lim D-K, Jeon K-S, Kim HM, Nam J-M, Suh YD. Nanogap-Engineered Raman-Active Nanodumbbells for Single-Molecule Detection. *Nat Mater.* 2010; 9 :60–67. [PubMed: 20010829]
11. Fan Z, Govorov AO. Plasmonic Circular Dichroism of Chiral Metal Nanoparticle Assemblies. *Nano Lett.* 2010; 10 :2580–2587. [PubMed: 20536209]
12. Kuzyk A, Schreiber R, Fan Z, Pardatscher G, Roller E-M, Högele A, Simmel FC, Govorov AO, Liedl T. DNA-Based Self-Assembly of Chiral Plasmonic Nanostructures with Tailored Optical Response. *Nature.* 2012; 483 :311. [PubMed: 22422265]
13. Schreiber R, Luong N, Fan Z, Kuzyk A, Nickels PC, Zhang T, Smith DM, Yurke B, Kuang W, Govorov AO. Chiral Plasmonic DNA Nanostructures with Switchable Circular Dichroism. *Nat Commun.* 2013; 4 :2948. [PubMed: 24336125]
14. Yan W, Xu L, Ma W, Liu L, Wang L, Kuang H, Xu C. Pyramidal Sensor Platform With Reversible Chiroptical Signals for DNA Detection. *Small.* 2014; 10 :4293–4297. [PubMed: 24989032]
15. Kuzyk A, Schreiber R, Zhang H, Govorov AO, Liedl T, Liu N. Reconfigurable 3D Plasmonic Metamolecules. *Nat Mater.* 2014; 13 :862–866. [PubMed: 24997737]
16. Funck T, Nicoli F, Kuzyk A, Liedl T. Sensing Picomolar Concentrations of RNA Using Switchable Plasmonic Chirality. *Angew Chem.* 2018; 130 :13683–13686.
17. Link S, El-Sayed MA. Spectral Properties and Relaxation Dynamics of Surface Plasmon Electronic Oscillations in Gold and Silver Nanodots and Nanorods. *J Phys Chem B.* 1999; 103 :8410–8426.
18. Becker J, Zins I, Jakab A, Khalavka Y, Schubert O, Sönnichsen C. Plasmonic Focusing Reduces Ensemble Linewidth of Silver-Coated Gold Nanorods. *Nano Lett.* 2008; 8 :1719–1723. [PubMed: 18454558]
19. Fernanda Cardinal M, Rodríguez-González B, Alvarez-Puebla RA, Pérez-Juste J, Liz-Marzán LM. Modulation of Localized Surface Plasmons and SERS Response in Gold Dumbbells through Silver Coating. *J Phys Chem C.* 2010; 114 :10417–10423.
20. Thompson DG, Enright A, Faulds K, Smith WE, Graham D. Ultrasensitive DNA Detection Using Oligonucleotide-Silver Nanoparticle Conjugates. *Anal Chem.* 2008; 80 :2805–2810. [PubMed: 18307361]
21. Huang X, Neretina S, El-Sayed MA. Gold Nanorods: From Synthesis and Properties to Biological and Biomedical Applications. *Adv Mater.* 2009; 21 :4880–4910. [PubMed: 25378252]
22. West PR, Ishii S, Naik GV, Emani NK, Shalae VM, Boltasseva A. Searching for Better Plasmonic Materials. *Laser Photonics Rev.* 2010; 4 :795–808.
23. Pal S, Deng Z, Ding B, Yan H, Liu Y. DNA-Origami-Directed Self-Assembly of Discrete Silver-Nanoparticle Architectures. *Angew Chem.* 2010; 49 :2700–2704. [PubMed: 20235262]
24. Weller L, Thacker VV, Herrmann LO, Hemmig EA, Lombardi A, Keyser UF, Baumberg JJ. Gap-Dependent Coupling of Ag–Au Nanoparticle Heterodimers Using DNA Origami-Based Self-Assembly. *ACS Photonics.* 2016; 3 :1589–1595.

25. Roller E-M, Besteiro LV, Pupp C, Khorashad LK, Govorov AO, Liedl T. Hotspot-Mediated Non-Dissipative and Ultrafast Plasmon Passage. *Nat Phys.* 2017; 13 :761. [PubMed: 28781603]
26. Yu D, Tian W, Sun B, Li Y, Wang W, Tian W. Preparation of Silver-Plated Wool Fabric with Antibacterial and Anti-Mould Properties. *Mater Lett.* 2015; 151 :1–4.
27. Liu M, Guyot-Sionnest P. Synthesis and Optical Characterization of Au/Ag Core/Shell Nanorods. *J Phys Chem B.* 2004; 108 :5882–5888.
28. Pande S, Ghosh SK, Praharaj S, Panigrahi S, Basu S, Jana S, Pal A, Tsukuda T, Pal T. Synthesis of Normal and Inverted Gold-Silver Core-Shell Architectures in β -Cyclodextrin and Their Applications in SERS. *J Phys Chem C.* 2007; 111 :10806–10813.
29. Chen H, Shao L, Li Q, Wang J. Gold Nanorods and Their Plasmonic Properties. *Chem Soc Rev.* 2013; 42 :2679–2724. [PubMed: 23128995]
30. Xu H, Kan C, Miao C, Wang C, Wei J, Ni Y, Lu B, Shi D. Synthesis of High-Purity Silver Nanorods with Tunable Plasmonic Properties and Sensor Behavior. *Photonics Res.* 2017; 5 :27–32.
31. Cho EC, Camargo PH, Xia Y. Synthesis and Characterization of Noble-Metal Nanostructures Containing Gold Nanorods in the Center. *Adv Mater.* 2010; 22 :744–748. [PubMed: 20217782]
32. Pietrobon B, McEachran M, Kitaev V. Synthesis of Size-Controlled Faceted Pentagonal Silver Nanorods with Tunable Plasmonic Properties and Self-Assembly of These Nanorods. *ACS Nano.* 2008; 3 :21–26.
33. Ramasamy P, Seo D-M, Kim S-H, Kim J. Effects of TiO₂ Shells on Optical and Thermal Properties of Silver Nanowires. *J Mater Chem.* 2012; 22 :11651–11657.
34. Sasikumar T, Ilanchelian M. Colorimetric Detection of Hypochlorite Based on the Morphological Changes of Silver Nanoprisms to Spherical Nanoparticles. *Anal Methods.* 2017; 9 :3151–3158.
35. Wiley BJ, Im SH, Li Z-Y, McLellan J, Siekkinen A, Xia Y. Maneuvering the Surface Plasmon Resonance of Silver Nanostructures through Shape-Controlled Synthesis. *J Phys Chem B.* 2006; 110 :15666–15675. [PubMed: 16898709]
36. Sun Y, Yin Y, Mayers BT, Herricks T, Xia Y. Uniform Silver Nanowires Synthesis by Reducing AgNO₃ with Ethylene Glycol in the Presence of Seeds and Poly (Vinyl Pyrrolidone). *Chem Mater.* 2002; 14 :4736–4745.
37. Park K, Vaia RA. Synthesis of Complex Au/Ag Nanorods by Controlled Overgrowth. *Adv Mater.* 2008; 20 :3882–3886.
38. Kottmann JP, Martin OJ, Smith DR, Schultz S. Plasmon Resonances of Silver Nanowires with a Nonregular Cross Section. *Phys Rev B.* 2001; 64 :235402
39. Xiang Y, Wu X, Liu D, Li Z, Chu W, Feng L, Zhang K, Zhou W, Xie S. Gold Nanorod-Seeded Growth of Silver Nanostructures: From Homogeneous Coating to Anisotropic Coating. *Langmuir.* 2008; 24 :3465–3470. [PubMed: 18294010]
40. Jana NR, Gearheart L, Murphy CJ. Seed-Mediated Growth Approach for Shape-Controlled Synthesis of Spheroidal and Rod-Like Gold nanoparticles Using a Surfactant Template. *Adv Mater.* 2001; 13 :1389–1393.
41. Liu B, Liu J. Freezing Directed Construction of Bio/Nano Interfaces: Reagentless Conjugation, Denser Spherical Nucleic Acids, and Better Nanoflakes. *J Am Chem Soc.* 2017; 139 :9471–9474. [PubMed: 28661156]
42. Koo KM, Sina AA, Carrascosa LG, Shiddiky MJ, Trau M. DNA-Bare Gold Affinity Interactions: Mechanism and Applications in Biosensing. *Anal Methods.* 2015; 7 :7042–7054.
43. Hu S, Huang P-JJ, Wang J, Liu J. Phosphorothioate DNA Mediated Sequence-Insensitive Etching and Ripening of Silver Nanoparticles. *Front Chem.* 2019; 7
44. Hartl C, Frank K, Amenitsch H, Fischer S, Liedl T, Nickel B. Position Accuracy of Gold Nanoparticles on DNA Origami Structures Studied with Small-Angle X-Ray Scattering. *Nano Lett.* 2018; 18 :2609–2615. [PubMed: 29498287]
45. Jette ER, Foote F. Precision Determination of Lattice Constants. *J Chem Phys.* 1935; 3 :605–616.
46. Berova N, Di Bari L, Pescitelli G. Application of Electronic Circular Dichroism in Configurational and Conformational Analysis of Organic Compounds. *Chem Soc Rev.* 2007; 36 :914–931. [PubMed: 17534478]

47. Guerrero-Martínez A, Auguie B, Alonso-Gómez JL, Džoli Z, Gómez-Graña S, Žini M, Cid MM, Liz-Marzán LM. Intense Optical Activity from Three-Dimensional Chiral Ordering of Plasmonic Nanoantennas. *Angew Chem.* 2011; 50 :5499–5503. [PubMed: 21506211]
48. Lee H-E, Ahn H-Y, Mun J, Lee YY, Kim M, Cho NH, Chang K, Kim WS, Rho J, Nam KT. Amino-acid- and peptide-directed synthesis of chiral plasmonic gold nanoparticles. *Nature.* 2018; 556 :360–365. [PubMed: 29670265]
49. Ye X, Jin L, Caglayan H, Chen J, Xing G, Zheng C, Doan-Nguyen V, Kang Y, Engheta N, Kagan CR, Murray CB. Improved Size-Tunable Synthesis of Monodisperse Gold Nanorods through the Use of Aromatic Additives. *ACS Nano.* 2012; 6 :2804–2817. [PubMed: 22376005]

Figure 1.

(a) Experimental extinction spectra Au/AgNRs@DNA synthesized using different AgNO₃ concentrations (10 – 40 mM) resulting in distinct Ag shell thicknesses from ~1.1 (red line) to ~5.4 nm (blue line), cf. Figure S4 for the respective non-normalized extinction spectra (b) Calculated theoretical extinction spectra of Au/AgNRs in water for the Ag shells of 1.1 (red line), 3.1 (yellow line), and 5.4 nm (blue line). (c) TEM images of Au/AgNRs@DNA with different Ag shell thicknesses (~1.1, ~3.1, and ~5.4 nm) tuned by raising AgNO₃ ratio (10, 25, 40 mM). Scale bars are 40 nm. (d) Field maps of Au/AgNRs synthesized with 25

mM AgNO₃ with a 3.1 nm shell, showing values of $|\mathbf{E}|/E_0$, or the near-field enhancement of the particles. The left panel shows the stronger longitudinal modes, while the right panel depicts the transversal modes. Here we also show schematically the charges induced by the plasmonic modes.

Figure 2.

(a) Small-angle x-ray scattering (SAXS) intensities (dots), with model fits based on cylindrical core-shell-shell particles (lines). Intensities were recorded at two detector positions, 5.1 m and 2.0 m from the sample, and the model was refined to both datasets simultaneously. Data were scaled for clarity. Inset: Schematic sketch of a core-shell-shell cylinder particle. (b) Wide-angle x-ray scattering (WAXS) data.

Figure 3. Agarose gel electrophoresis and TEM image of DNA-functionalized (a) 78×25 nm Au/AgNRs conjugated with a 14 HB DNA origami rod and (b) 64×25 nm Au/AgNRs conjugated with a 44 HB double-layered sheet DNA origami, and the respective illustration of the Au/AgNRs@DNA@origami conjugates. Scale bars are 50 nm.

Figure 4. CD spectra of chiral AuNRs (55×20 nm, red curve) and Au/AgNRs ($\sim 65 \times 30$ nm, Ag shell thickness ~ 5 nm, blue curve), assembled on a 44 HB double-layered sheet DNA origami (cf. Figure 3b) displaying the characteristic bisignate shape. The signal is blue-shifted and strongly increased for the Au/AgNR assembly compared to the AuNR assembly.

Scheme 1.

One-pot synthesis of DNA-functionalized Au/Ag core-shell nanorods denoted Au/AgNRs@DNA. We show that the particle's aspect ratio as well as their core and shell sizes and thus the respective localized surface plasmon resonance (LSPR) can be fine-tuned as desired. The particle's biocompatibility increases further by *in situ* conjugation to DNA. Since thiolated DNA is used as the initial stabilizing ligand, further ligand replacement steps become obsolete. We demonstrate that these particles can easily be assembled on DNA origami templates for studying and understanding cooperative and synergistic effects of such bimetallic plasmonic systems. We furthermore show, that besides DNA, other thiol-containing ligands can be incorporated in the ligand shell, rendering our approach simple and universal.